BDA The Association of UK Dietitians

THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION

Editor: **Simon Langley-Evans**

Journal of Human Nutrition and Dietetics

VOLUME 30 • ISSUE 3 • JUNE 2017

- INTERVENTION STUDIES
- CLINICAL NUTRITION
- PUBLIC HEALTH NUTRITION
- DIETETIC PRACTICE AND BEHAVIOUR CHANGE

WILEY

Journal of Human Nutrition and Dietetics

The Official Journal of the British Dietetic Association

Aims and editorial policy

Journal of Human Nutrition and Dietetics is an international peer reviewed journal publishing papers in applied nutrition and dietetics. Papers are therefore welcomed on:

- Clinical nutrition and the practice of therapeutic dietetics
- Public health nutrition and nutritional epidemiology
- Health promotion and intervention studies and their effectiveness
- Food choice and the psychology of eating behaviour
- Food intake and nutritional status
- Sociology of food intake

Further information on this journal can be accessed at wileyonlinelibrary.com/journal/jhn

The *Journal of Human Nutrition and Dietetics* is published by Blackwell Publishing Ltd: 9600 Garsington Road, Oxford OX4 2DQ, UK. Tel: +44 1865 776868; Fax: +44 (0)1865 714591. Blackwell Publishing was acquired by John Wiley & Sons in February 2007. Blackwell's programme has been merged with Wiley's global Scientific, Technical and Medical business to form Wiley Blackwell.

Production Editor: Patricia Joyce Tulayan (email: jhn@wiley.com)

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

INTERVENTION STUDIES

Dietary and nutrition interventions for the therapeutic treatment of chronic fatigue syndrome/myalgic encephalomyelitis: a systematic review

N. Campagnolo,[1,2] S. Johnston,[1,2] A. Collatz,[1,2] D. Staines[2] & S. Marshall-Gradisnik[1,2]

[1]School of Medical Science, Griffith University, Gold Coast, QLD, Australia
[2]National Centre for Neuroimmunology and Emerging Diseases, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia

Keywords
diet therapy, public health, supplements, systematic review, vitamins.

Correspondence
N. Campagnolo, National Centre for Neuroimmunology and Emerging Diseases, Menzies Health Institute Queensland, School of Medical Science, Griffith Health Centre (G40), 9.21, Griffith University, Gold Coast, Queensland 4222, Australia.
Tel: +61 (7) 5678 9283
E-mail: nadia.campagnolo@griffithuni.edu.au

How to cite this article
Campagnolo N., Johnston S., Collatz A., Staines D., & Marshall-Gradisnik S. (2017) Dietary and nutrition interventions for the therapeutic treatment of chronic fatigue syndrome/myalgic encephalomyelitis: a systematic review. *J Hum Nutr Diet*. **30**, 247–259
doi: 10.1111/jhn.12435

Abstract

Background: Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is characterised by unexplained fatigue for at least 6 months accompanied by a diverse but consistent set of symptoms. Diet modification and nutritional supplements could be used to improve patient outcomes, such fatigue and quality of life. We reviewed and discussed the evidence for nutritional interventions that may assist in alleviating symptoms of CFS/ME.
Methods: Medline, Cinahl and Scopus were systematically searched from 1994 to May 2016. All studies on nutrition intervention were included where CFS/ME patients modified their diet or supplemented their habitual diet on patient-centred outcomes (fatigue, quality of life, physical activity and/or psychological wellbeing).
Results: Seventeen studies were included that meet the inclusion criteria. Of these, 14 different interventions were investigated on study outcomes. Many studies did not show therapeutic benefit on CFS/ME. Improvements in fatigue were observed for nicotinamide adenine dinucleotide hydride (NADH), probiotics, high cocoa polyphenol rich chocolate, and a combination of NADH and coenzyme Q10.
Conclusions: This review identified insufficient evidence for the use of nutritional supplements and elimination or modified diets to relieve CFS/ME symptoms. Studies were limited by the number of studies investigating the interventions, small sample sizes, study duration, variety of instruments used, and studies not reporting dietary intake method. Further research is warranted in homogeneous CFS/ME populations.

Introduction

Chronic fatigue syndrome, also known as myalgic encephalomyelitis (CFS/ME), is a chronic, disabling illness characterised by unexplained persistent and debilitating fatigue, and this is accompanied by a diverse but consistent set of symptoms. CFS/ME has an unknown aetiology, as well as no known specific pathogenesis; therefore, there is no diagnostic pathology test. Rather, diagnosis is one of disease exclusion, and is made in accordance with symptom-specific criteria [1,2]. Prior to year 1994, many case definitions were used in research to aid in the diagnosis of this condition, thus limiting comparisons of published studies [3]. The Fukuda (1994) criteria was established to overcome inconsistency in the application of case definitions, as well as to assist in defining a distinct group of cases [2], and is now the most frequently used case definition. To meet the Fukuda [2]

criteria, a patient must have debilitating unexplained fatigue present for at least 6 months that is not explained by ongoing exertion or medical or psychiatric conditions, and is not alleviated by rest. In addition, the fatigue must be accompanied by four or more of the following symptoms: post-exertional malaise, difficulty with short-term memory or concentration, unrefreshed sleep, sore throat, muscle and/or joint pain, headaches, and tender lymph nodes [2]. A more recent, and alternative diagnostic criteria for CFS/ME, is the International Consensus Criteria (ICC) [1], which identifies distinct CFS/ME symptoms associated with neurological, immunological, gastrointestinal and energy production impairments [1].

The current worldwide prevalence of CFS/ME is estimated to be between 0.8% and 3.3% [4]. In the USA in 2008, the treatment and management of CFS/ME was estimated to cost US$319 million annually, with a direct cost of US$7406 per patient [5]. This syndrome is heterogeneous in nature, with CFS/ME patients reporting different accompanying symptoms, at different severities [1], frequency (continuous or intermittent symptoms) and duration [6]. Many CFS/ME patients experience significant cognitive and physical impairment and, consequently, a substantial decline in social, occupational, educational and personal activity [7]. Thus, CFS/ME significantly affects and interferes with everyday life and patients relationships.

Many CFS/ME patients complain of gastrointestinal symptoms, including but not limited to early satiety, abdominal distension and/or pain, nausea, vomiting and altered bowel habits [8,9]. Additionally, irritable bowel syndrome (IBS) [8–11], a functional disorder of the gastrointestinal tract, coeliac disease and food intolerance (e.g. wheat and dairy) [9] are frequently observed in CFS/ME patients.

CFS/ME patients report a high use of nutritional supplements [12,13] and approximately 50% of patients have reported food intolerances [9–12] and benefit from dietary modification [12]. Therefore, dietary therapy, including diet modification and/or provision of dietary supplements, may be beneficial in alleviating symptoms and reducing fatigue in CFS/ME. The present study aimed to systematically review original research investigating nutrition interventions in the symptom management of CFS/ME patients measured using patient-centred outcomes including fatigue, quality of life, physical activity and/or psychological wellbeing).

Materials and methods

Literature search

Three databases were utilised: Medline (EBSCOhost), Cinahl (EBSCO) and Scopus. The following terms were systematically searched as full-text and Medical Subject Headings (MeSH) terms (Medline and Cinahl): syndrome, chronic fatigue (which includes chronic fatigue syndrome and myalgic encephalomyelitis) and food, diet, nutrition therapy, diet therapy, vitamins, minerals, micronutrients, dietary supplements and/or nutritional supplements. Search results were limited to English language, publication date (year 1994–2016) and humans (all databases except Scopus). A secondary search was completed, whereby included studies and review articles were reviewed for forward citations and to identify other eligible studies. The final search was completed on 6 May 2016.

Inclusion and exclusion criteria

Studies that fulfilled the following criteria were eligible for inclusion: (i) all studies that were intervention research, defined as studies that evaluated the effectiveness of food and/or nutritional supplement on outcome measures; (ii) CFS/ME diagnosis according to Fukuda [2], Canadian (2003) [14] or International Consensus Criteria (ICC) (2011) [1]; (iii) adults aged 18 years and over; (iv) studies that had accessible full-text articles written in English; (v) year searched 1994 to present to exclude earlier studies prior to 1994 Fukuda criteria [2,15]; and (vi) studies comprising journal articles based on original research. The primary outcome of interest for this review was fatigue. Secondary outcomes evaluated were quality of life, physical activity and psychological wellbeing. Studies were excluded if they explicitly combined CFS/ME with other patient groups [e.g. CFS/ME and fibromyalgia (FMS)]. Although CFS/ME often co-occurs with FMS and other disorders such as IBS [1], the co-occurrence of FMS was excluded to understand the effect of nutrition interventions on outcome measures specifically in CFS/ME. Studies that used multi-treatments (e.g. nutrition and pharmaceutical treatment), duplicate studies, case reports/studies or review articles and studies not meeting the above inclusion criteria were also excluded.

Selection of studies

Titles and abstracts for each article were initially screened on the basis of eligibility criteria. Two review authors independently assessed full-text articles for suitability for inclusion in this review, and study quality, followed by a research meeting of all team members that confirm articles for inclusion in this review.

Data extraction and quality assessment

Eligible studies were read and the relevant data were extracted (Tables 1–3) including: (i) study design; (ii) CFS/ME case definition; (iii) country; (iv) sample size; (v) age of participants (vi) sex, percentage of female

Table 1 Summary of participant and study characteristics of the included studies

Reference	Study design	Dx	Country	Sample (*n*)		Age (years), mean (SD)		Sex, female %		Illness duration, Mean (SD)		BMI (kg m^{-2}), mean (SD)		Weight (kg), mean (SD)	
				Tx	Con	Tx	Con	Tx	Con	Tx	Con	Tx	Con	Tx	Con
Fukuda *et al.* [15]	RCT, PAR	F	Japan	17	14	34.8 (9.36)	39.5 (8.50)	76	86	NR	NR	NR	NR	NR	NR
Fukuda *et al.* [15] (Pilot)	Cohort, OPT	F	Japan	20	–	36.8 (6.88)	–	75	–	123 (64.2)	–	NR	–	NR	–
Ostojic *et al.* [26]	RCT, CO	F	Serbia	21	–	39.3 (8.8)	–	100	–	–	–	NR	NR	62.8 (8.5)	–
Castro-Marrero *et al.* [21]	RCT, PAR	F	Spain	39	34	NR	NR	100	100	15.4 (8.9)	14.7 (6.2)	26.7 (5.2)	25.9 (2.4)	68.5 (14.6)	72.1 (13.7)
Witham *et al.* [24]	RCT, PAR	F, C	Scotland	25	25	48.1 (12.0)	50.7 (13.1)	72	80	NR	NR	28.8 (7.9)	29.8 (5.4)	NR	NR
Maric *et al.* [30]	Cohort, PRO	F	Serbia	38	–	NR	–	100	–	NR	–	NR	–	NR	–
Sathyapalan *et al.* [32]	RCP, CO	F	UK	10	–	52 (8)	–	60	–	NR	–	28.3 (2)	–	NR	–
Sullivan *et al.* [33]	Cohort, OPT	F	Sweden	15	–	43	–	67	–	NR	–	NR	–	NR	–
Rao *et al.* [31]	RCP, PAR	Ca	Canada	19	16	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hobday *et al.* [27]	CS, NC	F	UK	25	27	44 (10.2)	42.3 (11.9)	88	78	NR	NR	NR	NR	NR	NR
The *et al.* [23]	RCT, PAR	F	NLD	30	27	40.9 (9.4)	43.4 (11.2)	77	59	NR	NR	NR	NR	NR	NR
McDermott *et al.* [22]	RCT, PAR	F	UK	37	34	42 (15)	43 (12)	76	68	NR	NR	NR	NR	NR	NR
Vermeulen *et al.* [28]	CS, NC	F	AMS	30	30 30	37 (11)	38 (11) 42 (12)	77	77 77	5.5 (1–23)*	3.0 (0.5–25)* 6.0 (1.0–21)*	NR	NR	NR	NR
Brouwers *et al.* [20]	RCT, PAR	F	NLD	27	26	40.0 (9.9)	38.9 (10.9)	74	65	8.0 (2–15)*	4.5 (2–10)*	NR	NR	NR	NR
Ockerman *et al.* [25]	RCT, CO	F	Sweden	22	–	50	–	86	–	NR	–	NR	–	NR	–
Plioplys *et al.* [29]	CS, NC, CO	F	USA	28	–	40 (18–67)*	–	57	–	5.0 (1–20)*	–	NR	–	NR	–
Forsyth *et al.* [6]	RCT, CO	F	USA	26	–	39.6	–	65	–	7.2	–	NR	–	NR	–

AMS, Amsterdam; BMI, body mass index; C, Canadian case definition [14]; Ca, Carruthers clinical case definition; CO, cross-over design; Con, control group; CS, comparative study design; Dx, case definition for CFS/ME diagnosis; F, Fukuda (1994)[2]; NC, no comparative controls; NLD, Netherlands, NR, not reported; OPT, Open labelled pilot trial; PAR, parallel design; PRO, prospective design; RCP, randomised control pilot study; RCT, randomised control study; Tx, treatment group; –, not applicable.
*Median (range) in years.

Table 2 Study intervention characteristic and results for the primary outcome of fatigue

Reference	Duration (weeks)*	Washout period^	Treatment intervention	Control intervention	Fatigue outcome	Fatigue result
Fukuda *et al.* [15]	12	–	Ubiquinol-10 (150 mg day^{-1} post meal)	Placebo	Chalder Fatigue Scale	NS
Fukuda *et al.* [15] (Pilot)	8	–	Ubiquinol-10 (150 mg day^{-1} post meal)	–	Chalder Fatigue Scale	NS
Ostojic *et al.* [26]	3 months	2 months	GAA (2.4 g day^{-1})	Placebo (cellulose)	Multidimensional Fatigue Inventory	NS for general or physical fatigue subscales. Significantly different for mental fatigue, activity and motivation ($P < 0.05$).
Castro-Marrero *et al.* [21]	8	–	NADH (200 mg day^{-1}) + CoQ_{10} (20 mg day^{-1})	Placebo	Fatigue Index Symptom Questionnaire (FIS-40)	Decreased ($P < 0.05$)
Witham *et al.* [24]	6 months	–	Vitamin D_3 (100 000 units orally every 2 months)	Placebo	Piper Fatigue Scale	NS
Maric *et al.* [30]	2 months	–	Multivitamin mineral supplement (Supradyn®)	–	Fibro Fatigue Scale (FFS)	Unclear, inconsistent data reported for total FFS. Fatigue subscale significant ($P = 0.0009$)
Sathyapalan *et al.* [32]	8	2 weeks	High cocoa liquor/ polyphenols rich chocolate (85% cocoa solids; 2.28 MJ/100 g; 15 g bar three times daily)	Cocoa liquor free/low polyphenols chocolate (2.29 MJ/100 g; 15 g bar three times daily)	Chalder Fatigue Scale	Decreased ($P = 0.01$)
Sullivan *et al.* [33]	4	–	Probiotic bacteria (2 dL of Cultura Dofilus natural yogurt twice daily for 30 days. *Lactobacillus* F19, *Lactobacillus acidophilus* NCFB 1748 and *Bifidobacterium lactis* Bb12)	–	Visual analogue Scales for general, muscle and neurocognitive fatigue	NS for general fatigue. Improved neurocognitive function ($P = 0.040$)
Rao *et al.* [31]	8		Probiotic bacteria (8 billion cfu of *Lactobacillus casei*, Shirota)	Placebo	NR	NR
Hobday *et al.* [27]	24	–	Low sugar low yeast diet + consult at baseline and 24 weeks + phone call monthly	Healthy eating diet† + consult at baseline and 24 weeks + phone call monthly	Chalder Fatigue Scale	NS
The *et al.* [23]	14		Acclydine	Placebo	CIS-fatigue Daily Observed Fatigue	NS
McDermott *et al.* [22]	8	–	BioBran MGN-3 (6 g; 2 g three times per day dissolved in water or milk)	Placebo	Chalder Fatigue Scale	NS

Table 2. Continued

Reference	Duration (weeks)*	Washout period^	Treatment intervention	Control intervention	Fatigue outcome	Fatigue result
Vermeulen *et al.* [28]	24	–	Acetyl-L-carnitine (ALC) (2 g day^{1}; post BF)	Propionyl-L-carnitine (PLC) (2 g day^{1}; post BF) OR ALC + PLC (2 g ALC + 2 g PLC day^{1}; post BF)	Multidimensional Fatigue Inventory	Within group analysis showed that ALC significantly improved mental fatigue ($P = 0.015$) PLC and ALC + PLC significantly improved general fatigue.
Brouwers *et al.* [20]	10	–	Multinutritional supplement, twice daily	Placebo	CIS-fatigue Daily Observed Fatigue	NS
Ockerman *et al.* [25]	3 months	2 weeks	Pollen and pistil extract (Polbax) (7 × tablets in 1 dose at BF)	Placebo	Subjective Symptom	No between group analyses. Decrease in self-reported symptoms in treatment group
Plioplys *et al.* [29]	8	2 weeks	L-carnitine (1 g three times daily)	Amantadine‡ (100 mg once daily (morning) for 4 weeks and then increased to 100 mg twice daily (morning and afternoon)	Fatigue Severity Scale	NS
Forsyth *et al.* [6]	4	4 weeks	NADH (10 mg day^{-1} with water, 45 min before BF, fasted)	Placebo	50 item questionnaire based on CDC criteria for CFS/ME	No between group comparisons. Within group analysis showed that 31% & 8% of patients benefited from NADH versus PBO, respectively ($P < 0.05$)

BF, Breakfast; CDC, Centers for Disease Control and Prevention; CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; CFU, colony-forming units; CIS-Fatigue, Checklist Individual Strength subscale fatigue severity; CoQ10, coenzyme Q10; GAA, guanidinoacetic acid; NADH, nicotinamide adenine dinucleotide hydride; NR, not reported; NS, not significant; PBO, placebo; VAS, Visual Analog Scale.

*Duration reported for cross-over trials is duration of each intervention, excluding washout period.

†Healthy eating diet as per Department of Health guidelines (COMA, 1991) for the general population.

‡Results are outside the scope of this systematic review.

^Washout period between crossover groups.

Table 3 Secondary outcome measure of psychological wellbeing, quality of life and physical activity level

Reference	Secondary outcome measure(s)	Results
Psychological wellbeing		
Fukuda *et al.* [15]	CES-D	NS
Fukuda *et al.* [15] (Pilot)	CES-D	NS Improvement in depressive symptoms was dependant on increase in total plasma CoQ10 levels
Witham *et al.* [24]	HADS	NS
Sathyapalan *et al.* [32]	HADS	Significant decrease in anxiety and depression ($P = 0.01$)
Rao *et al.* [31]	Beck Depression Inventory Beck Anxiety Inventory	NS Significant decrease in anxiety symptoms ($P = 0.01$)
Hobday *et al.* [27]	HADS	NS
McDermott *et al.* [22]	HADS	NS
Plioplys *et al.* [29]	Beck Depression Inventory Symptom Checklist 90-R	Significant decrease in depression at 8 weeks ($P = 0.22$) Significant decrease in somatisation ($P = 0.012$), obsessive-compulsive ($P = 0.036$), anxiety ($P = 0.006$) and depression ($P = 0.006$) subscale; and all summary scales including GSI ($P = 0.007$), PSDI ($P = 0.000$) and PSTI ($P = 0.038$) at 8 weeks
Quality of life		
Ostojic *et al.* [26]	SF-36	Significant improvement in physical ($P = 0.04$) and mental common scores ($P = 0.00$)
Maric *et al.* [30]	SF-36	NS
Sathyapalan *et al.* [32]	London Handicap Scale	Significant increase in residual function ($P = 0.01$)
Sullivan *et al.* [33]	SF-12 Health Survey	NS
Hobday *et al.* [27]	MOS SF-36	NS
The *et al.* [23]	SIP-8 score	NS
McDermott *et al.* [22]	WHO QOL-BREF	Social wellbeing subscale only ($P = 0.02$)
Vermeulen *et al.* [28]	CGI	Within group analysis showed improvement in ALC and PLC but not ALC + PLC
Brouwers *et al.* [20]	CDC symptom checklist SIP-8 score	NS NS
Plioplys *et al.* [29]	CFS Impairment Index CFS Severity Index	Within group analysis showed significant improvement in total function ($P = 0.001$) and physical ($P = 0.000$) and mental ($P = 0.038$) subsets at 8 weeks Significant improvement in function ($P = 0.031$)
Physical activity level		
Ostojic *et al.* [26]	Actigraphic assessment Energy expenditure, duration and intensity	NS NS
The *et al.* [23]	Actigraphic assessment	NS
Brouwers *et al.* [20]	Actigraphic assessment	NS

CDC, Centers for Disease Control and Prevention; CES-D, Centre for Epidemiologic Studies Depression Scale; CGI, Clinical Global Impression of Change; CoQ10, coenzyme Q10; GSI, Global Severity Index; HADS, Hospital Anxiety and Depression Score; LHS, London Handicap Scale; MOS SF-36, Medical Outcomes Survey Short Form; NS, no significant difference; PA, physical activity; PSDI, Positive Symptom Distress Index; PSTI, Positive Symptom Total Index; SF-36, Quality of Life Scale; SIP-8, Sickness Impact Scale.

participants; (vii) illness duration; (viii) body mass index; (ix) weight; intervention duration; washout period between trials; (x) nutrition intervention(s) being investigated; (xi) name of instrument to evaluate study outcomes; and (xii) result of intervention and level statistical significance.

To evaluate study quality and bias, the Rosendal scale [16], which combines the PEDro scale [17], Jadad scoring system [18] and Delphi List [19], was utilised (see Supporting information, Table S1). Items 15 and 16 of the Rosendal scale were excluded because outcomes associated with exercise performance (e.g. $VO_{2\ max}$) were not relevant to this review. Item 15 was replaced to included assessment of if an appropriate washout period was used for cross-over trials because of its relevance to nutrition interventions. A Rosendal score cut-off of 60% is classified as excellent methodological quality [16].

Results

Overview of studies and study quality

Figure 1 shows the PRISMA flow diagram with the number of included and excluded studies. Seventeen studies were included in this systematic review of nutrition and

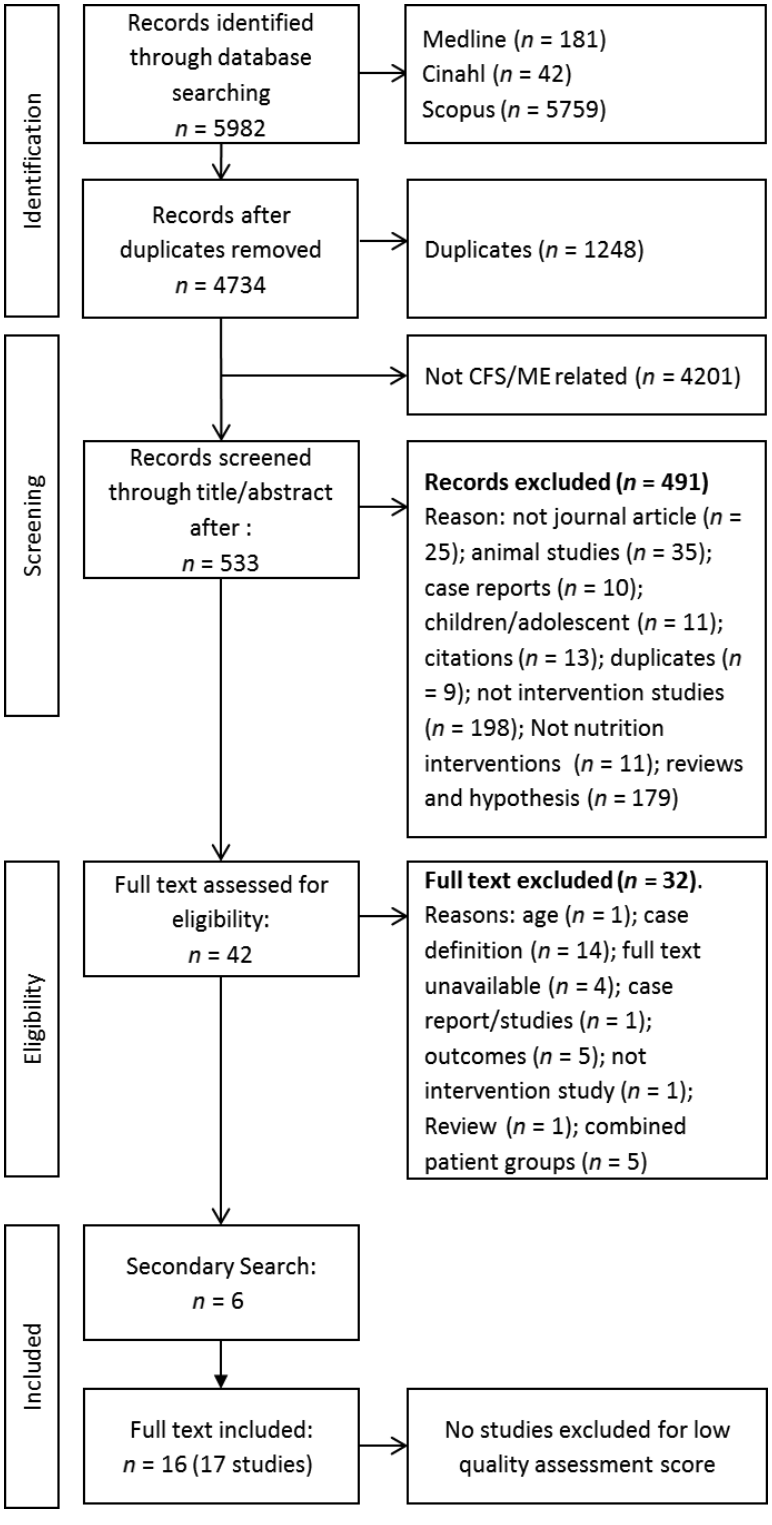

Figure 1 Flow diagram of database and secondary search for included studies in the review of nutrition intervention on chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) outcomes.

dietary interventions in CFS/ME patients. The included studies were six randomised control trial (RCT) parallel designs [15,20–24]; three RCT cross-over designs where patients were their own control [6,25,26]; three comparative studies without concurrent control (two- or three-arm parallel groups) [27–29]; one prospective cohort study (before and after) [30]; and four pilot studies [15,31–33]. All RCT (parallel and cross-over) [6,15,20–26] and two pilot

studies [31,32] reported using a double-blind design. Studies varied in study quality, with the Rosendal score ranging from 10% to 86%. Eleven of the 17 studies (65% of included studies) had a Rosendal score of >60% [6,15,20–24,26–28,32] (see Supporting information, Table S1).

Participant and study characteristics

The participant characteristics of the included studies are summarised in Table 1. For the diagnosis of CFS/ME, 15 studies used the Fukuda (1994) case definition [6,15,20–23,25–30,32,33], one study used Carruthers (2003) [31] and one combined Fukuda (1994) and the Canadian Clinical Case Definition [24]. Two studies also included participants with high fatigue severity measured with subjective fatigue subscale of the Checklist Individual Strength (CIS-fatigue ≥ 40) [20] and Chandler Fatigue Scale (score 10/11) [32]. The mean sample size for each study was approximately 24 participants.

All studies, except two, reported fatigue as the primary outcome, and one study did not report fatigue at all [31]. Of the 17 studies, five studies used the Chalder Fatigue Scale [15,22,27,32], three studies used an investigator designed symptom questionnaire [6,25,33], two studies used the Multidimensional Fatigue Inventory [26,28], and two studies used both the Checklist Individual Strength Fatigue Subscale and Daily Observed Fatigue [20,23]. All other studies used either the Fatigue Index Symptom Questionnaire (FIS-40) [21], Fatigue Severity Scale [29], Fibro Fatigue Scale [30] and Piper Fatigue Scale [24]. No trials reported a dietary intake method (diet record or food frequency questionnaires) for measurement at baseline or at the end of the study period to determine whether dietary changes occurred.

Interventions on fatigue

In total, 14 different interventions were evaluated (Table 2). All randomised control trials, including pilot studies, compared a form of nutritional supplement with a placebo control [6,15,20–26,31]. One RCT compared the combined effect of nicotinamide adenine dinucleotide hydride (NADH) and coenzyme Q10 (CoQ10) with a placebo, and found a significant reduction of fatigue after 8 weeks of treatment compared to placebo ($P < 0.05$) [21]. Another RCT observed a significant decrease in mental fatigue after guanidinoacetic acid (GAA) supplementation compared to a placebo [26]. One RCT tested the effect of pollen and pistil extract and a placebo after 3 months of supplementation on fatigue [25]. In the treatment group, a significant decrease in self-reported symptoms was observed, whereas no significant difference was observed for the placebo group from baseline [25]. A randomised control pilot study reported a significant decrease in self-reported fatigue after participants consumed high cocoa liquor (polyphenol rich chocolate) compared to iso-caloric chocolate for 8 weeks ($P = 0.01$) [32]. Other RCT either observed no significant difference between the nutrition intervention and placebo control [15,20,22–24] or did not report between group analysis [6,25]. One cohort study observed significant improvement in neurocognitive fatigue ($P = 0.040$) but not in general fatigue after probiotic bacteria supplementation [33]. All other studies reported no difference either compared to a comparative intervention [27] or after the intervention [15,29,30].

Interventions on secondary outcomes

Of the 17 studies, 14 studies evaluated the effect of the nutrition intervention on either quality of life ($n = 10$) [20,22,23,26–30,32,33], physical activity ($n = 3$) [20,23,26] and/or psychological wellbeing ($n = 8$) [15,22,24,27,29,31,32] (Table 3). Three studies observed improvement in psychological wellbeing [29,31,32]. One RCT study observed decrease in anxiety but not depression following probiotic supplementation [31]. Two comparative studies reported significant improvement in both anxiety and depression after consumption of polyphenol rich chocolate [32] and L-carnitine [29] compared to baseline. Consumption of L-carnitine also improved five other psychometric tests [29]. Two RCT observed significant difference in quality of life for supplementation of BioBran™ MGN-3 (Daiwa Pharmaceutical, Tokyo, Japan) (social wellbeing subscale only) [22] and guanidinoacetic acid [26]. Two comparative studies reported improvement in quality of life after supplementation of acetyl-L-carnitine and propionyl-L-carnitine [28], as well as L-carnitine [29]. No studies reported improvement in physical activity level; however, one study reported significant improvement in quadriceps isometric strength and $VO_{2\ max}$ after 3 months of supplementation of guanidinoacetic acid in patients compared with controls [26]. All other studies observed no difference of the nutrition intervention on any of the secondary outcomes [15,20,23,27,30,33].

Discussion

Elimination diets, dietary restriction and the addition of nutritional supplements to habitual diet are widely reported to be used by CFS/ME patients, and many individuals claim beneficial effects of these dietary interventions in reducing fatigue [12]. This systematic review has summarised the available evidence on dietary and nutritional interventions in CFS/ME on patient outcomes, including fatigue, quality of life, psychological wellbeing and physical activity level. Additionally, this review has

updated the literature search according to scientific accepted diagnostic criteria [34]. Our study highlights methodological limitations in the evidence and an overall lack of evidence that explores the therapeutic effect of diet and nutritional supplementation in CFS/ME.

Participant and study characteristics

Participants in this review were predominantly female, with a mean age of 35–50 years, residing in Europe. This finding is relatively consistent with epidemiological studies that report a higher prevalence of CFS/ME in females [7,9,35] and individuals aged 35 and 45 years [7,9]. However, this sex imbalance may be a consequence of studies recruiting participants from clinics or universities, and men being less likely to engage in help seeking behaviour from a healthcare professional [9].

The majority of studies in this review exclusively used the Fukuda (1994) case definition [2]. A common criticisms of this case definition include a combination of broad nonspecific symptoms [1,36], thus being less likely to identify a homogeneous patient population [1,36]. This may have contributed to a lack of sensitivity of the studies in this review to detect a beneficial effect of the nutritional treatment. Therefore, it is recommended that future research should employ more specific CFS/ME criteria to classify subgroups with similar symptoms and/or severity to identify those patients who may benefit from therapeutic nutrition treatment and counselling.

A variety of instruments, some validated and nonvalidated, were used to evaluate outcome measures in the included studies, thus highlighting a lack of agreement in the best instrument to measure improvement in CFS/ME symptoms, and limiting future comparisons between current and future studies. Therefore, to improve future intervention research, patient outcomes (e.g. fatigue, quality of life) need to be consistently measured using a single validated instrument for each outcome.

Additionally, many of the studies procedures indirectly excluded CFS/ME patients who were house and/or bed bound (i.e. severely affected patients) as a result of their inability to attend a clinic, hospital or university for screening and/or follow-up. Consequently, sampling bias is likely confounding results, and limits the results to CFS/ME patients with a mild severity of symptoms who have the ability to leave their home. Therefore, future research needs to consider the fluctuating nature and different severities of CFS/ME and thus design flexible protocols that are delivered in a variety of settings (e.g. in clinic or patient's homes) over the telephone or via the post. This will distinguish the effect of different dietary therapies, and identify whether patients with different severity of symptoms respond differently to the intervention.

Nutrition interventions

The aetiology of CFS/ME remains unclear; however, research suggests that this heterogeneous condition is likely a multisystem disorder involving the immune, gastrointestinal, neurological and metabolic systems [1]. A majority of the studies in this review investigated the effect of nutritional supplementation to initiate and promote ATP production with respect to reducing patient fatigue and cognitive dysfunction [6,15,21,29]. NADH alone [6] and in combination with CoQ10 [21] was observed to reduce fatigue in CFS/ME patients. Despite beneficial effects being observed, neither study reported a dietary intake method at baseline or at conclusion of the study to determine whether dietary changes occurred and potentially influenced the results. Furthermore, both studies are limited by small sample sizes and the duration of therapeutic treatment (4 and 8 weeks, respectively). Therefore, longitudinal studies with larger sample sizes are needed to determine whether NADH with and without CoQ10 has a prolonged therapeutic effect on CFS/ME patients. Furthermore, the study by Forsyth *et al.* [6] is also limited by the use of an investigator-developed questionnaire to measure symptom outcomes. Despite reproducibility testing being completed, it remains unclear whether this questionnaire was able to identify CFS/ME symptoms. It is recommended that future research uses validated instruments to enable confidence when interpreting results, and to allow comparison between studies.

Ubiquinol-10 (also known as coenzyme Q10) is an important nutrient for cellular energy production and for its antioxidant function [37]. Reduced levels of CoQ10 have been reported in CFS/ME patients compared to healthy controls [38]. Only one RCT has investigated supplementation of Ubiquinol-10 for 12 week in CFS/ME patients [15]. Despite no improvement in fatigue, measured with Chandler's Fatigue Scale, Ubiquinol-10 supplementation improved several other CFS/ME symptoms (e.g. night-time awakenings), which may have a longer-term effect on reducing fatigue in CFS/ME patients. To evaluate and demonstrate the benefits of ubiquinol-10 supplementation, longer-term controlled studies are needed.

Cocoa and dark chocolate consumption are known to have a number of positive health effect on chronic diseases [39–41]. This review has identified one randomised control cross-over study that investigated the therapeutic effect of cocoa with respect to decreasing fatigue and improving residual functions, as accessed by the London Handicap Scale, compared to when patients consumed an iso-caloric low polyphenol chocolate in CFS/ME patients [32]. Despite this positive result, the study is

limited by the small sample size (n = 10), treatment duration, and a lack of information on dietary habits and intake during the trial. Thus, it is unclear whether energy intake and the micro- and macronutrient composition of the diet influenced the result. Furthermore, long-term observational prospective research and well-designed RCTs are needed to confirm the clinical effects of cocoa in CFS/ME patients, as well as to understand the mechanism of different types of chocolate in this patient population.

Altered intestinal microbiota may contribute to pathogenesis of CFS/ME [42–44]. It has been postulated that the administration of probiotics may have therapeutic value in CFS/ME patients by decreasing pro-inflammatory cytokines and improving gut microbiota and mucosal barrier function [45]. This review identified two pilot studies each investigating different outcomes. These studies suggest that probiotic bacteria may improve neurocognitive function [33] and anxiety [31]. Furthermore, Sullivan *et al.* [33] observed individual differences regarding improvement in fatigue and physical activity, with patients reporting improvement in one or both areas. This provides further support for stratifying subgroups of CFS/ME patients likely to respond to a therapeutic nutrition treatment. As a result of the individual and preliminary benefits observed in these studies, the future research of probiotics is warranted in a larger homogeneous population.

Previous research suggests that oxidative stress results in CFS/ME patients as a result of diminished antioxidant capacity and/or reduced antioxidant enzymes activity [1,46]. The therapeutic treatment with antioxidants in CFS/ME is limited [20,25,30]. The studies identified in the present review suggest that the provision of pollen extract may improve CFS/ME symptoms and patients overall wellbeing [25]. However, no therapeutic benefits were observed in CFS/ME patients after multinutritional supplementation [20,30]. The results reported by Ockerman [25] are limited by a small sample size (n = 22) and an improvement in certain self-reported symptoms; thus, the reliability of results remains unclear.

CFS/ME is a complex condition with many symptoms, some of which may be related to the food and beverages consumed. This review identified only one study that evaluated an elimination diet in response to food sensitivities [27]. Therefore, future research may consider eliminating potential trigger foods in CFS/ME (e.g. alcohol, caffeine, fat, milk and dairy, gluten), followed by challenges to identify potential problem foods.

Quality assessment

Many of the studies included in this review were of high quality (Rosendal score >60%; Table S1), despite not reporting all methods (e.g. method of blinding, pre-trial conditions and method for assessing adverse effects). Those studies that received poorer quality scores were pilot or cohort or comparative studies and/or lacked full details of the methods. Future research should describe pretrial conditions and the method for evaluating blinding and accessing adverse effects, as well as the method of dietary assessment at baseline and at the trial conclusion.

Conclusions

Nutrition interventions may be used, in some chronic diseases (e.g. diabetes, cardiovascular disease, obesity), to manage or to minimise the progression of these condition. Therefore, it is reasonable to suggest that nutrition interventions may also improve patient outcomes, such as fatigue and quality of life, in CFS/ME patients. The conclusion of this review supports the current guidelines in that there is insufficient evidence for the use of nutritional supplements and elimination and modified diets with respect to relieving CFS/ME symptoms [47]. Therefore, the general prescription of supplements and long-term elimination diets is not recommended. Rather, recommendations are to eat a balanced diet and a variety of nutritious foods from the basic food groups [47] in accordance with the dietary guidelines for healthy people. However, supplementations may be considered as indicated [47]; for example, where CFS/ME patients have diagnoses of irritable bowel syndrome, lactose or gluten intolerance or coeliac disease; or have suspected inadequate nutrient intake; or in cases where nutrient deficiencies are identified via pathology tests.

Studies investigating nutritional interventions in CFS/ME remain very limited, with most interventions being evaluated in a single study. Furthermore, the present review emphasise that the interventions investigated have only been conducted in small sample sizes, and also lacked long-term follow-up (>6 months). Despite relative consistency in case definition, the studies differed with regard to inclusion and exclusion criteria and the reporting of participant characteristics (e.g. illness duration, BMI, weight). This heterogeneity in study design presents challenges when aiming to apply findings in the clinical environment. Therefore, longer-term randomised control trials in homogeneous populations that use more specific criteria are warranted.

Common comorbidities in CFS/ME include FMS and IBS [1]. The results of the present study are directly related to CFS/ME only because it did not assess effects on specific comorbid groups. Nutrition interventions targeted toward alleviating symptoms of FMS and IBS, for example, may also reduce the overall functional impact of CFS/ME. Hence, this is an important consideration for

future research to inform the dietetic practice, given the high prevalence of comorbidities among CFS/ME patients.

Dietary assessment methods and analysis were not described in any of the studies in the present review. Therefore, dietary variables, other than those being examined, may have confounded the results. The method of dietary assessment for future studies may be derived from a checklist devised by Nelson *et al.* [48] to adequately describe robust methodological approaches.

Furthermore, to control for dietary variables, it is recommended that future research: (i) assess the intervention for energy and nutrient content and (ii) assess habitual diet at baseline and study conclusion, as well as randomly throughout the trial. This will monitor compliance and minimise diet as a confounding factor in the interpretation of the results. Similarly, to control for physical activity, future research should also consider the use of wearable technology to monitor physical activity levels for the duration of the study.

From a clinical perspective, case studies derived from research with accredited dietitians may also provide additional evidence of benefits to specific cases of CFS/ME, as well as support patients with the dietary regime and fluctuating nature of CFS/ME symptoms.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
The Alison Hunter Memorial Foundation, Change for ME, Mason Foundation, the Stafford Medical Research Foundation, the Edward P Evans Foundation, Queensland Smart State and Advance Queensland provided continued support and funding.
NC designed the study and search strategy, and also performed primary and secondary searches, title and abstract screening, full-text screening, analysis, quality assessment, and wrote the draft and final manuscript. SJ provided consultation on the study design and data collection and critically reviewed the draft and final manuscript. AC performed the full-text screening and quality assessment, and also contributed to the final manuscript. DS supervised the study design and critically reviewed and contributed to the final manuscript. SMG supervised the study design and analysis and critically reviewed the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. Carruthers BM, van de Sande MI, De Meirleir KL *et al.* (2011) Myalgic encephalomyelitis: international consensus criteria. *J Intern Med* **270**, 327–338.
2. Fukuda K, Straus SE, Hickie I *et al.* (1994) The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* **121**, 953–959.
3. Sharpe M, Archard L, Banatvala J *et al.* (1991) A report – chronic fatigue syndrome: guidelines for research. *J R Soc Med* **84**, 118.
4. Johnston S, Brenu EW, Staines D *et al.* (2013) The prevalence of chronic fatigue syndrome/myalgic encephalomyelitis: a meta-analysis. *Clin Epidemiol* **5**, 105–110.
5. Jason LA, Benton MC, Valentine L *et al.* (2008) The economic impact of ME/CFS: individual and societal costs. *Dyn Med* **7**, 1.
6. Forsyth LM, Preuss HG, MacDowell AL *et al.* (1999) Therapeutic effects of oral NADH on the symptoms of patients with chronic fatigue syndrome. *Ann Allergy Asthma Immunol* **82**, 185–191.
7. Jason LA, Richman JA, Rademaker AW *et al.* (1999) A community-based study of chronic fatigue syndrome. *Arch Intern Med* **159**, 2129–2137.
8. Aaron LA, Burke MM & Buchwald D (2000) Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. *Arch Intern Med* **160**, 221–227.
9. Johnston SC, Staines DR & Marshall-Gradisnik SM (2016) Epidemiological characteristics of chronic fatigue syndrome/myalgic encephalomyelitis in Australian patients. *Clin Epidemiol* **8**, 97.
10. Scully P, McKernan DP, Keohane J *et al.* (2010) Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. *Am J Gastroenterol* **105**, 2235–2243.
11. Whitehead WE, Palsson O & Jones KR (2002) Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? *Gastroenterology* **122**, 1140–1156.
12. Nisenbaum R, Jones JF, Unger ER *et al.* (2003) A population-based study of the clinical course of chronic fatigue syndrome. *Health Qual Life Outcomes* **1**, 1.

13. Grant JE, Veldee MS & Buchwald D (1996) Analysis of dietary intake and selected nutrient concentrations in patients with chronic fatigue syndrome. *J Am Diet Assoc* **96**, 383–386.
14. Jain AK, Carruthers BM, van de Sande MI *et al.* (2003) Fibromyalgia syndrome: Canadian clinical working case definition, diagnostic and treatment protocols – a consensus document. *J Musculoskelet Pain* **11**, 3–107.
15. Fukuda S, Nojima J, Kajimoto O *et al.* (2016) Ubiquinol-10 supplementation improves autonomic nervous function and cognitive function in chronic fatigue syndrome. *BioFactors* **42**, 431–440.
16. van Rosendal SP, Osborne MA, Fassett RG *et al.* (2010) Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. *Sports Med* **40**, 113–139.
17. Maher CG, Moseley AM, Sherrington C *et al.* (2008) A description of the trials, reviews, and practice guidelines indexed in the PEDro database. *Phys Ther* **88**, 1068–1077.
18. Jadad AR, Moore RA, Carroll D *et al.* (1996) Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* **17**, 1–12.
19. Verhagen AP, de Vet HC, de Bie RA *et al.* (1998) The Delphi list: a criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus. *J Clin Epidemiol* **51**, 1235–1241.
20. Brouwers FM, Van Der Werf S, Bleijenberg G *et al.* (2002) The effect of a polynutrient supplement on fatigue and physical activity of patients with chronic fatigue syndrome: a double-blind randomized controlled trial. *QJM* **95**, 677–683.
21. Castro-Marrero J, Cordero MD, Segundo MJ *et al.* (2015) Does oral coenzyme Q10 plus NADH supplementation improve fatigue and biochemical parameters in chronic fatigue syndrome? *Antioxid Redox Signal* **22**, 679–685.
22. McDermott C, Richards SC, Thomas PW *et al.* (2006) A placebo-controlled, double-blind, randomized controlled trial of a natural killer cell stimulant (BioBran MGN-3) in chronic fatigue syndrome. *QJM* **99**, 461–468.
23. The GKH, Bleijenberg G & van der Meer JWM (2007) The effect of acclydine in chronic fatigue syndrome: a randomized controlled trial. *PLoS Clin Trials* **2**, e19.
24. Witham MD, Adams F, McSwiggan S *et al.* (2015) Effect of intermittent vitamin D3 on vascular function and symptoms in chronic fatigue syndrome – a randomised controlled trial. *Nutr Metab Cardiovasc Dis* **25**, 287–294.
25. Ockerman P (2000) Antioxidant treatment of chronic fatigue syndrome. *Clinical Practice of Alternative Medicine* **1**, 88–91.
26. Ostojic SM, Stojanovic M, Drid P *et al.* (2016) Supplementation with guanidinoacetic acid in women with chronic fatigue syndrome. *Nutrients* **8**, 1–9.
27. Hobday RA, Thomas S, O'Donovan A *et al.* (2008) Dietary intervention in chronic fatigue syndrome. *J Hum Nutr Diet* **21**, 141–149.
28. Vermeulen RCW & Scholte HR (2004) Exploratory open label, randomized study of acetyl- and propionylcarnitine in chronic fatigue syndrome. *Psychosom Med* **66**, 276–282.
29. Plioplys AV & Plioplys S (1997) Amantadine and L-carnitine treatment of chronic fatigue syndrome. *Neuropsychobiology* **35**, 16–23.
30. Maric D, Brkic S, Mikic AN *et al.* (2014) Multivitamin mineral supplementation in patients with chronic fatigue syndrome. *Med Sci Monit* **20**, 47–53.
31. Rao AV, Bested AC, Beaulne TM *et al.* (2009) A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathog* **1**, 1.
32. Sathyapalan T, Beckett S, Rigby AS *et al.* (2010) High cocoa polyphenol rich chocolate may reduce the burden of the symptoms in chronic fatigue syndrome. *Nutr J* **9**, 55.
33. Sullivan Å, Nord CE & Evengård B (2009) Effect of supplement with lactic-acid producing bacteria on fatigue and physical activity in patients with chronic fatigue syndrome. *Nutr J* **8**, 4.
34. Johnston S, Brenu EW, Staines DR *et al.* (2013) The adoption of chronic fatigue syndrome/myalgic encephalomyelitis case definitions to assess prevalence: a systematic review. *Ann Epidemiol* **23**, 371–376.
35. Lloyd AR, Hickie I, Boughton CR *et al.* (1990) Prevalence of chronic fatigue syndrome in an Australian population. *Med J Aust* **153**, 522–528.
36. Jason LA, Sunnquist M, Brown A *et al.* (2015) Defining essential features of myalgic encephalomyelitis and chronic fatigue syndrome. *J Hum Behav Soc Environ* **25**, 657–674.
37. Garrido-Maraver J, Cordero MD, Oropesa-Ávila M *et al.* (2014) Coenzyme q10 therapy. *Mol Syndromol* **5**, 187–197.
38. Maes M, Mihaylova I, Kubera M *et al.* (2009) Coenzyme Q10 deficiency in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is related to fatigue, autonomic and neurocognitive symptoms and is another risk factor explaining the early mortality in ME/CFS due to cardiovascular disorder. *Neuro Endocrinol Lett* **30**, 470–476.
39. Galleano M, Oteiza PI & Fraga CG (2009) Cocoa, chocolate and cardiovascular disease. *J Cardiovasc Pharmacol* **54**, 483.
40. Tokede O, Gaziano J & Djoussé L (2011) Effects of cocoa products/dark chocolate on serum lipids: a meta-analysis. *Eur J Clin Nutr* **65**, 879–886.
41. Desch S, Schmidt J, Kobler D *et al.* (2010) Effect of cocoa products on blood pressure: systematic review and meta-analysis. *Am J Hypertens* **23**, 97–103.
42. Lakhan SE & Kirchgessner A (2010) Gut inflammation in chronic fatigue syndrome. *Nutr Metab* **7**, 1.
43. Frémont M, Coomans D, Massart S *et al.* (2013) High-throughput 16S rRNA gene sequencing reveals alterations of intestinal microbiota in myalgic encephalomyelitis/chronic fatigue syndrome patients. *Anaerobe* **22**, 50–56.
44. Giloteaux L, Goodrich JK, Walters WA *et al.* (2016) Reduced diversity and altered composition of the gut

microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome* **4**, 1.
45. Logan AC, Rao AV & Irani D (2003) Chronic fatigue syndrome: lactic acid bacteria may be of therapeutic value. *Med Hypotheses* **60**, 915–923.
46. Logan AC & Wong C (2001) Chronic fatigue syndrome: oxidative stress and dietary modifications. *Altern Med Rev* **6**, 450–460.
47. Carruthers B, Van de Sande M, De Meirleir K *et al.* (2012) Myalgic encephalomyelitis – adult & paediatric: international consensus primer for medical practitioners.
48. Nelson M, Margetts BM & Black AE (1993) Checklist for the methods section of dietary investigations *Metabolism* **42**, 258–259.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Table S1. Methodology quality assessment summary and Rosendal score of studies included in the present systematic review.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

INTERVENTION STUDIES

Food-based anthocyanin intake and cognitive outcomes in human intervention trials: a systematic review

K. Kent,[1] K. E. Charlton,[1] M. Netzel[2] & K. Fanning[3]

[1]School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW, Australia
[2]Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia
[3]Department of Agriculture and Fisheries, Queensland Government, Brisbane, QLD, Australia

Keywords
anthocyanins, cognition, flavonoids, polyphenols.

Correspondence
Katherine Kent, School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW 2522, Australia.
Tel.: +61 2 4221 4754
Fax: +61 2 4221 3486
E-mail: kc582@uowmail.edu.au

How to cite this article
Kent K., Charlton K.E., Netzel M. & Fanning K. (2017) Food-based anthocyanin intake and cognitive outcomes in human intervention trials: a systematic review. *J Hum Nutr Diet*. **30**, 260–274
doi:10.1111/jhn.12431

Abstract

Background: Preclinical evidence suggests that the anthocyanins, which comprise a subclass of dietary flavonoids providing the purple and red pigmentation in plant-based foods, may have a beneficial impact on cognitive outcomes.
Methods: A systematic review was conducted to identify the published literature on food-based anthocyanin consumption and cognitive outcomes in human intervention trials. The literature search followed PRISMA guidelines and included six databases, as well as additional hand searching.
Results: Seven studies were included in this review, comprising acute trials ($n = 4$) and longer-term ($n = 3$) interventions that assessed multiple cognitive outcomes in children, adults and older adults with cognitive impairment. Six of seven studies reported improvements in either a single, or multiple, cognitive outcomes, including verbal learning and memory, after anthocyanin-rich food consumption. As a result of methodological limitations and the large clinical and methodological diversity of the studies, the pooling of data for quantitative analysis was not feasible.
Conclusions: The impact of food-based anthocyanin consumption on both acute and long-term cognition appears promising. However, adequately powered studies that include sensitive cognitive tasks are needed to confirm these findings and allow the translation of research into dietary messages.

Introduction

The rapidly ageing population has led to a major projected increase in the prevalence of neurodegenerative diseases such as dementia [(1)], which has resulted in intense research interest regarding the role of diet in neuroprotection. Dietary approaches are especially considered to comprise safe and effective methods for the prevention of cognitive disorders and the maintenance of cognitive function. The consumption of dietary flavonoids was shown to both enhance cognitive function [(2)] and prevent age-related cognitive decline in older adults [(3)]. The neuroprotective effects of flavonoids have been well-documented [(4–8)], although most of the research has focused on animal models, limiting translation to humans who utilise more complex cognitive domains, such as executive functioning. Because of their strong antioxidant potential [(9)], it has been hypothesised that flavonoids limit neurodegeneration by reducing neuro-inflammation and protecting the cellular architecture in the brain [(10)]. Flavonoids may also contribute to improved cognitive function by increasing the number and strength of neuronal signals [(11)] via increased brain blood flow [(7)] and an ability to initiate neurogenesis [(5)] in areas of the brain associated with learning and memory. A large proportion of this research has specifically highlighted the benefits of anthocyanin-rich foods [(12–15)].

Anthocyanins are a subclass of dietary flavonoids (i.e. different from proanthocyanidins, which are polymers of flavanols), which provide the purple, red and blue pigmentation in plant-based foods [16]. Typical food sources of anthocyanins are fruits such as apples, grapes, plums; berries such as strawberries, blueberries and blackberries; vegetables such as red cabbage, red onion and eggplant; and beverages such as red wine and red grape juice [17]. Approximately 640 individual anthocyanins have been identified to date [18]; however, anthocyanins naturally occur most ubiquitously in six common aglycones (pelargonidin, cyanidin, delphinidin, peonidin, petunidin, malvidin) in various glycosylated and acylated forms [18]. Anthocyanins are unique among flavonoids as a result of their ability to reversibly change their structures in response to changing pH values, from the stable red to orange coloured flavylium cation at pH < 2 to the colourless and less stable carbinol and chalcone forms at higher or neutral pH [18–20]. This unique feature may have a an impact on their stability and binding ability (e.g. to enzymes) throughout the gut, which has a dramatically changing pH environment ('acid' stomach to 'neutral' intestine).

The metabolism and absorption of anthocyanins has been documented previously [21]. Briefly, food-based anthocyanins are consumed and are either quickly absorbed as intact molecules via the stomach, or after extensive metabolism in the small and large intestine or the colon, to enter the circulation as metabolites such as phenolic acids [21]. In previous animal and human studies, only very low amounts of intact (non-metabolised) anthocyanin glycosides could be detected in plasma and urine after ingestion of pure anthocyanin compounds and/or anthocyanin-rich food and beverages; 15 animal and 17 human bioavailability studies were comprehensively reviewed by Pojer *et al.* [19]. However, recent human studies have clearly demonstrated that dietary anthocyanins undergo intensive metabolism after consumption and that those metabolites are the main transport forms *in vivo* [22–25]. For example, thirty-two anthocyanin-derived metabolites could be detected in the urine, serum and faeces of eight healthy volunteers after the consumption of 500 mg of ^{13}C-labelled cyanidin-3-glucoside [23]. In another study published by Ludwig *et al.* [22], 17 metabolites were quantified in urine collected from nine volunteers after the consumption of 300 g of blended raspberries [22]. Previously, the bioavailability of anthocyanins was considered to be very low (urinary recoveries <2% of intake in most studies), although it is now estimated as being at least 15% if detectable *in vivo* metabolites are also taken into account [22].

The main biological activity of anthocyanins *in vivo* was assumed to be their antioxidant capacity and protection against lipid peroxidation [26]. However, recent studies have identified more complex molecular effects of anthocyanins on signalling pathways in cells that may play a significant role in their protection against chronic diseases [19,26,27]. Modulation of neuronal functions by anthocyanin-rich food has been reported in several animal studies [19]. It is suggested that these neuroprotective effects (e.g. improved memory, learning, cognitive and motor functions) by anthocyanins (and/or their *in vivo* metabolites) are mediated via inhibition/suppression of interleukin-1b, tumor necrosis factor-α and nuclear factor kappa B. The modulatory effects of anthocyanins on important structural and synaptic plasticity markers have also been reported. Acute improvements in cognition after consuming anthocyanin-rich foods have been associated with increased cerebral blood flow (1–2 h and 6 h after consuming anthocyanin-rich blueberries) and improvements in endothelium-dependent vasodilation (as assessed by flow-mediated dilatation) [28,29].

A body of evidence concerning the benefits of anthocyanin consumption in human health, including protective effects against age-related neurodegeneration and cognitive decline, has accumulated over the recent years [16,19]. Animal studies have provided evidence that anthocyanin-rich food consumption can improve several cognitive functions, including long-term memory [30], spatial-working memory [31] and object-recognition memory [32]. In addition, improvements in motor performance
(i.e. balance and coordination) have been documented after receiving an anthocyanin-rich blueberry supplemented diet in an animal model [14]. Intact (non-metabolised) anthocyanins were detected in the brains of rats as soon as 10 min after their consumption, and these concentrations were found to be correlated with improvements in learning and memory tasks [13]. Of particular interest, supplementation of the diet with anthocyanin-rich fruits, such as blueberries and strawberries, was shown to not only maintain, but also reverse age-related cognitive decline in animal models [11,33,34].

A large epidemiological study of 16 010 older women enrolled in the Nurses' Health Study reported significant associations between anthocyanin-rich blueberry and strawberry consumption and the maintenance of cognitive function (a composite score of six cognitive tests, including various measures of memory) over 4 years [35]. It was reported that those individuals who consumed the highest amount of berries had delayed cognitive ageing by up to 2.5 years compared to those who ate the least [35]. Despite their known health benefits, anthocyanins are often overlooked in human intervention trials that investigate the impact of overall flavonoid intake on cognitive outcomes [36]. In a 2009 systematic review of human

intervention trials assessing the impact of flavonoids on cognitive outcomes [36], no studies related to anthocyanins were included.

There has been increasing interest in other areas of anthocyanin research, with a 15-fold rise in the number of published papers between 1990 and 2013 including the word 'anthocyanin' in their title and abstract [37]. Important advances in an increased understanding of the bioavailability and metabolism of anthocyanins (e.g. biotransformation by the gut microbiota), coupled with a greater focus on the role of plant foods in brain health (e.g. neuroprotective activities), warrant a targeted review aiming to summarise the evidence to date of human trials on anthocyanins and cognitive function.

Materials and methods

A systematic review of the literature was performed aiming to summarise the published literature on the effects of food-based anthocyanin consumption on cognitive function in human trials. The review also aimed to identify gaps in the literature and to consider the implications for future study designs. The search was conducted using methodology outlined in the PRISMA statement [38], according to the search strategy outlined below.

Types of studies

Studies were eligible for inclusion in this review if they:

- were randomised or nonrandomised controlled trials; cross-over trials (excluding case studies and observational studies)
- examined humans (any age, sex);
- administered a naturally occurring anthocyanin containing food or beverage (the anthocyanin content of the food needed to be objectively measured; foods fortified with additional anthocyanin were excluded);
- measured change in cognitive function using single or multiple instruments that had previously been validated in a similar population; and
- had full text available.

Only studies written in the English language were considered because of a lack of translational resources, although studies from any country were eligible if they met the rest of the inclusion criteria.

Search terms

The following search terms were developed:

- Anthocyan* and/or
- Flavonoid* and
- Cognit* and/or
- Memory

The * indicates a truncation, to ensure all variations of the search terms were included.

Databases

A three-stage search was conducted. First, a search of the following databases (abstract, title and keywords) was performed using the search terms using the Boolean terms AND/OR as appropriate:

- Scopus (1960 to Oct 2015)
- Medline (1960 to Oct 2015)
- CIHNAL (1960 to Oct 2015)
- Psychology and Behavioural Sciences Collection (1960 to October 2015)
- PsychInfo (1860 to October 2015)
- Web of Science (1965 to October 2015)

Second, hand searching of the reference list of identified studies was conducted. Third, an internet search was performed to identify any published studies outside the realms of the selected databases. The records were exported from the databases and managed in ENDNOTE X4 (Thomson Reuters, New York, NY, USA). After screening and selection of studies according to the inclusion criteria (Fig. 1), relevant summary data were extracted into a template made for this purpose, including the study design, intervention, dose, methods and results.

Results

The search returned 1684 articles and four other potential studies were identified through hand-sorting of reference lists. After duplicates were removed and the titles screened, 35 potential studies were identified. After screening abstracts, a further 25 were excluded and, after reading the full text articles of 10 studies, a further three were excluded according to the inclusion/exclusion criteria (Table 1), leaving seven papers in the final review (Fig. 1).

Description of studies

Statistical pooling of the data from the seven studies considered eligible for the review [39–45] was not possible because of the large clinical and methodological diversity of the studies. This heterogeneity resulted from variability in the characteristics of the participants and type of intervention (including food sources and dose of anthocyanins), varying study designs and study duration, as well as differences in choice of cognitive outcomes. The findings are therefore presented in narrative form. The characteristics and relevant information of each study are described in Table 2. Publication dates ranged from 2010 to 2015. Of the seven included studies,

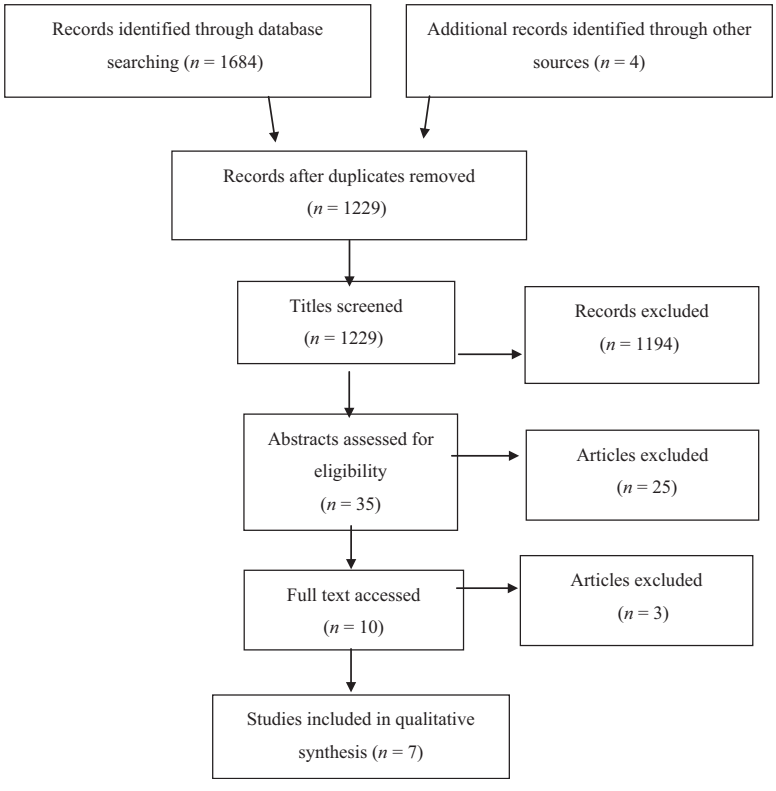

Figure 1 PRISMA flowchart for the search and study selection process of included and excluded studies.

three were randomised, double-blind, placebo-controlled studies [39,40] that assessed the daily consumption of anthocyanin-rich fruit juices on cognitive outcomes. The remaining four were placebo-controlled cross-over trials, either randomised ($n = 2$) [42,44] or nonrandomised ($n = 2$) [41,45] that assessed the acute impact of anthocyanin rich fruit juices on cognitive outcomes. Two studies investigated older adults with mild cognitive impairment [39,40], one study included older adults with dementia [43], one study was conducted in young, healthy adults [42], one study included a combination of older adults with and without dementia as well as healthy younger adults [44], and two studies were undertaken in children [41,45]. The studies were undertaken in New Zealand [42], the USA [39,40], the UK [41,45] and Australia [43,44].

Description of the interventions

In all seven studies, the vehicle for anthocyanin administration was a beverage. Four studies utilised a preformulated juice [39,40,43,44], one study [42] utilised a juice processed from fresh fruit (150 g of fruit yielded approximately 140 mL of juice), two studies used a powdered freeze-dried fruit made up with water [42] or cordial [45] and one study [41] blended fresh fruit into a smoothie. Two studies based the beverage serving size on the body weight of each participant [39,40] providing between 6 and 9 mL of juice kg^{-1} body weight of juice (providing between 428 and 598 mg anthocyanins day^{-1} divided over three servings). One study [42] altered the serving sizes of the juice to consistently deliver a mean (SD) of 525 (5) mg of polyphenols per 60 kg of bodyweight per participant. Four studies [41,43–45] administered a standard serving size to each participant, regardless of body size, providing total anthocyanins of between 55 mg [44] and 253 mg day^{-1} [45].

Outcome assessment

Cognitive outcomes were measured using valid, referenced instruments in all studies. Four studies measured acute change in cognition [41,42,44,45], whereas the other three studies measured longer-term change in cognition of between 12 and 16 weeks [39,40,43]. Detailed results, including significant effects, are reported below.

Longer-term anthocyanin supplementation and cognition

Two of the longer-term studies evaluated verbal learning and memory in older adults with mild cognitive impairment [39,40] using the California Verbal Learning Test and one study measured the same outcome using the Rey Auditory Verbal Learning Test (RAVLT) [43]. One study

Table 1 List of excluded studies

		Study reference	Reason
Excluded after abstract reviewed	1	(66)	No measure of cognition
	2	(67)	No measure of anthocyanin in apples
	3	(12)	Animal study
	4	(68)	Not primary research
	5	(69)	Not primary research
	6	(70)	Animal study
	7	(71)	Conference abstract
	8	(72)	Animal study and nonfood based
	9	(73)	Conference abstract and study design
	10	(74)	Review, not anthocyanin specific
	11	(75)	Conference abstract
	12	(76)	Editorial
	13	(77)	Review of blueberries, not focussed on cognition
	14	(78)	Full text cannot be found
	15	(79)	Animal study
	16	(80)	Pomegranate extract in pill form
	17	(81)	Narrative review focussed on animal studies (small section on Krikorian papers identified)
	18	(82)	Animal study
	19	(83)	Review of grape products, not focussed on cognition
	20	(84)	Review of animal studies
	21	(85)	Animal study
	22	(86)	Intervention not flavonoid related
	23	(87)	Review, cognitive outcomes not addresses
	24	(88)	Review, not food vehicle
	25	(89)	Animal study, black soy contains no anthocyanin
Excluded after full text retrieved	26	(57)	No measure of anthocyanin in juice
	27	(56)	No measure of anthocyanin in juice
	28	(58)	No measure of anthocyanin in juice

measured change in explicit episodic memory using the Paired Associate Learning Test [40] and two studies assessed change in mood using the Geriatric Depression Scale [39,43]. One study measured several other cognitive domains, including working memory (self-ordered pointing task), semantic memory (the Boston naming test), executive function (trail making test, category and letter verbal fluency) and short-term memory (digit span backwards task) [43]. One study [39] measured brain activity objectively, using functional magnetic resonance imaging (fMRI) brain imaging, which indicates stimulation and activity in specific regions of the brain.

Krikorian *et al.* [39] reported that, after 16 weeks of supplementation with concord grape juice, there was no between-group difference for performance on the CVLT learning task, although there was a reduction in interference on the CVLT memory task compared to baseline ($P = 0.04$). There was a nonsignificant effect on mood. Analysis of the fMRI brain analysis indicated that there was greater activation in the anterior and posterior regions of the brain for the intervention group only [39]. In another study, the same research team of Krikorian *et al.* [40] reported significant improvements on the verbal paired associate learning task ($P = 0.009$) after 12 weeks of supplementation with blueberry juice. However, both of these studies had small sample sizes of only 10 in the Concord grape juice intervention arm [39] and nine in the blueberry juice intervention arm [40].

Kent *et al.* [43] conducted a larger trial ($n = 21$ per group) in older adults with mild–moderate dementia and reported that, after 12 weeks of supplementation with cherry juice (138 mg day^{-1}), there were significant improvements in verbal fluency ($P = 0.014$), short-term memory (RAVLT) ($P = 0.014$) and long-term memory (RAVLT delayed) ($P \leq 0.001$).

Acute anthocyanin supplementation and cognition

The four acute feeding studies evaluated change in cognition using a variety of tests: the digital vigilance test (a measure of attention) [42], the rapid visual information processing test (attention and working memory) [42], the go-no-go test (response inhibition) [41,45], an objective location task (spatial memory) [41], a visual-n back test (attention and short-term memory) [41], a task switching test (higher executive function) [44], a pattern and letter comparison task (speed of processing) [44], a picture matching task (processing and response interference) [45] and the auditory verbal learning task (verbal learning and memory) [45]. Two studies used the Stroop test to measure attention and inhibition [41,42] and the RAVLT to measure verbal learning and memory [41,44].

Watson *et al.* [42] compared three conditions (placebo versus powdered blackcurrant extract with 7.7 mg anthocyanins kg^{-1} versus blackcurrant juice with 8 mg kg^{-1}, based on body weight) on acute cognition in 36 young adults by administering tasks seven consecutive times over 65 min after consumption of the beverages. Significant improvements in accuracy were reported, as measured by the rapid visual information processing test ($P = 0.011$), when participants consumed the blackcurrant extract versus placebo, as well as improvements in reaction time for some repetitions (1, 4 and 7) on the digital vigilance task compared to baseline tests

Table 2 Summary of included studies

	Reference	Participants	Design	Intervention	Dose	Cognitive tests	Results
Longer-term interventions	Krikorian *et al.* [39]	21 older adults, 68 years+, mild cognitive impairment (clinical dementia rating scale)	Randomised double-blind placebo-controlled trial	16 weeks, daily consumption of concord grape juice (n = 10) versus placebo (n = 11) (colour, taste, energy and sugar matched)	Anthocyanins: 425 mg L^{-1} (spectrophotometry). Serving size calculated to be between 6 and 9 mL kg^{-1} body weight over 3 servings day^{-1}	Baseline and 16 weeks: California Verbal Learning Test II (verbal learning and memory), Geriatric Depression Scale (mood), Sub-group (4 per group) fMRI brain imaging	No improvement in learning or retention. Interference on memory task was reduced for intervention group only. No change in mood. Greater activation in the anterior and posterior regions of the brain seen (fMRI) for intervention group
	Krikorian *et al.* [40]	9 older adults [5 men, mean (SD) age 76.2 (5.2) years] mild cognitive impairment (clinical dementia rating)	Randomised, double blind, placebo-controlled trial	12 weeks, daily consumption of blueberry juice (n = 9) versus placebo (n = 7) [not matched, control group from another study [56]]	Anthocyanins: 877 mg cyanidin 3-glucoside equiv L^{-1} (HPLC). Serving size between 6 and 9 mL kg^{-1} body weight over 3 servings day^{-1}	Baseline and 12 weeks: California Verbal Learning Test (verbal learning and memory), Verbal paired associate learning test (memory)	Significant improvement on verbal paired associate learning test (P = 0.009) at 12 weeks in the intervention group only. No significant improvement in other tasks
	Kent *et al.* [43]	42 older adults, 70+ years, mild-moderate dementia (geriatrician diagnosed)	Randomised double blind, placebo-controlled trial	12 weeks, daily consumption of cherry juice (n = 21) versus apple juice (n = 21)	Anthocyanins: 69 mg/100 g (HPLC); ORAC 3200 μmol Trolox equivalents g^{-1}. 200 mL day^{-1}	Baseline, 6 and 12 weeks: RAVLT (verbal learning and memory), the self-ordered pointing task (working memory), the Boston naming test (semantic memory), trail making test (executive function) digit span backwards task (short-term memory), category and letter verbal fluency (executive function)	Significant improvements in verbal fluency (P = 0.014), short-term memory (RAVLT) (P = 0.014) and long-term memory (RAVLT delayed) ($P \leq 0.001$) for intervention group

Table 2. Continued

	Reference	Participants	Design	Intervention	Dose	Cognitive tests	Results
Acute interventions	Watson *et al.* [42]	36 young adults [mean (SD) age 24.8 (3.9) years]	Acute randomised double-blind placebo-controlled crossover study	3 juices on different occasions: placebo juice, blackcurrant extract or blackcurrant juice	Placebo juice: 0 mg kg^{-1} anthocyanin; Blackcurrant extract: 7.7 mg kg^{-1} anthocyanin; Blackcurrant fruit juice: 8.0 mg kg^{-1} anthocyanin. Serving size 10 mL kg^{-1} body weight	7 repetitions over 70 m post-intervention: digital vigilance (attention), Stroop (attention, inhibition), RVIP (attention and working memory), Bond–Lader visual analogue (mood)	Significant improvement in RVIP accuracy after supplementation with anthocyanin-enriched blackcurrant extract versus control ($P = 0.011$). Significant improvement in reaction time on some repetitions (1, 4, 7) on digit vigilance task versus baseline for the cold-pressed blackcurrant fruit juice (overall effect $P = 0.044$). No significant effect on mood
	Whyte *et al.* [45]	21 children [9 male, mean (SD) age 8.7 (0.67) years]	Acute double blind cross-over placebo-controlled trial	Intervention: 15 or 30 g freeze-dried wild blueberry (WBB) powder	Intervention: 30-g WBB 253 mg anthocyanins, 15-g 127 mg anthocyanins (method not described)	Baseline, and 1,3,6 h post-intervention: AVLT (learning, memory and recall), modified Flanker task (response interference), Go-No Go (response inhibition), picture matching task (processing and response interference)	Consumption of the 30 g dose improved immediate word recall 1 h 15 m post-intervention, and delayed word recognition after 6 h (AVLT). No significant improvement was seen for other tasks

Table 2. Continued

Reference	Participants	Design	Intervention	Dose	Cognitive tests	Results
Whyte *et al.* [41]	16 children [10 male, mean (SD) age 9.17 (0.6) years]	Acute cross-over placebo-controlled trial	Intervention: 200 g fresh blueberries, 100 mL milk and 8 g sucrose. Control (sugar and vitamin C matched): 0.02 g vitamin C powder, 8.22 g sucrose, 9.76 g glucose and 9.94 g fructose and 100 mL milk	Intervention: 143 mg anthocyanins (method not described)	Baseline and 2 h post-intervention: Go-No Go (response inhibition), Stroop (verbal reaction and interference), RAVLT (verbal learning a nd memory), object location task (spatial memory and discrimination) and a visual b-back (visual attention and short-term memory)	Significant improvement in delayed recall of RAVLT (word learning and retention) for intervention group. No significant improvement for other tasks
Caldwell *et al.* [44]	16 adults: young adults, $n = 6$ [mean (SD) age 21.8 (0.97) years]; older adults, $n = 5$ [mean (SD) age 74.1 (7.9) years]; five older adults with dementia (geriatrician diagnosed), [mean (SD) age 79.8 (3.6) years]	Acute cross-over trial (dose-dependant response investigated)	Cherry juice administered either as a single 300 mL serving at 0 h or 3 × 100 mL servings at 0, 1, and 2 h	Anthocyanins: 18.6 mg/100 mL (HPLC); ORAC 58.99 μmol Trolox equivalent g^{-1}	Baseline and 6 h after intervention: RAVLT (verbal learning and memory); task switching test (higher executive function); pattern and letter comparison tasks (speed of processing)	Older adults only showed significant improvement in task switching test at 6 h compared to baseline (attributed to type 1 error). No significant change in acute cognition on any other test

AVLT, auditory verbal learning task; fMRI, functional magnetic resonance imaging; HPLC, high-performance liquid chromatgraphy; ORAC, oxygen radical absorbance capacity; RVALT, Rey Auditory verbal learning test; RVIP. rapid visual information processing

($P = 0.044$) for blackcurrant juice or extract versus placebo. No change in mood or other tasks was recorded.

