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EDITORIAL

The challenge of nutritional management in people with kidney disease

Dietary modification in renal disease

The impairment of metabolic functions associated with chronic kidney disease (CKD) requires dietary modification to prevent more rapid progression of disease and the development of comorbidities such as hypertension. In the early stages of disease, dietary modification such as reduction of sodium intake may be the only treatment offered to patients, but as disease progresses, such modifications become a critical adjunct to treatments such as haemodialysis. Dietary modifications in those with advanced kidney disease will extend to management of potassium and phosphorus intake and control over fluid balance for people requiring dialysis. Comorbidities which increase renal injury and promote disease progression, primarily diabetes, will also require dietary management. The nutrition of people with kidney disease is therefore highly complex, necessitating high levels of patient engagement with clinical input.

The challenge of a modified diet

Dietary changes in CKD are generally not required for those who have stage 1–3 CKD. However, once stage 4 CKD is reached and referral to secondary care has been initiated, the changes to diet can be lifelong, maintained outside clinical settings and self-managed by the patient. In the initial stages of predialysis care, there will be one of a number of factors which appear overwhelming for the individual. The psychological and behavioural challenges that accompany dietary change will persist for extended periods, particularly as the diet might need to vary as the disease progresses. Integrating the renal diet into normal life is particularly challenging in situations outside the home, such as at work or in social situations [(1)], but also necessitates changes within the family. This can require education and increased awareness among other family members who prepare and cook food, and wider accommodation to a restricted diet among the whole family if a child has renal disease [(2)].

Information provided to patients is clearly critical in helping to adjust to and maintain new dietary patterns. Increasingly the self-managing patient will utilise information available online. This is often of low quality, hard to understand and frequently inaccurate [(3)]. Dietitians therefore play a key role in educating patients on how to adhere to their therapeutic diet and, in addition to improving adherence with specific protocols [(4)], can help patients adapt to the challenges of lifestyle change [(1)].

Requirements, intake and malnutrition

Whereas patients who have advanced CKD are challenged by the need to change their established dietary habits, clinical management also poses problems. Due to poor appetite and metabolic disturbance, especially in end-stage kidney disease (ESKD) [(5)], patients are at high risk of protein–energy malnutrition [(6)] and require close monitoring of nutritional status. As with all dietary assessment approaches, investigating adherence to renal-nutrition regimens is problematic and becomes more complicated where patients have poor understanding of their condition or low literacy [(7)]. Ensuring that energy requirements are met depends on suitable tools to estimate total energy expenditure, where approximating expenditure through physical activity is particularly challenging [(8)].

As the use of dietary assessment tools and application of formulae to estimate requirements can be imprecise, monitoring the patients who have kidney disease for protein–energy malnutrition is an essential element of clinical management, particularly at the point where haemodialysis is required. Malnutrition is strongly associated with greater mortality risk. A number of approaches may be taken to monitoring malnutrition in this patient group, ranging from as simple as following appetite [(5)] to using indices that encompass serum albumin, mid-upper arm muscle area, skinfold thicknesses, protein catabolic rate [(6)], inflammatory markers [(9)] and indices of muscle strength (pinch grip or hand grip) [(10)].

Nutritional status and clinical outcomes

Malnutrition in those undergoing haemodialysis arises partly due to the anorexia associated with poor health, but is also driven by the nutrient losses that occur during dialysis, the impact of high infection rates and metabolic

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changes that occur as a consequence of the treatment. Malnutrition significantly increases the risk of comorbidities and mortality during treatment for ESKD. Simple measures such as body mass index are good predictors of this increased risk [(11)]. Patients are also at risk of other nutritional problems which are to some extent dependent on the nature of the dialysis treatment received. People with advanced CKD have disturbed calcium and phosphate status, which stems from insufficiency of serum 25-hydroxy vitamin D. Patients undergoing peritoneal dialysis are particularly prone to vitamin D deficiency, whereas haemodialysis can reduce this risk [(12)]. Anaemia is also an issue for patients with CKD and is one of the factors which impinges significantly on quality of life and cardiovascular complications. Managing iron status during dialysis has been shown to reduce inflammation, which will improve other clinical outcomes [(13)].

The nutritional management of people who have kidney diseases is important to consider, especially in terms of slowing the rate of renal deterioration and controlling the development and progression of comorbidities. The challenge is to find the balance between maintaining independence and a high quality of life in the early stages of disease and ensuring adequate monitoring and intervention to limit disease progression.

S. Langley-Evans[1] & N. Thomas[2]
[1]School of Biosciences, University of Nottingham, Loughborough, UK; [2]School of Health and Social Care, London South Bank University, London, UK
E-mail: Simon.Langley-Evans@nottingham.ac.uk

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LIPIDS, HEALTH AND DISEASE

Oral fish oil positively influences nutritional-inflammatory risk in patients with haematological malignancies during chemotherapy with an impact on long-term survival: a randomised clinical trial

T. R. Chagas,[1,2] D. S. Borges,[1] P. F. de Oliveira,[1] M. C. Mocellin,[1] A. M. Barbosa,[2,3] C. Q. Camargo,[1,2] J. Â. G. Del Moral,[4] A. Poli,[5] P. C. Calder,[6,7] E. B. S. M. Trindade[1] & E. A. Nunes[1,2,3]

[1]Graduate Program in Nutrition, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil
[2]Laboratory of Investigation in Chronic Diseases, Department of Physiological Sciences, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil
[3]Multicenter Post-Graduation Program in Physiological Sciences, Department of Physiology, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil
[4]Ambulatory Care Clinic and Oncologic Center, Professor Polydoro Ernani de São Thiago University Hospital, Florianópolis, Santa Catarina, Brazil
[5]Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil
[6]Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK
[7]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, University of Southampton, Southampton, UK

Keywords
fish oil, haematological malignancies, leukaemia, lymphoma, *n*-3 PUFA, nutritional status.

Correspondence
Everson Araújo Nunes, Laboratory of Investigation in Chronic Diseases, Department of Physiological Sciences, Biological Sciences Center, Federal University of Santa Catarina, Campus, Trindade, Florianópolis, Santa Catarina, 88040-900, Brazil.
Tel.: +5548 3721 2289
Fax: +5548 3721 2289
E-mail: everson.nunes@ufsc.br

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Abstract

Background: Studies suggest that the ingestion of fish oil (FO), a source of the omega-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can reduce the deleterious side-effects of chemotherapy. The aim of this randomised clinical trial was to evaluate the effect of supplementation with oral FO for 9 weeks on nutritional parameters and inflammatory nutritional risk in patients with haematological malignancies during the beginning of chemotherapy.
Methods: Twenty-two patients with leukaemia or lymphoma were randomised to the unsupplemented group (UG) ($n = 13$) or supplemented group (SG) ($n = 9$). SG received 2 g/day of fish oil for 9 weeks. Nutritional status, serum acute-phase proteins and plasma fatty acids were evaluated before (T0) and after (T1) the intervention period. Data were analysed using two models; model 1, comprising data from all patients included in the study, and model 2, comprising data from UG patients with no increase in the proportions of EPA and DHA in plasma and data from SG patients showing an at least 100% increase in plasma EPA and DHA.
Results: SG showed an increased plasma proportion of EPA and DHA in both models. In model 2, C-reactive protein (CRP) and CRP/albumin ratio showed larger reductions in the SG. Overall long-term survival in both models (465 days after the start of the chemotherapy) was higher in the group ingesting fish oil ($P < 0.05$).
Conclusions: These findings indicate an improved nutritional-inflammatory risk and potential effects on long-term survival in patients with haematological malignancies supplemented with FO during the beginning of chemotherapy.

Introduction

Haematological malignancies belong to a heterogeneous group of haematological diseases [1–2]. Inflammation is now recognised to be an important component in cancer prognosis, with several inflammatory markers being used to monitor disease progression and prognosis [3–5]. One of the main strategies used to treat haematological malignancies is chemotherapy. Such treatment not only aims to destroy neoplastic cells, but also affects healthy cells, such as cells of the gastrointestinal tract [6]. Adverse effects caused by antineoplastic therapy include giddiness, nausea, vomiting, mucositis, dysphagia, diaorrhea, and changes in taste and smell perception [7]. Such adverse effects are linked to metabolic and nutritional losses and a subsequent deterioration in the quality of life [7,8].

Dietary fatty acids (FAs) have profound physiological implications [9]. Studies suggest that ingestion of *n*-3 polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are found in fish oil, can be beneficial for cancer treatment by preserving nutritional status [10], decreasing inflammatory markers (e.g. positive acute-phase proteins) [11] and increasing survival [12]. However, studies using fish oil supplementation in haematological malignancies are scarce, and there is no clear information regarding any indication to use (or not use) such a nutritional strategy in these patients.

The present study aimed to evaluate the effect of oral supplementation with 2 g day^{-1} fish oil (a source of EPA and DHA) for 9 weeks on nutritional parameters and inflammatory status in newly diagnosed patients with haematological malignancies starting chemotherapy. Overall survival to 465 days from the start of fish oil supplementation was a secondary outcome. We hypothesised that patients diagnosed with haematological malignancies would show a reduction in overall nutritional inflammatory risk and improved survival when ingesting fish oil.

Materials and methods

Subjects

Patients with haematological malignancies who were treated at the Ambulatory and Hematology Clinical Center of the University Hospital of Santa Catarina – Florianópolis, Santa Catarina, Brazil, from November 2012 to December 2013, participated in the present randomised clinical trial (RCT). Eligibility criteria were: age ≥18 years, histopathological diagnosis of leukaemia or lymphoma and chemotherapy treatment indication. Exclusion criteria were: being in palliative care, taking statins or anti-inflammatory drugs, being allergic to fish and fish derivatives, being unable to perform oral ingestion of food, having received chemotherapy previously, being pregnant, and ingesting fish oil or other *n*-3 PUFA supplements in the 6 months before study initiation. A nonprobabilistic convenience sample was defined by time saturation

Diagnosis and disease staging were established according to routine biopsy, immunophenotyping, cytogenetics and immunohistochemistry, as conducted by the University Hospital Pathology Division.

Study design

Eligible patients were randomly allocated into one of two groups: supplemented group (SG) and unsupplemented group (UG). Randomisation was performed using an online tool: Research Randomizer (http://www.randomizer.org). Odd numbers meant that patients were allocated to SG and even numbers meant allocation to UG. The study was conducted in accordance with the Declaration of Helsinki [13]. All procedures involving human patients were approved by the Research Ethics Committee of the local Institution. All participants provided their written informed consent. The trial is registered at: www.ensaiosclinicos.gov.br, under the register RBR-7q6cqg.

The patients in the SG were instructed to ingest two capsules/day of fish oil (2 g day^{-1} in total) for 9 weeks. This dose has been tested effectively in previous studies [11,14,15]. Subjects were instructed to ingest capsules in a dose-fractionated form, approximately 20–30 min before lunch or dinner and accompanied by liquid. During the supplementation period, supplement intake was controlled by weekly telephone calls (for nonhospitalised subjects) or daily contact in the hospital. Also, subjects were instructed to record capsules intake in a provided form. The supplementation started on the first day of the chemotherapy (T0). The UG did not receive any supplement.

Personal data, lifestyle information and weight loss in the previous 6 months were obtained by interview. Clinical data (haematological malignant diagnosis, stage, other diseases and medication use) were obtained from patient records.

Dietary supplement

The fish oil supplement was in the form of gelatin capsules with 1000 mg oil/capsule (Omega 3, Phytomare; Governador Celso Ramos, SC, Brazil). The oil was extracted from salmon, mackerel and sardines. Two fish oil capsules provided a total of 71.13 kJ (17 kcal) and comprised 0.7 g of saturated fatty acids, 0.5 g of

monounsaturated fatty acids, 0.8 g of polyunsaturated fatty acids and 4 mg of cholesterol. Two capsules provided a total of 610 mg of *n*-3 PUFAs (367 mg of EPA and 243 mg of DHA). Analysis of the fatty acid profile of the fish oil capsule, by high-performance liquid chromatography (HPLC), was conducted and returned the investigated fatty acids in the proportions (%): EPA 25.06; DHA 14.58; palmitic 18.95; myristic 9.71; arachidonic <1; palmitoleic 12.94; oleic 9.73; linoleic 2.99; stearic 5.76; and α-linolenic <1.