Whyte *et al.* [45] assessed dose-dependent change in acute cognition after consumption of either 15 g or 30 g of reconstituted freeze-dried blueberries in 21 children. Consumption of a dose of 30 g (253 g of anthocyanin) resulted in significant improvements in word recall after five recall trials ($P < 0.001$) 95 min post-intervention for a dose of 30 g versus control, as measured by the auditory verbal learning test. Improved accuracy was reported during a flanker interference task (response inhibition; the ability to suppress responses that are inappropriate) after 3 h, with respect to a dose of 30 g versus control ($P = 0.035$). No improvement was seen for other tasks, except for the go–no go task, in which the placebo condition was associated with significantly better results.

Whyte *et al.* [41] assessed acute change in cognition after consumption of 200 g of fresh blueberries (143 mg of anthocyanins) provided in a milk-based smoothie in 16 children. There was a significant improvement in the delayed recall portion of the RAVLT task after consumption of the blueberry smoothie, although no improvements were seen for any other task.

Caldwell *et al.* [44] assessed dose-dependency related to change in acute cognition after the consumption of either a single 3000-mL serving of cherry juice (55 mg of anthocyanins) or three 100-mL servings at 0, 1 and 2 h. The researchers compared younger and older adults, with and without dementia. For the older adults only, a significant improvement was seen for the task switching test at 6 h compared to baseline ($P = 0.03$) (although this was attributed to type 1 error) and no changes in acute cognition were reported for any of the other tests.

Discussion

The aim of this systematic review was to assess the effects of anthocyanins consumed from food (not supplements) on domains of cognitive function, as well as to highlight gaps in the literature and address the applicability of the available evidence for development of future study designs. Despite more than a decade of preclinical evidence supporting the association of anthocyanin intake on cognitive outcomes [33], there remains a lack of experimental studies conducted in humans. Six of the seven studies included in the review reported positive benefits of anthocyanin consumption on some aspect of cognition; however, the generalisability of the findings to other clinical or healthy populations is limited by the small study sample sizes and inconsistent methodologies. There was a lack of homogeneity in the study designs that prevented pooling of data for a quantitative analysis of the magnitude of effect. From the available data, it is unclear in which population the effects are most likely or which doses of anthocyanins and/or their food sources are effective at inducing cognitive benefit.

The cognitive domains that appear to be acutely sensitive to consumption of anthocyanin-rich foods are verbal learning and memory, whereas, additionally, longer-term consumption also impacts upon attention and working memory. However, there is a lack of consensus regarding the choice of best instruments and methods to employ in studies that investigate potential change in cognitive function [36]. The seven identified studies used a wide range of tools to address multiple cognitive domains between studies. This limitation has been recognised and evaluated in a recent methodological review [46]. That review concluded that future studies should be guided by positive findings from previous similar research, and that it may be best to continue to include a battery of sensitive and specific tools that address multiple cognitive domains, until there is better understanding of specific brain functions that are influenced by these bioactive components in foods.

The duration of time needed to see an effect within both acute and longer-term trials is also poorly understood. The studies of Kent *et al.* [43] and Krikorian *et al.* [39,40] suggest that cognitive benefits are seen after 12 weeks of intervention, and that extending the study period to 16 weeks may not necessarily improve the strength of the association [39]. The timing related to the acute impact of food-based anthocyanins on cognition is hampered by the limited knowledge of whether the intact anthocyanins or their metabolites, or both, are responsible for the beneficial effects of an anthocyanin-rich diet [47]. Two of the acute studies [41,42] included in this review did not extend their trial times to encompass the secondary uptake of anthocyanin-related metabolites in the colon, and thus may have missed an important later interaction that these compounds have on cognition. The acute cross-over trial by Caldwell *et al.* [44] found no significant change in cognition 6 h post-intervention but the small numbers of participants suggest that it was likely to be underpowered to observe cognitive effects.

Another outcome from this systematic literature review is that biomarker data to confirm anthocyanin uptake in the included studies were lacking. Only one study [42] conducted an objective analysis of biological specimens to determine the uptake and metabolism of the anthocyanins present in the intervention foods. Such fundamental data are needed to confirm the bioavailability of anthocyanins from different foods and to determine which of the bioactive components are specifically associated with the observed health benefits. There is growing evidence to suggest that the biological activity of some flavonoids may improve during metabolism, in

comparison with their intact variants, which further complicates the interpretation of their biological effects of both the type and quantity found naturally in foods. In addition to metabolic conjugation and methylation of flavonoids in the human body, intestinal bacteria are also considered to play an integral role in their metabolism [48]. Studies that investigate the health effects of anthocyanins will need to consider new paradigms to understand their bioactivity *in vitro*, in an attempt to better explain their mechanisms of action [48]. A recent study [23] attempted to account for the fate of the remaining 99% of ingested anthocyanins that are not recovered in blood and urine. Rather than confirm that anthocyanins had low bioavailability resulting from poor absorption and chemical instability [48], the study identified an extensive biotransformation of anthocyanins that resulted in a relative bioavailability (i.e. the amount absorbed and found in blood, urine and breath) of 12%, rather than the previously assumed 1%. An extensive diversity of metabolites was identified (>30 unique structures), including phenolic, hippuric, phenylacetic and phenylpropenoic acids. These findings have important ramifications for future studies, in which urine and plasma sampling periods in short-term bioavailability studies should be extended because anthocyanin-derived metabolites are still being excreted up to 48 h post-ingestion [22,23,25]. As well as plasma/serum and urine, faeces should also be collected as an integral sampling 'matrix' in future human research to measure the metabolites produced by the gut/colonic microbiota. This information will be essential to further clarufy how dietary anthocyanins exert their biological activities *in vivo*.

In the case of the full complement of flavonoid metabolites not being able to be measured, other characteristic biomarkers of intake could be identified and assessed [49]. For example, one study measured change in nonspecific measures of inflammation [43] as a potential reflection of the anti-inflammatory mechanisms related to flavonoid bioactivity, and reported no significant impact on C-reative protein or interleukin-6 levels. However, the various other mechanisms by which flavonoids are purported to provide neuroprotection and improve memory and cognition [5,6] are not explained by anti-inflammatory biomarkers, and thus this choice of biomarker may not necessarily reflect the total bioactivities of flavonoids *in vivo*.

Ageing is associated with changes in the functional properties of the digestive system, such as delayed transit time, losses in absorptive function and the influence of prescription medication use on gut microbiota [50]. Therefore, the effect of age may influence the bioavailability and metabolism of anthocyanins [50]. This hypothesis has not been adequately tested but should be a consideration when comparing interventions across age groups. The studies included in the present review investigated groups ranging in age from children (8–10 years) to older adults (>68 years) with mild-cognitive impairment and dementia. Disease states that affect brain function, including vascular and nonvascular dementia, as well as Alzheimer's disease, may also result in different measureable outcomes in these groups [51], as shown by the limited impact of antioxidant clinical trials in these groups [51]. It is therefore important to clearly define patient groups and describe the differences in outcomes between children, as well as healthy young and older adults.

The vehicle for provision of anthocyanins also warrants consideration. All of the studies in the review provided anthocyanins in the form of a beverage, although the use of this vehicle was not well explained. It can be speculated that the provision of a preformulated juice is an ideal way to control the variation of anthocyanins in the fresh-fruit equivalent, which can be heavily influenced by growth conditions and can differ between seasons. Provision of a juice also reduces the impact of fruit seasonality on having sufficient fruit available year-round. The impact of food processing can also substantially alter the anthocyanin content of foods, especially with regard to heating or cooking. For example, heating of dark anthocyanin-rich blood plums during jam making resulted in losses of up to 70% of their total anthocyanin content [52]. Additionally, some studies administered relatively large doses of anthocyanin-rich beverages (up to over 600 mL), presumably to maximise the potential of the bioactive anthocyanins. The division of the anthocyanin-rich food over three servings per day in the longer-term trials [39,40] may have diminished their bioactivity, where the provision of smaller doses may not have provided a sufficient concentration of anthocyanin to exert a physiological effect, compared to an increased bioactivity if a larger serve is consumed at once. There is a trade-off in clinical trial methodology between reflecting the usual intake of anthocyanin-rich foods, as spread throughout the day, and ensuring that a sufficiently high dose is provided (in a single serving) to observe outcomes. The dose–response mechanisms for the impact of anthocyanin-rich food on cognition have not been well investigated, and the differences in anthocyanin intake across the intervention studies were vast. One study [44] that provided a juice much lower in anthocyanin content (18.6 mg/100 mL; 55 mg per serving) than the other trials reported no acute cognitive benefits, and therefore this dose may not be sufficiently high to induce acute cognitive change. Future trials are needed to further clarify the dose-dependent responses associated with the consumption of anthocyanin-rich foods. Interestingly, the

cognitive benefits associated with anthocyanin-rich food consumption do not appear to be limited to one food, with benefits documented after consuming blueberries, blackberries and cherries.

Berries, particularly blueberries and blackberries, have received the most interest in studies investigating the health impacts of anthocyanin-rich foods. Many other anthocyanin-rich sources have been overlooked, including vegetables such as red onions and cabbage. The potential for these foods to influence cognition needs to be confirmed because the large dependence in research on berries limits the translation of the outcomes to nutritional advice for the population. The consumption of large amounts of berries is not always feasible because berries are often expensive, seasonal (not available year-round) and can spoil quickly. The use of freeze-dried fruits to overcome these barriers may be a possibility, providing that the anthocyanin content of the fresh fruit equivalent is not spoiled. Freeze-dried fruit may be a nutritionally more preferable way in which to consume anthocyanins compared to the encapsulated anthocyanins or anthocyanins added to other processed foods. This is because anthocyanin pigments present within natural foods are unlikely to be working independently because plants typically contain a complex mixture of flavonoids that have synergistic bioactivities [53]. Some studies have found that anthocyanin bioactivity may be increased when they are consumed within a mixture of polyphenols (e.g. as they would naturally occur within a complex food matrix) [54]. This emphasises the importance of investigating whole food sources of anthocyanins, rather than isolated and encapsulated anthocyanins when studying health-related outcomes.

Background diet was considered by only one of the intervention studies [45], where recommendations were provided on which foods to avoid the evening prior to testing day. Diet was controlled on the day of the intervention in the longer acute trials [44,45] but was not controlled in the longer-term trials. Controlling the background diet, or at least monitoring it, is an important consideration in flavonoid trials [49] with respect to isolating the effect of the intervention. The provision of low flavonoid and/or washout diets before and during trials may be an effective way of reducing the impact of habitual diet on intervention outcomes, although monitoring habitual diet throughout the period of longer-term interventions may also be useful for identifying potential dietary confounders [49]. Alternatively, excluding potential participants with abnormally high (vegans, vegetarians) or low flavonoid intake may be an easier way of reducing the influence of background diet on the study results [49]. The potential benefit of an anthocyanin intervention to improve cognitive outcomes when the background diet of an individual is already rich in dietary flavonoids and/or anthocyanins needs to be considered in future research. Ideally, an intervention would contain an anthocyanin dosage that can feasibly be achieved in a habitual diet. The ideal protocol for standardising the background diet among participants, either by providing a controlled diet or providing recommendations regarding the intake of flavonoid rich foods, including the length of run-in periods in flavonoid trials, has not been investigated and identified, although a minimum of 3 days has been suggested [49]. However, manipulation of the background diet of participants limits the usefulness of the intervention findings in a real world setting.

The implication of study design (i.e. a cross-over design versus parallel groups) is important when considering flavonoid trials. As a result of the high inter-individual variability in the metabolism of flavonoids [55], a cross-over design where each individual serves as their own control is arguably more appropriate, especially in trials with small sample sizes. In these study designs, however, the influence of learned practice effects on performance in the cognitive tasks associated with repeated testing would need to be accounted for.

Three studies were excluded on the basis of not having a measure of anthocyanin content of the intervention juice [56–58]. Hendrickson *et al.* [58] measured total phenolic content but did not report anthocyanin content specifically. Previous research has shown that both cranberry [59] and grape juice [39] contain anthocyanins but, without an objective measure of their anthocyanin content, the extent to which their bioactivity relates to the anthocyanin content, specifically, cannot be speculated. These studies showed that, in older adults ($n = 5$), daily concord grape juice consumed (4–6 mL kg^{-1} body weight divided over three servings per day) over 12 weeks was associated with significant improvements ($P = 0.04$) in verbal learning and memory on the California Verbal Learning Test [56]. In young adults ($n = 35$), grape juice (10 mL kg^{-1} body weight) had no impact on acute cognition [58] (implicit memory as measured by a word fragment completion task) and cranberry juice consumption (470 mL consumed twice per day) had no significant impact on cognitive outcomes [57].

Lastly, it is important to note here the distinction between the terms anthocyanins and anthocyanidins, which are sometimes used interchangeably but incorrectly in the literature. Anthocyanins are glycosylated anthocyanidins (aglycones), which are the naturally occurring water-soluble pigments in coloured fruits and vegetables, whereas anthocyanidins are rarely found in nature because of their poor stability [18,19] and differ in terms of molecular size and polarity [18]. Anthocyanins are more water soluble, whereas anthocyanidins are more hydrophobic [18,19]. Anthocyanidins are the initial

degradation products in the gut microbial metabolism of anthocyanins, which can be transported to the colon as matrix-bound glycosides [60]. After hydrolysis by microbiota, the released and unstable aglycones are rapidly degraded to their respective phenolic acids depending on their aglycone structure (e.g. cyanidin to protocatechuic acid, malvidin to syringic acid and peonidin to vanillic acid) [60]. There are contradicting reports in the literature about the (bio)activity of anthocyanins and anthocyanidins. Tsuda *et al.* [61] measured a stronger *in vitro* antioxidative activity of cyanidin (anthocyanidin) than cyanidin-3-glucoside (anthocyanin) in an experimental liposome/rabbit erythrocyte membrane model. However, a similar *in vitro* radical scavenging activity was reported for eight common anthocyanidins and seven anthocyanins [62], whereas a weaker inhibitory effect on cell transformation could be observed for delphinidin glycosides (anthocyanins) compared to their sugar-free aglycone delphinidin (anthocyanidin) [63]. However, it is unlikely that intact (non-metabolised) anthocyanidins exert any significant biological effects because of their instability at neutral and physiological pH conditions [64,65].

In conclusion, the impact of food-based anthocyanin consumption on both acute and long-term cognition appears to be promising, with six of seven studies reporting improvements in either single, or multiple, cognitive outcomes after anthocyanin-rich food consumption. However, adequately powered studies that utilise sensitive cognitive tasks previously shown to detect changes in cognition in anthocyanin trials (e.g. the RAVLT for measuring change in verbal learning and memory) are needed to confirm these findings. Future research should focus on follow current recommendations for designing, implementing and reporting of flavonoid trials [49]. Studies should focus on clarifying which sources of anthocyanin-rich foods are most beneficial and the dosage of anthocyanin intake needed to induce acute and longer-term cognitive effects. Ultimately, this will allow statistical comparison between studies, and potentially lead to the translation of research into dietary messages about dietary anthocyanins for acute and longer-term cognitive benefits.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
No funding declared.
KK designed and conducted the review and prepared the manuscript. KEC reviewed the methods comprising the review and contributed to the preparation of the manuscript. MN and KF contributed to the preparation of the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. Jorm AF, Dear KBG & Burgess NM (2005) Projections of future numbers of dementia cases in Australia with and without prevention. *Aust NZ J Psychiatry* **39**, 959.
2. Nurk E, Refsum H, Drevon CA *et al.* (2009) Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. *J Nutr* **139**, 120–127.
3. Letenneur L, Proust-Lima C, Le Gouge A *et al.* (2007) Flavonoid intake and cognitive decline over a 10-year period. *Am J Epidemiol* **165**, 1364–1371.
4. Williams RJ & Spencer JPE (2012) Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radic Biol Med* **52**, 35–45.
5. Spencer JPE (2008) Flavonoids: modulators of brain function? *Br J Nutr* **99**, ES60–ES77.
6. Spencer JPE (2010) The impact of fruit flavonoids on memory and cognition. *Br J Nutr* **104**, S40–S47.
7. Vauzour D, Vafeiadou K, Rodriguez-Mateos A *et al.* (2008) The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr* **3**, 115–126.
8. Youdim KA, Spencer JPE, Schroeter H *et al.* (2002) Dietary flavonoids as potential neuroprotectants. *Biol Chem* **383**, 503–519.
9. Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**, 933–956.
10. Spencer JPE, Vauzour D & Rendeiro C (2009) Flavonoids and cognition: the molecular mechanisms underlying their behavioural effects. *Arch Biochem Biophys* **492**, 1–9.
11. Joseph JA, Shukitt-Hale B & Casadesus G (2005) Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* **81**, 313S–316S.
12. Cho J, Kang JS, Long PH *et al.* (2003) Antioxidant and memory enhancing effects of purple sweet potato anthocyanin and cordyceps mushroom extract. *Arch Pharm Res* **26**, 821–825.
13. Andres-Lacueva C, Shukitt-Hale B, Galli RL *et al.* (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutr Neurosci* **8**, 111–120.
14. Joseph JA, Shukitt-Hale B, Denisova NA *et al.* (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with

blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* **19**, 8114–8121.
15. Shukitt-Hale B, Cheng V & Joseph JA (2009) Effects of blackberries on motor and cognitive function in aged rats. *Nutr Neurosci* **12**, 135–140.
16. Tsuda T (2012) Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. *Mol Nutr Food Res* **56**, 159–170.
17. Wu X, Beecher GR, Holden JM *et al.* (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J Agric Food Chem* **54**, 4069–4075.
18. He J & Giusti MM (2010) Anthocyanins: natural colorants with health-promoting properties. *Ann Rev Food Sci Technol* **1**, 163–187.
19. Pojer E, Mattivi F, Johnson D *et al.* (2013) The case for anthocyanin consumption to promote human health: a review. *Compr Rev Food Sci Food Saf* **12**, 483–508.
20. Lapidot T, Harel S, Akiri B *et al.* (1999) pH-dependent forms of red wine anthocyanins as antioxidants. *J Agric Food Chem* **47**, 67–70.
21. Fang J (2014) Bioavailability of anthocyanins. *Drug Metab Rev* **46**, 503–520.
22. Ludwig IA, Mena P, Calani L *et al.* (2015) New insights into the bioavailability of red raspberry anthocyanins and ellagitannins. *Free Radic Biol Med* **89**, 758–769.
23. de Ferrars RM, Czank C, Zhang Q *et al.* (2014) The pharmacokinetics of anthocyanins and their metabolites in humans. *Br J Pharmacol* **171**, 3268–3282.
24. Kalt W, Liu Y, McDonald JE *et al.* (2014) Anthocyanin metabolites are abundant and persistent in human urine. *J Agric Food Chem* **62**, 3926–3934.
25. Czank C, Cassidy A, Qingzhi Z *et al.* (2013) Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a 13C-tracer study. *Am J Clin Nutr* **97**, 995–1003.
26. Crozier A, Jaganath IB & Clifford MN (2009) Dietary phenolics: chemistry, bioavailability and effects on health. *Nat Prod Rep* **26**, 1001–1043.
27. Del Rio D, Rodriguez-Mateos A, Spencer JPE *et al.* (2013) Dietary (Poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* **18**, 1818–1892.
28. Rodriguez-Mateos A, Rendeiro C, Bergillos-Meca T et al. (2013) Intake and time dependence of blueberry flavonoid–induced improvements in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity. *Am J Clin Nutr* **98**, 1179–1191.
29. Zhu Y, Xia M, Yang Y et al. (2011) Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clin Chem* **57**, 1524–1533.
30. Barros D, Amaral OB, Izquierdo I *et al.* (2006) Behavioral and genoprotective effects of vaccinium berries intake in mice. *Pharmacol Biochem Behav* **84**, 229–234.
31. Williams CM, El Mohsen MA, Vauzour D *et al.* (2008) Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radic Biol Med* **45**, 295–305.
32. Goyarzu P, Malin DH, Lau FC *et al.* (2004) Blueberry supplemented diet: effects on object recognition memory and nuclear factor-kappa B levels in aged rats. *Nutr Neurosci* **7**, 75–83.
33. Galli RL, Shukitt-Hale B, Youdim KA *et al.* (2002) Fruit polyphenolics and brain aging: nutritional interventions targeting age-related neuronal and behavioral deficits. *Ann NY Acad Sci* **959**, 128–132.
34. Youdim KA & Joseph JA (2001) A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radic Biol Med* **30**, 583–594.
35. Devore EE, Kang JH, Breteler MMB *et al.* (2012) Dietary intakes of berries and flavonoids in relation to cognitive decline. *Ann Neurol* **72**, 135–143.
36. Macready AL, Kennedy OB, Ellis JA *et al.* (2009) Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. *Genes Nutr* **4**, 227–242.
37. Santos-Buelga C, Mateus N & De Freitas V (2014) Anthocyanins. Plant pigments and beyond. *J Agric Food Chem* **62**, p6879–p6884.
38. Stewart LA, Clarke M, Rovers M *et al.* (2015) Preferred reporting items for systematic review and meta-analyses of individual participant data: the PRISMA-IPD statement. *JAMA* **313**, 1657–1665 9p.
39. Krikorian R, Boespflug EL, Fleck DE *et al.* (2012) Concord grape juice supplementation and neurocognitive function in human aging. *J Agric Food Chem* **60**, 5736–5742.
40. Krikorian R, Shidler MD, Nash TA *et al.* (2010) Blueberry supplementation improves memory in older adults. *J Agric Food Chem* **58**, 3996–4000.
41. Whyte AR & Williams CM (2015) Effects of a single dose of a flavonoid-rich blueberry drink on memory in 8 to 10 y old children. *Nutrition* **31**, 531–534.
42. Watson AW, Haskell-Ramsay CF, Kennedy DO *et al.* (2015) Acute supplementation with blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in healthy young adults. *J Funct Foods* **17**, 524–539.
43. Kent K, Charlton K, Roodenrys S *et al.* (2015) Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. *Eur J Nutr* Oct 19. [Epub ahead of print].
44. Caldwell K, Charlton KE, Roodenrys S *et al.* (2015) Anthocyanin-rich cherry juice does not improve acute cognitive performance on RAVLT. *Nutr Neurosci* Feb 23. [Epub ahead of print].

45. Whyte A, Schafer G & Williams C (2016) Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. *Eur J Nutr* **55**, 2151–2162.
46. de Jager CA, Dye L, de Bruin EA *et al.* (2014) Criteria for validation and selection of cognitive tests for investigating the effects of foods and nutrients. *Nutr Rev* **72**, 162–179.
47. Jenner A (2012) The importance of bioavailability and metabolism on the health benefits of dietary flavonoids – advances in biomarkers of intake. *Australas Med J* **5**, 683.
48. Kay CD (2015) Rethinking paradigms for studying mechanisms of action of plant bioactives. *Nutr Bull* **40**, 335–339.
49. Balentine DA, Dwyer JT, Erdman JW Jr *et al.* (2015) Recommendations on reporting requirements for flavonoids in research. *Am J Clin Nutr* **101**, 1113–1125.
50. Meskin MS, Bidlack WR & Randolph RK (2008) *Phytochemicals: Aging and Health*. Boca Raton, FL: CRC Press.
51. Polidori MC (2014) Antioxidant clinical trials in mild cognitive impairment and Alzheimer's disease – challenges and perspectives. *Curr Pharm Des* **20**, 3083–3092.
52. Fanning KJ, Topp B, Russell D *et al.* (2014) Japanese plums (*Prunus salicina* Lindl.) and phytochemicals – breeding, horticultural practice, postharvest storage, processing and bioactivity. *J Sci Food Agric* **94**, 2137–2147.
53. Lila MA (2004) Anthocyanins and human health: an in vitro investigative approach. *J Biomed Biotechnol* **2004**, 306–313.
54. Liu R (2003) Health benefits of fruit and vegetables are from additive and synergistic. *Am J Clin Nutr* **78**, 517S–520S.
55. Radtke J, Linseisen J & Wolfram G (2002) Fasting plasma concentrations of selected flavonoids as markers of their ordinary dietary intake. *Eur J Nutr* **41**, 203–209.
56. Krikorian R, Nash TA, Shidler MD *et al.* (2010) Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *Br J Nutr* **103**, 730–734.
57. Crews WD Jr, Harrison DW, Griffin ML *et al.* (2005) A double-blinded, placebo-controlled, randomized trial of the neuropsychologic efficacy of cranberry juice in a sample of cognitively intact older adults: pilot study findings. *J Altern Complement Med* **11**, 305–309.
58. Hendrickson SJ & Mattes RD (2008) No acute effects of grape juice on appetite, implicit memory and mood. *Food Nutr Res* **52**, doi:10.3402/fnr.v52i0.1891.
59. Bhagwat S, Haytowitz DB & Holden JM (2013) *USDA Database for the Flavonoid Content of Selected Foods*. Beltsville, MD: Nutrient Data Laboratory, US Department of Agriculture.
60. Keppler K & Humpf H-U (2005) Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. *Bioorg Med Chem* **13**, 5195–5205.
61. Tsuda T, Watanabe M, Ohshima K *et al.* (1994) Antioxidative activity of the anthocyanin pigments Cyanidin 3-O-.beta.-D-glucoside and cyanidin. *J Agric Food Chem* **42**, 2407–2410.
62. Ali HM, Almagribi W & Al-Rashidi MN (2016) Antiradical and reductant activities of anthocyanidins and anthocyanins, structure–activity relationship and synthesis. *Food Chem* **194**, 1275–1282.
63. Sogo T, Kumamoto T, Hisanaga A *et al.* (2015) Comparison of the inhibitory effects of delphinidin and its glycosides on cell transformation. *Planta Med* **81**, 26–31.
64. Felgines C, Talavera S, Gonthier MP *et al.* (2003) Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. *J Nutr* **133**, 1296–1301.
65. Fleschhut J, Kratzer F, Rechkemmer G *et al.* (2006) Stability and biotransformation of various dietary anthocyanins in vitro. *Eur J Nutr* **45**, 7–18.
66. Bagchi D, Sen CK, Bagchi M *et al.* (2004) Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. *Biochemistry (Mosc)* **69**, 75.
67. Bondonno CP, Downey LA, Croft KD *et al.* (2014) The acute effect of flavonoid-rich apples and nitrate-rich spinach on cognitive performance and mood in healthy men and women. *Food Funct* **5**, 849–858.
68. Franz M (2011) Your brain on blueberries. *Sci Am Mind* **21**, 54–59.
69. Galli RL, Shukitt-Hale B, Youdim KA *et al.* (2002) Fruit polyphenolics and brain aging: nutritional interventions targeting age-related neuronal and behavioral deficits. *Ann NY Acad Sci* **959**, 128–132.
70. Ho L, Chen LH, Wang J *et al.* (2009) Heterogeneity in red wine polyphenolic contents differentially influences Alzheimer's disease-type neuropathology and cognitive deterioration. *J Alzheimers Dis* **16**, 59–72.
71. How PS, Cox R, Ellis JA *et al.* (2007) The impact of plant-derived flavonoids on mood, memory and motor skills in UK adults. *Proc Nutr Soc* **66**, 87A.
72. Lafay S, Fouéré L, Lux V *et al.* (2014) Interest of anthocyanin standardized red fruit extract in formulation for delay age-related cognitive degeneration. *Phytotherapie* **12**, 156–163.
73. Macready AL, Butler LT, Kennedy OB *et al.* (2011) Carotenoids but not flavonoids are associated with improvements in spatial working memory in younger adults in a flavonoid-rich v. -poor fruit and vegetable intervention study. *Proc Nutr Soc* **70**, E133.
74. Malar DS & Devi KP (2014) Dietary polyphenols for treatment of Alzheimer's disease – future research and development. *Curr Pharm Biotechnol* **15**, 330–342.
75. Milbury PE (2012) Anthocyanin effects in cognition and dementia: probable mechanisms and future directions. *Abstr Pap Am Chem Soc* **244**.
76. Panickar KS (2013) Bioactive components of plant products including polyphenols exert neuroprotective effects and benefit neural function. *Cent Nerv Syst Agents Med Chem* **13**, 2.

77. Patel S (2014) Blueberry as functional food and dietary supplement: the natural way to ensure holistic health. *Med J Nutrition Metab* **7**, 133–143.
78. Ramirez MR, Geracitano L, Marti Barros D *et al.* (2009) Beneficial properties of red berries extracts and their anthocyanins. *Bol Latinoam Caribe Plant Med Aromat* **8**, 456–468.
79. Rendeiro C, Vauzour D, Rattray M *et al.* (2013) Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor. *PLoS One* **8**, e63535.
80. Ropacki SA, Patel SM & Hartman RE (2013) Pomegranate supplementation protects against memory dysfunction after heart surgery: a pilot study. *Evid Based Complement Alternat Med* **2013**, 932401.
81. Shukitt-Hale B (2012) Blueberries and neuronal aging. *Gerontology* **58**, 518–523.
82. Shukitt-Hale B, Carey A, Simon L *et al.* (2006) Effects of concord grape juice on cognitive and motor deficits in aging. *Nutrition* **22**, 295–302.
83. Vislocky LM & Fernandez ML (2010) Biomedical effects of grape products. *Nutr Rev* **68**, 656–670.
84. Vauzour D & Spencer JPE (2011) The impact of blueberry flavonoids on learning and memory. *Agro Food Ind Hi Tech* **22**, 13–15.
85. Wattanathorn J, Muchimapura S, Thukhammee W et al. (2012) Mulberry fruits protects against age-related cognitive decline. *Am J Appl Sci* **9**, 1503–1511.
86. Wouters-Wesseling W, Wagenaar LW, Rozendaal M et al. (2005) Effect of an enriched drink on cognitive function in frail elderly persons. *J Gerontol A Biol Sci Med Sci* **60**, 265–270.
87. Xia E-Q, Deng G-F, Guo Y-J & Li H-B (2010) Biological Activities of Polyphenols from Grapes. *International Journal of Molecular Sciences* **11**(2), 622–646.
88. Zafra-Stone S, Yasmin T, Bagchi M et al. (2007) Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol Nutr Food Res* **51**, 675–683.
89. Jeong JH, Kim HJ, Park SK et al. (2014) An investigation into the ameliorating effect of black soybean extract on learning and memory impairment with assessment of neuroprotective effects. *BMC Complement Altern Med* **14**, 14:482.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

INTERVENTION STUDIES

Effects of Dietary Approach to Stop Hypertension diet on androgens, antioxidant status and body composition in overweight and obese women with polycystic ovary syndrome: a randomised controlled trial

M. Azadi-Yazdi,[1,2] M. Karimi-Zarchi,[3] A. Salehi-Abargouei,[1,2] H. Fallahzadeh[4] & A. Nadjarzadeh[1,2]

[1]Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2]Department of Nutrition, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Obstetric & Gynecology Department, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4]Research center for Prevention and Epidemiology of non-communicable disease, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Keywords
androgens, dietary approach to stop hypertension, female, oxidative stress, polycystic ovary syndrome, randomised controlled trial.

Correspondence
A. Nadjarzadeh, Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. 8915173160
Tel.: +98 35 38209114
Fax: +98 35 38209119
E-mail: azadehnajarzadeh@gmail.com

How to cite this article
Azadi-Yazdi M., Karimi-Zarchi M., Salehi-Abargouei A., Fallahzadeh H. & Nadjarzadeh A. (2017) Effects of Dietary Approach to stop Hypertension diet on androgens, antioxidant status and body composition in overweight and obese women with polycystic ovary syndrome: a randomised controlled trial. *J Hum Nutr Diet.* **30**, 275–283
doi: 10.1111/jhn.12433

Clinical Trial Registration Number:
IRCT2014121610826N16

Abstract

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disease in reproductive age women. The present study aimed to determine the effects of Dietary Approaches to Stop Hypertension (DASH) diet on reproductive hormones, plasma total antioxidant status and anthropometric indices in overweight and obese PCOS women.
Methods: In this randomised controlled clinical trial, 60 women with PCOS were randomly assigned to one of two diets with energy restriction: the DASH diet and a control diet. The DASH and control diets consisted of 50–55% carbohydrate, 15–20% protein and 25–30% total fat. The DASH diet was designed to be rich in vegetables, fruits, whole grains and low-fat dairy products, as well as low in saturated fats, cholesterol, refined grains and sweets. In the present study, the anthropometric indices, body composition, total testosterone, androstenedione, sex hormone binding globulin (SHBG), free androgen index and 2,2′-diphenyl-1-picryylhydrazyl (DPPH) scavenging activity were measured before and after 3 months.
Results: The consumption of DASH diet compared to the control diet was associated with a significant reduction in weight [−5.78 (1.91) kg versus −4.34 (2.87) kg, $P = 0.032$], body mass index (BMI) [−2.29 (0.15) kg m^{-2} versus −1.69 (0.20) kg m^{-2}, $P = 0.02$], fat mass [−3.23 (1.66) kg versus −2.13 (1.26) kg, $P = 0.008$] and serum androstenedione [−1.75 (1.39) ng mL^{-1} versus −1.02 (0.72) ng mL^{-1}, P-value = 0.019]. Increased concentrations of SHBG [28.80 (21.71) versus 11.66 (18.82) nmol L^{-1}, $P = 0.003$) and DPPH scavenging activity [30.23% (19.09) versus 12.97% (25.12) were also found in the DASH group.
Conclusions: The DASH diet could improve weight loss, BMI and fat mass. Furthermore, it could result in a significant reduction in serum androstenedione and a significant increase in antioxidant status and SHBG.

Introduction

Polycystic ovary syndrome (PCOS) is a complex heterogeneous endocrine disorder [(1)]. Approximately 20% of women of reproductive age show morphological evidence of polycystic ovaries (PCO) [(2)]. The prevalence of PCOS in Iran has been reported to be 7–15% [(3)]. This condition is characterised by the presence of at least two of the three following features: (i) oligo- or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism;

and (iii) polycystic ovaries [4]. Women with PCOS have a clustering of disorders, including insulin resistance, impaired glucose tolerance, dyslipidaemia, dysfunctional bleeding, being overweight or obesity, an increased risk of infertility and cardiovascular diseases [5]. Being overweight or obesity might influence hyperandrogenism in women with PCOS, by various mechanisms [6]. Approximately, 30–70% of women with PCOS worldwide are overweight or obese [7] and lifestyle modifications that target weight reduction, including increased physical activity, are advised as the primary strategy in the management of PCOS [8]. Sex hormone binding globulin (SHBG), comprising the primary plasma transport system controlling the availability of androgens, is reduced in obese women with PCOS, leading to an increase in free testosterone levels [6,9]. In addition, as a result of the effects of insulin on hepatic SHBG production, insulin insensitivity may affect ovulation and fertility [10]. Studies have shown that weight loss of as little as 5% improves reproductive and metabolic abnormalities [11].

It has been suggested that diets low in carbohydrates and high in protein can produce a clinically relevant weight loss and reduce insulin resistance and insulin concentrations in overweight and hyperinsulinaemic individuals [12,13].

The DASH (dietary approaches to stop hypertension) diet, comprising a low-glycaemic index, low energy-dense diet including higher amounts of dietary fibre, phyto-oestrogens, potassium, magnesium, folic acid and other beneficial nutrients, was primarily designed for lowering blood pressure [14], although it is now suggested as a diet that is not only beneficial for people with hypertension, but also improves the blood lipid profile and the glycaemic index [15], as well as promotes weight reduction, which in turn might reduce testosterone levels [16]. Therefore, this dietary strategy appears to be potentially beneficial for women with PCOS. To the best of our knowledge, no study has examined the effect of the DASH diet on the androgenic profile in patients with PCOS. Therefore, the present study aimed to examine the effect of the DASH diet on the androgenic profile, total antioxidant capacity, body weight and composition in women with PCOS in the context of a randomised controlled clinical trial. In the present study, it was hypothesised that, independent of weight loss, the DASH diet would improve the androgenic profile in overweight and obese women with PCOS.

Materials and methods

Study design

On the basis of the previous study by Sørensen *et al.* [17], to enable detection of a decrease in total testosterone at 0.12 nmol L^{-1}, with 80% power, a significance level of 0.05 and SD of 0.15, a total of 25 subjects in each study group was required. Sixty subjects were originally recruited to allow for drop-outs. The present study comprised a parallel randomised controlled clinical trial with a 12-week follow-up carried out in Yazd, Iran, from May to August 2015. The participants were selected from Baghaeipoor Gynaecology clinic. Overweight (body mass index; BMI: 25–29.9 kg m^{-2}) and obese participants (BMI $\geq$ 30 kg m^{-2}) were selected based on inclusion criteria and then equally randomised into DASH diet and control diet groups using block randomisation. Random assignment was conducted using computer-generated random numbers (DASH group, control group). The participants were unaware of their diets. Dietary intakes, anthropometric measurements and serum androgens were assessed at baseline and at the end of the follow-up period. The primary outcome was the difference in testosterone and secondary outcomes were differences in androstenedione and SHBG, body composition and total antioxidant capacity. The study was performed in accordance with the Helsinki II Declaration and its protocol was approved by the ethical committee of Shahid Sadoughi University of Medical Sciences (SSU) and registered in the Iranian Registry of Clinical trials (www.irct.ir) under registry number: IRCT2014121610826N16. Each subject provided their written informed consent form before beginning the study.

Eligibility criteria

Overweight or obese (BMI = 25–40 kg m^{-2}) women aged 20–40 years who were newly diagnosed with PCOS based on the Rotterdam criteria [4], did not use hormonal contraception or other medications that could alter the concentration of androgens, did not use hormones as medication for 3 months prior to the study, were without type 1 diabetes, and were not using anti-obesity medications or engaging in a specific physical activity program, were included in the trial. PCOS was identified by ultrasonography (12 or more small follicles observed in an ovary on ultrasound examination) in participants with menstrual dysfunction (the presence of chronic amenorrhea or a menstrual cycle length of less than 21 days or more than 35 days, or more than 4 days of variation between cycles) and/or hirsutism. Participants were excluded from the study if hormonal therapy or other medications that could affect PCOS or weight were initiated for them during the study.

Intervention protocol

The macronutrient compositions of intervention and control diets were the same, containing 50–55% carbohydrate,

15–20% protein and 25–30% total fat. The DASH diet was designed to be rich in fruits, vegetables, whole grains and low-fat dairy products, as well as low in saturated fats, cholesterol, refined grains and sweets. The amount of sodium intake was also designed to be less than 2400 mg day^{-1}. The number of servings for both intervention and control groups for a 6.69 MJ day^{-1} (1600 kcal day^{-1}) diet is presented in Table 1. A 7-day menu cycle for each participant was planned. The energy requirements of each participant were estimated based on physical activity level and resting energy expenditure by the use of the Harris–Benedict equation [18]. Both diets were designed to be energy-restricted: 1.46–2.09 MJ (350–500 kcal) less than the computed energy requirement for each participant; 1.46 MJ (350 kcal) for subjects with BMI in the range 25–29.9 kg m^{-2}; 2.09 MJ (500 kcal) for those with the BMI in the range 30–39.9 kg m^{-2}. To monitor adherence to the diet, participants were asked to record their dietary intakes for 3 days (2 week days and 1 weekend) in each month. Moreover, every month, a 24-h dietary recall was completed by dietitian and each participant received a bimonthly visit from a dietitian.

Anthropometric measurements

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer at the screening visit. Body weight was measured to the nearest 0.05 kg at the baseline and after 12 weeks of intervention in a gynaecology clinic without shoes in a minimal clothing state using a digital scale (model 703; Seca, Hamburg, Germany). Waist circumference was recorded to the nearest 0.5 cm using a nonstretch plastic tape placed midway between the iliac crest and lowest rib when participants were in a standing position. In addition, the hip circumference was measured over the largest part of the buttocks to an accuracy of 0.5 cm. Body composition was estimated using a Body Composition Analyzer (BC-418; Tanita Corporation, Tokyo, Japan).

Table 1 Description of the DASH and control diets used in the present study

Food group (serving day^{-1})	Control diet	DASH diet
Grains*	9	6
Simple sugar	4	2
Vegetables	2	4
Fruits	2	4
Dairy†	1	2
Meats, poultry and fish	3	3
Nuts, seeds and legumes	1	2
Fats and oils	2	3

DASH, Dietary Approaches to Stop Hypertension. Data are presented for an energy intake of 6.69 MJ day^{-1} (1600 kcal day^{-1}). *At least three servings from whole grains in the DASH diet. †Low-fat (< 2%) in the DASH diet.

Blood sampling

Fasting blood samples (10 mL) were taken after fasting for at least 12 h at baseline and after 12 week of intervention. Serum and plasma were isolated by centrifugation for 10 min and aliquots were stored at −80 °C.

Biochemical assessments

Total testosterone was assayed using monobind kit by an enzyme-linked immunosorbent assay (ELISA). The sensitivity limit was 0.05 nmol L^{-1}. SHBG and androstenedione were analysed by ELISA, using kits from DiaMetra (Milan, Italy). The sensitivity limits were 0.1 nmol L^{-1} and 0.1 ng mL^{-1}, respectively. The free androgen index is calculated as the ratio of serum total testosterone divided by SHBG.

For total antioxidant capacity (TAC), the 2,2′-diphenyl-1-picryylhydrazyl (DPPH) method was used. The DPPH reduction assay was based on the method of Janaszewska and Bartosz [19].

Statistical analysis

Randomisation and statistical analyses were performed using SPSS, version 16 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are reported as the mean (SD) or SEM. Normal distribution and homoscedasticity of outcome variables were checked by means of Kolmogrov–Smirnov and Levene's tests, respectively. An independent samples *t*-test was used to detect differences in general characteristics, body composition, dietary intakes, androgenic profile and TAC between two groups. The differences or changes in outcome variables between the intervention and control groups were analysed using analysis of covariance (ANCOVA) in which the test diets were considered as factors and variables such as participants' age, fat mass and weight changes and baseline values were adjusted as covariates. $P < 0.05$ (two-tailed) was considered statistically significant. To obtain the nutrient intakes of participants based on these 3-day food diaries, NUTRITIONIST IV (First Databank, San Bruno, CA, USA) modified for Iranian foods. The energy-adjusted dietary intakes of nutrients were computed using ANCOVA.

Results

A total of 95 women were screened for PCOS. Sixty women were recruited in the present study and were randomly allocated to the DASH ($n = 30$) and control

(n = 30) diets. In the end, 55 participants [control diet (n = 27) and the DASH diet (n = 28)] completed the trial (Fig. 1). The mean (SD) age was 31.95 (6.07) years and was not statistically different between the DASH and control groups (Table 2).

Food intake

After testing for equality of variances using Levene's test, an independent sample t-test showed that dietary intakes of energy, carbohydrates, protein, sodium, saturated fatty acids, polyunsaturated fatty acids, cholesterol, monounsaturated fatty acids and sucrose intake were not significantly different between two groups. However, significant differences were found in the dietary intakes of fat, potassium, magnesium, calcium, dietary fibre, Soluble fibre and vitamin C (Table 3).

Body weight and composition

Participants' weight and BMI did not differ between two groups before and after treatment (Table 2). However, greater mean (SD) reductions in both weight [−5.78 (1.91) kg versus −4.34 (2.87) kg, P = 0.032] and BMI [−2.29 (0.15) kg m^{-2} versus −1.69 (0.20) kg m^{-2}, P = 0.02) were found in the DASH group compared to the control group. Fat mass decreased more in the DASH diet [−3.23 (1.66) kg versus −2.13 (1.26) kg, P = 0.008). There was no difference in lean body mass, waist circumferences, hip circumferences or waist-to-hip ratios between the two groups.

Sex hormones and total antioxidant capacity

The reduction of androstenedione was greater with DASH diet than with the control diet in unadjusted and adjusted models ($P < 0.05$). The SHBG and antioxidant capacity (DPPH reduction) increased more in the DASH diet group compared to the control group in unadjusted and adjusted models ($P < 0.05$). In comparison with the control diet, the consumption of DASH eating pattern resulted in a significant mean (SD) reduction in serum testosterone [−0.39 (0.28) ng mL^{-1} versus −0.14 (0.12) nmol L^{-1}, $P \leq 0.001$] and free androgen index [−4.51 (4.5) versus −1.59 (0.66), P = 0.003]. However, after adjustment for potential confounding variables, these differences did not reach statistical significance (Table 4).

Discussion

The results of the present study indicate that the DASH diet had beneficial effects on weight, BMI, fat mass, androstenedione, SHBG and antioxidant capacity.

The benefits of weight loss in obese and overweight women with PCOS have been well documented [20,21]. However, in the present study, independent of the degree

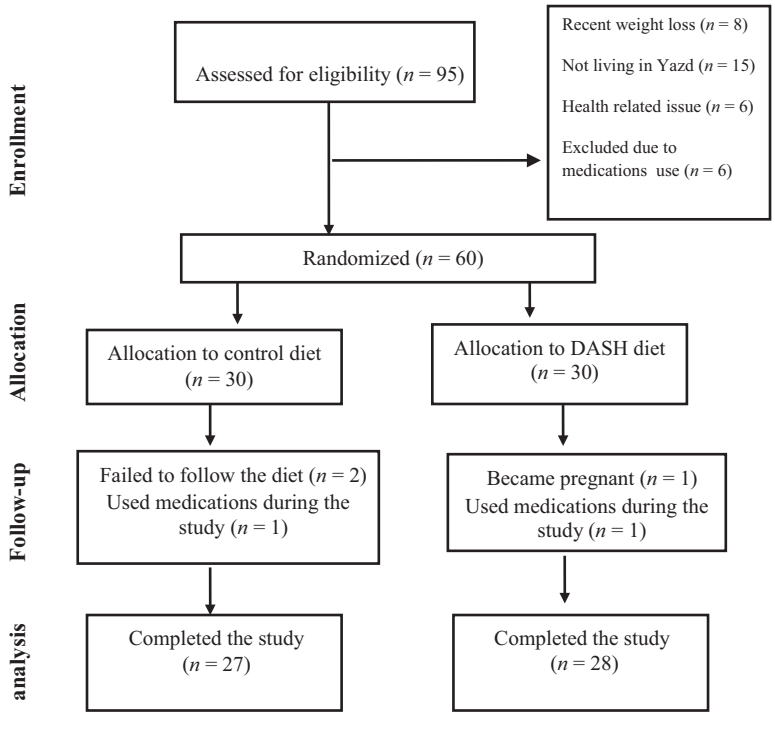

Figure 1 Summary of participants flow.

Table 2 General characteristics and body composition of the study participants[†]

	Control diet (n = 27)		DASH diet (n = 28)		P-value[¶]		
Age (yrs)	31.76 (6.24)		32.13 (5.99)		0.817		
Height (Cm)	159.78 (7.01)		159.11 (5.28)		0.681		
	Baseline	**Δ 12 weeks[§]**	**Baseline**	**Δ 12 week[§]**	**week0**	**week 12**	**Group difference**
Weight (kg)	77.68 (12.47)	−4.34 (2.86)*	80.91 (11.53)	−5.78 (1.90)*	0.302	0.569	0.032
BMI (kg m^{-2})	30.20 (3.25)	−1.69 (1.06)*	31.92 (4.16)	−2.29 (0.78)*	0.08	0.253	0.02
Waist circumference (cm)	99.67 (10.72)	−3.77 (3.09)*	100.95 (11.58)	−4.97 (3.77)*	0.660	0.910	0.205
Hip circumference (cm)	110.25 (6.75)	−3.96 (2.89)*	112.70 (9.18)	−4.14 (3.38)*	0.244	0.495	0.833
Waist-to-hip ratio	0.89 (0.073)	−0.0063 (0.024)	0.89 (0.065)	−0.0117 (0.0138)	0.748	0.775	0.315
Lean body mass (kg)	46.79 (5.57)	−0.51 (1.27)*	48.61 (5.61)	−0.82 (1.64)*	0.372	0.378	0.434
Fat mass (kg)	30.35 (8.28)	−2.13 (1.26)*	32.40 (9.32)	−3.23 (1.66)*	0.213	0.603	0.008

*P < 0.05, paired t-test. BMI, body mass index. [†]Values are the mean (SD). [§]Change from baseline in unadjusted model. [¶]Obtained from an independent t-test.

of weight loss, adherence to the DASH diet had more beneficial effects on androstenedione, SHBG and antioxidant capacity compared to the conventional energy-restricted diet.

Several studies have reported the beneficial effect of different diets except for the DASH diet on androgenic status in women with PCOS [11,17,22,23].

The design of the present study is similar to that employed by Asemi *et al.* [24], who reported the beneficial effects of a hypocaloric DASH diet on overweight and obese women with PCOS after 8 weeks. However, Asemi *et al.* [24] did not examine the effect of the DASH diet on androgenic profile. To the best of our knowledge, the present study provides the first clinical evidence revealing the effects of the DASH diet on androgenic profile in PCOS. The results show that consumption of the DASH diet resulted in a significant reduction in weight, BMI and fat mass changes. These findings are in line with those reported by Asemi *et al.* [24]. A recent meta-analysis showed that DASH diet has a significant effect on weight, BMI and waist circumference in overweight and obese people [25]. Weight loss decreased insulin resistance [26] and this reduction is associated with a decrease in the P450c17a enzyme activity and, consequently, a decrease in ovarian androgen production [27]. In addition, weight loss in combination with a reduction of leptin resulted in deactivation of the neuroendocrine control of ovarian steroid secretion [28]. Moreover, a low energy diet and weight loss affected the family of insulin-like growth factors (IGFs) and their binding proteins [29]. During short-term energy restriction, decreased insulin levels were associated with increased IGF-binding protein-1 [30]. On the other hand, adiponectin might be responsible for the metabolic and neuroendocrine characteristics of obesity and obesity-related disease, such as PCOS [31]. Serum adiponectin is inversely correlated with the severity of insulin resistance [32]. The beneficial results of the DASH diet could be partly attributed to its effects on weight and metabolic parameters. However, the results of the present study revealed that the effects of the DASH diet on the androgen profile might be independent of weight loss.

Several mechanisms can explain the beneficial effects of the DASH diet on the androgenic profile. In the present study, the calcium content of the DASH diet was approximately 1.5 times greater than that of the control diet. The metabolic changes in PCOS patients are related to dysfunction of vitamin D and calcium metabolism, which is also important in follicular development [33] and normal glucose metabolism [34]. Some studies have shown that PCOS patients, particularly if obese, have lower serum vitamin D levels [35,36]. Lou *et al.* [37] showed that 1,25-dihydroxyvitamin D mediates aromatase activity, which converts androgen derivatives to oestrogen in granulosa cells of the ovaries. Therefore, a dysfunction of this mechanism affects the menstrual cycle and ovulation by altering the hormone levels mediated and suppressed by oestrogen, luteinising hormone and follicle-stimulating hormone. Impaired calcium regulatory system could cause follicular arrest. Moreover, animal studies in this field revealed that calcium plays a crucial role in oocyte maturation, as well as in the resumption and progression of follicular development [33].

PCOS is associated with decreased levels of adiponectin concentrations, which in turn, leads to disturbances of mitogen-activated protein kinases and the nuclear factor kappa-light-chain-enhancer of activated B cells signalling pathway. These conditions are associated with increased inflammatory markers and biomarkers of oxidative stress [38]. A decrease in body weight increases adiponectin concentrations [39], suggesting that adiponectin expression is down-regulated by adipose tissue [40]. Apart from weight loss, the DASH diet is high in antioxidants contained in fruit, vegetables and whole grains. In the present study, the DASH diet increased the antioxidant capacity compared to the control diet after controlling for age, weight loss, fat

Table 3 Energy and nutrient intakes of study participants throughout the present study*

		Control diet (n = 27)	DASH diet (n = 28)	P-value†	P-value‡
Energy (MJ day^{-1}) [kcal day^{-1}]		7.01 (0.58) [1697.66 (138.13)]	6.99 (0.43) [1671.98 (103.4)]	0.437	0.164
Protein (g day^{-1})	Crude	65.39 (12.06)	70.05 (9.26)	0.224	0.669
	Model 1	64.66 (1.61)	70.75 (1.58)	0.01	
Carbohydrates (g day^{-1})	Crude	244.57 (26.02)	243.63 (23.32)	0.888	0.936
	Model 1	242.60 (3.23)	245.531 (3.171)	0.522	
Fat (g day^{-1})	Crude	57.06 (11.40)	51.22 (8.03)	0.032	0.496
	Model 1	56.41 (1.51)	51.84 (1.45)	0.036	
Cholesterol (mg day^{-1})	Crude	197.54 (71)	180.77 (62.26)	0.355	0.154
	Model 1	198.45 (12.68)	179.82 (12.91)	0.309	
MUFA (g day^{-1})	Crude	13.17 (3.01)	13.75 (3.00)	0.474	0.556
	Model 1	13.24 (0.51)	13.59 (0.59)	0.707	
SFA (g day^{-1})	Crude	19.45 (7.72)	16.20 (3.31)	0.172	0.198
	Model 1	19.11 (0.97)	16.53 (0.95)	0.252	
PUFA (g day^{-1})	Crude	23.87 (12.21)	24.84 (14.33)	0.135	0.833
	Model 1	24.45 (2.55)	25.56 (2.64)	0.163	
Dietary fibre (g day^{-1})	Crude	13.00 (2.19)	18.36 (3.09)	< 0.001	0.308
	Model 1	12.96 (0.51)	18.40 (0.51)	< 0.001	
Soluble fibre (g day^{-1})	Crude	5.14 (0.18)	7.34 (0.28)	0.001	0.094
	Model 1	5.16 (0.04)	7.33 (0.04)	0.002	
Vitamin C (mg day^{-1})	Crude	126.16 (44.79)	229.76 (75.74)	< 0.001	0.006
	Model 1	123.70 (11.89)	231.16 (11.67)	< 0.001	
Magnesium (mg day^{-1})	Crude	194.38 (44.50)	248.20 (55.88)	< 0.001	0.599
	Model 1	192.66 (9.35)	249.87 (9.18)	< 0.001	
Calcium (mg day^{-1})	Crude	852.20 (260.72)	1164.96 (182.84)	< 0.001	0.422
	Model 1	846.99 (42.70)	1169.98 (41.93)	< 0.001	
Potassium (mg day^{-1})	Crude	2278.33 (320.39)	3276.32 (455.01)	< 0.001	0.153
	Model 1	2265.25 (74.00)	3288.93 (72.65)	< 0.001	
Sodium (mg day^{-1})	Crude	943.40 (205.68)	846.56 (166.51)	0.061	0.222
	Model 1	939.43 (35.67)	850.39 (35.02)	0.082	
Sucrose (g day^{-1})	Crude	23.16 (6.61)	20.65 (7.28)	0.187	0.413
	Model 1	23.14 (1.35)	20.67 (1.33)	0.202	
Grains (servings day^{-1})		9.3 (0.5)	6.1 (0.5)	< 0.001	0.024
Simple sugar (servings day^{-1})		5.11 (1.00)	2.21 (0.2)	< 0.001	< 0.001
Vegetables (servings day^{-1})		2.5 (1.00)	4.3 (0.40)	< 0.001	0.045
Fruits (servings day^{-1})		2 (1.00)	4.75 (1.00)	< 0.001	0.11
Dairy (servings day^{-1})		2 (1.00)	3 (1.00)	< 0.001	0.442
Meats, poultry and fish (servings day^{-1})		4 (1.00)	4 (1.00)	0.674	0.442
Nuts, seeds and legumes (servings day^{-1})		1 (0.30)	2 (0.30)	< 0.001	0.087
Fats and oils (servings day^{-1})		3 (1.00)	3 (0.40)	0.142	< 0.001

DASH, Dietary Approaches to Stop Hypertension; MUFA, monounsaturated fatty acids; PUFA, Polyunsaturated fats; SFA, Saturated fatty acids.*Data are the mean (SD). †Independent t test. ‡Levene's test. Model 1: adjusted for energy intake; data are the mean (SE).

mass and baseline value. Al-Solaiman *et al.* [41] reported the beneficial effects of the DASH diet on the F2-isoprostane level, which is a measure of oxidative stress, and is associated with a significant increase in plasma TAC and total glutathione levels in obese patients with PCOS [24], as well as in pregnant women with gestational diabetes [42]. However, adherence to the DASH eating plan for 3 months did not lead to a significant increase in plasma TAC in healthy individuals [43]. The DASH diet is a rich source of dietary magnesium, which increases the antioxidant capacity of the serum and tissues [44], restores the activity of anti-oxidative enzymes [45], scavenges oxygen radicals [46] and reduces insulin resistance [47]. The total vitamin C intake in the DASH group was 1.8-times greater than that in the control group. Vitamin C, a major component of TAC, has been reported to decrease the nicotinamide adenine dinucleotide phosphate-oxidase activity, which is a major superoxide-generating enzyme [48]. Finally, calcium can act as a DNA damage reducing agent, which in turn, results in free radical scavenging and an increase in plasma TAC [49].

In the present study design, the control of some potential confounding variables, such as age, weight loss, fat mass and baseline variables, showed an effect of the DASH diet independent of weight and fat mass reduction.

Table 4 Serum androgenic profiles and total antioxidant capacity (TAC) at baseline and after the intervention‡

	Control diet (n = 27)		DASH diet (n = 28)		Group difference, P†	
	Baseline‡	Δ 12 weeks§	Baseline‡	Δ 12 week§	Crude	Model 1
Total testosterone (nmol L^{-1})	1.02 (0.20)	−0.14 (0.12)*	1.45 (0.77)	−0.39 (0.28)*	< 0.001	0.946
Androstenedione (ng mL^{-1})	3.38 (1.14)	−1.02 (0.72)*	3.36 (1.84)	−1.75 (1.39)*	0.019	< 0.001
SHBG (nmol L^{-1})	38.88 (20.8)	11.66 (18.82)*	28.93 (18.08)	28.80 (21.71)*	0.003	0.006
FAI	3.45 (2.17)	−1.58 (2.09)*	6.79 (4.83)	−4.51 (4.5)*	0.003	0.698
DPPH scavenging activity (%)	29.24 (3.32)	12.97 (25.12)*	23.30 (13.06)	30.23 (19.09)*	0.005	0.008

DASH, Dietary Approaches to Stop Hypertension; SHBG, sex hormone binding globulin; FAI, free androgen index; DPPH, 2,2′-di phenyl-1-picryyl-hydrazyl. *P < 0.05, paired t-test. †Unadjusted model compares the raw change over 12 weeks between the control diet and the DASH diet groups; model 1 adjusted for age, fat mass and weight changes and baseline values. ‡Values are the mean (SD). §Change from baseline.

The compliance of participants was maximised by frequent visits to the dietitian and the provision of detailed food plans with menu. Although controlled feeding is a gold standard study design, studies conducted in free-living populations are more reflective of nutritional management in routine clinical practice. In the present study, physical activity among participants was not measured. However, participants were asked not to change their routine physical activity during the study. We could not examine the effect of the DASH diet on other biochemical indicators of oxidative stress and factors of peroxidation. Therefore, further studies are required to assess the effects of the DASH diet on oxidative stress. Furthermore, the present study did not investigate the effect of this kind of diet on insulin resistance. Moreover, our laboratory did not validate the kits used against the gold standard liquid (or gas) chromatography-tandem mass spectrometry. Another limitation of the present study is that it is impossible to determine whether the positive effects are essentially attributable to a higher intake of fruits and vegetables or to other variables of the diet.

In conclusion, the DASH energy-restricted diet could be a clinically useful plan in the management of overweight women with PCOS. The results of the present study suggest that the DASH dietary pattern with energy restriction lowers androstenedione levels and increases antioxidant activity, independent of its weight loss effects. The results oof the present study warrant further investigation via long-term randomised clinical trials in either hypocaloric or isocaloric conditions to fully clarify the effect of the DASH diet in PCOS.

Acknowledgments

We are grateful to all the women participants in the present study and also to the research deputy of Shahid Sadoughi University of Medical sciences for providing funding. This work has been performed as a thesis toward a master's degree of public Health Nutrition.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained. The reporting of this work is compliant with CONSORT guidelines.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
This study was financially supported by Shahid Sadoughi University of Medical Sciences as a MsPH thesis.
MAY conducted the study, wrote the manuscript, carried out the statistical analyses and contributed to the explanation of the findings. MKZ contributed to the diagnosis of polycystic ovary syndrome. ASA contributed to the statistical analyses and edited the manuscript. HF assisted in the design and statistical analyses. AN contributed to the conception and design and helped with the interpretation of the findings. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. Lobo RA & Carmina E (2000) The importance of diagnosing the polycystic ovary syndrome. *Ann Intern Med* **132**, 989–993.
2. Farquhar CM, Birdsall M, Manning P *et al.* (1994) The prevalence of polycystic ovaries on ultrasound scanning in a population of randomly selected women. *Aust NZ J Obstet Gynaecol* **34**, 67–72.

3. Mehrabian F, Khani B, Kelishadi R *et al.* (2011) The prevalence of polycystic ovary syndrome in Iranian women based on different diagnostic criteria. *Endokrynol Pol* **62**, 238–242.
4. Rotterdam E, ASRM-Sponsored P (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* **81**, 19.
5. Azziz R, Carmina E, Dewailly D *et al.* (2009) The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* **91**, 456–488.
6. Pasquali R, Casimirri F & Vicennati V (1997) Weight control and its beneficial effect on fertility in women with obesity and polycystic ovary syndrome. *Hum Reprod* **12**, 82–87.
7. Yucel A, Noyan V & Sagsoz N (2006) The association of serum androgens and insulin resistance with fat distribution in polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* **126**, 81–86.
8. Costello MF, Misso ML, Wong J *et al.* (2012) The treatment of infertility in polycystic ovary syndrome: a brief update. *Aust NZ J Obstet Gynaecol* **52**, 400–403.
9. Martínez-García MÁ, Gambineri A, Alpañés M *et al.* (2012) Common variants in the sex hormone-binding globulin gene (SHBG) and polycystic ovary syndrome (PCOS) in Mediterranean women. *Hum Reprod* **27**, 3569–3576.
10. Cobin R, Futterweit W, Nestler J *et al.* (2005) American Association of Clinical Endocrinologists position statement on metabolic and cardiovascular consequences of polycystic ovary syndrome. *Endocr Pract* **11**, 125–134.
11. Stamets K, Taylor DS, Kunselman A *et al.* (2004) A randomized trial of the effects of two types of short-term hypocaloric diets on weight loss in women with polycystic ovary syndrome. *Fertil Steril* **81**, 630–637.
12. McAuley K, Hopkins C, Smith K *et al.* (2005) Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. *Diabetologia* **48**, 8–16.
13. Gannon MC & Nuttall FQ (2004) Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. *Diabetes* **53**, 2375–2382.
14. Azadbakht L, Fard NRP, Karimi M *et al.* (2011) Effects of the dietary approaches to stop hypertension (DASH) eating plan on cardiovascular risks among type 2 diabetic patients a randomized crossover clinical trial. *Diabetes Care* **34**, 55–57.
15. Shirani F, Salehi-Abargouei A & Azadbakht L (2013) Effects of Dietary Approaches to Stop Hypertension (DASH) diet on some risk for developing type 2 diabetes: a systematic review and meta-analysis on controlled clinical trials. *Nutrition* **29**, 939–947.
16. Luo X & Xu L (2012) Association of fat distribution with metabolic syndrome in patients with polycystic ovary syndrome. *Nan Fang Yi Ke Da Xue Xue Bao* **32**, 1325–1327.
17. Sørensen LB, Søe M, Halkier KH *et al.* (2012) Effects of increased dietary protein-to-carbohydrate ratios in women with polycystic ovary syndrome. *Am J Clin Nutr* **95**, 39–48.
18. Trumbo P, Schlicker S, Yates AA *et al.* (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc* **102**, 1621–1630.
19. Janaszewska A & Bartosz G (2002) Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest* **62**, 231–236.
20. Norman RJ, Davies MJ, Lord J *et al.* (2002) The role of lifestyle modification in polycystic ovary syndrome. *Trends Endocrinol Metab* **13**, 251–257.
21. Moran LJ, Ko H, Misso M *et al.* (2013) Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *J Acad Nutr Diet* **113**, 520–545.
22. Nybacka Å, Carlström K, Ståhle A *et al.* (2011) Randomized comparison of the influence of dietary management and/or physical exercise on ovarian function and metabolic parameters in overweight women with polycystic ovary syndrome. *Fertil Steril* **96**, 1508–1513.
23. Nadjarzadeh A, Firouzabadi RD, Vaziri N *et al.* (2013) The effect of omega-3 supplementation on androgen profile and menstrual status in women with polycystic ovary syndrome: a randomized clinical trial. *Iran J Reprod Med* **11**, 665.
24. Asemi Z, Samimi M, Tabassi Z *et al.* (2014) Effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized clinical trial. *Nutrition* **30**, 1287–1293.
25. Soltani S, Shirani F, Chitsazi MJ *et al.* (2016) The effect of dietary approaches to stop hypertension (DASH) diet on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. *Obes Rev* **17**, 442–454.
26. Pasquali R, Antenucci D, Casimirri F *et al.* (1989) Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. *J Clin Endocrinol Metab* **68**, 173–179.
27. Jakubowicz DJ & Nestler JE (1997) 17α-Hydroxyprogesterone responses to leuprolide and serum androgens in obese women with and without polycystic ovary syndrome after dietary weight loss 1. *J Clin Endocrinol Metab* **82**, 556–560.
28. Pasquali R, Gambineri A, Biscotti D *et al.* (2000) Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *J Clin Endocrinol Metab* **85**, 2767–2774.
29. Hamilton-Fairley D, Kiddy D, Anyaoku V *et al.* (1993) Response of sex hormone binding globulin and insulin-like

growth factor binding protein-1 to an oral glucose tolerance test in obese women with polycystic ovary syndrome before and after calorie restriction. *Clin Endocrinol* **39**, 363–367.
30. Moran L, Noakes M, Clifton P *et al.* (2003) Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. *J Clin Endocrinol Metab* **88**, 812–819.
31. Orio F Jr, Palomba S, Cascella T *et al.* (2003) Adiponectin levels in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* **88**, 2619–2623.
32. Weyer C, Funahashi T, Tanaka S *et al.* (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**, 1930–1935.
33. Thys-Jacobs S, Donovan D, Papadopoulos A *et al.* (1999) Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. *Steroids* **64**, 430–435.
34. Firouzabadi Rd, Aflatoonian A, Modarresi S *et al.* (2012) Therapeutic effects of calcium & vitamin D supplementation in women with PCOS. *Complement Ther Clin Pract* **18**, 85–88.
35. Yildizhan R, Kurdoglu M, Adali E *et al.* (2009) Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Arch Gynecol Obstet* **280**, 559–563.
36. Hahn S, Haselhorst U, Tan S *et al.* (2006) Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes* **114**, 577–583.
37. Lou Y-R, Murtola T & Tuohimaa P (2005) Regulation of aromatase and 5α-reductase by 25-hydroxyvitamin D 3, 1α, 25-dihydroxyvitamin D 3, dexamethasone and progesterone in prostate cancer cells. *J Steroid Biochem Mol Biol* **94**, 151–157.
38. Essick EE, Wilson RM, Pimentel DR *et al.* (2013) Adiponectin modulates oxidative stress-induced autophagy in cardiomyocytes. *PLoS ONE* **8**, e68697.
39. Yang W-S, Lee W-J, Funahashi T *et al.* (2001) Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* **86**, 3815–3819.
40. Matsubara M, Maruoka S & Katayose S (2002) Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab* **87**, 2764–2769.
41. Al-Solaiman Y, Jesri A, Zhao Y *et al.* (2009) Low-Sodium DASH reduces oxidative stress and improves vascular function in salt-sensitive humans. *J Hum Hypertens* **23**, 826–835.
42. Asemi Z, Samimi M, Tabassi Z *et al.* (2013) A randomized controlled clinical trial investigating the effect of DASH diet on insulin resistance, inflammation, and oxidative stress in gestational diabetes. *Nutrition* **29**, 619–624.
43. Miller ER, Erlinger TP, Sacks FM *et al.* (2005) A dietary pattern that lowers oxidative stress increases antibodies to oxidized LDL: results from a randomized controlled feeding study. *Atherosclerosis* **183**, 175–182.
44. King JL, Miller RJ, Blue JP *et al.* (2009) Inadequate dietary magnesium intake increases atherosclerotic plaque development in rabbits. *Nutr Res* **29**, 343–349.
45. Yang Y, Gao M, Nie W *et al.* (2012) Dietary magnesium sulfate supplementation protects heat stress-induced oxidative damage by restoring the activities of anti-oxidative enzymes in broilers. *Biol Trace Elem Res* **146**, 53–58.
46. Hans CP, Chaudhary DP & Bansal DD (2003) Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. *Magnes Res* **16**, 13–19.
47. Rowe W (2012) Correcting magnesium deficiencies may prolong life–Space Station Feb 2012. *Clin Interv Aging* **7**, 51–54.
48. Chen X, Touyz RM, Park JB *et al.* (2001) Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension* **38**, 606–611.
49. Fedirko V, Bostick RM, Long Q *et al.* (2010) Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: a randomized clinical trial. *Cancer Epidemiol Biomarkers Prev* **19**, 280–291.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

CLINICAL NUTRITION

Body composition assessed by dual-energy X-ray absorptiometry predicts early infectious complications after liver transplantation

C. Lindqvist,[1] A. Majeed[2,3] & S. Wahlin[2]

[1]Department of Clinical Nutrition and Dietetics, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[2]Department of Gastroenterology and Hepatology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[3]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Keywords
body composition, dual-energy X-ray absorptiometry, fat free mass index, liver transplantation, outcome.

Correspondence
C. Lindqvist, Department of Clinical Nutrition and Dietetics, Karolinska University Hospital, 141 86 Stockholm, Sweden.
Tel.: +46 8 585 896 96
Fax: +46 8 585 823 35
E-mail: catarina.lindkvist.1@ki.se

How to cite this article
Lindqvist C., Majeed A. & Wahlin S. (2017) Body composition assessed by dual-energy X-ray absorptiometry predicts early infectious complications after liver transplantation. *J Hum Nutr Diet*. **30**, 284–291
doi: 10.1111/jhn.12417

Abstract

Background: It is challenging to identify malnutrition, which is a risk factor for poor outcome in patients with liver cirrhosis. In the present study, we aimed to investigate the prevalence of malnutrition among patients listed for liver transplantation, as assessed by different methods, and also to relate dual-energy X-ray absorptiometry (DXA) to short-term post-transplant outcomes.
Methods: In this retrospective cohort study, we reviewed the medical records of 106 patients who underwent liver transplantation in 2009–2012. Body composition was assessed by the fat-free mass index (FFMI) and fat mass index (FMI) obtained using DXA. Severe infections within 1 month, length of stay in intensive care unit and length of hospital stay were end-points of primary interest.
Results: The prevalence of malnutrition was 2–20% depending on sex and the assessment method. Thirty-nine (37%) patients developed severe infections within 1 month after liver transplantation. In multivariate analysis with logistic regression, body composition was significantly associated with post-operative infection when measured with FFMI ($P = 0.043$) but not with FMI ($P = 0.087$). Post-operative dialysis ($P = 0.004$) and post-operative infections ($P < 0.001$) were significantly associated with length of stay in hospital. Post-operative bleeding ($P = 0.015$), duration of mechanical ventilation ($P < 0.001$) and the need for dialysis ($P < 0.001$), but not body composition, were significant predictors of the length of stay in the intensive care unit.
Conclusions: The prevalence of malnutrition depends on assessment method. FFMI is an independent predictor for early post-transplant infections. Body composition measured by DXA during the pretransplant evaluation provides valuable information about nutritional status in patients with liver cirrhosis.

Introduction

Malnutrition is common in chronic liver disease. The nutritional status is of importance for quality of life [(1)] and has an impact on the duration of hospitalisation, complication rates, costs and survival in patients with severe or prolonged illness [(2–4)]. Sarcopenia has been shown to impair the prognosis in liver cirrhosis [(5)]. For patients awaiting liver transplantation, malnutrition affects the length of stay (LOS), rate of serious infections and mortality [(2,6,7)]. In efforts to optimise resource utilisation and provide efficient care, it is important to

identify malnutrition and attempt to mitigate this potentially reversible risk factor for early complications after liver transplantation.

The prevalence of malnutrition increases with severity of liver disease, from 20% to 46% in patients with Child-Pugh A to 60–95% in those with Child-Pugh C [8,9], depending on the assessment method and definition of malnutrition. Reported prevalence ranges from 6% (body mass index; BMI) up to 81% (handgrip dynamometry) [10]. In liver cirrhosis, oedema, over- and under-nutrition can coexist, and this makes nutritional assessment demanding. There is no gold standard for screening or assessing nutritional status in patients with liver cirrhosis. Common nutritional parameters such as serum albumin, BMI and weight loss may be misleading because of water retention, ascites, oedema, impaired protein synthesis and altered renal function [7]. BMI provides no information on body composition. Measuring body composition may be a better way of assessing nutritional status in patients with liver cirrhosis and may yield better information about outcome and prognosis [11,12].

Recent studies have focused on assessing malnutrition by measuring cross-sectional muscle area at the lumbar level with computed tomography (CT). This method provides an objective and reproducible measure of muscularity. However, abdominal CT or magnetic resonance imaging (MRI) are not always carried out as parts of the pretransplant work-up, unless malignancy is present or suspected, or the transplant indication is primary sclerosing cholangitis (PSC).

Dual-energy X-ray absorptiometry (DXA) is a non-invasive procedure that involves minimal radiation exposure and is less expensive than CT or MRI. It is routinely used for pretransplant osteoporosis screening and can provide information about fat mass (FM), lean soft tissue (LST) and bone mineral content (BMC), of which LST and BMC together constitute the fat-free mass (FFM) [13,14]. These objective parameters on body composition are potentially valuable for predicting malnutrition-related early post-transplant complications.

In the present study, we aimed to investigate the prevalence of malnutrition among patients listed for liver transplantation and to relate body composition parameters measured with DXA to the length of hospital stay and the occurrence of severe infections after liver transplantation.

Materials and methods

Setting and study population

Data on all consecutive liver transplantations between 2009 and 2012 were reviewed retrospectively. The inclusion criteria were: patients aged 19 years or older, with chronic liver diseases, and who underwent DXA at our centre during the pretransplant work-up. Patients with hepatocellular carcinoma (HCC) within the University of California, San Francisco transplant criteria [15], as well as patients with PSC and premalignant changes in the biliary tree, were included. Patients with previous liver transplantations and those planned for multi-organ transplantation were excluded. Data on patient characteristics, including demographics, transplant indication, the presence of ascites, Child-Pugh and model of end-stage liver disease (MELD) scores, as well as laboratory values at the time of transplantation work-up, were collected from the local transplant registry and from patient medical records.