Anthropometric data

Weight and height were measured with an electronic platform scale with a coupled vertical stadiometer (Toledo; Toledo Company of Brazil, São Bernardo do Campo, SP, Brazil). Usual weight was self-reported by the patient. Triceps skinfold (TS) was measured with a Compass Lange Skinfold Caliper (Beta Technology Incorporated, Santa Cruz, CA, USA) and mid-upper arm circumference (MUAC) was measured with an inelastic tape (TBW; São Paulo, SP, Brazil). TS and arm circumference (AC) were obtained from the arithmetic mean of three measures. All anthropometric measurements were made in accordance with standard techniques [16].

Blood collection

Blood samples (30 mL) were collected after approximately 8 h of fasting at two-time points: on the day prior to the first chemotherapy (baseline) and 9 weeks later (week 9). Blood was collected into vacuum tubes (Vacutainer System; BD Biosciences, Abingdon, UK) containing lithium–heparin. Plasma was prepared and stored at −80 °C until thawing for determination of the fatty acid profile. Blood was collected into separating gel tubes and serum was isolated for determination of -reactive protein (CRP) and albumin concentrations.

Determination of haemogram, albumin and C-reactive protein concentration

Haemogram parameters were obtained from whole blood using an automated method (Sysmex Xe-2100; Roche, Kobe, Japan); the values are expressed as cells mm^{-3}. Albumin was quantitatively determined using an automated colorimetric method (Siemens Healthcare Diagnostics Inc., Newark, DE, USA) employing bromocresol purple as a colour reagent [17] and is expressed as g dL^{-1}. Serum CRP was quantified by a nephelometric method (Siemens Dade Behring Inc., Newark, DE, EUA) [18] and is expressed as mg L^{-1}.

Nutritional status

The variation of weight related to the previous study period was assessed by calculating the weight loss percentage, resulting from the division between ΔW (i.e. the difference between usual weight and current weight) and the usual weight multiplied by 100 [19].

Several variables were assessed to evaluate nutritional status. Arm circumference (AC), mid-upper arm circumference (MUAC) and triceps skinfold (TS) (expressed in cm and mm, respectively). Mid-upper arm muscle circumference (MUAMC) was calculated according to the expression: MUAC − (π × TS)/10. Body mass index (BMI) was calculated by the ratio of weight (kg) to the square of the height (m). The cut-off points for classification were proposed by the World Health Organization [16,20,21]. Nutritional Risk Index (NRI) was calculated as: 1.519 × serum albumin (g dL^{-1}) + 41.7 × current weight (kg)/usual weight (kg). The classification adopted was: No nutrition risk, >100; Borderline nutrition risk, 99.9–97.5; Mild nutrition risk, 83.5–97.5; and Severe nutritional risk, <83.5 [22,23].

The CRP/albumin index was applied to categorise the inflammatory-nutritional prognosis of the patient. The classification adopted was: without risk, <0.4; low risk, 0.4–1.2; moderate risk, 1.2–2.0; and high risk, >2.0 [24].

Plasma fatty acids profile

The plasma fatty acid profile was determined by HPLC according to the method described by Nishiyama-Naruke *et al.* [25]. Plasma fatty acid constituents of phospholipids, triacylglycerols, cholesterol esters and free fatty acids were extracted using chloroform : methanol (2 : 1, v/v), adapting the method described by Folch *et al.* [26]. Lipid extracts were suspended in methanol and the pH was adjusted to ≥12 with 5 mol L^{-1} NaOH. The aqueous solution was acidified with hydrochloric acid (pH ≤ 3) and subjected to a new lipid extraction using hexane, followed by evaporation in gas N_2 at 37 °C. Fatty acids were derivatised with 4-bromomethyl-7-coumarin and acetonitrile as described by Abushufa *et al.* [27] and subsequently separated on a reversed phase analytical column (Discovery BIO Wide Pore, C8, 5 microns particles, 250 × 4.6 mm; Supelco/Sigma-Aldrich, St Louis, MO, USA). The chromatographic analysis was performed with an Alliance BIO Separation Module e2796 (Waters Corp., Milford, MA, USA). Sixteen microlitres of derivatised fatty acids were injected and then eluted by the binary gradient of acetonitrile: water from 70 : 30–90 : 10 at 0.5 mL min^{-1} in an 80-min run at a temperature of between 18 and 21 °C. Compounds were detected by fluorescence detection (Waters 2475 Multi λ Fluorescence Detector;

Waters Corp.), with excitation at 325 nm and emission at 398 nm. Chromatographic data were recorded and integrated into EMPOWER PRO, version 2.0 (Waters Corp.). The fatty acids investigated were: DHA, EPA, arachidonic, stearic, oleic, linoleic, α-linolenic, palmitic, myristic and lauric. Data are expressed as a percentage of total fatty acids.

Overall survival, hospital readmissions and number of chemotherapy sessions

Overall survival (OS) was defined as the time elapsed between baseline (the day of the first chemotherapy) and death from any cause or censored if alive at follow-up date [the follow-up was standardised to 465 days (15.5 months) after study entry]. The date of death, hospital readmissions and the number of chemotherapy sessions were recorded from patient medical records. OS curves were computed using the Kaplan–Meier method and compared using log-rank tests.

Statistical analysis

In the statistical analysis, the intake of fish oil was considered as the exposure variable. Data normality was tested by applying the Shapiro–Wilk test. A Student's unpaired *t*-test or a Mann–Whitney test were used to test for differences between groups at each time point. A Student's paired *t*-test or a Wilcoxon test for paired data were used to test the differences between time points within a study group.

All analyses were performed in STATA, version 11.0 (StataCorp, College Station, TX, USA) and images were prepared using PRISM, version 5.01 (Graphpad Inc., La Jolla, CA, USA). $P < 0.05$ was considered statistically significant.

Two models for statistical analyses were applied: Model 1 included all eligible patients who agreed to participate and finished the 9 weeks of follow-up; Model 2 included patients from SG who presented ≥100% increment in proportions of plasma EPA and DHA at 9 weeks compared to baseline and UG patients who did not show an increment (<50% increase) in plasma EPA and DHA at 9 weeks compared to baseline. Such a strategy was applied to reduce any potential heterogeneity related to the absorption and incorporation of fatty acids caused by the chemotherapy.

Results

Eighty-one new cases of haematological diseases were identified at University Hospital Professor Polydoro Ernani de São Thiago/Federal University of Santa Catarina between November 2012 and December 2013. Fifty patients were not eligible for participation in the study according to the inclusion/exclusion criteria. Therefore, 31 patients were invited to take part in the trial. Three patients chose not to participate. Finally, 28 patients (90.3% of the eligible patients) were randomised to two study groups (Model 1). Subsequently, according to the analysis of the plasma fatty acid profile, 14 patients were randomised to the same two study groups (Model 2), as shown in Fig. 1. Six participants were withdrawn from the study (three per group). The reasons for withdrawal are given in Fig. 1.

In both analysis models, plasma EPA and DHA concentrations did not change in the UG ($P > 0.05$) (see Supporting information, Table S1). In the SG, EPA and DHA increased in both analysis models. However, the increase in plasma DHA for model 1 reached a *P* value of 0.07, which can be interpreted as a statistical tendency (see Supporting information, Table S1).

Characteristics of the study participants

Baseline characteristics of the 22 randomised patients for Model 1 and the 14 patients in Model 2 did not differ between UG and SG (Table 1). Acute leukaemia and non-Hodgkin lymphoma were the main diagnoses in patients included in both analysis models. The distribution of patients between groups according to sex was not different. The concomitant diseases presented by the patients were: osteoporosis, type 2 diabetes mellitus, hypertension, rheumatoid arthritis, depression, gastritis, gastric ulcer, hiatal hernia, oesophagitis and hypothyroidism, and hyperthyroidism. The percentage of weight loss in the last 6 months before chemotherapy was numerically higher in the UG compared to the SG and was statistically significant.

Anthropometric parameters and nutritional status

No significant changes were observed in either analysis model for weight, MUAC, TS and MUAMC (Table 2). For Model 2, BMI was lower in SG than in UG at the end of the 9 weeks ($P < 0.05$). However, NRI was higher in SG than in UG using Model 2 ($P < 0.05$) (Table 2).

The CRP/albumin ratio for both models of analysis is shown in Fig. 2. Both groups had a significant decrease in the inflammatory-nutritional risk from baseline to the ninth week (T1) for analysis model 1. UG patients changed their classification from high to the medium and low risk of complications categories. In the group ingesting fish oil, most patients were categorised as low risk or no risk after supplementation [median (interquartile range): UG = 5.1 (2.0–31.6) to 1.4 (1.0–9.0); SG = 12.6 (2.8–18.1) to 1.1 (0.9–6.8); $P < 0.05$]. There were no

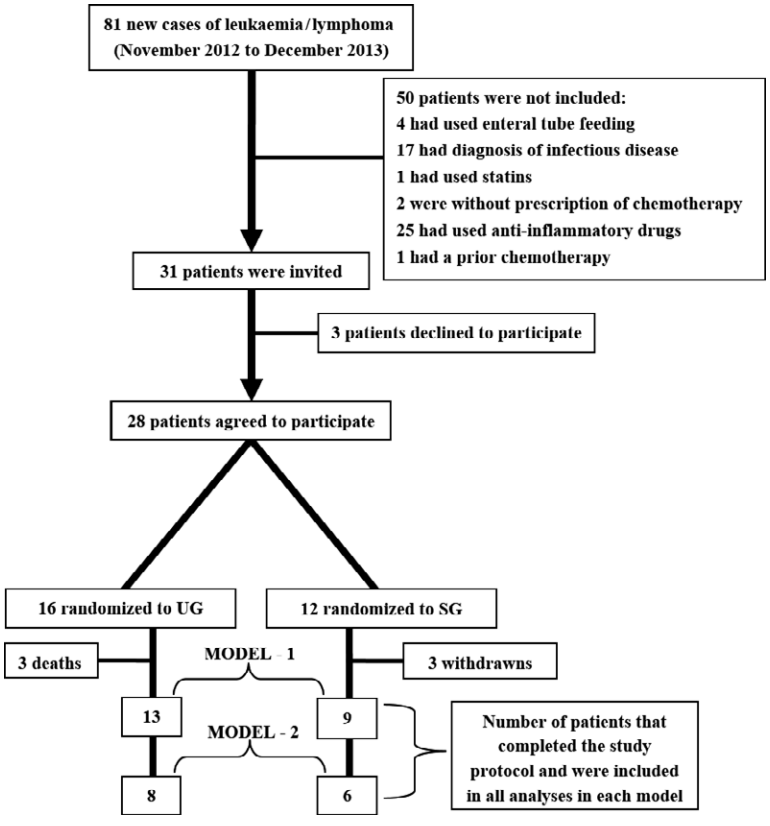

Figure 1 Flowchart of patients in the present study. Model 1 included all eligible patients who agreed to participate and finished the 9 weeks of follow-up. Model 2 included patients from SG who presented ≥100% increment in the proportion of plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 9 weeks compared to baseline and UG patients who did not show an increment (<50%) in plasma EPA and DHA at 9 weeks compared to baseline.

significant differences between groups when analysis model 2 was applied, in addition to the same changes as the lower risk category (Fig. 2).

Blood, serum and plasma parameters

UG showed a significant increase in red blood cell count with both analysis models (Table 3). Haematocrit and leukocytes increased significantly after 9 weeks for model 1 ($P < 0.05$). These changes did not occur in the SG. Additionally, using Model 1, the SG had a significant reduction in serum levels of CRP ($P < 0.05$), which did not occur in patients in the UG (Table 3).