Body composition

DXA and nutritional assessment by a dietitian were used to assess the nutritional status of the patients. BMC, FM, LST and the sum of body tissues were measured using a fan-beam DXA (GE Lunar iDXA; system number ME +200030; GE Lunar Corp., Madison, WI, USA). The reported precision for this DXA model is coefficients of variation (CV) 0.5% for lean tissue mass and CV 0.82% for total fat mass [16]. FFM was calculated as the sum of LST and BMC. FFM and FM were adjusted for height, FMI = FM in kg $\times$ m^2 and FFMI = FFM in kg $\times$ m^2. Fat and fat-free mass depletion (i.e. malnutrition) were defined as FMI and FFMI indices <5th percentile, according to standard tables from a reference population [17].

Nutritional assessment

A dietitian evaluated the nutritional status during the pretransplant investigation. In the assessment, the factors considered were: the presence of eating difficulties, the presence of weight loss and BMI. The nutritional status was categorised into three different groups: well nourished (no eating difficulties, no weight loss or had spontaneously recovered lost weight and BMI above 20); at risk for malnutrition (eating difficulties and weight loss <10%, or weight loss >10% but no eating difficulties); and malnourished (eating difficulties and weight loss > 10% or BMI < 20). In the few cases where there was a discrepancy between the recorded clinical assessment by the dietitian and what the criteria oulined above stipulate, the former was used.

Outcome measures

A poor nutritional status is likely to have greatest impact on the early post-operative course. We therefore focused on short-term (30 day) outcome measures during the early post-operative period: incidence of severe infections, length of stay in the intensive care unit (ICU) and total

hospital stay, as well as 30-day all-cause mortality. Severe infections were defined as systemic or requiring intravenous or prolonged courses of antimicrobials. Minor infections, such as uncomplicated urinary tract infections, are generally of limited clinical consequence and are difficult to determine in a retrospective study. We recorded post-operative bleedings, need for dialysis or prolonged ventilator support, although we chose not to include outcomes such as acute rejection, bile leaks, early re-operation, etc. These latter complications often have multifactorial causes and are less likely to be directly related to the preoperative condition and nutritional status of patients.

Statistical analysis

Continuous variables with skewed distribution are presented as medians and inter-quartile ranges (IQR). Univariate analysis was conducted using chi-squared or Fisher's exact test for categorical data, and the Mann–Whitney test for continuous variables with skewed distribution. The selection of variables for the multivariate analysis was an *a priori* hypothesis based on literature search for known predictors of the outcome of interest. Variables significantly associated with the outcome of interest in univariate analysis were also included in the multivariate model.

A logistic regression model was fitted for the dichotomous outcome variable of post-operative infections up to 1 month after liver transplantation. The model adjusted for age, sex, body composition (measured as FMI or FFMI), indication for liver transplantation, causes of end-stage liver disease, weight loss, nutritional assessment by dietitian, time on the waiting list, MELD score at the time of liver transplantation, per-operative bleeding, need for dialysis, duration of mechanical ventilation, and length of stay in the ICU and hospital. Model fitness was assessed with the Hosmer–Lemeshow test.

Multiple linear regression analyses, with the length of stay in the ICU in the first model and duration of hospitalisation in the second as outcome variables, were constructed separately. The models adjusted for age, sex, body composition (measured as FMI or FFMI), indication for transplantation, causes of end-stage liver disease, weight loss, nutritional assessment by dietitian time on the waiting list, MELD score at the time of liver transplantation, post-operative bleeding, need for dialysis and duration of mechanical ventilation. In the model with the duration of hospitalisation, adjustments for the length of stay in the ICU and post-operative infections up to 1 month were also included. Preliminary analyses were performed to test for violation of linear regression assumptions of normality and multicollinearity. Logarithmic transformation of the dependent variables (length of stay in hospital and in intensive care unit) was carried out to correct for non-normality of the residuals in the regression model. Unstandardised betas and their SEs are reported together with their associated *P*-values. Standardised betas were also calculated to allow for comparison of effect size between the predictors. The sample size and number of cases per variable in the regression models was in line with recommendations derived from published simulation studies [18,19].

In selecting variables for the multivariate analysis, we used a combination of predefined predictors based on literature search, and any significant variable in the univariate analyses. Thus, we did not include only significant predictors from the univariate analyses in the final multivariate model because such an approach might wrongly reject potentially important variables [20].

Testing for influential cases and outliers in regression models was also performed, and such cases were excluded from the primary analysis. A further sensitivity analysis was then carried out with the inclusion of these outliers to check for changes in the significance level of predictors. Patients with missing data were excluded from the regression analysis. $P < 0.05$ (two-sided) was considered to be statistically significant. Statistical analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Ethical considerations

The study procedure was approved by the Regional Ethics Committee in Stockholm, (2012/2118-31/1). The study follows the Declaration of Helsinki.

Results

Study population

Data on 228 consecutive liver transplantations between 2009 and 2012 were reviewed retrospectively. In total, 109 patients met the inclusion and exclusion criteria (Fig. 1). When the quality of the DXA data was analysed, three of 109 DXA scans were excluded as a result of a ≥3% percent difference in the sum of BMC, LST and FM compared to the weight measured at the time of DXA. The baseline characteristics of the remaining 106 patients are presented in Table 1. The majority of patients received grafts from deceased donor. One received a partial graft from a living donor. The most common cause for end-stage liver disease was viral hepatitis (n = 37, 35%), followed by autoimmune (n = 33; 31%) and alcoholic liver disease (n = 23; 22%). The median (IQR) Child-Pugh and MELD scores at the time of transplantation were 9 (4) and 13.4 (7.3), respectively. No patient expired within the first post-transplant month but total mortality at

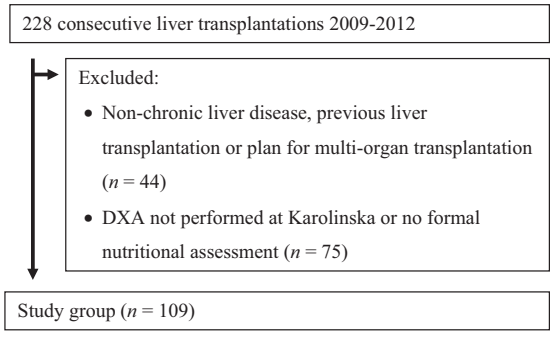

Figure 1 Study population. DXA, dual-energy X-ray absorptiometry.

12 months was 6.6% (n = 7). Causes of death in these seven patients were liver failure, sepsis, tumour recurrence and heart failure.

Body composition and nutritional status

FMI and FFMI in the study population at the time of wait listing are shown in Table 1. In total, 15% and 9% were classified as having a low FFMI and FMI, respectively (Fig. 2). In the nutritional assessment, five (5%) patients were classified as malnourished, and another 41 (39%) as being at risk of developing malnutrition. As shown in Fig. 1, the proportion of malnourished patients in the study group was highly dependent on sex and on which assessment method we used. FMI and nutritional assessment, but not FFMI, categorised more women as malnourished. A low FFMI was found in 20% of the men but in only 5% of the women. A low FMI, on the other hand, was found in 16% of the women but in only 6% of the men.

Post-operative infection

Thirty-nine (37%) patients developed severe post-operative infections (sepsis, pneumonia, cytomegalovirus or herpesvirus infections, wound infections, abdominal abscesses, soft tissue infections or fungal infections) within 1 month after liver transplantation. The results of the univariate analyses are provided in Table 1. In the multivariate analysis with logistic regression, body composition was significantly associated with post-operative infection when measured with FFMI [odds ratio (OR) = 0.67; 95% confidence interval (CI) = 0.45–0.99] but not FMI (OR = 0.85; 95% CI = 0.70–1.02) (Table 2). Longer length of stay in hospital was associated with a higher risk of infection (OR = 1.2; 95% CI = 1.1–1.3), whereas increasing age was associated with a lower risk of post-operative infection (OR = 0.91; 95% CI = 0.85–0.99). The other variables analysed were not significant predictors in the multivariate analysis (see Supporting information, Table S1).

Table 1 Basic characteristics of the study population

	Infection within 1 month after liver transplantation		
	No (n = 67)	Yes (n = 39)	P*
Age (years), median (IQR)	56 (15)	54 (21)	0.17
Sex, male (%)	44 (66)	24 (62)	0.68
Weight (kg), median (IQR)	78 (23)	69.1 (23.6)	0.38
Body composition, n (%)			
FMI	7.7 (3.7)	6.7 (3.2)	0.10
FFMI	18.5 (3.3)	17.4 (3.9)	0.16
Child-Pugh, median (IQR)	9 (4)	8 (4)	0.86
MELD, median (IQR)	12.7 (7.1)	14 (7.5)	0.52
Ascites, n (%)	28 (42%)	16 (41%)	0.94
Dietitian assessment, n (%)			
Good	41 (61)	19 (49)	
Risk	23 (34)	18 (46)	0.43
Poor	3 (5)	2 (5)	
Weight loss (kg)			
5–10	11 (16.42)	11 (28.21)	
<5	41 (61.19)	23 (58.97)	0.24
>10	15 (22.39)	5 (12.82)	
Cause of liver disease, n (%)			
Viral	22 (32.84)	15 (38.46)	
Alcohol/viral	11 (16.42)	2 (5.13)	
Autoimmune	22 (32.84)	11 (28.21)	0.17
Alcohol	7 (10.45)	3 (7.69)	
Other	5 (7.46)	8 (20.51)	
Indication for transplantation, n (%)			
Cirrhosis	37 (55)	20 (51)	
HCC	23 (34)	14 (36)	0.89
PSC	7 (10)	5 (13)	
Time on waiting list (days), median (IQR)	56 (102)	85 (139)	0.03
Dialysis, n (%)	3 (4.5)	6 (15.4)	0.07
Per-op bleeding volume (L), median (IQR)	3.2 (4.9)	7.1 (8.7)	0.03
Mechanical ventilation time (days), median (IQR)	0 (1)	0 (1)	0.91
Length of stay in ICU (days), median (IQR)	1 (1)	1 (1)	0.27
Length of stay in hospital (days), median (IQR)	16 (7)	28 (26)	<0.001

FFMI, fat-free mass index; FMI, fat mass index; HCC, hepatocellular carcinoma; ICU, intensive care unit; IQR, inter-quartile range; MELD, model of end-stage liver disease; PSC, primary sclerosing cholangitis.
*P-value for univariate analysis. Calculated using chi-squared for categorical variables, and the Mann–Whitney test for continuous ones.

Hospitalisation after liver transplantation

In the model for the length of stay in hospital after liver transplantation (Table 3), only post-operative dialysis (regression coefficients: beta = 0.53; 95% CI = 0.14–0.91, P = 0.008) and post-operative infections within 1 month after surgery (beta = 0.42; 95% CI = 0.25–0.59, P < 0.001) were significantly associated with the outcome.

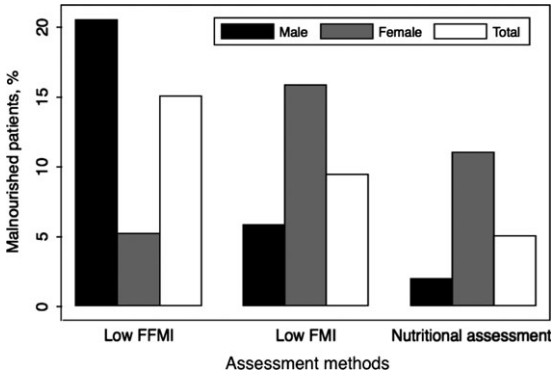

Figure 2 Proportion of malnourished patients depending on choice of assessment method. FFMI, fat-free mass index; FMI, fat mass index.

Table 2 Logistic regression models for post-operative infections within one month after surgery*

	Odds ratio	95% CI Low	95% CI Up	$P^{‡}$
Model with FFMI				
Age	0.91	0.84	0.99	0.026
FFMI	0.67	0.45	0.99	0.043
LOS hospital	1.20	1.09	1.33	<0.001
Goodness of fit‡				0.26
Model with FMI				
Age	0.92	0.85	0.99	0.029
FMI	0.85	0.70	1.02	0.087
LOS hospital	1.18	1.08	1.29	<0.001
Goodness of fit†				0.55

CI, confidence interval; FFMI, fat-free mass index; FMI, fat mass index; LOS, length of stay.
*Showing only body composition and other significant predictors from the model. The model adjusted otherwise for sex, indication for liver transplantation, causes of end stage liver disease, weight loss, nutritional assessment by dietitian, model of end-stage liver disease at the time of liver transplantation, post-operative bleeding, dialysis, duration of mechanical ventilation, and length of stay in the intensive care unit.
†Wald chi-squared test.
‡Hosmer–Lemeshow test.

Two patients were excluded from this analysis because they had duration of stay in hospital of 180 and 302 days, respectively, and constituted outliers. When these two patients were included in the sensitivity analysis, the length of stay in the ICU became an additional significant predictor of duration of hospitalisation (beta = 0.02, 95% CI = 0.00–0.04, $P = 0.013$). Other predictors in the model were not significantly associated with the duration of hospitalisation (see Supporting information, Table S2).

In the analysis of length of stay in the intensive care unit, significant predictors were the indication for liver transplantation, post-operative bleeding, duration of mechanical ventilation and the need for dialysis (Table 4). Time on the waiting list was also significantly, although negatively, associated with duration of stay in the ICU. The other predictors included in the analysis were not significantly associated with the length of stay in the ICU (see Supporting information, Table S3). Including the two outliers in the sensitivity analysis caused the effect of post-operative bleeding to be attenuated and to become not significant.

Table 3 Multiple linear regression models for the log of duration of hospitalisation after liver transplantation*

Covariate	Beta	95% CI Low	95% CI Up	*P*
Model with FMI (r^2 = 0.49)				
FMI	0.02	−0.01	0.04	0.126
Dialysis	0.50	0.12	0.89	0.011
Post-operative infection	0.44	0.27	0.62	<.001
Model with FFMI (r^2 = 0.50)				
FFMI	0.02	−0.02	0.06	0.370
Dialysis	0.53	0.14	0.91	0.008
Post-operative infection	0.42	0.25	0.59	<.001

CI, confidence interval; FFMI, fat-free mass index; FMI, fat mass index.
*Showing only body composition and significant predictors from the model. Other variables in the model were: age, sex, model of end-stage liver disease, indication for liver transplantation, cause of end-stage liver disease, dietitian assessment, bleeding, weight loss within 1 year, and length of stay in the intensive care unit; all of which were not significant.

Discussion

Liver transplantation is a multifaceted surgical treatment and many different factors influence the outcome. An increasing body of evidence supports the clinical relevance of poor nutritional status as a risk factor for complications and worse outcome after liver transplantation [2,21,22].

The present study of body composition measured with DXA yielded several noteworthy findings. Most notably, we found a correlation between FFMI and the risk of severe infections in the first month after liver transplantation. Earlier studies [7,23] have also shown that poor nutritional status influences the risk of developing infections. Infections were associated with a 37% increase in the length of stay in the present study, which suggests that malnutrition may indirectly increase costs and might have an impact on mortality. The low 30-day mortality (0%) and 12-month mortality (6; 6%) in our cohort precluded meaningful conclusions about any associations between nutritional status and mortality.

It was more common for women to have a low FMI, whereas men tended to have a low FFMI (Fig. 2). These findings are in accordance with those reported by Riggio *et al.* [24], who found that lean tissue is maintained longer than fat stores in women, whereas lean tissue is reduced

Table 4 Multiple linear regression models for the log of duration of stay in the intensive care unit after liver transplantation*

Covariate	B	95% CI Low	95% CI Up	*P*
Model with FMI (r^2 = 0.73)				
FMI	0.01	−0.01	0.04	0.305
Waiting list	−0.00	−0.00	−0.00	0.001
Liver transplantation indication				
Cirrhosis	0.37	0.14	0.60	0.006
PSC	0.09	−0.28	0.46	
HCC	–	–	–	
Bleeding	0.02	0.01	0.03	0.005
Mechanical ventilation time	0.16	0.08	0.24	<0.001
Dialysis	1.14	0.78	1.49	<0.001
Model with FFMI (r^2 = 0.73)				
FFMI	0.02	−0.03	0.07	0.373
Waiting list	−0.00	−0.00	−0.00	0.003
Liver transplantation indication				
HCC	−0.13	−0.51	0.25	0.013
Cirrhosis	0.22	−0.15	0.59	
HCC	−0.13	−0.51	0.25	
Bleeding	0.02	0.00	0.03	0.011
Mechanical ventilation time	0.17	0.09	0.25	<0.001
Dialysis	1.13	0.78	1.49	<0.001

CI, confidence interval; FFMI, fat-free mass index; FMI, fat mass index; HCC, hepatocellular carcinoma; PSC, primary sclerosing cholangitis.
*Showing only body composition and significant predictors from the model. Other variables in the model were: age, sex, model of end-stage liver disease, cause of end-stage liver disease, dietitian assessment, and weight loss within 1 year; all of which were not significant.

earlier in men. There was no sex difference in the risk of developing post-transplant infections, although we found that older patients had a lower risk. This might be counterintuitive but has also been noted in a previous study, in which lower recipient age and sarcopenia were significant risk factors for postoperative sepsis [22]. In another study, age did not influence the risk of infections [23].

Unlike earlier studies [4,7,21], we found no correlation between nutritional status and LOS in hospital or ICU, although post-operative dialysis increased LOS in hospital with 56%. Time on the waiting list was also significantly, although negatively, associated with LOS in the ICU. The practice to prioritise the sickest patients is likely to explain this. Our statistical model explained 73% of the variation in the duration of ICU stay (r^2 = 0.73) (Table 4), although only 47% of the variation in the total duration of hospitalisation. Many important factors were not considered in the present study, such as rejection and surgical complications.

Nutritional assessment by a dietitian was not useful for predicting post-transplant complications in the present study. Many factors that are considered in nutritional assessments, such as dietary intake and anthropometric measures, were not available. Adding such factors might have influenced the nutritional assessment. Patients at risk of malnutrition or defined as malnourished received intensified nutritional treatment during their time on the waiting list and this may have influenced the analysis.

Sarcopenia has been shown to be negatively associated with survival both before and after liver transplantation [3,22,25,26] and to be a risk factor for severe post-transplant infections [2,22,23] and post-transplant LOS in hospital [21].

Muscular mass, as often assessed by computed tomography at the level of the third lumbar vertebrae (L3) to determine the L3 skeletal muscle index or the transverse total psoas muscle area, has attracted much recent attention. CT and MRI are expensive procedures, have additional risks associated with radiation and contrast, and are performed routinely only on patients with HCC and PSC in our centre. We therefore looked at alternative cost-effective methods to assess the nutritional status of patients. DXA is non-invasive, relatively inexpensive and comprises a routine examination for pre-transplant osteoporosis screening in all patients.

A few studies have evaluated body composition by DXA in cirrhosis [13,24,27]. DXA performs well with respect to assessing FM and FFM [13,27]. In a recent study by Guisto *et al.* [28], muscularity measured with CT correlated with mid-arm circumference as measured with anthropometry, as well as with FFMI and appendicular skeletal mass index measured with DXA. FFMI depletion had prognostic value for developing hepatic encephalopathy in one study [29]. Possibly, the validity of DXA would be greater if DXA was combined with a measurement of fluid volume in patients with liver disease, although this is likely to pertain to most methods. Cirrhotic patients are volume overloaded compared to healthy individuals [13,30], although small changes in volume have limited impact on FFM measured by DXA [31]. FM did not change significantly before and after paracentesis in a small study on six patients [32]. In the present study, 42% of the population had ascites. Because ascites is included in FFM, some patients may have had a false high FFM. Possibly, additional patients would be classified as malnourished if we had a measure to evaluate any influence of fluid overload on FFMI.

The proportion of malnourished patients in the present study was low (15% had low FFMI and 9% had low FMI) compared to many other studies where 41–68% of patients were sarcopenic/cachectic [2,21,22,26]. This calls for a cautious interpretation of our results. The high number of patients with HCC or PSC in our cohort is likely to explain this. Recipients with HCC have been reported to have a higher muscle mass [33].

It is not only malnutrition that is important with respect to worse outcomes. An increasing body of evidence shows that obese patients can present with sarcopenic obesity, which may affect mortality [26,34].

We consider that the present study is the first to investigate whether body composition measured with DXA can predict short-term post-transplant outcome after liver transplantation. The significant association between FFMI and severe post-operative infections suggests a potential role for DXA as a cost-effective method for investigating the nutritional status of liver transplant recipients.

In conclusion, the assessment of nutritional status and body composition is demanding in liver transplant recipients. FFMI by DXA is correlated with the incidence of severe infections in the first post-transplant month. Our results suggest that DXA provides valuable information. There is a need to establish the objective tool that best identifies those malnourished patients who would benefit from intense pre-transplant nutritional intervention. CT/MRI is valuable but might not be the most cost-effective method. The results of the present study imply that there is a need for further comparative studies on the different assessment methods for body composition in patients with chronic liver disease.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained. The reporting of this work is compliant with STROBE guidelines.

Conflict of Interests, sources of funding and authorship

The authors declare that they have no conflicts of interest.
The study was supported by general grants from the Department of Clinical Nutrition and Dietetics and from Karolinska Institutet.
CL designed the study, performed study, collected data, analysed data and wrote the paper. AM analysed data and wrote the paper. SW designed the study, analysed data and wrote the paper.

References

1. Norman K, Kirchner H, Lochs H *et al.* (2006) Malnutrition affects quality of life in gastroenterology patients. *World J Gastroenterol* **12**, 3380–3385.
2. Tandon P, Ney M, Irwin I *et al.* (2012) Severe muscle depletion in patients on the liver transplant wait list: its prevalence and independent prognostic value. *Liver Transpl* **18**, 1209–1216.
3. Montano-Loza AJ, Meza-Junco J, Baracos VE *et al.* (2014) Severe muscle depletion predicts postoperative length of stay but is not associated with survival after liver transplantation. *Liver Transpl* **20**, 640–648.
4. Correia MI & Waitzberg DL (2003) The impact of malnutrition on morbidity, mortality, length of hospital stay and costs evaluated through a multivariate model analysis. *Clin Nutr* **22**, 235–239.
5. Hanai T, Shiraki M, Nishimura K *et al.* (2015) Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition* **31**, 193–199.
6. Plauth M, Merli M, Kondrup J *et al.* (1997) ESPEN guidelines for nutrition in liver disease and transplantation. *Clin Nutr* **16**, 43–55.
7. Merli M, Giusto M, Gentili F *et al.* (2010) Nutritional status: its influence on the outcome of patients undergoing liver transplantation. *Liver Int* **30**, 208–214.
8. Carvalho L & Parise ER (2006) Evaluation of nutritional status of nonhospitalized patients with liver cirrhosis. *Arq Gastroenterol* **43**, 269–274.
9. Nutritional status in cirrhosis (1994) Italian multicentre cooperative project on nutrition in liver cirrhosis. *J Hepatol* **21**, 317–325.
10. Ferreira LG, Anastacio LR, Lima AS *et al.* (2011) Assessment of nutritional status of patients waiting for liver transplantation. *Clin Transplant* **25**, 248–254.
11. Thibault R, Genton L & Pichard C (2012) Body composition: why, when and for who? *Clin Nutr* **31**, 435–447.
12. Madden AM & Morgan MY (1997) The potential role of dual-energy X-ray absorptiometry in the assessment of body composition in cirrhotic patients. *Nutrition* **13**, 40–45.
13. Strauss BJ, Gibson PR, Stroud DB *et al.* (2000) Total body dual X-ray absorptiometry is a good measure of both fat mass and fat-free mass in liver cirrhosis compared to "gold-standard" techniques. Melbourne Liver Group. *Ann NY Acad Sci* **904**, 55–62.
14. Carey EJ (2014) Sarcopenia in solid organ transplantation. *Nutr Clin Pract* **29**, 159–170.
15. Yao FY, Ferrell L, Bass NM *et al.* (2001) Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* **33**, 1394–1403.
16. Hind K, Oldroyd B & Truscott JG (2011) *In vivo* precision of the GE Lunar iDXA densitometer for the measurement of total body composition and fat distribution in adults. *Eur J Clin Nutr* **65**, 140–142.
17. Coin A, Sergi G, Minicuci N *et al.* (2008) Fat-free mass and fat mass reference values by dual-energy X-ray absorptiometry (DEXA) in a 20-80 year-old Italian population. *Clin Nutr* **27**, 87–94.

18. Austin PC & Steyerberg EW (2015) The number of subjects per variable required in linear regression analyses. *J Clin Epidemiol* **68**, 627–636.
19. Vittinghoff E & McCulloch CE (2007) Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol* **165**, 710–718.
20. Sun GW, Shook TL & Kay GL (1996) Inappropriate use of bivariable analysis to screen risk factors for use in multivariable analysis. *J Clin Epidemiol* **49**, 907–916.
21. DiMartini A, Cruz RJ Jr, Dew MA *et al.* (2013) Muscle mass predicts outcomes following liver transplantation. *Liver Transpl* **19**, 1172–1180.
22. Masuda T, Shirabe K, Ikegami T *et al.* (2014) Sarcopenia is a prognostic factor in living donor liver transplantation. *Liver Transpl* **20**, 401–407.
23. Krell RW, Kaul DR, Martin AR *et al.* (2013) Association between sarcopenia and the risk of serious infection among adults undergoing liver transplantation. *Liver Transpl* **19**, 1396–1402.
24. Riggio O, Andreoli A, Diana F *et al.* (1997) Whole body and regional body composition analysis by dual-energy X-ray absorptiometry in cirrhotic patients. *Eur J Clin Nutr* **51**, 810–814.
25. Durand F, Buyse S, Francoz C *et al.* (2014) Prognostic value of muscle atrophy in cirrhosis using psoas muscle thickness on computed tomography. *J Hepatol* **60**, 1151–1157.
26. Cruz RJ Jr, Dew MA, Myaskovsky L *et al.* (2013) Objective radiologic assessment of body composition in patients with end-stage liver disease: going beyond the BMI. *Transplantation* **95**, 617–622.
27. Morgan MY, Madden AM, Jennings G *et al.* (2006) Two-component models are of limited value for the assessment of body composition in patients with cirrhosis. *Am J Clin Nutr* **84**, 1151–1162.
28. Giusto M, Lattanzi B, Albanese C *et al.* (2015) Sarcopenia in liver cirrhosis: the role of computed tomography scan for the assessment of muscle mass compared with dual-energy X-ray absorptiometry and anthropometry. *Eur J Gastroenterol Hepatol* **27**, 328–334.
29. Kalaitzakis E, Josefsson A, Castedal M *et al.* (2013) Hepatic encephalopathy is related to anemia and fat-free mass depletion in liver transplant candidates with cirrhosis. *Scand J Gastroenterol* **48**, 577–584.
30. Peng S, Plank LD, McCall JL *et al.* (2007) Body composition, muscle function, and energy expenditure in patients with liver cirrhosis: a comprehensive study. *Am J Clin Nutr* **85**, 1257–1266.
31. Pietrobelli A, Formica C, Wang Z *et al.* (1996) Dual-energy X-ray absorptiometry body composition model: review of physical concepts. *Am J Physiol* **271**, E941–E951.
32. Haderslev KV, Svendsen OL & Staun M (1999) Does paracentesis of ascites influence measurements of bone mineral or body composition by dual-energy x-ray absorptiometry? *Metabolism* **48**, 373–377.
33. Tsien C, Garber A, Narayanan A *et al.* (2014) Post-liver transplantation sarcopenia in cirrhosis: a prospective evaluation. *J Gastroenterol Hepatol* **29**, 1250–1257.
34. Hara N, Iwasa M, Sugimoto R *et al.* (2016) Sarcopenia and sarcopenic obesity are prognostic factors for overall survival in patients with cirrhosis. *Intern Med* **55**, 863–870.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Logistic regression model for post-operative infection at 1 month.

Table S2. Multiple linear regression models for the log of duration of hospitalisation after liver transplantation.

Table S3. Multiple linear regression models for the log of duration of stay in the intensive care unit after liver transplantation.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

CLINICAL NUTRITION

The challenge of nutritional profiling of a protein-free feed module for children on low protein tube feeds with organic acidaemias

A. Daly, S. Evans, C. Ashmore, S. Chahal, S. Santra & A. MacDonald

Birmingham Children's Hospital, Birmingham, UK

Keywords
enteral feeding, feed module, low protein, organic acidaemias.

Correspondence
A. Daly, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, UK.
Tel.: +44 07979592648
Fax: 0121 333 8021
E-mail: anne.daly@bch.nhs.uk

How to cite this article
Daly A., Evans S., Ashmore C., Chahal S., Santra S., MacDonald A. (2017) The challenge of nutritional profiling of a protein-free feed module for children on low protein tube feeds with organic acidaemias. *J Hum Nutr Diet*. **30**, 292–301
doi: 10.1111/jhn.12455

Abstract

Background: Enteral tube feeding for children with organic acidaemias (OA) is recommended. Protein restriction, providing minimum safe levels of protein intake, is advocated. Standard paediatric tube feeding formulae provide more than the minimum safe protein requirements and are unsuitable in OA without modification. Modified paediatric enteral feeds consist of several modular ingredients. The aim of this prospective longitudinal interventional study was to assess the efficacy of a premeasured novel protein-free module developed for children aged over 12 months compared to conventional practice.
Methods: In total, 15 children with OA (11.6–31 kg) needing enteral feeding were recruited. The protein-free module, from either a protein-free infant feed or modular ingredients, was replaced by the study feed. To ensure metabolic stability, energy and protein intake were unchanged. Dietary intake, anthropometry and nutritional biochemistry were recorded at baseline and week 26.
Results: Dietary intakes of magnesium ($P = 0.02$), sodium ($P = 0.005$), vitamin D ($P = 0.04$), docosahexaenoic acid ($P = 0.01$) and arachidonic acid ($P = 0.001$) significantly improved; plasma selenium ($P = 0.002$) and whole blood glutathione peroxidase ($P = 0.02$) significantly increased. Feed preparation accuracy as measured by composition analysis showed consistent errors both in pre- and study feeds.
Conclusions: A protein-free module improved nutritional intake and biochemistry, although feed preparation errors remained a common finding

Introduction

In children with inherited organic acidaemias [propionic acidaemia (PA), B_{12} nonresponsive methylmalonic acidaemia (MMA B_{12}nr), isovaleric acidaemia (IVA) and glutaric acidaemia type I (GA1)], the primary treatments are: (i) natural protein restriction, aiming to provide minimum safe levels of protein intake [1] World Health Organization (WHO)/Food and Agriculture Organization (FAO)/United Nations University (UNU) 2007, and (ii) adjunctive compounds (e.g. carnitine) to dispose of toxic metabolites. Some inherited metabolic disease centres also prescribe precursor-free L-amino acids to supplement natural protein intake, although their long-term value remains uncertain [2,3]. To meet energy requirements and to avoid prolonged fasting (in MMA/PA), enteral tube feeding is a common intervention [4].

The composition of paediatric low protein enteral formulae is problematic. 'Complete' ready-to-feed protein-free enteral formulae are unavailable and enteral feeds for this population usually consist of multiple, 'modular' feed ingredients. Individual modular ingredients usually supply their energy, micronutrients and electrolyte content, or these are derived from supplemented protein-free infant formula. Feed recipes are carefully calculated, requiring professional time and skill, although, in clinical practice,

feed composition commonly fails to meet age related requirements for all nutrients. We have previously reported [5] that a median of four (range 3–5) ingredients was added to enteral feeds for children with organic acidaemias.

Multiple modular feed ingredients are associated with nutrient feed composition inaccuracies [6]. In a home-based study, modular home tube feed ingredients were commonly measured incorrectly and non-native speaking caregivers failed to read and understand English written feed recipe instructions [7]. Inaccuracy of feed nutrient composition was increased with a higher number (six compared to two) of feed ingredients [6]. Gokmen-Ozel [8] also showed that the method of measuring feed ingredients influenced accuracy. When one powdered ingredient was measured by three different methods (scoops, scales or pre measured sachets), premeasured sachets resulted in a greater accuracy compared to scoop measurements.

There are no dietetic guidelines that define the optimal nutrient composition of paediatric protein-free enteral formula. To help improve nutritional composition, minimise the number of feed ingredients and thereby reduce preparation error and improve ease of use, a protein-free, powdered module that is premeasured in a sachet has been developed. Its nutritional composition is similar to whole protein standard enteral formula designed for children aged 1–10 years of age, although it does not contain protein. The protein-free module is designed to be used both with natural and precursor-free L-amino acid sources or it can be given as a protein-free feed only.

To assess the efficacy and preparation accuracy of this premeasured protein-free module (Basecal 200; Vitaflo Ltd, Liverpool, UK), we conducted a longitudinal, prospective study in children with organic acidaemias.

Materials and methods

Subjects

Fifteen children [eight boys and seven girls; median (range) age of 4.5 years (15 months to 12 years)] with organic acidaemias were recruited (PA, $n = 8$; MMA B_{12} nr, $n = 4$; IVA $n = 2$; and GA1, $n = 1$). Ten children were of Asian Pakistani origin, one was Afro-Caribbean, one was Arabic, one was Bangladeshi and two were European. All children received ≥75% of their enteral nutritional intake via a tube feed (nutritional intake: ≥90%, $n = 14$; 75%, $n = 1$).

Patient characteristics

Thirteen children had a gastrostomy tube/button and two had nasogastric feeding tubes. Thirteen families had one caregiver who was a non-native speaker but spoke and read English fluently. Diagnosis was mainly in the neonatal period (including three siblings of index cases) ($n = 13$), although two were diagnosed later in infancy (7 months, $n = 1$; 10 months, $n = 1$).

Inclusion/exclusion criteria

Children were recruited to the study if they had a proven organic acidaemia, were aged between 1 and 10 years or weighed between 8 and 31 kg, and were taking a modular feed as part of their treatment. Exclusion criteria included children over 31 kg, and use of emergency feeds in the 2 weeks prior to the study entry.

Enteral feeds pre-study

Pre-study (baseline), the energy was supplied by a combination of protein-free infant formula (Energivit; Nutricia, Schiphol, The Netherlands) ($n = 12$); glucose polymer (Super Soluble Maxijul; Nutricia) ($n = 11$) and a 50% fat emulsion (Calogen; Nutricia) ($n = 1$) (Table 1). Thirteen children (exception two children with IVA) were prescribed precursor-free L-amino acids added to their enteral feeds. L-amino acid supplements were either added as premeasured sachets with no additional vitamins and minerals ($n = 8$) or as an infant precursor-free L-amino acid formula ($n = 5$).

The median number of daily medications was 3 (1–9): including L-carnitine ($n = 15$), metronidazole ($n = 7$), domperidone ($n = 4$), ondansetron ($n = 1$) and omeprazole ($n = 5$). Four subjects were prescribed regular laxatives (movicol and lactulose). Three children were prescribed sodium and potassium supplements as a result of previous low blood concentrations. Drugs such as sodium benzoate or sodium phenylbutyrate were used during metabolic decompensation only; no patients were given these drugs when well.

A favourable ethical opinion was obtained from the local research ethics committee and written informed consent was obtained from all participants.

Product description

Basecal 200 (Vitaflo Ltd), a premeasured (sachet) protein-free powdered module, contains sources of carbohydrate, fat (including long chain polyunsaturated fatty acids), vitamins, minerals and trace elements, and has a nutritional profile that meets the nutritional requirements of 1–10-year-old children (Table 2). Each sachet (43 g) mixed with 200 mL of water, contained 200 kcal (1 kcal mL^{-1}).

Table 1 Type of feed ingredients used and method of measurement in the pre-study and study feed

	Feed ingredient	Measurement	Pre-study feed	Study feed
Natural protein source	Nutrini multifibre[1]	Liquid	$n = 10$	$n = 11$
	Tentrini multifibre[1]	Liquid	$n = 2$	$n = 2$
	Standard whey based infant formula (Cow and Gate)[4]	Scales	$n = 2$	$n = 1$
	Whey based lactose free infant formula (SMA lactose free)[5]	Scales	$n = 1$	$n = 1$
Precursor-free L-amino acids	MMA/PA amino[5,2]	Sachet	$n = 7$	$n = 11$
	GA 1 gel[2]	Sachet	$n = 1$	$n = 1$
	MMA/PA Anamix Infant	Scales	$n = 5$	$n = 1$
Energy source	Energivit[1]	Scales	$n = 12$	–
	Study feed[2]	Sachets	–	$n = 15$
	Glucose polymer Maxijul[1]	Scales	$n = 11$	–
	SOS glucose polymer[2]	Sachets	–	$n = 8$
	Calogen[1]	Liquid	$n = 1$	$n = 1$
Vitamins and mineral supplement	Vitamin and mineral supplement (Paediatric Seravit)[1]	Scales	$n = 1$	–
Fibre	Soluble fibre Optifibre[3]	Sachet	$n = 1$	$n = 1$
Median number (range) of feed ingredients			$n = 4$ (1–5)	$n = 3$ (1–4)

Feed ingredients: Nutricia Ltd[1,4]; Vitaflo Ltd[2]; Nestle Ltd (Vevy, Switzerland)[3,5].
GA, glutaric acidaemia type I; MMA, methylmalonic acidaemia; PA, propionic acidaemia.

Study design

In this longitudinal prospective open-label, interventional study, subjects took their usual feed from enrolment week −8 to week 0 (baseline). At baseline, they were changed to the study feed (a feed containing the protein-free pre-measured module together with protein-containing feed to meet minimum safe levels of protein intake) until week 26. To ensure metabolic stability, a number of precautions were taken with the introduction of the study feed. The whole protein source remained unchanged [Nutrini (Nutricia) multifibre, $n = 10$; Tentrini (Nutricia) multifibre, $n = 2$; lactose free formula, $n = 1$; whey dominant infant formula, $n = 2$]. The total daily energy, carbohydrate and fat intake also remained unchanged from the pre-study amounts. To meet the same energy content, the protein free infant formula (Energivit, $n = 12$) was replaced with the protein-free formula sachets (Basecal, $n = 15$); the precursor free infant L-amino acid infant formula was replaced with sachets of precursor-free L-amino acids ($n = 4$ of 5 children); and the weighed glucose polymer (Maxijul $n = 11$) was replaced by pre-measured sachets of glucose polymer (SOS) in eight children and was discontinued in three children. The use of protein-free module as a core ingredient in the low protein feed is referred to as study feed in the present study.

Dietary assessment

For 3 days during weeks −8, 0, 12 and 26, caregivers recorded the volume of modular feed actually consumed and any oral food and drink intake ($n = 1$) by weighing dietary intake. Dietary analysis was calculated using ELECTRONIC DIETETIC MANAGER (EDM 2000; MicroMan2000 Ltd, Newport Pagnell, UK) and *McCance and Widdowson's The Composition of Foods*. The intake of energy, total protein (natural protein and protein equivalent from precursor-free L-amino acids), fibre, fat, carbohydrate, calcium, magnesium, iron, zinc, selenium, sodium, potassium, vitamin B_{12}, vitamin D and fluid was assessed. Each nutrient (except protein) was compared as a percentage of the DH 1991 [9] reference nutrient intakes or estimated average requirement for energy. Protein intake was compared with the WHO/FAO/UNU safe levels of protein intake [1].

Anthropometric measurements

Anthropometric measurements (weight, height/length, *Z*-scores) were collected at weeks −8, 0, 12 and 26 by a health professional. Weight and height were measured in children ≥2 years of age when clothed: weight by standing and sitting electronic scales (accurate to two decimal places); and height by a calibrated stadiometer (accurate to one decimal place). In children <2 years of age, measurements were taken naked: weight using infant scales accurate to two decimal places and supine length on a measuring mat accurate to one decimal place.

Feed tolerance

Caregivers kept a record of any changes in their child's usual symptoms (stool frequency, vomiting or abdominal discomfort).

Table 2 A comparison of the nutritional composition (per 100 mL and per 100 kcal) of study protein-free module ingredient (Basecal) with a protein-free infant feed (Energivit)

	Basecal 200 (Vitaflo Ltd) per 100 kcal equivalent to 100 mL	Energivit (Nutricia) per 100 mL (15% dilution as recommended by the manufacturer)	Energivit (Nutricia) per 100 kcal equivalent to 20% dilution (i.e. in excess of manufacturer's recommendation)
Energy (kcal kJ^{-1})	100/420	74/309	100/420
Protein (g)	0	0	0
Carbohydrate (g)	15	10	13.3
Fat (g)	4.5	3.8	5.1
Vitamin A (μg)	65	58.8	78.4
Vitamin D (μg)	1.7	1.3	1.7
Vitamin E (mg)	2	1	1.3
Vitamin C (mg)	15	7.4	10
Vitamin K (μg)	5.7	5.6	7.5
Thiamin (mg)	0.11	0.08	0.1
Riboflavin (mg)	0.16	0.08	0.1
Niacin (mg)	1.1	1.1	1.5
Vitamin B_6 (mg)	0.13	0.08	0.1
Folic acid (μg)	18.5	8.3	11.1
Vitamin B (12 μg)	0.28	0.2	0.27
Iron (mg)	1	1.2	1.6
Zinc (mg)	1	0.9	1.2
Copper (mg)	0.07	0.07	0.09
Selenium (μg)	3	2.3	3
Magnesium (mg)	10.2	8.7	11.6
Manganese (mg)	0.1	0.06	0.08
Biotin (μg)	3.5	2.7	3.6
Pantothenic acid (mg)	0.55	0.4	0.5
Sodium (mmol)	1.8	1.2	1.6
Potassium (mmol)	2.5	1.9	2.5
Chloride (mmol)	1.6	1.5	2
Phosphorous (mg)	1.3	1.5	2
Iodine (μg)	17	12.5	17
Molybdenum (μg)	4.8	1.8	2.4
Choline (mg)	20.6	13.7	18.3
Inositol (mg)	11	14.7	19.6
DHA (mg)	16	0	0
AA (mg)	16	0	0

AA, arachidonic acid; DHA, docosahexaenoic acid.

Illness episodes

Any illness (e.g. diarrhoea, vomiting), the number of hospital admissions, reason for admission and length of stay were recorded from the time of recruitment. The use of protein-free emergency regimens was also documented.

Blood biochemistry

Venous blood samples for glucose, electrolytes, full blood count, plasma selenium, glutathione peroxidase, copper, zinc, iron, ferritin, vitamin B_{12}, plasma MMA, C-reactive protein and total blood glutathione peroxidase were taken at weeks −8, 0, 12 and 26. Blood samples were taken after a minimum of 3 h of fasting.

Home feed nutrient analysis

The nutrient composition (fat, vitamin B_1, zinc, sodium and potassium) of the enteral feeds prepared by the main caregiver at home was analysed. Feed samples (500 mL) were collected monthly (week −8 to week 26) and stored at −20 °C. From each sample feed, the actual nutrient concentrations were analysed and compared with the ideal calculated composition. At the end of the study, one health professional observer (AD) objectively evaluated the feed preparation technique of each caregiver, assessing the accuracy of liquid measurement, ability to use scales, following of a feed recipe, mixing of ingredients, and suitability of preparation equipment.

Nutrient feed analysis methodology

Fat was determined by hydrolysing each sample with hydrochloric acid, cooling, filtering and drying. Fat was then extracted from the residue with petroleum ether and the dried fat determined gravimetrically. The level of measurement uncertainty given at a 95% confiedence interval was 7.34 g/100 ± 0.36 g/100 g. For potassium, sodium and zinc analysis, 1 g of homogenised sample was digested with concentrated nitric acid using microwave-assisted accelerated digestion. The amounts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Measurement uncertainty level (using ICP-OES), for zinc was ±8.9%, and that of sodium and potassium was ±7.7%. Vitamin B_1 was measured by extracting a sample by hot acid digestion followed by enzyme digestion. The solution was then analyzed for vitamin B_1 by reverse phase high-performance liquid chromatography with fluorescence detection.

Statistical analysis

For statistical analysis, the actual nutritional compositions of the two feed preparations (pre-study and study feed) were compared with the calculated nutrient composition assuming correct measurement and preparation of the modular ingredients. The statistical tests used comprised nonparametric paired Wilcoxon signed rank tests. The same statistical test was applied to analyse for significant differences in nutritional and haematological results between pre-study and protein-free feed.

Results

Feed preparation changes

The use of protein-free premeasured sachets as a core module ingredient in the study feed minimised the use of scales and scoops and decreased feed ingredient number (median ingredient number: pre-study 4 (1–5) versus study feed 3 (1–4). With the exception of two children, all powdered ingredients were dispensed as sachets (scales/scoop use: pre-study feeds, $n = 15$; with study feed, $n = 2$; $P = 0.002$).

Nutrient intake: with prebaseline and study feed

Energy, protein and fluid intake

No significant differences were observed for enteral feed energy intake between pre-study and study feed (Table 3). Similarly, there were no significant differences for total fat and carbohydrate intake. The amount of additional glucose polymer required to achieve iso-energy intake was significantly reduced between baseline and study feed at week 26, and three subjects stopped using glucose polymer completely [median percentage of additional carbohydrate provided by glucose polymer was: pre-study feed 29% (0–48); week 26 study feed 14% (0–34); $P \leq 0.001$].

Total protein intake was similar between pre-study and study feeds and met WHO/FAO/UNU [1] safe levels of protein intake. Fluid intake and fibre intake remained unchanged but fibre intake was consistently low [median (range) intake was 4 g day^{-1} (0–12)] on pre-study and study feed.

Vitamin, mineral and long chain fatty acid intake

Using the study feed, the median percentage reference nutrient intake (RNI) increased statistically for magnesium ($P = 0.02$) and sodium ($P = 0.005$) (Table 3). Vitamin D intake also significantly increased ($P = 0.04$), although there is no UK RNI for vitamin D intake after the age of 3 years, thereby limiting statistical comparisons with RNI. The intake of both docosahexaenoic acid (DHA) ($P = 0.01$) and arachidonic acid (AA) ($P = 0.001$) significantly improved.

The intake of calcium, iron, zinc, selenium and phosphorous met the RNI [9] in both the pre-study and study feeds. Intakes of these minerals were higher in the study feed, although this did not reach statistical significance.

Nutritional biochemical and haematological results

Although median baseline results were within reference values, the study feed significantly increased plasma selenium ($P = 0.002$) and whole blood glutathione peroxidase ($P = 0.02$) (Table 4). There was no statistically significant change for copper, zinc, plasma glutathione peroxidase, ferritin, mean corpuscular volume, haemoglobin, vitamin B_{12} and plasma MMA.

Anthropometry

There were no significant changes in weight and height z-scores over the study period [median height *Z*-score: baseline, −1.3 (−2.6 to 1.5), week 26, −1.3 (−2.3 to 0.7); median weight z-score baseline, 0.3 (−3.1 to 2.5), week 26, 0.05 (−3 to 2.8)].

Hospital admissions

There was a median of 3 (2–12) days spent in hospital from a total of 15 hospital admissions ($n = 11$ children) as a result of vomiting or chest infections causing metabolic decompensation during the 26 weeks on the study feed. Protein-free emergency regimens, based on glucose polymer were given a median of 12 (0–14) times ($n = 15$). No hospital admissions were reported as a result of changing to the new low-protein feed.

Table 3 Median (range) daily nutritional intake compared with reference intakes [estimated average requirements (EAR) for energy, WHO/FAO/UNU 2007 recommendations for safe protein intakes and reference nutrient intake (RNI) (DH 1991) for all other nutrients] measured pre-study (week −8) and study feed (week 26)

Nutrients	Pre-study feed (week −8), median (range)	Study feed (week 26), median (range)	Significance	Comparison expressed as a percentage of reference intake		Significance
				Pre-study feed	Study feed	
Energy (kcal kg^{-1})* kcal (kg^{-1} day^{-1})	59 (32–82)	61 (34–87)	$P = 0.87$	76 (47–103)	78 (46–98)	$P = 0.06$
Total protein (g kg^{-1}) (natural and precursor-free L-amino acids) (kg^{-1} day^{-1})	1.2 (0.7–1.5)	1.2 (0.8–1.4)	$P = 0.48$	129 (78–153)	127 (88–164)	$P = 0.32$
Natural protein (g kg^{-1} day^{-1})†	0.85 (0.5–1.2)	0.85 (0.5–1.2)	$P = 0.3$	94 (56–141)	91 (56–118)	$P = 0.56$
Precursor-free L-amino acids (g kg^{-1} day^{-1})	0.35 (0.2–0.7)	0.4 (0.2–0.6)	$P = 0.8$	NA	NA	
Calcium (mg day^{-1})‡	693 (426–848)	718 (498–1102)	$P = 0.39$	145 (89–242)	152 (95–247)	$P = 0.43$
Iron (mg day^{-1})‡	12 (6–17)	12 (6–18)	$P = 0.75$	142 (87–232)	147 (87–197)	$P = 0.75$
Zinc (mg day^{-1})‡	10.5 (6–14)	11.5 (6–17)	$P = 0.15$	149 (92–240)	179 (120–243)	$P = 0.15$
Selenium (μg day^{-1})‡	30.2 (15–46)	35.5 (17–68)	$P = 0.12$	148 (87–247)	168 (84–213)	$P = 0.1$
Phosphorous (mg day^{-1})‡	508 (308–749)	553 (291–832)	$P = 0.37$	144 (93–214)	148 (95–211)	$P = 0.36$
Potassium (mmol day^{-1})‡	26.9 (16–46)	28 (15–48)	$P = 0.2$	91 (28–164)	102 (33–164)	$P = 0.06$
Vitamin D (μg day^{-1})‡	13 (8–17)	15.7 (12–24)	$P = 0.04$	179 (114–242)	214 (171–257)	$P = 0.1$
Magnesium (mg day^{-1})‡	100 (67–173)	121 (57–278)	$P = 0.02$	77 (43–144)	102 (49–135)	$P = 0.02$
Vitamin B (12 μg day^{-1})‡	2.7 (1.7–3.6)	3.0 (2–5)	$P = 0.01$	339 (200–580)	388 (280–580)	$P = 0.04$
Sodium (mmol day^{-1})‡	20 (8–33)	25 (9–36)	$P = 0.002$	61 (29–110)	72 (34–110)	$P = 0.005$
Docosahexaenoic Acid (mg)§	0 (0–340)	96 (32–340)	$P = 0.01$	0 (0–170)	48 (16–170)	$P = 0.005$
Arachidonic acid (mg)	0 (0–91)	96 (32–128)	$P = 0.001$	n/a	n/a	
Fluid (mL kg^{-1} day^{-1})¶	1087 (630–1430)	1100 (700–1430)	$P = 0.1$	68 (39–89)	69 (44–89)	$P = 0.2$
Fibre (g day^{-1})**	4 (0–12)	4 (0–12)	$P = 1$	20% (0–60)	20% (0 = 60)	

NA, not available.
*Estimated average requirement.
†WHO/FAO/UNU 2007 recommendations.
‡Reference nutrient intake.
§Food and Agriculture Organization of United Nations (FAO).
¶Based on Holliday Segar formula for a 20-kg child.
**SCAN 2015.

Table 4 Median biochemical and haematological (range) values with pre-study feed (week −8) and study feed (week 26)

	Pre-study (week −8) Median (range)	Study feed (week 26) Median (range)	Reference range	Statistical significance
Copper (μmol L^{-1})	18.5 (14–24)	18 (14–25)	10–25	$P = 0.89$
Plasma zinc (μmol L^{-1})	10.9 (8.9–14.6)	10.7 (7–15.3)	10–18	$P = 0.98$
Plasma glutathione peroxidase (IU L^{-1})	489 (283–946)	492 (288–651)	186–762	$P = 0.95$
Ferritin (μg L^{-1})	34 (5.3–121)	39.2 (7.8–167)	32–233	$P = 0.12$
Vitamin B_{12} (ng L^{-1})	957 (208–1049)	977 (166–2121)	259–823	$P = 0.95$
Haemoglobin (g dL^{-1})	122.9 (103–139)	123.8 (114–136)	112–130	$P = 0.84$
Mean corpuscular volume (g L^{-1})	75 (65–83)	76 (68–84)	75–90	$P = 0.22$
C-reactive protein (mg L^{-1})	2.6 (1–13)	2.9 (1–19)	<10	$P = 0.9$
Plasma MMA (μmol L^{-1})	0.9 (0.15–3066)	0.4 (0.16–2629)	≥0.42	$P = 0.5$
Plasma selenium (μmol L^{-1})	0.96 (0.6–1.2)	1.11 (0.8–1.4)	0.8–8.2	$P = 0.002$*
Whole blood glutathione peroxidase (IU gHb^{-1})	53.4 (21–69)	54.9 (33–73)	15–50	$P = 0.02$*

MMA, methylmalonic acidaemia.
*Significant value.

Feed composition analysis of the feeds prepared by caregivers

Measurement of nutrient composition within 10% or 20% of the ideal calculated composition failed to show any significant differences between the pre- or study feeds as prepared by carers (Fig. 1). However, a significant difference ($P = 0.03$) was observed when measuring the consistency of nutrient composition between the two feeds. Feeds prepared using scales had a significantly greater variation of nutrient composition compared to those prepared using premeasured sachets. Caregivers made a number of errors during feed preparation with both the pre-study and study feed, particularly with liquid measurement. Nine of 15 (60%) caregivers used unsuitable equipment to measure liquids (e.g. jugs had faded measurement lines) and four (27%) caregivers did not measure water at eye level. No caregiver checked the scale accuracy, ensuring they were on 'zero' when individual ingredients were measured for feeds.

Evaluation of new premeasured sachets

All caregivers ($n = 15$) preferred the premeasured sachets to using scales or scoops for feed ingredient measurement. With the study feed, they reported that feed preparation was easier, and caregivers also said that they were able to remember the feed recipe more easily because it was less complex.

Discussion

This is the first prospective open-label longitudinal study to investigate a protein-free premeasured sachet specifically developed as a modular ingredient for children with metabolic disorders over 12 months of age, on low protein enteral feeds. Overall, fewer ingredients were added to the study feed and caregivers found it easier to recall the feed recipe. The nutritional profile of the protein-free sachets (per 100 mL when prepared) replicated the formulation of standard enteral paediatric feeds, although it was without protein. This clearly led to significant increases in the intake of several micronutrients, notably magnesium and sodium, and lowered the variability of errors in feed preparation. This is a particularly important observation for this group of children who, during illness, stop regular feeds, replacing them with an emergency feed based on glucose polymer only without vitamin and mineral supplementation.

Designing the ideal nutritional profile for a protein-free enteral feed module is challenging. Using the baseline (pre-study) formulae, although some nutrients met recommended intakes, others did not. The study feed module was designed to accompany a separate protein-containing enteral feed (the latter to provide protein requirements). Our study protein-free module provided energy, vitamins, minerals and also a source of long chain fatty acids. Considering long chain fatty acid deficiency has been reported in children with organic acidaemia [11], their addition is beneficial in enteral feeding formula and, in the present study, this led to improved intake of DHA and arachidonic acid, although biochemical fatty acid status was not studied. Both *n*3 fatty acids (DHA) and *n*6 (AA) are important components in brain development and may influence brain function throughout life by changing neuronal membrane fluidity, production of neurotransmitters and brain peptides. [10,11]. The development of vitamin D deficiency is of particular concern. A new global consensus suggests that all children aged over 1 year should receive 15 μg day^{-1} of vitamin D [12], and

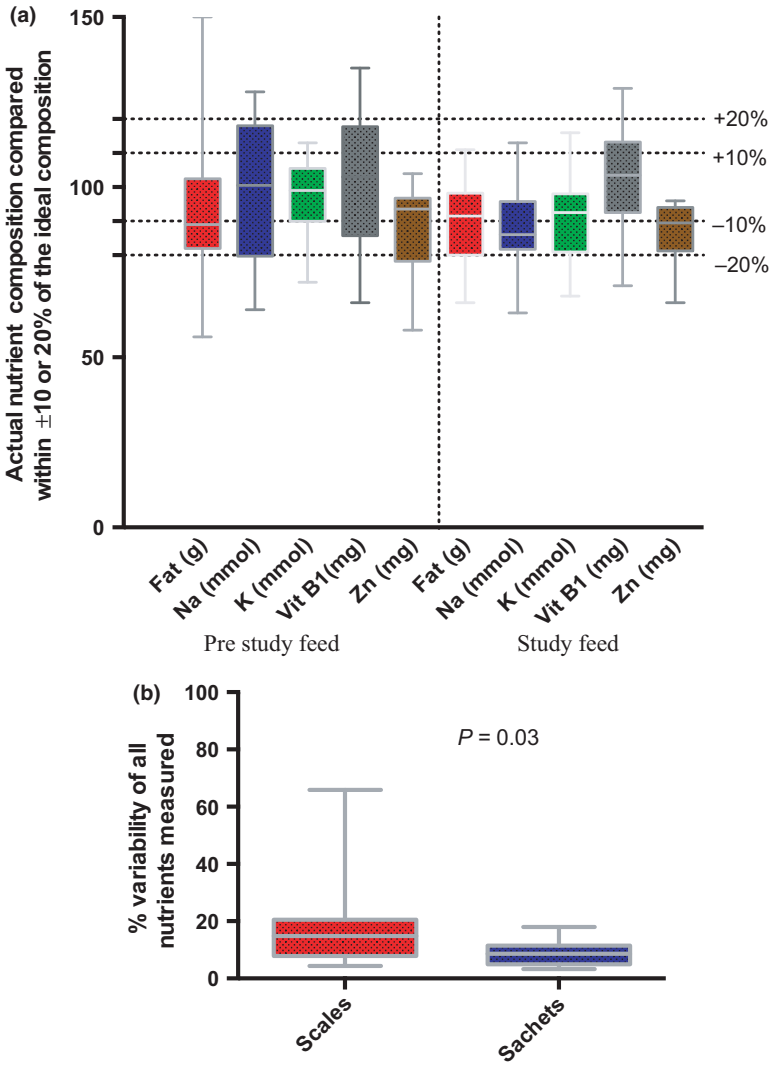

Figure 1 (a) Median composition of nutrients analysed and compared within ±10% and 20% of the ideal feed composition in pre-study and study feed. (b) Percentage variability of all nutrients measured by scales in pre-study feed and sachets in study feed.

our low protein feed provided this requirement. It is essential that the low protein feeds provide adequate vitamin D because many of our patients are of Asian origin, have limited exposure to the sunshine, and may have interrupted periods of using a glucose based emergency feed only when unwell.

Some nutrients were still below the RNI, even with the new study formula, particularly sodium [9] and fibre [13]. It is commonly assumed that children with MMA and PA obtain additional sodium intake from medications (e.g. sodium benzoate), although this was not the case in this group of children. The chronic deficiency of sodium may be a rate-limiting factor in achieving optimal nutritional status because it is an important nutrient for growth, stimulating cell proliferation and protein synthesis [14,15]. This protein-free module did not contain fibre and this may be a potential disadvantage. The ideal fibre content of a feed designed for children with MMA and PA is unknown. Fermentation of carbohydrate, proteins, amino acids and fibre modifies gut propionic acid production, which is toxic in PA and MMA [16,17], and can lead to metabolic instability. Consequently, there is hesitation in adding additional fibre to feeds designed for this patient group because any increased production of propionic bacteria may trigger metabolic decompensation. However, constipation is a common issue and the use of laxatives is high [5]. SCAN 2015 [13] recommendations for fibre intake in healthy children aged 2–5 years and 5–11 years are 15 g and 20 g day^{-1} respectively, and so a median intake of 4 g day^{-1} fibre is well below this amount. By identifying the correct amount, blend and composition of fibre, the production of toxic propiogenic bacteria could be reduced and a feed with added fibre may be clinically beneficial.

The protein-free enteral module in premeasured sachets both increased micronutrient intake and significantly decreased the spread of error measured by the variability of nutrient composition compared to the use of scales, although inaccuracies in feed preparation still occurred. One concern was that some of the mixing equipment used was 'not fit for purpose' and any preparation of feeds that required addition of water or other liquids was inaccurate [6,8]. Indeed, many of our caregivers had large families and were managing the time demands of caring for a child with chronic illness, leading to feeds being prepared quickly and sometimes without attention to accuracy.

The present study did have some limitations. It could not be conducted as a randomised controlled trial because of concerns about precipitating metabolic instability. The introduction of the study feed was deliberately slow and systematic, which also led to a protracted withdrawal of other module ingredients such as glucose polymer. This group of children appear to be sensitive to feed changes, although there was no evidence of feed intolerance with the study feed. Although an independent researcher did not observe caregiver feed preparation, a strict set of objective measurements was studied to limit observer bias.

This prospective open-label longitudinal, 6-month study demonstrated that using a composite, protein-free module (in premeasured sachets) as a core ingredient in low protein feeds improved nutritional intake, decreased the number of ingredients used, was well tolerated, and was preferred by caregivers. However, the main aim of the present study was to deliver a more accurate feed composition, and it was disappointing to still observe significant errors in feed preparation resulting in over or under delivery of nutrients. The principal dietetic challenge when feeding children with organic acidaemias is to maintain metabolic stability, maximise linear growth and prevent nutritional deficiency, at the same time as ensuring that nutrition is administered in a safe and convenient package. A new generation of feeds ensuring nutritional adequacy and safety is necessary for children with inherited metabolic disease on protein restrictions.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained. The reporting of this work is compliant with STROBE guidelines.

Conflict of interests, source of funding and authorship

Professor Anita MacDonald, Anne Daly, Sharon Evans, Satnam Chalal and Catherine Ashmore declare that they have undertaken evaluation work for the nutritional companies: Vitaflo Ltd, Nutricia Ltd, and First play dietary foods. Dr Si Santra has no conflicts of interest with respect to the present study.
We received funding to carry out this piece of original work, and declare that the work is in agreement with the Transparency Declaration.
AD was the main researcher for the paper, collected data, assimilated data and wrote the paper. SE, CA, SC, SS and AM helped with the data collection, interpretation of results and made critical reviews and changes to the text as appropriate. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. Joint, W.H.O.F.A.O.U.N.U.E.C. (2007) Protein and Amino Acid Requirements in Human Nutrition. World Health Organ Tech Rep Ser 935. pp. 1–265, back cover.
2. Manoli I, Myles JG, Sloan JL *et al.* (2016) A critical reappraisal of dietary practices in methylmalonic acidemia raises concerns about the safety of medical foods. Part 1: isolated methylmalonic acidemias. *Genet Med* **18**, 386–395.
3. Touati G, Valayannopoulos V, Mention K *et al.* (2006) Methylmalonic and propionic acidurias: management without or with a few supplements of specific amino acid mixture. *J Inherit Metab Dis* **29**, 288–298.
4. North KN, Korson MS, Gopal YR *et al.* (1995) Neonatal-onset propionic acidemia: neurologic and developmental profiles, and implications for management. *J Pediatr* **126**, 916–922.
5. Daly A, Evans S, Gerrard A *et al.* (2016) The nutritional intake of patients with organic acidaemias on enteral tube feeding: can we do better? *JIMD Rep* **28**, 29–39.
6. Evans S, Daly A, Ashmore C *et al.* (2013) Nutritional content of modular feeds: how accurate is feed production? *Arch Dis Child* **98**, 184–188.
7. Evans S, Preston F, Daly A *et al.* (2011) Accuracy of home enteral feed preparation for children with inherited metabolic disorders. *J Hum Nutr Diet* **24**, 68–73.
8. Gokmen-Ozel H, Daly A, Davies P *et al.* (2010) Errors in emergency feeds in inherited metabolic disorders: a randomised controlled trial of three preparation methods. *Arch Dis Child* **95**, 776–780.
9. Dietary reference values for food energy and nutrients for the United Kingdom (1991) Report of the panel on dietary

reference values of the committee on medical aspects of food policy. *Rep Health Soc Subj (Lond)* **41**, 1–210.
10. Martinez M (1992) Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr* **120**(Pt 2), S129–S138.
11. Yehuda S (2003) Omega-6/omega-3 ratio and brain-related functions. *World Rev Nutr Diet* **92**, 37–56.
12. Munns CF, Shaw N, Kiely M *et al.* (2016) Global consensus recommendations on prevention and management of nutritional rickets. *Horm Res Paediatr* **85**, 83–106.
13. Scientific Advisory Committee on Nutrition (SACN) (2015) *Carbohydrates and Health Report*. London: Public Health England.
14. Butterworth SA, Lalari V & Dheensaw K (2014) Evaluation of sodium deficit in infants undergoing intestinal surgery. *J Pediatr Surg* **49**, 736–740.
15. Haycock GB (1993) The influence of sodium on growth in infancy. *Pediatr Nephrol* **7**, 871–875.
16. Prasad C, Nurko S, Borovoy J *et al.* (2004) The importance of gut motility in the metabolic control of propionic acidemia. *J Pediatr* **144**, 532–535.
17. Henningsson AM, Nyman EM & Bjorck IM (2001) Content of short-chain fatty acids in the hindgut of rats fed processed bean (*Phaseolus vulgaris*) flours varying in distribution and content of indigestible carbohydrates. *Br J Nutr* **86**, 379–389.
18. McCance RA, et al. (1978) *McCance and Widdowson's The composition of Foods*. 4th rev. and extended edn. pp. xii, 417. London, Amsterdam; New York, NY: H.M. Stationery Off.; Elsevier/North Holland Biomedical Press.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

CLINICAL NUTRITION

Nitrogen balance in patients with hemiparetic stroke during the subacute rehabilitation phase

A. Wada,[1] M. Kawakami,[2] T. Otsuka,[1] H. Aoki,[3] A. Anzai,[1] Y. Yamada,[1] F. Liu,[2] E. Otaka,[1] K. Akaboshi[4] & M. Liu[2]

[1]Department of Rehabilitation Medicine, National Higashisaitama Hospital, Hasuda, Japan
[2]Department of Rehabilitation Medicine, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan
[3]Nutrition Management Office, National Higashisaitama Hospital, Hasuda, Japan
[4]Department of Rehabilitation Medicine, Ichikawa City Rehabilitation Hospital, Ichikawa, Japan

Keywords
energy metabolism, nitrogen balance, rehabilitation.

Correspondence
M. Kawakami, Department of Rehabilitation Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
Tel: +81 3 5363 3832
Fax: +81 3 3225 5476
E-mail: michiyukikawakami@hotmail.com

How to cite this article
Wada A., Kawakami M., Otsuka T., Aoki H., Anzai A., Yamada Y., Liu F., Otaka E., Akaboshi K. & Liu M. (2017) Nitrogen balance in patients with hemiparetic stroke during the subacute rehabilitation phase. *J Hum Nutr Diet*. **30**, 302–308
doi: 10.1111/jhn.12457

Abstract

Background: In highly invasive diseases, metabolism commonly changes. Hypercatabolism is frequent in acute stroke, and nitrogen balance tends to be negative. However, there has been no study describing nitrogen balance in subacute and chronic stroke patients. The present study aimed to examine nitrogen balance in the subacute and chronic phases and to identify the factors related to it.
Methods: Nitrogen balance was calculated from the collected urine of 56 patients with subacute stroke [mean (SD) 53.8 (18.4) days post-stroke] who were admitted for rehabilitation for their first-ever ischaemic or nonsurgical haemorrhagic stroke. In the first experiment, their nitrogen balance was measured during the rehabilitation phase, and factors (type, severity of hemiparesis, activities of daily living, dysphagia and malnutrition status) related to it were evaluated. The second experiment was performed to describe the time course of nitrogen balance in 31 consecutive patients, with assessments made at admission and at discharge.
Results: Nitrogen balance was positive in all patients in the subacute phase. A significant difference was seen in nitrogen balance between high and low fat-free mass in male patients. In the chronic phase, nitrogen balance was positive in 96% of the patients. There was no significant difference in nitrogen balance between discharge and admission.
Conclusions: In the subacute and chronic phases of stroke, it was confirmed that hypercatabolism had resolved and that intensive rehabilitation is possible in the convalescent period of stroke.

Introduction

Nutrition therapy during the subacute phase is especially important because patients with hemiparetic stroke require intensive rehabilitation [1,2], which includes activities such as resistance training. As for the metabolic rate, the resting metabolic rate (RMR) of stroke patients during the acute phase was reported to be 10–26% higher than that predicted by the Harris–Benedict equation (HB) [3,4] and RMR in subacute stroke patients was approximately the same as that predicted from the HB [5,6]. Kawakami *et al.* [6] showed that resting energy expenditure in stroke patients was not hypermetabolic during the subacute and chronic phases. These studies indicate that the impact of stress after the acute phase in stroke patients might show a gradual decline and is restored to its prior state.

It is well known that severe inflammatory states cause catabolism [7]. It is therefore important that patients should be provided with proper nutritional support based

on a nutritional/metabolic assessment [7]. Hypercatabolism is common in acute stroke, and nitrogen balance tends to be negative [8]. However, to the best of our knowledge, there has been no study of nitrogen balance in patients with stroke during the subacute and chronic phases; we therefore aimed to determine whether such patients are catabolic or anabolic.

Measurement of nitrogen balance is useful for documenting the effectiveness of nutritional therapy. Nitrogen balance is positive if the trend is anabolic and negative if it is catabolic. The major portion of urinary nitrogen is lost as urea, which is measured as urinary urea nitrogen (UUN). Measurement of 24-h UUN excretion enables an estimation of the actual metabolic expenditure to evaluate the degree of hypermetabolism. Urinary nitrogen loss therefore provides the most useful index of the protein needs of the patient in a clinically feasible manner [9].

The present study aimed to examine nitrogen balance in subacute and chronic stroke patients and to identify the factors related to it.

Materials and methods

Participants

A total of 170 consecutive patients who had been treated for subacute stroke at a rehabilitation unit of National Higashisaitama Hospital between June 2014 and September 2015, after having been transferred from acute care hospitals, were recruited.

Criteria for inclusion in the study were admission for rehabilitation for a first-ever ischaemic or nonsurgical haemorrhagic stroke, as confirmed either by computed tomography or magnetic resonance imaging, and enteral nutrition either as oral or tube feeding. Patients were excluded if: (i) they were recurrent cases of stroke; (ii) urine collection was difficult because they could not control urination and were incontinent; (iii) they had major systemic illnesses including sepsis, carcinoma with or without chemotherapy, chronic renal failure requiring dialysis, congestive heart failure with pulmonary oedema, respiratory disorders, such as severe asthma or pneumonia, or a prolonged severe disturbance of consciousness; and (iv) if the serum C-reactive protein (CRP) level was more than 20 mg L^{-1}. This exclusion related to the CRP level means that the results do not apply to stroke patients with ongoing inflammatory states. After excluding 114 patients based on the above criteria, 56 patients were included in the present study.

The study was approved by the institutional ethics review board (#13-2) and was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants or their proxies.

Assessment

Nitrogen metabolism

All participants were inpatients in a rehabilitation unit of National Higashisaitama Hospital for the duration of the experimental phase of the study, allowing for control and monitoring of energy intake and energy expenditure. All participants were given foods that provided the same energy and protein intakes for the 4 days before urine nitrogen collection was performed. The participants were encouraged to eat all of the food provided, and this was recorded. The caloric and protein goals were basically 25–30 (nonprotein) kcal kg^{-1} day^{-1} [8] and 1.0–1.5 g protein kg^{-1} day^{-1} [10] and the amounts of energy and protein provided were adjusted using the HB. Each patient's ideal body weight rather than actual weight was used. Ideal body weight was calculated as: [height (m)] × [height (m)] × 22. As a result, the energy and protein amounts [mean (SD) g day^{-1}] that were actually provided for participants were 22–38 (nonprotein) kcal kg^{-1} day^{-1} and 0.8–1.5 g kg^{-1} day^{-1} [62.2 (8.4) g day^{-1}].

A 24-h urine nitrogen collection was performed. Urine was collected in containers containing a preservative. Toluene was used as the preservative. The nurses in charge and also the patients themselves double-checked that the urine collections were complete. Nitrogen balance, fat-free mass (FFM) and the creatinine height index (CHI) were calculated using the formulae [7,10–12]:

$$\text{Nitrogen balance}\,(\text{g day}^{-1}) = \text{nitrogen intake}\,(\text{g day}^{-1}) - \text{nitrogen excretion}\,(\text{g day}^{-1})$$

$$\text{Nitrogen intake} = \text{intake amount of protein}/6.25\,(\text{g day}^{-1})$$

$$\text{Nitrogen excretion} = \text{urinary urea nitrogen excretion} \times 1.25\,(\text{g day}^{-1})$$

$$\text{FFM}\,(\text{kg}) = 23.3 \times \text{urinary creatinine excretion}\,(\text{g day}^{-1}) + 21.1$$

$$\text{CHI}\,(\%) = \text{actual urinary creatinine}/\text{ideal urinary creatinine}\,(\%)$$

The CHI is an effective measure of muscle mass and allows an estimation of lean body mass [7]. In addition, the patients were divided into two groups (low FFM and high FFM) according to the mean FFM score for each sex.

Energy consumption at rest

RMR was measured using indirect calorimetry (Metavine; Vine, Tokyo, Japan) [13]. This indirect calorimeter calculates RMR from measured VO_2 and an assumed

respiratory quotient of 0.82. Several studies have reported that Metavine measurement of RMR gives a result similar to other indirect calorimetry methods using *in vivo* gas (O_2 and CO_2) analyses. Measurements were performed in the supine position at least 2 h after meal consumption and after the patient had been recumbent for at least 30 min, as described in previous reports involving stroke patients [6]. A facemask was used for gas collection, with calibration performed before every measurement. Measurements were performed within 2 days from the day when the 24-h urine nitrogen collection was performed. The patients were divided into two groups (low RMR and high RMR) according to the mean RMR. The predicted RMR was also calculated using the HB [5].

Severity of hemiparesis

The severity of hemiparesis was assessed with the motor items of the Stroke Impairment Assessment Set (SIAS) [14]. The scores range from 0–5, with 0 indicating complete paralysis, 3 indicating the ability to complete the task with difficulty, and 5 indicating no paresis. The motor items consist of five items (knee-mouth, finger function, hip-flexion, knee-extension and foot-pat), with a full score of 25 points. The patients were divided into two groups (severe and mild) according to the severity of hemiparesis as assessed with the median SIAS motor score of the study population.

Activities of daily living

Activities of daily living (ADL) were assessed with the Functional Independence Measure (FIM) by trained doctors or physiotherapists. Patients were divided into a low-ADL or a high-ADL group by the median FIM score of this study population [15].

Other background information

Age, sex, height, weight and body mass index (BMI) were collected. Serum albumin was obtained from the blood tests. In addition, dysphagia was assessed by clinical examination and the patients were divided into two groups (no dysphagia and dysphagia). Patients in the no dysphagia group were eating a normal diet or porridge and an incremental diet and those in the dysphagia group were eating a puree or soft diet, or oral ingestion was not possible.