Overall survival of the patients who completed the study

During the 9 weeks of the trial, three patients in the UG died (Fig. 1). Patients who completed the 9 weeks of the trial were followed for additional 14 months; outcomes were survival (Fig. 3), hospital readmissions and the number of chemotherapy cycles. No significant differences were observed in the number of hospital readmissions. However, the number of chemotherapy cycles was significantly higher in the SG independent of the model of analysis (see Supporting information, Table S2). During the follow-up, four patients of the UG started palliative chemotherapy treatment. In the SG, two patients relapsed after 1 year of remission and were submitted to further chemotherapy sessions (data not shown).

There were no reported deaths during the 465 days of follow-up for the patients in the SG independent of the analysis model. By contrast, the total number of reported deaths in the UG was eight when analysis Model 1 was used (log-rank $P = 0.005$ compared to the SG group) (Fig. 3a). Appling Model 2 analysis, there were six reported deaths in the UG (log=rank $P = 0.008$ compared to the SG group) (Fig. 3b).

Discussion

The present randomised clinical trial has shown that ingesting 2 g day^{-1} supplemental fish oil improved long-term survival in those patients with haematological malignancies who were receiving chemotherapy. Furthermore, patients receiving fish oil were also able to undertake a greater number of cycles of chemotherapy. Fish oil containing 367 mg of EPA and 243 mg of DHA for 9 weeks was sufficient to alter the fatty acid composition of plasma lipid constituents, leading to an approximately two-fold increment in the proportion of EPA and a 1.8-fold increase for DHA. The same finding has been reported in previous studies [14,28,29].

Table 1 Characteristics of the patients at baseline

Characteristic	Model 1			Model 2		
	UG (n = 13)	SG (n = 9)	P	UG (n = 8)	SG (n = 6)	P
Sex (n/%)						
Female	5 (22.7)	5 (22.7)	0.66‡	3 (21.4)	2 (14.3)	1.00‡
Male	8 (36.4)	4 (18.2)		5 (35.7)	4 (28.6)	
Age (years)*	53.8 (15.8)	43.8 (23.3)	0.24§	54.8 (16.1)	39.0 (22.2)	0.15§
Diagnosis (n/%)						
Acute leukaemia	5 (22.7)	4 (18.2)	*NA*	3 (21.4)	2 (14.3)	*NA*
Non-Hodgkin lymphoma	6 (27.2)	2 (9.1)		3 (21.4)	2 (14.3)	
Hodgkin lymphoma	1 (4.5)	3 (13.6)		1 (7.1)	2 (14.3)	
Chronic leukaemia	1 (4.5)	0 (0)		1 (7.1)	0 (0)	
Other diseases (n/%)						
Absent	6 (27.3)	7 (31.8)	0.20‡	4 (28.6)	6 (42.8)	0.15‡
Present	7 (31.8)	2 (9.1)		4 (28.6)	0	
Percentage of weight loss in the previous 6 months (%)†	4.3 (0.0–13.3)	1.9 (1.5–6.3)	0.44¶	9.1 (3.2–17.2)	3.0 (1.5–6.3)	0.16¶

Data are n (%).

*Mean (SD).

†Median (interquartile range).

Model 1 included all eligible patients who agreed to participate and finished the 9 weeks of follow-up. Model 2 included patients from SG who presented ≥ 100% increment in the proportion of plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 9 weeks compared to baseline and UG patients who did not show an increment (< 50%) in plasma EPA and DHA at 9 weeks compared to baseline.

NA, not applicable; SG, supplemented Group; UG, unsupplemented group.

‡Chi-squared and Fisher's exact tests.

§Student's t-test.

¶Mann–Whitney test.

Studies testing the effects of EPA and DHA in patients diagnosed with haematological malignancies are scarce. One previous study was performed in early-stage chronic lymphocytic leukaemia [29] and another in acute myeloid leukaemia [30]. Both studies used fish oil as a nutritional strategy in parallel with chemotherapy. However, neither of these previous studies investigated the same outcomes as those assessed in the present study. Hence, it is difficult to compare our findings with those of these two previous studies.

In vitro and review studies have concluded that EPA and DHA can induce apoptosis in leukaemic cell lineages [32–36]. Furthermore, *in vitro* studies performed with leukaemic cell lines have shown an increment in the antineoplastic action of drugs used in chemotherapy when EPA and DHA are present in the culture medium [28,37,38]. Additionally, in dogs with lymphoblastic lymphoma, EPA and DHA ingestion increased survival and decreased the plasma concentration of interleukin-6 [39].

RCTs conducted in different types of cancer patients receiving chemotherapy and supplemented with n-3 PUFAs have shown the positive effects of this strategy on inflammatory and nutritional outcomes. For example, a double-blind RCT in patients with lung cancer reported increased body weight and a reduction in inflammatory indices in the group receiving fish oil containing 510 mg of EPA and 310 mg of DHA [40]. A RCT testing colorectal cancer patients found that fish oil containing 367 mg of EPA and 243 mg of DHA, given daily for 9 weeks, decreased serum levels of CRP [15], reduced inflammatory and nutritional risk [15,41], and maintained or increased BMI and body weight during chemotherapy [41].

A systematic review regarding the effects of n-3 PUFA in cancer patients receiving chemotherapy concluded that the main beneficial effect of this supplementation is the preservation of body weight and body composition [10]. However, in the present study, there were no significant changes in body weight or BMI with fish oil supplementation. Nevertheless, we observed a trend increment in MUAMC in the subjects supplemented with fish oil, suggesting the preservation or a gain of lean mass. In addition, NRI was higher in patients receiving fish oil.

Studies with different cancer patients have demonstrated a decrease in inflammation after supplementation with n-3 PUFAs [42–45]. These effects may be partly a result of inflammatory and immune response modulation by n-3 PUFAs because of an altered pattern of production of lipid mediators including eicosanoids, such as prostaglandins, leukotrienes, thromboxanes, resolvins (E and D)

Table 2 Nutritional status parameters of patients with haematological malignancies supplemented or not with fish oil during chemotherapy

	Model 1			Model 2		
Parameters	UG (n = 13)	SG (n = 9)	$P^{\dagger}$	UG (n = 8)	SG (n = 6)	$P^{\dagger}$
Body weight (kg)						
T0	72.4 (11.6)	68.1 (10.3)	0.38§§	74.0 (13.5)	69.3 (11.4)	0.51§§
T1	69.9 (11.7)	68.0 (8.2)	0.68§§	72.5 (11.0)	69.0 (9.1)	0.55§§
Δ	−1.0 (−5.6 to 5.6)	−1.0 (−2.0 to 2.4)	0.50††	−0.9 (−3.5 to 0.8)	−1.5 (−9.8 to 6.0)	0.85††
$P^{\ddagger}$	0.20‡‡	0.96‡‡		0.49‡‡	0.90‡‡	
BMI (kg m^{-2})						
T0	25.7 (4.0)	24.6 (4.1)	0.52§§	26.7 (3.9)	23.1 (3.4)	0.10§§
T1	24.8 (3.8)	24.5 (3.4)	0.85§§	26.1 (2.9)	22.9 (1.9)	0.04§§*
Δ	−0.4 (−2.2 to 0.0)	−0.4 (−0.8 to 1.0)	0.52††	−0.4 (−1.4 to 0.3)	−0.5 (−0.8 to 1.8)	0.80††
$P^{\ddagger}$	0.17‡‡	0.91‡‡		0.47‡‡	0.85§§	
MUAC (cm)						
T0	29.8 (3.4)	30.2 (3.5)	0.81§§	30.4 (3.4)	29.2 (3.4)	0.50§§
T1	29.1 (3.8)	30.9 (3.2)	0.25§§	30.0 (3.2)	30.1 (3.4)	0.96§§
Δ	−1.0 (−2.0 to 0.0)	1 (−0.5 to 1.5)	0.06††	−1.0 (−3.5 to 1.0)	1.3 (−0.5 to 1.5)	0.21††
$P^{\ddagger}$	0.27‡‡	0.24‡‡		0.65‡‡	0.32‡‡	
TS (mm)						
T0	24.5 (20.0–29.0)	22.0 (20.5–23.0)	0.26††	26.3 (21.5–37.0)	21.3 (17.0–23.0)	0.07††
T1	23.0 (17.0–31.0)	21.0 (20.0–25.0)	0.95††	28.0 (18.0–32.0)	20.5 (18.0–22.0)	0.24††
Δ	−3.0 (−6.0 to −1.0)	−2.0 (−3.0 to 5.0)	0.27††	−3.0 (−5.0 to −0.2)	0.3 (−3.0 to 5.0)	0.36††
$P^{\ddagger}$	0.08**	0.72**		0.12**	0.83**	
MUAMC (cm)						
T0	21.7 (2.7)	23.3 (3.3)	*0.21*§§	21.4 (3.0)	22.8 (3.3)	0.43§§
T1	22.0 (1.8)	23.7 (4.1)	*0.95*††	22.0 (1.6)	23.9 (3.0)	0.14§§
Δ	−0.1 (−0.7v1.4)	0.6 (0.2–1.6)	*0.52*††	0.1 (−0.6 to 1.2)	1.1 (0.2–2.4)	0.30††
$P^{\ddagger}$	0.15‡‡	0.72**		0.49‡‡	0.07‡‡	
NRI§						
T0	84.2 (11.9)	90.6 (7.6)	0.17§§	83.6 (10.0)	93.3 (6.7)	0.07§§
T1	86.9 (9.0)	94.7 (10.8)	0.09§§	86.7 (7.2)	97.4 (10.5)	0.04§§*
Δ	2.4 (–3.8 to 8.5)	2.3 (–0.8 to 7.0)	0.66††	2.0 (–3.0 to 6.8)	1.6 (–2.2 to 10.8)	0.70††
$P^{\ddagger}$	0.25‡‡	0.14**		0.32‡‡	0.23‡‡	

Data are presented as the mean (SD) or median (interquartile range).

Model 1 included all eligible patients who agreed to participate and finished the 9 weeks of follow-up. Model 2 included patients from SG who presented ≥100% increment in the proportion of plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 9 weeks compared to baseline and UG patients who did not show an increment (<50%) in plasma EPA and DHA at 9 weeks compared to baseline.

BMI, body mass index; MUAC, mid-upper arm circumference; MUAMC, mid-upper arm muscle circumference; NRI, nutritional risk SG; supplemented group; T0, data before the first session of chemotherapy (baseline); T1, data after 9 weeks of chemotherapy; TS, triceps skinfold; UG, unsupplemented group; Δ, difference between the final and initial value.

*$P < 0.05$.

†Comparison between groups at the respective time points.

‡Comparison within the same group for different time points.

§Values represent a score. Not malnourished, >100; mildly malnourished, 97.5–100; moderately malnourished, 83.5–97.5; severely malnourished, <83.5.

**Wilcoxon test. ††Mann–Whitney test. ‡‡t-test for paired data. §§Student's t-test.

and D1 protectins [46–48]. Additionally, some anti-inflammatory effects of n-3 PUFAs appear to be exerted through the decreased activation of the pro-inflammatory transcription factor nuclear factor-kappa B and perhaps an increased activation of the anti-inflammatory transcription factor peroxisome proliferator activated receptor-γ [49–51].

In the present study, chemotherapy affected the CRP/albumin ratio. In the UG, the risk classification changed from high to moderate. However, in patients who received fish oil, the risk classification changed from high to low. This risk classification decline can be indicative of a positive effect of supplementation with fish oil. The same effect was reported previously in patients with colorectal cancer [15,41]. Thus, the CRP/albumin ratio may be a sensitive marker of the ability of fish oil supplementation to improve the inflammatory-nutritional status in these patients.