Study design

Experiment 1: Cross-sectional study

The experiment was performed to investigate nitrogen balance in stroke patients during the subacute rehabilitation phases and to examine the factors related to nitrogen balance. All 56 patients participated and were assessed at admission. The subacute phase in the study was defined as the days from stroke onset between 30 and 90 days using a previous study as a reference [6].

Experiment 2: Prospective cohort study

The experiment was performed to describe the time course of nitrogen balance. Thirty-one consecutive patients participated in the study between January 2015 and September 2015. They were assessed at admission (subacute phase) and at discharge (chronic phase). The chronic phase in the study was defined as 90 days after stroke onset using a previous study as a reference [16].

Statistical analysis

Experiment 1

The nitrogen balance of the two groups was compared by stroke type, hemiparesis severity, ADL limitation, nutrition status by FFM, RMR and dysphagia severity using the Mann–Whitney *U*-test. The correlations between UUN and hemiparesis severity, ADL limitation, nutrition status, and dysphagia severity were assessed using the Spearman rank correlation test.

Experiment 2

Thirty-one patients were evaluated at admission and at discharge. The difference in nitrogen balance was analysed at admission and discharge using the Wilcoxon signed-rank test. $P < 0.05$ was considered statistically significant. All data analyses were performed using JMP, version 11 (SAS Institute Inc., Cary, NC, USA).

Results

Experiment 1

Demographics and relevant clinical features are summarised in Table 1. The mean (SD) time from stroke onset to the urine test was 53.8 (18.4) days. All patients were fed orally when the nitrogen balance was assessed, and the mean (SD) energy and protein goals were 27.8 (3.2) (nonprotein) kcal kg^{-1} day^{-1} and 1.10 (0.14) g protein kg^{-1} day^{-1}. The comorbidities of the participants included hypertension ($n = 44$), diabetes mellitus ($n = 16$), hyperlipidaemia ($n = 18$), coronary artery disease ($n = 3$), renal dysfunction ($n = 6$) and depression ($n = 6$).

The results for nitrogen balance are summarised in Table 2. No patient demonstrated a negative nitrogen balance, and anabolism (nitrogen balance >2 g) [8] was seen in 50 of the 56 patients (89%). There was no significant difference in nitrogen balance between groups classified by type, severity of hemiparesis, ADL and dysphagia (Table 3). There was a significant difference in nitrogen

Table 1 Participant characteristics

Number of patients	56
Age (years)	62.8 (12.5)
Sex (male: female)	36: 20
Stroke type (ischaemic: haemorrhagic)	36: 20
Height (cm)	160.5 (10.7)
Body weight (kg)	59.0 (14.0)
Body mass index (kg m^{-2})	22.7 (3.9)
RMR (kcal day^{-1})	1394.5 (410.6)
Predicted RMR (kcal day^{-1})	1273.4 (272.2)
Median of the SIAS motor items scores (range)	17 (0–25)
Median FIM score (range)	89 (21–125)

FIM, Functional Independence Measure; RMR, resting metabolic rate; SIAS, Stroke Impairment Assessment Set.
RMR was measured using indirect calorimetry, and predicted RMR was calculated using the Harris–Benedict equation.
Data are the mean (SD) or median (range).

Table 2 Nitrogen balance in stroke patients during the subacute phase

Urinary urea nitrogen (g day^{-1})	4.7 (1.4) (2.2–8.5)
Mean nitrogen excretion (g day^{-1})	5.8 (1.8) (2.7–10.7)
Mean nitrogen balance (g day^{-1})	4.1 (1.3) (1.1–7.3)
Patients achieving anabolism (number)	50 of 56
Fat-free mass (kg)	42.3 (7.0)
Creatinine height index (%)	90.1 (17.8)

Anabolism is defined as nitrogen balance >2 g.
Data are the mean (SD) (range).

balance between high and low FFM in male patients. The mean (SD) protein amounts that were actually provided for high FFM in male, low FFM in male, high FFM in female and low FFM in female patients were 67.3 (6.9), 63.5 (9.1), 56.9 (5.3) and 56.1 (4.8) g day^{-1}, respectively. Table 4 shows the correlations between UUN and clinical factors; the FFM had a moderate positive correlation with UUN ($\rho = 0.62$, $P < 0.001$).

Experiment 2

Thirty-one patients were evaluated both at admission and at discharge. The mean (SD) duration between the two assessments was 96.5 (42.6) days. The results of each parameter are summarised in Table 5. For BMI, RMR, UUN, nitrogen excretion, nitrogen balance, SIAS motor item scores and other nutritional parameters, there were no significant differences between the admission and discharge values. The only significant difference was observed with respect to the FIM score.

Discussion

This is the first study to report nitrogen balance in patients with stroke during the subacute phase. The primary findings are that nitrogen balance during the subacute phase was not negative and urea nitrogen excretion was not very high. Typically, measurement of 24-h UUN excretion enables an estimation of the actual metabolic expenditure to evaluate the degree of hypermetabolism [(7)]. The more severe trauma or disease is, the more the urea nitrogen excretion is increased. For example, during stress, it measures 7–9 g day^{-1} in elective surgery [(7)], 9–11 g day^{-1} in infection [(7)], 13 g day^{-1} in mechanically ventilated critically ill patients [(17)], 11–14 g day^{-1} in severe sepsis [(7)] and 17 g day^{-1} in multiple trauma patients [(18)]. In the present cases, it was 5.8 ± 1.8 g day^{-1}, which is not a particularly high value.

Hypercatabolism is common in acute stroke, and there is a tendency for nitrogen excretion to increase and nitrogen balance to become negative [(8)]. This could be a mixture of the acute brain injury and secondary infection that is common in this population, although patients with catabolic illnesses (malignancy, AIDs, sepsis, burns, fractures, pancreatitis) were excluded in a study examining this issue [(8)]. By contrast, nitrogen excretion did not show high values, and nitrogen balance was positive in subacute stroke patients, with a minimal inflammatory response (CRP < 20 mg L^{-1}), in the present study. Thus, it was found that an anabolic tendency is common in subacute stroke patients. It is considered that patients with subacute stroke had escaped from the highly invasive stress during the acute phase, and the nutrition status

Table 3 Nitrogen balance at admission and its relationship to clinical factors (Mann–Whitney *U* test)

		n	Nitrogen balance		*n*	Nitrogen balance	*P* value
Stroke type	Ischaemic	36	4.26 (1.44)	Heamorrhagic	20	3.89 (1.14)	0.24
SIAS motor items	Mild hemiparesis	29	4.43 (1.17)	Severe hemiparesis	27	3.79 (1.49)	0.10
FIM	High ADL	28	4.07 (1.44)	Low ADL	28	4.19 (1.31)	0.67
RMR	High RMR	27	3.93 (1.28)	Low RMR	29	4.40 (1.32)	0.15
FFM Male	High FFM	18	3.44 (1.40)	Low FFM	18	4.61 (1.17)	<0.01
FFM Female	High FFM	10	4.20 (1.52)	Low FFM	10	4.43 (0.94)	0.54
Dysphagia	No dysphagia	53	4.18 (1.34)	Dysphagia	3	3.12 (1.21)	0.14

FFM, fat-free mass; FIM, Functional Independence Measure; RMR, resting metabolic rate; SIAS, Stroke Impairment Assessment Set.
Data are the mean (SD).

Table 4 Spearman's rank correlation coefficients between urinary urea nitrogen and clinical factors

	Spearman's rank correlation coefficient	*P* value
SIAS motor items	0.06	0.65
FIM	0.33	0.02
CHI	0.29	0.03
FFM	0.62	<0.001
Serum albumin level	0.31	0.02
RMR	0.43	<0.001
Dysphagia	0.004	0.97

CHI, creatinine height index; FFM, fat-free mass; FIM, Functional Independence Measure; RMR, resting metabolic rate; SIAS, Stroke Impairment Assessment Set.

Table 5 Comparisons of nutritional parameters, SIAS and FIM at admission (during the subacute phase) and at discharge (during the chronic phase)

	Admission	Discharge	*P* value
Duration from onset (days)	53.8 (18.4)	140.7 (44.3)	
Body mass index (kg)	23.8 (4.4)	22.9 (3.9)	0.32
Predicted RMR (kcal day^{-1})	1291.6 (308.1)	1261.7 (298.4)	0.76
Urinary urea nitrogen (g day^{-1})	4.38 (1.3)	5.03 (1.4)	0.06
Nitrogen excretion (g day^{-1})	5.5 (1.6)	6.3 (1.8)	0.06
Nitrogen balance (g day^{-1})	4.3 (1.4)	4.2 (1.5)	0.49
SIAS motor items	17 (0–25)	21 (3–25)	0.11
FIM score	88 (21–121)	111 (45–126)	0.002
Serum albumin level (mg dL^{-1})	3.8 (0.4)	4.0 (0.4)	0.37
FFM (kg)	33.0 (9.6)	33.1 (9.6)	0.94
CHI (%)	91.7 (21.3)	88.2 (19.1)	0.50

CHI, creatinine height index; FFM, fat-free mass; FIM, Functional Independence Measure; RMR, resting metabolic rate; SIAS, Stroke Impairment Assessment Set.
Data are the mean (SD) or median (range).

was in the recovery process. It was also suggested that exercise load can be applied during their rehabilitation without the risk of proteolysis induced by catabolism. That is, patients with no severe secondary infection reaching the sub-acute stage of stroke are no longer catabolic, and exercise may therefore facilitate anabolism. On the other hand, the results for nitrogen balance must be interpreted with caution because, if nitrogen balance had been positive, there should eventually have been an increase in the fat-free mass. This did not happen, and so it is more likely that these patients were in nitrogen equilibrium than in positive nitrogen balance. However, the implication is that, if protein intake had been increased, nitrogen balance would have been improved. The over-riding point is that patients in this phase of stroke care had escaped from hypercatabolism.

The results of the present study showed that only FFM in males was related to nitrogen balance, although stroke type, severity of hemiparesis and FFM in females were not related to it. This might be explained by the greater muscle mass in males than in females, which makes males more susceptible to changes in muscle mass. It appears that patients with a higher FFM are more prone to catabolism and may therefore need a higher protein input. Similarly, the FFM had a positive moderate correlation with UUN. This result suggests that a higher FFM necessitates higher protein input to achieve nitrogen balance.

As described previously, hypercatabolism is common in acute stroke. It has been reported that FFM was low in chronic stroke survivors with permanent feeding tubes and in a bed-bound state as a result of hemiparesis and changes associated with prolonged bed rest [19]. In the present study, patients with low FFM were in an anabolic state in the subacute phase. Thus, we proposed the hypothesis that the hypercatabolism of the acute phase results in gradual loss of FFM in stroke patients, although the anabolic tendency restores the temporary loss of the FFM after the acute phase. If patients do not receive enough nutrition and physical therapy, their muscle mass is reduced.

Ryan *et al.* [20] reported that myostatin mRNA expression, which is a regulator of skeletal muscle development and size, is higher in paretic muscle than in nonparetic muscle, and that resistance training can reduce myostatin expression in stroke survivors. This suggests that myostatin is a key molecular regulator for the paretic side muscle atrophy that can respond favourably to intensive resistance training [20]. Bos *et al.* [21] reported that short-term protein and energy supplementation in malnourished elderly patients was associated with an increase in FFM and stimulation of nitrogen kinetics. Increasing energy and protein intakes and the ratio of protein to energy in these subjects led to a positive protein balance with a concomitant FFM gain [21]. The present results support these previous studies. Because patients with low FFM showed an anabolic tendency, adequate nutrition and exercise might increase muscle mass, especially in malnourished patients.

At approximately 5 months after stroke onset, nitrogen balance was generally positive. Nitrogen balance at admission (subacute phase) was not different from that at discharge (chronic phase). This suggests that metabolism in stroke patients who had no adverse events did not become catabolic after the acute phase.

The only change from admission to discharge during the convalescent phase was observed in the FIM, which can reasonably be interpreted as a rehabilitation effect. There was no significant change in other nutritional parameters. In the present study, there was no significant change in the FFM. It is assumed that the reduction of muscle mass on the paralytic side [19,20] was suppressed by sufficient nutrition and adequate resistance training during the anabolic phase [20–23].

The present study had several limitations. The study was conducted at only one institution, and thus the results must be generalised with caution. Because the present study was performed in a subacute rehabilitation unit, the study sample does not include the severest bedbound patients and relatively mild cases where patients were discharged home directly from the acute hospital. Furthermore, the study excluded those patients with urinary incontinence, many of whom might have been affected by cognitive impairment; thus, the study mainly included patients with good cognitive function. We consider, however, that the present study population represented patients treated at general subacute stroke wards fairly well because it covered patients with wide ranges of paralysis severity and nutritional status. Furthermore, using the assumption that UUN is 80% of total urea nitrogen was a limitation. The proportion of non-urea nitrogen may be more variable and higher if patients are in more catabolic states, such as burns [11]. However, in general, the excretion of non-urea nitrogen (such as skin or stool) is regarded as minimal [10]. This assumption was considered appropriate in the present study population patients they did not show hypercatabolic states.

Despite these limitations, we consider that the findings of the present study provide important information for improving nutritional management during the subacute rehabilitation phase in patients with stroke.

Conclusions

In the subacute and chronic phases of stroke, it was confirmed that hypercatabolism had resolved and that intensive rehabilitation is possible in the convalescent period of stroke.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interests.
No funding is declared.
AW, MK and ML contributed to conception and design, data acquisition, analysis and interpretation, and drafting of the manuscript. TO, HA, AA, YY, FM and EO contributed to data acquisition and analysis. KA contributed to drafting the manuscript. All authors revised the article critically and approved the final version submitted for publication.

References

1. Rabadi MH, Coar PL, Lukin M *et al.* (2008) Intensive nutritional supplements can improve outcomes in stroke rehabilitation. *Neurology* **71**, 1856–1861.
2. Nii M, Maeda K, Wakabayashi H *et al.* (2016) Nutritional improvement and energy intake are associated with functional recovery in patients after cerebrovascular disorders. *J Stroke Cerebrovasc Dis* **25**, 57–62.
3. Finestone HM, Greene-Finestone LS, Foley NC *et al.* (2003) Measuring longitudinally the metabolic demands of stroke patients: resting energy expenditure is not elevated. *Stroke* **34**, 502–507.
4. Esper DH, Coplin WM & Carhuapoma JR (2006) Energy expenditure in patients with nontraumatic intracranial hemorrhage. *JPEN J Parenter Enteral Nutr* **30**, 71–75.
5. Harris JA & Benedict FG (1919) *A Biometric Study of Basal Metabolism in Man*. Washington, DC: Carnegie Institute of Washington.
6. Kawakami M, Liu M, Wada A *et al.* (2015) Resting energy expenditure in patients with stroke during the subacute phases – relationships with stroke types, location, severity of paresis, and activities of daily living. *Cerebrovasc Dis* **39**, 170–175.
7. Blackburn GL, Bistrian BR, Maini BS *et al.* (1977) Nutritional and metabolic assessment of the hospitalized patient. *JPEN J Parenter Enteral Nutr* **1**, 11–22.
8. Chalela JA, Haymore J, Schellinger PD *et al.* (2004) Acute stroke patients are being underfed: a nitrogen balance study. *Neurocrit Care* **1**, 331–334.
9. Clifton GL, Robertson CS, Grossman RG *et al.* (1984) The metabolic response to severe head injury. *J Neurosurg* **60**, 687–696.
10. Japanese Society for Parenteral and Enteral Nutrition. (2011) *Japanese Society for Parenteral and Enteral Nutrition Parenteral and Enteral Nutrition Handbook*. Tokyo: Nankodo.
11. Milner EA, Cioffi WG, Mason AD Jr *et al.* (1993) Accuracy of urinary urea nitrogen for predicting total urinary nitrogen in thermally injured patients. *J Parenter Enteral Nutr* **17**, 411–416.
12. Welle S, Thornton C, Totterman S *et al.* (1996) Utility of creatinine excretion in body-composition studies of healthy men and women older than 60 y. *Am J Clin Nutr* **63**, 151–156.

13. Kato M, Tajika M, Miwa Y *et al.* (2002) Validation of a portable indirect calorimeter (Metavine) for measuring energy expenditure in an elderly population. *Clin Exp Pharmacol Physiol Suppl* **29**, S9–S12.
14. Tsuji T, Liu M, Sonoda S *et al.* (2000) The stroke impairment assessment set: its internal consistency and predictive validity. *Arch Phys Med Rehabil* **81**, 863–868.
15. Data Management Service of the Uniform Data System for Medical Rehabilitation and the Center for Functional Assessment Research. (1990) *Guide for Use of the Uniform Data Set for Medical Rehabilitation*, Version 3.0. Buffalo: State University of New York.
16. Cui L, Murikinati SR, Wang D *et al.* (2013) Reestablishing neuronal networks in the aged brain by stem cell factor and granulocyte-colony stimulating factor in a mouse model of chronic stroke. *PLoS ONE* **8**, e64684.
17. Dickerson RN, Tidwell AC, Minard G *et al.* (2005) Predicting total urinary nitrogen excretion from urinary urea nitrogen excretion in multiple-trauma patients receiving specialized nutritional support. *Nutrition* **21**, 332–338.
18. Japur CC, Monteiro JP, Marchini JS *et al.* (2010) Can an adequate energy intake be able to reverse the negative nitrogen balance in mechanically ventilated critically ill patients? *J Crit Care* **25**, 445–450.
19. Leone A & Pencharz PB (2010) Resting energy expenditure in stroke patients who are dependent on tube feeding: a pilot study. *Clin Nutr* **29**, 370–372.
20. Ryan AS, Ivey FM, Prior S *et al.* (2011) Skeletal muscle hypertrophy and muscle myostatin reduction after resistive training in stroke survivors. *Stroke* **42**, 416–420.
21. Bos C, Benamouzig R, Bruhat A *et al.* (2000) Short-term protein and energy supplementation activates nitrogen kinetics and accretion in poorly nourished elderly subjects. *Am J Clin Nutr* **71**, 1129–1137.
22. Campbell WW, Haub MD, Wolfe RR *et al.* (2009) Resistance training preserves fat-free mass without impacting changes in protein metabolism after weight loss in older women. *Obesity* **17**, 1332–1339.
23. Walrand S, Short KR, Bigelow ML *et al.* (2008) Functional impact of high protein intake on healthy elderly people. *Am J Physiol Endocrinol Metab* **295**, E921–E928.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

PUBLIC HEALTH NUTRITION

Health literacy, literacy, numeracy and nutrition label understanding and use: a scoping review of the literature

L. Malloy-Weir[1] & M. Cooper[2]

[1]College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada
[2]Bureau of Nutritional Sciences, Health Canada, Ottawa, ON, Canada

Keywords
health literacy, literacy, numeracy, nutrition labels.

Correspondence
L. Malloy-Weir, College of Medicine, University of Saskatchewan, 104 Clinic Place, Box 23, Saskatoon, SK S7N 5E5, Canada.
Tel.: 306-966-8796
Fax: 306-966-8799
E-mail: lem074@mail.usask.ca

How to cite this article
Malloy-Weir L., Cooper M. (2017) Health literacy, literacy, numeracy and nutrition label understanding and use: a scoping review of the literature. *J Hum Nutr Diet*. **30**, 309–325
doi:10.1111/jhn.12428

Abstract

Background: Low health literacy, literacy and numeracy have been identified as barriers to consumer understanding and the interpretation of nutrition-related information. To inform policy and dietetic practice, we examined the extent, range and nature of research on empirical relationships between health literacy, literacy or numeracy and the understanding and use of nutrition labels.
Methods: A scoping review of the literature was conducted. A search of eight databases on 15 April 2014 and 26 May 2016 returned 651 and 173 records, respectively. After de-duplication and two levels of relevance screening, 16 studies were deemed eligible for inclusion in the present review.
Results: The majority of studies were conducted in the USA and focused primarily on the use of back-of-pack nutrition labels. Empirical relationships reported between health literacy and nutrition label use were inconsistent and, in some cases, contradictory. The findings from studies examining empirical relationships between literacy, numeracy and nutrition label use suggest that consumers with lower literacy and numeracy: (i) differ from those with higher levels in some of the judgements that they make about food and (ii) may benefit from interventions designed to improve their understanding and use of nutrition label information. Measurement-related issues were identified, such as a reliance on self-reports of nutrition label use, as well as a lack of independence between some measures of health literacy and nutrition label understanding and use.
Conclusions: The empirical relationships between health literacy, literacy, numeracy and nutrition label understanding and use have not been well-studied. Additional attention is needed regarding the measurement-related issues identified in the present review.

Introduction

One of the goals of nutrition labelling is to better inform consumers and promote healthier eating [(1–3)]. An evaluation of the effects of nutrition labelling policies on consumer food choices reveals contradictory findings, with some studies showing modest effects and other studies showing no effect [(1,2)]. Given these findings, and a trend toward the use of both front-of-pack (FOP) labels and back-of-pack (BOP) labels, research is needed to determine how consumers can be best helped to make use of labelling [(1)].

One factor that may affect a consumer's ability to make use of nutrition labels is health literacy: 'the ability to access, comprehend, evaluate and communicate information as a way to promote, maintain and improve health in a variety of settings across the life-course' [(4)]. Low health literacy has been reported to be a barrier to the understanding of nutrition labels [(5)] and an important consideration for nutrition education [(6)]. Literacy

and numeracy (or math ability), which are widely considered to be sub-components of health literacy, have also been identified as important to the interpretation of nutrition information [2]. To help inform nutrition labelling policy and dietetic practice, we aimed to determine what is known from the existing literature about the empirical relationships between health literacy, literacy or numeracy and the understanding and use of nutrition labels. To be as inclusive as possible, we defined 'nutrition label' as any type of nutrition-related information found on the front or back of food products. 'Use' of nutrition labels was defined to include: (i) visual attention allocated to entire, or sections of, labels and (ii) application of label information for making product choices or purchases. We considered both subjective and objective understandings of nutrition labels. We kept the definition of 'health literacy' open because of the lack of agreement over its definition [7].

Materials and methods

A scoping review of the literature was conducted using the framework approach described by Arksey and O'Malley [8]. Scoping reviews allow researchers to: (i) 'examine the extent, range, and nature of research activity' in a specific topic area; (ii) summarise and disseminate research findings; and (iii) 'identify research gaps in the existing literature' [8].

To identify primary studies relevant for inclusion, an electronic search of eight databases (Table 1) was undertaken by a librarian at Health Canada on 15 April 2014. The search terms used in these databases are provided in Appendix 1. A total of 651 records was returned from the first search. A second search of the eight databases was performed on 26 May 2016 to identify studies published after our initial search. This search returned 173 records. All records underwent two levels of relevance screening using the process shown in Fig. 1. Using this process, 16 studies were considered eligible for inclusion in the present review.

A data charting form was used by both investigators to chart specific information from each of the 16 studies: author(s), publication date; purpose; characteristics of the study population; research design; type of labels examined (FOP, BOP); measure(s) of understanding; measure(s) of use; and results relevant for answering the scoping review question. Definitions and measures of health literacy, literacy and numeracy were also charted. Any conflicts that arose during the entire relevance screening and data charting process were resolved by both investigators. A narrative account of the findings is also provided.

Table 1 Academic databases searched

Academic database	Temporal period covered
Embase	1974 – 26 May 2016
Ovid Medline (R)	1946 – 26 May 2016
CAB Abstracts	1973 – 26 May 2016
Econolit	1886 – 26 May 2016
Food Science & Technology Abstracts	1969 – 26 May 2016
Global Health	1973 – 26 May 2016
PsychINFO	1806 – 26 May 2016
Social Policy & Practice	1981 – 26 May 2016

Results

Temporal and geographical distribution of the 16 studies

The majority of studies (n = 10; 63%) were conducted in the USA. Two studies were conducted in Australia, two in Switzerland, one in Canada and one in Singapore. All of the studies were published between the 2004 and 2015.

Research designs and study populations

Eight studies included in the review used experimental or quasi-experimental designs. The remaining eight studies employed a correlational (n = 6), qualitative (n = 1) or mixed-methods designs (n = 1). Nonprobabilistic sampling was used in 11 studies. Across studies, the number participants ranged from 11 to 1180. The mean age of participants, where reported, ranged from children in elementary school to individuals aged 53.5 years. Females were featured more frequently than males.

Location of nutrition labels

Back-of-pack nutrition information was the focus in six studies [9–15]. Five studies examined both BOP and FOP information [5,15–18]. In five studies, the location of nutrition information was unclear or was not stated by the authors [19–23].

Definitions and measures of health literacy, literacy and numeracy

Health literacy was mentioned and defined in six [5,9,10,15,19,23] of the 16 studies included in the present review (Table 2,5 column). Common elements across these definitions include the ability (or capacity) to obtain (or acquire), understand (or interpret) and use (e.g. for decision making purposes) information (e.g. nutrition information panel, basic health information). The Newest Vital Sign, which contains questions about numerical and non-numerical information contained on an ice cream label [24], was used to assess health literacy in five of the seven studies [5,9,15,18,19]. Only two of the

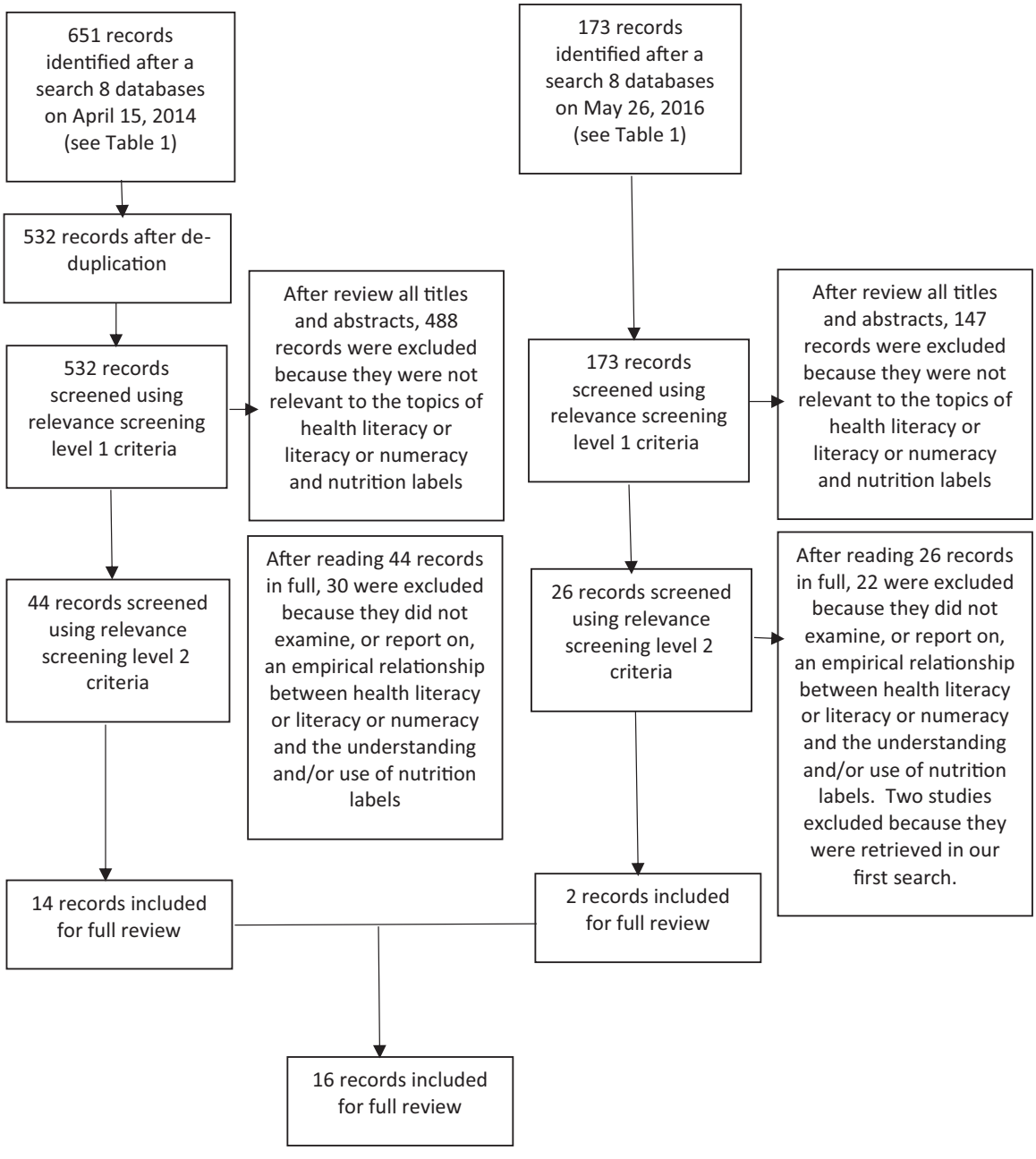

Figure 1 Summary of how primary studies were identified for inclusion in the present review.

six questions from the Newest Vital Sign were utilised in one of these studies [5]. Four questions based on nutrition information contained on a product were used to assess health literacy in one study [23]. Five multiple choice questions were used in another study [22]. The Short Test of Functional Health Literacy in Adults, which assesses a person's ability to read and understand numerical and non-numerical information contained on prescription bottles and appointment slips [25], was used in the remaining study [10].

Six of the 16 studies included in this review examined literacy, nutrition literacy, nutrition and food label literacy, food label literacy or media literacy [11–13,16,17,20]. The instruments used to assess these literacies are listed in Table 2 (column 6). Numeracy was assessed in two studies [14,21] using items derived from the Subjective Numeracy Scale, which is a subjective assessment of quantitative ability [26]. Eight items from the Subjective Numeracy Scale were used in one of these studies [22], whereas only one item was used in the other [15].

Table 2 Empirical relationships between health literacy, literacy, or numeracy and the understanding and/or use of nutrition labels

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Cha (2014) [19] United States (Atlanta, GA)	'To examine relationships among health literacy, self-efficacy, food label use, and dietary quality in young adults aged 18–29.' (p. 331)	*N* = 103 Female: 78.6% Mean age: NR Sampling method: nonprobabilistic	Correlational cross-sectional study design Year of data collection NR	'Health literacy refers to an individual's capability to obtain and understand health information to make appropriate health decisions.' (p. 332)	NVS	NR		Food label use was assessed using a self-reported item: 'How often do you use the food label when making a food selection?' 'It was measured on a 5-point Likert scale ranging from never (1) to always (5).' (p. 333)	No statistically significant difference in young adults' self-reported use of food labels by level of health literacy (i.e. low versus high, and medium versus high); $P > 0.05$
Speirs (2012) [15] United States (Maryland)	'To explore the relationship between health literacy and nutrition behaviors using a low-income sample.' (p. 1082)	*N* = 142 low income adults Female: 76% Mean age: 37 years Sampling method: nonprobabilistic	Face-to-face cross-sectional survey Year of data collection NR	Health literacy defined as 'the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions. (p. 1082)	NVS	FOP and BOP List of ingredients, short phrases on label (e.g. 'low fat'), nutrition panel (e.g. calories, protein, fat), information about serving size, statements describing health benefits of food		Food label use assessed by asking: 'When you buy foods, how often do you use each of the following sections of the label?' (p. 1087) Sections of the label included: list of ingredients; short phrases on label (e.g. 'low fat'); nutrition panel that tells the amount of calories, protein, fat, and such in a serving of the food; information about the size of a serving; statements describing health benefits of nutrients in the food. Self-reported use of each section assessed on a Likert scale: 5 = always and 1 = never	No statistically significant differences in self-reported use of food label by level of health literacy (i.e. adequate health literacy, possible limited health literacy, high likelihood of limited health literacy)

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Sinclair (2013) [5] Canada (Ontario)	'To examine comprehension of nutrition labels across sociodemographic groups using a measure of health literacy.' (p. 767)	*N* = 639 Female: 55.1% Mean age: 44.7 years Sampling method: nonprobabilistic	Cross-sectional survey was 'administered with groups of up to 10 subjects at a time During the first part of the study session, participants were randomly assigned to view menus with different types of nutrition information' (p. 768) Data collected in 2010–2011	Health literacy defined as: 'the degree to which individuals have the capacity to obtain, process, and understand basic health information needed to make health decisions.' (p. 1)	Two questions from the NVS: (1) calorie calculator question, and (2) %DV question	BOP (nutrition facts table or number of calories) and FOP (any of the other nutrition information)		Label use assessed by asking 'frequency of reading labels (1 = 'Never/Only the first time I buy a product/ sometimes'; 2 = 'Usually/ always') and types of nutrition information looked at (1 = none; 2 = nutrition facts table or number of calories; and 3 = any of the other nutrition information)' (p. 2)	'participants who indicated that they looked at nutrition facts or calories on labels were significantly more likely to correctly answer the calorie question versus participants who did not look at any information on labels ($P = 0.01$; odds ratio, 2.59; 95% confidence interval, 1.25–5.35).' (p. 4)
Graham *et al.* (2015) [18] United States (Minnesota)	'To quantify Nutrition Facts and front-of-package nutrition label viewing among American adult consumers.' (p. 1636)	*N* = 123 Female: 86.4% Mean age: 38.2 years Sampling method: nonprobabilistic	Intervention: 'Participants were randomized to conditions in which front-of-package nutrition labels were present or absent, and signage explaining front-of-package nutrition labels was present or absent.' (p. 1636) Data collected in 2012–2013	Health literacy – no definition provided	NVS – all six questions	FOP (Traffic light labels and Facts up Front Labels) and BOP (Nutrition Facts Labels)		Eye-tracking glasses that recorded video of what participants looked and audio of what participants said	When aisle signage describing FOP nutrition labels was present participants with both high literacy ($P < 0.001$) and low literacy ($P = 0.003$) were more likely to view FOP labels than BOP labels (i.e. Nutrition Facts Labels) When aisle signage was not present, there was no difference in the viewing of FOP and BOP labels by participants with high and low health literacy

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Mackert (2013) [9] United States	'To explore how individuals with different levels of health literacy visualize health-related information.' (p. 185)	*N* = 49 Female: 75.5% Mean age: NR Sampling method: stratified	Design: NR Year data collected NR computerized eye-tracking technology was used 'to measure the amount of time each participant spent fixing their view at nutrition label information that was relevant to the questions being asked and the amount of time they spent viewing nonrelevant information.' (p. 185)	Health literacy: '... an individual's ability to acquire, interpret, and use health information properly' (p. 186)	NVS	BOP		Eye-tracking used to examine participants' fixation on 18 areas of interest on the NVS nutrition label	'The total amount of time spent fixating on the NVS nutrition label was not correlated with NVS score, nor did total fixation duration differ significantly across levels of health literacy.' (p. 192) '... for each point decrease in NVS score, the amount of time looking at nonrelevant information increased by 4.21 s. This association was statistically significant ($P < 0.005$)' (p. 192) 'Health literacy score was not significantly associated with fixation duration or fixation count for relevant information [for answering NVS questions] or for fixation count on nonrelevant information.' (p. 192)

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Vijaykumar (2013) (23) Singapore	'To examine factors influencing food label use among Singapore's supermarket shoppers using the Theory of Planned Behavior.' (p. 204)	*N* = 199 shoppers Female: 63.5% Mean age: NR Sampling method: nonprobabilistic	'Point-of-purchase survey among general shoppers in 2 supermarkets' (p. 204) Data collected in 2010	'Health literacy was operationalized as an individual's ability to understand and use information from the nutrition information panel.' (p. 206)	Measure of health literacy based on nutrition information provided on a product: How many grams of sugar are there in two servings of this product? How many grams of fat are there in 50% of this pack? How many servings are there in this product? Is this product high in fat?	NR – 19 different elements in food labels		'Overall food label use was measured through an index of respondents' use of 19 different elements in food labels.' (p. 207)	Authors found high-food label use among shoppers, but low levels of health literacy
Jay (2009) (10) United States (New York)	'To study a multimedia intervention to improve food label comprehension in a sample of low income patients in NewYork City.' (p. 25)	*N* = 23 intervention group (IG) – *N* = 19 control group (CG) Female: 74% IG; 89% CG Mean age: 52 years IG; 49 years CG Sampling method: nonprobabilistic	RCT Conducted in 2005–2007' intervention group received a Nutrition Facts Label Pocket Card and viewed an 8 minute video explaining card use.' (p. 27) 'control group received a print version of publicly available FDA materials explaining the Nutrition Facts Label' (p. 28)	Health literacy: 'the degree to which individuals have the capacity to obtain, process and understand basic health information and services for appropriate health decisions' (p. 26)	Short Test of Functional Health Literacy in Adults (STOFHLA)	BOP	Understanding of food labels was measured by a nutrition food label quiz authors assessed pre- and post-intervention		'... the multimedia intervention group participants with adequate literacy had a higher test score on the post-[nutrition label food] quiz than those individuals in the control group with adequate literacy [($P < 0.05$)].' (p. 29) '... there was no improvement in either intervention group for patients with limited health literacy.' (p. 25)

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Viswanathan (2009) [11] United States	'To examine the effect of different formats for presenting nutrition information on low-literate consumers' judgments of healthfulness' (p. 137)	N = 214 (120 participants registered at adult education centres + 94 undergraduate students) Female: NR Mean age: NR Sampling method: stratified	'Respondents with varying levels of literacy were randomly assigned to each of the four summary conditions [%DV, average, graphic rating, graphic range].' (p. 140) Year data collected NR	Literacy: 'the ability to exhibit all of the behaviors a person needs in order to respond properly to all possible reading tasks' (p. 136) Numeracy: the 'capacity and propensity to effectively and critically interact with the quantitative aspects of the adult world' (p. 136)	Literacy assessed only in participants attending adult education centres only: 'tests consist of several multiple choice questions (40 questions for reading level, 50 questions for math) . . .' (p. 139)	BOP		Use of information contained in Nutrition Facts panels for rating products in terms of their healthfulness Information presented in graphic formats (i.e. rating, range) and non-graphic formats (%DV, Average)	Consumers with higher levels of literacy were more effective in judging product healthfulness based on nutrition information than consumers with lower levels of literacy (F = 8.42, P < 0.01) Consumers with lower and medium levels of literacy benefited more from graphic formats than consumers with higher literacy levels for information about calories (P = 0.02), fat (P = 0.02), and carbohydrates (P = 0.02)

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Watson (2012) [16] Australia	'To investigate nutrition literacy among adult grocery buyers regarding energy-related labelling terms on food packaging.' (p. 409)	*N* = 40 (qualitative) *N* = 405 (quantitative) Female: 79% Mean age: NR Sampling method: interviews (nonprobabilistic drawn from an existing database); survey (quota drawn from 2 shopping centres)	Face-to-face interviews and shopper-intercept surveys Data collected in 2010–2011	Nutrition literacy: 'can mean the extent to which people access, understand and use nutrition information.' (p. 410)		FOP and BOP	Participants were asked: (1) what the term 'high energy' meant to them and whether it was 'a good or a bad thing', and (2) a true/false question: 'kilojoules and calories measure the same thing'; and if kilojoules measure (i) sugar content only; (ii) fat content only; or (iii) the energy content of food.' (p. 411)	Participants were asked about situations when they looked for a product high or low in energy	'Understanding of kilojoules and calories was generally poor.' (p. 413–414) 'When asked how they would determine if a product was high in energy, almost half (15/34) mentioned they would look at the panel on the back of the product, and some would rely on claims about energy on the front of the pack (8/34).' (p. 415)
Wilson (2010) [17] Australia (Melbourne)	'To explore African migrant communities living in North-West Melbourne, Australia, Conceptualise and interpret the Australian food system from an intergenerational perspective and how this impacts on their attitudes and beliefs about food in Australia' (p. 969)	*N* = 15 adolescents and 25 parents – African migrants Female: 66.7% (youth), 77.7% (parents) – interviews Mean age: 15.6 years (youth), 37.3 years (parents) – interviews Sampling method: nonprobabilistic	Exploratory qualitative research design Data collected in 2007–2008	Literacy – no definition provided	Literacy emerged as a theme during analysis of qualitative data	FOP and BOP (labels illustrating health claims and nutrition information)	Not measured quantitatively	Not measured quantitatively	Reading and understanding food labels difficult for African migrants with limited English and literacy

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Katz (2011) (12) United States (Missouri)	'To evaluate the effects of a nutrition education programme designed to teach elementary school students and their parents, and to distinguish between more healthful and less healthful choices in diverse food categories.' (p. 21)	N = 1180 elementary students (n = 628 intervention, n = 552 control) and their parents Female: 50.3% intervention, 52.2% control Mean age: NR Sampling method: nonprobabilistic	Students were recruited from 5 elementary schools, matched 'based on their demographic characteristics and then randomly assigned to intervention or control group' (p. 22) Data collected in 2007–2008 Nutrition Detectives program: sought to educate students 'regarding the selection of healthful foods' (i.e. 'minimally processed and close to nature; relatively high in intrinsic nutrients as compared to calories; relatively low in added sugars and trans-fat; and relatively rich in desirable constituents, such as fiber.') (p. 22)	Nutrition and food label literacy mentioned – no definitions provided but captured under the heading of nutrition knowledge	Food Label Quiz: 'The test instrument consists of 10 questions, with 10 representing the highest possible score on the test. Each question asks students to select which is the more healthful of the 2 food products based on the Nutrition Facts panel and ingredient list for each food product.' (p. 23) assessed pre- and post- intervention	BOP (nutrition facts panel and ingredient list)		Measure of food label literacy and nutrition-related knowledge assessed participant's use of information for making food choices	'... students' nutrition knowledge improved significantly (18.1% ± 26.9; $P < 0.01$) compared to baseline. Students in grade 3 showed the greatest improvement of nutrition knowledge compared to baseline among the 3 grade levels (23.3% ± 26.1). The parents of the students in the intervention group also showed statistically significant improvement in their nutrition knowledge compared to baseline... (7.9% ± 19.9; $P < 0.01$).' (p. 24) '... a booster session significantly improved the students' nutrition knowledge from the initial session (18.1% ± 28.1; $P < 0.01$)' (p. 24)

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Katz (2014) [13] United States	'To investigate the effectiveness of a 45 minute version of the programme [Nutrition Detectives].' (p. 1)	*N* = 212 5th grade students from five schools Female: 58% Mean age: NR Sampling method: nonprobabilistic	Pre-post Data collected in 2010 Intervention: 'Nutrition Detectives conveys the idea of a link between food choices and health, in addition to information on how and what nutritious foods to choose.' (p. 2)	Food label literacy	Food Label Quiz described in Katz (2011) study assessed pre- and post- intervention	BOP (nutrition facts panel and ingredient list)		Measure of food label literacy and nutrition-related knowledge assessed participant's use of information for making food choices	'... a significant improvement overall (all 5 schools combined) of 16.2 percentage points was observed in students' scores ($P < 0.001$) on the quiz between pretest and post-test measures ... Girls' scores improved significantly more than boys' scores ($P = 0.04$)' (p. e4)
Hindin (2004) [20] United States (New York)	'To evaluate whether a media literacy nutrition education curriculum about the effects of television advertising on children's food choices influenced the behavior, attitudes, and knowledge of Head Start parents.' (p. 192)	*N* = 35 parents of children aged 3–6 years Female: 97% Mean age: NR Sampling method: Nonprobabilistic	'Pretest–post-test, comparison condition and intervention condition design' (p. 193) Data collected in 2000.' The same sample first participated in a four-week comparison condition involving a food safety nutrition education curriculum and, immediately following, a four-week intervention condition involving a media-based nutrition education curriculum designed for the study.' (p. 193)	Media literacy: not defined, but participants were provided with media literacy nutrition education intervention		NR		Knowledge scale assessed 'parents' ability to read labels to distinguish between claims and truths in television commercials' (p. 194) – administered at three points in time (baseline, comparison condition, post-intervention)	Programme had significant effects in terms of parents' understanding of, and ability to read, food labels at post-intervention ($P < 0.001$)

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Balance & Webb (2015) [22] United States (Georgia)	'To answer: Could a programme on nutrition be designed that teachers and directors would embrace and that would result in healthier choices at the center?' (p. 3)	*N* = 11 teachers of children from 6 weeks through preschool age Female: NR Mean age: NR Sampling method: nonprobabilistic	Pretest-post-test Data collected in 2013–2014 Intervention: five 60 minutes sessions on topics taught by a dietitian or librarian (i.e. use of iPad, apps, and Medline Plus; adult nutrition; child and infant nutrition) One session was on 'reading nutrition labels, defining and identifying whole wheat and products containing whole grains' (p. 5)	Health literacy – no definition provided	Five multiple choice questions admini-stered pre-and post-intervention	NR	NR		'The teachers indicated they gained increased personal understanding of formerly confusing nutrition issues (e.g. how to read a nutrition label and what defines a whole grain)' (p. 8)
Hess (2012) [21] Switzerland (German-speaking part)	'To a comprehensive model of food label use consisting of sociodemo-graphic, health-related and motivating variables' (p. 407)	*N* = 1013 Female: 55% Mean age: 53.5 years Sampling method: probability	Cross-sectional mail survey Data collected in 2009	Numeracy – definition not provided	1 item from the Subjective Numeracy Scale: 'How good are you at working with percentages?' (p. 410) Assessed on a Likert scale: 1 = not at all good to 6 = very good (p. 410)	NR		Self-reported label used assessed by asking: 'How often do you use labels when you buy a product for the first time?' (p. 410) Respondents assessed self-reported label use using a Likert scale: 1 = never to 6 = very often	Numeracy was positively associated with self-reported food label use ($\beta = 0.114$, $P < 0.001$) after socio-demographic, health-related, and motivational variables were controlled for

Table 2. Continued

First author (year), country	Purpose	Sample (not reported)	Study design and year data collected (not reported)	Definition(s) of health literacy, literacy, and numeracy	of health literacy, literacy, and numeracy	Type of label(s) examined: FOP, BOP, NR (not reported)	Measure(s) of nutrition label under-standing (subjective, objective)	Measure(s) of nutrition label use	Relevant findings
Visschers (2010) [14] Switzerland	'To investigate how fat reduction in foods affects people's food choices' (p. 730)	*N* = 80 Female: 51% Mean age: 42 years Sampling method: probabilistic	Within-subjects design Year data collected NR 'Respondents received two nutrition tables for two crisps products and had to indicate which of these products or neither of them they wanted to buy for themselves. This procedure was repeated twice (Choices 2 and 3). In each following choice, the fat levels of crisps were reduced. The instructions mentioned that both products contained less fat due to production changes' (p. 731). Similar procedure used for a yoghurt product	Numeracy – definition not provided	Eight items from the Subjective Numeracy Scale	BOP (Nutrition tables)		Use of information about grams of fat to make product choices about crisps and yogurts	*Crisps:* When asked to choose between crisps with more and less fat, participants lower in numeracy were more likely to choose crisps that were higher in fat than participants with higher numeracy ($P = 0.02$) This difference did not persist when both groups asked to choose between zero fat and reduced fat crisps. *Yoghurt:* When fat content was adjusted, consumers with lower and higher numeracy made similar choices

%DV, percent daily value; BOP, back-of-pack; FOP, front-of-pack; NR, not reported; NVS, Newest Vital Sign.

Assessment of nutrition label understanding and use

Table 2 (column 9) summarises the ways in which nutrition label use was assessed in studies included in this review. Different self-report measures of nutrition label use were used in six studies [5,15,16,19,21,23]. Eye-tracking technology was employed in two studies to assess the length of time participants' eyes fixated on different areas of a nutrition label [9,18]. Six studies evaluated participants' use of nutrition label information for rating the healthfulness of food products [11,12], evaluating claims made about foods [21] and making food choices [12–14]. Participants' objective understanding of nutrition labels was measured in two studies [10,16]. Subjective reports of understanding were provided in two studies [17,22]. The instruments used to assess nutrition label understanding in studies are listed Table 2 (column 8).

Empirical relationships examined between health literacy, nutrition literacy, literacy and nutrition label understanding

The findings from the studies that examined empirical relationships between health literacy, nutrition literacy, literacy and nutrition label understanding are summarised in Table 2 (column 10). Limited literacy was found, in one Australian study, to make the reading and understanding of food labels difficult for adolescent and adult African migrants [17]. Nutrition literacy appeared to be equated with nutrition label understanding in an Australian study [16]. In one American study, a multimedia intervention to improve the comprehension of food labels had a positive effect on American consumers with adequate health literacy, although it had no effect on those with limited health literacy [10]. In the remaining study, a nutrition programme for teachers and directors was found to increase teachers' understanding of nutrition label information that was previously found to be confusing [22].

Empirical relationships between health literacy, literacy, numeracy and nutrition label use

The empirical relationships examined between health literacy, literacy, numeracy and nutrition label use are summarised in Table 2(column 7). A review of Table 2 reveals variable, and in some cases contradictory, findings with regard to relationships between health literacy and nutrition label use. A positive relationship was found between health literacy and nutrition label use in one Canadian study [5]. An inverse relationship between health literacy and nutrition label use was observed in one Singaporean study [23]. In one American study, health literacy was significantly associated with the use of some information on the nutrition label, but not other information [9]. No relationships were observed between health literacy and nutrition label use in two American studies [15,19]. In another American study, participants with high and low literacy were more likely to use FOP labels than BOP labels when aisle signage describing FOP labels was present. However, there was no difference in the use of FOP and BOP labels by participants with high and low health literacy when aisle signage was not present [18].

Studies examining relationships between other literacies and nutrition label use were more consistent in terms of their findings. Consumers with higher levels of literacy were significantly better at using nutrition information to judge the healthfulness of food products than those with lower levels of literacy in one study [11]. Consumers with lower and medium levels of literacy were also found to benefit (and significantly more so than consumers with higher levels of literacy) from graphic formats (e.g. rating, range) of calorie, fat and carbohydrate information. Two studies found that a nutrition and food label literacy intervention significantly increased the food label literacy scores of both elementary grade students and their parents [12,13], with girls achieving greater gains in scores than boys in one [13]. A media literacy education intervention was also found to significantly increase the ability of parents to use information on foods to evaluate claims made about them on television [20].

Only two studies included in the present review examined empirical relationships between numeracy and nutrition label use. In one of these studies, numeracy was positively associated with self-reported label use after controlling for socio-demographic, health-related and motivational variables in one study [21]. In the other study, participants with lower numeracy differed from participants with higher numeracy on some food choices (but not others) when the fat content of two food products was adjusted [14].

Discussion

This scoping review aimed to determine what is known from the existing literature about the empirical relationships between health literacy, literacy or numeracy and the understanding and use of nutrition labels. The inconsistent, and sometimes contradictory, relationships observed between health literacy and nutrition label understanding and use may be attributable to a number of factors, including (but not limited to) differences in study design, sampling method and sample characteristics (i.e. age, sex), a lack of consistency in the measurement of health literacy and nutrition label understanding and use across studies, and differences in the nutrition label

information examined (i.e. BOP and FOP). A lack of independence may pose another problem. By definition, health literacy includes the ability to read (or comprehend) and use information that should, in theory, be positively correlated with nutrition label understanding and use. The fact that one study [16] included in this review appeared to equate health literacy with nutrition label understanding partially supports this hypothesis and may explain the dearth of research in this topic area. We chose to include, rather than exclude, studies that assessed health literacy because a lack of agreement exists over its meaning and measure [7]. Some measures of health literacy may thus be appropriate in studies, whereas others may not. Furthermore, the scoping review framework [8] approach that we adopted does not include an assessment of study quality. The use of the framework, however, does not prevent us from drawing attention to measurement-related problems for the purpose of informing future research in this topic area.

The reliance on self-reports of nutrition label in studies may also explain the variability in the relationships observed. Self-report measures are susceptible to social desirability [27] and recall [28] bias, and this was identified as a limitation in one study [16]. The use of eye-tracking technology may help to overcome limitations associated with the use of self-report data; particularly, if eye-tracking technology is able to validate self-report data.

Studies examining relationships between literacy, nutrition literacy, nutrition and food label literacy, food label literacy, media literacy, numeracy and nutrition label understanding and use were more consistent in terms of their findings. As a group, these studies suggest that literacy and numeracy influence the ways in which consumers both understand and use nutrition label information, with higher levels leading to a greater understanding.

The use of nutrition labels by consumers with low levels health literacy [23] suggests that, although efforts to raise consumers' awareness and use of nutrition labels may be working, problems related to comprehension may be more difficult to address. To this end, some of the literacy interventions included in this review show promise [11–13,20,22] for increasing nutrition label understanding and use, although further studies are needed to determine the extent to which their findings are generalisable to other contexts. The effect(s) of these interventions on both short- and long-term nutrition-related behaviour(s) and health outcomes should be examined using research designs that allow for causal inferences to be drawn, or when controlling for potentially confounding variables. Research is also needed to identify, and address, the reason(s) for disparities in intervention outcomes, such as when females benefit more than males [13] and when persons with adequate health literacy benefit more than those with limited health literacy [10].

Strengths and Limitations

The strengths of this review include the use of the Arksey and O'Malley [8] scoping review framework, the identification and search of relevant databases by a librarian, the screening of all articles for relevance by both investigators, and the charting of data by both investigators. The exclusion of studies not published in French or English is a limitation.

Conclusions

Empirical relationships between health literacy, literacy, numeracy and nutrition label understanding and use have not been well-studied. This finding may be explained by the fact that health literacy and nutrition label understanding and use may, in some studies, be overlapping rather than independent variables, particularly if the Newest Vital Sign is used to assess health literacy. Careful attention is needed to this issue in future studies, as well as the limitations associated with the use of self-report data. Disparities arising from the implementation of nutrition label-related interventions that confer greater benefits to females and those with adequate health literacy must also be addressed in research, policy and practice.

Acknowledgments

The authors would like to thank Melanie Weger, Reference Librarian at Health Canada, for her assistance in searching multiple databases for this review.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
This research was supported by a Canadian Institutes of Health Research Science Policy Fellowship awarded to Leslie J. Malloy-Weir.
LMW conceived the study, charted and analysed the data, and wrote the manuscript. MC charted and analysed the data and contributed to the writing of the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. European Food Information Council (2015) *Global Update on Nutrition Labelling*. Available at: http://www.eufic.org/upl/1/default/doc/GlobalUpdateExecSumJan2015.pdf (accessed February 2016).
2. National Collaborating Centre for Healthy Public Policy (2011) *Public Policies on Nutrition Labelling: Effects and Implementation Issues – A Knowledge Synthesis – Highlights*. Available at: https://www.inspq.qc.ca/pdf/publications/1256_ApplicationPolPubliquesAffichageNutrition_Highlights.pdf (accessed February 2016).
3. Baltas G (1999) Nutrition labelling: issues and policies. *Eur J Mark* **5/6**, 708–721.
4. Public Health Agency of Canada (2014) *About Health Literacy*. Available at: http://www.phac-aspc.gc.ca/cd-mc/hl-ls/index-eng.php (accessed October 2014).
5. Sinclair S, Hammond D & Goodman S (2013) Sociodemographic differences in the comprehension of nutritional labels on food products. *J Nutr Educ Behav* **45**, 767–772.
6. Carbone ET & Zoellner JM (2012) Nutrition and health literacy: a systematic review to inform nutrition research and practice. *J Acad Nutr Diet* **112**, 254–265.
7. Baker DW (2006) The meaning and measure of health literacy. *J Gen Intern Med* **21**, 878–883.
8. Arksey H & O'Malley L (2005) Scoping studies: towards a methodological framework. *Int J Soc Res Methodol* **8**, 19–32.
9. Mackert M, Champlin SE, Pasch KE *et al.* (2013) Understanding health literacy measurement through eye tracking. *J Health Commun* **18**, 185–196.
10. Jay M, Adams J, Herring SJ *et al.* (2009) A randomized trial of a brief multimedia intervention to improve comprehension of food labels. *Prev Med* **48**, 25–31.
11. Viswanathan M, Hastak M & Gau R (2009) Understanding and facilitating the usage of nutrition labels by low-literate consumers. *J Public Policy Market* **28**, 135–145.
12. Katz DL, Katz CS, Treu JA *et al.* (2011) Teaching healthful food choices to elementary school students and their parents: the nutrition detectivesTM program. *J Sch Health* **81**, 21–28.
13. Katz DL, Treu CS, Ayettey RG *et al.* (2014) Testing the effectiveness of an abbreviated version of the nutrition detectives program. *Prev Chronic Dis* **11**, 130161.
14. Visschers VH & Siegrist M (2010) When reduced fat increases preference. How fat reduction in nutrition tables and numeracy skills affect food choices. *Appetite* **55**, 730–733.
15. Speirs KE, Messina LA, Munger AL *et al.* (2012) Health literacy and nutrition behaviors among low-income adults. *J Health Care Poor Underserved* **23**, 1082–1091.
16. Watson, WL, Chapman, K, King, L *et al.* (2012) How well do Australian shoppers understand energy terms on food labels? *Public Health Nutr* **16**, 409–417.
17. Wilson A, Renzaho AM, McCabe M *et al.* (2010) Towards understanding the new food environment for refugees from the Horn of Africa in Australia. *Health Place* **16**, 969–976.
18. Graham DJ, Heidrick C & Hodgin K (2015) Nutrition label viewing during a food-selection task: front-of-package labels vs nutrition facts labels. *J Acad Nutr Diet* **11**, 1636–1646.
19. Cha E, Kim KH, Lerner HM *et al.* (2014) Health literacy, self-efficacy, food label use, and diet in young adults. *Am J Health Behav* **38**, 331–339.
20. Hindin TJ, Contento IR & Gussow JD (2004) A media literacy nutrition education curriculum for head start parents about their effects of television advertising on their children's food requests. *J Am Diet Assoc* **104**, 192–198.
21. Hess R, Visschers V & Siegrist M (2012) The role of health-related, motivational and sociodemographic aspects in predicting food label use: a comprehensive study. *Public Health Nutr* **15**, 407–414.
22. Balance D & Webb N (2015) For the mouths of babes: nutrition literacy outreach to a child care center. *J Consum Health Internet* **19**, 1–12.
23. Vijaykumar S, Lwin MO, Chao J *et al.* (2013) Determinants of food label use among supermarket shoppers: a Singaporean perspective. *J Nutr Educ Behav* **45**, 204–212.
24. Weiss BD, Mays MZ, Martz W *et al.* (2005) Quick assessment of literacy in primary care: the newest vital sign. *Ann Fam Med* **3**, 514–522.
25. Baker DW, Williams MV, Parker RM *et al.* (1999) Development of a brief test to measure functional health literacy. *Patient Educ Couns* **38**, 33–42.
26. Fagerlin A, Zikmund-Fisher BJ, Ubel PA *et al.* (2007) Measuring numeracy without a math test: development of the subjective numeracy scale. *Med Decis Making* **27**, 672–680.
27. van de Mortel TF (2008) Faking it: social desirability response bias in self-report research. *Aust J Adv Nurs* **25**, 40–48.
28. Hassan E (2006) Recall bias can be a threat to retrospective and prospective research designs. *Internet J Epidemiol* **3**, 4.

Appendix 1
Search terms used in eight electronic databases on 15 April 2014 and 26 May 2016
Database(s): Embase 1974 to 2014 April 15

Search strategy:

#	Searches
1	exp food packaging/
2	((food* adj3 (label* or packag*)) or (nutrition* adj2 facts* adj2 (table* or label* or packag* or panel*)) or (nutrition* adj3 (facts*or table* or label* or packag* or panel*))).tw.
3	exp health literacy/ or ((health* or food* or nutrit*) adj3 (litera* or numerac*)).tw.
4	(1 or 2) and 3
5	exp cognition/ or exp perception/ or exp decision making/
6	exp consumer/ or exp consumer attitude/ or exp consumer health information/
7	(1 or 2) and 5 and 6
8	exp health education/
9	exp information/ or exp nutrient content/ or exp nutritional value/ or exp public health/ or exp nutritional status/ or exp nutritional parameters/
10	(1 or 2) and (5 or 6) and 8 and 9
11	4 or 7 or 10
12	limit 11 to (yr=2003-2014 and (english or french))

Database(s): Ovid MEDLINE(R) In-Process and Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) 1946 to Present.

Search strategy:

#	Searches
1	exp Food Packaging/
2	((food* adj3 (label* or packag*)) or (nutrition* adj2 facts* adj2 (table* or label* or packag* or panel*)) or (nutrition* adj3 (facts*or table* or label* or packag* or panel*))).tw.

Appendix 1. Continued

#	Searches
3	exp health literacy/ or ((health* or food* or nutrit*) adj3 (litera* or numerac*)).tw.
4	(1 or 2) and 3
5	exp Cognition/ or exp perception/ or exp decision making/
6	exp Consumer Participation/ or consumer*.tw.
7	(1 or 2) and 5 and 6
8	exp health education/ or exp Health Knowledge, Attitudes, Practice/
9	(1 or 2) and 6 and 8
10	4 or 7 or 9
11	limit 10 to (yr=2003-2014 and (english or french))

Database(s): CAB Abstracts 1973 to 2014 Week 14, Econlit 1886 to March 2014, Food Science and Technology Abstracts 1969 to 2014 April Week 2, Global Health 1973 to 2014 Week 14, PsycINFO 1806 to April Week 3 2014, Social Policy and Practice 201401.

Search strategy:

#	Searches
1	((food* adj3 (label* or packag*)) or (nutrition* adj2 facts* adj2 (table* or label* or packag* or panel*)) or (nutrition* adj3 (facts*or table* or label* or packag* or panel*))).tw.
2	((health* or food* or nutrit*) adj3 (litera* or numerac*)).tw.
3	1 and 2
4	limit 3 to (yr=2003-2014 and (english or french)) [Limit not valid in Social Policy and Practice; records were retained]
5	remove duplicates from 4

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

PUBLIC HEALTH NUTRITION

An evaluation of diabetes targeted apps for Android smartphone in relation to behaviour change techniques

C. D. Hoppe,[1,2] J. E. Cade[1] & M. Carter[1]

[1]Nutritional Epidemiology Group, School of Food Science and Nutrition, University of Leeds, Leeds, UK
[2]Department of Nutrition and Dietetics, King's College London, London, UK

Keywords
behaviour change techniques, diabetes, mobile apps, smartphone, nutritional epidemiology.

Correspondence
J. E. Cade, Nutritional Epidemiology Group, School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK.
Tel.: +44 (0)113 343 6946
Fax: +44 (0)113 343 2982
E-mail: j.e.cade@leeds.ac.uk

How to cite this article
Hoppe C.D., Cade J.E. & Carter M. (2017) An evaluation of diabetes targeted apps for Android smartphone in relation to behaviour change techniques. *J Hum Nutr Diet*. **30**, 326–338
doi:10.1111/jhn.12424

Abstract

Background: Mobile applications (apps) could support diabetes management through dietary, weight and blood glucose self-monitoring, as well as by promoting behaviour change. The present study aimed to evaluate diabetes apps for content, functions and behaviour change techniques (BCTs).
Methods: Diabetes self-management apps for Android smartphones were searched for on the Google Play Store. Ten apps each from the following search terms were included; 'diabetes', 'diabetes type 1', 'diabetes type 2', 'gestational diabetes'. Apps were evaluated by being scored according to their number of functions and BCTs, price, and user rating.
Results: The mean (SD) number of functions was 8.9 (5.9) out of a possible maximum of 27. Furthermore, the mean (SD) number of BCTs was 4.4 (2.6) out of a possible maximum of 26. Apps with optimum BCT had significantly more functions [13.8; 95% confidence interval (CI) = 11.9–15.9] than apps that did not (4.7; 95% CI = 3.2–6.2; $P < 0.01$) and significantly more BCTs (5.8; 95% CI = 4.8–7.0) than apps without (3.1; 95% CI = 2.2–4.1; $P < 0.01$). Additionally, apps with optimum BCT also cost more than other apps. In the adjusted models, highly rated apps had an average of 4.8 (95% CI = 0.9–8.7; $P = 0.02$) more functions than lower rated apps.
Conclusions: 'Diabetes apps' include few functions or BCTs compared to the maximum score possible. Apps with optimum BCTs could indicate higher quality. App developers should consider including both specific functions and BCTs in 'diabetes apps' to make them more helpful. More research is needed to understand the components of an effective app for people with diabetes.

Introduction

Diabetes mellitus is becoming increasingly prevalent worldwide. Currently, 387 million people are diagnosed with diabetes, representing 8.3% of the global population [(1)], and this prevalence is expected to rise to 592 million by the year 2035. Affected individuals have to manage diabetes for the rest of their lives. A number of long-term complications are associated with diabetes [(2)], and the effective control of blood pressure and blood glucose reduces the risk of both macrovascular and microvascular diseases [(3–5)]. It is therefore important to carefully manage the disease to minimise its impact on morbidity and mortality.

In 2015, 76% of the UK population owned a smartphone [(6)] and it is predicted that, by 2017, 2.5 billion people worldwide will own a smartphone [(7)]. Smartphones therefore have the potential to be used to manage disease using 'mHealth' (mobile health) applications [(8)]. There were over 6000 medical apps available on the Android market in 2013 [(9)] and this has subsequently almost quadrupled to 23 000 apps [(10)]. Many apps aim to support self-management for people with diabetes; however, although mHealth apps may benefit people suffering

from chronic disease, there are also problems associated with them. These problems include lack of evidence on clinical effectiveness, lack of integration into the health care system and potential threats to safety [9]. A recent study found that health apps in the UK NHS Health Apps Library had poor compliance with data protection principles [11]. For an app to be recommended to patients by health professionals, its effectiveness should be scientifically proven. Most apps do not have a strong evidence base demonstrating their effectiveness. The US Food and Drug Administration (FDA) defined a mobile app to be a medical device if it was intended to diagnose, cure, mitigate, treat or prevent a disease [12], needing FDA approval before being released to the market. Unapproved apps could lead to adverse health effects if users replaced a visit to the doctor by consulting an app [9].

There is substantial research investigating new technology in the use of managing disease [13]. However, in relation to diabetes-linked conditions, these are mostly focused on weight loss, and focus on web-based programmes rather than mobile apps [14,15]. Additionally, these studies have not looked at BCTs, but rather measure body mass index or body weight as outcomes. Although these are appropriate outcomes to measure effectiveness of diabetes management interventions, it is also important to understand which BCTs are promoting effective behaviour change. Some diabetes management apps have been evaluated, although these were web-based rather than mobile app-based [16,17] and measure user satisfaction or usability [18,19] rather than BCTs. A qualitative study on the usability of apps for weight loss [20] concluded that app designers should employ BCTs to improve effectiveness. Furthermore, a Cochrane review [21] investigated which computer-based intervention would be most effective at improving HbA1c levels in adults with diabetes, and found that mobile apps were more effective than computer programmes used in hospitals or at home. It was considered that this was a result of the inclusion of control theory techniques such as self-regulation.

Twenty-six distinct, theory-linked BCTs have been described and tested [22]. BCTs are theory-based methods to facilitate change in individuals, and examples include 'Prompt intention formation' and 'Model or demonstrate behaviour', which could be incorporated into mobile apps. A meta-analysis [23] was undertaken to assess the effectiveness of these 26 BCTs in promoting physical activity and healthy eating. It found that interventions combining self-monitoring with at least one other technique derived from control theory were significantly more effective than the other interventions. The present study aimed to evaluate Android apps for people with diabetes in terms of which functions they included and which BCTs they employed to encourage behaviour change. To our knowledge, there is no research assessing the inclusion of BCTs in interventions used in diabetes mobile apps. This research could provide a basis for improving 'diabetes apps' in the future.

Methods

App selection

Google Play Store (UK) for Android was used as a database to search for relevant apps on 27 October 2014. Because there is no existing appropriate category, these specific search terms were used: 'diabetes', 'diabetes type 1', 'diabetes type 2' and 'gestational diabetes'. The apps were initially prescreened for suitability before being downloaded. Inclusion criteria were (i) to be intended for patients with type 1, 2 or gestational diabetes; (ii) to be addressing any aspect of management of diabetes (e.g. blood glucose monitoring, medication, healthy diet); (iii) to have stand-alone functionality (i.e. not requiring membership in a specific programme or website to function); and (iv) to be available in English. The exclusion criteria were (i) to be for self-diagnosis for the user and (ii) to be intended for education of medical personnel. Apps that did not function properly on the test phone (e.g. they would not open or would not advance past the introduction screen) were also excluded). This prescreening was based on the app descriptions and screenshots provided in Google Play Store. The number of medical apps available on Google Play Store is 23 000 [10], with only a small proportion of these of relevance to people with diabetes. The exact number of 'diabetes apps' could not be determined because Google Play Store does not state the number of search results. Each search only shows 200 app results. Because of restraints in time and resources, the number of apps included had to be restricted. The first 10 apps passing the prescreening from each search term were included, giving 40 apps in total. App ranking is partly determined by App Store Optimisation, which, among other aspects, takes into account keyword alignment (i.e. how the user's search term matches with words in the app title and description) and app performance (e.g. app ratings and number of downloads) [24]. An algorithm is used to determine the exact ranking, which is not available to the general public and undergoes continuous change [25]. For the purposes of the present study, it is therefore not possible to determine the total number and ranking of all available diabetes-related apps.

Following identification, the apps were downloaded and evaluated again based on the same inclusion and exclusion criteria stated above. At this point, some of the apps were excluded, and therefore a second stage of searches and screenings was performed to meet the study aim of evaluating 40 apps, 10 from each search term

(Fig. 1). This second search was performed on 9 June 2015. Five apps were independently evaluated by another assessor to determine the repeatability and relative validity of the assessments.

App testing

Each app that met the inclusion and exclusion criteria was used by one of the investigators (CH) to identify the functions and BCTs included. The results were recorded in a data extraction form (Table 1) recording the functions and BCTs included in each app. A possible 27 functions were categorised into 'Provision of information', 'Allows self-recording', 'Generates output from self-recording', 'Data management' and 'Other'. The 26 BCTs identified by Michie and Abraham [22], were categorised into 'Motivation enhancing', 'Planning and preparation' and 'Goal striving and persistence' (a list of these is provided in Figs 2 and 3). Therefore, a maximum score of 53 could be obtained by each app. Each app was downloaded immediately before assessment using the investigator's private mobile phone. The majority of apps were evaluated between the 3 November and 10 December 2014, and apps identified in the second search stage were evaluated between the 9 June and 15 June 2015. Some apps had data collection functions, such as recording blood glucose readings or food intake, and, where this was the case, they were used for 2 days to enable sufficient data for graph generation. Apps that did not have data collection functions were explored to extract information on all other functions and BCTs present.

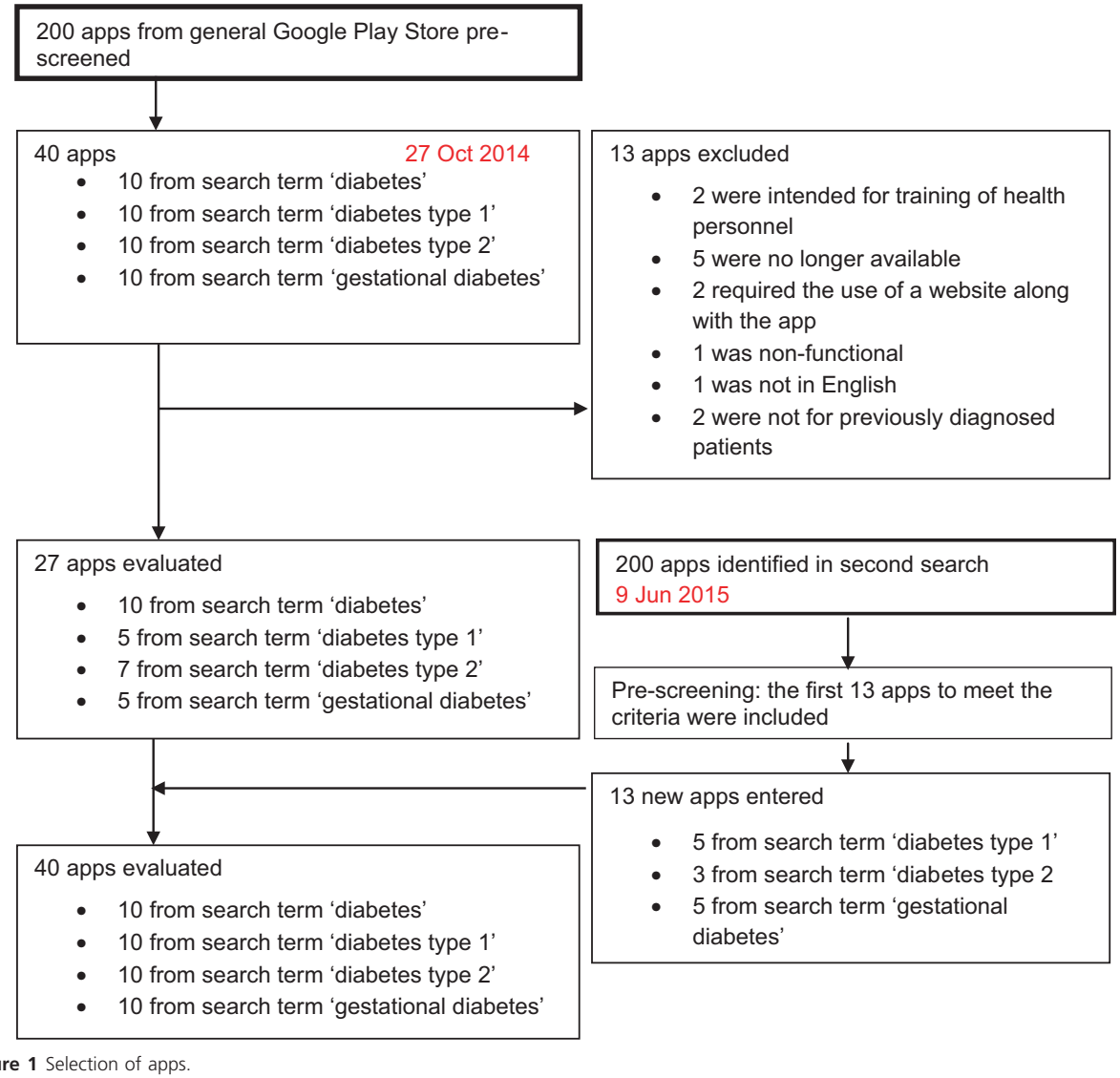

Figure 1 Selection of apps.

Table 1 Data extraction form

General
Name of app
Version
Developer
Last updated
Number of downloads
Price (currency)
Average number of stars given by users (user rating)
Aimed at (diabetes type 1,2, gestational, or any)

Functions of app	Yes (= 1)	No (= 0)	Comments
Provision of information			
General information about the condition			
Recipes for meals that are beneficial to blood glucose level			
Glycaemic index of foods			
Allows self-recording			
Enter blood glucose values			
Enter HbA1c values			
Enter height			
Enter weight			
Enter other values (e.g. blood lipids, blood pressure, etc.)			
Enter carbohydrates consumed (manually)			
Enter food consumed (from database)			
Activity diary			
Enter how much medication taken			
Generates output from self-recording			
Creates graph of blood glucose over time			
Creates graph of HbA1c over time			
Calculates body mass index			
Generate table of nutrients consumed			
Calculate how much insulin is needed following a meal			
Provides feedback (on any values entered)			
Data management			
Technological additional feature: connect glucose meter to Smartphone to transfer data			
Export data to Smartphone/ computer			
Medium to communicate with general practitoner/send data			
Other			
Reminders to measure blood glucose			
Other reminders (e.g. check feet, attend doctor's appointment, etc.)			

Table 1. Continued

Functions of app	Yes (= 1)	No (= 0)	Comments
Provides general encouragement to adhere to new lifestyle			
Link to social media			
Set target ranges for any data			
Any other (describe)			
Sum (/27)			

Behaviour change techniques Do the functions of the app include any of the 26 behaviour change techniques developed by Abraham and Michie [22]?	Yes (= 1)	No (= 0)
Motivation enhancing		
Provide information about behaviour-health link		
Provide information on consequences		
Motivational interviewing		
Prompt intention formation		
Provide information about others' approval		
Planning and preparation		
Provide instruction		
Provide opportunities for social comparison		
Prompt specific goal setting		
Plan social support or social change		
Set graded tasks		
Model or demonstrate behaviour		
Prompt barrier identification		
Time management		
Agree on behavioural contract		
Prompt identification as a role model		
Goal striving and persistence		
Prompt self-monitoring of behaviour		
Provide feedback on performance		
Provide general encouragement		
Teach to use prompts or cues		
Prompt practice		
Provide contingent rewards		
Prompt review of behavioural goals		
Use follow-up prompts		
Prompt self-talk		
Relapse prevention		
Stress management		
Sum (/26)		

Which tasks have been performed on the app? Tasks performed	Yes: tick	Description/output
Entered blood glucose values		
Entered blood HbA1c values		
Entered other values (e.g. blood lipids, blood pressure, etc.)		
Entered weight		
Entered height		
Entered food intake		
Entered physical activity		
Entered medication taken		

Based on the meta-analysis by Michie *et al.* [23], the most effective combination of BCTs is 'Prompt self-monitoring of behaviour' in combination with at least one of four other self-regulatory techniques: 'Prompt intention formation', 'Prompt specific goal setting', 'Provide feedback on performance' and 'Prompt review of behavioural goals'. This was evaluated as 'optimum BCT' in the present study'.

Statistical analysis

The results were analysed using the statistical software STATA/IC, version 13.1 (Stata Corp, College Station, TX, USA). A *t*-test was performed to assess the difference in mean number of functions, number of BCTs, overall score, price and user rating according to inclusion of 'optimum BCT', price (free or paid) and user rating. For the latter, user rating, normally ranging from one to five, was divided into two groups: low = 1.0–4.0 and high = 4.1–5.0. The uneven division of user rating was a result of the average app rating for the majority of apps being greater than 4. Regression was performed to determine whether there was a relationship between number of functions, number of BCTs and overall score versus price (£) and user rating. Regression models for price adjusted for user rating and vice versa. Cohen's kappa was calculated to determine the inter-rater reliability from the duplicate extracted data.

Results

App selection

The initial prescreening gave a list of 40 apps to be further evaluated for eligibility. Of these, 13 apps were excluded because of nonconformity with inclusion criteria (Fig. 1). The excluded apps were either intended for training of health personnel ($n = 2$), no longer available at the point of download ($n = 5$), required the use of a website along with the app ($n = 2$), nonfunctional ($n = 1$), not in English ($n = 1$) or not for previously diagnosed patients ($n = 2$). This initially gave 27 apps to be included in the present study. However, to improve the generalisability of the study, 13 further apps were added from a repeated search to give 40 apps in total. These were individually prescreened before inclusion.

App testing

Based on overall score (i.e. the sum of number of functions and BCTs), *Diabetes Tracker* by *Mig Super, Diabetes:*

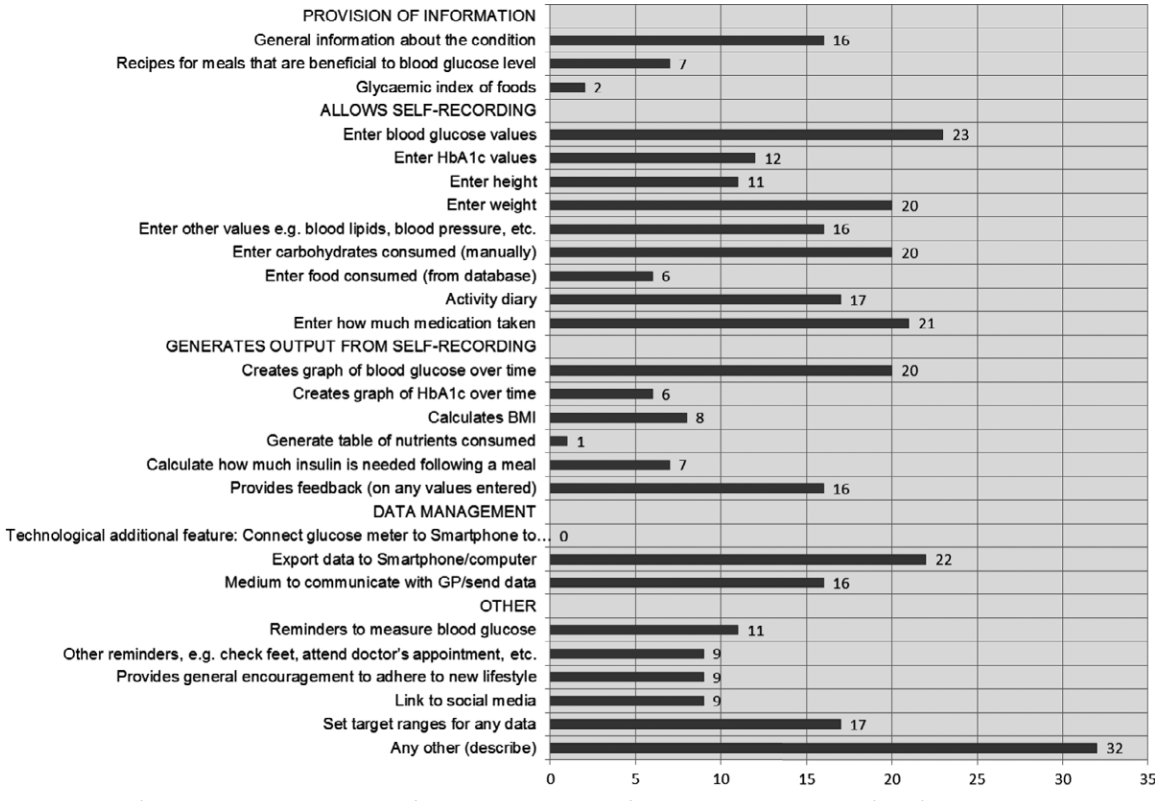

Figure 2 Functions of apps, as well as the number of the 40 apps with each function. A list is provided of the functions examined in the present study, organised into five subgroups. The bars show how many apps contained each function.

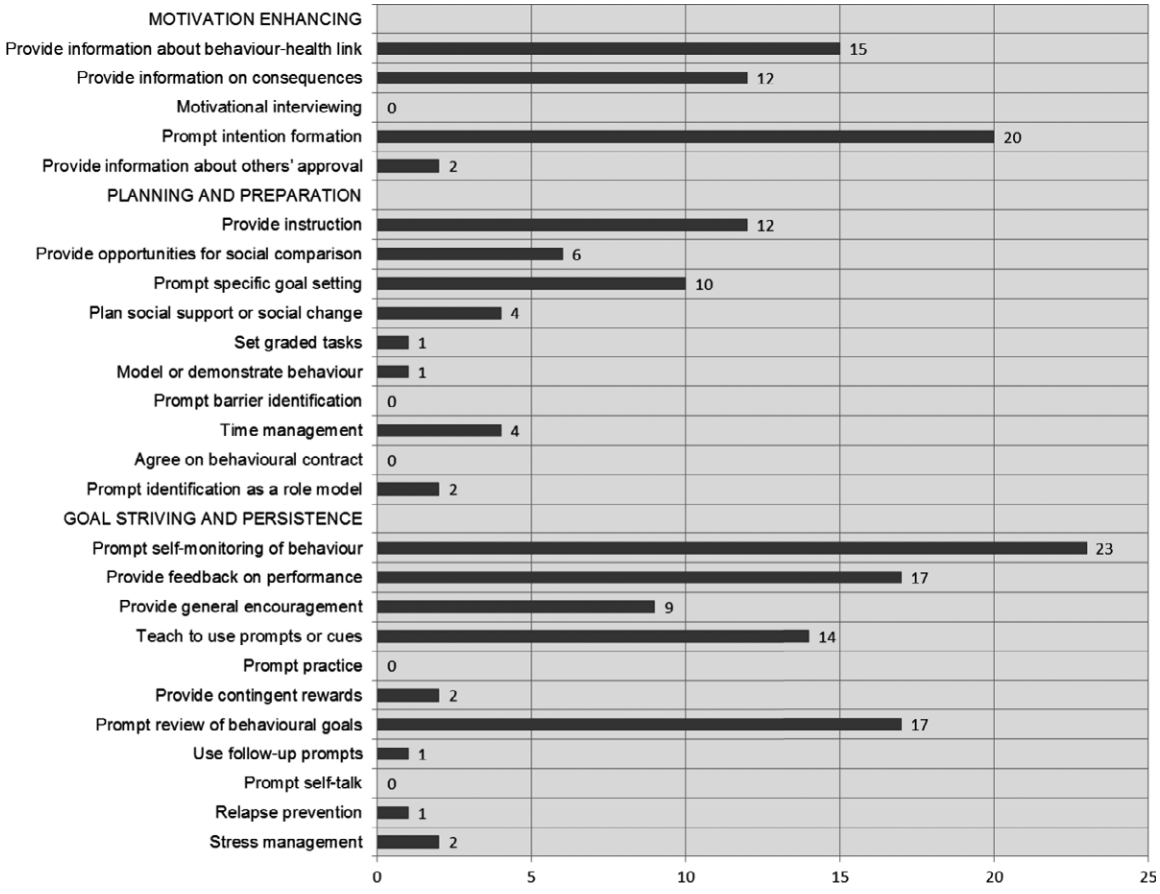

Figure 3 Behaviour change techniques (BCTs) as identified by Michie and Abraham [22]; number of the 40 apps with each BCT. A list is provided of the BCTs examined in the present study, as identified by Michie and Abraham [22] and organised into three subgroups. The bars show how many apps contained each BCT.

M by *Rossen Varbanov* and *Diabetes Companion* by *mySugr GmbH* ranked highest, scoring 29, 27 and 26 out of 53, respectively. These were all apps that offered recording of various physical measures (e.g. blood glucose, weight and height). They all included 'optimum BCT'. The apps scoring lowest overall were *Type 1 Diabetes* by *Colby Taylor*, *Recipes for Diabetes* by *University of Illinois Extension* and *Diabetic Diet Samples* by *Awesomeappcenter LLC*, with scores of 2, 3 and 4 and out of 53, respectively. These apps focused on giving information and advice about the disease and how to manage it. The mean (SD) overall score was 13.2 (7.4) out of 53 (Table 2).

The mean (SD) number of functions included in the apps was 8.9 (5.9) out of 27 (Table 2).

The most common functions were 'Enter blood glucose values' and 'Export data to Smartphone/send data', which were included in 23 and 22 of the apps, respectively. This involved downloading data or graphs to the Smartphone directly; sending it to a specified email address; or uploading it to a cloud based storage system. Other common functions included enter medication; weight; and carbohydrates consumed. Thirty-two out of the 40 apps included 'Any other (describe)', a mixed group of functions including anything that was not included in the rest of the list. These ranged from offering a forum to communicate with other people with diabetes; a game including a point system for performing a beneficial activity; making a shopping list for meals; and information on which McDonald's meals were 'diabetic-friendly', and few were found in more than one app. Only one app included the potential to generate a table of nutrients consumed. None of the apps included 'Technological additional feature: Connect glucose meter to Smartphone to transfer data' (Fig. 2).

The inclusion of BCTs in apps was far less common than the inclusion of functions. The mean (SD) number of BCTs was 4.4 (2.7) out of 26 (Table 2). The most commonly included technique was 'Prompt self-monitoring of behaviour' ($n = 23$) and 'Prompt intention formation' ($n = 20$). These techniques are both among the self-regulatory techniques that were identified as most

Table 2 Relative ranking of the mobile apps according to their total score

Name of app	Developer	Number of functions	Number of behaviour change techniques	Overall sum
Diabetes Tracker	Mig Super	18	11	29
Diabetes:M	Rossen Varbanov	20	7	27
Diabetes Companion	mySugr GmbH	14	12	26
MyDiabetes	Rossen Varbanov	19	5	24
Diabetes PA (Diabetes Manager)	Diabetes Digital Media Ltd	18	6	24
Social Diabetes	Social Diabetes	18	5	23
Glucool Diabetes (Premium)	3qubits	16	5	21
OnTrack Diabetes	Medivo	14	6	20
Carbs & Cals – Diabetes & Diet	Chello Publishing Limited	13	7	20
BG Monitor Diabetes Pro	Gordon Wong	13	6	19
DiaLog: Diabetes Logbook	David Froehlich	13	6	19
SiDiary: Diabetes Management	SINIVO Ltd & Co KG	14	4	18
Diabetes Connect	SquareMed Software GmbH	13	5	18
Glucose Buddy: Diabetes Log	Azumio, Inc.	14	4	18
Diabetes Plus	SquareMed Software GmbH	11	5	16
Diabetes Diary	Jeschua Schang	11	5	16
Diabetes UK Tracker	Diabetes UK	12	3	15
T1DM – Manage Type 1 Diabetes	Dheryta	12	2	14
Diabetes Forum	Diabetes Digital Media Ltd	4	8	12
Diabetes Journal	Suderman Solutions	11	1	12
Diabetes Tools – Glucose	TopAppsFor – Health	10	1	11
Diabetes Recipes Free	Riafy Technologies	5	5	10
Diabetes Forecast®	GTxcel	5	5	10
Diabetes Guide	Avinash Kulkarni	3	6	9
SugarSense – Diabetes App	MedHelp, Inc. – TopHealthApps	6	3	9
Easy Diabetes	EasyMobileApp	5	4	9
Diabetes Forum for Diabetics	moveforward	3	6	9
Gestational Diabetes Disease	Naster Solomon	3	4	7
Diabetes Type 2	Personal Remedies	5	2	7
Diabetes Type 2 Guide	Kool Appz	2	5	7
Diabetes – Glucose Diary	Klimaszewski Szymon	6	1	7
Diabetic Recipes!	NetSummitApps	3	4	7
Diabetic Assistant	Peter Wescott	4	2	6
Audiobook – Diabetes	Twayesh Projects	2	4	6
Pregnant with diabetes	heyworld.dk	2	3	5
Gestational Diabetes	Apps Den	3	2	5
Diabetic Grocery List	LISERE MEDIA LLC	2	3	5
Diabetic Diet Samples	Awesomeappcenter LLC	3	1	4
Recipes for Diabetes	University of Illinois Extension	2	1	3
Type 1 Diabetes	Colby Taylor	2	0	2
Mean (SD)		8.9 (5.9)	4.4 (2.7)	13.2 (7.4)

effective when used in combination with each other [23]. However, fewer apps ($n = 18$) had 'optimum BCT' defined as 'Prompt self-monitoring of behaviour' with at least one other self-regulatory technique (i.e. 'optimum BCT' 'Prompt intention formation', 'Prompt specific goal setting', 'Provide feedback on performance' and 'Prompt review of behavioural goals'). Five BCTs were not used in any of the apps: 'Prompt barrier identification', 'Agree on behavioural contract', 'Prompt practice', 'Prompt self-talk' and 'Motivational interviewing' (Fig. 3).

App characteristics

Apps including 'optimum BCT' had more functions [13.8; 95% confidence interval (CI) = 11.9–15.9] than apps that did not (4.7; 95% CI = 3.2–6.2; $P < 0.01$). This was also true in all the subgroups of functions. The same was found to be true with regard to the BCTs themselves, with more BCTs (5.8; 95% CI = 4.8–7.0) in apps with 'optimum BCT' than in apps without (3.1; 95% CI = 2.2, 4.1; $P < 0.01$). Logically, apps with 'optimum BCT' also had an overall higher score (19.8; 95% CI = 17.1–22.5)

Table 3 Characteristics of reviewed apps

		Optimum BCT			Price			User rating		
	Overall Mean (95% CI)	With optimum BCT Mean (95% CI)	Without optimum BCT Mean (95% CI)	*t*-test *P* value	Free Mean (95% CI)	Paid Mean (95% CI)	*t*-test *P* value	Low Mean (95% CI)	High Mean (95% CI)	*t*-test *P* value
All apps reviewed:	*n* = 40	*n* = 18	*n* = 22		*n* = 27	*n* = 13		*n* = 13	*n* = 25	
Functions (maximum of 27)†	8.9 (7.0, 10.7)	13.8 (11.9, 15.9)	4.7 (3.2, 6.2)	<0.01	8.6 (6.3, 11.0)	9.3 (5.8, 12.8)	0.74	6.2 (3.0, 9.5)	10.6 (8.3, 13.9)	0.03
Provision of information	0.6 (0.4, 0.9)	0.2 (−0.1, 0.4)	1.0 (0.7, 1.3)	<0.01	0.6 (0.3, 0.9)	0.7 (0.2, 1.2)	0.70	1.0 (0.7, 1.3)	0.4 (0.1, 0.7)	0.02
Allows self-recording	3.7 (2.6, 4.8)	6.5 (5.5, 7.5)	1.4 (0.3, 2.5)	<0.01	3.7 (2.2, 5.1)	3.7 (1.9, 5.5)	0.98	2.1 (0.2, 4.0)	4.7 (3.3, 6.1)	0.03
Generates output from self-recording	1.5 (1.0, 1.9)	2.7 (2.1, 3.4)	0.4 (0.1, 0.7)	<0.01	1.3 (0.7, 2.0)	1.7 (0.9, 2.5)	0.49	0.7 (0.0, 1.4)	1.9 (1.3, 2.6)	0.02
Data management	1.0 (0.7, 1.3)	1.7 (1.3, 2.0)	0.4 (0.1, 0.7)	<0.01	0.9 (0.5, 1.2)	1.1 (0.5, 1.7)	0.56	0.5 (−0.0, 0.9)	1.3 (0.9, 1.7)	0.01
Other	2.2 (1.8, 2.6)	2.8 (2.2, 3.5)	1.6 (1.3, 2.0)	<0.01	2.2 (1.7, 2.6)	2.2 (1.4, 3.1)	0.84	2.0 (1.4, 2.6)	2.4 (1.8, 2.9)	0.38
BCTs (maximum of 26)†	4.4 (3.6, 5.2)	5.8 (4.8, 7.0)	3.1 (2.2, 4.1)	<0.01	3.9 (3.1, 4.7)	5.3 (3.3, 7.2)	0.11	4.5 (2.8, 6.1)	4.5 (3.6, 5.3)	0.98
Motivation enhancing	1.2 (1.0, 2.6)	1.1 (0.8, 1.3)	1.4 (0.9, 1.8)	0.27	1.1 (0.8, 1.5)	1.5 (1.0, 1.9)	0.23	1.5 (1.0, 2.1)	1.0 (0.7, 1.3)	0.07
Planning and preparation	1.0 (0.6, 1.3)	0.9 (0.4, 1.4)	1.1 (0.6, 1.6)	0.56	1.0 (0.6, 1.4)	1.0 (0.3, 1.7)	1.00	1.4 (0.8, 2.0)	0.8 (0.4, 1.3)	0.14
Goal striving and persistence	2.2 (1.6, 2.8)	3.9 (3.4, 4.5)	0.7 (0.4, 1.0)	<0.01	1.8 (1.2, 2.4)	2.9 (1.4, 4.3)	0.10	1.5 (0.4, 2.6)	2.7 (2.0, 3.4)	0.05
Overall score (maximum of 53)	13.2 (10.9, 15.6)	19.8 (17.1, 22.5)	7.9 (6.3, 9.4)	<0.01	12.6 (9.8, 15.3)	14.6 (9.4, 19.8)	0.42	10.7 (6.2, 15.2)	15.1 (12.1, 18.1)	0.08
Price (£)	1.6 (0.4, 2.9)	3.2 (0.6, 5.9)	0.3 (−0.0, 0.5)	0.01	–	–	–	1.0 (−0.2, 2.1)	1.9 (−0.0, 3.9)	0.49
User rating	4.2 (4.0, 4.4)	4.4 (4.2, 4.5)	4.0 (3.6, 4.4)	0.07	4.3 (4.1, 4.4)	4.0 (3.2, 4.7)	0.16	–	–	–

†For examples of these characteristics, see data extraction form in Table 1. BCTs, behaviour change techniques; CI, confidence interval.

Table 4 Evaluation of price and user rating of apps on functions, behaviour change techniques (BCTs) and total score

	Price		Increase in score with paid apps*		User rating		Increase in score with highly rated apps*	
App score	Paid (n = 13) mean (SD)	Free (n = 27) mean (SD)	Regression coefficient (95% CI)	P-value	Low (n = 13) mean (SD)	High (n = 25) mean (SD)	Regression coefficient (95% CI)	P-value
Functions	9.3 (5.8)	8.6 (6.0)	2.5 (−1.6, 6.6)	0.22	6.23 (5.34)	10.64 (5.69)	4.8 (0.9, 8.7)	0.02
BCTs	5.3 (3.3)	3.9 (2.1)	1.9 (0.1, 3.8)	0.04	4.46 (2.67)	4.48 (2.60)	0.3 (−1.5, 2.1)	0.73
Overall score	14.6 (8.6)	12.6 (6.9)	4.4 (−0.7, 9.6)	0.09	10.69 (7.42)	15.12 (7.11)	5.1 (0.1, 10.0)	0.04

*Regression models adjusted for user rating with price; and price with user rating. CI, confidence interval.

than those that did not have 'optimum BCT' (7.9; 95% CI = 6.3–9.4; $P < 0.01$). Furthermore, apps with 'optimum BCT' had a higher price (£) (3.2; 95% CI = 0.6–5.9) than those without (0.3; 95% CI = −0.0 to 0.5; $P = 0.01$) (Table 3).

Apps with a high user rating had more functions (10.6; 95% CI = 8.3–13.9) than those that had a low rating (6.2; 95% CI = 3.0–9.5; $P = 0.03$). This was also true for the functions subgroups, except 'Other'. Conversely, the number of BCTs included was not related to user rating (high user rating number of BCTs 4.5; 95% CI = 2.8–6.1) versus (low user rating number of BCTs 4.5; 95% CI = 3.6–5.3; $P = 0.98$). Only BCTs in the subgroup 'Goal striving and persistence' were significantly more common in highly rated apps (2.7; 95% CI = 2.0–3.4) compared to low user rated apps (1.5; 95% CI = 0.4–2.6; $P = 0.05$). However, there was an indication of a higher user rating in apps with 'optimum BCT' (4.4; 95% CI = 4.2–4.5) than in those without (4.0; 95% CI = 3.6–4.4; $P = 0.07$) (Table 3).

The regression analysis also resulted in a significant association between number of functions (but not BCTs) and user rating (Table 4). In the adjusted models, highly rated apps had an average of 4.8 (95% CI 0.9–8.7; $P = 0.02$) more functions than lower rated apps. However, payment for an app was significantly related to a higher number of BCTs; paid apps had a higher number of BCTs by 1.9 (95% CI = 0.1–3.8; $P = 0.04$) than free ones. Price did not affect the overall score, although user rating was associated with overall score. Highly rated apps had a higher overall score by 5.1 (95% CI = 0.1–10.0; $P = 0.04$).

The inter-rater reliability gave an average agreement of 86% and kappa was 0.68, corresponding to a substantial or good agreement between raters.

Discussion

The inclusion of 'optimum BCT' has been used as a proxy for app quality because this combination of BCTs is most effective at changing behaviour [23] and is therefore potentially most beneficial to a person with diabetes using the app. The analysis showed that both the number of functions and the number of BCTs included in the apps were quite low. The mean (SD) number of BCTs was only 4.4 (2.6) out of 26. Therefore, BCTs were probably not actively considered in the development of the apps. Diabetes is a chronic disease requiring lifelong management; changing behaviour is key to achieving this successfully [26]. The combination of BCTs that was found to be most effective [23], was only included in 18 of the 40 apps. It is clear that there is still considerable potential for improvement of BCT inclusion in 'diabetes apps'.

Apps with optimum BCT had significantly more functions and BCTs, indicating that these could be predictors of app quality. Furthermore, user rating significantly predicted the number of functions included, whereas price was linked to an increased number of BCTs. There was a nonstatistically significant suggestion of a higher user rating in apps with 'optimum BCT' compared to apps without the optimum combination of BCTs. The validity of user rating as a predictor of app effectiveness is uncertain because most users are unlikely to base their rating on whether they managed to change behaviour. Research on user reviews [27] found that the most common causes of complaint, among others, were attractiveness, stability and compatibility. None of the causes listed were related to the apps' ability to change behaviour. Apps with 'Optimum BCT' cost more than others. West *et al.* [28], who appraised a number of apps based on their potential to influence behaviour change, found that more expensive apps were more likely to be scored as intending to promote health or prevent disease.

The small sample size of the study (i.e. only 40 apps were evaluated) could have limited our ability to determine predictors of app quality. With approximately 23 000 [10] health apps available, the total number of 'diabetes apps' is likely to be much greater than 40 and the sample size therefore presents a limitation to the study. Additionally, iTunes Store was not searched for apps, and

it is possibile that there are some key diabetes management apps that were missed. However, we did undertake an independent evaluation of a subsample of the apps included and found good agreement between reviewers. Resource implications precluded duplicate extraction of all apps, which is another limitation of the present study.

Diabetes Tracker by *Mig Super*, which scored highest in the present study, is an app that includes the recording of blood glucose, carbohydrate consumption and activity, as well as providing tips for recipes and physical exercises, dietary guidelines for each type of diabetes, and information on so-called 'superfoods'. The app that scored lowest, *Type 1 Diabetes* by *Colby Taylor* included different types of functions. They were more informative and advisory; giving rather limited information about the condition and about healthy meals that could keep blood sugar levels stable. It is clear that apps directed at people with diabetes include a range of different functions, making comparisons between them challenging. This variation in intended use creates a heterogeneity that might impact on the results.

As previously noted, there were five BCTs that were not included in any app. It might be unrealistic to think that all of the BCTs can feasibly be fitted into a mobile phone app. Some techniques would be more challenging to include because there was no link to a human decision maker (e.g. deciding when the target behaviour has been reached, or if the participant has relapsed). Peer or health care professional support would be possible through links to social media or downloads to surgery records. 'Agree on behavioural contract' could have been included in an app, for example, as behavioural goals written by the user themselves within the app or for the user to agree to prewritten goals.

The function 'Connect glucose meter to Smartphone to transfer data' was not included in any apps. The list of possible functions was developed by the investigators, partly based on similar research done by Chen [29], as well as knowledge about which elements are important when managing diabetes. However, expecting the inclusion of this function is not unreasonable. There are 'diabetes apps' currently on the market, and not identified by our search, that do have the possibility of being connected to a blood glucose meter either via a USB cable (e.g. *Apps Glooko* by *Glooko* and *iBGstar* by *Sanofi Diabetes*) or wirelessly via Bluetooth (e.g. *iHealth Gluco-Smart* by *iHealth Lab Inc.*), thereby allowing transfer of glucose readings directly to the diabetes management app. This is a great advantage to the user because it eliminates the burden of manually entering blood glucose values into the app.

As briefly noted previously, one app included a game where the user could earn points for undertaking health behaviours (*Diabetes Companion* by *mySugr GmbH*). Gamification is a term describing the use of game elements in a nongame setting [31]. There is some evidence that gamification is useful in the management of diabetes [30,31], and *Diabetes Companion* is also one of the highest scoring apps in this study, possibly as a result of the greater facilitation of some BCTs. Similarly, social support has repeatedly been shown to have a beneficial effect on diabetes management [32,33], although only nine out of the 40 apps provided at forum for the users to communicate among each other ('Link to social media'). Again, this aspect could be worth including in a 'diabetes app' to improve outcomes for the user.

A weakness of the present study is that it did not measure actual behaviour change as an outcome. Instead, the inclusion of specific BCTs was used as a proxy for effectiveness [23]. The optimum BCT score was derived from a peer reviewed meta-analysis including 122 papers. Although this was not focussed on diabetes management, and, instead, on diet and physical activity, these are both factors important in the management of type 2 diabetes. More recent evidence, as published after the main part of the present study was conducted, is conflicting. Avery *et al.* conducted a meta-analysis to determine which BCTs were most effective at increasing levels of physical activity, and consequently improving HbA1c levels in adults with diabetes type 2 [34]. The four most effective techniques that they found were 'Prompt focus on past success', 'Barrier identification/problem-solving', 'Use of follow-up prompts' and 'Provide information on where and when to perform physical activity'. 'Prompt focus on past success could be perceived as included within 'self-monitoring of behaviour' provided that this behaviour was indeed a success. Apart from that, the techniques found to be most effective differed completely. This suggests that finding BCTs that can be generalised to behaviour change interventions is difficult and may be behaviour or condition specific. Future work may include different interpretations of the most effective BCTs or undertaking a randomised controlled trial of apps including measurement of behaviour change as an outcome.

The aim of the present study was to evaluate 'diabetes apps' with regard to behaviour change techniques. The same taxonomy of BCTs has previously been used in relation to mobile apps for physical activity and diet [35]. However, we consider this to be the first study focusing on BCTs in 'diabetes apps'. The mobile app market is quickly changing and can be perceived as rather chaotic [36]. Health apps that have not been approved by a professional body may be problematic if users are not instructed correctly. The European Directory of Health Apps (2012) reviewed approximately 200 health apps in cooperation with patient groups [37]. The 'diabetes apps' included that overlapped with the apps evaluated here were *Carbs & Cals* by *Chello Publishing, Diabetes UK*

Tracker by *Diabetes UK*, *Glucose Buddy* by *Azumio, Inc.* and *OnTrack Diabetes* by *Medivo*. The Directory did not quantitatively evaluate the apps; included apps were recommended by patient groups. These four apps ranked within the upper half of the apps evaluated in the present study. Demidowich *et al.* assessed 42 'diabetes apps' in 2011 [19], although they did not include BCTs. Their highest ranking apps were *Glucool Diabetes*, *OnTrack Diabetes*, *Dbees* and *Track3 Diabetes Planner*. This agrees with the results from the present study that also evaluated *Glucool Diabetes* by *3qubits* and *OnTrack Diabetes* by *Medivo*, ranking them seventh and eighth overall.

In conclusion, we have conducted a study evaluating diabetes self-management apps with regard to BCTs. This is highly relevant in today's society because both Smartphone usage and diabetes is becoming increasingly prevalent. Behaviour change is an essential aspect of successful diabetes management, and incorporating BCTs in 'diabetes apps' represents a great opportunity to provide people with diabetes with a self-management tool. However, the 'diabetes apps' on the Android market were found to generally include few functions and even fewer BCTs. The three apps scoring most highly in the present study were *Diabetes Tracker by Mig Super, Diabetes:M by Rossen Varbanov and Diabetes Companion by mySugr GmbH*; these had the most functions and BCTs and including the combination of BCTs thought to be most effective at changing behaviour. Health professionals may want to recommend these apps to people with diabetes. More research on the effectiveness of BCTs in mobile apps is needed, although this time including more tangible outcomes of behaviour change techniques (e.g. HbA1c levels or weight change). With effectiveness established, app developers could work in conjunction with doctors, dietitians and psychologists, who have expert knowledge in the field, to include more BCTs in apps and make them as beneficial to patients as possible.

Acknowledgments

The authors acknowledge the support received from Marion Hery in the duplicate evaluation of the apps.

Conflict of interests, source of funding and authorship

JEC and MC have developed a smartphone app My Meal Mate, which aims to support weight loss.
No funding declared.
JEC had the initial idea of the study and had a major role in its design and execution. CDH undertook the evaluation of the apps, the statistical analysis, and the majority the of drafting the paper. MC contributed to the draft of the paper and reviewed its content. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. International Diabetes Federation (2014) IDF Diabetes Atlas - 7th Edition. Brussels, Belgium: International Diabetes Federation 2015.
2. van Dieren S, Beulens JWJ, van der Schouw YT *et al.* (2010) The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil* **17**, s3–s8.
3. Gaede P, Lund-Andersen H, Parving HH *et al.* (2008) Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* **358**, 580–591.
4. Lyakishev AA (2006) Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. Results of the DCCT/EDIC study. *Kardiologiia* **46**, 73.
5. UK Prospective Diabetes Study Group (1998) Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *Br Med J* **317**, 703–713.
6. Deloitte (2015) Mobile Consumer 2015: The UK cut. Available at: http://www.deloitte.co.uk/mobileuk/ (accessed 16 September 2016).
7. EMarketer (2014) 2 billion consumers worldwide to get smart(phones). by 2016. Available at: http://www.emarketer.com/newsroom/index.php/emarketer-2-billion-consumers-worldwide-smartphones-2016/ (accessed 16 September 2016).
8. World Health Organisation (2011) mHealth: New horizons for health through mobile technologies. Geneva: WHO Press.
9. Eng DS & Lee JM (2013) The promise and peril of mobile health applications for diabetes and endocrinology. *Pediatr Diabetes* **14**, 231–238.
10. AppBrain (2015) Most popular Google Play categories. Available at: http://www.appbrain.com/stats/android-market-app-categories (accesesed 16 September 2016).
11. Huckvale K, Prieto JT, Tilney M *et al.* (2015) Unaddressed privacy risks in accredited health and wellness apps: a cross-sectional systematic assessment. *BMC Med* **13**, 1–13.
12. US Food and Drug Administration (2015) Mobile Medical Applications – Guidance for Industry and Food and Drug Administration Staff. Rockville, USA: Food and Drug Administration.
13. Chen J, Cade JE & Allman-Farinelli M. (2015) The Most Popular Smartphone Apps for Weight Loss: A Quality Assessment *JMIR Mhealth Uhealth* **3**, e104.

14. Harvey-Berino J, Pintauro S, Buzzell P *et al.* (2002) Does using the internet facilitate the maintenance of weight loss? *Int J Obes Relat Metab Disord* **26**, 1254–1260.
15. Aguilar-Martínez A, Solé-Sedeño JM, Mancebo-Moreno G *et al.* (2014) Use of mobile phones as a tool for weight loss: a systematic review. *J Telemed Telecare* **20**, 339–349.
16. McKay H, Feil E, Glasgow R *et al.* (1998) Feasibility and use of an internet support service for diabetes self-management. *Diabetes Educ* **24**, 174–179.
17. Balas EA, Krishna S, Kretschmer RA *et al.* (2004) Computerized knowledge management in diabetes care. *Med Care* **42**, 610–621.
18. Bain TM, Jones ML, O'Brian CA *et al.* (2015) Feasibility of smartphone-delivered diabetes self-management education and training in an underserved urban population of adults. *J Telemed Telecare* **21**, 58–60.
19. Demidowich AP, Lu K, Tamler R *et al.* (2012) An evaluation of diabetes self-management applications for Android smartphones. *J Telemed Telecare* **18**, 235–238.
20. Tang J, Abraham C, Stamp E *et al.* (2015) How can weight-loss app designers' best engage and support users? A qualitative investigation. *Br J Health Psychol* **20**, 151–171.
21. Pal KES, Michie S, Farmer AJ *et al.* (2013) Computer-based diabetes self-management interventions for adults with type 2 diabetes mellitus. *Cochrane Database Syst Rev*28, CD008776.
22. Abraham C & Michie S (2008) A taxonomy of behavior change techniques used in interventions. *Health Psychol* **27**, 379–387.
23. Michie S, Abraham C, Whittington C *et al.* (2009) Effective techniques in healthy eating and physical activity interventions: a meta-regression. *Health Psychol* **28**, 690–701.
24. Briggs J (2015) Everything You Need to Know About Mobile App Search. In *Moz*, vol. 2015. Available at: https://moz.com/blog/mobile-app-search (Accessed 16 September 2016).
25. Gauchet S (2013) Optimize your google play store app details page. In *apptamin*. Available at: http://www.apptamin.com/blog/optimize-play-store-app/ (Accessed 16 September 2016).
26. Peyrot M & Rubin RR (2007) Behavioral and psychosocial interventions in diabetes: a conceptual review. *Diabetes Care* **30**, 2433–2440.
27. Fu B, Lin J, Li L *et al.* (2013) Why people hate your app: making sense of user feedback in a mobile app store. In: *Proceedings of the 19th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*. pp. 1276–1284. Chicago, IL: ACM.
28. West JHHP, Hanson CL, Barnes MD *et al.* (2012) There's an app for that: content analysis of paid health and fitness apps. *J Med Internet Res* **14**, e72.
29. Chen JCJ & Allman-Farinelli M (2015) The most popular smartphone apps for weight loss: a quality assessment. *JMIR Mhealth Uhealth* **3**, e104.
30. Cugelman B (2013) Gamification: what it is and why it matters to digital health behavior change developers. *JMIR Serious Games* **1**, e3.
31. Kamel Boulos MN, Gammon S, Dixon MC *et al.* (2015) Digital games for type 1 and type 2 diabetes: underpinning theory with three illustrative examples. *JMIR Serious Games* **3**, e3.
32. Toma T, Athanasiou T, Harling L *et al.* (2014) Online social networking services in the management of patients with diabetes mellitus: systematic review and meta-analysis of randomised controlled trials. *Diabetes Res Clin Pract* **106**, 200–211.
33. van Dam HA, van der Horst FG, Knoops L *et al.* (2005) Social support in diabetes: a systematic review of controlled intervention studies. *Patient Educ Couns* **59**, 1–12.
34. Avery L, Flynn D, Dombrowski SU *et al.* (2015) Successful behavioural strategies to increase physical activity and improve glucose control in adults with Type 2 diabetes. *Diabet Med* **32**, 1058–1062.
35. Brannon EE & Cushing CC (2014) Is there an app for that? Translational science of pediatric behavior change for physical activity and dietray interventions: a systematic review. *J Pediatr Psychol* **40**, 373–384.
36. Nass I (2015) The Mobile App Market Is Changing In 2015, Try And Keep Up. In *Dazeinfo*, vol. 2015. Available at: http://dazeinfo.com/2015/03/19/the-mobile-app-market-is-changing-in-2015-try-and-keep-up/ (Accessed 16 September 2016).
37. European Commission (2012–2013) *European Directory of Health Apps 2012–2013: a Review by Patient Groups and Empowered Consumers*. London: PatientView.

The mobile applications

1. 3qubits. 2013. *Glucool Diabetes (Premium)* (1.4.3.1). [mobile app]. [Date accessed: 06.12.2014]
2. Apps Den. 2014. *Gestational Diabetes* (1.0). [mobile app]. [Date accessed: 07.12.2014]
3. Avinash Kulkarni. 2012. *Diabetes Guide* (1.0). [mobile app]. [Date accessed: 10.12.2014]
4. Awesomeappcenter LLC. 2013. *Diabetic Diet Samples* (1.0). [mobile app]. [Date accessed: 26.11.2014]
5. Azumio, Inc. 2012. *Glucose Buddy: Diabetes Log* (1.0). [mobile app]. [Date accessed: 06.11.2014]
6. Colby Taylor. 2012. *Type 1 Diabetes* (1.0). [mobile app]. [Date accessed: 19.11.2014]
7. Chello Publishing Limited. 2014. *Carbs & Cals – Diabetes & Diet* (2.11). [mobile app]. [Date accessed: 12.06.2015]
8. David Froehlich. 2012. *DiaLog: Diabetes Logbook* (1.3.12). [mobile app]. [Date accessed: 10.06.2015]
9. Dheryta. 2013. *T1DM – Manage Type 1 Diabetes* (3.0). [mobile app]. [Date accessed: 20.11.2014]

10. Diabetes Digital Media Ltd. 2015. *Diabetes PA (Diabetes Manager)* (1.1.0). [mobile app]. [Date accessed: 11.06.2015]
11. Diabetes Digital Media Ltd. 2014. *Diabetes Forum* (3.11.34). [mobile app]. [Date accessed: 26.11.2014]
12. Diabetes UK. 2013. *Diabetes UK Tracker* (1.4). [mobile app]. [Date accessed: 19.11.2014]
13. EasyMobileApp. 2014. *Easy Diabetes* (1.7.0). [mobile app]. [Date accessed: 26.11.2014]
14. Gordon Wong. 2015. *BG Monitor Diabetes Pro* (6.8.4). [mobile app]. [Date accessed: 12.06.2015]
15. Gtxcel. 2014. *Diabetes Forecast®* (4.41). [mobile app]. [Date accessed: 11.6.2015]
16. Heyworld.dk. 2014. *Pregnant with diabetes* (1.1.0). [mobile app]. [Date accessed: 10.12.2014]
17. Jeschua Schang. *Diabetes Diary* (1.2.2). [mobile app]. [Date accessed: 12.06.2015]
18. Klimaszewski Szymon. 2014. *Diabetes – Glucose Diary* (1.3.04). [mobile app]. [Date accessed: 16.11.2014]
19. KoolAppz. 2011. *Diabetes Type 2 Guide* (1.0). [mobile app]. [Date accessed: 20.11.2014]
20. LISIERE MEDIA LLC. 2013. *Diabetic Grocery List* (2.1). [mobile app]. [Date accessed: 26.11.2014]
21. Medhelp, Inc. – Top Health Apps. 2014. *Sugar Sense – Diabetes App* (1.0.1). [mobile app]. [Date accessed: 06.12.2014]
22. Medivo. 2014. *OnTrack Diabetes* (3.2.5). [mobile app]. [Date accessed: 03.11.2014]
23. Mig Super. 2014. *Diabetes Tracker* (1.8). [mobile app]. [Date accessed: 09.06.2015]
24. moveforward. 2012. *Diabetes Forum for Diabetics* (1.3.18). [mobile app]. [Date accessed: 15.06.2015]
25. mySugr GmbH. 2014. *Diabetes Companion* (2.3.4). [mobile app]. [Date accessed: 13.11.2014]
26. Naster Solomon. 2014. *Gestational Diabetes* (1.0). [mobile app]. [Date accessed: 08.12.2014]
27. NetSummitApps. 2015. *Diabetic Recipes!* (1.2). [mobile app]. [Date accessed: 12.06.2015]
28. Personal Remedies. 2014. *Diabetes Type 2* (1.0). [mobile app]. [Date accessed: 20.11.2014]
29. Peter Wescott. 2011. *Diabetic Assistant* (2.0). [mobile app]. [Date accessed: 11.06.2015]
30. Riafy Technologies. 2014. *Diabetes Recipes Free* (7.0.0). [mobile app]. [Date accessed: 11.11.2014]
31. Rossen Varbanov. 2015. *Diabetes:M* (3.0.2). [mobile app]. [Date accessed: 09.06.2015]
32. Rossen Varbanov. 2014. *My Diabetes* (2.3.5). [mobile app]. [Date accessed: 12.11.2014]
33. SINIVO Ltd & Co KG. 2014. *SiDiary Diabetes Management* (1.27). [mobile app]. [Date accessed: 28.11.2014]
34. Social Diabetes. 2014. *Social Diabetes* (2.8.4). [mobile app]. [Date accessed: 03.11.2014]
35. SquareMed Software GmbH. 2014. *Diabetes Connect* (2.0.2). [mobile app]. [Date accessed: 19.11.2014]
36. SquareMed Software GmbH. 2014. *Diabetes Plus* (1.0.4). [mobile app]. [Date accessed: 16.11.2014]
37. Suderman Solutions. 2014. *Diabetes Journal* (1.4.4). [mobile app]. [Date accessed: 11.11.2014]
38. TopAppsFor-Health. 2014. *Diabetes Tools – Glucose* (1.2). [mobile app]. [Date accessed: 10.12.2014]
39. Twayesh Projects. 2014. *AudioBook – Diabetes* (26.0). [mobile app]. [Date accessed: 10.06.2015]
40. University of Illinois Extension. 2013. *Recipes for Diabetes* (1.1). [mobile app]. [Date accessed: 11.06.2015]

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

PUBLIC HEALTH NUTRITION

Trends in added sugar intake and food sources in a cohort of older Australians: 15 years of follow-up from the Blue Mountains Eye Study

H. Moshtaghian,[1] J. C. Y. Louie,[2,3] K. E. Charlton,[1] Y. C. Probst,[1,4] B. Gopinath,[5] P. Mitchell[5] & V. M. Flood[6,7]

[1]School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW, Australia
[2]School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, NSW, Australia
[3]School of Biological Sciences, Faculty of Science, The University of Hong Kong, Pokfulam, Hong Kong
[4]Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia
[5]Center for Vision Research, Department of Ophthalmology and Westmead Millennium Institute, University of Sydney, Sydney, NSW, Australia
[6]Faculty of Health Sciences, University of Sydney, Sydney, NSW, Australia
[7] Westmead Hospital, Western Sydney Local Health District, NSW, Australia

Keywords
added sugar intake, energy from added sugar, Blue Mountain Eye Study, older adults, time trend.

Correspondence
H. Moshtaghian, School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW 2522, Australia.
Tel.: + 61 2 4221 4584
E-mail: hm389@uowmail.edu.au

How to cite this article
Moshtaghian H., Louie J.C.Y., Charlton K.E., Probst Y.C., Gopinath B., Mitchell P. & Flood V.M. (2017) Trends in added sugar intake and food sources in a cohort of older Australians: 15 years of follow-up from the Blue Mountains Eye Study. *J Hum Nutr Diet*. **30**, 339–348
doi: 10.1111/jhn.12425

Abstract

Background: The trend of added sugar (AS) intake has not been investigated in the Australian population, including in older adults. The present study aimed to assess trends and food sources of AS intake among a cohort of older Australians during 15 years of follow-up.
Methods: Dietary data from participants of the Blue Mountains Eye Study (1264 men and 1614 women), aged ≥49 years at baseline, were collected. Dietary intakes were assessed at 5-yearly intervals (1992–94 to 2007–09) using a 145-item food frequency questionnaire (FFQ). AS content of FFQ food items was estimated using a stepwise systematic method. Trends for AS intake between baseline and the three follow-up periods were assessed using linear mixed modelling.
Results: In men, the mean (SEM) percentage of energy provided by AS intake (EAS%) declined from 10.4% (0.1%) at baseline to 9.4% (0.2%) at 15-year follow-up (P_{trend} = 0.028). Women's mean (SEM) EAS% intake at baseline and 15-year follow-up was 9.2% (0.1%) and 8.8% (0.2%), respectively (P_{trend} = 0.550). EAS% intake of men was significantly higher than that of women for 10 years ($P < 0.05$). Sugar products (table sugar, syrup, jam and honey) were the major sources of AS at all-time points and contributed to more than 40% and 35% of AS intake in men and women, respectively. Intake of sugar products decreased in men during follow-up ($P_{trend} < 0.001$).
Conclusions: An overall downward trend was observed in AS intake in both genders, however, was only significant for men during 15 years of follow-up. Table sugar and sugar-containing spreads represent the major source of AS in this cohort of older Australians.

Introduction

Added sugar (AS) is defined as sugar and syrups that are added to foods during processing or preparation [(1)]. This also includes the naturally occurring sugars that are isolated from the whole food and concentrated (e.g. fruit juice concentrates) [(2)]. Adverse health consequences associated with a high consumption of AS are currently the focus of many health debates, particularly with regard to the aetiology of obesity and its associated metabolic and cardiovascular complications [(3,4)]. Ageing populations face an increased risk from several metabolic and

cardiovascular diseases [5]. Various health authorities recommend that populations decrease their intake of energy provided from added sugar (EAS%) to below 10% of total energy intake and, for additional health benefits, reduce this intake to 5% [6,7].

Cross-sectional studies have described AS intakes across age groups, including older adults [8,9]. AS intakes of older adults have been found to be less than those of children and adolescents [8,10]. For example, the EAS% intake of older Australians was reported to be less than 10%, whereas the EAS% intake of younger Australians was reported to be more than 10% [8,11]. The AS intake of the Australian population has only been assessed twice: in the 1995 national nutrition survey (NNS) [8] and the National Nutrition and Physical Activity Survey (NNPAS) in 2011–12 [11]. According to these two surveys, the EAS % intake of Australians was 11.2% and 10.8%, respectively [8,11]. These surveys used different methods to assess AS content of foods; therefore, change and trends in AS intake cannot be compared statistically over this timeframe.

Trends in AS consumption over time have been reported in the USA using ongoing nationally representative cross-sectional data from National Health and Nutrition Examination Survey (NHANES) [12,13]. Americans, including older Americans, were found to have decreased their EAS% intake between 1999 and 2008, although their intake remained above 10% of energy intake [12]. This downward trend may not be the same as observed in other countries. A 5-year follow-up of South African adults reported that their EAS% increased from less than 10% to more than 10% between 2005 and 2010 [14]. Differences in the sugar content of processed foods in the food supply of different countries over time and cultural influences of food consumption patterns limit the generalisation of findings between countries [15].

In most of the published studies, sugar sweetened beverages were the major sources of AS intake [12,14]. However, there is some evidence that food sources of AS differ between age groups, even within the same population, because of different food preferences. In both the USA and Australia, sugar sweetened beverages are the major source of AS in young adults, although their consumption is lower in older adults [10,11]. In the US national survey, age-related changes in AS intake over time were partly a result of reductions in sugar sweetened beverages [12], suggesting that dietary preferences related to sweet foods may change across the lifespan.

Cohort studies with long follow-up periods can provide an improved understanding of changes to adults' dietary preferences as they age. This provides valuable information for the development of appropriate public health nutrition messages to older sectors of the population who may be at high risk of nutrient deficiencies [16] and cardiometabolic diseases [5]. To inform international dietary guidelines related to AS consumption, it is necessary to identify trends of AS and changes in its food sources at country-specific levels. Because there is no information available about trends of AS consumption in Australians, particularly the older population, the present study aimed to assess the trend of AS intake and its contributory food sources during a 15-year follow-up in the same cohort of older Australians.

Materials and methods

The present study analysed dietary data from participants of the Blue Mountains Eye Study (BMES). BMES participants were non-institutionalised permanent residents, aged ≥49 years at baseline (1992–1994), who lived in two postcodes of the Blue Mountains area, New South Wales, Australia. In total, 4433 eligible residents were identified in a door to door census, of whom 3654 attended an eye examination (response rate 82.4%) [17]. Response rates among survivors in BMES2, 3 and 4 were 75.1%, 75.6% and 55.4%, respectively [18]. The cohort has been followed up every 5 years and the present study reports on BMES1 (1992–1994), BMES2 (1997–1999), BMES3 (2002–2004) and BMES4 (2007–2009). A summary of BMES is provided in Fig. 1. BMES had ethics approval from the Sydney West Area Health Services and the University of Sydney Human Research Ethics Committees. Written informed consent was obtained from all participants.

Details of BMES have been described elsewhere [19]. Briefly, dietary data were collected using a 145-item semi-quantitative food frequency questionnaire (FFQ). Prior to having an eye examination, the self-administrated FFQ was sent by mail to participants and the completed FFQ was returned at the time of the face-to-face examination. The BMES FFQ is a modified Australian version of the Willett FFQ [20]. For nutritional data cleaning, a protocol was developed based on the epidemiological approaches in similar nutritional studies [21,22]. According to this protocol [19,23,24], participants with extreme energy intakes (<2500 kJ and >18 000 kJ) and more than 12 unanswered questions or entire blank page(s) were excluded. Implausible nutrient intakes were screened by inspecting values in the upper and lower 2% of the distribution [25,26] to locate and correct any data entry errors and to check for plausibility by dietitians. For the purpose of the present study, extreme outliers (mean ± 4SD) [27] of AS intake were excluded from the analysis, in accordance with a similar protocol used previously in the BMES [23]. The number of participants who fulfilled these criteria and were included in the current analysis at BMES1, 2, 3 and 4 was 2878, 1978, 1523 and 971, respectively.

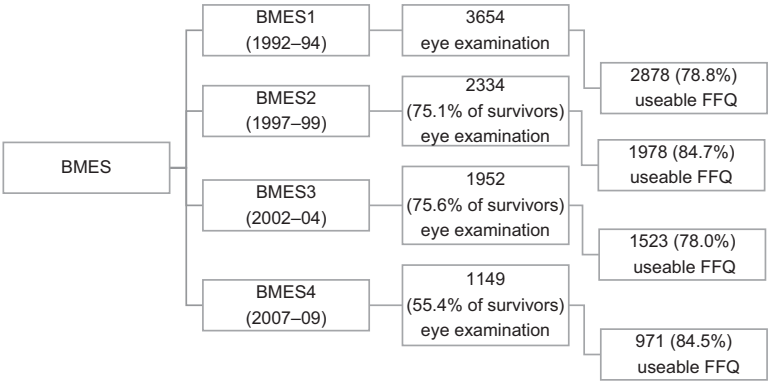

Figure 1 Flowchart of Blue Mountain Eye Study (BMES) during 15 years of follow-up. FFQ, food frequency questionnaire.

Australian nutrient tables (NUTTAB) were used for assessing food and nutrient intake from the FFQ [19]: NUTTAB 1990 [28], 1995 [29] and 2010 [30] were used to assess nutrient intake for relevant BMES time points (NUTTAB1990 for BMES1, NUTTAB1995 for BMES2 and 3, NUTTAB2010 for BMES4). There is no analytical laboratory method to measure AS content of foods [8,31] and NUTTAB does not include AS values. Therefore, a stepwise systematic method [31] was used to estimate the AS content of the food items for these NUTTABs. According to this stepwise systematic method, foods with zero total sugar content and natural (unprocessed) foods were assigned a zero AS value [31]. The AS content of high sugar-containing foods (i.e.100% AS food group such as table sugar and regular soft drinks) was considered to be equivalent to their total sugar content [31]. The AS content of other foods was calculated by using their recipes or comparing them with their unsweetened varieties [31]. Other steps in this method included an estimation of the AS content of foods based on analytical data of individual sugar types, adoption from overseas' databases and the ingredients list on food labels [31]. This systematic stepwise method was applied to food items in each time-relevant NUTTAB for BMES1, 2, 3 and 4 and thus AS values were estimated for FFQ items in each time point, separately. Participants' food and nutrient intakes including AS were calculated from these NUTTABs using a customized data processing system [19] and purpose-built queries in ACCESS 2013 (Microsoft Corp., Redmond, WA, USA).

Grouping of food items included in the FFQ was based on the hierarchical groupings of the 1995 Australian National Nutrition Survey [32]. Specific details of major and minor food groups that have been identified for this cohort have been published elsewhere [33]. Food subgroups likely to contribute to AS were created by combining of the predefined minor food groups. The subgroups included: sugar sweetened beverages (sweetened juices, cordial and soft drink), cereal products (breakfast cereals), cereal-based products and dishes (biscuits, cakes, buns and scones, pastries, mixed dishes), dairy products and dishes (yoghurt, custard, ice-cream and dairy-based desserts), sugar products and dishes (discretionary sugar, honey, jam and syrup), confectionary (sweets and chocolate), savoury sauces, meat (processed) and vegetables (processed/canned varieties).

Statistical analyses were conducted using SPSS, version 21 (IBM Corp., Armonk, NY, USA). Percentage energy (E%) for macronutrients was calculated based on multiplying the intake (g) by Atwater general factors: carbohydrate including AS (17 kJ g^{-1}), protein (17 kJ g^{-1}), fat (37 kJ g^{-1}) and alcohol (29 kJ g^{-1}) [34,35] and then dividing the results by total energy intake. It should be noted that, in the assessment of daily nutrient intake, the sum of E% from these macronutrients may exceed 100% because of the use of Atwater general factors [35]. Energy adjusted AS was reported as grams of AS intake expressed per 1 MJ of energy intake. Trends of energy, AS, E% from macronutrients including percentage of energy from AS (EAS%), energy adjusted AS (gram per 1 MJ) and AS intake from major food sources were considered for this analysis. Descriptive analyses were used to provide the mean (SEM) dietary intakes for each time point. Statistical significance of the change in intakes over 15 years (P for trend) was assessed using linear mixed modelling (random effects). The unstructured covariance model was used to assess random effects of time. Because the effect of gender in the linear mixed model was statistically significant, gender stratified analyses were conducted. The difference between intakes for men and women were assessed by an independent t-test. $P < 0.05$ was considered statistically significant.

Results

Of the 2878 participants at baseline, 662 provided dietary data at 5-year, 10-year and 15-year follow-up. These 662 participants who provided dietary data at all time points were more likely to be younger, married, employed, non-smokers, home-owners and to have qualifications after leaving school compared to those with incomplete data

Table 1 Baseline characteristics of the Blue Mountains Eye Study participants with complete dietary data at all-time points versus participants with incomplete data during 15-year follow-up

	Participants with complete data	Participants with incomplete data	*P**
n	662	2216	–
Age, mean (SEM)	60.7 (0.3)	66.8 (0.2)	0 < 0.001
≤59 years, *n* (%)	283 (42.7)	558 (25.2)	0 < 0.001
60–69 years, *n* (%)	316 (47.7)	789 (35.6)	0 < 0.001
70–79 years, *n* (%)	61 (9.2)	653 (29.5)	0 < 0.001
≥80 years, *n* (%)	2 (0.3)	216 (9.7)	0 < 0.001
Female, *n* (%)	391 (59.1)	1223 (55.2)	0.076
Qualification after leaving school, *n* (%)	443 (66.9)	1196 (54.0)	0 < 0.001
Employed, *n* (%)	242 (36.6)	403 (18.2)	0 < 0.001
Married, *n* (%)	487 (73.6)	1419 (64.0)	0 < 0.001
Pension, *n* (%)	236 (35.6)	1330 (60.0)	0 < 0.001
Home ownership, *n* (%)	621 (93.8)	1922 (86.7)	0 < 0.001
Live alone, *n* (%)	126 (19.0)	580 (26.2)	0 < 0.001
Smoker, *n* (%)	295 (44.6)	1167 (52.7)	0 < 0.001
Poor self-rated health, *n* (%)	5 (0.8)	82 (3.7)	0 < 0.001
Energy intake (kJ)†, mean (SEM)	8526.7 (93.5)	8574.4 (55.5)	0.661
Added sugar intake (g), mean (SEM)	46.0 (1.2)	51.1 (0.7)	0 < 0.001
Energy intake from added sugar (EAS%), mean (SEM)	8.9 (0.2)	9.9 (0.1)	0 < 0.001
EAS% ≤ 5, *n* (%)	153 (23.1)	399 (18.0)	0.003
EAS% ≤ 10, *n* (%)	430 (65.0)	1241 (56.0)	0 < 0.001

*All *P* values were assessed using Pearson's chi-squared, except for age, energy, added sugar and energy from added sugar where *P* values were assessed using an independent *t*-test.

†Energy intake is expressed as kilojoules (kJ). 1 kJ is equivalent to 0.239 kcal.EAS, energy intake from added sugar.

(i.e. missing data during follow-up) (Table 1). The AS and EAS% intakes of those with complete data were lower than those with incomplete data during follow-up. Compared to participants with complete data, the proportion of those who had EAS% ≤ 10 and EAS% ≤ 5 was lower in people with incomplete data.

Mean (SEM) AS intakes of the overall cohort at BMES1, 2, 3 and 4 were 49.9 (0.6), 49.8 (0.7), 49.0 (0.7) and 46.6 (0.9) g, respectively (P_{trend} = 0.226). In the overall cohort, baseline EAS% consumption was 9.7%. The EAS% intake of this cohort at subsequent follow-ups was 9.8%, 9.6% and 9.1%, respectively (P_{trend} = 0.335). The proportion of participants who had EAS% ≤ 5 was less than 20% at all time points. The proportion of participants who had EAS% ≤ 10 at BMES1, 2, 3 and 4 was 58.1%, 58.8%, 59.7% and 63.9%, respectively.

In gender-specific analysis, mean energy intake of women increased, whereas energy intake of men decreased, over 15 years of follow-up (Table 2). In women, mean E% intake from protein increased, whereas E% intake from alcohol and carbohydrate decreased, over the 15-year follow-up (P_{trend} < 0.05). In men, mean E% intake from alcohol decreased, whereas E% intake of protein increased, over the 15-year follow-up (P_{trend} < 0.05). In women, there was no significant change in trend over time for energy adjusted AS and EAS%, although the intake in men decreased significantly between BMES1 and BMES4 (P_{trend} < 0.05). Men had a higher EAS% intake compared to women at all-time points and this difference was significant in BMES1, 2 and 3 (P < 0.001, P = 0.004 and P = 0.008, respectively).

Within the 15-year time period, sugar products and dishes (sugar, syrup, honey and jam) remained the main sources of AS in both men and women (Table 3). The consumption of this food group decreased over-time, in particular for men (P_{trend} < 0.001). Confectionary was the second most important source of AS at the majority of time points, except BMES2, where this ranking was replaced by sugar sweetened beverages. Sugar sweetened beverages remained the second highest source of AS in men for BMES3. Intake of AS from cereal and cereal-based products, savoury sauces and meat dishes changed significantly over-time for both men and women (P_{trend} < 0.05). In women, AS intake from dairy products and dishes increased over time (P_{trend} < 0.001). In both genders, sugar sweetened beverage consumption increased in BMES2 and decreased in BMES4. Men had higher intakes of AS from sugar products and dishes, cereal-based product and dishes and meat dishes compared to women at all-time points (P < 0.05).

In terms of the contribution of different food sources to AS intake (Fig. 2), sugar products and dishes were the major contributors at all time points for both men (>40% of total AS intake) and women (>35% of total AS

Table 2 Selected Macronutrient intake in Blue Mountain Eye Study population in four time points

	BMES1 1992–1994		BMES2 1997–1999		BMES3 2002–2004		BMES4 2007–2009		
	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	P_{trend}
Women									
n	1614		1136		898		560		–
Age (years)	65.3	(0.2)	69.1	(0.3)	74.0	(0.2)	76.4	(0.3)	–
Energy intake (kJ)†	7998.8	(58.1)	8131.1	(67.1)	8281.6	(79.5)	8281.2	(103.4)	<0.001
Total sugar (g per 1 MJ)	15.2	(0.1)	15.4	(0.1)	15.3	(0.1)	14.9	(0.2)	0.275
Added sugar (g per 1 MJ)	5.4	(0.1)	5.6	(0.1)	5.5	(0.1)	5.2	(0.1)	0.550
Total sugar (E%)	25.9	(0.2)	26.2	(0.2)	26.0	(0.2)	25.4	(0.3)	0.275
Added sugar (E%)	9.2	(0.1)	9.5	(0.2)	9.3	(0.2)	8.8	(0.2)	0.550
Carbohydrate (E%)	47.5	(0.2)	49.0	(0.2)	47.7	(0.2)	45.8	(0.3)	<0.001
Protein (E%)	18.2	(0.1)	18.3	(0.1)	18.6	(0.1)	19.3	(0.1)	<0.001
Fat (E%)	32.9	(0.2)	31.4	(0.2)	32.4	(0.2)	33.1	(0.2)	0.383
Alcohol (E%)	2.6	(0.1)	2.4	(0.1)	2.2	(0.1)	2.1	(0.2)	<0.001
Men									
n	1264		842		625		411		–
Age (years)	65.4	(0.3)	69.2	(0.3)	73.4	(0.3)	76.3	(0.3)	–
Energy intake (kJ)†	9284.2*	(75.0)	9030.0*	(88.1)	9053.7*	(99.4)	9130.9*	(124.7)	0.006
Total sugar (g per 1 MJ)	14.6*	(0.1)	14.3*	(0.1)	14.4*	(0.2)	14.0*	(0.2)	0.008
Added sugar (g per 1 MJ)	6.1*	(0.1)	6.0*	(0.1)	5.9*	(0.1)	5.5	(0.1)	0.028
Total sugar (E%)	24.8*	(0.2)	24.4*	(0.2)	24.5*	(0.3)	23.9*	(0.3)	0.008
Added sugar (E%)	10.4*	(0.1)	10.2*	(0.2)	10.0*	(0.2)	9.4	(0.2)	0.028
Carbohydrate (E%)	45.9*	(0.2)	47.3*	(0.3)	46.6*	(0.3)	44.5*	(0.4)	0.074
Protein (E%)	17.0*	(0.1)	17.3*	(0.1)	17.5*	(0.1)	17.9*	(0.2)	<0.001
Fat (E%)	33.0	(0.2)	31.4	(0.2)	32.0	(0.2)	33.1	(0.3)	0.491
Alcohol (E%)	5.3*	(0.2)	5.0*	(0.2)	5.0*	(0.2)	4.8*	(0.3)	0.001

*Significant difference between men and women ($P < 0.05$).
†Energy intake is expressed as kilojoules (kJ). 1 kJ is equivalent to 0.239 kcal.

intake). During the 15-year follow-up, AS contributions from sugar products and dishes decreased and from dairy products and dishes increased for both men and women ($P_{trend} < 0.05$). During this follow-up, the proportion of AS intake from confectionary increased in men and proportion of AS intake from cereal-based products and dishes decreased in women ($P_{trend} < 0.05$).

Discussion

This 15-year assessment of AS consumption in the same cohort of older Australians from the BMES has demonstrated fluctuations in AS and EAS% intake. In women, there was a slight but nonsignificant decline in EAS% intake from 9.2% to 8.8%, whereas EAS% intake in men decreased significantly from 10.4% to 9.4%. In terms of food sources, sugar products and dishes (sugar, syrup, honey and jam) remained the major source of AS intake in this older cohort across all time points.

In Australia, data from apparent sugar consumption, which provide an indication of primary sugar production and export [36], have shown a decrease between 1992 and 2009 [37]. According to national survey data in 1995 (NNS 1995) and 2011–12 (NNPAS 2011–12), the EAS% intake of Australians was reported to be 11.2% [8] and 10.8%, respectively [11]. The EAS% intake of older adults was approximately 9% in both the 1995 and 2011–12 surveys [8,11]. These data suggest that the AS intake of the Australian population has not changed considerably over the past two decades. However, a direct comparison of findings from these national data is difficult to undertake because AS estimation methods were not identical between these two surveys. In the NNPAS 2011–12, the AS content of foods was determined by the same systematic method used in the BMES study, whereas the AS estimation method for NNS 1995 data was different from our systematic method and the details have not been outlined in a reproducible manner.

The application of different dietary assessment methods over time is also one of the reasons why trends of AS intake have been reported inferably rather than statistically [38]. The EAS% intake of Danish adults appeared stable (approximately 9%) between 1985 and 2006, whereas the EAS% intake of Norwegian adults appeared to be declining from 9% to 7.3% over 18 years (1993–2011) [38]. However, because different dietary data

Table 3 Mean (standard error) added sugar intake from major food sources in overall cohort of Blue Mountain Eye Study at all-time points

	Added sugar (g)								
	BMES1		BMES2		BMES3		BMES4		
	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	P_{trend}
Women									
n	1614		1136		898		560		–
Total added sugar	44.1	(0.7)	46.1	(0.9)	45.8	(0.9)	43.0	(1.1)	0.051
Sugar products and dishes	18.9	(0.4)	19.1	(0.5)	18.4	(0.5)	16.8	(0.7)	0.062
Cereal-based product and dishes	5.5	(0.1)	5.2	(0.1)	5.0	(0.2)	4.6	(0.2)	0.001
Confectionary	7.5	(0.4)	6.7	(0.4)	7.2	(0.4)	7.6	(0.5)	0.370
Dairy products and dishes	4.1	(0.1)	4.5	(0.1)	5.4	(0.2)	5.6	(0.2)	<0.001
Sugar-sweetened beverages	4.9	(0.2)	6.9	(0.4)	6.4	(0.4)	4.8	(0.4)	0.083
Cereal product	1.3	(0.1)	1.1	(0.1)	0.8	(0.1)	1.3	(0.1)	0.003
Vegetables	1.3	(0.1)	1.3	(0.1)	1.4	(0.1)	1.3	(0.1)	0.480
Savoury sauces	0.3	(0)	0.8	(0)	0.8	(0)	0.6	(0)	<0.001
Meats	0.2	(0)	0.2	(0)	0.2	(0)	0.3	(0)	<0.001
Men									
n	1264		842		625		411		–
Total added sugar	57.4*	(1.0)	54.7*	(1.1)	53.6*	(1.2)	51.4*	(1.5)	<0.001
Sugar products and dishes	28.4*	(0.6)	26.5*	(0.8)	25.1*	(0.8)	22.9*	(1.0)	<0.001
Cereal-based product and dishes	6.9*	(0.2)	6.2*	(0.2)	6.0*	(0.2)	6.0*	(0.3)	0.001
Confectionary	7.4	(0.4)	6.0	(0.4)	6.5	(0.4)	8.5	(0.6)	0.367
Dairy products and dishes	4.7*	(0.1)	4.6	(0.2)	5.0	(0.2)	5.2	(0.2)	0.056
Sugar-sweetened beverages	6.8*	(0.3)	7.9	(0.4)	7.5	(0.5)	5.4	(0.4)	0.196
Cereal product	1.5	(0.1)	1.2	(0.1)	0.9	(0.1)	1.1	(0.1)	<0.001
Vegetables	1.2	(0)	1.2	(0.1)	1.3	(0.1)	1.1	(0.1)	0.537
Savoury sauces	0.3*	(0)	0.6*	(0)	0.7*	(0)	0.6	(0)	<0.001
Meats	0.3*	(0)	0.3*	(0)	0.3*	(0)	0.4*	(0)	<0.001

*Significant difference between men and women ($P < 0.05$).

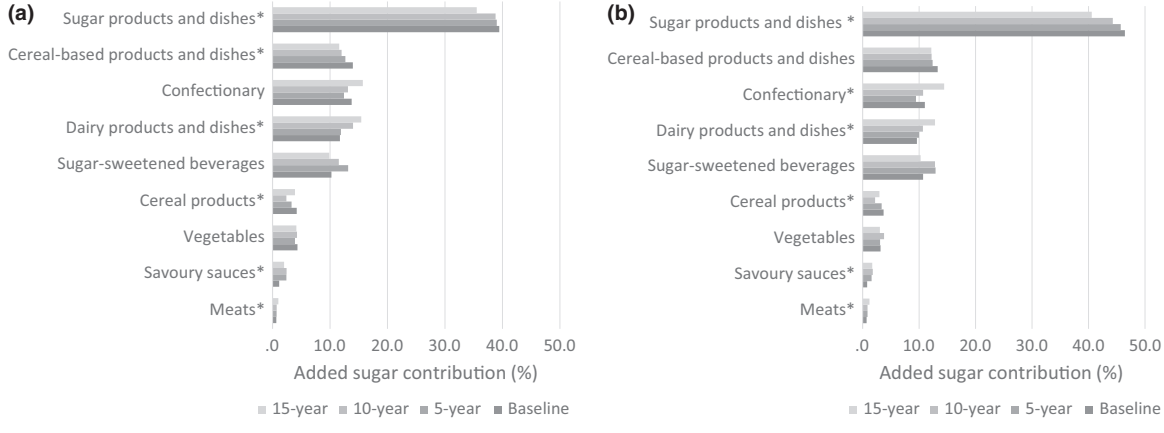

Figure 2 Contribution of different food sources in added sugar intake (%) in Blue Mountains Eye Study participants: (a) women and (b) men. *$P_{trend} < 0.05$.

collection methods were used during these time points, the statistical significance of this trend cannot be assessed. For example, in Norway, dietary data were collected by semi-quantitative FFQ and 24-h recalls in 1990s and 2010–11 surveys, respectively [38].

Notably, the present study could capture the intake of the same participants over four time points to assess trends in their intake, by applying the same FFQ and systematic method for assessment of AS intake at all time points. The method of dietary assessment in the present study was different from national surveys: the NNS 1995 and NNPAS 2011–12 used the 24-h recall method and our longitudinal cohort study of older people used the FFQ. Despite the different dietary assessment methods used in the present study and the two national surveys, both similarly reported little change in AS intake of older adults over time.

The trend of AS intake over time was assessed statistically in the USA [12,13] and this trend was not similar to the trends in BMES. During the 2000s, the EAS% intake of the older Americans decreased from 13.3% to 11.8% ($P_{trend} < 0.01$) [12]; however, their EAS% intake remained higher than the BMES population (>10% in older Americans versus 9% in BMES). This could be a result of differences in the amount of major sweeteners used by the food industry in each of the countries or differences in dietary patterns of intakes of major AS food sources, including sugar sweetened beverages. Methodological differences in assessing the intake may also limit comparisons. We have used FFQs from a longitudinal study, whereas the US national survey (NHANES) used 24-h recalls in repeated cross-sectional surveys (i.e. not necessarily the same participants sampled each time). The assessment of AS content of foods was also different between these two countries. While we applied a systematic method for the assessment of AS content of foods, the US study used AS values provided from the MyPyramid Equivalents database, which expresses AS content of foods in teaspoon measures (i.e. each teaspoon is equivalent to 4.2 g of table sugar) [39]. For estimation of AS values in that database, the AS component was identified in the ingredient list of multi-ingredient foods and total AS content was calculated using a two-step formula [39].

In the BMES study, the mean EAS% for women was in line with the 10% reference cut-off value [7] at all time points. For men, the EAS% exceeded the recommendation for 10 years. In rural South Africa, the EAS% intake of men and women was 6.9% and 7.6% in 2005 but, during a 5-year follow-up, the intakes increased to 10.5% and 11.3%, respectively [14]. In older Americans, the EAS % intake has remained above 10% despite a decline in EAS% consumption from 13.3% to 11.8% over an 8-year period [12]. The reported intake of BMES participants was above the American Heart Association recommendation of 100 kcal day^{-1} (418.4 kJ day^{-1}) from AS intake in women and 150 kcal day^{-1} (627.6 kJ day^{-1}) in men [6]. This recommendation is approximately equivalent to an EAS% intake of 5%. Currently, health agencies provide recommendations to reduce AS intake to less than 5% of total energy, although they also emphasise that high quality evidence is required to investigate health-related outcomes in populations with 5% EAS intakes [40,41]. In addition, the feasibility of meeting the 5% recommendation in everyday diet has not yet been demonstrated [42]. Australian dietary guidelines recommend limiting the intake of food sources of AS [43], but this recommendation does not provide a quantifiable target. To this end, our analysis has identified the major AS sources that can be used for food-based dietary guidance purposes in older Australian adults.

Sugar products and dishes were the major source of AS in the BMES cohort. In NNS 1995, the major sources of AS in adults were sugar sweetened beverages, followed by sugar products and dishes (sugar, honey and jam) [8]; however, no specific information was available for older Australians. Sugar and sweet spreads were reported to be the major source of AS in older Australians in NNPAS 2011–12 [11]. Our data are the first to provide a comprehensive analysis of major sources of AS in older Australians over a prolonged period of time. By contrast to our findings, sugar sweetened beverages were the main source of AS intake in the US older population in the early 2000s, although the intakes of these beverages decreased in subsequent years and they were replaced by sweetened grains as the major source of AS in the late 2000s [12]. In the present study, sugar sweetened beverages were the second most common source of AS in the late 1990s but their intake declined over time. The apparently lower intake of sugar sweetened beverages in our Australian cohort could be a result of preferences for alternative beverages such as tea, coffee and alcohol. The NNS 1995 data support this hypothesis by showing that older Australians had lower soft drink consumption and higher sugar product intake compared to other age groups [44]. Another explanation may be related to the higher socio-economic status of the BMES cohort compared to the general Australian population [45]. The soft drink consumption of lower socio-economic groups tends to be higher than the rest of the population [46].