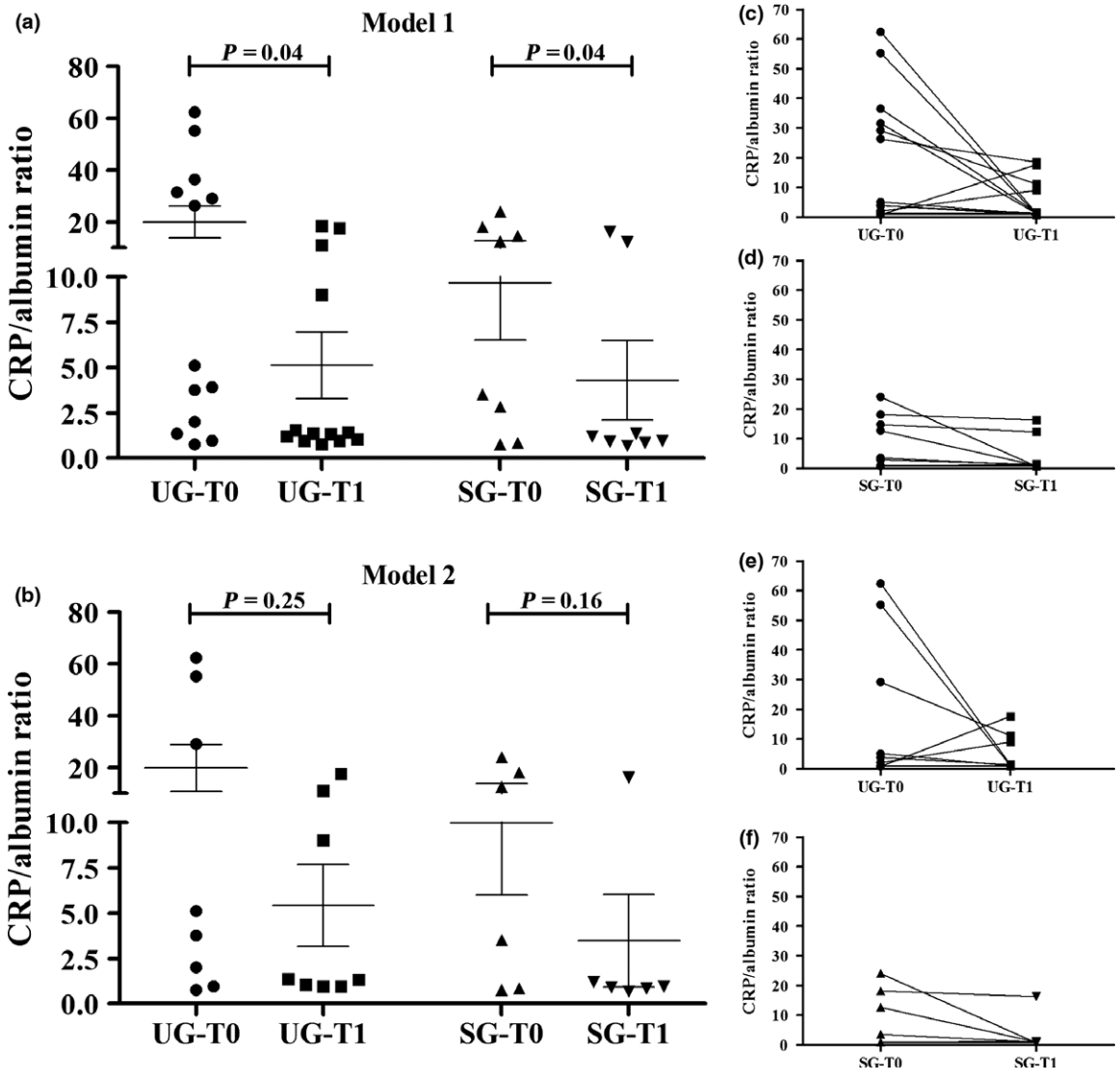

Figure 2 C-reactive protein (CRP)/albumin ratio of patients with haematological neoplasms supplemented with fish oil during chemotherapy on different moments: T0, data before the first session of chemotherapy; T1, data after 9 weeks of chemotherapy. (a) Model 1 included all eligible patients who agreed to participate and finished the 9 weeks of follow-up; Model 1: UG, unsupplemented group (n = 13); SG, supplemented Group (n = 9). (b) Model 2: included patients from SG who presented ≥100% increment in the proportion of plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 9 weeks compared to baseline and UG patients who did not show an increment (<50%) in plasma EPA and DHA at 9 weeks compared to baseline. Model 2: UG group (n = 8); SG (n = 6). (c, d) Model 1 and (e, f) Model 2 line representation to visualise individual changes in 9 weeks. CRP/albumin ratio: values represent an inflammatory – nutritional risk index. Without risk, <0.4, low risk, 0.4–1.2; moderate risk, 1.2–2.0, high risk, >2.0. #*P* value for Wilcoxon test for paired data.

Survival up to 465 days of follow-up was greater when patients ingested fish oil independent of the model of analysis. Similarly, some previous studies showed prolonged survival after *n*-3 PUFA supplementation in patients with pancreatic cancer [12], advanced nonsmall cell lung cancer [52] and metastatic breast cancer [53]. The longer survival might be attributed to the effects of fish oil with respect to the nutritional-inflammatory risk. Additionally, the increment of the antineoplastic action and reduction of the toxicity of the chemotherapy [54] could explain such observations.

The data reported in the present study suggest that it will be important for future trials to perform medium- or long-term follow-up of some variables relevant to the clinical environment (e.g. survival, hospital readmissions, etc.). Furthermore, it may be possible for researchers involved in previous trials to retrospectively check clinical variables from the enrolled patients with

Table 3 Blood parameters of study's patients supplemented or not with fish oil during chemotherapy.

	Model 1			Model 2		
Parameters	UG (n = 13)	SG (n = 9)	$P^{\dagger}$	UG (n = 8)	SG (n = 6)	$P^{\dagger}$
Red blood cell count (10^6 mm^{-3})						
T0	3.1 (2.4–4.1)	3.2 (2.2–4.5)	0.89‡‡	3.2 (2.6–4.2)	4.1 (2.3–4.6)	0.56‡‡
T1	4.2 (2.8–4.5)	3.7 (3.6–4.0)	0.59‡‡	4.4 (4.0–4.6)	3.8 (3.6–4.5)	0.33‡‡
Δ	0.7 (0.4–1.1)	0.4 (−0.4 to 1.3)	0.47‡‡	0.9 (0.6–1.2)	−0.4 (−0.5 to 1.4)	0.40‡‡
$P^{\ddagger}$	<0.01††*	0.26††		0.01††*	0.69††	
Haemoglobin (g dL^{-1})						
T0	9.0 (8.6–11.0)	9.9 (6.8–13.6)	0.83‡‡	9.6 (8.7–11.5)	13.4 (6.7–13.8)	0.38‡‡
T1	11.5 (9.2–12.6)	11.9 (11.3–12.8)	0.16‡‡	11.5 (9.0–12.6)	12.7 (11.3–13.4)	0.20‡‡
Δ	1.0 (−0.2 to 3.2)	2.9 (−0.4 to 3.7)	0.49‡‡	1.9 (−1.2 to 3.4)	3.3 (−0.6 to 3.8)	0.38‡‡
$P^{\ddagger}$	0.12††	0.09††		0.21††	0.22††	
Haematocrit (%)						
T0	27.7 (5.9)	30.7 (8.9)	0.21§§	27.7 (6.2)	33.2 (9.2)	0.23§§
T1	31.7 (5.5)	35.0 (3.8)	0.31§§	32.6 (5.9)	36.2 (3.6)	0.21§§
Δ	3.1 (−1.2 to 9.9)	2.6 (−1.4 to 9.5)	0.88‡‡	6.0 (0.1–10.3)	2.5 (−1.7 to 2.7)	0.56‡‡
$P^{\ddagger}$	0.04§*	0.10§		0.10§	0.38§	
Leucocytes (10^6 mm^{-3})						
T0	4.6 (3.4–6.2)	5.1 (4.0–16.3)	0.56‡‡	4.5 (2.8–5.8)	5.3 (5.0–27.4)	0.14‡‡
T1	4.7 (3.5–6.6)	7.0 (4.2–9.6)	0.30‡‡	4.8 (4.1–6.1)	8.3 (5.2–12.5)	0.12‡‡
Δ	0.7 (−2.1 to 2.2)	−0.6 (−7.6 to 3.2)	0.72‡‡	1.5 (−0.7 to 2.6)	−2.3 (−12.7 to 2.5)	0.46‡‡
$P^{\ddagger}$	0.03††*	0.16††		0.40††	0.50††	
CRP (mg L^{-1})						
T0	16.9 (6.8–75.9)	35.3 (10.2–54.4)	0.66‡‡	14.3 (4.9–103.4)	27.1 (3.2–54.4)	0.90‡‡
T1	4.3 (3.7–28.9)	4.3 (3.2–19.1)	0.72‡‡	4.3 (3.7–30.0)	3.4 (3.2–5.1)	0.36‡‡
Δ	−13.7 (−47.4 to −1.1)	−4.7 (−23.8 to −1.0)	0.35‡‡	−10.5 (−85.8 to 11.1)	−6.8 (−38.0 to 0.2)	0.80‡‡
$P^{\ddagger}$	0.06††	0.04††*		0.33††	0.12††	
Albumin (g dL^{-1})						
T0	3.0 (0.7)	3.4 (0.5)	0.25§§	3.1 (0.5)	3.5 (0.3)	0.12§§
T1	3.3 (0.5)	3.7 (0.6)	0.18§§	3.4 (0.4)	3.8 (0.5)	0.08§§
Δ	0.2 (0.1–0.7)	0.2 (0.0–0.4)	0.72‡‡	0.2 (−0.1 to 0.4)	0.2 (−0.1 to 0.5)	0.90‡‡
$P^{\ddagger}$	0.08§	0.06§		0.13§	0.13§	

Data are presented as the mean (SD) or median (interquartile range).

Model 1 included all eligible patients who agreed to participate and finished the 9 weeks of follow-up. Model 2 included patients from SG who presented ≥100% increment in the proportion of plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 9 weeks compared to baseline and UG patients who did not show an increment (<50%) in plasma EPA and DHA at 9 weeks compared to baseline.

CRP, C-reactive protein; SG, supplemented Group; T0, data before the first session of chemotherapy (baseline); T1, data after 9 weeks of chemotherapy; UG, unsupplemented group; Δ, difference between the final and initial value.

*$P < 0.05$.

†Comparison between groups at the respective time points.

‡Comparison within the same group for different time points.

††Wilcoxon test. ‡‡Mann–Whitney test. §t-test for paired data. §§Student's t-test.

the aim of identifying medium- or long-term changes that were not part of the original follow-up in some trials.

Studies with oral fish oil supplementation have a specific limitation: the odour and aftertaste of regular fish oil affect the performance of double-blind placebo-controlled trials. Although some studies try to minimise this limitation using deodorised fish oil capsules, in medium to low-income countries, regular fish oil is easily assessable compared to other forms available in the market. Therefore, no placebo was offered to the control group. Other limitations include the study sample, which was composed of patients with different onco-haematological diagnosis, as well as with different disease staging, chemotherapy regimes and co-morbidities. However, the cohort was a result of a careful screening according to our inclusion and exclusion criteria. We consider that our criteria were important with respect to reducing additional confounding factors. Nevertheless, despite these limitations, the data from the present study provide relevant information to guide future research regarding these health conditions.

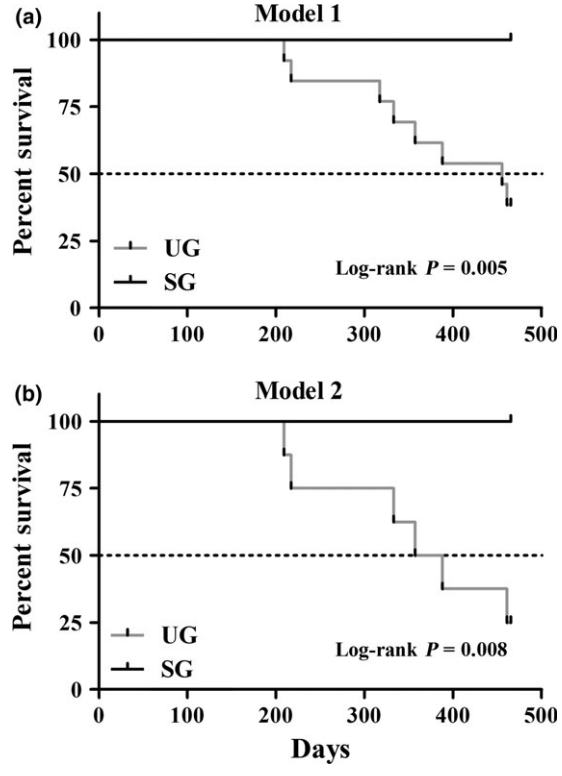

Figure 3 Overall survival (OS) was defined as the time elapsed between baseline (i.e. the day of the first chemotherapy) and death from any cause until 465 days of follow-up. OS curves according to fish oil supplementation were computed using the Kaplan–Meier method and compared using log-rank tests. (a) Model 1: US (n = 13); SG (n = 9) included all eligible patients who agreed to participate and finished the 9 weeks of follow-up. (b) Model 2: UG (n = 8); SG (n = 6) included patients from SG who presented ≥100% increment in the proportion of plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 9 weeks compared to baseline and UG patients who did not show an increment (<50%) in plasma EPA and DHA at 9 weeks compared to baseline. SG, supplemented Group; UG, unsupplemented group.

In conclusion, the ingestion of fish oil concomitant with chemotherapy increases long-term survival (465 days after the beginning of the chemotherapy), potentially by reducing the inflammatory-nutritional risk, in patients with haematological malignancies. Larger clinical trials focusing on these patients need to be conducted to test such findings globally.

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Conflicts of interest, source of funding and authorship

PCC is an advisor to Pronova BioPharma, Aker Biomarine, Smartfish, DSM, Sancilio, Solutex and Danone/Nutricia. The other authors have no conflicts of interest to declare.
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TRC, EAN and EBSMT were responsible for the research design. TRC, DSB, PFO, CQC, AMB and JAGM conducted the research and collected the data. TRC and MCM performed the statistical analysis. TRC, PCC and EAN reviewed the data and drafted the manuscript. TRC and EAN had primary responsibility for the final content. EAN and EBSMT were responsible for obtaining funding sources.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Table S1 Percentage (%) of plasma fatty acids in patients with haematological malignancies supplemented (or not) with fish oil during chemotherapy.
Table S2 Number of hospital readmissions and chemotherapy cycles during 465 days of follow-up.

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LIPIDS, HEALTH AND DISEASE

Algal supplementation of vegetarian eating patterns improves plasma and serum docosahexaenoic acid concentrations and omega-3 indices: a systematic literature review

J. C. Craddock,[1] E. P. Neale,[1,3] Y. C. Probst[1,3] & G. E. Peoples[2]

[1]Faculty of Science Medicine and Health, School of Medicine, University of Wollongong, Wollongong, NSW, Australia
[2]Faculty of Science Medicine and Health, Graduate School of Medicine, School of Medicine, University of Wollongong, Wollongong, NSW, Australia
[3]Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia

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Correspondence
J. C. Craddock, School of Medicine, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia.
Tel.: +61422369633
Fax: +61242214844
E-mail: jcc256@uowmail.edu.au

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Abstract

Background: Vegetarians are likely to have lower intakes of preformed docosahexaenoic acid (DHA) than omnivorous populations who consume fish and animal products. As such, vegetarian populations have omega-3 indices up to 60% lower than those who consume marine products. Algae, the primary producer of DHA in the marine food chain, offer an alternative source of DHA for those who do not consume marine or animal products. This systematic review aims to examine the evidence for the relationship between supplementation with algal forms of DHA and increased DHA concentrations in vegetarian populations.
Methods: The SCOPUS, Science Direct and Web of Science scientific databases were searched to identify relevant studies assessing the effect of algal DHA consumption by vegetarian (including vegan) populations.
Results: Four randomised controlled trials and two prospective cohort studies met the inclusion criteria. All included studies reported algal sources of DHA significantly improve DHA concentrations (including plasma, serum, platelet and red blood cell fractions), as well as omega-3 indices, in vegetarian populations. An evident time or dose response was not apparent given the small number of studies to date.
Conclusions: Future studies should address long chain *n*-3 polyunsaturated fatty acid deficiencies in vegetarian populations using algal DHA and explore the potential physiological and health improvements in these individuals.

Introduction

Vegetarian eating patterns are increasing in popularity as the associated health benefits become evident [1–3]. Protection against chronic disease has been observed in those individuals following a vegetarian eating pattern, including coronary heart disease, hypertension, diabetes mellitus, obesity and some cancers [1,2,4]. Eating patterns of this nature are typically higher in fruits and vegetables leading to increased oligo-and polysaccharides, fibre and phytochemicals, at the same time also being lower in saturated fat and cholesterol compared to omnivorous eating patterns [5,6]. Approaches to vegetarian eating range from the complete exclusion of animal products through to their inclusion at varying levels; eggs or dairy may be consumed [7] referred to as ovo-and lacto- forms

of vegetarian eating, respectively. Individuals adhering to a vegan eating pattern exclude all products of an animal origin.

Although many beneficial characteristics have been attributed to vegetarian eating patterns, vegetarian populations generally have lower plasma concentrations of docosahexaenoic acid (DHA; 22 : 6*n*-3; percentage of total fatty acids) [8] and lower omega-3 indices compared to those who eat fish [9]. The index is calculated using the sum of eicosapentaenoic acid (EPA; 22 : 5*n*-3) and DHA in erythrocyte membranes expressed as a percentage of total fatty acids [10].

DHA is one of the two most prevalent polyunsaturated fatty acids in brain and retinal phospholipids (along with arachidonic acid) and plays a key role in normal neurotransmission [11] and visual function [12] and can be incorporated into cardiac [13] and skeletal muscle [14,15]. DHA is a long-chain omega-3 polyunsaturated fatty acid with a range of proposed health benefits, including assisted foetal development [16], improved cardiovascular function [16,17], a reduced incidence of dementia [18] and improved cognitive functioning [11,16].

Vegetarian eating patterns are typically higher in α-linolenic acid (ALA), a precursor to DHA, compared to omnivorous eating patterns; however, conversion of ALA to DHA is limited within the human body [9,19]. Several studies have reported varying conversion rates of ALA to DHA in humans, from no detectable conversion, to 9% conversion [20–22]. Nonetheless, long-term vegetarian populations may have an increased capacity for synthesising long chain omega-3 fatty acids (LCn-3FA) particularly DHA as a result of evolutionary pressures for improved ALA metabolism [23,24].

The richest dietary sources of DHA are fatty fish and seafood; however, fish are not the originators of DHA. Fish, similar to humans, do not readily synthesise DHA but feed on zooplankton, which feeds on algae, the primary producer of the omega-3 DHA in the marine food chain [25]. Algae and algal supplements are rich in DHA, with some supplements containing no EPA at all [26]. Human consumption of algal DHA rather than fish or seafood forms of DHA may provide adequate intakes of DHA for those who do not eat fish or seafood, although a consensus on the effect of algal supplementation on circulating DHA concentrations and incorporation into membranes has not been established.

With the proposed health benefits and the limited research exploring the bioavailability of algal DHA compared to fish and fish oil, an important question arises: does supplementation with algal forms of DHA in vegetarian populations improve their DHA concentrations? The present review aims to address this question in relation to reported DHA fractions and/or omega-3 indices as markers of membrane incorporation.

Materials and methods

Study protocol

A systematic review of the literature was conducted using the SCOPUS, Science Direct and Web of Science scientific databases (all years to February 2016). The review was registered with PROSPERO, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO, registration number CRD42015020724). The search strategy used the following keyword and Boolean combinations; 'delta-6 desaturase enzyme' OR 'docosahexaenoic acid' OR 'DHA' OR 'omega 3' OR 'eicosapentaenoic acid' OR 'EPA' OR 'linolenic acid' OR 'LA' OR 'alpha-linoleic acid' OR 'ALA' OR 'essential fatty acid' OR 'α-linolenic acid' OR 'omega 3 index' AND 'vegetarian*' OR '*vegetarian' OR 'vegan*' OR 'plant-based' AND 'human' AND 'supplemen*' in the article, keywords or abstract.

Study selection

Included publications met certain requirements: the studies (i) assessed the effect of consumption of algal sources of DHA in vegetarian (vegan, ovo-lacto-, ovo-, lacto-) populations aged 18 years or over and (ii) reported DHA fractions including plasma, serum, platelet, fat, red blood cell (RBC) concentrations and/or omega-3 indices. Studies where vegetarian populations were included alongside omnivorous groups were included. Publications that met the following exclusion criteria were omitted: (i) not published in the English language; (ii) conference papers, short surveys, letters, notes, editorials, articles in press, book series, erratum and conference proceedings; (iii) intervention studies focused on fatty acids other than DHA (such as ALA, EPA); and (iv) populations following a pesco-vegetarian, flexitarian (plant-based eating patterns consuming fish or meat) or semi-vegetarian eating pattern. Duplicate publications were removed using ENDNOTE, version X7 (Thomson Reuters, Philadelphia, PA, USA). Where results from the same study were reported in multiple publications, the first published study was included to avoid duplication of results.

Screening of titles and abstracts was initially applied to exclude irrelevant papers, followed by retrieval of full-text publications. Reference lists of all included publications were also examined for relevant studies. Data extraction included information related to: the publication year, study design/quality, total sample size, population type, intervention and results. National Health and Medical Research Council levels of evidence [27] were applied to

the included studies. Study quality was assessed using the quality criteria checklist of the Evidence Analysis Library (http://www.andeal.org) of the Academy of Nutrition and Dietetics (2012) [28].

Results

The literature search identified 695 publications (Fig. 1). After duplicates were removed, 626 publications were excluded by title and abstract, whereas a further five were excluded following full text assessment. Seven publications (describing six studies) met the inclusion criteria, which included two prospective cohort studies and five intervention studies (Fig. 1) [29–35]. Two publications used the same data from one study and therefore the second publication (by date) was excluded [33], resulting in a total of six publications being included in the review. All studies received positive scores after the quality criteria checklist of the Evidence Analysis Library study was applied [28] (data not shown).

The doses of DHA provided via algal supplements ranged from 172 mg day^{-1} to 2.14 g day^{-1} in the included studies (Table 1). Five studies [30–32,34,35] investigated the effects of DHA supplementation on vegetarians, with one study including vegans, exclusively [29]. The study durations ranged from 2 weeks to 4 months, with sample sizes ranging from 20 to 108 participants.

DHA outcome measurements varied among studies and included serum total phospholipid (PL) DHA[30,31,34], platelet total PL DHA[30,31], RBC total lipid DHA, RBC-phosphatidylethanolamine (RBC-PE) DHA, RBC-phosphatidylcholine (RBC-PC) DHA, plasma-PL DHA[32], low-density lipoprotein (LDL) DHA concentrations [35] and omega-3 indices [29,32]. All included studies reported increases in serum, plasma, platelet and RBC DHA fractions and/or omega-3 indices following algal DHA supplementation. Increases in omega-3 indices were reported to range from 55% to 82% [29,32] within groups supplemented with algal DHA. Similarly, DHA serum total phospholipids and platelet phospholipids were also elevated after algal supplementation, with increases ranging from 238% to 246% [30,31] and 209–225% [30,31] in total and platelet phospholipids, respectively. The sole study exploring DHA plasma as a percentage of total fatty acids reported a 59% increase from baseline [34]. Geppert *et al.* [32] reported increases within groups in RBC total lipids (80% increase), RBC-PE wt% (86% increase), RBC-PC (174% increase) and plasma PL (164% increase) ($P < 0.001$).