According to national Australian dietary data, the consumption of sugar products and dishes decreased in both older men and women, whereas confectionary consumption increased between 1995 and 2011–12 [47,48]. In BMES participants, AS consumption from sugar products and dishes decreased for men, and AS intake from confectionary remained the same in both sexes during follow-up. During the 2000s, in older Americans, the AS intake from sugar sweetened beverages, and sugar and syrups decreased [12]. In a different timeframe (between 1980s and 1990s), middle and older aged Danes (both men and women) did not alter their consumption of sugar sweetened beverages and sweet spreads, although their intake of confectionary increased [49]. These data suggest that food preferences of older adults, with regard to sugar-containing foods, differ by gender, country and time.

The major strengths of the present study were the ability to follow the same participants over a 15-year time period and the use of the same FFQ across all time points. Inclusion of the quantity of discretionary sugar in the FFQ and the use of a systematic, stepwise, reproducible method for the assessment of AS content of foods are other strengths of the present study. Response rates were moderately good among survivors (75%), up to the

fourth wave of the data collection period, when the response rate dropped to 55% among survivors. In this ageing cohort, a lack of participation at follow-up includes death, moving from the area or being too frail to participate [50]. Efforts were made to use multiple contacts for each participant (e.g. contact children and spouse) aiming to increase the chances of being able to contact participants at each follow-up. Given that the present study assessed the AS intake of old people during 15 years, the subsequent number included is a relatively low proportion of original cohort and may represent a survivor cohort where individuals are likely to have healthier lifestyles. Limitations include the potential recall bias related to memory difficulties (i.e. overestimating or underestimating of food intakes), where participants are asked to consider their usual intake over the prior 12-month period in relation to the listed food items on the FFQ. There may have been errors introduced into some of the steps included in the AS systematic method where some measure of subjective judgement was required [31]. However, any measurement error would have been consistent across all time points, and thus would not have affected the time trend analyses overall.

In conclusion, AS intake decreased over 15 years in older Australian men but not in women. In this ageing Australian cohort, rather than sugar sweetened beverages, the main contributors to AS intake were sugar and sweet spreads, such as jam, honey and syrup. Public health programmes aiming to encourage dietary modification in older adults should focus on gender- and age-specific preferred food sources of AS.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest. The Blue Mountains Eye Study was supported by funding from the Australian National Health and Medical Research Council, Canberra, Australia.
HM conducted the research, estimated added sugar values, analysed the data and drafted the manuscript. VMF and PM were involved in collection of original BMES data. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

The authors affirm that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained. The reporting of this work is compliant with STROBE guidelines.

References

1. Institute of Medicine (2005) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: National Academies Press.
2. US Department of Health Human Services (Food and Drug Administration) (2014) Food labeling: revision of the nutrition and supplement facts labels; proposed rule. *Federal Register [Internet] Food and Drug Administration*. Available at: https://www.federalregister.gov/articles/2014/03/03/2014-04387/food-labeling-revision-of-the-nutrition-and-supplement-facts-labels (accessed November 2015).
3. Schulze MB, Manson JE, Ludwig DS *et al.* (2004) Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *JAMA* **292**, 927–934.
4. Dhingra R, Sullivan L, Jacques PF *et al.* (2007) Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the Community. *Circulation* **116**, 480–488.
5. Yang Q, Zhang Z, Gregg EW *et al.* (2014) Added sugar intake and cardiovascular diseases mortality among us adults. *JAMA Intern Med* **174**, 516–524.
6. Johnson RK, Appel LJ, Brands M *et al.* (2009) Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. *Circulation* **120**, 1011–1020.
7. US Department of Agriculture and US Department of Health and Human Services (2015) Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Available at: https://ods.od.nih.gov/About/2015_DGAC_Report.aspx (accessed July 2015).
8. Cobiac L, Record S, Leppard P *et al.* (2003) Sugars in the Australian diet: results from the 1995 National Nutrition Survey. *Nutr Diet* **60**, 152–173.
9. Marriott BP, Olsho L, Hadden L *et al.* (2010) Intake of added sugars and selected nutrients in the United States, National Health and Nutrition Examination Survey (NHANES) 2003—2006. *Crit Rev Food Sci Nutr* **50**, 228–258.
10. Popkin BM (2010) Patterns of beverage use across the lifecycle. *Physiol Behav* **100**, 4–9.
11. Lei L, Rangan A, Flood VM *et al.* (2016) Dietary intake and food sources of added sugar in the Australian population. *Br J Nutr* **115**, 868–877.
12. Welsh JA, Sharma AJ, Grellinger L *et al.* (2011) Consumption of added sugars is decreasing in the United States. *Am J Clin Nutr* **94**, 726–734.

13. Wang H, Steffen LM, Zhou X *et al.* (2013) Consistency Between Increasing Trends in Added-Sugar Intake and Body Mass Index Among Adults: The Minnesota Heart Survey, 1980–1982 to 2007–2009. *Am J Public Health* **103**, 501–507.
14. Vorster HH, Kruger A, Wentzel-Viljoen E *et al.* (2014) Added sugar intake in South Africa: findings from the Adult Prospective Urban and Rural Epidemiology cohort study. *Am J Clin Nutr* **99**, 1479–1486.
15. Cust AE, Skilton MR, van Bakel MME *et al.* (2009) Total dietary carbohydrate, sugar, starch and fibre intakes in the European prospective investigation into cancer and nutrition. *Eur J Clin Nutr* **63**, S37–S60.
16. Charlton KE, Kolbe-Alexander TL & Nel JH (2005) Micronutrient dilution associated with added sugar intake in elderly black South African women. *Eur J Clin Nutr* **59**, 1030–1042.
17. Cugati S, Wang JJ, Rochtchina E *et al.* (2007) Ten-year incidence of diabetes in older Australians: the Blue Mountains Eye Study. *Med J Aust* **186**, 131–135.
18. Gopinath B, Russell J, Flood VM *et al.* (2014) Adherence to dietary guidelines positively affects quality of life and functional status of older adults. *J Acad Nutr Diet* **114**, 220–229.
19. Flood VM, Smith W, Rochtchina E *et al.* (2008) Assembling a nutrient database for a large cohort study: The Blue Mountains Eye Study. *Food Australia* **60**, 37–40.
20. Willett WC, Sampson L, Browne ML *et al.* (1988) The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* **127**, 188–199.
21. Willett W (1998) Issues in analysis and presentation of dietary data. *Nutr Epidemiol* **2**, 321–345.
22. Fogli-Cawley JJ, Dwyer JT, Saltzman E *et al.* (2007) The 2005 dietary guidelines for americans and insulin resistance in the framingham offspring cohort. *Diabetes Care* **30**, 817–822.
23. Flood VM (2004) Folate, Vitamine B12 and homocysteine status among older Australians.Dissertation, University of Sydney.
24. Smith W, Mitchell P, Reay EM *et al.* (1998) Validity and reproducibility of a self-administered food frequency questionnaire in older people. *Aust NZ J Public Health* **22**, 456–463.
25. Mendez MA, Popkin BM, Buckland G *et al.* (2011) Alternative methods of accounting for underreporting and overreporting when measuring dietary intake-obesity relations. *Am J Epidemiol* **173**, 448–458.
26. Seo S (2006) A review and comparison of methods for detecting outliers in univariate data sets, University of Pittsburgh.
27. Rebello SA, Chen CH, Naidoo N *et al.* (2011) Coffee and tea consumption in relation to inflammation and basal glucose metabolism in a multi-ethnic Asian population: a cross-sectional study. *Nutr J* **10**, 61.
28. Food Standards Australia New Zealand (1990) *NUTTAB 1990*. Canberra: FSANZ .
29. Food Standards Australia New Zealand (1995) *NUTTAB 1995*. Canberra: FSANZ .
30. Food Standards Australia New Zealand (2010) *NUTTAB 2010*. Canberra: FSANZ .
31. Louie JCY, Moshtaghian H, Boylan S *et al.* (2015) A systematic methodology to estimate added sugar content of foods. *Eur J Clin Nutr* **69**, 154–161.
32. McLennan, W, Podger, A (1998) *National Nutrition Survey Nutrient Intakes and Physical Measurements*, vol. ABS Catalogue No. 4805.0.. Canberra: Australian Bureau of Statistics.
33. Louie JCY, Flood VM, Rangan AM *et al.* (2013) Higher regular fat dairy consumption is associated with lower incidence of metabolic syndrome but not type 2 diabetes. *Nutr Metab Cardiovasc Dis* **23**, 816–821.
34. Slimani N, Deharveng G, Unwin I *et al.* (2007) The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr* **61**, 1037–1056.
35. Maclean W, Harnly J, Chen J *et al.* (2003) Food energy – methods of analysis and conversion factors. Food and Agriculture Organization of the United Nations Technical Workshop Report 77. Available at http://www.fao.org/uploads/media/FAO_2003_Food_Energy_02.pdf (accessed May 2016).
36. Rikkers W, Lawrence D, Hafekost K *et al.* (2013) Trends in sugar supply and consumption in Australia: is there an Australian Paradox? *BMC Public Health* **13**, 668.
37. McNeill TJ & Shrapnel WS (2015) Apparent consumption of refined sugar in Australia (1938–2011). *Eur J Clin Nutr* **69**, 1233–1237.
38. Wittekind A & Walton J (2014) Worldwide trends in dietary sugars intake. *Nutr Res Rev* **27**, 330–345.
39. Bowman, S, Friday, J & Moshfegh, A (2008) MyPyramid Equivalents Database, 2.0 for USDA Survey Foods, 2003–2004 Food Surveys Research Group. Available at: http://www.ars.usda.gov/SP2UserFiles/Place/80400530/pdf/mped/mped2_doc.pdf (accessed April 2013).
40. World Health Organization (2015) *Guideline: Sugars Intake for Adults and Children.* Geneva: WHO.
41. Anderson AS (2014) Sugars and health – risk assessment to risk management. *Public Health Nutr* **17**, 2148–2150.
42. Erickson J & Slavin J (2015) Total, added, and free sugars: are restrictive guidelines science-based or achievable? *Nutrients* **7**, 2866–2878.
43. National Health Medical Research Council (2013) *Dietary Guidelines for Australians*. Canberra: Australian Government Publishing Service.
44. Rangan AM, Schindeler S, Hector DJ *et al.* (2008) Consumption of/'extra/' foods by Australian adults: types, quantities and contribution to energy and nutrient intakes. *Eur J Clin Nutr* **63**, 865–871.

45. Flood VM, Burlutsky G, Webb KL *et al.* (2010) Food and nutrient consumption trends in older Australians: a 10-year cohort study. *Eur J Clin Nutr* **64**, 603–613.
46. Darmon N & Drewnowski A (2008) Does social class predict diet quality? *Am J Clin Nutr* **87**, 1107–1117.
47. McLennan W & Podger A (1997) *National Nutrition Survey, Selected Highlights*. Canberra,: Australian Government Publishing Service.
48. Australian Bureau of Statistics (2014) Australian Health Survey: Nutrition First Results – Food and Nutrients, 2011–12. Available at: http://www.ausstats.abs.gov.au/ausstats/subscriber.nsf/0/4683FD7315DFDFDBCA257D080014F9E0/$File/australian%20health%20survey%20nutrition%20first%20results%20-%20food%20and%20nutrients,%202011-12.pdf (accessed November 2015).
49. Osler M, Heitmann BL & Schroll M (1997) Ten year trends in the dietary habits of Danish men and women. Cohort and cross-sectional data. *Eur J Clin Nutr* **51**, 535–541.
50. Liew G, Mitchell P, Wong T *et al.* (2013) The association of aspirin use with age-related macular degeneration. *JAMA Intern Med* **173**, 258–264.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

PUBLIC HEALTH NUTRITION

Vegetarianism and breast, colorectal and prostate cancer risk: an overview and meta-analysis of cohort studies

J. Godos,[1] F. Bella,[1] S. Sciacca,[1] F. Galvano[2] & G. Grosso[1]

[1]Integrated Cancer Registry of Catania-Messina-Siracusa-Enna, Azienda Ospedaliero Universitaria Policlinico Vittorio Emanuele, Catania, Italy
[2]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

Keywords
breast cancer, colorectal cancer, epidemiology, meta-analysis, prostate cancer, vegetarian diet, vegetarianism.

Correspondence
G. Grosso, Integrated Cancer Registry of Catania-Messina-Siracusa-Enna, Via S. Sofia 85, 95123 Catania, Italy.
Tel.: +39 0953782182
Fax: +39 0953782177
E-mail: giuseppe.grosso@studium.unict.it

How to cite this article
Godos J., Bella F., Sciacca S., Galvano F. & Grosso G. (2017) Vegetarianism and breast, colorectal and prostate cancer risk: an overview and meta-analysis of cohort studies. *J Hum Nutr Diet*. **30**, 349–359
doi: 10.1111/jhn.12426

Abstract

Background: Vegetarian diets may be associated with certain benefits toward human health, although current evidence is scarce and contrasting. In the present study, a systematic review and meta-analysis of prospective cohort studies was performed with respect to the association between vegetarian diets and breast, colorectal and prostate cancer risk.
Methods: Studies were systematically searched in Pubmed and EMBASE electronic databases. Eligible studies had a prospective design and compared vegetarian, semi- and pesco-vegetarian diets with a non-vegetarian diet. Random-effects models were applied to calculate relative risks (RRs) of cancer between diets. Statistical heterogeneity and publication bias were explored.
Results: A total of nine studies were included in the meta-analysis. Studies were conducted on six cohorts accounting for 686 629 individuals, and 3441, 4062 and 1935 cases of breast, colorectal and prostate cancer, respectively. None of the analyses showed a significant association of vegetarian diet and a lower risk of either breast, colorectal, and prostate cancer compared to a non-vegetarian diet. By contrast, a lower risk of colorectal cancer was associated with a semi-vegetarian diet (RR = 0.86, 95% confidence interval = 0.79–0.94; I^2 = 0%, $P_{\text{heterogeneity}}$ = 0.82) and a pesco-vegetarian diet (RR = 0.67, 95% confidence interval = 0.53, 0.83; I^2 = 0%, $P_{\text{heterogeneity}}$ = 0.46) compared to a non-vegetarian diet. The subgroup analysis by cancer localisation showed no differences in summary risk estimates between colon and rectal cancer.
Conclusions: A summary of the existing evidence from cohort studies on vegetarian diets showed that complete exclusion of any source of protein from the diet is not associated with further benefits for human health.

Introduction

Cancer is a major malignancy worldwide and a common cause of death in both men and women [(1)]. Among the most frequent and deadly cancers, colorectal cancer, breast cancer in women, and prostate cancer in men, are the most representative [(1)]. Current knowledge suggests that extrinsic environmental factors are the main contributors to carcinogenesis and, among them, diet could play a key role in modifying the risk of cancer [(2)]. Compounds characterising dietary content may potentially exert protective and adverse action toward cancer risk as a result of direct and indirect effects [(3)]. Plant-based dietary patterns high in fruit and vegetables are rich in fibre [(4)], antioxidant compounds and healthy fatty acids, both monounsaturated fatty acids and polyunsaturated fatty acids (PUFA), which are associated with a decreased risk of cancer [(5,6)]. By contrast, diets characterised by processed fat foods, alcoholic beverages and sweets, rich in trans-fatty acids, alcohol and refined carbohydrates, have been related with an increased risk of cancer, mainly but not limited to digestive tract cancers [(7–9)].

Following the International Agency for Research on Cancer (IARC) classification of consumption of red meat

as 'probably carcinogenic to humans' and of processed meat as 'carcinogenic to humans', vegetarian diets have gained particular attention from the general population, despite current scientific evidence of their effects on human health still being scarce [10]. A vegetarian diet is defined as a dietary profile characterised by abstention from consuming meat (including red meat, fish and poultry) [11]. From a public health point of view, it is unclear whether the restrictive approach related to vegetarian diets (i.e. 'no meat consumption') would result in better health outcomes than plant-based dietary guidelines. Indeed, there is no comprehensive evidence demonstrating that individuals would better benefit from a vegetarian rather than other 'healthy' and sustainable dietary patterns. A dated pooled analysis of cohort studies conducted to explore the association between vegetarian diets and mortality showed no significant differences between vegetarians and non-vegetarians [12]. A more recent study attempted to investigate noncommunicable disease risk and mortality-related outcomes in relation to vegetarian diets, suggesting that an association with decreased incidence and mortality of cardiovascular disease and cancer may exist [13]. However, updated findings have been published recently and there is no summary analysis comparing meat eaters and vegetarians in relation to risk of individual cancers. Thus, the present study aimed to summarise evidence on vegetarian diets and the risk of breast, colorectal and prostate cancer in cohort studies.

Materials and methods

Study search

A systematic search on PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and EMBASE (http://www.embase.com/) databases of English language studies published up to March 2016 was performed. Search terms included the following keywords, used in combination: 'vegetarian', 'vegetarianism', 'vegetarian diet', 'dietary pattern', 'cancer' and their variants. Inclusion criteria were: (i) having a prospective cohort study design; (ii) evaluating the association between adoption of vegetarian compared to a non-vegetarian diet and the risk of breast, colorectal, or prostate cancer; (iii) assessed and reported a measure of association for the outcome considered as hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs). Reference lists of included studies were scrutinised for any article not previously identified. The selection process was independently performed by two investigators.

Data extraction and study quality assessment

Data were extracted from selected studies using a standardised extraction form. The information collected comprised: (i) author name; (ii) year of publication; (iii) cohort name, length of follow-up, country; (iv) number, sex, and age of participants and cases; (v) reference category and HR and 95% CI for the other categories of exposure; and (vi) covariates used in adjustments.

The Newcastle-Ottawa Quality Assessment Scale was used to assess the quality of each study [14]. The instrument consists of three domains indicating the study quality as: selection (4 points), comparability (2 points) and outcome (3 points) for a total score of 9 points (with 9 representing the highest quality). Studies scoring 7–9 points, 3–6 points and 0–3 points were identified as high, moderate and low quality, respectively.

Statistical analysis

When more than one study was published on the same cohort or group of patients, only the most comprehensive or the most updated was selected to perform the meta-analyses. Random-effects models were used to calculate risk ratios (RRs) with 95% CIs for various qualitative categories of exposure (i.e., meat eaters versus vegetarians). The risk estimate from the most fully adjusted models in the analysis of the pooled RR was used. Heterogeneity was assessed using the Q test and I^2 statistic. The level of significance for the Q test was defined as $P < 0.10$. The I^2 statistic represented the amount of total variation that could be attributed to heterogeneity. $I^2 > 50$ indicates high heterogeneity. When data were sufficient to perform subgroup analyses, the following variables were tested as a potential source of heterogeneity: menopausal status for breast cancer, cancer topography (i.e. colon and rectum) and sex for colorectal cancer, and stadium (i.e. advanced) for prostate cancer risk. Publication bias was evaluated by a visual investigation of funnel plots for potential asymmetry. All analyses were performed using REVMAN, version 5.2 (The Nordic Cochrane Centre, Copenhagen, Denmark).

Results

Out of the 93 unique citations, 75 were excluded after abstract examination and nine were excluded after full-text reading for the following reasons: five studies explored cancer mortality; two studies explored total cancer incidence risk; and two studies were overlapping (Fig. 1). This selection process led to the consideration of nine studies [15–23] to be included in this meta-analysis.

Study characteristics

The main characteristics of the study included are presented in Table 1. Cohorts investigated included the

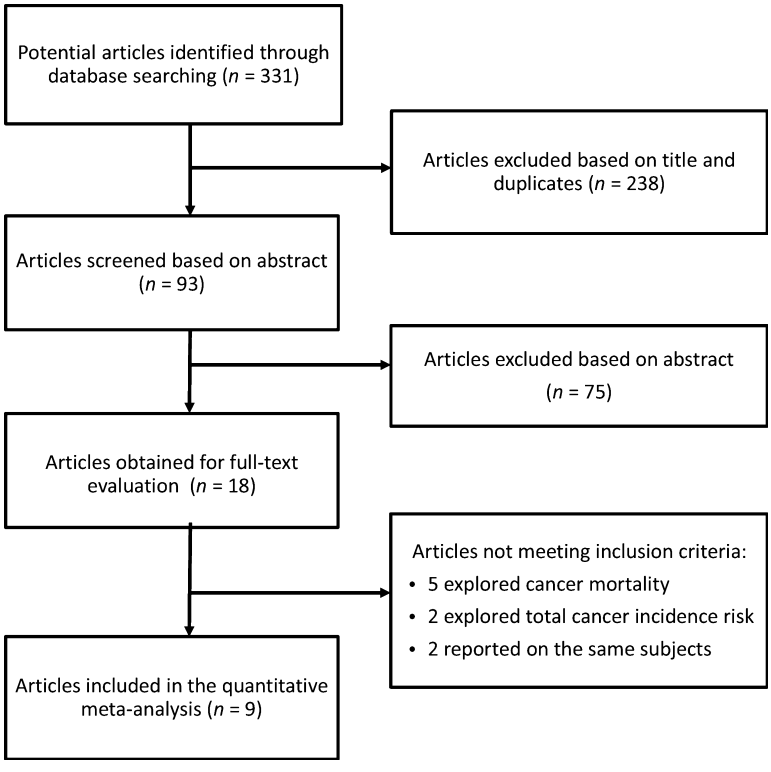

Figure 1 Study selection flow chart.

National Institutes of Health/American Association of Retired Persons (NIH-AARP) Diet and Health Study [16], the Adventist Health Study-2 (AHS-2) [20,22,23], the Oxford Vegetarian Study (OVS) and the European Prospective Investigation into Cancer and Nutrition (EPIC)-Oxford cohort [15,18], the UK Women Cohort Study (UKWHS) [17], and the Netherlands Cohort Study-Meat Investigation Cohort (NLCS-MIC) [19,21], accounting for a total of 686 629 individuals. Two studies on breast cancer [15,18] were conducted on the same cohort but were included in two different analysis: one [18] had a larger sample, which was suitable for the general analysis; the other [15] had specific information on pre- and post-menopausal cancer risk, which was used for the subgroup analysis. The follow-up periods ranged between 5 and 20 years. All studies adjusted for variables potentially related with cancer incidence, including sex, body mass index, smoking status, physical activity, energy intake, and hormonal and parity status in women. Study quality was high for all articles included in the analyses.

Cohort profiles

Two cohorts were conducted in the North America and four in Europe. Among the former, the NIH-AARP Diet and Health Study was the largest included in this meta-analysis [16]: the cohort was established in 1995–1996 and involved 492 306 men and women above 50 years of age from six US states. Incident cases of cancer were identified by linkage between the NIH-AARP cohort membership and cancer registry databases of the targeted states. The AHS-2 was a cohort study of 90422 members of the Seventh-Day Adventist churches over all US states and Canada [20,22,23]; their religious beliefs discourage the consumption of meat (especially pork) and recommend abstinence from alcoholic beverages, tobacco and illegal drugs. Cancer cases were identified by computer-matching identifying information from AHS-2 study subjects to lists of cases in state cancer registries. Among the cohorts conducted in Europe, participants of the OVS (n = 11 140) were recruited throughout the UK between 1980 and 1984 through advertisements, the news media and word of mouth, whereas the EPIC-Oxford ones were recruited between 1993 and 1999 by general practitioners (n = 7421) and by mail (n = 57 990) [15,18]. Participants in both studies were followed until 31 December 2010 by record linkage with the UK National Health Service (NHS) Central Register, which provides information on cancer diagnoses and all deaths. Women of the UKWHS were responders to a direct mail survey of the World Cancer Research Fund (WCRF), with 35 372 women aged 35–69 years responders from England, Wales and Scotland [17]. Subjects were flagged with the NHS Central Register for cancer and death notification. The NLCS-MIC started in September 1986 and included 12 852 men and women who were 55–69 years at baseline and

Table 1 Main characteristics of the cohort studies conducted on vegetarian diets and cancer risk included in the meta-analysis

Author (year)	Cancer site	Study cohort, f-up (country)	Number of individuals or controls	Number of cases	Age range, years	Dietary assessment	Factors adjustment	Study quality
Travis (2008) [15]	Breast	EPIC–Oxford cohort, 7.4 years (UK)	37 643	585	20–89	FFQ, 45 items	Height, BMI, age at menarche, age at first birth and parity, alcohol consumption, daily energy intake, menopausal status, and current hormone replacement therapy	High
Wirfalt (2009) [16]	Colon and rectal	NIH-AARP Diet and Health Study 1995–2000, 5 years (USA)	492 306 (293 576 men and 198 730 women)	In men, 631 rectal and 1539 olon. In women 258 rectal and 707 colon	50–71	FFQ, 181 items	Age, BMI, education, ethnicity, smoking, leisure time physical activity and total energy. In women also adjusted for hormone replacement therapy	High
Cade (2010) [17]	Breast	UKWCS, 9 years (UK)	33 725	783	35–69	FFQ, 217 items	Age, energy intake, menopausal status, calorie adjusted fat, BMI, physical activity, hormone replacement therapy use, smoking status, parity, age at menarche, ethanol, total days breast feeding, socioeconomic class and level of education	High
Key (2014)* [18]	Colorectal, breast, and prostate	OVS and EPIC–Oxford cohort, 14.9 years (UK)	61 647 (15 594 men and 46 053 women)	579 colorectal, 1454 breast, 457 prostate	20–89	FFQ, 45 items (Oxford Vegetarian Study); FFQ, 130 items (EPIC-Oxford)	Smoking, alcohol consumption, physical activity level, BMI; + parity and oral contraceptive use for breast cancer	High
Orlich (2015) [20]	Colorectal	AHS-2, 7.3 years (USA and Canada)	77 659	490	≥25	FFQ, 200 items	Age, sex, race, educational level, moderate or vigorous exercise, smoking, alcohol, family history of colorectal cancer, history of peptic ulcer, history of inflammatory bowel disease, treatment for diabetes mellitus within the past year, aspirin use, statin therapy, prior colonoscopy or flexible sigmoidoscopy, supplemental calcium consumption, supplemental vitamin D, dietary energy, hormone therapy among menopausal women, BMI, fibre intake	High

Table 1 Continued

Author (year)	Cancer site	Study cohort, f-up (country)	Number of individuals or controls	Number of cases	Age range, years	Dietary assessment	Factors adjustment	Study quality
Tantamango-Bartley (2016)	Prostate	AHS-2, 7.8 years (USA and Canada)	33 715	1079 (237 advanced)	≥30	FFQ, 200 items	Race, family history of prostate cancer, education, screening for prostate cancer, energy intake and BMI	High
Gilsing (2015) [19]	Colorectal	NLCS-MIC, 20.3 years (Netherlands)	10 210	437 colorectum (307 colon, 92 rectum)	55–69	FFQ, 150 items	Age, sex, total energy intake, cigarette smoking, alcohol consumption, BMI, non-occupational physical activity and level of education	High
Gilsing (2016) [21]	Prostate and breast†	NLCS-MIC, 20.3 years (Netherlands)	11 082	312 breast, 399 prostate (136 advanced)	55–69	FFQ, 150 items	Age, total energy intake, cigarette smoking, frequency of smoking, duration of smoking, alcohol consumption, BMI, non-occupational physical activity, and level of education; + family history of breast cancer, age menarche, age menopause, age first child, hormone replacement therapy, use of oral contraceptives and number of children for breast cancer; + family history of prostate cancer for prostate cancer	High
Penniecook-Sawyers (2016) [22]	Breast	AHS-2, 7.8 years (USA and Canada)	50 404	892	≥30	FFQ, 200 items	Race, height, physical activity, family history of cancer, mammography in the last 2 years after age 42 years, age at menopause, age at menarche, birth control pills, hormone replacement therapy, age at first child, number of children, breastfeeding, educational level, smoking, alcohol, BMI	High

AHS-2, Adventist Health Study 2; BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; NIH-AARP, National Institutes of Health/American Association of Retired Persons; NLCS-MIC, Netherlands Cohort Study – Meat Investigation Cohort; OVS, Oxford Vegetarian Study; UKWCS, UK Women's Cohort Study.
*Also included total cancers and several other cancer sites.
†Also included lung cancer.

monitored for cancer occurrence by repeated record linkage to the Netherlands Cancer Registry, the Dutch Pathology Registry and the cause of death registry [19,21].

Dietary patterns

All cohort studies used food frequency questionnaires to characterise the diet of participants. In all studies but one, diet characteristics were based on the response frequencies of key dietary components: pure vegetarian diet characterised by eating meat less than once per month; semi-vegetarian diet characterised by low consumption of meat (more than once per month but less than once per week); pesco-vegetarian diet characterised by consumption of fish more than once per month; and non-vegetarian diet characterised by eating meat more than once per week [17–23]. One study explored dietary patterns through cluster analysis and compared the consumption of 'several foods' with a dietary pattern characterised by 'fruit and vegetables' that we considered as 'semi-vegetarian'. In this meta-analysis, we compared a non-vegetarian diet with: (i) pure vegetarian; (ii) semi-vegetarian; and (ii) pesco-vegetarian diets.

Vegetarian diets and breast cancer risk

Four datasets from four studies [17,18,21,22] were analysed to test the association of vegetarian compared to a non-vegetarian diet and breast cancer risk (Fig. 2). The cohorts included the OVS and the EPIC-Oxford cohort [18], the UKWCS [17], the NLCS-MIC [21] and the AHS-2 [22], accounting for more than 35 000 individuals (the exact number was not calculated because one study did not provide the number of women) and 3441 breast cancer cases examined. The analysis showed a nonsignificant decreased risk of breast cancer for vegetarian compared to a non-vegetarian diet (RR = 0.96, 95% CI = 0.88–1.05). There was no evidence of heterogeneity (I^2 = 0%, $P_{heterogeneity}$ = 0.93) or publication bias as asymmetry of funnel plot (see Supporting information, Fig. S1). Subgroup analysis by menopausal status showed similar findings, with no significant results in premenopausal (RR = 0.99, 95% CI: 0.82, 1.20; I^2 = 0%, $P_{heterogeneity}$ = 0.63) and postmenopausal women (RR = 0.93, 95% CI: 0.81, 1.06; I^2 = 0%, $P_{heterogeneity}$ = 0.55).

The same cohorts [17,18,21,22] also evaluated the association of pesco-vegetarian compared to a non-vegetarian diet and breast cancer risk (Fig. 2). The analysis showed mainly null results, with no heterogeneity or asymmetry of funnel plot (see Supporting information, Fig. S1) also in the subgroup analysis by menopausal status (data not shown). The analysis on semi-vegetarian compared to a non-vegetarian diet was conducted on only two studies [21,22] with no significant findings (Fig. 2; see also Supporting information, Fig. S1).

Vegetarian diets and colorectal cancer risk

The association of vegetarian compared to a non-vegetarian diet and colorectal cancer risk was explored in three

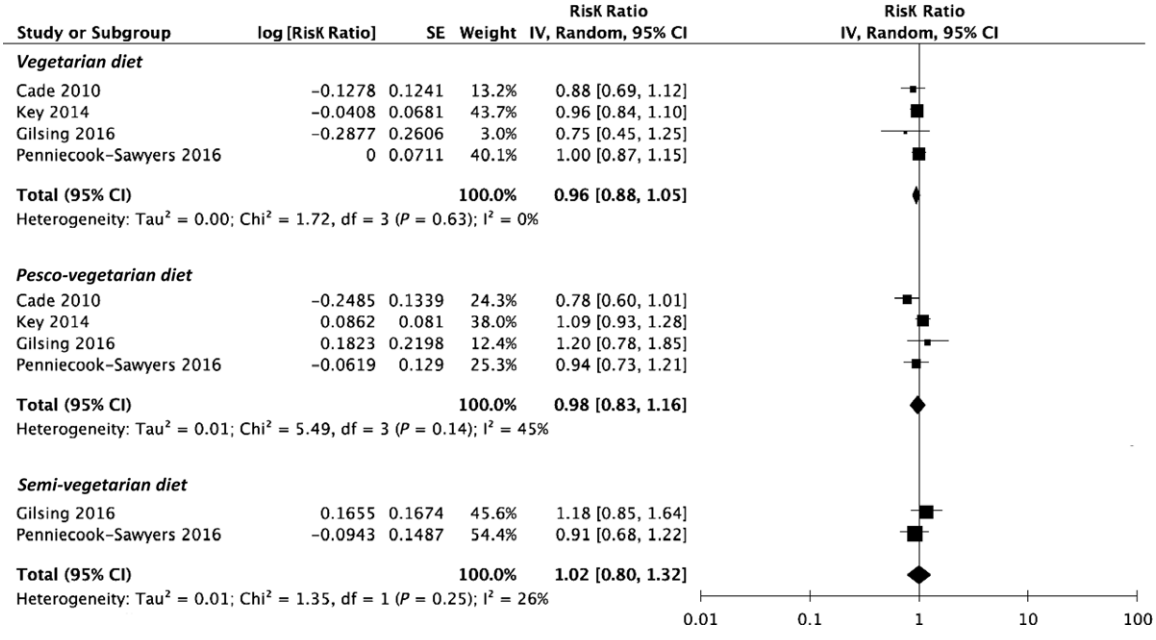

Figure 2 Forest plot of prospective cohort studies evaluating summary risk ratios of breast cancer by adoption of vegetarian, pesco-vegetarian and semi-vegetarian versus non-vegetarian diet (reference). CI, confidence interval.

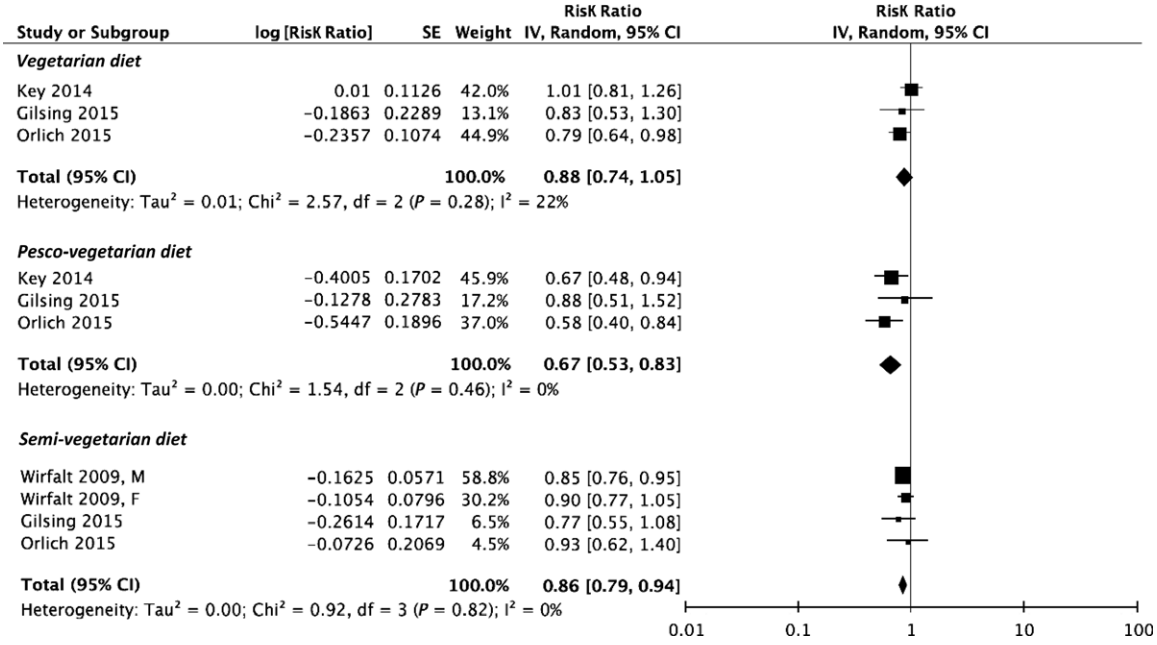

Figure 3 Forest plot of prospective cohort studies evaluating summary risk ratios of colorectal cancer by adoption of vegetarian, pesco-vegetarian and semi-vegetarian versus non-vegetarian diet (reference). CI, confidence interval.

studies [18–20], including the OVS and the EPIC-Oxford cohort, the NLCS-MIC and the AHS-2, for a total of 61 647 individuals and 1056 cases (Fig. 3). The analysis showed a nonsignificant decreased risk of colorectal cancer (RR = 0.88, 95% CI = 0.74–1.05) with no evidence of heterogeneity (I^2 = 22%, $P_{heterogeneity}$ = 0.28) or asymmetry of funnel plot (see Supporting information, Fig. S1). A subgroup analyses by cancer localisation showed no significant difference for colon (RR = 0.91, 95% CI = 0.77–1.08; I^2 = 0%, $P_{heterogeneity}$ = 0.51) and rectal cancer risk (RR = 0.78, 95% CI = 0.46–1.33; I^2 = 63%, $P_{heterogeneity}$ = 0.07). Heterogeneity was a result of the study on the OVS and the EPIC-Oxford cohort [18], despite there being no apparent reasons to explain this; however, exclusion of the study did not lead to significant results.

The association of a pesco-vegetarian diet with colorectal cancer risk was evaluated in three studies, including the four aforementioned cohorts (Fig. 3). The analysis showed the lowest summary risk estimates for adherence to a pesco-vegetarian compared to a non-vegetarian diet (RR = 0.67, 95% CI = 0.53–0.83) with either no evidence of heterogeneity (I^2 = 0%, $P_{heterogeneity}$ = 0.46) or asymmetry of funnel plot (see Supporting information, Fig. S1). By contrast, the subgroup analysis by cancer localisation led to inconclusive results, with a nonsignificant decreased risk of colon (RR = 0.74, 95% CI = 0.52–1.06; I^2 = 6%, $P_{heterogeneity}$ = 0.30) and rectal cancer (RR = 0.70, 95% CI = 0.43–1.13; I^2 = 0%, $P_{heterogeneity}$ = 0.97).

The analysis on semi-vegetarians included four datasets from three cohorts (the NLCS-MIC, the AHS-2, and the NIH-AARP Diet and Health Study) accounting for a total of 580 175 individuals and 4062 cases of colorectal cancer (Fig. 3). The analysis showed a significant association with reduced cancer risk (RR = 0.86, 95% CI = 0.79–0.94) with no evidence of heterogeneity (I^2 = 0%, $P_{heterogeneity}$ = 0.82) or asymmetry of funnel plot (see Supporting information, Fig. S1). However, the summary risk estimates were mainly driven by the two datasets (men and women) provided by the NIH-AARP Diet and Health Study; when these were excluded, the results of the other two cohorts were not significant. Findings were consistent also when considering colon (RR = 0.89, 95% CI = 0.81–0.98; I^2 = 0%, $P_{heterogeneity}$ = 0.86) and rectal cancer risk (RR = 0.81, 95% CI = 0.68–0.96; I^2 = 0%, $P_{heterogeneity}$ = 0.38) independently.

Vegetarian diets and prostatic cancer risk

The analysis on prostate cancer risk was conducted summarising risk estimates from four cohorts [18,21,23] (the OVS and the EPIC-Oxford cohort, the NLCS-MIC and the AHS-2) (Fig. 4) accounting for more than 50 000 individuals (the exact number was not calculated because one study did not provide the number of men), 1935 prostate and 373 advanced prostate cases. A vegetarian diet was associated with a nonsignificant decreased risk of prostate cancer (RR = 0.83, 95% CI = 0.63–1.10)

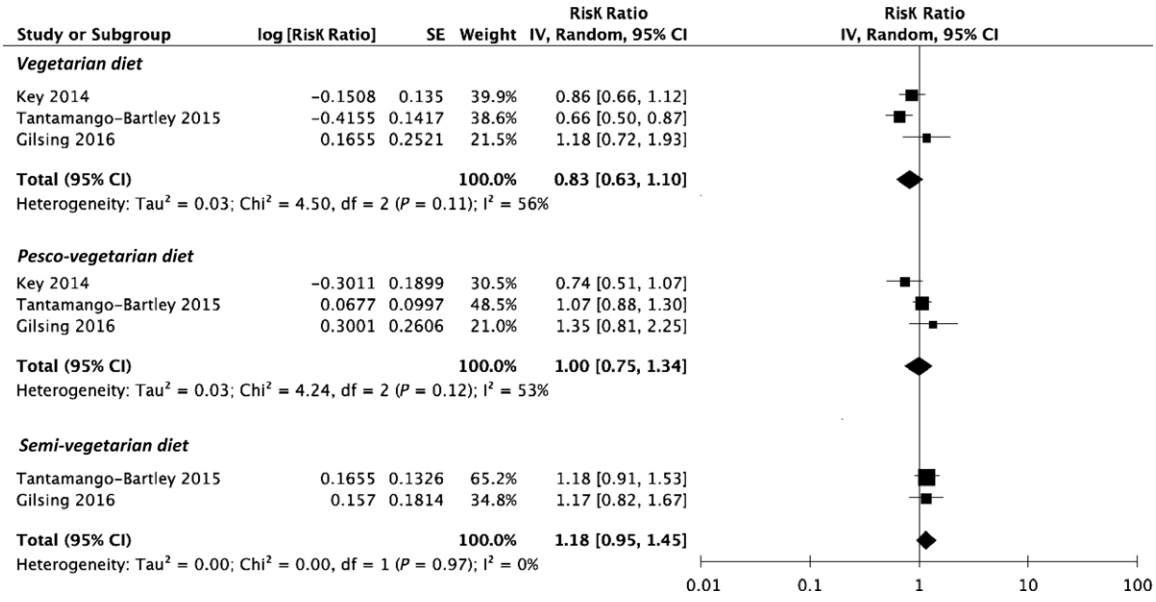

Figure 4 Forest plot of prospective cohort studies evaluating summary risk ratios of prostate cancer by adoption of vegetarian, pesco-vegetarian and semi-vegetarian versus non-vegetarian diet (reference). CI, confidence interval.

with some evidence of heterogeneity ($I^2 = 56\%$, $P_{heterogeneity} = 0.11$) but not of asymmetry of funnel plot (see Supporting information, Fig. S1). Risk estimates ranged from significantly lower to a higher prostate cancer risk associated with a vegetarian compared to a non-vegetarian diet with no particular difference between the cohorts. Data from the same four cohorts [18,21,23] were used to calculate summary risk of prostate cancer for a pesco-vegetarian compared to a non-vegetarian diet, resulting in null findings (Fig. 4; see Supporting information, Fig. S1). Finally, data on semi-vegetarians were available only for two cohorts [21,23] (the NLCS-MIC and the AHS-2) resulting in no significant findings (Fig. 4; see Supporting information, Fig. S1).

A further analysis was conducted on advanced prostate cancer [21,23]. Vegetarian, semi- and pesco-vegetarian diets were associated with the risk of advanced prostate cancer (data not shown).

Discussion

The summary of existing evidence on the association between a pure vegetarian diet and the risk of cancer demonstrated scarce findings from prospective cohort studies. The results from the present meta-analysis are in line with previous pooled analyses on vegetarian diets and mortality risk (including some of the cohorts also presented in the present analysis) reporting no increased length of survival in vegetarians compared to non-vegetarians. However, we found that some benefits may be related to the adoption of a mainly plant-based dietary pattern because semi- and pesco-vegetarian diets were associated with a lower risk of colorectal cancer compared to a non-vegetarian diet. This finding confirm the corroborated hypothesis that dietary patterns rich in fruit and vegetables, accompanied by the consumption of fish, may exert benefits toward human health and, in particular, may lower the risk of colorectal cancer.

Reports in the scientific literature on vegetarian diets is scarce. Summary of risk estimates derived by existing cohort studies on vegetarian diets were weaker than those obtained from pesco-vegetarian dietary patterns, suggesting that the complete exclusion of any source of protein from the diet is not associated with further benefits for human health. These findings are supported by biological plausibility. Vegetarian diets are rich in fibre, magnesium, phytochemicals, antioxidants, vitamins C and E, Fe^{3+} , folic acid and *n*-6 PUFA, whereas they are low in cholesterol, total fat and saturated fatty acid, sodium, Fe^{2+}, zinc, vitamins A, B_{12} and D, and *n*-3 PUFA [24]. Antioxidant vitamins, phenolic compounds and PUFA may exert anti-inflammatory effects, as well as protective effects toward DNA damage by preventing oxidation and improving biological pathways related to cancer initiation, such as cell signalling, cell cycle regulation, angiogenesis and inflammation [25,26]. Thus, unbalanced vegetarian diets may be deleterious in terms of nutritional adequacy, and the exclusion of major food groups from the diet (i.e. not limited to meat, but also fish and animal-derived foods) may be likely to result in nutrient deficiencies [24]. However, compared to uncontrolled omnivorous diets, vegetarian diets have been reported to have a better quality [27], suggesting that nutritional adequacy may not be strictly related to the dietary profile but rather to the overall balance of food consumed.

The rationale behind the hypothesis that a plant-based dietary pattern could protect against cancer relies on the benefits of a high fibre and antioxidant intake characterising the dietary content of fruit, vegetables and whole grains [28–30]. Dietary fibre may exert anti-carcinogenic effects through a direct action in the gastrointestinal tract, by reducing transit time and the contact of carcinogens with the colonic mucosa, and increasing the binding of carcinogens and the production of short-chain fatty acids [31]. Fish may provide an adequate amount of *n*-3 PUFA, which exert anti-oxidant effects at a systemic level [32,33]. Nutraceuticals and functional food ingredients may also improve vascular health and improve metabolic disorders, which may be associated with an increased cancer risk [34]. Mechanistic studies are promising and support evidence from epidemiological studies. A decreased risk of colorectal cancer has been associated with the consumption of fruit and vegetables [35]. Findings from a pooled analysis of 14 cohorts show that fruit and vegetable intake was associated with rectal cancer risk [36]. In addition, the consumption of fish has been associated with a decreased risk of colorectal cancer, despite stratified analyses showing most of the association being with rectal cancer [37]. Studies on dietary patterns are able to capture various aspects of the dietary experience that is adopted by a population and may provide insights into the synergistic effect of several components of a diet [38]. The findings on colorectal cancer and adenomas (a cancer precursor) appear to be consistent in that high compared to low adherence to a plant-based dietary pattern could be associated with a decreased risk of disease, whereas high compared to low adherence to a 'Western' dietary pattern may result in the opposite outcomes [39,40]. Finally, adherence to the Mediterranean dietary pattern, which is characterised by the high consumption of plant-derived foods, olive oil and fish as the main source of fats, as well as low intakes of meat, has been associated with a decreased risk of cancer, including colorectal cancer [41,42]. When considering animal protein intake, dietary sources suggest that other components in protein-rich foods (i.e. sodium, nitrates and nitrites in processed red meat), in addition to protein content *per se*, may have a critical health effect [43].

The main issue when examining the role of dietary patterns is represented by the potential confounding factors related to the overall lifestyle associated with dietary choices. Lifestyle behaviours are complex and multidimensional, generallying tend to cluster into healthy or unhealthy, including with respect to smoking and alcohol drinking habits, physical activity levels, and dietary choices [44,45]. The outcome mostly affected by diet is body weight, which in turn is a major contributor to cancer risk [46]. In this context, exploring the relationship between diet and cancer is challenging because most of the aforementioned factors generally cluster and, more or less directly contribute to modifying cancer risk. For example, the consumption of animal protein has been associated with an increased risk of mortality only in participants with at least one of the unhealthy behaviours, including smoking, heavy alcohol intake, being overweight or obesity, and physical inactivity [43]. By contrast, when considering observational studies, a common feature found across populations is that vegetarians are likely to exhibit an overall healthier lifestyle compared to the general population [47]. Despite being far from definitive, the results of the present study provide some insights regarding this issue because the significant association between dietary patterns high in vegetables, fruit and fish and a lower risk of gastrointestinal cancers suggests that a qualitative role of the diet may affect locally the risk of malignancy; for example, by limiting oxidative stress, inflammation and the effects of carcinogens [48].

There are some other limitations that should be addressed. The main issue when considering results from the present study is that the number of studies was generally limited for all the meta-analyses performed. Despite the results are not definitive, the studies included large samples and the findings are quite consistent across datasets with no evidence of heterogeneity and publication bias, which could somehow offset this issue. Second, differences in the background characteristics of the populations included in the cohort studies may weaken the results. For example, part of the existing evidence on a vegetarian diet is derived by the AHS-2, which involved a group of individuals with religious beliefs influencing their lifestyle choices, according to which it is uncertain whether the observed outcomes could be replicated or even applied to the general population. Third, despite all studies used validated instruments to collect dietary data, with most of them not providing repeated measurements during the follow-up periods, thus not registering any possible change in diet over time. Finally, vegetarian diets may differ each other for type and content of fruit/vegetables and dietary sources of proteins; we were unable to add further variation of vegetarian diets (e.g. lacto-ovo-vegetarian) as a result of the limited data available, and future studies on variants of vegetarian diets are needed to improve current evidence and to better define the dietary profiles more likely to be associated to positive health outcomes.

In conclusion, plant-based and fish-based dietary patterns represent a healthy dietary choice compared to meat-based dietary patterns when considering cancer as an outcome. Significant associations were found with respect to risk of colorectal cancers, suggesting a possible direct role in the aetiological pathway of gastrointestinal

cancers. However, the limited evidence retrieved regarding pure vegetarian diets highlights the possibility that very low meat intake can be associated with a lower risk of cancer, despite there being no strong evidence that a total depletion of sources of protein from the diet improves the outcome under consideration. Further studies are needed to provide more evidence and to better investigate the potential causative roles. However, from a public health perspective, claims regarding vegetarian diets should be evaluated carefully before informing the general population.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
No funding declared.
JG and FB performed the searches and study analysis, and wrote the manuscript (equal contributions). SS and FG designed the study and provided critical revision. GG designed the study, performed the analysis and wrote the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with PRISMA guidelines.

References

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, Pain A *et al.* (2015) The Global Burden of Cancer 2013. *JAMA Oncol* **1**, 505–527.
2. Wu S, Powers S, Zhu W *et al.* (2016) Substantial contribution of extrinsic risk factors to cancer development. *Nature* **529**, 43–47.
3. Martinez ME, Marshall JR & Giovannucci E (2008) Diet and cancer prevention: the roles of observation and experimentation. *Nat Rev Cancer* **8**, 694–703.
4. Guine RP, Ferreira M, Correia P *et al.* (2016) Knowledge about dietary fibre: a fibre study framework. *Int J Food Sci Nutr* **67**, 707–714.
5. Giacosa A, Barale R, Bavaresco L *et al.* (2013) Cancer prevention in Europe: the Mediterranean diet as a protective choice. *Eur J Cancer Prev* **22**, 90–95.
6. D'Alessandro A, De Pergola G & Silvestris F (2016) Mediterranean Diet and cancer risk: an open issue. *Int J Food Sci Nutr* **67**, 593–605.
7. Bagnardi V, Rota M, Botteri E *et al.* (2015) Alcohol consumption and site-specific cancer risk: a comprehensive dose-response meta-analysis. *Br J Cancer* **112**, 580–593.
8. Wiseman M (2008) The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc* **67**, 253–256.
9. Bouvard V, Loomis D, Guyton KZ *et al.* (2015) Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* **16**, 1599–1600.
10. Li D (2014) Effect of the vegetarian diet on non-communicable diseases. *J Sci Food Agric* **94**, 169–173.
11. Leitzmann C (2014) Vegetarian nutrition: past, present, future. *Am J Clin Nutr* **100**(Suppl 1), 496S–502S.
12. Key TJ, Fraser GE, Thorogood M *et al.* (1999) Mortality in vegetarians and nonvegetarians: detailed findings from a collaborative analysis of 5 prospective studies. *Am J Clin Nutr* **70**, 516S–524S.
13. Dinu M, Abbate R, Gensini GF *et al.* (2016) Vegetarian, vegan diets and multiple health outcomes: a systematic review with meta-analysis of observational studies. *Crit Rev Food Sci Nutr* Feb 6:0. [Epub ahead of print].
14. Wells GASB, O'Connell D, Peterson J *et al.*, editors (1999) *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses*. Ottawa: Ottawa Health Research Institute.
15. Travis RC, Allen NE, Appleby PN *et al.* (2008) A prospective study of vegetarianism and isoflavone intake in relation to breast cancer risk in British women. *Int J Cancer* **122**, 705–710.
16. Wirfalt E, Midthune D, Reedy J *et al.* (2009) Associations between food patterns defined by cluster analysis and colorectal cancer incidence in the NIH-AARP diet and health study. *Eur J Clin Nutr* **63**, 707–717.
17. Cade JE, Taylor EF, Burley VJ *et al.* (2010) Common dietary patterns and risk of breast cancer: analysis from the United Kingdom Women's Cohort Study. *Nutr Cancer* **62**, 300–306.
18. Key TJ, Appleby PN, Crowe FL *et al.* (2014) Cancer in British vegetarians: updated analyses of 4998 incident cancers in a cohort of 32 491 meat eaters, 8612 fish eaters, 18 298 vegetarians, and 2246 vegans. *Am J Clin Nutr* **100** (Suppl 1), 378S–385S.
19. Gilsing AM, Schouten LJ, Goldbohm RA *et al.* (2015) Vegetarianism, low meat consumption and the risk of colorectal cancer in a population based cohort study. *Sci Rep* **5**, 13484.
20. Orlich MJ, Singh PN, Sabate J *et al.* (2015) Vegetarian dietary patterns and the risk of colorectal cancers. *JAMA Intern Med* **175**, 767–776.
21. Gilsing AM, Weijenberg MP, Goldbohm RA *et al.* (2016) Vegetarianism, low meat consumption and the risk of

lung, postmenopausal breast and prostate cancer in a population-based cohort study. *Eur J Clin Nutr* **70**, 723–729.
22. Penniecook-Sawyers JA, Jaceldo-Siegl K, Fan J *et al.* (2016) Vegetarian dietary patterns and the risk of breast cancer in a low-risk population. *Br J Nutr* **115**, 1790–1797.
23. Tantamango-Bartley Y, Knutsen SF, Knutsen R *et al.* (2016) Are strict vegetarians protected against prostate cancer? *Am J Clin Nutr* **103**, 153–160.
24. McEvoy CT, Temple N & Woodside JV (2012) Vegetarian diets, low-meat diets and health: a review. *Public Health Nutr* **15**, 2287–2294.
25. Grosso G, Bei R, Mistretta A *et al.* (2013) Effects of vitamin C on health: a review of evidence. *Front Biosci (Landmark Ed)* **18**, 1017–1029.
26. Fantini M, Benvenuto M, Masuelli L *et al.* (2015) *In vitro* and *in vivo* antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: perspectives on cancer treatment. *Int J Mol Sci* **16**, 9236–9282.
27. Clarys P, Deliens T, Huybrechts I *et al.* (2014) Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet. *Nutrients* **6**, 1318–1332.
28. Turati F, Rossi M, Pelucchi C *et al.* (2015) Fruit and vegetables and cancer risk: a review of southern European studies. *Br J Nutr* **113**(Suppl 2), S102–S110.
29. Mourouti N, Kontogianni MD, Papavagelis C *et al.* (2015) Diet and breast cancer: a systematic review. *Int J Food Sci Nutr* **66**, 1–42.
30. Chen CY, Kamil A & Blumberg JB (2015) Phytochemical composition and antioxidant capacity of whole wheat products. *Int J Food Sci Nutr* **66**, 63–70.
31. Grosso G, Buscemi S, Galvano F *et al.* (2013) Mediterranean diet and cancer: epidemiological evidence and mechanism of selected aspects. *BMC Surg* **13**(Suppl 2), S14.
32. Marventano S, Kolacz P, Castellano S *et al.* (2015) A review of recent evidence in human studies of n-3 and n-6 PUFA intake on cardiovascular disease, cancer, and depressive disorders: does the ratio really matter? *Int J Food Sci Nutr* **66**, 611–622.
33. Hirotani Y, Ozaki N, Tsuji Y *et al.* (2015) Effects of eicosapentaenoic acid on hepatic dyslipidemia and oxidative stress in high fat diet-induced steatosis. *Int J Food Sci Nutr* **66**, 569–573.
34. Scicchitano P, Cameli M, Maiello M *et al.* (2014) Nutraceuticals and dyslipidaemia: beyond the common therapeutics. *J Funct Foods* **6**, 11–32.
35. Aune D, Lau R, Chan DS *et al.* (2011) Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies. *Gastroenterology* **141**, 106–118.
36. Koushik A, Hunter DJ, Spiegelman D *et al.* (2007) Fruits, vegetables, and colon cancer risk in a pooled analysis of 14 cohort studies. *J Natl Cancer Inst* **99**, 1471–1483.
37. Wu S, Feng B, Li K *et al.* (2012) Fish consumption and colorectal cancer risk in humans: a systematic review and meta-analysis. *Am J Med* **125**, 551–559. e5
38. Edefonti V, Randi G, La Vecchia C *et al.* (2009) Dietary patterns and breast cancer: a review with focus on methodological issues. *Nutr Rev* **67**, 297–314.
39. Magalhaes B, Peleteiro B & Lunet N (2012) Dietary patterns and colorectal cancer: systematic review and meta-analysis. *Eur J Cancer Prev* **21**, 15–23.
40. Godos J, Bella F, Torrisi A *et al.* (2016) Dietary patterns and risk of colorectal adenoma: a systematic review and meta-analysis of observational studies. *J Hum Nutr Diet* Jul 14. doi: 10.1111/jhn.12395. [Epub ahead of print].
41. Schwingshackl L & Hoffmann G (2015) Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis of observational studies. *Cancer Med* **4**, 1933–1947.
42. Grosso G, Biondi A, Galvano F *et al.* (2014) Factors associated with colorectal cancer in the context of the Mediterranean diet: a case–control study. *Nutr Cancer* **66**, 558–565.
43. Song M, Fung TT, Hu FB *et al.* (2016) Association of animal and plant protein intake with all-cause and cause-specific mortality. *JAMA Intern Med* Aug 1. doi: 10.1001/jamainternmed.2016.4182. [Epub ahead of print].
44. Boniol M & Autier P (2010) Prevalence of main cancer lifestyle risk factors in Europe in 2000. *Eur J Cancer* **46**, 2534–2544.
45. Alvarez-Cubero MJ, Pascual-Geler M, Rivas A *et al.* (2015) Lifestyle and dietary factors in relation to prostate cancer risk. *Int J Food Sci Nutr* **66**, 805–810.
46. Baena R & Salinas P (2015) Diet and colorectal cancer. *Maturitas* **80**, 258–264.
47. Singh PN, Sabate J & Fraser GE (2003) Does low meat consumption increase life expectancy in humans? *Am J Clin Nutr* **78**, 526S–532S.
48. Bultman SJ (2016) Interplay between diet, gut microbiota, epigenetic events, and colorectal cancer. *Mol Nutr Food Res* May 3. doi: 10.1002/mnfr.201500902. [Epub ahead of print].

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1. Funnel plots of prospective cohort studies evaluating summary risk ratios of breast, colorectal and prostate cancer by adoption of vegetarian, pesco-vegetarian and semi-vegetarian versus non-vegetarian diet (reference).

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

PUBLIC HEALTH NUTRITION

Dietary intake in people consuming a reduced-carbohydrate diet in the National Diet and Nutrition Survey

N. Guess[1,2]

[1]Division of Diabetes and Nutritional Sciences, Kings College London, London, UK
[2]Department of Medicine, Imperial College London, London, UK

Keywords
fibre, fish, fruit, low-carbohydrate, meat, vegetables.

Correspondence
N. Guess, Division of Diabetes and Nutritional Sciences, Kings College London, Room 4.13, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK.
Tel.: +44 (0) 20 7848 4356
Fax: +44 (0) 20 7848 4171
E-mail: nicola.guess@kcl.ac.uk

How to cite this article
Guess N. (2017) Dietary intake in people consuming a reduced-carbohydrate diet in the National Diet and Nutrition Survey. *J Hum Nutr Diet*. **30**, 360–368
doi:10.1111/jhn.12429

Abstract

Background: Diets reduced or low in carbohydrates are becoming increasingly popular. The replacement foods and their accompanying nutrients determine the health effects of such diets. However, little is known about the dietary intake of people consuming reduced or low carbohydrate diets.
Methods: In this cross-sectional study, the dietary and nutrient intake of individuals aged 16–75 years consuming less than 40% of energy from carbohydrate (n = 430) was compared with individuals consuming equal to or more than 40% energy from carbohydrate (n = 1833) using the UK National Diet and Nutrition Survey.
Results: Those consuming less than 40% of total energy from carbohydrate reported a higher consumption of red and processed meat, butter, oily fish and vegetables, as well as a lower consumption of soft drinks and pulses, than those with a normal carbohydrate intake. After adjusting for socio-economic status, only red meat intake was different between the groups, and reached the maximum recommended daily intake daily intake. There were no significant differences in micronutrient intakes between the groups, although magnesium, selenium and potassium, along with fibre, were lower than recommended amounts across the cohort.
Conclusions: Individuals consuming reduced or low carbohydrate diets could benefit from replacing some red meats with white meats and vegetable sources of protein, and increasing vegetable intake.

Introduction

There has been widespread interest in low- or reduced carbohydrate (CHO) diets in the popular media [(1)] and increasingly within clinical research [(2,3)]. Terms such as low- or reduced CHO diets are limited because CHOs are a heterogeneous group of compounds, and the quality of CHO largely determines its metabolic effects [(4,5)]. Nevertheless, the majority of CHO consumed in most parts of the world is rapidly available starch [(4,6)], and there is accumulating data suggesting that limiting its consumption aids weight loss, at least over the short term [(2,3,7)] and may have some favourable metabolic effects, including decreased triglyceride and glucose concentrations, and increased high-density lipoprotein-cholesterol [(2,7)]. Nevertheless, some concerns about their long-term safety remain [(7,8)].

In reduced- or low-CHO diets, the CHO may be replaced by some protein but usually fat [(2,3,7)]. The replacement macronutrient or food may augment, or attenuate the beneficial effect of reducing carbohydrate on metabolic risk factors. For example, replacement of CHOs such as white rice or bread with fish, nuts or olive oil lowers low-density lipoprotein-cholesterol (LDL-C) and fasting triglycerides [(9,10)], whereas replacement with foods high in saturated fat such as butter, red meat or cream can increase LDL-C [(11)]. Furthermore, the consumption of foods high in saturated fat can lead to increases in visceral and liver fat, and decreases in insulin sensitivity even in the context of a very-low carbohydrate (ketogenic) diet, whereas foods high in unsaturated fat such as olive or vegetable oils have a neutral or beneficial effect on these important risk factors [(12–14)].

In addition, other nutritive and non-nutritive components of the replacement foods may be as (or more) important than the fatty acid composition. For example, the presence of nitrates and haem iron [15] in red meat may explain the association between their consumption and increased risk of colon cancer [16]. Similarly, the sodium and nitrate content of processed red meats may explain the relationship with increased risk of cardiovascular disease [17]. Conversely, the presence of micronutrients and non-nutritive compounds such as polyphenols may at least partly mediate the beneficial effects of nuts and extra-virgin olive oil on multiple aspects of human health [18].

Despite the importance of the replacement food to health and well-being, little is known about the types of foods consumed by individuals restricting dietary CHOs outside of clinical research. An online nonvalidated survey of people following a low-CHO diet [19] found that more than half of respondents reported increasing their intake of salad and greens, although the study did not measure absolute intake, micronutrient composition of the diet or other dietary factors. The present study therefore aims to determine the dietary and nutrient intake of individuals consuming fewer than 40% of energy from CHO.

Materials and methods

This is a cross-sectional study comparing dietary intake in individuals with a reduced carbohydrate diet (RCHO) with those with a normal intake (NCHO) in the National Diet and Nutrition Survey (NDNS) [20]. The 40% cut-off was chosen because this is below the current and previous recommendations of 50% and 47% of total energy from CHOs by the 2015 Scientific Advisory Committee on Nutrition's report on Carbohydrates and Health and Committee on the Medical Aspects of Food Policy report, respectively [21]. It is also the proportion of energy from carbohydrate used in a recent report on dietary treatment of obesity of a 'moderate low-CHO diet' [22].

National diet and nutrition survey

Subjects and study design

The NDNS is a national dietary survey carried out every 1.5 years across the UK. Full details of the methodology can be found in Chapter 2 of the full report [20]. In brief, a sample of people representative of the UK population was drawn from the Postcode Address File, a list of all the addresses in the UK. Information about the survey was posted to all selected addresses, followed by a face-to-face visit by an interviewer to recruit participants in the eligible age range. From each household, the interviewer randomly selected up to one adult and one child to take part. For the present study, only individuals aged 16–75 years were included. Other groups excluded included women who were pregnant or breastfeeding. This report presents the combined results from Years 1, 2, 3 and 4 of the NDNS (2008/2009–2011/2012. Ethical approval for the present study was obtained from the Oxfordshire A Research Ethics Committee. Research governance approval was sought for all participating NHS laboratories and obtained where required by each Research and Development Committee [20].

Dietary data

Participants were provided with a diary and asked to keep a record of everything they ate and drank in and outside the home over 4 days including both weekend days [20]. To ensure compliance, interviewers contacted the participants on the second or third day of data collection in person or over the telephone, aiming to provide encouragement and to collect any missing detail for foods [20]. Portion sizes were estimated using household measures and weights from labels. The diaries included photographs of 10 frequently consumed foods and pictures of life-size spoons and a life-size glass to aid accurate recording [20]. If participants did not know what type of food they had consumed, default foods were used; for example, for milk, this was semi-skimmed; for fat spread, this was reduced fat spread (not polyunsaturated). This method has been described in detail elsewhere [20,23].

Response rates

In total, 46% of the 21 573 addresses issued to interviewers were eligible for selection, although 9% of these refused to take part before the household selection could be carried out. Of 9858 eligible households, 56% of selected participants completed three or four dietary recording days in Year 1, 57% in Year 2, 53% in Year 3 and 55% in Year 4. Including only those aged 16–75 years of age left 2263 participants.

Socio-economic status

The NDNS dataset includes the Index of Multiple Deprivation (IMD), which is separated into quintiles. The IMD data are only available for England. Therefore, in the present study, adjustment for socio-economic status (SES) was carried out for the 1885 individuals residing in England.

Dietary analysis

Food and nutrient intakes were calculated using DINO (Diet In Nutrients Out), a dietary assessment system developed at the Human Nutrition Research Centre at Cambridge University [23]. The databank is based on

McCance and Widdowson's Composition of Foods series [24], Food Standards Agency *Food Portion Sizes* [25] and manufacturer's data where applicable. The database is also updated every year by the Food Standards Agency, including the creation of new food codes for novel or fortified food products, updates to existing food codes and the removal of foods that are no longer sold.

Food consumption and nutrient intake were analysed by sex and age group (11–18 years and 19–64 years) to provide percentages of reference nutrient intakes. Data were weighted to account for bias due to non-response or to differences in the probability of households or individuals being selected to take part [20,23].

Statistical analysis

The independent variable was created by splitting the dataset into those with a percentage of total energy of CHO less than or equal to/more than 40%. For normally distributed dependent variables, independent *t*-tests were used. The Mann–Whitney *U*-test was used to examine differences in median intake for nonparametric variables, such as particular foods and food groups. Where there were ties in the data, the asymptomatic *P* value is reported. Proportions between groups were compared using chi-squared tests. Analysis of covariance was used to assess differences in foods and food groups with SES as a covariate, for which the dependent variable was log-transformed, and histogram plots were used to check for normal distribution of residuals. The NDNS dataset provides information on whether individuals were following a particular diet, including vegetarian, vegan, diets for weight reduction and gain, cholesterol-reducing diets, low allergy diets, and others. These individuals were included but sensitivity analyses conducted with (n = 2263) and without (n = 2159) their data. Data analysis was carried out using SPSS, version 23 (IBM Corp., Armonk, NY, USA) $P < 0.0001$ was considered statistically significant throughout the present study to take into account the multiple testing of dependent variables.

Results

Participants

Baseline characteristics are shown in Table 1. Dietary data were provided by 2263 individuals, of whom 430 (20%) had a reported CHO intake of less than 40% of total energy (RCHO) and 1833 (80%) reported equal or more than 40% of total energy from CHO (NCHO). Less than half the NDNS sample agreed to provide biochemical data. Exact numbers for whom biochemical and anthropometric data are available are shown in Table 1. The NCHO group was significantly younger and had a higher body mass index and fasting plasma glucose compared to the RCHO group ($P < 0.0001$ for all comparisons). There were no differences in SES ($P = 0.12$).

Table 1 Baseline characteristics of study subjects

	RCHO (<40% energy from CHO)			NCHO (≥40% energy from CHO)			*P* value
	n			*n*			
Age (years)*		47	(37–59)		40	(25–55)	<0.0001
Sex (M : F)†	430	50 : 50		1833	43 : 57		0.011
SES (IMD score)†	358			1527			
0.5–8.5		27			22		0.12
8.5–13.8		23			21		
13.8–21.4		15			19		
21.4–34.2		17			19		
34.2–87.8		18			20		
Weight (kg)‡	397	79.1	(16.3)	1716	75.7	(16.7)	<0.0001
BMI (kg m^{-2})‡	394	27.8	(5.3)	1708	26.8	(5.6)	<0.0001
FPG (mmol L^{-1})*§	195	5.1	(4.8–5.6)	832	4.9	(4.6–5.3)	0.0001
HbA1c (mmol mol^{-1})*§	196	5.5	(5.3–5.8)	878	5.4	(5.2–5.7)	0.218
LDL (mmol L^{-1})*§	191	3.2	(2.6–3.8)	875	3.0	(2.4–3.7)	0.003
HDL (mmol L^{-1})*§	198	1.5	(1.2–1.9)	888	1.4	(1.2–1.7)	0.001
TG (mmol L^{-1})*§	197	1.1	(0.8–1.7)	888	1.1	(1.1–1.5)	0.085

BMI, body mass index; CHO, carbohydrate; FPG, fasting plasma glucose; HbA1c, Haemoglobin A1c; HDL, high-density lipoprotein cholesterol; IMD, Index of Multiple Deprivation; LDL, low-density lipoprotein cholesterol; NCHO, ≥40% of total energy from carbohydrate; RCHO, <40% of total energy from carbohydrate; SES, socio-economic status; TG, fasting triglycerides.

*Data shown as the median (interquartile range).

†Data shown as the percentage of study subjects.

‡Data shown as the mean (SD).

§Biochemical data were collected for approximately 46% of the RCHO group and 49% of the NCHO group.

Consumption of foods

People in the RCHO group not only reported a higher consumption of red meat, processed meat and butter, but also reported a higher consumption of vegetables and oily fish (Table 2). The RCHO group also reported a lower intake of soft drinks (not low-energy versions) and pulses ($P < 0.0001$ for all comparisons). After adjusting for multiple comparisons, there were no differences in reported intakes of other foods or food groups. There were no differences in the proportion of individuals consuming five or more fruits and vegetables per day (RCHO: 27% versus NCHO: 30%, $P = 0.102$).

Intake of macro- and micronutrients

As expected, people in the RCHO group reported a higher percentage of energy from protein, total fat and each fat class ($P < 0.0001$ for all comparisons) (Table 3). The RCHO group consumed less dietary fibre than the NCHO group, although both were under current recommendations. Non-milk extrinsic sugar (NMES) consumption was higher in both groups than current (2015) recommendations [21], although the RCHO group was within previous recommendations for NMES (<10% energy) in contrast to the NCHO group.

After controlling for multiple comparisons, there were no differences in intakes of micronutrients between the RCHO and NCHO diets. Consumption of magnesium, potassium, and selenium were below current recommended nutrient intakes (RNIs) for both groups (Fig. 1).

Adjustment for socio-economic status

After adjusting for SES, only the difference in red meat intake between groups remained significant ($P = 0.003$). The differences in vegetable intake were explained entirely by SES.

Sensitivity analysis

The results were not different when excluding individuals on restrictive diets.

Discussion

In this cross-sectional study, individuals consuming less than 40% of total energy from CHO (reduced carbohydrate; RCHO) consumed more red meat, processed meat, butter, oily fish and vegetables, as well as fewer soft drinks and pulses than those with a normal (equal or more than 40% of total energy) carbohydrate (NCHO) intake. After adjusting for SES, only red meat remained significantly different. There were no significant differences in white meat, total fibre, nuts and seeds or total fruit intake between the groups.

A widespread concern regarding low-CHO diets is that the replacement of CHO with foods high in saturated fat may result in an increase of LDL-C [8,11]. In the present study, the percentage of energy from saturated fat was significantly higher in the RCHO group. The most likely explanation for the increase or lack of LDL-C reduction despite the significant weight loss observed in low-CHO clinical studies is a result of the increased intake of foods such as butter and red meat. However, interpretation of

Table 2 Intakes of foods and food groups (grams per day) amongst study subjects

	RCHO (<40% energy) Median (g day^{-1})	IQR	NCHO (>40% energy) Median (g day^{-1})	IQR	*P* value
White meat	31.8	0.1–58.7	26.2	0–51.6	0.031
Red meat	91.0	48.4–131.4	54.5	25–89.9	<0.0001
Processed red meat	15.0	1.65–31.6	11.0	0–23.5	<0.0001
Bacon and ham	11.5	0–27.9	5.8	0–18.7	<0.0001
Sausages	0	0–30.0	5.8	0–15.0	<0.0001
Oily fish	0	0–25.0	0	0–314.7*	<0.0001
Nuts and seeds	0	0–137.5*	0	0–84.5*	0.002
Butter	0	0–7	0	0–2.5	<0.0001
Polyunsaturated oils	0	0–4.13*	0	0–11.6*	0.125
Fats and oils	0	0–17.8*	0	0–28.0*	0.014
Beans	0	0–11.1	0.8	0–20.0	<0.0001
Fruits	52.9	14.5–114.8	75	20.3–153.8	0.001
Vegetables	171.2	116.5–247.3	156.2	98.6–224.6	<0.0001
Soft drinks (not low-energy)	0	0–81.6	50	0–208.8	<0.0001

IGQ, interquartile range; NCHO, ≥40% of total energy from carbohydrate; RCHO, <40% of total energy from carbohydrate.
*Where the IQR was 0–0, the total range is shown.

Table 3 Intake of major nutrient groups amongst study subjects

	RCHO (n = 430) Mean	(SEM)	NCHO (n = 1833) Mean	(SEM)	P value
Energy intake [MJ (kcal)]	7.9 (1907)	6.4–9.6 (1516–2283)	7.1 (1705)	5.8–8.8 (1396–2103)	<0.0001
CHO (g)*	175.7	(141.6–216.4)	219.1	(178.8–270.8)	<0.0001
CHO%	35.3	0.2	48.6	0.1	<0.0001
Starch (g)*	104.2	(79.1–133)	125.2	(100–155.5)	<0.0001
Starch%	20.6	0.2	27.8	0.1	<0.0001
NSP (Englyst) (g)	12.3	0.2	13.8	0.1	<0.0001
Total sugar (g)*	71.6	(52.1–94.5)	92.3	(67.4–123.2)	<0.0001
Total sugar%	14.7	0.2	20.8	0.2	<0.0001
NMES (g)*	37.1	(24.6–60)	52.7	(33.4–80.7)	<0.0001
NMES%*	7.7	(5.5–10.7)	11.7	(8.1–16.2)	<0.0001
Fat (g)*	75.6	(56.9–94.0)	62.2	(47.2.–79.6)	<0.0001
Fat%	36.3	0.4	32.4	0.1	<0.0001
SFA (g)*	27.1	(20.1–35.6)	22.5	(16.7–29.8)	
SFA%	14.8	0.2	12.3	0.1	<0.0001
MUFA (g)*	27.3	(21.1–35.2)	22.5	(16.8–29.2)	
MUFA%	14.8	0.1	12.1	0.1	<0.0001
PUFA n-3 (g)*	2.0	(1.5–3.0)	1.7	(1.2–2.4)	
PUFA n-3%*	1.1	(0.8–1.5)	0.9	(0.7–1.1)	<0.0001
PUFA n-6 (g)*	10.1	(7.5–13.4)	8.7	(6.4–11.6)	
PUFA n-6%*	5.4	(4.5–6.5)	4.6	(3.9–5.6)	<0.0001
Protein (g)*	78.9	(66.7–94.1)	16.3	(14.6–19.6)	<0.0001
Protein%*	16.8	(14.6–19.6)	15.7	(13.7–18.1)	<0.0001
Alcohol (%)*	9.8	(3.4–15.8)	0	(0–4.4)	<0.0001

CHO, carbohydrate; MUFA, monounsaturated fat; NCHO, normal carbohydrate intake; NMES, non-milk extrinsic sugars; NSP, nonstarch polysaccharides; PUFA, polyunsaturated fat; RCHO, reduced carbohydrate; SFA, saturated fat; n-6, omega-6; n-3, omega-3.
*Data shown as the median (interquartile range).

low-CHO clinical studies is challenging because many do not report detailed food intake. Furthermore, a 28-day randomised control trial comparing red meat versus white meat in the context of a very low-CHO diet (<20% energy from CHO) found no differences in LDL-C between the groups [26]. It is also worth noting that

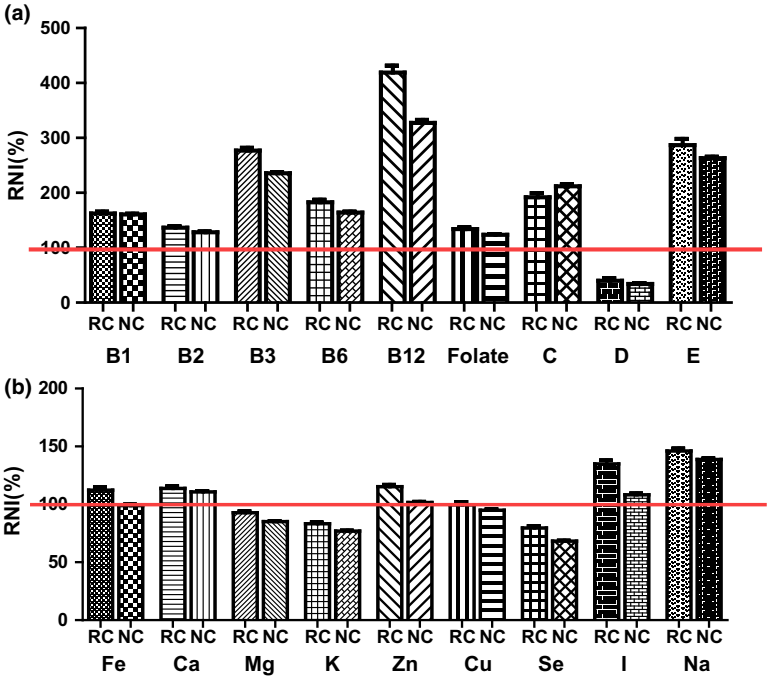

Figure 1 Percentage of reference nutrient intake for (a) vitamins and (b) minerals amongst the reduced-carbohydrate and normal-carbohydrate groups. Vitamins: B_1, thiamin; B_2, riboflavin; B_3, niacin; B_6, pyridoxine; B_{12}, cobalamin. Minerals: Fe, iron; Ca, calcium; Mg, magnesium; K, potassium; Zn, zinc; Cu, copper; Se, selenium; I, iodine; Na, sodium. RC, reduced carbohydrate group; NC, normal carbohydrate. Data are presented as the mean (SEM).

attenuation of or increases in total LDL-C may be offset by favourable alterations in cholesterol fractions, alongside increases in HDL-cholesterol and reductions in triglycerides when refined CHO is replaced with foods high in saturated fat [27,28].

In addition to a significantly higher consumption of red meat, individuals consuming a RCHO diet also reported significantly higher intake of processed meats, such as bacon and ham. The presence of compounds such as nitrites, advanced glycation end-products and aromatic hydrocarbons [28] in red and processed meat products have been linked to type 2 diabetes and some cancers, and illustrate the importance of the consideration of foods beyond their effects on the lipid profile [5,6,29,30]. Therefore, although red meat such as beef provides nutrients commonly lacking in the UK diet, such as iron, zinc and selenium, its overconsumption, particularly when processed, should be discouraged. The UK government recommends people who regularly consume >90 g day^{-1} of red meat should cut down to 70 g day^{-1} [31]. In the present study, the median intake of red meat was 91 g day^{-1} in the RCHO group, suggesting that these individuals could benefit from replacing some red meat with white meat such as chicken or fish.

In contrast to the relative controversy of replacing refined CHO with saturated fat, replacement of refined CHO with foods high in monounsaturated fats such as olive oil and avocado, or polyunsaturated fats, including sunflower oil, nuts and oily fish, has neutral or beneficial effects on a multitude of health factors [32–34]. Again, in addition to the fatty acid profile of these foods, nutritive and non-nutritive components such as polyphenols are also likely to be as important [18,35]. In the present study, the reported intakes of total oils, including oils high in unsaturated fats such as sunflower and olive oil, was relatively low (and lower than butter for both groups). This supports previous reports of the limited consumption of olive oil in the UK, which was 16.0 mL per week in 2013 [36]. Individuals choosing to minimise dietary CHOs may benefit from the replacement of some butter with a variety of unsaturated oils.

Other concerns expressed about the long-term health effects of low-CHO diets include the lack of essential nutrients [37]. In the present study, the RCHO group had a higher percentage RNI of most micronutrients (apart from vitamin C) than the NCHO group, although no differences were significant. Magnesium, potassium and selenium were lacking in both groups and may reflect the low intake of fruits, vegetables, seeds and nuts across the cohort. However, the RNIs for these minerals are also based on limited data [20] and thus should be interpreted with caution. It is again worth noting that the micronutrient content of the diet represents one aspect of nutrition, and non-nutrient components of a variety of foods are also vital to health [18–21,35].

The benefits of consuming a variety of high-fibre foods are well documented [38], and concerns have been raised that low or reduced CHO diets may be deficient in fibre [2]. In the present study, both groups consumed lower than recommended amounts of fibre, even without considering the recent increase in the dietary reference value to 30 g day^{-1} using the Association of Official Analytical Chemists (AOAC) method [21]. In the UK, the major sources of dietary fibre are breads, cereals and vegetables [39], whereas, per portion size, legumes, some fruits such as raspberries, apples and pears, vegetables such as artichokes, peas and broccoli, and high-bran cereals provide the most fibre in the diet [23]. Fewer than 30% of either group reported a consumption of five or more fruits and vegetables per day, and white bread, pasta, rice and potatoes were the largest contributors to the CHO content of both diets. It is also worth noting that recent high-profile studies examining the Mediterranean diet pattern also included more fibre than currently consumed in Western diets [3,40] and a very high fibre [28 g per 4.2 (28 g per 1000 kcal)] diet has been shown to improve glucose homeostasis compared to a reduced-CHO high-monounsaturated fat diet in people with type 2 diabetes [41]. Furthermore, there is some evidence to suggest that dietary fibre can offset some the increased risk of high-meat consumption on colon cancer [42]. Therefore, individuals who choose to consume a low-CHO diet should plan carefully to ensure they benefit from a range of dietary fibre from a variety of sources. This may be more important in low-income groups who consume more red meat, as well as fewer fruits and vegetables.

The detrimental impact of excessive NMES (now termed free sugar) consumption on multiple aspects of health was recognised by the 2015 SACN report, which now recommends that individuals aged >11 years consume no more than 5% energy from such sugars [21]. In the present study, both groups exceeded this recommendation, although the reported intake of NMES was significantly greater in the NCHO group, largely reflecting the differences in soft drink consumption. Although the RCHO group reported a consumption of total CHO g day^{-1} that was approximately 80% of that reported in the NCHO, the reported consumption of NMES g/day was approximately 70% of that in the NCHO group. This suggests that restriction of dietary CHO is associated with a preferential reduction in free sugar intake, which will likely be beneficial for health. Future studies should examine whether further restriction of CHO leads to

intakes of added sugars to levels within the current recommendations.

In the present study, the RCHO group consumed more energy from protein than the NCHO group, with a median intake of 16.8% of total energy. The long-term safety of high-protein diets has been questioned, based on the posited deleterious effect on renal function and bone density [37]. Although emerging evidence suggests that the effect of total protein on both bone and renal health may be overstated, this remains an understudied area [42]. Given the reported food intake in the present study, it is likely the majority of protein came from animal sources, with minimal vegetable protein from foods such as pulses. Diets high in animal protein have been linked with increased risk of morbidity, including inflammatory bowel disease and type 2 diabetes [43,44]. By contrast, diets high in pulses and other sources of vegetable protein have been linked to positive health outcomes and decreased mortality [45]. Given the association between plant-based diets and good health, both groups in the present study would benefit from increasing vegetable intake, to include where possible, vegetable sources of protein.

The RCHO had a higher body mass index and fasting plasma glucose than the NCHO group. The direction of this relationship cannot be established from a cross-sectional analysis. It may be the case that the RCHO group had altered their diets to reduce their body weight. Given the rising interest in RCHO diets in the UK [1], this is a real possibility. On the other hand, the RCHO (which was higher in energy) may have led to an increase in weight, alongside a slight but significant increase in fasting glucose. Although RCHO diets appear to be as effective as (if not superior to) NCHO diets for weight loss [2,3], some studies have suggested that RCHO may also lead to weight gain [46]. The available data do not suggest that RCHO diets increase the risk of obesity [47]. However, given the interest in RCHO diets, the longitudinal association of RCHO diets with the risk of obesity, type 2 diabetes and cardiovascular disease should be studied further.

This cross-sectional analysis has some strengths including a large sample size, which is representative of the UK population. The collection of both nutrient and food information also allows a practical analysis of the results. Given the recent interest in low carbohydrate diets, this in-depth analysis of food and nutrient intake, which is the first of its kind, may also provide some insight into typical dietary intake and, as such, can help public health education efforts. However, some weaknesses must be acknowledged. First, the categorisation of the RCHO and NCHO diets was based on observed differences at a point in time, and may not reflect the intakes of people who deliberately choose to consume a low carbohydrate diet. For example, the RCHO group was significantly older than the NCHO group and the differences may therefore reflect changes in dietary intake across the life course. Furthermore, differences in dietary intake from the NCHO may be greater in individuals consuming a very low-CHO or ketogenic diet. Future research should explore these questions. Furthermore, as a self-reported survey, the NDNS dataset suffers from under-reporting, with energy intake under-reported by approximately one-third in the doubly-labelled water method validation of this survey; see Appendix X in Tedstone *et al.* [20]. Validation studies in normal and overweight individuals suggest that fat and carbohydrate foods are particularly under-reported [48]. Although it might be suggested that under-reporting would be equally distributed across the RCHO and NCHO groups, individuals aiming to reduce foods such as carbohydrates (RCHO group) may under-report these to a greater degree because individuals may under-report food items they consider unhealthy [48]. Given that the reported median daily intake of absolute grammes of carbohydrate was 175.7 g day^{-1} (141.6–216.4 g day^{-1}), in the present study, the actual intake may therefore reach approximately 260 g day^{-1}, which is twice the recommended intake of a low-CHO [49]. The individuals included in the RCHO group in the present study may therefore not represent individuals who are aiming to reduce dietary CHO. A lower cut-off to <26% energy from CHO to define the RCHO group may have generated a group more likely to be intentionally reducing CHO intake, although this would have resulted in a RCHO group of 15 subjects. Finally, in contrast to the under-reporting of perceived unhealthy foods, perceived healthier foods such as fruits and vegetables may be over-reported [48], and thus the true intake across this cohort may be even lower than reported in the presented study.

In conclusion, the present study finds that people consuming less than 40% energy from CHO consume more red and processed meat, oily fish, butter and vegetables but fewer soft drinks and pulses than people consuming a normal CHO diet. After controlling for SES, only the consumption of red meat was different between the groups.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with STROBE guidelines.

Conflict of interests, source of funding and authorship

The author declares that there are no conflicts of interest.
The author has received grant funding from Diabetes UK and the National Obesity Forum and fellowship funding from Diabetes UK, the Winston Churchill Memorial Trust and the American Overseas Dietetics Association.
NG is solely responsible for the planning, analysis and write-up of the study. NG critically reviewed the manuscript and approved the final version submitted for publication.

References

1. Atkins and the Never-Ending Battle over Carbs. Vanessa Barford, BBC News Magazine. http://www.bbc.co.uk/news/magazine-22145709. Accessed July 2016.
2. Foster GD, Wyatt HR, Hill JO, *et al.* (2003) A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* **348**, 2082–2090.
3. Schwarzfuchs D, Golan R & Shai I (2012) Four-year follow-up after two-year dietary interventions. *N Engl J Med* **367**, 1373–1374.
4. Sun Q, Spiegelman D, van Dam RM, *et al.* (2010) White rice, brown rice, and risk of type 2 diabetes in US men and women. *Arch Intern Med* **170**, 961–969.
5. Livesey G, Taylor R, Hulshof T, *et al.* (2008) Glycemic response and health – a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. *Am J Clin Nutr* **87**, 258S–268S.
6. Malik VS, Willett WC & Hu FB (2013) Global obesity: trends, risk factors and policy implications. *Nat Rev Endocrinol* **9**, 13–27.
7. Nordmann AJ, Nordmann A, Briel M, *et al.* (2006) Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med* **166**, 285–293.
8. Bravata DM, Sanders L & Huang J (2003) Efficacy and safety of low-carbohydrate diets: a systematic review. *JAMA* **289**, 1837–1850.
9. Jenkins DJ, Wong JM, Kendall CW, *et al.* (2009) The effect of a plant-based low-carbohydrate ('Eco-Atkins') diet on body weight and blood lipid concentrations in hyperlipidemic subjects. *Arch Intern Med* **169**, 1046–1054.
10. Garg A, Bonanome A, Grundy SM, *et al.* (1988) Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* **319**, 829–834.
11. McAuley KA, Hopkins CM, Smith KJ, *et al.* (2005) Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. *Diabetologia* **48**, 8–16.
12. Bozzetto L, Prinster A, Annuzzi G, *et al.* (2012) Liver fat is reduced by an isoenergetic MUFA diet in a controlled randomized study in type 2 diabetic patients. *Diabetes Care* **35**, 1429–1435.
13. Summers LK, Fielding BA, Bradshaw HA, *et al.* (2002) Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* **45**, 369–377.
14. Fuehrlein BS, Rutenberg MS, Silver JN, *et al.* (2004) Differential metabolic effects of saturated versus polyunsaturated fats in ketogenic diets. *J Clin Endocrinol Metab* **89**, 1641–1645.
15. Bingham SA, Hughes R & Cross AJ (2002) Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. *J Nutr* **132**, 3522S–3525S.
16. English DR, MacInnis RJ, Hodge AM, *et al.* (2004) Red meat, chicken, and fish consumption and risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* **13**, 1509–1514.
17. Micha R, Wallace SK & Mozaffarian D (2010) Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. *Circulation* **121**, 2271–2283.
18. Konstantinidou V, Covas MI, Muñoz-Aguayo D, *et al.* (2010) In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. *FASEB J.* **24**, 2546–2557.
19. Feinman RD, Vernon MC & Westman EC (2006) Low carbohydrate diets in family practice: what can we learn from an internet-based support group. *Nutr J* **5**, 26.
20. Tedstone A, Lund V, Sanderson P, *et al.* (2015) *Carbohydrates and Health*. London: The Stationery Office.
21. Swedish Council on Technology Assessment in Health Care (SBU), Dietary Treatment in Obesity. Available at: http://www.sbu.se/globalassets/publikationer/content1/1/dietary_treatment_obesity.pdf. Accessed June, 2016.
22. Bates B, Lennox A, Prentice A, *et al.* (2014) National diet and nutrition survey results from years 1, 2, 3 and 4 (combined) of the rolling programme (2008/2009-2011/2012): A Survey Carried Out on Behalf of Public Health England and the Food Standards Agency: London, UK.
23. Whitton C, Nicholson SK, Roberts C, *et al.* (2011) National diet and nutrition survey: UK food consumption and nutrient intakes from the first year of the rolling programme and comparisons with previous surveys. *Br J Nutr* **106**, 1899–1914.

24. Food Standards Agency (2002) *McCance and Widdowson's The Composition of Foods*. 6th edn. Cambridge: Royal Society of Chemistry.
25. Food Standards Agency (1988) *Food Portion Sizes*. 3rd edn. London: The Stationery Office.
26. Cassady BA, Charboneau NL, Brys EE, *et al.* (2007) Effects of low carbohydrate diets high in red meats or poultry, fish and shellfish on plasma lipids and weight loss. *Nutr Metab* **4**, 23.
27. Westman EC, Yancy WS Jr, Olsen MK, *et al.* (2006) Effect of a low-carbohydrate, ketogenic diet program compared to a low-fat diet on fasting lipoprotein subclasses. *Int J Cardiol* **110**, 212–216.
28. Bueno NB, de Melo IS, de Oliveira SL, *et al.* (2013) Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *Br J Nutr* **110**, 1178–1187.
29. Abid Z, Cross AJ & Sinha R (2014) Meat, dairy, and cancer. *Am J Clin Nutr* **100**, 386S–393S.
30. Schulze MB, Manson JE, Willett WC, *et al.* (2003) Processed meat intake and incidence of Type 2 diabetes in younger and middle-aged women. *Diabetologia* **46**, 1465–1473.
31. Department of Health (2011) Red meat link to bowel cancer.
32. Brehm BJ, Lattin BL, Summer SS, *et al.* (2009) One-year comparison of a high-monounsaturated fat diet with a high-carbohydrate diet in type 2 diabetes. *Diabetes Care* **32**, 215–220.
33. Berglund L, Lefevre M, Ginsberg HN, *et al.* (2007) Comparison of monounsaturated fat with carbohydrates as a replacement for saturated fat in subjects with a high metabolic risk profile: studies in the fasting and postprandial states. *Am J Clin Nutr* **86**, 1611–1620.
34. Siri-Tarino PW, Sun Q, Hu FB, *et al.* (2010) Saturated fat, carbohydrate, and cardiovascular disease. *Am J Clin Nutr* **91**, 502–509.
35. Chiva-Blanch G, Urpi-Sarda M, Ros E, *et al.* (2013) Effects of red wine polyphenols and alcohol on glucose metabolism and the lipid profile: a randomized clinical trial. *Clin Nutr* **32**, 200–206.
36. Olive oil consumption in the UK. Available at: http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures. Accessed, June 2016.
37. Dyson P (2015) *Diabetes Ther* **6**, 411–424.
38. Fardet A (2010) New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr Res Rev* **23**, 65–134.
39. Hoare J, Henderson L, Bates CJ, *et al.* (2004) National diet and nutrition survey: adults aged 19 to 64 years volume 5: summary report. The Stationary Office, London, UK.
40. Estruch R, Ros E, Salas-Salvadó J, *et al.* (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* **368**, 1279–1290.
41. De Natale C, Annuzzi G, Bozzetto L, *et al.* (2009) Effects of a plant-based high-carbohydrate/high-fiber diet versus high-monounsaturated fat/low-carbohydrate diet on postprandial lipids in type 2 diabetic patients. *Diabetes Care* **32**, 2168–2173.
42. Johnstone AM (2012) Safety and efficacy of high-protein diets for weight loss. *Proc Nutr Soc* **71**, 339–349.
43. Jantchou P, Morois S, Clavel-Chapelon F, *et al.* (2010) Animal protein intake and risk of inflammatory bowel disease: the E3N prospective study. *Am J Gastroenterol* **105**, 2195–2201.
44. Sluijs I, Beulens JW, van der A DL, *et al.* (2010) Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. *Diabetes Care* **33**, 43–48.
45. Sievenpiper JL, Kendall CW, Esfahani A, *et al.* (2009) Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. *Diabetologia* **52**, 1479–1495.
46. Mobbs CV, Mastaitis J, Yen K, *et al.* (2007) Low-carbohydrate diets cause obesity, low-carbohydrate diets reverse obesity: a metabolic mechanism resolving the paradox. *Appetite* **48**, 135–138.
47. Mozaffarian D, Hao T, Rimm EB, *et al.* (2011) Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med* **364**, 2392–2404.
48. Macdiarmid J & Blundell J (1998) Assessing dietary intake: who, what and why of under-reporting. *Nutr Res Rev* **11**, 231–253.
49. Feinman RD, Pogozelski WK, Astrup A, *et al.* (2015) Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition* **31**, 1–13.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

PUBLIC HEALTH NUTRITION

Comparisons of physical activity, adipokines, vitamin D status and dietary vitamin D intake among adolescents

K. V. Giudici, R. M. Fisberg, D. M. Marchioni & L. A. Martini

Nutrition Department, School of Public Health, University of São Paulo, São Paulo, Brazil

Keywords
adolescence, dietary assessment, physical activity, sun exposure, vitamin D.

Correspondence
K. V. Giudici, Nutrition Department, School of Public Health, University of São Paulo, Avenida Doutor Arnaldo, 715 / CEP 01246-904 / Cerqueira César, São Paulo, SP, Brazil.
Tel.: +55 11 3061 7859
Fax: +55 11 3061 7130
E-mail: kellygiudici@gmail.com

How to cite this article
Giudici K.V., Fisberg R.M., Marchioni D.M.L. & Martini L.A. (2017) Comparisons of physical activity, adipokines, vitamin D status and dietary vitamin D intake among adolescents.
J Hum Nutr Diet. **30**, 369–377
doi: 10.1111/jhn.12434

Abstract

Background: Considering that lifestyle and diet are key factors responsible for the increases in adiposity in youth, it is important to understand how vitamin D, adipokines and markers of glucose metabolism are related to physical activity level (PAL) during growth. The present study aimed to investigate associations between physical activity level, adiponectin/leptin ratio, vitamin D status and dietary vitamin D intake among adolescents.
Methods: A cross-sectional study was conducted with adolescents aged 14–18 years old who were living in São Paulo, Brazil. Serum 25 hydroxyvitamin D [25(OH)D], adiponectin (A), leptin (L), glucose and insulin were obtained after 12 h of fasting. Dietary calcium and vitamin D intake were measured by 24-h food record, as repeated in 62.6% of the sample. PAL was measured by the International Physical Activity Questionnaire (IPAQ). Pearson's chi-square test, Pearson correlation and linear regression analysis were performed.
Results: A total of 198 subjects, mean (SD) age 16.3 (1.4) years, 51% male, were enrolled in the study. Some 9% of participants were sedentary, 22% were insufficiently active (IA), 51% were active and 18% were very active (VA). The A/L ratio was lower among sedentary/IA subjects [2.2 (4.0) versus 5.6 (12.3); $P = 0.01$] compared to active/VA subjects. PAL was not associated with vitamin D status or markers of glucose metabolism. Serum 25 (OH)D positively associated with vitamin D intake, after adjusting for sex, sun exposure and season of the year in regression analysis (partial r^2=0.026, $P = 0.02$).
Conclusions: Low PAL was associated with a lower A/L ratio. Vitamin D status was not associated with sun exposure habits, although it was positively correlated with vitamin D intake.

Introduction

Vitamin D deficiency and insufficiency are highly prevalent conditions in many countries and are related to the risk of several chronic diseases [1,2]. Circulating vitamin D is a sum of the amount ingested with the diet (calciferol) or produced by skin when exposed to sunlight [2]. Because few food sources naturally contains great amounts of vitamin D (fish, fish oil, oysters, eggs, milk), most of this vitamin found in circulation is produced endogenously [3]. In Brazil, food fortification with this nutrient is not mandatory, and foods naturally containing vitamin D are not highly frequent in the Brazilian diet [4]. A study reviewing vitamin D data among children and adolescents showed that low vitamin D intake is frequent in many different populations [5]. Thus, it is difficult to determine how vitamin D intake contributes to serum 25 hydroxyvitamin D [25(OH)D] concentrations.

Besides vitamin D deficiency and insufficiency, a sedentary lifestyle and low physical activity level are becoming

more common among populations all over the world [6,7] and studies indicate that such conditions are associated with each other. Among adults, vitamin D status was related to physical activity levels [8], as well as to the frequency of outdoor physical activity [9], although studies evaluating this relationship among adolescents [10,11] are less frequent.

Physical inactivity is a main risk factor for cardiovascular disease [12] and insulin resistance [13] among children and adolescents. In addition, a high physical activity level has been shown to favour the maintenance of adequate levels of total body fat, lower visceral body fat and normal insulin sensitivity by increasing energy expenditure and improving peripheral insulin resistance and cardiovascular fitness [14,15]. Being overweight is of particular concern in public health for favouring insulin resistance, a potent risk factor for diabetes mellitus and other chronic diseases in children [16]. Energy homeostasis is regulated by a complex neuroendocrine system that includes hypothalamic signalling and adipokines such as leptin [17] and adiponectin [18]. Although higher levels of circulating leptin are found among overweight and obese individuals, adiponectin is normally inversely correlated with adiposity measures [19]. Adipokine concentrations may vary dramatically according to physical activity level [20] and physical fitness parameters [21] among adolescents. In addition, the adiponectin/leptin (A/L) ratio (or leptin/adiponectin ratio) is considered to comprise a good parameter for estimating metabolic alterations and the risk of developing chronic diseases, such as diabetes mellitus, hypertension and cardiovascular diseases [22–24], even in youth [25].

Considering that changes in lifestyle and diet are the key factors responsible for the increases in adiposity in children, it is important to understand how vitamin D, adipokines and markers of glucose metabolism are related to physical activity levels during adolescence. Accordingly, we hypothesised that higher physical activity level is associated with better nutritional status and vitamin D status, higher A/L ratio, higher calcium and calciferol intake, and a lower risk of disturbances in glucose homeostasis among adolescents. In addition, serum 25(OH)D concentrations should be associated with sun exposure habits and with calciferol from the diet. Thus, the present study aimed to investigate associations between physical activity level, A/L ratio, vitamin D status and dietary calcium and vitamin D intake among Brazilian adolescents.

Materials and methods

Study design and population

A total of 198 adolescents aged 14–18 years old living in São Paulo, Brazil (latitude −23.5 degrees South) were evaluated in this cross-sectional study. The sample comprised participants from the Health Survey – São Paulo (ISA-SP; http://www.fsp.usp.br/isa-sp), a multicentre and population-based study of people from the city of São Paulo, Brazil, as well as individuals from the League of Childhood Obesity of the School of Medicine of the University of São Paulo and from the Primary Care Center Horácio Geraldo de Paula Souza of the University of São Paulo. Individuals with chronic diseases (besides obesity) or taking supplements of calcium or vitamin D were not included. From the total of 437 eligible individuals, 241 agreed to participate and provided their written informed consent; however, only 198 came to blood collection, even after rescheduling. Measurements of fasting serum glucose and insulin were available for 63 subjects.

Ethical standards disclosure

Subjects who agreed to participate were informed about the objectives and procedures of the research and provided their written informed consent (in the case of those aged <18 years, the document was signed by their legal guardian). The present study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics in Research Committee from the School of Public Health of the University of São Paulo (FSP/USP) under protocol number 2307/2011.

Study variables

Height, weight, waist and hip circumferences were measured in duplicate by trained personnel. Participants were weighed and had their height measured wearing light clothes and no shoes. Weight was determined within 0.1 kg using a manual scale (Filizola®, São Paulo, Brazil). Height was determined using a fixed wall-scale stadiometer to the nearest 0.1 cm. Waist (WC) and hip circumferences (HC) were measured with an inextensible 150 cm measuring tape. Body mass index (BMI) was defined as weight (kg)/height^2 (m^2). Nutritional status was determined according to BMI-for-age Growth Charts [26]. Adolescents between percentiles 3 and 85 (equivalent to *Z*-score −2 and 1) were considered to have normal weight; those between percentiles 85 and 97 (or between *Z*-score 1 and 2) were considered overweight; and those above percentile 97 (*Z*-score > 2) were considered obese.

Blood collection after 12 h of fasting was performed for biochemical assessment of 25(OH)D (measured by high-performance liquid chromatography; Immunodiagnostik AG, Bensheim, Germany), parathyroid hormone [enzyme-linked immunosorbent assay (ELISA); IBL America, Minneapolis, MN, USA], leptin (ELISA; Enzo

Life Sciences, Farmingdale, NY, USA), adiponectin (ELISA; Enzo Life Sciences), glucose (enzymatic colorimetric method; Quibasa, Belo Horizonte, Brazil) and insulin (chemiluminescence; Beckman Coulter, Brea, CA, USA). Vitamin D status is currently classified by different reference intervals based on 25(OH)D concentrations, without consensus in the literature. In the present study, vitamin D status was classified in accordance with a well accepted reference in literature [27], in which subjects with 25(OH)D concentrations <20 ng mL^{-1} were considered deficient and those between 20 and 30 ng mL^{-1} were considered insufficient. The Quantitative Insulin Sensitivity Check Index (QUICKI) was determined as: 1/(log insulin + log glucose). The Homeostatic Model of Assessment Estimate of β Cell Function (HOMA-β) was calculated as: [360× fasting insulin (μU mL^{-1})]/[fasting glucose (mg dL^{-1}) − 63]. The Homeostasis Model of Assessment Estimate of Insulin Resistance (HOMA-IR) was calculated as: fasting glucose (mg dL^{-1}) × fasting insulin (μU mL^{-1})/405.

Physical activity level (PAL) was determined according to the International Physical Activity Questionnaire (IPAQ) [28], and individuals were classified as sedentary, insufficiently active, active and very active. Sun exposure was measured in accordance with a questionnaire adapted from Szklo *et al.* [29]. Another sun exposure questionnaire, proposed by Hanwell *et al.* [30] was also answered by a subset sample of 65 participants. This weekly sun exposure recall questionnaire is a 7-day record about sun exposure habits of the individual and gives a score according to the time (<5 min, 5–30 min or >30 min) and extent of body parts exposed (hands and face; hands, face and arms; hand, face and legs; bathing suit) to the sun in the prior 7 days before the interview. All questionnaires were completed by trained interviewers.

Total energy, vitamin D (calciferol) and calcium intake were assessed by 24-h Food Record (R24 h). To correct intrapersonal variability of intake, a second R24 h was answered in nonconsecutive days, with a more than 1-week interval, in 62.6% (n = 124) of the sample. Subjects were interviewed by trained personnel, in person or by telephone (in the case of subjects who were unable to come to a second interview). Conducting food records by telephone has become a usual and valid way of collecting dietetic information in studies evaluating food intake [31], especially when repeated measures are carried out to obtain more than 1 day of food intake.

Subjects were solicited to report everything they have eaten or drunk in the day before the interview. The Multiple Pass Method, as developed by the United States Department of Agriculture (USDA), was used to obtain detailed information [32]. This method is based on five steps to help guiding the interviewed person to describe her food intake (quick list, forgotten foods, time and occasion, detailed cycle and final probe) [33]. A detailed description of all types of foods and servings consumed was obtained using rigorous standardisation in accordance with a specific guide of household measures transformation [34]. Preparations were dismembered by their ingredients and quantities of each ingredient were standardised according to a Brazilian manual for calculating dietary surveys [35]. Dietetic information was calculated using the Nutrition Data System for Research software (NDSR, version 2007) (Nutrition Coordinating Center, University of Minnesota, MN, USA), which includes food composition data from USDA because Brazilian data of food composition for vitamin D are still not available. To minimise errors in estimating calcium intake (a nutrient not mandatorily fortified in Brazilian foods), food composition from NDSR was then compared with the Brazilian data (from the Brazilian Table of Food Composition; TACO) [36] and every ingredient with more than 20% of difference was corrected in accordance with the values from TACO [37].

Statistical analysis

Descriptive analyses were used for characterisation of the samples. The normality of the distribution of each variable was tested by the Shapiro–Wilk test, and logarithmic transformation was used when needed. The comparison between subgroups for quantitative variables with normal distribution was made using Student's t-test. Categorical variables were analysed by Pearson's chi-square test. Correlations between continuous variables were tested using Pearson correlation. Linear regression analysis was performed with vitamin D intake as the independent variable and serum 25(OH)D as the dependent variable, and sex, BMI, PAL, the season of the year in which blood was collected, and the habit of being exposed to sunlight for more than 30 min were used as variables of adjustment. The observed statistical power for this regression model, when considering a probability level of 5% ($\alpha = 0.05$), was 0.978, confirming that our sample size was adequate for the analysis conducted in the present study. Statistical analysis was performed using the SPSS, version 22.0 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Based on repeated measures of 24-h Food Records, intrapersonal variability of intake was corrected using the Multiple Source Method software (MSM) [38], an online tool developed by the Epidemiology Department of the German Institute of Human Nutrition Postdam-Rehbrücke (DIfE) that statistically estimates habitual intake. To estimate the prevalence of nutrient intake

below or above the recommendations, the intakes of calcium and vitamin D were compared with their estimated average requirement (EAR), which are 1100 mg day^{-1} and 10 μg day^{-1}, respectively, for males and females aged 14–18 years [39]. The analyses of estimated inadequate intake were conducted using PC-SIDE, version 1.0 (Iowa State University, IA, USA). Studies evaluating food intake usually exclude diet reports that exceed 16.74 MJ day^{-1} (4000 kcal day^{-1}) or that are lower than 2.09 MJ day^{-1} (500 kcal day^{-1}), with the aim of minimising over-report or under-report of ingestion [31]. In our sample, no food record was excluded from analysis once minimum and maximum individual energy intake were 4.4 MJ (1052 kcal) and 14.29 MJ (3414 kcal), respectively.

Results

The mean (SD) age of the studied population was 16.3 (1.4) years and 49% of participants were female. Overall, 16.2% of participants were overweight (BMI-for-age *Z* score between 1 and 2) and 26.4% were obese (BMI-for-age *Z* score > 2). Although 9.3% of participants were classified as sedentary, 21.8% were considered insufficiently active, 50.8% active and 18.1% very active. Physical activity level was not associated with nutritional status, nor with markers of glucose metabolism.

Mean (SD) A/L ratio was 4.5 (10.4), and lower among individuals classified as sedentary or insufficiently active [2.2 (4.0) versus 5.6 (12.3); $P = 0.013$], compared to active or very active subjects (Table 1). No correlations,

Table 1 Characterisation of the studied sample according to physical activity level

		Physical activity level (*n* = 193)	
	Total (*n* = 198) Mean (SD)	Sedentary or insufficiently active (*n* = 60) Mean (SD)	Active or very active (*n* = 133) Mean (SD)
Age (years)	16.3 (1.4)	16.4 (1.4)	16.2 (1.3)
Anthropometric measures			
Weight (kg)	71.4 (26.3)	72.1 (30.5)	70.9 (23.9)
Height (m) (*n* = 197)	1.68 (0.09)	1.68 (0.08)	1.68 (0.09)
BMI (kg m^{-2}) (*n* = 197)	25.2 (8.7)	25.5 (9.7)	25.0 (8.2)
Waist circumference (cm) (*n* = 186)	84.3 (17.8)	85.7 (20.1)	83.5 (16.4)
Hip circumference (cm) (*n* = 69)	113.1 (20.8)	117.7 (20.7)	111.2 (20.9)
Sun exposure			
Time of exposure score (*n* = 65)	6.8 (3.3)	6.0 (3.1)	7.3 (3.4)
Skin exposure score (*n* = 65)	14.2 (4.1)	13.4 (4.0)	14.7 (4.2)
Total score (*n* = 65)	21.1 (6.6)	19.4 (6.4)	22.0 (6.7)
Nutrient intake			
Energy (MJ^{-1} day^{-1}) [kcal day^{-1}]	8.8 (1.89) [2101.7 (452.3)]	8.8 (1.9) [2096.1 (446.8)]	8.8 (1.9) [2108.0 (462.6)]
Calcium (mg day^{-1})	583.1 (199.0)	581.8 (207.7)	584.4 (198.4)
Calcium (mg per 1000 kcal or 4.19 MJ)	281.1 (86.7)	277.8 (79.8)	282.0 (89.8)
Vitamin D (μg day^{-1})	3.0 (0.9)	2.9 (0.8)	3.1 (0.9)
Vitamin D (μg per 1000 kcal or 4.19 MJ)	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)
Vitamin D and PTH			
25 hydroxyvitamin D (ng mL^{-1})	25.2 (13.4)	25.7 (11.7)	25.0 (14.1)
PTH (pg mL^{-1}) (*n* = 195)	43.6 (27.9)	48.5 (34.5)	41.5 (24.4)
Adipokines			
Leptin (ng mL^{-1})	29.5 (21.4)	33.6 (19.7)	27.7 (22.0)*
Adiponectin (μg mL^{-1})	27.2 (17.5)	25.8 (18.3)	28.1 (17.3)
Adiponectin/leptin ratio	4.5 (10.4)	2.2 (4.0)	5.6 (12.3)*
Markers of energy metabolism			
Glucose (mg dL^{-1}) (*n* = 69)	90.7 (9.3)	91.3 (10.4)	90.5 (8.3)
Insulin (mUI L^{-1}) (*n* = 63)	10.8 (7.1)	13.0 (7.3)	10.2 (7.0)
HOMA-IR (*n* = 63)	2.4 (1.6)	2.9 (1.6)	2.3 (1.7)
HOMA-β (*n* = 63)	165.8 (134.3)	209.3 (178.1)	148.8 (104.4)
QUICKI (*n* = 63)	0.35 (0.04)	0.34 (0.03)	0.35 (0.04)

BMI, body mass index; HOMA-β, Homeostatic Model of Assessment Estimate of β Cell Function; HOMA-IR, Homeostatic Model of Assessment Estimate of Insulin Resistance; PTH, parathyroid hormone; QUICKI, Quantitative Insulin Sensitivity Check Index.
*$P < 0.05$ (Student's *t*-test).

however, were observed between A/L ratio and vitamin D status. A negative correlation was observed between serum leptin and serum adiponectin ($r = -0.175$, $P = 0.014$). Such inverse association was stronger among those with vitamin D deficiency ($n = 72$) ($r = -0.385$; $P = 0.001$).

Subjects presented mean calcium and vitamin D intake of 281.1 (86.7) mg per 1000 kcal (or per 4.19 MJ) and 1.5 (0.4) μg per 1000 kcal (or per 4.19 MJ), respectively. Analysis of inadequate food intake based on EAR values revealed that the intake of calcium and vitamin D were lower than the recommended among 97%, 8% and 100%, respectively, of the sample. Despite this high prevalence of inadequate intake, vitamin D intake positively correlated with serum 25(OH)D ($r = 0.175$, $P = 0.014$), and this association persisted after adjusting for sex, BMI, PAL, sun exposure and season of the year in linear regression analysis (partial $r^2 = 0.026$, $P = 0.018$) (Fig. 1).

Although 88.4% of the sample have declared to usually expose themselves to sunlight for a minimum period of 30 min and 68.8% reported rarely or never applying sunscreen when exposed to sunlight for >30 min, 63.3% frequently or always choose to stay in the shade when is a sunny location (Table 2) and 71.8% were classified as vitamin D deficient or insufficient [25(OH)D concentrations below 30 ng mL^{-1}]. Vitamin D status was not correlated with sun exposure habits, nor was it associated with physical activity level among the studied population.

A sun-exposure score was determined to a subset of 65 individuals, who answered a questionnaire with a maximum score of 42 (for a condition in which someone report to expose himself to sunlight every day in the prior 7 days for more than 30 min using a bathing suit). The mean (SD) score was 21.1 (6.6), although this measure was not associated with serum 25(OH)D, nutritional status, physical activity level or other variables.

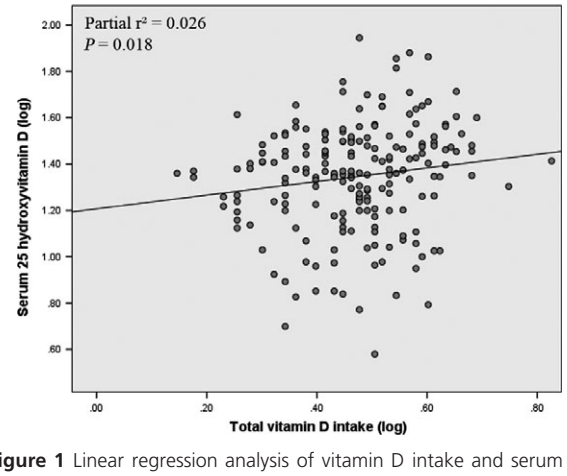

Figure 1 Linear regression analysis of vitamin D intake and serum 25 hydroxyvitamin D adjusted for sex, body mass index, physical activity level, sun exposure and season of the year among adolescents.

Table 2 Sun exposure habits and physical activity level according to sex among Brazilian adolescents

	Total *n* (%)	Sex: Male *n* (%)	Sex: Female *n* (%)
Sun exposure			
Usual exposure to sunlight >30 min (*n* = 173)			
Yes	153 (88.4)	79 (88.8)	74 (88.1)
No	20 (11.6)	10 (11.2)	10 (11.9)
Use of sunscreen when exposed >30 min (*n* = 173)			
Never/rarely	119 (68.8)	69 (77.5)	50 (59.5)
Sometimes	29 (16.8)	9 (10.1)	20 (23.8)
Frequently/always	25 (14.4)	11 (12.3)	14 (16.7)
Use of hat or similar when exposed >30 min (*n* = 172)			
Never/rarely	104 (60.4)	42 (47.1)	62 (74.7)
Sometimes	19 (11.0)	12 (13.5)	7 (8.4)
Frequently/always	49 (28.5)	35 (39.3)	14 (16.8)
Choice of staying in the shadow when in a sunny location (*n* = 173)			
Never/rarely	26 (15.0)	14 (15.7)	12 (14.3)
Sometimes	37 (21.4)	23 (25.8)	14 (16.7)
Frequently/always	110 (63.6)	52 (58.4)	58 (69.1)
Time of exposure score (*n* = 65)*	6.8 (3.3)	7.3 (3.8)	6.3 (2.8)
Skin exposure score (*n* = 65)*	14.2 (4.1)	15.4 (4.6)	13.3 (3.4)
Total score (*n* = 65)*	21.1 (6.6)	22.7 (7.6)	19.6 (5.3)
Physical activity level (*n* = 193)			
Sedentary	18 (9.3)	10 (10.2)	8 (8.4)
Insufficiently active	42 (21.8)	16 (16.3)	26 (27.4)
Active	98 (50.8)	49 (50.0)	49 (51.6)
Very active	35 (18.1)	23 (23.5)	12 (12.6)

*Mean (SD).

Discussion

The present study, evaluating lifestyle factors (such as physical activity, sun exposure, calcium and vitamin D intake) and their relationship withh vitamin D status and adipokine concentrations among a sample of adolescents, revealed that more than 70% of the population were vitamin D deficient or insufficient, despite living in a sunny country and reporting usual exposure to sunlight for >30 min (88%) and not wearing sunscreen when exposed to sunlight for >30 min (69%). Vitamin D status did not correlate with sun exposure habits. Park & Johnson [40], when reviewing some studies conducted in American populations, concluded that living in lower latitude regions does not appear to protect from poor vitamin D status. Indeed, over 60% of our sample also declared frequently or always choosing to stay in the shade when in a sunny location.

Sun exposure habits were also evaluated with an instrument proposed by Hanwell *et al.* [30], who have developed a weekly sun exposure recall questionnaire which, among healthy Caucasian adults from Italy, was correlated with 25(OH)D concentrations in the summer but not in the winter. The observed correlation was almost entirely driven by time spent in the sun rather than the amount of exposed skin, suggesting that the categories for time exposed to sunlight proposed by them (<5 min, 5–30 min and >30 min) are easily recalled and likely to be of biological significance. Positive (but moderate) correlations of sun exposure score and 25(OH)D concentrations were also seen among North-American youths (1–21 years old) [41]. Wakayo *et al.* [42], when evaluating Ethiopian adolescents with this questionnaire, did not report the results obtained using the scores, although the duration of exposure to sunlight and the extent of body parts exposed to sunlight was found to predict vitamin D deficiency. The present study, however, did not find any association with this questionnaire, nor did that of Forney *et al.* [43] investigating North-American college-aged youths or that of Sham *et al.* [44] investigating young Canadian adults (18–35 years old).

Although it is widely recognised that major circulating 25(OH)D originates from skin production driven by sunlight exposure [3], our analysis found a weak but positive correlation among vitamin D intake and serum 25(OH) D, even after adjusting for sex, sun exposure and season of the year. This is an important finding, given that we were able to show that, even in a population with vitamin D intake below the recommendations and with the majority of individuals presenting vitamin D insufficiency or deficiency, vitamin D intake from diet (not from supplements) was significantly associated with higher 25 (OH)D levels. Other studies with Brazilian adolescents have also observed a high proportion of inadequate vitamin D intake: less than 16% of individuals reached the recommendations for this nutrient; however, in both studies, an association between vitamin D from diet and 25(OH)D concentrations was not found [45,46]. On the other hand, Xu *et al.* [47] found that consuming foods naturally containing vitamin D was associated with higher 25(OH)D levels among children and adolescents from Hong Kong. The present study found the intake of calcium was also inadequate for almost the entire sample. Such a condition has already been observed among other populations [48] and is also of concern because it has been associated with several chronic diseases, such as obesity, osteoporosis, hypertension cervical cancer and autoimmune diseases [49].

The present study found a prevalence of low physical activity level of 31.1% (with 9.3% of our sample being sedentary and 21.8% being insufficiently active) among Brazilian adolescents aged 14–18 years old. Other studies from different countries have also shown considerable frequencies of physical inactivity among adolescents: 50.3% of males and 67.8% of females in a sample of 7982 Canadian youth (mean age 15.6 years) were classified as inactive [6]. Data from the 2003–2004 National Health and Nutritional Examination Survey (NHANES) show that only 5.6–8% of North-American adolescents (12–19 years old) achieved the national recommendations of physical activity [7]. In addition to concerns of youths being insufficiently active, there is evidence to show that infrequent participation in sports and low grades in school sports during adolescence were associated with physical inactivity in adulthood [50].

Substantial evidence highlights the importance of physical exercise with respect to protecting from obesity in childhood and adolescence [15,51]. Despite the usual positive correlation between low physical activity level and obesity [52] and the risk for diabetes observed in other studies [53], our results do not show an association between physical activity and nutritional status, insulin resistance or β-cell function. On the other hand, the A/L ratio, a good parameter for disturbances on glucose homeostasis and adiposity [22,24], was shown to be higher among active and very active adolescents compared to sedentary and insufficiently active subjects.

We found no other studies evaluating the relationship between physical activity level and A/L (or L/A) ratio among adolescents. Jürimäe [54], in a review study, analysed adiponectin and leptin responses to acute exercise and sport training in children and adolescents, and also found few publications among people this age. It was concluded that acute exercise does not apppear to impact adiponectin and leptin concentrations among children and adolescents. On the other hand, chronic training appears to influence lower leptin levels, given that leptin concentrations are lower in young athletes compared to untrained controls. The studies reported in the review by Jürimäe [54] were focused on specific (acute or chronic) training, especially among athletes, which differs from the objectives of the present study, which aimed to evaluate regular physical activity level among normal (non-athlete) adolescents.

The present study has some limitations. Its cross-sectional design does not enable the inference of causality or temporal associations between the measured variables. Serum glucose, insulin and part of the sun exposure score were measured in a subset sample. Opting for the 24-h recall as a method of estimating food intake was another limiting factor because, despite being an appropriate tool to estimate average values of food intake in a population sample, it depends on the memory of the individuals who are interviewed, and refers to the current diet, not the

habitual one. Moreover, it is not the most precise instrument for evaluating vitamin D intake, given that food sources of this nutrient are few, favouring an irregular consumption (i.e. large day-by-day variability). For such reasons, repetition of the 24-h recall was performed in a subsample of participants to evaluate more than 1 day of intake and adequate statistical adjustments considering these measures [55] were applied, which made it possible to reduce the effects of variabilities from diet and to improve accuracy of ingestion estimates. In addition, we were unable to use measures of biological maturation (as Tanner stages of pubertal development). To limit possible bias as a result of variation in stages of puberty, we only included adolescents older than 14 years, and girls who declared have already having had their menarche. Lastly, the sun exposure questionnaire was not validated for the present study specifically, although it has been created to evaluate sun exposure habits among a population-based random sample of 16 999 individuals ≥15 years old from all five regions of Brazil [29].

The number of studies evaluating the relationship between physical activity level and adipokines concentrations in children and adolescents is still limited. Further prospective research, especially intervention studies, is needed to better understand how low physical activity levels may influence vitamin D status, circulating adipokines and markers of energy metabolism. This may help to create preventive actions favouring the achievement of better health status and a lower risk of developing chronic diseases in adolescence and other life stages.

In conclusion, we have shown that poor physical activity level was associated with a lower adiponectin/leptin ratio among Brazilian adolescents. Vitamin D status was not associated with physical activity levels or with sun exposure habits, although it was positively correlated with vitamin D intake. Despite being a weak correlation, this result is relevant and illustrates the importance of stimulating an adequate intake of vitamin D via natural food sources, or with fortified products, if available. Additionally, the adoption of a healthy lifestyle, comprising the usual amount of physical activity, safe (but regular) sun exposure and a diversified healthy diet, should also be encouraged.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with STROBE guidelines.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo/São Paulo Research Foundation (FAPESP), grant numbers 2011/22768-2 and 2012/11061-8. FAPESP had no role in the design, analysis or writing of this article.

Conflict of interest, source of funding and authorship

The authors declare that they have no conflicts of interest.
This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo/São Paulo Research Foundation (FAPESP), grant numbers 2011/22768-2 (KV Giudici's PhD scholarship) and 2012/11061-8 (project funding). FAPESP had no role in the design, analysis or writing of this article.
KV Giudici performed the subject recruitment and was involved in data collection and computation, statistical analysis, interpretation of the results, and writing the manuscript. LA Martini defended the research grant, managed the study, contributed to the interpretation of the results, and assisted in the preparation and review of the manuscript. DML Marchioni and RM Fisberg assisted in the interpretation of the results, as well as the review of the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. Fonseca VM, Sichieri R & Veiga GV (1998) Fatores associados à obesidade em adolescentes. *Rev Saude Publica* **32**, 541–549.
2. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
3. Holick MF (2003) Vitamin D: a millenium perspective. *J Cell Biochem* **88**, 296–307.
4. Souza AM, Pereira RA, Yokoo EM *et al.* (2013) Most consumed foods in Brazil: National Dietary Survey 2008-2009. *Rev Saude Publica* **47**(Suppl 1), S190–S199.
5. Kimball S, Gel-H Fuleihan & Vieth R (2008) Vitamin D: a growing perspective. *Crit Rev Clin Lab Sci* **45**, 339–414.
6. Koezuka N, Koo M, Allison KR *et al.* (2006) The relationship between sedentary activities and physical inactivity among adolescents: results from the Canadian Community Health Survey. *J Adolesc Health* **39**, 515–522.
7. Troiano RP, Berrigan D, Dodd KW *et al.* (2008) Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc* **40**, 181–188.

8. Nakamura K, Kitamura K, Takachi R *et al.* (2015) Impact of demographic, environmental, and lifestyle factors on vitamin D sufficiency in 9084 Japanese adults. *Bone* **74**, 10–17.
9. Scragg R & Camargo CA Jr (2008) Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* **168**, 577–586.
10. Oberg J, Jorde R, Almås B *et al.* (2014) Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population. *Scand J Public Health* **42**, 593–602.
11. Colao A, Muscogiuri G, Rubino M *et al.* (2015) Hypovitaminosis D in adolescents living in the land of sun is correlated with incorrect life style: a survey study in Campania region. *Endocrine* **49**, 521–527.
12. Väistö J, Eloranta AM, Viitasalo A *et al.* (2014) Physical activity and sedentary behaviour in relation to cardiometabolic risk in children: cross-sectional findings from the Physical Activity and Nutrition in Children (PANIC) Study. *Int J Behav Nutr Phys Act* **11**, 55.
13. Rizzo NS, Ruiz JR, Oja L *et al.* (2008) Associations between physical activity, body fat, and insulin resistance (homeostasis model assessment) in adolescents: the European Youth Heart Study. *Am J Clin Nutr* **87**, 586–592.
14. Duncan GE, Perri MG, Theriaque DW *et al.* (2003) Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. *Diabetes Care* **26**, 557–562.
15. Reichert FF, Baptista Menezes AM, Wells JC *et al.* (2009) Physical activity as a predictor of adolescent body fatness: a systematic review. *Sports Med* **39**, 279–794.
16. Roemmich JN, Clark P, Lusk M *et al.* (2002) Pubertal alterations in growth and body composition: pubertal insulin resistance – relationship to adiposity, body fat distribution and hormone release. *Int J Obes Relat Metab Disord* **26**, 701–709.
17. Hinoi E, Gao N, Jung DY *et al.* (2008) The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity. *J Cell Biol* **183**, 1235–1242.
18. Díez JJ & Iglesias P (2003) The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* **148**, 293–300.
19. Matsubara M, Maruoka S & Katayose S (2002) Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* **147**, 173–180.
20. Jiménez-Pavón D, Ortega FB, Artero EG *et al.* (2012) Physical activity, fitness, and serum leptin concentrations in adolescents. *J Pediatr* **160**, 598–603.
21. Martinez-Gomez D, Eisenmann JC, Gomez-Martinez S *et al.* (2012) Associations of physical activity and fitness with adipocytokines in adolescents: the AFINOS Study. *Nutr Metab Cardiovasc Dis* **22**, 252–259.
22. Inoue M, Yano M, Yamakado M *et al.* (2006) Relationship between the adiponectin-leptin ratio and parameters of insulin resistance in subjects without hyperglycemia. *Metabolism* **55**, 1248–1254.
23. Norata GD, Raselli S, Grigore L *et al.* (2007) Leptin: adiponectin ratio is an independent predictor of intima media thickness of the common carotid artery. *Stroke* **38**, 2844–2846.
24. Jung CH, Rhee EJ, Choi JH *et al.* (2010) The relationship of adiponectin/leptin ratio with homeostasis model assessment insulin resistance index and metabolic syndrome in apparently healthy korean male adults. *Korean Diabetes J* **34**, 237–243.
25. Donoso MA, Muñoz-Calvo MT, Barrios V *et al.* (2013) Increased leptin/adiponectin ratio and free leptin index are markers of insulin resistance in obese girls during pubertal development. *Horm Res Paediatr* **80**, 363–370.
26. World Health Organization (WHO) (2006) Multicentre Growth Reference Study Group. *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development*. Geneva: WHO.
27. Holick MF, Binkley NC, Bischoff-Ferrari HA *et al.* (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* **96**, 1911–1930.
28. Craig CL, Marshall AL, Sjöström M *et al.* (2003) International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* **35**, 1381–1395.
29. Szklo AS, Almeida LM, Figueiredo V *et al.* (2007) Comportamento relativo à exposição e proteção solar na população de 15 anos ou mais de 15 capitais brasileiras e Distrito Federal, 2002-2003. *Cad Saúde Pública* **23**, 823–834.
30. Hanwell HE, Vieth R, Cole DE *et al.* (2010) Sun exposure questionnaire predicts circulating 25-hydroxyvitamin D concentrations in Caucasian hospital workers in southern Italy. *J Steroid Biochem Mol Biol* **121**, 334–337.
31. Willett WC. (1998) 24-hour dietary recall and food record methods. In: *Nutritional Epidemiology*. 2nd ed. pp. 305–333. [Willett WC, editor]. New York, NY: Oxford University Press.
32. Buzzard IM, Faucett CL, Jeffery RW *et al.* (1996) Monitoring dietary change in a low-fat diet intervention study: advantages of using 24-hour dietary recalls vs food records. *J Am Diet Assoc* **96**, 574–579.
33. Moshfegh AJ, Rhodes DG, Baer DJ *et al.* (2008) The US Department of Agriculture automated multiple-pass method reduces bias in the collection of energy intakes. *Am J Clin Nutr* **88**, 324–332.
34. Fisberg RM, Villar BS. (2002) *Manual de receitas e medidas caseiras para cálculo de inquéritos alimentares*. São Paulo: Signus.
35. Pinheiro ABV, Lacerda EMA, Bezencry EH *et al.* (2004) *Tabela para Avaliação do Consumo Alimentar em Medidas Caseiras*. 5ª edn. São Paulo: Atheneu.

36. NEPA Núcleo de Estudos e Pesquisas em Alimentação da Universidade Estadual de Campinas (2011). *Tabela brasileira de composição de alimentos (TACO),* 4th edn revisada e ampliada. Campinas: NEPA-UNICAMP. 161 p.
37. Fisberg RM, Marchioni DML. (2012) *Manual de avaliação do consumo alimentar em estudos populacionais: a experiência do Inquérito de Saúde em São Paulo (ISA)*. São Paulo: Universidade de São Paulo. Faculdade de Saúde Pública. Grupo de Pesquisa de Avaliação do Consumo Alimentar. 197 p.
38. Hoffmann K, Boeing H, Dufour A *et al.* (2002) Estimating the distribution of usual dietary intake by short-term measurements. *Eur J Clin Nutr* **56**(Suppl 2), S53–S62.
39. Institute of Medicine (IOM) (2011) US Committee to review dietary reference intakes for vitamin D and calcium. In: *Dietary Reference Intakes for Calcium and Vitamin D*. pp. 349–363. [Ross AC, Taylor CL, Yaktine AL & Del Valle HB, editors]. Washington, DC: National Academies Press (US).
40. Park S & Johnson MA (2005) Living in low-latitude regions in the United States does not prevent poor vitamin D status. *Nutr Rev* **63**, 203–209.
41. Simmons J, Sheedy C, Lee H *et al.* (2013) Prevalence of 25-hydroxyvitamin D deficiency in child and adolescent patients undergoing hematopoietic cell transplantation compared to a healthy population. *Pediatr Blood Cancer* **60**, 2025–2030.
42. Wakayo T, Belachew T, Vatanparast H *et al.* (2015) Vitamin D deficiency and its predictors in a country with thirteen months of sunshine: the case of school children in central Ethiopia. *PLoS One* **10**, e0120963.
43. Forney LA, Earnest CP, Henagan TM *et al.* (2014) Vitamin D status, body composition, and fitness measures in college-aged students. *J Strength Cond Res* **28**, 814–824.
44. Sham L, Yeh EA, Magalhaes S *et al.* (2015) Evaluation of fall Sun Exposure Score in predicting vitamin D status in young Canadian adults, and the influence of ancestry. *J Photochem Photobiol, B* **145**, 25–29.
45. Peters BS, dos Santos LC, Fisberg M *et al.* (2009) Prevalence of vitamin D insufficiency in Brazilian adolescents. *Ann Nutr Metab* **54**, 15–21.
46. Oliveira RM, Novaes JF, Azeredo LM *et al.* (2014) Association of vitamin D insufficiency with adiposity and metabolic disorders in Brazilian adolescents. *Public Health Nutr* **17**, 787–794.
47. Xu C, Perera RA, Chan YH *et al.* (2015) Determinants of serum 25-hydroxyvitamin D in Hong Kong. *Br J Nutr* **114**, 144–151.
48. Wang Y & Li S (2008) Worldwide trends in dairy production and consumption and calcium intake: is promoting consumption of dairy products a sustainable solution for inadequate calcium intake? *Food Nutr Bull* **29**, 172–185.
49. Peterlik M & Cross HS (2005) Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest* **35**, 290–304.
50. Tammelin T, Näyhä S, Laitinen J *et al.* (2003) Physical activity and social status in adolescence as predictors of physical inactivity in adulthood. *Prev Med* **37**, 375–381.
51. Flodmark CE, Marcus C & Britton M (2006) Interventions to prevent obesity in children and adolescents: a systematic literature review. *Int J Obes (Lond)* **30**, 579–589.
52. Abbott RA & Davies PS (2004) Habitual physical activity and physical activity intensity: their relation to body composition in 5.0-10.5-y-old children. *Eur J Clin Nutr* **58**, 285–291.
53. Rubin DA, McMurray RG & Harrell JS (2008) Insulin and weight status in adolescents: independent effects of intensity of physical activity and peak aerobic power. *Pediatr Exerc Sci* **20**, 29–39.
54. Jürimäe J (2014) Adipocytokine and ghrelin responses to acute exercise and sport training in children during growth and maturation. *Pediatr Exerc Sci* **26**, 392–403.
55. Guenther PM, Kott PS & Carriquiry AL (1997) Development of an approach for estimating usual nutrient intake distributions at the population level. *J Nutr* **127**, 1106–1112.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

DIETETIC PRACTICE AND BEHAVIOUR CHANGE

Weekend days are not required to accurately measure oral intake in hospitalised patients

L. S. Chapple,[1,2] A. M. Deane,[1,2,3] K. Lange,[2] A. J. Kranz,[1] L. T. Williams[4] & M. J. Chapman[1,2,3]

[1]Discipline of Acute Care Medicine, University of Adelaide, Adelaide, SA, Australia
[2]National Health and Medical Research Council of Australia Centre of Research Excellence in Translating Nutritional Science to Good Health, Adelaide, SA, Australia
[3]Intensive Care Unit, Level 4, Emergency Services Building, Royal Adelaide Hospital, Adelaide, SA, Australia
[4]Menzies Health Institute of Queensland, Griffith University, Southport, QLD, Australia

Keywords
dietary intake methodology, head injured patients, nutrition, oral intake, weighed food record.

Correspondence
L. Chapple, Discipline of Acute Care Medicine, University of Adelaide, Adelaide, SA, Australia.
Tel.: +61 8 8222 4624
Fax: +61 8 8223 3870
E-mail: lee-anne.costello@adelaide.edu.au

How to cite this article
Chapple L.S., Deane A.M., Lange K., Kranz A.J., Williams L.T. & Chapman M.J. (2017) Weekend days are not required to accurately measure oral intake in hospitalised patients. *J Hum Nutr Diet*. **30**, 378–384
doi: 10.1111/jhn.12432

Abstract

Background: Nutrition studies in patients admitted to hospital frequently disregard oral intake because measurement is time-intensive and logistically challenging. In free-living populations, weighed food records (WFR) are the gold-standard and are conducted on weekend and weekdays to capture variations in intake, although this may not translate during hospitalisation. The present study aimed to determine whether oral intake differs between weekends and weekdays in hospitalised patients.
Methods: For adult patients initially admitted to the intensive therapy unit with a moderate-severe head injury over a 12-month period, WFR were conducted each week on Tuesday, Thursday and Saturday throughout hospitalisation. Meal components were weighed before and after consumption, and energy and protein intakes were calculated using specialised software. Data are reported as the mean (SD). Differences were assessed using paired *t*-tests and agreement using Bland–Altman plots.
Results: Thirty-two patients had WFR collected on 220 days, 68% (n = 149) on weekdays and 32% (n = 71) on weekends. Overall, daily intakes were 5.72 (3.67) MJ [1367 (877) kcal] and 62 (40) g protein. There were no differences in intake across all days (P = 0.937 energy, P = 0.797 protein), nor between weekdays and weekends, in weeks 1–3 of oral intake (all $P > 0.1$). Limits of agreement between mean intakes across days were wide for energy [range −11.20 to 9.55 MJ (−2680 to 2283 kcal)] and protein (range −125 to 110 g).
Conclusions: Grouped energy and protein intakes from WFR in hospitalised patients are similar on weekdays and weekends, although large intra-patient variations occur. Future quantification of oral intake during hospitalisation should include as many days as feasible, although not necessarily weekend days, to reflect true intake.

Introduction

Studies of nutrition practices in a hospitalised setting frequently disregard oral intake because of the time-intensive nature and logistic challenges associated with obtaining accurate intake measures. This may explain the absence of recommendations on oral intake in critical illness [(1,2)]. Excluding orally ingested nutrient may lead to a considerable underestimation of energy and protein intakes over the entire hospitalisation. Of note, inferences from nutrition interventions conducted within the intensive therapy unit (ITU) have not measured oral intake either in the

ITU or after ITU discharge [3–7]. The lack of detailed information regarding oral intake means that much of the nutritional intake throughout hospitalisation remains an unmeasured confounder, which could lead to incorrect inferences for both observational and interventional studies [8]. Accordingly, a precise but efficient and feasible method to measure oral intake in hospitalised patients is required.

Up to now, hospital-based nutrition studies have used a range of methods to assess oral intake, including recall, measures of waste and estimates of intake [9–11]. These methods contain inherent inaccuracies, which may limit their use in high-quality research [12]. Methods that rely on patient memory are prone to recall bias and exclude patients with acute delirium and those with certain presentations, such as head injury, reducing the generalisability of the results. Similarly, methods that provide an indirect measure of intake lack precision [12].

Weighed food records (WFR) are considered the gold-standard technique for precise measurement of nutrient intakes in free-living populations [13]. It is recommended these be conducted at least 3 days per week, comprising 2 weekdays and 1 weekend day, to account for social variations and to provide a reliable representation of actual intake [14]. The extent to which this is true in an institutionalised setting, such as a hospital, where variations in intake may differ from free-living individuals, has never been evaluated. If, similar to free-living individuals, intake varies between week days and weekends, WFR will need to be conducted for both days to accurately quantify intake in hospitalised patients. Such a requirement has considerable cost and feasibility implications for studies of nutritional therapies in critically ill and hospitalised patients, whereas, if variations in intake do not occur across different days of the week, the need to conduct WFR on a range of days, or outside of usual work days, may be unnecessary. Finally, a rigorous evaluation of oral intake over time as patients recover from time in the ITU has not been carried out previously.

It was therefore hypothesised that, as a result of fewer fasting periods for procedures and greater periods when family were present to assist with feeding, head-injured patients recovering from critical illness would consume more energy and protein on weekends. The primary aim of the present study was to compare energy and protein intakes derived from investigator-led WFR conducted on weekdays and weekends in patients with a head injury. The secondary aim was to determine whether energy and protein intakes increased over time as patients recovered from their primary illness.

Materials and methods

Adult patients consecutively admitted with a moderate-severe head injury (Glasgow Coma Scale score 3–12) to the ITU of the major neuro-trauma referral centre in the state of South Australia over a 12-month period were screened for eligibility. Data on energy and protein consumed orally throughout the hospital stay were collected prospectively and censored at 90 days.

To precisely quantify oral intake, WFR were conducted once tube feeding was ceased and on three predetermined days per week, including 2 weekdays (Tuesday and Thursday) and 1 weekend (Saturday), by trained research dietitians. Patients received hospital meals in accordance with usual practices, based on individual meal selection or standard menu choices. Individual meal components (e.g. meat portion, mashed potato, gravy) provided from breakfast to 1 h post-dinner were weighed prior to delivery to the patient. Meal components were weighed separately as they were plated on the plating line using Model 405 digital scales (Salter Brecknell, Springvale North, VIC, Australia) to the nearest 0.1 g. After consumption, individual components of waste were weighed and deducted from the preweights to calculate the total proportion consumed in grammes. Items provided outside of observation times were estimated using collection of wrappers and nursing notes, as well as by communication with patients, families and nursing staff.

Recorded weights were entered into FOODWORKS, version 8 dietary analysis software (Xyris, Brisbane, QLD, Australia) using pre-entered hospital recipes to calculate energy and protein intakes. WFR were excluded if they were partial days (as a result of hospital discharge or day leave) or if patients also received artificial nutrition simultaneous (e.g. overnight enteral tube feeding). Energy and protein requirements prescribed by the hospital dietitians as part of standard care were recorded.

Ethical approval

The present study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human patients were approved by the Royal Adelaide Hospital Human Research Ethics committee (HREC/14/RAH/100). Written informed consent was obtained from all patients or their legal authorised guardian where appropriate.

Statistical analysis

Data are presented as the mean (SD). For weekend versus weekday analyses, mean intake data from the 2 weekdays per week were combined to be representative of a single

Table 1 Patient demographics (n = 32)

	Total
Age (years), mean (SD)	44 (16)
Sex (male), *n* (%)	28 (88)
Initial GCS	
GCS 3–8, *n* (%)	20 (63)
GCS 9–12, *n* (%)	12 (37)
APACHE II score, median [IQR]	18 [13–21]
SOFA score, mean (SD)	6 (3)
Body mass index (kg m^{-2}), mean (SD)	26 (6)
ITU LOS (day), median (IQR)	12 [6–17]
Hospital LOS (day), median (IQR)	30 [19–50]
Days to commence oral intake, median (IQR)	13 [5–25]
Days received oral intake, median (IQR)	15 [9–23]

APACHE II, acute physiology and chronic health evaluation; GCS, Glasgow Coma Scale; IQR, interquartile range; ITU, intensive therapy unit; LOS, length of stay; SOFA, sequential organ failure assessment.

weekday and then compared with the corresponding weekend day for that week. Differences between energy and protein intakes on week versus weekend days were assessed using linear mixed models with a fixed effect for day of week and random effects to account for clustering of multiple days within subjects. Agreement between energy and protein intakes assessed on week versus weekend days was determined using Bland–Altman plots with the bias (mean difference) and limits of agreement calculated at two SDs from the derived mean and difference variables. Based on these results, all analyses were repeated for individual days. Poor agreement was defined *a priori* to be approximately 25% of the patient's requirements [15] [>2.09 MJ day^{-1} (>500 kcal day^{-1}) and >25 g protein day^{-1}] as a result of prior research suggesting that weight loss occurs when less than 75% of daily requirements are delivered and therefore this is considered inadequate [10].

Results

Consent to participate was obtained in 32 patients admitted to the ITU following a moderate–severe head injury and who were subsequently ingesting food orally when hospitalised. Baseline patient characteristics are shown in Table 1.

A total of 220 days of WFR were included: 68% (n = 149) on a weekday and 32% (n = 71) on a weekend. Patients contributed data from a mean of 6.9 (range 1–26) days, 5.0 (range 1–21) weekdays and 2.3 (range 1–7) weekends.

Overall, mean daily oral intake was 5.72 (3.67) MJ day^{-1} [1367 (877) kcal day^{-1}] and 62 (40) g protein day^{-1}, with similar amounts consumed on week and weekend days: 5.72 (3.74) MJ day^{-1} [1368 (894) kcal day^{-1}] versus 5.71 (3.54) MJ day^{-1} [1364 (845) kcal day^{-1}] and 61 (41) g protein day^{-1} versus 63 (39) g protein day^{-1}. Total mean prescribed energy and protein requirements were 9.41 (1.72) MJ [2249 (412) kcal] and 105 (20) g protein, respectively. Days with interruptions were similar between groups (49.4% for weekdays and 50.6% for weekends).

There were no differences in energy or protein intakes received on any individual day (P = 0.937 energy, P = 0.797 protein) or between combined week versus weekend days (P = 0.913 energy, P = 0.567 protein). There were no differences in energy or protein intakes consumed across days in the first (P = 0.665 energy, P = 0.433 protein; n = 83 WFR), second (P = 0.529 energy, P = 0.907 protein; n = 56 WFR) or third (P = 0.426 energy, P = 0.110 protein; n = 30 WFR) week of oral intake. In patients who remained in hospital for at least two full weeks, mean oral energy and protein intakes increased over time (Table 2).

Bland–Altman plots of mean difference in energy and protein intake across individual days are shown in Fig. 1. There were wide limits of agreement for both energy and protein, although these were consistent across days. There were consistently wide limits of agreement across days and between combined weekdays and weekend days in both weeks 1 and 2 (Table 3).

Discussion

The present study is the first to explore variations in oral intake in hospitalised patients measured on weekdays

Table 2 Mean (SD) energy and protein intakes per day for weeks 1 and 2 for patients with complete data (n = 15)

	Energy (kcal), mean (SD)			Protein (g), mean (SD)		
	Week 1	Week 2	*P*-value	Week 1	Week 2	*P*-value
Saturday	1073 (858)	1313 (729)	0.31	52 (44)	64 (36)	0.29
Thursday	935 (907)	1440 (914)	**0.035**	39 (35)	69 (45)	**0.005**
Tuesday	1140 (772)	1600 (945)	0.91	55 (41)	68 (44)	0.39
Mean for all days	1050 (833)	1451 (856)	**0.004**	49 (40)	67 (41)	**0.009**

Paired-samples *t*-test for means comparison. P-values that are statistically significant are shown in bold text.