Discussion

The results of the present review suggest that consumption of algal sources of DHA in vegetarian populations

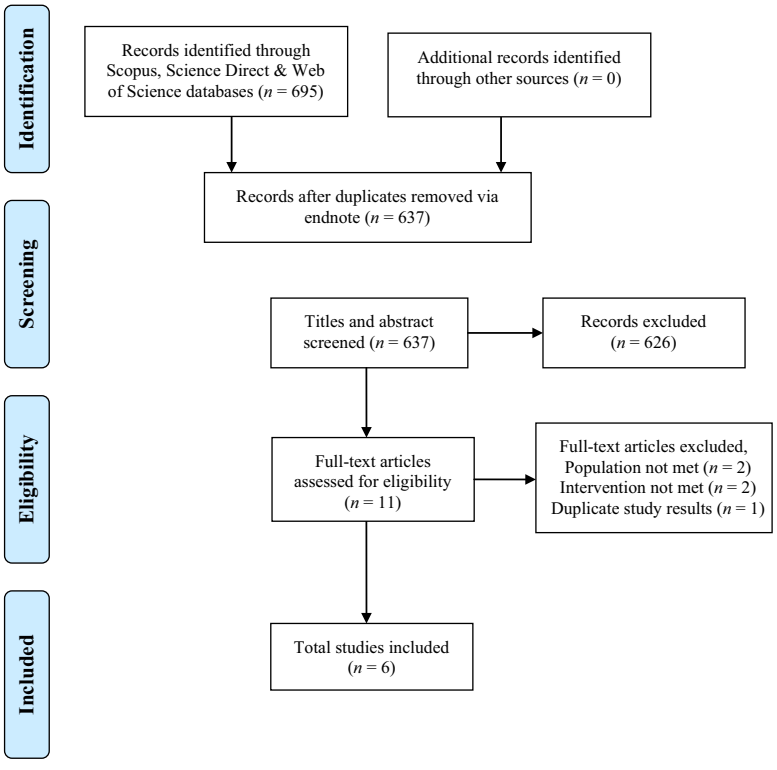

Figure 1 PRISMA flowchart showing the initial and final number of studies obtained.

Table 1 Summary of included studies using algal DHA supplementation

Reference	Study design (Level of evidence)	Sample size	Length of vegetarianism/ Population	Age (mean ± SD or range), years	DHA dose/day, mg	Duration, weeks	Study results	DHA outcomes
Sarter et al (2015)[29]	Prospective Cohort (III – 2)	*n* = 46	• Minimum 1 year vegan • omega-3 index <4%	22–85	172	16	• Omega-3 index ↑ 3.1% ± 0.6% to 4.8% ± 0.8[§]	Increased
Conquer & Holub (1997)[31]	Prospective Cohort (III – 2)	*n* = 20	• Minimum 6 months vegetarian	26.8 ± 1.6	1620	6	• DHA serum total PL ↑ from 2.1 ± 0.2 to 7.1 ± 0.4 mol%[¶] • DHA Platelet PL ↑ from 1.1 ± 0.1 to 3.4 ± 0.2 mol%[¶]	Increased
Ryan & Symington (2014)[34]	A pseudorandomised controlled trial (III – 1)	*n* = 12	• Vegetarian/vegan (length not reported)[‡]	18–65	200	2	• DHA plasma (% of total fatty acids) ↑ 2.76 ± 1.13 to 5.08 ± 0.45[¶]	Increased
Conquer & Holub (1996)[30]	RCT (II)	*n* = 24	• Minimum 6 months vegetarian	29.6 ± 1.7	1620*	6	• DHA serum total PL ↑ from 2.4 ± 0.2 to 8.3 ± 0.2 g/100 g[¶] • DHA platelet PL ↑ from 1.2 ± 0.1 to 3.9 ± 0.2 g/100 g[¶]	Increased
Geppert et al (2005)[32]	RCT (II)	*n* = 108	• Minimum 1 year vegetarian	25.9 ± 5.6	940[†]	8	• Omega 3 index ↑ from 4.8 to 8.4wt%[§,**] • DHA RBC total lipids ↑ from 4.4 ± 0.2 to 7.9 ± 0.2 wt%[§,**] • DHA RBC PE ↑ from 6.5 ± 0.3 to 12.1 ± 0.3 wt[§,**] • DHA RBC PC ↑ from 1.38 ± 0.07 to 3.78 ± 0.13[§,**] • DHA Plasma PL ↑ from 2.8 ± 0.1 to 7.4 ± 0.2 wt%[§,**]	Increased
Wu et al (2006)[35]	RCT (II)	*n* = 25	• Minimum 1 year vegan and/or lacto-ovo vegetarian	52.3 ± 5.1 (control group) 52.6 ± 4.4 (intervention group)	2140*	6	• LDL-DHA ↑ from 1.35 ± 0.54 to 3.71 ± 1.03**	Increased

Prospective Cohort study, (III-2) - Non-randomised, experimental trial.
Pseudorandomised controlled Trial (III-1) - Alternate allocation or some other method.
RCT (Randomized Controlled Trial; II) - A study of test accuracy with: an independent, blinded comparison with a valid reference standard, among consecutive patients with a defined clinical presentation.
*Control – Corn Oil.
[†]Control – Olive Oil.
[‡]Other study arms described in study – Nil control.
[§]Significant within group ($P < 0.001$).
[¶]Significant within group ($P < 0.05$).
**Significant between control group ($P < 0.001$).

considerably increased levels of circulating DHA, including those measured in plasma, serum, platelet and RBC DHA fractions, as well as omega-3 indices. This review highlights algal supplementation as a viable method of addressing the low DHA levels often seen in vegetarians and vegans [8,9]; however, given the varying doses, supplement periods and tissues, a clear dose response to recommend a threshold for effective increases in omega-3 status was not apparent. Providing DHA in the diet is clearly associated with positive health outcomes, including cardiovascular disease. As such, optimising the tissue concentrations of DHA in vegetarian populations by using algal oil will be an important avenue of further research in nutrition translation and practice, and may also be relevant for omnivorous populations.

When interpreting the results of this systematic literature review, it is important to note that, although all of the included studies were assessed as being of positive quality, certain studies exhibited stronger methodology. Von Shacky *et al.* [36] demonstrated that, for cardiovascular protection to be observed as a health benefit of omega-3 fatty acids, evidence of tissue incorporation is required and study design is imperative. For example, Sarter *et al.* [29] and Geppert *et al.* [32] used durations of 4 and 2 months, respectively, reporting both baseline and post-supplementation omega-3 indices, which provided a more rigorous depiction of algal supplementation in vegetarian populations. Conversely, four of the included studies [30,31,34,35] reported the effects of short-term supplementation (2–6 weeks) on serum total phospholipid DHA, platelet phospholipid DHA or LDL-DHA concentrations. The results may have been indicative of circulating concentrations and it is unclear whether these elevated DHA concentrations would have been incorporated into heart, skeletal muscle and brain tissue for health benefits to be observed.

One included prospective cohort study [31] compared total phospholipid DHA in platelets between omnivorous and vegetarian populations after both groups were supplemented with algal oil. Similar increases in total phospholipid DHA were observed in both groups, suggesting that algal oil supplementation has relevance for both omnivorous and vegetarian populations. Only one included study [34] explored the bioequivalence between fish oil and algal oil in a three-arm randomised controlled trial with no significant differences being observed between groups. There appears to be a paucity of literature evaluating the efficacy between fish and algal forms of DHA; however, from the limited human studies, it appears algal sourced DHA may have comparable bioavailability to fish sourced DHA [37,38]. Comparing algal DHA to fish DHA supplements is an area that warrants further investigation.

Recently, concern has been expressed for omega-3 fatty acid controlled trials exploring cardiac outcomes [39,40]. Much of the criticism surrounds the disparity in study design when comparing standard pharmacological controlled trials to fish oil trials. In drug trials, the intervention is given to those in the experimental group but not to those in the control group. In fish oil trials, both experimental and control groups are exposed to DHA as a result of background dietary intakes and baseline levels [39]. Nonetheless, because the present review explored DHA supplementation in vegetarian populations, this flaw is somewhat negated in that the control groups would theoretically be consuming none, or very small amounts of DHA and EPA. Furthermore, from a systems physiology perspective, there is no doubt that the consumption of long chain omega-3 can provide support for optimising heart function [17]; however, the translation of these benefits to a vegetarian population is currently unknown. A meta-analysis by Huang *et al.* [41] showed that vegetarians already have a 29% reduced risk of death from ischaemic heart disease compared to omnivores, although there is a lack of research exploring whether vegetarians would acquire even further protection with an increased omega-3 indices.

Algae and algal supplements tend to have a higher DHA to EPA ratio, with many supplements containing no EPA [26]. Thus, the present review focused on the effect of algal supplements on DHA levels; however, the increased levels of DHA observed in the studies in this review may also have additional benefits on other fatty acids. Supplementation with DHA may result in improved EPA concentrations via retro-conversion from DHA to EPA. For example, Conquer and Holub [30] used an EPA-free preparation of DHA that was supplemented daily by an omnivorous group and vegetarian group. DHA concentrations (serum and platelet PL) significantly increased in both groups, as did EPA via retro-conversion of DHA to EPA. The increase in serum PL EPA concentration was 9.4% overall, with no significant difference between omnivores and vegetarians. EPA has been linked to favourable health characteristics, such as cardiovascular protection [42] and reduced inflammation [43]. Given that EPA concentrations (plasma PL) have also been reported to be significantly lower in vegetarian populations compared to groups who consume moderate to large amounts of meat [44], supplementation with algal DHA may provide an alternate method of increasing EPA levels for those who do not consume fish.

Although the present review has provided insight into the effect of algal DHA supplementation in vegetarians, there are some limitations. Large double-blinded randomised controlled trials measuring baseline and post-

supplementation omega-3 indices with algal forms of DHA would be beneficial to substantiate these results. Publication bias may also have influenced the results of this review. Regardless of the limitations, the findings were consistent across studies, with all six included studies reporting increases in various plasma and serum fractions and/or erythrocyte LDL-DHA concentrations and/or omega-3 indices across a range of doses and intervention durations.

Conclusions

The present review has consolidated existing studies and reports that supplementation with algal forms of DHA can increase an array of serum and platelet DHA concentrations and omega-3 indices in vegetarians. This finding is relevant given that this population is known to have lower serum and plasma DHA concentrations than omnivorous individuals. Further research is warranted aiming to determine the appropriate algal DHA threshold doses/supplement durations that achieve clinically relevant elevations in omega-3 indices. Additionally, research investigating whether there are grounds for DHA supplementation in vegetarian populations, which may potentially further optimise their cardiac protection and reduce chronic disease, is indicated.

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 PRISMA guidelines.

Conflicts of interest, Source of funding, authorship

The authors declare that they have no conflicts of interest.
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JCC designed the study. JCC collected and analysed the data. JCC, EPN, YCP and GEP interpretated the data and were responsible for the preparation of the manuscript. All authors approved the final version of the paper submitted for publication.

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LIPIDS, HEALTH AND DISEASE

Post-lunch triglyceridaemia associates with HDLc and insulin resistance in fasting normotriglyceridaemic menopausal women

A. Sanz-Paris,[1] A. Rodriguez-Valle,[2] M. A. Navarro,[3,4,5] J. Puzo-Foncillas[2] & J. M. Arbones-Mainar[4,5,6]

[1]Nutrition Department, University Hospital Miguel Servet, Zaragoza, Spain
[2]Clinical Biochemistry Department, University Hospital Miguel Servet, Zaragoza, Spain
[3]Department of Biochemistry and Molecular Biology, University of Zaragoza, Zaragoza, Spain
[4]Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain
[5]Centro de Investigación Biomédica en Red Fisiopatología Obesidad y Nutrición (CIBERObn), Instituto Salud Carlos III, Madrid, Spain
[6]Adipocyte and Fat Biology Laboratory (AdipoFat), Translational Research Unit, Instituto Aragonés de Ciencias de la Salud (IACS), University Hospital Miguel Servet, Zaragoza, Spain

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glucose metabolism, hipertriglyceridaemia, Mediterranean Pattern, menopause, receiver operative characteristics.

Correspondence
Jose M. Arbones-Mainar, Adipocyte and Fat Biology Laboratory (AdipoFat), Unidad de Investigación Traslacional, Hospital Universitario Miguel Servet, Zaragoza 50009, Spain.
Tel.: +34 976 769 565
Fax: +34 976 769 566
E-mail: jmarbones.iacs@aragon.es

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Abstract

Objectives: Post-prandial hypertriglyceridaemia (P-HTG) is associated with cardiovascular disease. This association is of paramount importance during menopause, which is also related to reduced high-density lipoprotein-cholesterol (HDLc) and elevated triglyceride (TG) levels. We aimed to provide a self-assesing tool to screen for P-HTG in menopausal women who were normotriglyceridaemic at fasting and adhered to a Mediterranean-style eating pattern.

Methods: We performed oral fat loading tests (OFLT) in combination with self-measurements of diurnal capillary TG at fixed time-points (DC-TG) in 29 healthy menopausal women. TG levels >220 mg dL^{-1} at any given time during the OFLT served as diagnostic criteria for P-HTG. Subsequently, DC-TG profiles were examined to determine the best mealtime (breakfast, lunch or dinner), as well as optimal cut-off points to classify these women as having P-HTG according to the OFLT. Insulin resistance was defined as the upper tertile of the homeostatic model assessment of insulin resistance.

Results: We found that, despite having normal fasting TG levels, P-HTG was highly prevalent (approximately 40%). Moreover, self-assessed 3-h post-lunch TG levels >165 mg dL^{-1} increased the odds of having hypo-HDL cholesterolaemia by 14.1-fold ($P = 0.026$) and the odds of having insulin resistance by 31.6-fold ($P = 0.007$), adjusted for total fat intake in women adhering to a Mediterranean eating pattern having their highest energy intake at lunch.

Conclusions: Self-assessed 3-h post-lunch TG can be used to study post-prandial TG metabolism in Southern European menopausal women who are normotriglyceridaemic at fasting. Characterising an individual's post-prandial response may help menopausal women to evaluate their risk of cardiovascular disease.

Introduction

Post-prandial hypertriglyceridaemia (P-HTG) has been established as an independent risk factor for cardiovascular disease (CVD) [1–4]. This association is of paramount importance in the menopausal period during which reduced high-density lipoprotein-cholesterol (HDLc) and elevated triglyceride (TG) levels may coexist, both contributing toward the augmented CVD risk in women [5,6].

P-HTG has been commonly assessed by measuring the response of plasma TG to an oral fat loading test (OFLT) over a period of time (3–12 h). However, the OFLT is time consuming and requires specialised facilities and trained personnel, making it infeasible for regular clinical practice or large population studies. OFLT also fails to reflect life style and eating habits. Alternatively, self-measurements of TG in normal-life situations may overcome those issues [7,8]. Indeed, self-measurements of diurnal capillary TG at fixed time-points (DC-TG) were strongly associated with post-prandial TG assessed by OFLT [9], and correlated well with obesity, insulin sensitivity [9] and coronary artery disease [10]. The DC-TG method requires measures of fasting and post-prandial TG for each major food intake, considering that lipid intake is approximately the same for each meal. However, a wide variation of energy intake has been demonstrated for different geographical regions. Specifically, citizens of Mediterranean countries consume approximately 40–50% of the amount of their daily lipid intake at lunch [10,11].

In the present study, we aimed to provide a simple self-assessment tool to screen for the risk of P-HTG in menopausal women adhering to a Mediterranean eating pattern. Accordingly, we first performed OFLT in combination with DC-TG measures in healthy menopausal women who were normotriglyceridaemic at fasting. OFLT was used as reference method to detect P-HTG. Subsequently, DC-TG profiles were studied to determine (i) the optimal meal-time (breakfast, lunch, or dinner) and (ii) a threshold for post-meal TG to achieve the maximum specificity and sensitivity with respect classifying these women as having P-HTG according to the OFLT. Additionally, we also aimed to investigate whether or not altered TG post-prandial profiles were also associated with low HDLc and/or insulin resistance in menopausal women.

Materials and methods

Subjects

Thirty healthy post-menopausal (no menses for ≥12 months) women with baseline plasma triglycerides <150 mg dL^{-1} were recruited. Exclusion criteria were (i) acute or chronic disease; (ii) diabetes; (iii) smoking; and (iv) the use of lipid lowering, antihypertensive or antidiabetic medications. All subjects provided their written informed consent and the study was approved by the Ethics Committee for Clinical Research of Aragon.

Laboratory data and dietary intake

Blood samples were collected following a 12-h overnight fast. Glucose, TG, total cholesterol, and HDLc were determined by enzymatic assay with an LX20 auto-analyser (Beckman Coulter, Fullerton, CA, USA). Low-density lipoprotein-cholesterol (LDLc) was estimated by the Friedewald formula. The chemiluminescent Inmulite-One system (Euro/Dpc, Gwynedd, UK) was used to determine the concentration of C-peptide and insulin. C-reactive protein was assessed by immunonephelometry (Immage 800; Beckman Coulter). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose (mg dL^{-1}) × fasting insulin (mU mL^{-1})/405 [12]. HbA1c was measured using high-performance liquid chromatography (ARKRAY ADAMS A1c HA-8180V analyser; Menarini Diagnostics, Florence, Italy). Apolipoprotein E (APOE) genotype was determined using a reverse hybridisation method in accordance with the manufacturer's instructions (INNO-LIPA Apo-E typing test; Innogenetics, Ghent, Belgium).

Dietary intake was self-reported using a 24-h food recall. Subjects were requested to follow their regular diet during the study. Questionnaire data were converted into nutrient and energy intake using the Spanish Food Composition Database (BEDCA) http://www.bedca.net/bdpub/index_en.php.

Post-prandial tests

Measurement of capillary TG and glucose was performed with a TG-specific point-of-care testing device (Accutrend GCT; Roche Diagnostics, Mannheim, Germany). The OFLT was carried out after 10-h overnight fast in a clinical setting. A baseline measurement of TG was performed and then participants were given 100 mL of a long chain triglyceride fat emulsion (Supracal; Nutricia, Madrid, Spain). The standardised fat meal consisted of 1.88 MJ (450 kcal) of energy: 50 g of fat (of which 30.4 g was monounsaturated, 5.3 g saturated and 14.3 g polyunsaturated). Subsequently, TG and glucose were determined at 1, 2 and 3 h after ingestion. For the DC-TG measurement, subjects in free-living conditions were asked to record their levels of DC-TG in a diary at the following time-points: before and 3 h after breakfast, lunch and dinner as described previously [9].

Statistical analysis

Chi-squared tests were used to test associations among categorical variables. A Student's *t*-test or a Mann–

Whitney–Wilcoxon test were used to compare continuous normally or non-normally distributed variables, respectively. Spearman correlation coefficients (ρ) were used to examine relationships between variables. Areas under receiver operating characteristic (ROC) curves were computed using the trapezoidal rule. Multivariate logistic regression was used to analyse the association of HDLc and HOMA adjusted by dietary fat intake. Data were analysed using R, version 3.1.3 (http://www.r-project.org) and the required packages. $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of participants during fasting conditions

Thirty post-menopausal women provided their informed consent. One participant was excluded because of a fasting TG of >150 mg dL^{-1}. Table 1 shows the baseline characteristics of the study sample. The mean (SD) age, body mass index (BMI) and waist circumference were 52.5 (6.50) years, 26.8 (3.97) kg m^{-2} and 88.3 (11.5) cm, respectively. Waist circumference was >88 cm in 37.9% of participants. Regarding APOE genotype, 6.9%, 69% and 24.1% of participants were E2E3, E3E3 or E3E4 respectively.

Table 1 Baseline characteristics of the study subjects during the fasting state

	Mean (SD) or *n* (%) (*N* = 29)
Age (years)	52.5 (6.50)
Body mass index (kg m^{-2})	26.8 (3.97)
Waist circumference (cm)	88.3 (11.5)
Apolipoprotein E genotype	
E2/E3	2 (6.90%)
E3/E3	20 (69.0%)
E3/E4	7 (24.1%)
Tot. cholesterol (mg dL^{-1})	216 (38.3)
LDL-cholesterol (mg dL^{-1})	137 (31.0)
HDL-cholesterol (mg dL^{-1})	64.8 (17.6)
Triglycerides (mg dL^{-1})	69.2 (24.7)
Glucose (mg dL^{-1})	94.7 (15.0)
HbA1c (%)	5.50 (0.37)
Insulin (μUI mL^{-1})	8.75 (5.19)
C-peptide (ng mL^{-1})	2.42 (1.15)
HOMA	2.15 (1.64)
Dietary fat (g)	
Breakfast	11.4 (1.86)
Lunch	33.4 (5.11)
Dinner	22.5 (5.23)
Total	75.1 (10.3)

HDL, high-density lipoprotein; HOMA, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.

The mean (SD) levels of total cholesterol, LDLc, HDLc and TG were 216 (38.3), 137 (31.0), 64.8 (17.6) and 69.2 (24.7) mg dL^{-1}, respectively, with a prevalence of reduced HDLc (<50 mg dL^{-1}) of 20.7%. Fasting plasma glucose and glycated haemoglobin concentrations were 94.7 (15.0) mg dL^{-1} and 5.50% (0.37%), respectively. The mean (SD) levels of insulin and C-peptide were 8.75 (5.19) (μUI mL^{-1}) and 2.42 (1.15) ng mL^{-1}, respectively. Mean (SD) glucose tolerance, as assessed by HOMA, was 2.15 (1.64) among all participants.

All subjects ingested three meals a day and their major daily energy intake was at lunch. In the present study, lunch contributed to approximately 45% of daily fat intake compared to 15% and 30% during breakfast and dinner, respectively.

Response to oral fat load tests

Fasted participants were given 50 g of lipids orally. No changes in the mean plasma glucose concentrations were observed during the fat challenge (Fig. 1a). However, plasma TG rose in all individuals (Fig. 1b) after the lipid load, reaching a mean (SD) peak at 193 (93) mg dL^{-1}. The time needed to achieve these peaks was highly variable among individuals; 17%, 28% and 55% of the participants achieved maximum values of TG after 1, 2 or 3 h after the lipid load, respectively.

Post-prandial hypertriglyceridaemia (P-HTG) was defined as TG values >220 mg dL^{-1} during the standardised OFLT [13]. Among the studied women, we detected two subgroups of participants: approximately 40% ($n = 11$) experienced an exaggerated response, reaching peak values of TG >220 mg dL^{-1} during the test, whereas, in the other subgroup, post-challenge TG always remained <220 mg dL^{-1}.