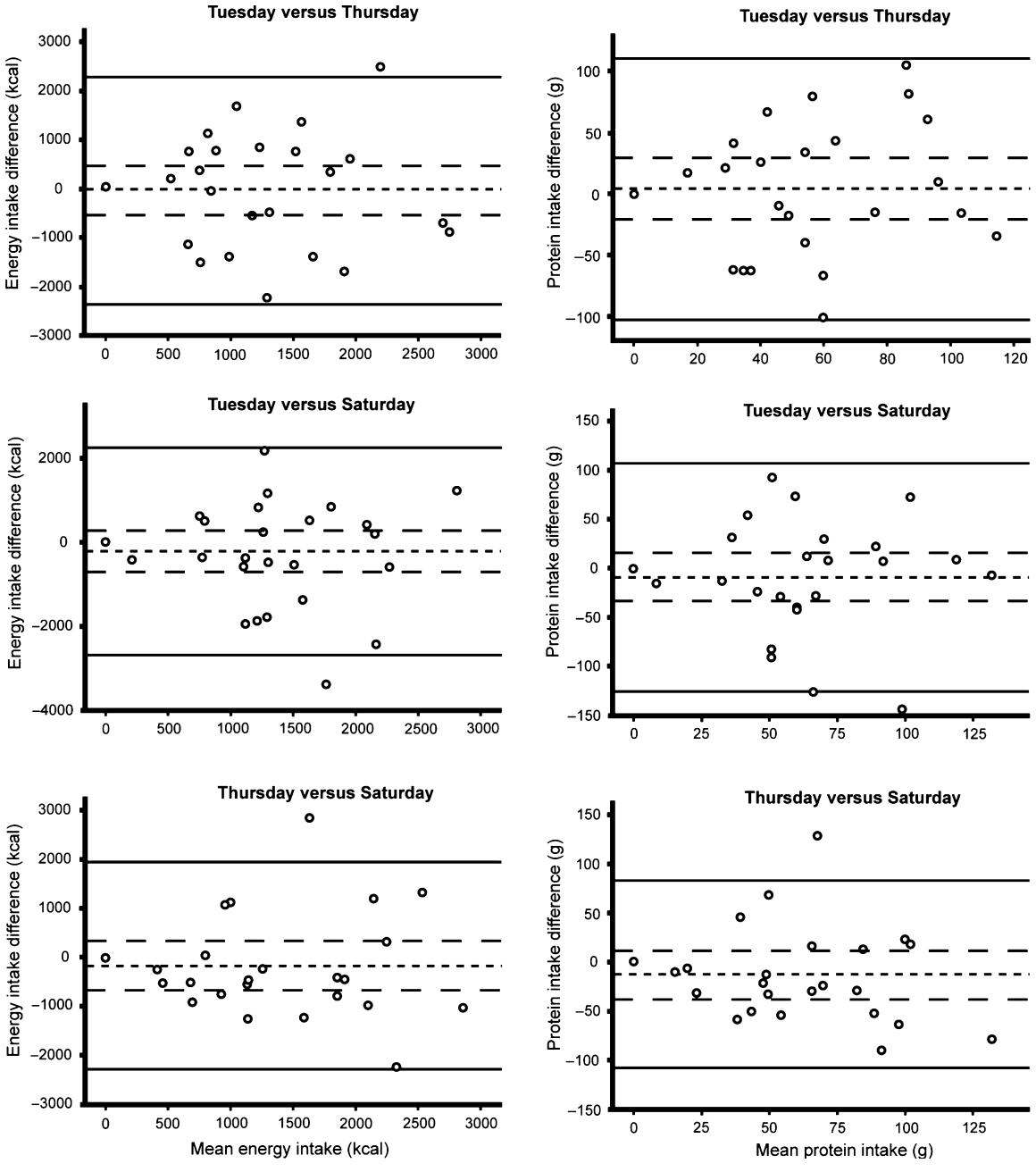

Figure 1 Bland–Altman plots of difference versus mean of energy and protein intake measured across days. --- Mean intake. — – Clinical agreement [defined as 2.09 MJ (500 kcal) and 25 g protein from the mean]. —— Two standard deviations from the mean (Bland–Altman limits of agreement).

compared to weekends using the gold standard technique: investigator-led WFR. At the population level (e.g. mean intake for all patients), there were no differences in mean energy or protein intakes between weekday and weekends, or individual days per week. Hence, these results negate the hypothesis of the present study, which proposed that head-injured patients recovering from critical illness would consume more energy and protein on weekends. This observation is important for future population-based research, such as observations of intake in specific patient groups, because representative intake could be collected on any day of the week.

Only one other study has previously attempted to quantify oral intake in a critically ill population. Peterson *et al.* [9] reported that energy and protein intakes from oral intake were less than 50% of estimated requirements

Table 3 Mean energy and protein intakes and limits of agreement across days from Bland–Altman plots

	Energy (kcal)		Protein (g)	
	Mean difference intake	Limits of agreement (mean ± 2SD)	Mean difference intake	Limits of agreement (mean ± 2SD)
Week 1 (n = 25)				
Tuesday versus Thursday	−36	−2355, 2283	4	−103, 110
Thursday versus Saturday	−175	−2281, 1931	−13	−108, 83
Tuesday versus Saturday	−211	−2680, 2258	−9	−125, 107
Weekend versus weekday	−153	−2135, 1828	−11	−90, 67
Week 2 (n = 15)				
Tuesday versus Thursday	161	−958, 1279	−2	−56, 53
Thursday versus Saturday	126	−1779, 2031	5	−88, 98
Tuesday versus Saturday	287	−1055, 1629	3	−59, 66
Weekend versus weekday	155	−1398, 1708	−2	−97, 94

each day; however, their data were limited to the week following weaning from ventilatory support. Given the lack of data the need to collect precise dietary intake information, post-ITU has been identified by several authorities as an important objective [16,17]. Given the scarcity of research that includes the measurement of nutrition consumed orally in both critically ill and head-injured patients, the finding that representative intake does not require measurement on weekend days provides an achievable, sufficiently accurate methodology for quantifying energy and protein intakes, with positive inferences for funding.

However, at the individual patient-level, there was considerable day-to-day variation in energy and protein intakes, irrespective of the measurement day, and large intra-patient variation across days. Accordingly, in studies exploring the influence of oral intake in individual hospitalised patients (e.g. interventional or observational studies exploring associations between variables) [18,19], a single day is an imprecise measure of mean intake for that patient. Accordingly, WFR should be collected on as many days as possible to precisely reflect actual intake. This finding also has implications for clinical practice where estimates of oral intake made on a single day will need to be repeated as frequently as possible to improve accuracy. Although, in free-living populations, a small selection of both week and weekend days is required to provide a true reflection of intake, in this patient group, large intra-patient variation occurred across all days, demonstrating the need to include measurements on as many days as possible. The finding that there was no difference in oral intake between week and weekend days also has implications for staffing a clinical service because this information does not require measurement at the weekend.

In the present study, there was also an increase in mean energy and protein intakes over time. This requires consideration, both for research and clinical practice. Based on these results, a longer duration of measurement is required when evaluating the nuanced relationships between oral intake and recovery [15,20].

Although the present study was conducted in a specific population, the measurement of energy and protein consumed via the oral route has been described in other patient groups. These studies have all used single day snapshots of oral intake, with data from self-reported intakes [21], visual reports of categorised food waste (e.g. nothing, <1/2, >1/2, all) [11,22] or measures of plate waste but not the portion provided [23]. Although these studies have not been able to demonstrate changes in intake longitudinally, when taken together, they are consistent with the concept that patients consuming nutrients orally receive suboptimal nutrition [11,21,23,24], which is associated with adverse outcomes such as increased infection rates and mortality [11,21,22]. Therefore, the assessment of ingested nutritional intake is important, and greater emphasis on accurate measurement of nutrients consumed via this route should take place both in clinical practice and nutrition research.

The present study has a number of strengths. It is the first to investigate the variance and agreement between energy and protein intakes quantified using WFR on weekdays versus weekends in an institutional setting. Compared to WFR in free-living populations where the individual completes the measurements, these WFR were conducted by two independent study investigators who were not open to respondent bias. The main limitation was that 2 weekdays and 1 weekend were considered representative of weekly intake, as is common practice in dietary methodology. Additionally, any items consumed overnight were not included in the observation period. However, the latter was consistent for all days. Finally, these data were obtained from a small, defined group of patients who were admitted to a single-centre and so the results may not be generalisable to all healthcare settings and patient groups.

Conclusions

In hospitalised head-injured patients recovering from critical illness, mean energy and protein intakes were observed to be similar across days but with large intra-patient variations. This suggests that future quantification of oral intake in hospitalised populations, but not individuals, could be performed using investigator-led WFR on any day of the week. This has significant implications for resourcing nutrition research. Studies of individual treatment effects should include as many days as feasible, but not necessarily weekends, aiming to adequately reflect the true intake.

Acknowledgments

The authors thank Annie Chung for her contributions to the data entry of weighed food records.

Conflict of interests, sources of funding and authorship

The authors declare that they have no competing interests, such as employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, grants or other funding.
AMD has received honoraria for participation on Clinical Advisory Board to treat nutritional insufficiency (Medtronic). This research was funded by an Australasian Society of Parenteral and Enteral Nutrition (AuSPEN) small project grant and a Neurosurgical Research Foundation grant. The first author was supported by an Australian Post-Graduate Award and Royal Adelaide Hospital Dawes Top-Up Scholarship.
LSC was responsible for the study design, recruitment, data collection, data entry, data analysis, statistical analysis, interpretation of the data and drafting of the manuscript. She is guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the accuracy of the data analysis. MJC, AMD and LTW contributed to the study design, interpretation of the data and the critical revision of the manuscript for important intellectual content. KL was responsible for analysis and interpretation of the data, and contributed to the study design and the critical revision of the manuscript for important intellectual content. AK contributed to data collection, data entry, interpretation of the data and the critical revision of the manuscript for important intellectual content. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. McClave SA, Taylor BE, Martindale RG *et al.* (2016) Guidelines for the provision and assessment of nutrition support therapy in the adult critically Ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr* **40**, 159–211.
2. Jacobs D, Jacobs D, Kudsk K *et al.* (2004) Practice management guidelines for nutritional support of the trauma patient. *J Trauma* **57**, 660–679.
3. Casaer MP, Mesotten D, Hermans G *et al.* (2011) Early versus late parenteral nutrition in critically ill adults. *N Engl J Med* **365**, 506–517.
4. Heyland D, Muscedere J, Wischmeyer PE *et al.* (2013) A randomized trial of glutamine and antioxidants in critically ill patients. *N Engl J Med* **368**, 1489–1497.
5. Doig GS, Simpson F, Sweetman EA *et al.* (2013) Early parenteral nutrition in critically ill patients with short-term relative contraindications to early enteral nutrition: a randomized controlled trial. *JAMA* **309**, 2130–2138.
6. Harvey SE, Parrott F, Harrison DA *et al.* (2014) Trial of the route of early nutritional support in critically ill adults. *N Engl J Med* **371**, 1673–1684.
7. Peake SL, Young PJ, Chapman M *et al.* (2015) Permissive underfeeding or standard enteral feeding in critical illness. *N Engl J Med* **373**, 1173–1174.
8. Braunschweig CA, Sheean PM, Peterson SJ *et al.* (2015) Intensive nutrition in acute lung injury: a clinical trial (INTACT). *JPEN J Parenter Enteral Nutr* **39**, 13–20.
9. Peterson SJ, Tsai AA, Scala CM *et al.* (2010) Adequacy of oral intake in critically ill patients 1 week after extubation. *J Am Diet Assoc* **110**, 427–433.
10. Kondrup J (2001) Can food intake in hospitals be improved? *Clin Nutr* **20**, 153–160.
11. Dupertuis Y (2003) Food intake in 1707 hospitalised patients: a prospective comprehensive hospital survey. *Clin Nutr* **22**, 115–123.
12. Rutishauser IH (2005) Dietary intake measurements. *Public Health Nutr* **8**, 1100–1107.
13. Wrieden, W, Peace, H, Armstrong, J *et al.* (2003) A short review of dietary assessment methods used in National and Scottish Research Studies [Monitorig of Scottish Dietary Targets Workshop].
14. Yang PH, Black JL, Barr SI *et al.* (2014) Examining differences in nutrient intake and dietary quality on weekdays versus weekend days in Canada. *Applied Physiol Nutr Metab* **39**, 1413–1417.
15. Chapple LS, Deane AM, Heyland DK *et al.* (2016) Energy and protein deficits throughout hospitalization in patients admitted with a traumatic brain injury. *Clin Nutr* DOI: 10.1016/j.clnu.2016.02.009.
16. Merriweather J, Smith P & Walsh T (2014) Nutritional rehabilitation after ICU – does it happen: a qualitative

interview and observational study. *J Clin Nurs* **23**, 654–662.
17. Tan T, Brett SJ, Stokes T *et al.* (2009) Rehabilitation after critical illness: summary of NICE guidance. *BMJ* **338**, b822.
18. Bruce D, Laurance I, McGuiness M *et al.* (2003) Nutritional supplements after hip fracture: poor compliance limits effectiveness. *Clin Nutr* **22**, 497–500.
19. Neumann M, Friedmann J, Roy MA *et al.* (2004) Provision of high-protein supplement for patients recovering from hip fracture. *Nutrition* **20**, 415–419.
20. Hackett AF, Yeung CK & Hill GL (1979) Eating patterns in patients recovering from major surgery – a study of voluntary food intake and energy balance. *Br J Surg* **66**, 415–418.
21. Hiesmayr M, Schindler K, Pernicka E *et al.* (2009) Decreased food intake is a risk factor for mortality in hospitalised patients: the NutritionDay survey 2006. *Clin Nutr* **28**, 484–491.
22. Thibault R, Makhlouf AM, Kossovsky MP *et al.* (2015) Healthcare-associated infections are associated with insufficient dietary intake: an observational cross-sectional study. *PLoS ONE* **10**, e0123695.
23. Barton AD, Beigg CL, Macdonald IA *et al.* (2000) High food wastage and low nutritional intakes in hospital patients. *Clin Nutr* **19**, 445–449.
24. Thibault R, Chikhi M, Clerc A *et al.* (2011) Assessment of food intake in hospitalised patients: a 10-year comparative study of a prospective hospital survey. *Clin Nutr* **30**, 289–296.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

DIETETIC PRACTICE AND BEHAVIOUR CHANGE

Dietitians' practice in giving carbohydrate advice in the management of type 2 diabetes: a mixed methods study

P. D. McArdle,[1] S. M. Greenfield,[2] A. Avery,[3] G. G. Adams[3] & P. S. Gill[2]

[1]Birmingham Community Healthcare NHS Foundation Trust (BCHC), Birmingham, UK
[2]Primary Care Clinical Sciences, College of Medical & Dental Sciences, University of Birmingham, Birmingham, UK
[3]Faculty of Science, The University of Nottingham, Sutton Bonington, UK

Keywords
diabetes, dietitian, carbohydrate, dietary advice.

Correspondence
P. D. McArdle, Birmingham Community Nutrition, BCHC, St Patricks Centre for Community Heath, Frank Street, Birmingham B12 0YA, UK.
Tel.: +44 (0)121 4461110
Fax: +44 (0)121 4461020
E-mail: paul.mcardle@nhs.net

How to cite this article
McArdle P.D., Greenfield S.M., Avery A., Adams G.G. & Gill P.S. (2017) Dietitians' practice in giving carbohydrate advice in the management of type 2 diabetes: a mixed methods study. *J Hum Nutr Diet*. **30**, 385–393
doi: 10.1111/jhn.12436

Abstract

Background: Carbohydrate is accepted as the principal nutrient affecting blood glucose in diabetes; however, current guidelines are unable to specify the optimal quantity of carbohydrate for glycaemic control. No studies exist that describe current practice amongst healthcare professionals giving carbohydrate advice in type 2 diabetes. The present study aims to improve understanding of the degree of variation in the current practice of UK registered dietitians (RDs) by describing how RDs advise patients.
Methods: UK RDs were contacted through national networks and asked to complete an online survey, which was analysed using STATA, version 12 (StataCorp, College Station, TX, USA). Three consultations between dietitians and patients with type 2 diabetes were observed, followed by semi-structured interviews with the dietitians.
Results: In total, 320 complete survey responses were received. Dietitians' advice varied according to expertise, training and confidence, and the complexity of the patient's blood glucose treatment. Some 48% (n = 154) of respondents advised patients to restrict carbohydrate intake either occasionally or frequently, with 35.6% (n = 114) considering 30–39% of total energy from carbohydrate to be a realistic expectation. The overall theme from the interviews was 'Conflicting Priorities', with three sub-themes: (i) how treatment decisions are made; (ii) the difference between empowerment and advice; and (iii) contradictory advice. A disparity existed between what was observed and interview data on how dietitians rationalise the type of carbohydrate advice provided.
Conclusions: Dietitians' advice varies for a number of reasons. Consensus exists in some areas (e.g. carbohydrate awareness advice); however, clear definitions of such terms are lacking. Clarification of interventions may improve the consistency of approach and improve patient outcomes.

Introduction

Diabetes affects over 3 million people in the UK and 415 million worldwide [(1)], most of whom are diagnosed with type 2 diabetes. Diabetes is a complex condition requiring a multidisciplinary approach, including physicians, nurses and dietitians [(2)]. Treating diabetes and its complications costs the National Health Service (NHS) approximately £10 billion ($14 billion) per year, accounting for approximately 10% of the budget [(3)]. The effective management of type 2 diabetes should include nutritional advice from those individuals with specific expertise and competencies in nutrition [(4)], such as a RD.

In clinical trials, nutrition interventions have achieved reductions in HbA1c of between 0.5% and 2.3% (6–25 mmol mol^{-1}) [(5)]. A range of dietary approaches may be effective in managing blood glucose in type 2 diabetes, including low fat, low carbohydrate, low glycaemic

index, high protein and Mediterranean diets [6]. However, as a result of limited published evidence, current UK and international guidelines are unable to recommend a particular regimen and, instead, highlight weight loss as the principal strategy for managing blood glucose in those who are overweight [7]. Carbohydrate is accepted as the only nutrient with a direct effect on blood glucose and, as such, attracts significant attention in the literature, with recent reviews being unable to conclude the optimal quantity of carbohydrate [8,9]. The ideal proportion of macronutrients in the diet, particularly carbohydrate, is consequently the subject of debate [10]. Therefore, in the absence of evidence, UK and US guidelines suggest that people with diabetes follow general population guidelines for 'healthy eating' [11], which recommend obtaining 50% of energy from carbohydrate, although with an emphasis on monitoring individual responses to carbohydrate intake to achieve glycaemic control. Previous guidelines recommended a specific proportion of total energy from carbohydrate [30].

Dietitians are the only statutory registered diet and nutrition specialists in the UK and play an important role in advising people with diabetes [11]. The lack of strong evidence or a professional consensus on the optimal quantity of carbohydrate in people with type 2 diabetes means that there is the potential for wide variations in dietetic practice [12]. However, this has not been studied previously.

Research is lacking regarding current advice and dietary management in type 2 diabetes, and little is known specifically about advice given by dietitians. As a result of the lack of a definitive guideline on the quantity of carbohydrate in type 2 diabetes, we consider that this area warrants further investigation. Therefore, the present study aims to describe and explore the practice of UK dietitians with respect to carbohydrate advice in type 2 diabetes, focussing on the degree of variation in advice.

Materials and methods

A mixed methods approach was taken, adopting a convergent, parallel design [13]. A national, cross-sectional survey was used to provide quantitative data on the practice of dietitians, together with qualitative data (from observations and interviews) to provide further insights into how dietitians advise patients about carbohydrate. Sponsorship was provided by The University of Nottingham. Ethical approval was obtained from NHS REC (13/SW/0120).

Cross-sectional survey of registered dietitians

Subjects and survey administration

The survey was constructed using Bristol Online Surveys [14], which automatically codes responses, and was distributed by e-mail using national networks of dietitians; principally members of The British Dietetic Association (BDA) whose membership represents >80% of UK dietitians [15]. Data on the number of dietitians working in diabetes in 1996 identified a population of 512 dietitians [16]; however, there is no official estimate of the current figure (1). The survey was also promoted using social media (Twitter, Facebook, LinkedIn) and personal contacts. The population reach was conservatively estimated at 3000 dietitians and approximately half were likely to have been eligible to take part, using data available from The BDA [17], suggesting a target population estimate of 1500. Based on these figures, the target sample size was calculated as 341 respondents, using a 95% confidence level and 5% confidence intervals. Before completing the survey, respondents were asked to confirm their eligibility to participate by answering two screening questions (if they were a UK RD and consult patients with type 2 diabetes in one-to-one clinic settings). An optional prize draw for a £50 shopping voucher was offered as an incentive to participate.

Survey design

The survey was piloted by asking two colleagues to check the clarity of questions and the length of time taken to complete the survey. Feedback was also obtained from academic supervisors, following which minor changes were made to the layout and wording of the questions relating to the types of advice given, aiming to improve clarity of the terms used. The survey contained questions about dietitians' training and confidence in advising patients about carbohydrate before asking about advice given regarding glycaemic index, glycaemic load, frequency and level of carbohydrate restrictions used, as well as their definition of carbohydrate awareness advice. Dietitians were then asked to state how frequently different types of carbohydrate advice were given to different patient types. For the purposes of the survey, five categories of patient type were defined simply by the level of complexity of treatment for blood glucose control: (i) no medication; (ii) oral medication only; (iii) once or twice daily background insulin; (iv) once or twice daily premixed insulin; and (v) multiple daily injections. These patients are referred to as patient types 1–5 accordingly.

Statistical analysis

Response data were extracted, together with a codebook detailing the descriptive meaning of each coded answer, from Bristol Online Surveys and imported into STATA, version 12 [18] for generation of descriptive statistics. Non-parametric tests (chi-squared, Fisher's exact) were undertaken to check for independence. A linear regression model was developed to establish how much of the variation in the carbohydrate advice given could be attributed

to the different patient types (i.e. the complexity of the treatment for blood glucose control). Dummy variables were created to represent the Likert scores for carbohydrate counting advice in each patient type. These were then used in a stack form to combine each patient type and other predictor variables (previous training, confidence, etc.) in the regression model.

Nonparticipant observation and interviews

Subjects and sampling

Purposive sampling [19] was used to identify dietitians who specialise in or see patients with type 2 diabetes in two England NHS sites (one community-based and one incorporating both acute and community). The second site was included to minimise bias because the RDs working there were not known to the researcher. Sample size was determined by data saturation, which is the point at which no new information or themes emerged [20] and can be achieved with as few as 10 participants [21]. Each dietitian was observed in consultation with one patient and then interviewed. Written informed consent was first obtained from the dietitians who were then asked to identify clinics with patients with a confirmed diagnosis of type 2 diabetes. Written, informed consent was obtained from patients by the researcher at the clinic.

Observation of consultations

Nonparticipant observations of consultations between dietitians and patients with type 2 diabetes were undertaken immediately prior to semi-structured interviews with the dietitian. Observations of health consultations can be a useful method for understanding the components of care and decision-making [22]. The highly contextual nature of qualitative research interviews means that conducting the interview immediately following the observation should allow for a richer account of the nature of the phenomenon [23]. The purpose of the observation in this setting was to observe how dietitians advised patients about carbohydrate, aiming to inform the framing of the questions in the interview and enable comparisons to be made between what is reported in the interviews and what is actually observed in practice. Observed consultations typically lasted 30–45 min and were not recorded, although field notes were made, consisting primarily of the researchers' reflections and areas to explore in questioning in the interviews.

Interviews with dietitians

The interviews were allocated 30 min each to minimise pressure on the clinician's time and none of the interviews required longer than this to fully explore the topic. An interview schedule was used and interviews were recorded using a digital audio recorder. Interview questions initially focussed on exploring the dietitian's aims and focus of the observed consultation, as well as their rationale for these. Subsequent questions asked about how the dietitian usually advises patients about carbohydrate, and what they consider to be the essential knowledge and skills required by different types of patients.

Data analysis

Data collection took place throughout July 2013. All observations and interviews were conducted by the lead author (PM). Interviews were recorded, transcribed verbatim and underwent thematic analysis [24]. Texts were read and re-read, and then coded for meaning using an inductive, iterative process [25]. Codes were then grouped into meaning units and themes generated. The notes from observations primarily captured what the observer felt was influencing the dietitians' decision-making process during the consultation. The field notes from observations were not subjected to the same thematic analysis but were used to guide specific questions during the interview and were later reviewed during the analysis of interview transcripts as an aide memoire to assist in interpreting the contextual meaning of the texts [26].

Results

Cross-sectional survey of dietitians

A total of 377 survey responses were received; however, only 320 were complete and used in the analysis, representing a 21.3% response rate based on the estimated population of 1500 dietitians. Respondent characteristics are described in Table 1. The sample was largely female and mostly comprised experienced dietitians in NHS pay bands 6 and 7, who identified as specialists in diabetes and were UK trained.

Table 2 summarises participant responses for questions relating to general advice about carbohydrate. Advice about the glycaemic index (GI) and glycaemic load (GL) is not covered in detail by most dietitians, although advice to avoid specific high-GI and, conversely, to include specific low-GI foods is given by most of the dietitians surveyed. Carbohydrate restrictions are advised occasionally or frequently in 48% of respondents, and the most popular restriction is 30–39.9% of total energy from carbohydrate.

Table 3 shows that Diabetes Specialist Dietitians (DSDs) were more confident and more likely to recommend a restriction in carbohydrate quantity than non-DSDs ($P < 0.01$, $n = 320$). In addition, DSDs considered that a greater restriction in the proportion of energy from carbohydrate was more realistic compared to non-DSDs ($P = 0.01$).

Carbohydrate awareness advice was reportedly given 'almost always' in all patient types by ≥78% of respondents.

Table 1 Characteristics of respondents (n = 320)

Characteristic	Findings		
	Variable	*n*	%
Sex	Male	14	4.4
	Female	306	95.6
Age	18–24 years	15	4.7
	25–34 years	109	34.1
	35–44 years	89	27.8
	45–54 years	73	22.8
	55–64 years	33	10.3
	65 years and over	1	0.3
Years qualified as a registered dietitian	Less than 12 months	1	0.3
	1–3 years	39	12.2
	4–6 years	64	20.0
	7–9 years	37	11.6
	10 years or more	179	55.9
NHS pay band	Band 5	25	7.8
	Band 6	131	40.9
	Band 7	128	40.0
	Band 8	15	4.7
	Non-NHS	21	6.6
Diabetes specialist dietitian	Yes	176	55.0
	No	144	45.0
Dietetic training in the UK?	UK	297	92.8
	Overseas	23	7.2

The most popular definition was 'Education about identifying foods and drinks that contain carbohydrate'. Respondents were allowed to select more than one definition, and many did so, indicating either a plurality of definitions or some uncertainty amongst the profession.

A linear regression model was developed to examine the relationship between increasing complexity of advice (i.e. likelihood of giving detailed carbohydrate counting advice) and patient type. After accounting for the confidence and training of dietitians, the analysis demonstrated there was a 24% increase in the likelihood of the patients being offered carbohydrate counting advice comparing patient type 5 with patient type 1 ($P < 0.05$). Therefore, increasing complexity of blood glucose treatment does not fully explain the likelihood of patients receiving more complex carbohydrate advice. Carbohydrate awareness and GL advice increased to a lesser extent between patient types 1 and 5 (8.4%, $P < 0.05$), and the association with GI advice was even smaller.

Nonparticipant observation and interviews

In total, three out of 10 dietitians approached took part (three dietitians from site one and none from site two). Dietitians who did not take part cited a reluctance to be interview and observed. Two specialist dietitians and one nonspecialist were included. The non-DSD had no specific training in carbohydrate counting and the two DSDs had been trained and both had more than 4 years of experience working in diabetes. The purposive sampling was intended to include specialists and nonspecialists to reflect the survey respondents; however, comparisons between the two groups would not be appropriate as a result of the sample size.

The analysis resulted in the generation of one overarching theme, 'Conflicting priorities', and three sub-themes linked to this.

Overall theme: conflicting priorities – carbohydrate versus other advice

Registered dietitians appeared to have difficulty in differentiating the various types of carbohydrate advice and separating it from other forms of advice. For example, where the definition of carbohydrate awareness may overlap with the definition of carbohydrate counting, or the difference between discussing carbohydrate for blood glucose management and for obtaining a balance of nutrients or for controlling weight.

> 'I think the basic skill is basically carbohydrate awareness. Which means basically education on what exactly carbohydrate foods are. Identify what are carbohydrate foods. Not only identify carbohydrate but at the same time the amount of carbohydrate as well and what will be the implication of eating that amount of carbohydrate on blood glucose' (RD1 – DSD)

> 'Obviously, within healthy eating, we can't talk about healthy eating without bringing in carbohydrate advice but for, certainly at my level, keeping it relatively straight forward, basic and portion sizing being correct but overall, looking at overall energy intake rather than just focusing on one food group' (RD2 – non-DSD)

> 'So, people who are wanting to control their weight they know that it's the carbs that they need to inject for so, for example, if they want to have any carbs at lunch time, because sometimes you know, people, patients, have said to me that the insulin puts weight on and we keep saying that well actually insulin is non calories, its what you're eating that would put the weight on . . . So they can manipulate it to that advantage really so if they were having something like a chicken salad then they would say, what's the carbohydrate content there, and if there is nothing you say then you don't need to inject for that. So, I think it is a good skill to have in terms of balancing the meals as well' (RD3 –DSD)

There was a description of a patient-centred approach alongside a contradictory account of how the approach is chosen by the Dietitian, following their assessment, as outlined below.

Table 2 General advice regarding carbohydrate (n = 320)

Question	Responses	Results	
		n	%
What advice do you usually give regarding *Glycaemic Index (GI)*?*	I cover GI as a general concept only	200	NA
	I advise patients to avoid specific high-GI foods	80	NA
	I advise patients to include specific low-GI foods	148	NA
	I educate patients about the specific GI values of certain foods	37	NA
	I don't usually cover GI	30	NA
What advice do you usually give regarding *Glycaemic Load (GL)*?*	I cover GL as a general concept only	164	NA
	I advise patients to avoid specific high-GL foods	26	NA
	I advise patients to include specific low-GL foods	30	NA
	I educate patients about the specific GL values of certain foods or their overall diet.	34	NA
	I don't usually cover GI	119	NA
How often do you advise or support patients to implement a *carbohydrate restriction?*	Never	47	14.7
	Rarely (5% of the time)	56	17.5
	Sometimes (10% of the time)	63	19.7
	Occasionally (25% of the time)	60	18.8
	Frequently (50% of the time)	94	29.4
For you, what would represent a *realistic carbohydrate restriction* in type 2 diabetes?	Roughly 50% of total energy from carbohydrate	55	17.2
	40–49.9% of total energy from carbohydrate	100	31.2
	30–39.9% of total energy from carbohydrate	114	35.6
	Less than 30% of total energy from carbohydrate	21	6.6
	Ketogenic amounts of 20 g of carbohydrate per day or less	1	0.3
What does the term *Carbohydrate Awareness* mean to you?	Education about identifying foods & drinks that contain carbohydrate	281	NA
	Education about portions of common foods that contain equivalent amounts of carbohydrate	228	NA
	Education about the actual quantities of carbohydrate in common foods	161	NA
	Other	43	NA

*'Usually' was defined as approximately 50% of the time. NA, respondents were able to select more than one answer and so percentages are not applicable.

> 'I think it depends on the individual so I wouldn't force a low carbohydrate diet on my patient because it depends on what stage of change they're at and what they want from the consultation. So what is their priority, what is their aim?'

> '... when they come to clinic you'll see them and when you do the whole assessment process, you've taken all the details, you will then be able to identify which way you are going to go with them, whether its going to be looking at very low carb diets or is it going to be looking at carbohydrate portion control to begin with, then gain their confidence ...' (RD1 – DSD)

This overall theme highlights the conflict between the evidence-based guidelines and everyday practice. Weight loss and overall calorie reduction is highlighted as best practice, yet dietitians acknowledge quantity of carbohydrate as an important factor in managing blood glucose.

Sub-theme one: the difference between empowerment and advice

Registered dietitians in interviews highlighted the importance of patient 'empowerment' and offering support, at the same time as distinguishing this from the giving of advice. Empowerment was not seen as advice, yet, in observations, RDs were seen questioning patients about how they feel, what they understand and what they do, at the same time as giving carbohydrate advice to patients. This advice about types and quantities of carbohydrate was termed 'education' by the dietitians, and therefore appeared to fall outside their definition of 'advice'.

> '... with talk about empowerment, empowerment is much more important just to educate people and then once we educate people and we work together with them we are not basically making things changing for them we are basically facilitating decision making, making them decide for themselves what's basically good for them and what changes are more really suitable for them in the long term and things like that. So having that thing in mind [empowerment] and, like her really poor understanding of healthy eating, carbohydrate awareness and things like that, it was much more important for me to give her some education, to inform her, to be aware of carbohydrate food' (RD1 – DSD)

Table 3 Differences between specialist and non-specialist dietitians

n = 320	Diabetes Specialist Dietitian % (*n*)						Non-Diabetes Specialist Dietitian % (*n*)						*P* for between groups*
I feel confident in teaching patients with type 2 diabetes about the quantity of carbohydrate in food	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Other	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Other	<0.01
	3.1 (10)	0.1 (3)	0.0 (1)	17.2 (55)	33.4 (107)	N/A	2.2 (7)	7.8 (25)	7.8 (25)	20.3 (65)	6.9 (22)	N/A	
How often do you advise or support patients to implement a carbohydrate restriction?	Never	Rarely	Sometimes	Occasionally	Frequently	Other	Never	Rarely	Sometimes	Occasionally	Frequently	Other	<0.01
	3.8 (12)	6.6 (21)	11.3 (36)	12.8 (41)	20.6 (66)	N/A	10.9 (35)	10.9 (35)	8.4 (27)	5.9 (19)	8.8 (28)	N/A	
For you, what would represent a realistic carbohydrate restriction in type 2 diabetes?	50% energy from CHO	40–49.9% energy from CHO	30–39% energy from CHO	<30% energy from CHO	20 g CHO or less	Other (see free text)	50% energy from CHO	40–49.9% energy from CHO	30–39% energy from CHO	<30% energy from CHO	20 g CHO or less	Other (see free text)	0.01
	6.6 (21)	15.9 (51)	22.5 (72)	4.4 (14)	<0.0 (1)	5.3 (17)	10.6 (34)	15.3 (49)	13.1 (42)	2.2 (7)	0.0 (0)	3.8 (12)	

*Chi-squared test for independence.

[Correction added on 1 March 2017, after first online publication: The 'Other' columns in Table 3 were previously missing and have now been added in this current version.]

The importance of 'support' rather than advice and the use of behaviour change skills were emphasised by one dietitian.

> 'She has ... there are things going on at home, I think there are issues of going into the kitchen, the kitchen is all upside down and what have you and that is not really advice, that's "how can I help you ..." how can we problem solve that really' (RD2 – non-DSD)

Sub-theme two: how treatment decisions are made

It was unclear from the dietitians' accounts whether the dietary approaches were intended to support the medical management or vice versa. There was a contradiction between what the dietitians reported in interviews and what was observed during the consultations. Interviewees reported the patient as the driving force for decisions over the type of carbohydrate advice at the same time as stating that it was a team approach in collaboration with other healthcare professionals, or that the type of medication patients take will largely decide what carbohydrate advice is given.

> 'She had heard about this approach [carbohydrate counting] and ... so she sort of brought that up and we said okay if that's what you would like to do we will try that ... I think it just depends because we work closely with the DSNs [diabetes nurse] and its kind of like a joint decision and we will say, well actually, I think before we try anything else, and this person doesn't like multiple injections, then maybe we will go with the twice a day insulin ...' (RD3 – DSD)

> '... because of all her symptoms of poor diabetes control I mean we don't have many options available in terms of medication. The only option is basically dietary intake ...' (RD1 – DSD)

Sub-theme three: contradictory advice

Dietitians were inconsistent or unclear both within and between interviews when describing the various forms of advice relating to carbohydrate. For example, low carbohydrate is referred to both in terms of being 'good' (useful) and 'bad' (not healthy), whereas the message is mixed as part of an overall calorie or portion reduction. They had difficulty assigning the relative importance of carbohydrate advice versus advice to reduce portion sizes or reduce total calories. There was ambiguity over the use of 'low carbohydrate' approaches and the terms 'restrict' and 'reduce' were used when referring to carbohydrate, in preference to use of the term 'low'.

> 'Low carbohydrate is quite good. Good and bad. Good for those patients if they can just manage reducing their total portion intake and as part of that total, that reduction in portion, if they reduce carbohydrate intake that's absolutely fine ...' (RD1 – DSD)

> 'I think if this patient gets to grips with carb counting and knows, you know, that some days it's okay if she didn't fancy any carbs with her lunch, in terms of weight loss, I wouldn't always promote, you know, don't have a carb free day because we know carbs provide you with energy but she's got that flexibility to have less to control her weight. Because the carbs are not necessarily in isolation, it could be fat and sugar with them you see, so that's why we kind of say with this regime you've got that flexibility to control your weight really' (RD3 – DSD)

Discussion

The aim of the present study was to improve understanding of the degree of variation in the current practice of UK RDs by exploring and describing how dietitians advise patients with type 2 diabetes about carbohydrate.

This is the first study of its kind and the findings obtained show that there is variation in practice, which could be accounted for partly by the imperative to provide patient-centred and individualised care. However, it could also be a result of the lack of a clear evidence base and guidelines relating to carbohydrate advice in type 2 diabetes. Specialist dietitians were more likely to recommend a carbohydrate restriction and to recommend a greater restriction in carbohydrate than nonspecialist dietitians, thereby suggesting less reliance on specific guidelines by more experienced dietitians.

Nonspecialist dietitians reported a lack of confidence in teaching people with type 2 diabetes about the quantity of carbohydrate in food, which is likely related to a lack of specific training in carbohydrate counting or diabetes education. Considering the low uptake of structured patient education in diabetes [27] and the limited resources with regard to access to diabetes specialist dietitians, it is vital that nonspecialist dietitians are equipped with a good level of knowledge and skills and are confident in advising people with type 2 diabetes about carbohydrate in food.

Dietitians reported almost universally providing 'carbohydrate awareness' advice, yet were unable to coherently define and in some cases distinguish this from advice about portion-control in general. The frequency with which dietitians give carbohydrate awareness advice highlights the importance of this term being properly described and defined as an intervention for the dietetic profession and others working with people with type 2

diabetes. The qualitative strand of the present study corresponds with the survey findings and provides further narrative regarding the difficulty that dietitians have in defining this term. The recent media and professional focus regarding the balance of specific macronutrients, namely carbohydrate and fat, in the diet of people with type 2 diabetes [9] requires that RDs are able to speak confidently and coherently both to other healthcare professionals and patients regarding the evidence base in this important area.

The mixed methods study design allowed for a deeper understanding of the factors that may influence how RDs determine which patients should receive what form of carbohydrate advice. The benefit of the mixed methods approach in the present study is the way in which the qualitative data informed the interpretation of the survey data [28]. The survey had an estimated reach of approximately one-third of UK RDs [29] and provided a national view of dietetic practice. The interviews and observations helped add meaning to this. The survey results suggested an increase in the likelihood of patients being offered more complex carbohydrate counting advice with increasingly complex treatment regimens; however, the regression model suggests this only accounts for a small proportion of the variation in advice. Understanding of this finding was enhanced by the observations and interviews, which reveal a number of influences on the decision for what type of advice the patient should be offered, including collaboration with other team members. Without the qualitative strand to the present study, interpretation of the variation in advice shown in the regression model would have been more challenging.

The sampling approach may have led to a risk of selection bias in the present study, for the quantitative and qualitative elements. More experienced dietitians and those specialising in diabetes may have been more likely to take part, which may explain some of the participant characteristics for the survey. However, the characteristics suggest a representative sample in terms of sex distribution (BDA membership 3.9% male as of September 2012) [16]. Although the qualitative sample did not allow for saturation, there was consistency amongst the participants in terms of overall themes. The qualitative data helped to expand on the quantitative data, despite the relatively small sample. In addition, the recruitment and selection for the survey, being entirely conducted via electronic means, may have excluded a particular section of the dietitian population. It is likely that practice will always vary as a result of the imperative to provide patient-centred care, and dietitians are skilled in individualising advice for each patient. However, there is also a need to provide advice that has a clear rationale and can be explicated clearly and concisely. This warrants further studies that aim to gain a deeper understanding of this decision-making process amongst dietitians with respect to aiding the development of future interventions.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with STROBE guidelines.

Conflict of interests, source of funding and authorship

PM is a member of the Professional Practice Board of the BDA and a Committee Member of the Diabetes Specialist Group of the BDA.
PM is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. This report is independent research arising from a Clinical Doctoral Research Fellowship, awarded to PM CDRF-2014-05-030, supported by the National Institute for Health Research (NIHR) & Health Education England. SG is part funded by the NIHR Collaboration for Leadership in Applied Health Research and Care West Midlands (CLAHRC WM). The views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. We would like to thank Parth Narendran (Birmingham, UK) for reviewing the manuscript.
PM conceived and designed the study, conducted the data collection and analysis, and wrote the paper. GA and AA were involved in the design and planning of the research, in revising the manuscript, and supervising the conduct of the research. PG and SG undertook several manuscript revisions. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

1. IDF (2015) *Internation Diabetes Federation Diabetes Atlas*. 7th edn, 2015 Update. Brussels, Belgium: IDF.

2. IDF (2012) Clinical Guidelines Task Force. Global Guideline for Type 2 Diabetes [Internet]. Brussels. https://www.idf.org/sites/default/files/IDF T2DM Guideline.pdf (accessed August 2016).
3. Diabetes UK (2015) *Diabetes: Facts and Stats*. London, UK: Diabetes UK.
4. NICE (2015) *Clinical Guideline NG28. Type 2 Diabetes in Adults: Management*. London: National Institute for Health and Care Excellence.
5. Franz MJ, Boucher JL, Green-Pastors J *et al.* (2008) Evidence-based nutrition practice guidelines for diabetes and scope and standards of practice. *J Am Diet Assoc* **108**(4 Suppl 1), S52–S58.
6. Ajala O, English P & Pinkney J (2013) Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *Am J Clin Nutr* **97**, 505–516.
7. Dyson PA, Kelly T, Deakin T *et al.* (2011) Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes. *Diabet Med* **28**, 1282–1288.
8. van Wyk HJ, Davis RE & Davies JS (2016) A critical review of low-carbohydrate diets in people with Type 2 diabetes. *Diabet Med* **33**, 148–157.
9. Dyson P (2015) Low carbohydrate diets and type 2 diabetes: what is the latest evidence? *Diabetes Ther* **6**, 411–424.
10. Mann J & Morenga LT (2015) Carbohydrates in the treatment and prevention of Type 2 diabetes. *Diabet Med* **32**, 572–575.
11. Franz MJ, Powers MA, Leontos C *et al.* (2010) The evidence for medical nutrition therapy for type 1 and type 2 diabetes in adults. *J Am Diet Assoc* **110**, 1852–1889.
12. Wennberg JE (2010) *Tracking Medicine*. New York, NY: Oxford Univeristy Press.
13. Creswell JW & Plano Clark VL (2011) *Designing and Conducting Mixed Methods Research*. 2nd edn. London: SAGE.
14. UoB (2013) Bristol: Bristol Online Surveys, University of Bristol. http://www.survey.bris.ac.uk/?op=login (accessed April 2013).
15. BDA (2015) *The British Dietetic Association Annual Report 2014/15*. Birmingham, UK: The British Dietetic Association.
16. Nelson M, Lean ME, Connor H *et al.* (2000) Survey of dietetic provision for patients with diabetes. *Diabet Med* **17**, 565–571.
17. BDA (2013) Membship survey demographics. In: *Report of the age and sex Distribtion of Members of The British Dietetic Association at September 2012 ed*. pp. 2. Birmingham, UK: The British Dietetic Association.
18. StataCorp (2011) *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP.
19. Marshall MN (1996) Sampling for qualitative research. *Fam Pract* **13**, 522–525.
20. Dworkin SL (2012) Sample size policy for qualitative studies using in-depth interviews. *Arch Sex Behav* **41**, 1319–1320.
21. Mason M (2010) Sample size and saturation in phd studies using qualitative interviews. forum: qualitative. *Qual Soc Res* **11**, 8.
22. Ford S, Schofield T & Hope T (2006) Observing decision-making in the general practice consultation: who makes which decisions? *Health Expect* **9**, 130–137.
23. Graneheim UH & Lundman B (2004) Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. *Nurse Educ Today* **24**, 105–112.
24. Braun V & Clarke V (2006) Using thematic analysis in psychology. *Qual Res Psychol* **3**, 77–101.
25. Wicks A & Whiteford G (2006) Conceptual and practical issues in qualitative research: reflections on a life-history study. *Scand J Occup Ther* **13**, 94–100.
26. Decrop A (1999) Triangulation in qualitative tourism research. *Tour Manag* **20**, 157–161.
27. Diabetes UK (2016) Diabetes education: the big missed opportunity in diabetes care [Internet]. London. https://www.diabetes.org.uk/Upload/Get involved/campaigning/Taking Control/Diabetes UK_Diabetes education – the big missed opportunity_updated June 2016.pdf (accessed August 2016).
28. Mason J (2006) *Six Strategies for Mixing Methods and Linking Data in Social Science Research*. Southampton, UK: ESRC National Centre for Research Methods.
29. HCPC (2013) HCPC - Health & Care Professions Council – Professions. http://www.hpc-uk.org/aboutregistration/professions/index.asp?id=5 (accessed August 2013).
30. Connor H, Annan F, Bunn E *et al.* (2003) The implementation of nutritional advice for people with diabetes. *Diabet Med.* **20**, 786–807.

BDA The Association of UK Dietitians
THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
Journal of Human Nutrition and Dietetics

DIETETIC PRACTICE AND BEHAVIOUR CHANGE

Accessing hospital packaged foods and beverages: the importance of a seated posture when eating

A. Bell,[1] L. Tapsell,[1] K. Walton[1] & A. Yoxall[2]

[1]School of Medicine, Faculty of Science, Medicine & Health, University of Wollongong, Wollongong, NSW, Australia
[2]Art & Design Research Centre, Sheffield Hallam University, Sheffield, UK

Keywords
access, hospital food, older adults, packaging.

Correspondence
A. Bell, School of Health & Society, Faculty of Social Science, University of Wollongong, New South Wales 2522 Australia.
Tel.: +61 2 4221 4095
Fax: +61 4221 5945
E-mail: abell@uow.edu.au

How to cite this article
Bell A., Tapsell L., Walton K., Yoxall A. (2017) Accessing hospital packaged foods and beverages: the importance of a seated posture when eating. *J Hum Nutr Diet*. **30**, 394–402
doi:10.1111/jhn.12430

Abstract

Background: Hospitalised and community dwelling older people (aged 65 years and over) have difficulties opening certain food and beverage items (e.g. cheese portions and tetra packs) served in public hospitals. Previously, the role of hand strength on successful pack opening has been explored in a seated position. However, because many people in hospital eat in bed, the present laboratory study examined the differences between participants opening a selection of products in a hospital bed and a chair.
Methods: The present study used a qualitative method (satisfaction) and quantitative methods (grip and pinch strength, dexterity, time and attempts) in two conditions (bed; chair) in a sample of well older community dwelling adults (n = 34). Packs tested included foil sealed thickened pudding, foil sealed thickened water, tetra pack, dessert, custard, jam, cereal, honey sachet and cheese portions.
Results: Honey sachets, cheese portions, foil sealed thickened pudding and tetra packs were the most difficult packs to open, with 15% of cheese portions unable to be opened in either the bed or chair posture. Although grip strength was consistent for each posture, pinch grips and dexterity were adversely affected by the bed posture. Lying in a hospital bed required greater pinch strength and dexterity to open packs.
Conclusions: Eating in a seated position when in hospital has been shown to improve intake. The present study demonstrates that eating in a seated posture is also advantageous for opening the food and beverage packs used in the NSW hospital food service and supports the notion that patients should sit to eat in hospital.

Introduction

Food and beverages in public hospitals are routinely served in a packaged format to deliver standardised portion sizes and cost effective nutrition [(1)]. The population is rapidly ageing [(2)] and meeting their nutritional needs is challenging in hospitals where patients are 'unwilling customers' [(3)], often malnourished [(4)], and experience physical, organisational and environmental barriers to eating [(5)]. A great deal of research has been undertaken to suggest and test interventions that aim to improve the situation, such as changes to food service [(6)], food fortification [(7)] and volunteer feeding programmes [(8)].

Positioning patients to eat by sitting them in a chair is one of the strategies shown to increase intakes by older people in the hospital environment [(9)]. However, despite the importance of eating in a chair, many patients continue to eat in bed as a result of an absence of dining areas and the low priority of nutrition in nursing and medical care [(10)].

Previous studies have demonstrated that hospital packaged foods and beverage packs are difficult to open in a seated posture and that the importance of grip and pinch strength in efficient pack opening is limited, suggesting that dexterity was likely to be the critical aspect of efficient pack opening [(11)].

Standardised testing for grip and pinch strength is conducted in seated postures [12]. The few studies that have examined grip strength in other postures have reported conflicting results and no other research was identified that examined pinch strength or dexterity in any posture other than sitting [13,14]. Because hospital food is mostly served in sealed packaging and many patients eat in bed, it is important to examine the use of food and beverage packaging by the older person and the way in which it is accessed in the hospital environment. The present laboratory study aimed to compare the openability of a selection of hospital food and beverage items when both lying in a hospital bed and sitting, as well as to examine the role of grip strength, pinch strength and dexterity in successful and efficient pack opening by older people.

Materials and methods

This research was conducted in a simulated hospital laboratory setting. Quantitative data collection included demographic data; time and attempts to open packs; grip and pinch strength; and dexterity measures. Qualitative measures included ratings and questions on ease of opening (satisfaction). All measures were conducted in both lying and seated postures with each participant and the order of posture was randomised throughout the sample. The use of a mixed methodology approach such as this has been found to be advantageous when addressing health and human service research [15]. Ethics approval was obtained through the University of Wollongong.

Participants

The present study involved a nonprobability convenience sample of well older adults living in the Illawarra region of NSW, Australia. Criteria to participate included being 65 years or older, well and living independently in the community. Written consent was obtained from all participants. Participants who normally wore reading glasses were asked to wear them for the study. A biostatistician was consulted regarding suitable sample size and 30 participants in total was considered appropriate for statistical power because we expected dexterity to have a large effect for opening packs ($P < 0.05$ and 80%).

Setting

The study was conducted at the University of Wollongong in Nursing Simulation Laboratories. The facilities allowed for two simulation rooms: one set up with a table and chair and the other with a hospital bed and table. *In situ* recording devices are installed in each room with the control centre located between the two rooms (Figs 1 and 2).

Figure 1 Simulation room 1: bed posture.

Figure 2 Simulation room 2: seated posture.

Posture

Bed posture

Bed angle and bed table height were standardised for the study. The distance between the mattress and top of bedside table was 27 cm to enable leg clearance and reasonable eating height. The bed angle was set at 60%,

comprising a 'modified' Fowler's bed position [16] with two standard hospital pillows. In this way, participants were given the optimum posture for eating in bed.

Chair posture

A standard waiting room style chair was used for the study (Fig. 2). The chair had no arms, allowing participants to sit close to the table for dexterity testing and opening of products, as well as complete the standard protocol for grip and pinch strength testing, with the chair at right angles to the table and away from it to ensure good elbow clearance.

Hand function testing

Grip and pinch strength

Grip and pinch strength testing was conducted on each participant using a standardised protocol [17] with the Jamar Grip Strength Dynamometer (Lafayette Instruments, Lafayette, IN, USA) and the B & L Pinch Gauge (B & L Engineering, CA, Santa Ana, USA). Both instruments were calibrated prior to the study. For standardisation, the dynamometer's adjustable handle was set on the second handle position for all participants with single effort and hand dominance recorded. The B & L pinch gauge measured tip, three point and lateral pinch strength for a single effort. These two hand assessment tools are commonly used and considered to produce the most reliable and valid measurements of grip and pinch strength [18].

Dexterity

The dexterity of participant's hands was analysed using the Purdue Pegboard Test [19]. This test was initially developed to assess suitability to factory assembly tasks but is now used for a variety of purposes, including the assessment of brain impairment and learning disabilities. The test consists of a battery of four different tasks administered in a standardised protocol with the participant seated at a table. The sum of tests 1, 2 and 3 determine a macrodexterity score. Macrodexterity was used in the present study to correlate with opening time and attempts because this measure has been identified as the critical dexterity component related to successful pack opening [20].

Food and beverage packs

Nine packs were sourced from a local hospital for testing. These included: foil sealed items (thickened pudding, thickened water, custard); tetra packs; condiment packs (jams, marmalade); individual honey 'squeeze' sachets; single serve cereal boxes; sealed desserts; and cheese portions. These items were selected because previous studies had found them to be difficult to open, participants had reported the packaging as 'fiddly' with poor correlations between faster opening times and grip strength, indicating that dexterity may have been the key factor in openability [21]. As a result of the range and numbers of products supplied by the hospital, each participant opened seven of the nine in the two postures. Products were consistent in the two postures for each participant to ensure that each participant was their own control. The participants had no choice in pack selection.

An example of a participant's tray is shown in Fig. 3. The range of products tested is shown in Fig. 4.

Video capture (timing and attempts)

Researchers independently reviewed the video footage of three participants to jointly determine consistent criteria for the beginning and end of opening, as well as the number of attempts. Opening the pack was measured from the time of gripping the tab or pack; end of timing was the release of the tab/pack from grasp. The number of attempts to open the pack was determined by changing grips, orientations and manipulations of the pack.

Interview

Participants were interviewed with a questionnaire used previously in packaging research [21]. Ratings of opening ability were organised by answering 'yes' or 'no', followed by a scale of 'no difficulty/easy', 'some difficulty', 'moderately difficult', 'very difficult' and 'impossible', as well as general comments on the pack.

Statistical analysis

Data for all phases were analysed using SPSS, version 21 [22]. Questionnaires and sample meal tray recordings were analysed with descriptive statistics. Correlations using Spearman's rho were performed to determine whether or not a relationship existed between participant's hand function elements (grip, pinch strengths and dexterity)

Figure 3 Participant and example testing tray in bed posture.

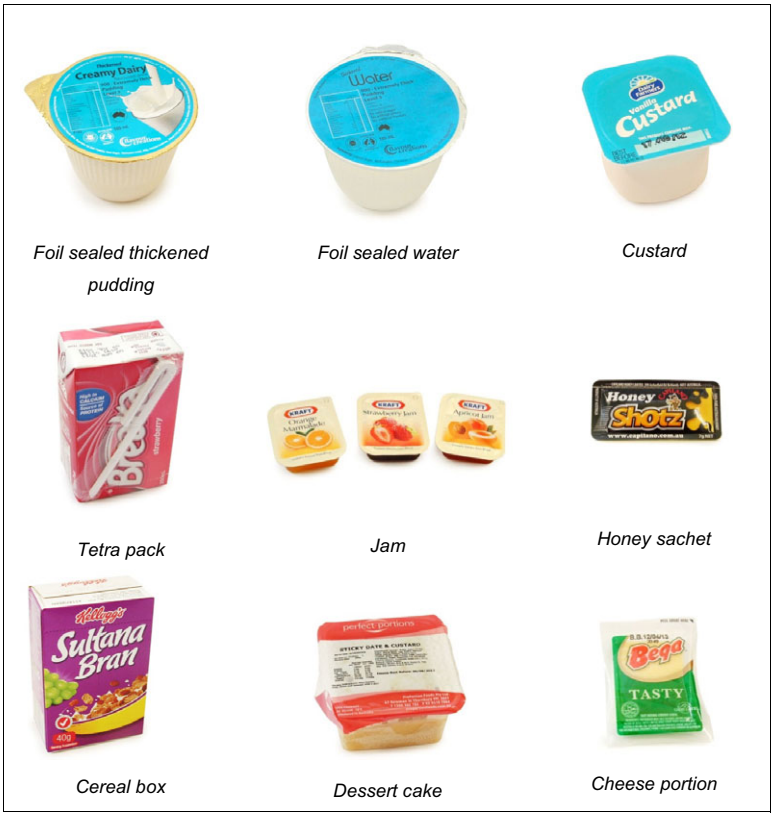

Figure 4 Range of products in the present study; each participant tested seven of the nine products.

and the time taken to open the items in the lying down and seated postures. Significant differences between the two postures for hand function tests and the time taken to open the products were analysed using paired samples *t*-tests and Wilcoxon signed rank tests. The effect size of the differences between the two postures for hand function on the paired samples *t*-tests was determined using the eta squared statistic. Cohen [23] states that an eta squared value of 0.01 is a small effect, 0.06 a moderate effect and 0.14 is a large effect. Effect size for the Wilcoxon signed rank test items was determined by r [24], where 0.1 represents a small association, 0.3 is a medium association and 0.5 is a large association.

Results

Participants

There were 34 participants aged between 65 and 86 years with a mean (SD) age of 73 (5.4) years (23 females and 11 males).

Hand function tests: bed versus chair

Grip and pinch strength

Grip and pinch strength scores for the total study population were normally distributed in both postures, with the exception of dominant three point pinch strength in the bed posture, and nondominant grip and nondominant lateral pinch in the chair posture. Mean grip strength for the bed and chair posture is shown in Table 1. No significant differences were found for grip strength between the two postures.

Significant differences were found for all pinch grip measures, with stronger pinch grips in the chair posture. Table 2 shows the dominant and nondominant pinch strength data and significance values (two-tailed) between the postures and outlines the effect size. Participants exerted less pinch strength in the bed posture compared to the chair posture, with a large negative effect for all pinch grips, except the dominant three point pinch grip, which had a medium negative effect ($Z = -2.93$, $P = 0.003$, $r = -0.36$), and the nondominant lateral pinch grip, with a medium negative effect ($Z = -2.82$, $P = 0.005$, $r = -0.34$).

Dexterity

Dexterity measures were normally distributed for the bed posture. Dominant and nondominant dexterity was not normally distributed in the chair posture. The bed posture had a large negative effect on macrodexterity [mean (SD) = 32.36 (5.59)] compared to the chair posture [mean (SD) 35.29 (5.54), $Z = -4.15$, $P < 0.001$, $r = -0.71$].

Table 1 Grip strength data, bed and chair posture (n = 34)

	Dominant grip (kg/f)			Nondominant grip (kg/f)		
	Bed posture	Chair posture	Significance	Bed posture	Chair posture	Significance
Mean	29.19	28.58	0.195	27.46	27.56	0.694
Standard deviation	10.61	10.29		11.52	10.85	

Table 2 Dominant and Nondominant pinch grip strength data in bed and chair posture (kg/f) (n = 34)

	Dominant tip pinch*				Dominant 3 point pinch*				Dominant lateral pinch†			
	Bed	Chair	Significance	Eta squared	Bed	Chair	Significance	r	Bed	Chair	Significance	Eta squared
Mean	3.82	4.31	0.001	0.30	5.76	6.21	0.003	−0.36	6.87	7.25	0.016	0.16
(SD)	(1.58)	(1.32)			(2.28)	(2.07)			(2.50)	(2.28)		

	Nondominant tip pinch†				Nondominant 3 point pinch*				Nondominant lateral pinch*			
	Bed	Chair	Significance	Eta squared	Bed	Chair	Significance	Eta squared	Bed	Chair	Significance	r
Mean	3.79	4.16	0.044	0.12	5.29	5.88	0.005	0.21	6.20	6.66	0.005	−0.34
(SD)	(1.43)	(1.3)			(1.69)	(1.85)			(2.42)	(2.52)		

*Spearman rho correlation is significant at the 0.05 level (two-tailed).
†Spearman rho correlation is significant at the 0.01 level (two-tailed).

Food products

The time taken to open the products by each participant in each posture was calculated. The item with the maximum opening time was the honey sachet in the bed posture (144 s) followed by the cheese portion in the chair posture (133 s). Figure 5 shows the median time to open each product in each posture. The thickened water, custard and condiments are the only products with a longer median opening time in the bed posture. No significant differences in opening times between postures were observed.

Attempts to open products in each posture

The number of attempts to open each product was also calculated from the video footage to further explore the interaction of the person and package (Table 3). The differences in the maximum amount of attempts in the bed and chair posture reflect the median time differences for the postures in Fig. 5 for the thickened water, custard and condiments, although not for other items such as the thickened pudding, honey sachet and cheese portions. The packages that took the longest time to open in each posture (cheese, honey and tetra pack) also demonstrated

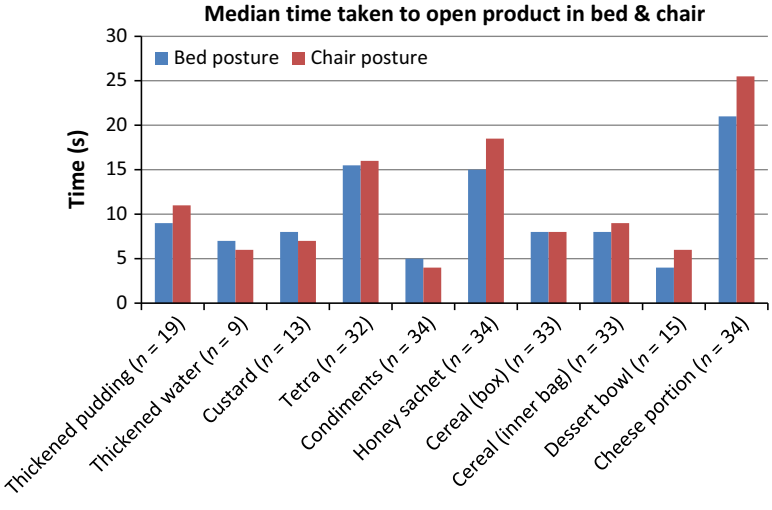

Figure 5 Median time taken to open product in the bed and chair postures.

Table 3 Number of attempts to open each item in each posture

Item	*N*	Posture	Mean	Median	SD	Minimum	Maximum
Thickened pudding	19	Bed	5	4	3	1	10
		Chair	5	4	3	1	13
Thickened water	9	Bed	2	2	1	1	3
		Chair	3	2	2	1	7
Custard	13	Bed	2	2	1	1	4
		Chair	2	2	1	1	5
Tetra: remove straw	32	Bed	5	4	3	2	13
		Chair	5	5	3	1	16
Tetra: insert straw	32	Bed	1	1	1	1	2
		Chair	1	1	2	1	10
Condiments	34	Bed	1	1	1	1	3
		Chair	1	1	0	1	5
Honey sachet	34	Bed	3	3	3	1	13
		Chair	2	1	3	1	12
Cereal (box)	33	Bed	3	2	1	1	6
		Chair	3	2	2	1	7
Cereal (inner bag)	33	Bed	3	2	3	1	13
		Chair	3	2	2	1	7
Dessert	15	Bed	1	1	0	1	2
		Chair	2	1	2	1	7
Cheese portion	34	Bed	5	3	5	1	30
		Chair	4	2	5	1	20

a high number of attempts to open. For example the mean number of attempts to open a cheese portion in the bed posture is five attempts, with a maximum of 30 attempts to open the pack.

A number of participants were unable to open the honey sachet and cheese portion in either posture: Honey 18% (bed), 24% (chair) and cheese 15% (bed and chair).

Questionnaire

Ratings were consistent between the two postures, with the cheese portion, thickened pudding, honey sachet and tetra packs found ro be the more difficult packs to open, scoring 'some difficulty–moderately difficult'. Participants were also asked to comment on their experience with the packs and the reasons for any difficulty.

Packaging and hand function

Grip and pinch strength

A significant correlation only was found for nondominant grip strength and the opening of thickened foil sealed water in the bed posture ($r = -0.71$, $n = 9$, $P = 0.032$). No other significant correlations were found for grip strength and the time to open the packs. A significant correlation was found between a shorter opening time for the thickened water and the dominant tip pinch grip in the bed posture only ($r = -0.71$, $n = 9$, $P = 0.031$). No other significant relationships between pinch grips and more efficient opening times were found.

Dexterity

Significant negative correlations were found between macrodexterity (Right, Left, Both on the Purdue Pegboard Test) and time taken to open for six of the nine packs in both postures as shown in Table 4. A negative correlation indicates that macrodexterity was associated with shorter opening times. Consistent relationships are

Table 4 Significant correlations between dexterity and time to open packages in two postures

Food/ beverage item (time to open)	Sample size (*n*)	Macrodexterity (Right, Left, Both)			
		Chair		Bed	
		Significance (two-tailed)	*r*	Significance (two-tailed)	*r*
Thickened pudding	19	NS		0.047	−0.46
Custard	13	0.001	−0.80	0.001	−0.82
Tetra pack	32	0.010	−0.49	0.010	−0.45
Condiments	34	0.015	−0.41	0.001	−0.63
Honey sachet	34	0.000	−0.65	0.031	−0.38
Cereal inner bag	33	0.038	−0.36	0.002	−0.54

NS, not significant.

demonstrated in both postures for dexterity and the custard and the tetra pack. Macrodexterity has a stronger relationship with efficient pack opening in the bed posture for the thickened pudding ($r = -0.46$, $n = 19$, $P = 0.047$); condiments ($r = -0.63$, $n = 34$, $P = 0.001$); and cereal inner bag ($r = -0.54$, $n = 33$, $P = 0.002$). Conversely, macrodexterity is strongly correlated with faster opening of the honey sachet in the seated posture ($r = -0.65$, $n = 34$, $P = 0.000$).

Discussion

The purpose of food service in hospitals is to deliver the nutrition required for recovery and to encourage patients to eat [25]. This is a challenging proposition with cost pressures from government and large numbers of patients, who are increasingly older people with complex medical issues [26]. Additionally, food service is conducted in an environment where there are conflicting priorities of medical procedures over meal times, lack of meal choice, increasing use of cook-chill options and lack of assistance to eat and open packaging [27]. Previous research has examined the association between grip and pinch strength and the time taken to open hospital food and beverage items and has highlighted that dexterity was likely a critical aspect of hand function for 'openability' of these items and yet to be measured [21]. The present study explores the role of dexterity to open the items found to be 'fiddly' in these previous studies [11,21] by testing the packs with well older people (aged 65 years and above) in a controlled laboratory setting. The study also reviews the impact of a bed posture on hand function and time to open packs, attempts to open packs, and satisfaction with a selection of pack types.

Studies into postural differences in grip strength are very limited and have conflicting results [13,14] and no studies were found that examined pinch strength or dexterity in different postures such as undertaken in this research. No significant difference was found for grip strength between the bed and chair postures in the present study. It is likely that grip strength was unaffected because the participant was seated in a supported and almost upright posture with the trunk stable in the bed as determined by our protocol, and therefore able to exert maximum effort in comfort. However, the present study demonstrated that a bed posture negatively affects both pinch grips and macrodexterity, with both elements of hand function being required to successfully open packaging used in hospitals. Future research is warranted to examine the strength and dexterity of older hospital patients and comparing them with well community dwelling populations for whom packaging is designed.

The correlations between hand function elements and efficient pack opening suggest that the bed posture required the recruitment of more elements of hand function to open packs compared to the seated posture, and that macrodexterity was more important than strength. For example, stronger nondominant grip and dominant tip pinch grip were associated with faster opening times for the thickened water in the bed posture. This is likely a result of the need for greater stabilisation of the pack with the nondominant hand and greater tip pinch strength to pull the tab with the dominant hand compared to opening the pack in a seated posture. Macrodexterity was associated with efficient pack opening in the bed posture for thickened pudding, condiments and the cereal inner bag. Similarly, macrodexterity was associated with faster opening times for the honey sachet in the chair posture. However, macrodexterity was associated with efficient pack opening in both postures for thickened pudding, custard, tetra pack, condiments, honey sachet and cereal inner bag, illustrating the importance of macrodexterity in opening packs generally.

The present study has found that the seated posture facilitates better pinch grip strength and macrodexterity ability than lying in a hospital bed. Nutrition researchers have found that being seated for meals in hospital is beneficial and improves intake, as well as improves the eating experience for patients [9,10]. Sitting to eat requires less 'effort' (in terms of hand function) to open packs, and this supports the notion that it should be the preferred posture for the patient to eat in because less effort is better when the person is feeling unwell and the effort of eating itself can be a burden [9]. Although sitting is the optimal posture for eating, it is not always possible because patients may be too unwell. Additionally, positioning patients to eat in an optimal posture requires a coordinated multidisciplinary approach, which may take time for an organisation to implement.

As in the previous studies [11,21], the tetra and cheese portions were found to take a long time to open, required repeated effort, and were rated more poorly on the 'ease of opening' scale. Again, as in the previous papers, a number of participants could not open the cheese portion (15%). Interestingly, this was unaffected by posture, indicating that the cheese portion is poorly designed for 'openability'. Cheese portions are an important source of protein, comprising a quick and easy (once opened) way for the patient to access valuable nutrition, and are served as a between meal snack for this purpose. Tetra packs are provided in hospitals to deliver supplements to frail and unwell older patients who are malnourished or at risk of malnourishment. Further research is required to investigate the impact of packaging on intake in older people because these products are

routinely used in hospitals, care facilities and the community.

There are a number of limitations to this research. First, for study efficiencies, the sampling approach and testing location were controlled by the researcher. The participants were recruited using a purposive sampling approach with researcher-directed inclusion and exclusion criteria [28]. As such, they were not a random sample and may not represent the wider population. No formal assessment was made of cognition, vision or health, relying on participants to self-select. However, because participants were required to attend the university, making their own way to and from the venue, they may represent a more 'able' group than the general population. Indeed, the participants in the present study were able to use both hands to access the packs, whereas hospitalised older adults may experience medical conditions or interventions such as an *in situ* cannula impeding their hand function. However, the artificial setting of the simulated hospital laboratory could have affected the results through central location bias [29]. Ideally, the present study would be conducted in a hospital setting with larger subject numbers. However, such a study would require greater resources and administrative organisation and it would be difficult to access patients as a result of medical conditions, medical interventions and nursing activities. Second, although the bed posture was controlled by maintaining the bed angle and table height, participants varied their posture by sitting further forward or removing a pillow for greater comfort. This may have affected the results in the bed posture. Finally, many participants were unfamiliar with the honey sachet pack type and this may have affected the time to open the pack. This could have been overcome by providing a 'practice' pack as used in the European technical specification for packaging ease of opening [30].

Conclusions

This research has two key findings. First, pinch grip strength and macrodexterity ability for the older adult are better in a seated position than in a semi-recumbent hospital bed posture. Second, macrodexterity ability is associated with faster opening times for a range of hospital food and beverage items routinely served in hospitals and care facilities. These findings support the advice from nutrition experts: older patients should sit to eat to maximise intake and meal-time enjoyment.

Improvement of pack design for the cheese portion, an important protein snack source, as well as for the honey sachet and, most importantly, the tetra pack, which is routinely used to provide supplementary nutrition, is indicated. Involvement of older consumers and understanding the capacities and abilities of this population is integral to better design. Within the broader hospital foodservice literature, this research has highlighted the need to consider not only pack design and procurement, but also how the patient is positioned, assisted and encouraged to eat.

The implications for effective food service delivery in hospital is clear: food is an essential 'treatment' in hospital, delivering the nutritional elements necessary for recovery and is best delivered in an environment allowing a seated eating position, promoting social interaction, and, wherever packaged food and beverages are used, presented in more easily accessible pack formats.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
The main author (A Bell) was a PhD student; Prof Linda Tapsell and A Prof Karen Walton were my supervisors; Dr Yoxall an advisor and review author.
This research was funded by a University of Wollongong Early Career Researcher Grant.

References

1. Rechbauer C (2013) Healthcare NSW plates up 22 million meals. *Hospital & Aged Care*. Available at: http://www.simonbryant.com.au/files/176_ha_food_nutrition_sepoct_2013_feature_cover.pdf (accessed 30 June 2014).
2. Australian Institute of Health and Welfare (2013) *Australian Hospital Statistics 2011–12*. Canberra, ACT: Australian Government.
3. Johns N, Edwards JSA & Hartwell HJ (2013) Hungry in hospital, well-fed in prison? A comparative analysis of food service systems. *Appetite* **68**, 45–50.
4. Gout BS, Barker LA & Crowe TC (2009) Malnutrition identification, diagnosis and dietetic referrals: are we doing a good enough job? *Nutr Diet* **66**, 206–211.
5. Naithani S, Whelan K, Thomas J *et al.* (2008) Hospital inpatients' experiences of access to food: a qualitative interview and observational study. *Health Expect* **11**, 294–303.
6. Hartwell HJ, Edwards JSA & Symonds C (2006) Foodservice in hospital: development of a theoretical model for patient experience and satisfaction using one hospital in the UK National Health Service as a case study. *J Food Serv* **17**, 226–238.
7. Walton K, Williams P & Tapsell L (2012) Improving food services for elderly, long-stay patients in Australian hospitals: adding food fortification, assistance with packaging and feeding assistance. *Nutr Diet* **69**, 137–144.

8. Manning F, Harris K, Duncan R *et al.* (2012) Additional feeding assistance improves the energy and protein intakes of hospitalised elderly patients. A health services evaluation. *Appetite* **59**, 471–477.
9. Walton K, Williams P, Tapsell L *et al.* (2013) Observations of mealtimes in hospital aged care rehabilitation wards. *Appetite* **67**, 16–21.
10. Edwards JSA & Hartwell HJ (2004) A comparison of energy intake between eating positions in a NHS hospital —a pilot study. *Appetite* **43**, 323–325.
11. Bell AF, Walton KL & Tapsell LC (2016) Easy to open? Exploring the 'openability' of hospital food and beverage packaging by older adults. *Appetite* **98**, 125–132.
12. Innes E (1999) Handgrip strength testing: a review of the literature. *Aust Occup Ther J* **46**, 120–140.
13. Richards LG (1997) Posture effects on grip strength. *Arch Phys Med Rehabil* **78**, 1154–1156.
14. Teraoka T (1979) Studies on the peculiarity of grip strength in relation to body positions and aging. *Kobe J Med Sci* **25**, 1–17.
15. DePoy E & Gitlin LN (1998) *Introduction to Research: Understanding and Applying Multiple Strategies*, 2nd edn. pp. xii. St Louis, MO: Mosby.
16. Metzler DJ & Har J (1996) Positioning your patient properly. *Am J Nurs* **96**, 33–37.
17. American Society of Hand Therapists (1992) *Clinical Assessment Recommendations*, 2nd edn. Chicago, IL: American Society of Hand Therapists.
18. Crosby CA (1994) Hand strength: normative values. *J Hand Surg* **19**, 665–670.
19. Tiffin J & Asher EJ (1948) The Purdue Pegboard: norms and studies of reliability and validity. *J Appl Psychol* **32**, 234–247.
20. Rodriguez-Falcon EM, Yoxall, A (2011) Beyond a spoonful of syrup: understanding physical barriers to medical packaging, vol. *Proceedings of the First European Conference on Design 4 Health*, 13–15 July 2011. pp. 266–286. Sheffield: Sheffield Hallam University.
21. Bell AF, Walton K, Chevis JS *et al.* (2013) Accessing packaged food and beverages in hospital. Exploring experiences of patients and staff. *Appetite* **60**, 231–238.
22. IBM (2012) *SPSS, Version 21 for Windows*. Chicago, IL: SPSS Inc.
23. Cohen J (2005). Chapter 16. In: *SPSS Survival Manual*. p. 212. [Pallant J, editor]. Crows Nest: Allen & Unwin.
24. Pallant J (2005) *SPSS Survival Manual*. pp. 224–225. Crows Nest: Allen & Unwin.
25. British Association of Parenteral and Enteral Nutrition BAPEN (2012) *Hospital Food as Treatment*. Redditch: BAPEN.
26. Garling P (2008) *Final Report Special Commission of Enquiry Acute Care Services in NSW Public Hospitals: Overview*. Sydney: NSW Government.
27. Walton K (2012) Improving opportunities for food service and dietetics practice in hospitals and residential aged care facilities. *Nutr Diet* **69**, 222–225.
28. Hesse-Biber SN (2010) *Mixed Methods Research: Merging Theory with Practice. [electronic resource]*. New York, NY: Guilford Press.
29. Kozlowska K, Jeruszka M, Matuszewska I *et al.* (2003) Hedonic tests in different locations as predictors of apple juice consumption at home in elderly and young subjects. *Food Qual Prefer* **14**, 653–661.
30. European Committee for Standardisation (CEN) (2011) *CEN/TS 15945. Packaging – Ease of Opening – Criteria and Test Method for Evaluating Consumer Packaging*. Brussels: European Committee for Standardisation.