Diurnal capillary triglycerides

All women followed a three-meal-a-day pattern, mean (SD) 3-h post-prandial DC-TG was 114 (55), 154 (73) and 139 (72) mg dL^{-1} after breakfast, lunch and dinner, respectively. All post-prandial concentrations were significantly higher than the fasting concentration ($P < 0.001$) (Fig. 2). Subsequently, participants were stratified into the aforementioned subgroups on the basis of their response to the OFLT. Those women classified as having P-HTG (represented by crosses in Fig. 2) also had elevated DC-TG concentrations after breakfast and lunch compared to those with normal response to the OFLT (filled dots). The largest difference between these groups was observed 3 h after lunch (137 mg dL^{-1} for the normal responders versus 210 mg dL^{-1} for those with an exaggerated response, $P < 0.001$) compared to 3 h post-

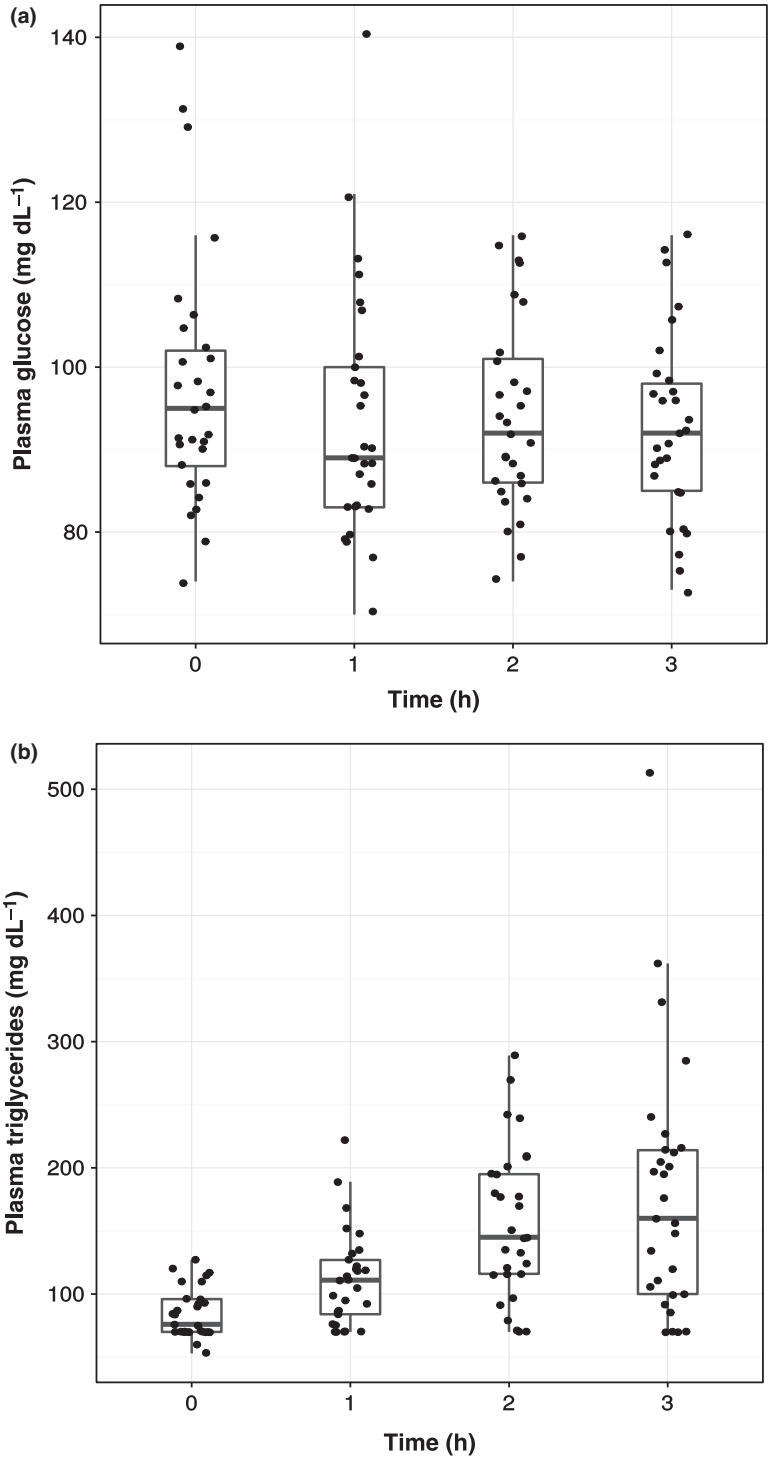

Figure 1 Post-prandial glucose (a) and triglycerides (b) responses in normotriglyceridaemic menopausal women after administration of oral fat load tests. Dots represent individuals. Boxes represent interquartile ranges (between the 25th and 75th quartiles); whiskers represent the range; and lines within boxes represent the median. Dots outside whiskers are outliers (beyond 1.5 times the interquartile range).

breakfast (103 mg dL^{-1} for the normal responders versus 149 mg dL^{-1} for those with P-HTG, $P = 0.16$) and to 3 h post-dinner (134 mg dL^{-1} for the normal responders versus 156 mg dL^{-1} for those with an exaggerated response, $P = 0.53$).

Cut-off points to determine post-prandial hypertriglyceridaemia

We used ROC analysis to examine the ability of post-prandial DC-TG concentrations to discriminate P-HTG

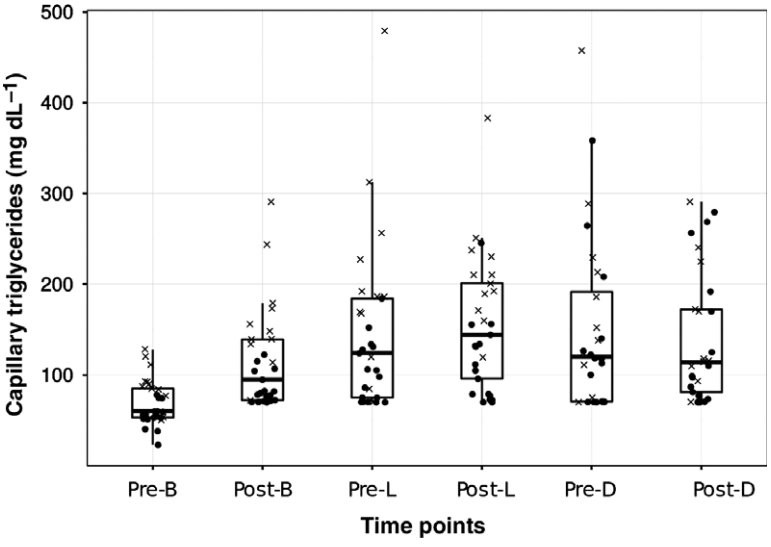

Figure 2 Diurnal capillary triacylglycerol (DC-TG) concentrations in in normotriglyceridaemic menopausal women 3 h after the different daily meals. Crosses represent women who reached values of TG greater than 220 mg dL^{-1} during the oral fat loading test (OFLT). Filled dots correspond to women whose TG always remained below 220 mg dL^{-1} during the OFLT. Boxes represent interquartile ranges (between the 25th and 75th quartiles); whiskers represent the range; and lines within boxes represent the median. Dots outside whiskers are outliers (beyond 1.5 times the interquartile range). B, breakfast; L, lunch; D, dinner.

from normal post-prandial triglyceridaemia. TG values ≥220 mg dL^{-1} at any given point of the OFLT were the diagnostic criteria for P-HTG. Figure 3 depicts ROC curves plotting the sensitivity and specificity for different cut-off points of post-prandial DC-TG concentrations. The areas under the ROC curves were 69.5%, 87.7% and 58.8% for the prediction of P-HTG based on the TG response to breakfast, lunch and dinner, respectively. The post-lunch triglyceridaemia values were more informative and so we balanced specificity and sensitivity to select the optimal cut-off point of 165 mg dL^{-1} as the threshold value to predict P-HTG. Accordingly, a single 3-h post-lunch DC-TG measure >165 mg dL^{-1} will classify a subject as having P-HTG 100% of the time when, in reality, the subject has P-HTG, although it will misclassify them as P-HTG 18.2% of the time.

Finally, we used the calculated cut-off point to split the participants into two subgroups according to the dichotomisation threshold of 165 mg dL^{-1} of post-lunch DC-TG levels. We then studied whether or not this tool was able to stratify the study group based on relevant CVD prognostic reference standards, particularly those related to lipid and glucose metabolism. We note that these subgroups are reduced and some of those parameters might be underpowered to detect significant variations. After classification, age ($P = 0.045$), BMI ($P = 0.023$) and waist circumference ($P = 0.004$) were higher than in women with a high risk for P-HTG compared to low-risk counterparts (Table 2). APOE genotypes did not contribute toward P-HTG risk because they appeared to be evenly distributed in both risk groups. P-HTG was also associated with an impaired fasting lipid profile with increased TG and a 14.1-fold increased odds of having low HDLc ($P = 0.026$). No differences were observed in HbA1c between the risk groups. An elevated fasting glucose, although not reaching statistical significance, was observed in the higher risk group. Interestingly, markers of insulin resistance appeared to be elevated in the latter group [i.e. increased fasting insulin ($P = 0.039$) and C-peptide ($P = 0.007$)], in addition to a higher HOMA ($P = 0.045$). No differences between groups were observed regarding daily intake of fat, although high-risk women ingested more fat during lunch. We next attempted to correct for the possible confounding effects of a higher fat intake during the lunch. The strength of the association of HOMA-IR with our classification tool, as well as its dependence on the daily fat intake during lunch, was assessed by logistic regression. Self-assessed 3-h post-lunch TG levels >165 mg dL^{-1} increased the odds of being in the upper tertile of the HOMA-IR by 31.6-fold ($P = 0.007$), adjusted for total fat intake in women adhering to a Mediterranean eating pattern having their highest energy intake at lunch.

Discussion

Women are affected by metabolic changes during menopause that are able to increase their CVD risk. These metabolic changes include dyslipidaemia and impaired glucose tolerance, which are both important risk factors of CVD [14]. In the present study, we investigated a cohort of healthy menopausal women and found that (i) despite having normal fasting TG levels, P-HTG was highly prevalent (approximately 40%) and associated with insulin resistance and low HDL levels and (ii) a single

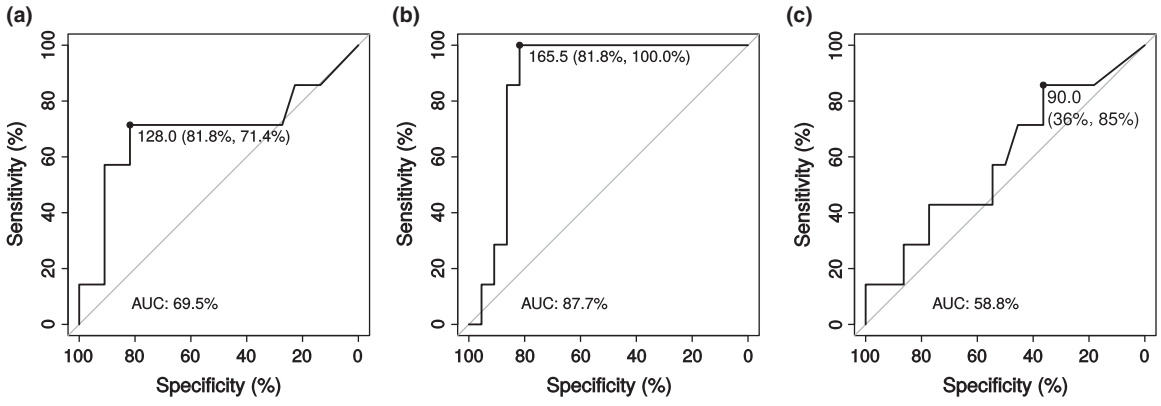

Figure 3 Receiver-operating characteristics (ROC) curves representing the discriminant performance of the post-prandial capillary triglyceride levels during breakfast (a), lunch (b) and dinner (c) for identifying post-prandial hypertriglyceridaemia in fasting normotriglyceridaemic menopausal women. The cut-off points correspond to the best trade-off between specificity and sensitivity (in parentheses). AUC, area under the curve.

Table 2 Characteristics of the study subjects at fasting after categorisation employing the post-prandial hypertriglyceridaemia (P-HTG) diagnosis tool

	Low risk (n = 18)	High risk (n = 11)	P
Age (years)	50.7 (6.62)	55.5 (5.32)	0.045
Body mass index (kg m^{-2})	25.3 (2.22)	29.3 (4.94)	0.023
Waist circumference (cm)	82.9 (6.68)	97.0 (12.6)	0.004
Apolipoprotein E genotype			1.000
E2/E3	1 (5.56%)	1 (9.09%)	
E3/E3	13 (72.2%)	7 (63.6%)	
E3/E4	4 (22.2%)	3 (27.3%)	
Total cholesterol (mg dL^{-1})	216 (40.2)	216 (37.1)	0.998
LDL-cholesterol (mg dL^{-1})	134 (33.1)	143 (27.5)	0.399
HDL-cholesterol (mg dL^{-1})	70.9 (18.1)	55.0 (11.8)	0.008
Low HDL	1 (5.56%)	5 (45.5%)	0.018
Triglycerides (mg dL^{-1})	57.6 (21.2)	88.2 (17.5)	<0.001
Glucose (mg dL^{-1})	90.5 (6.14)	101 (22.0)	0.136
HbA1c (%)	5.47 (0.32)	5.55 (0.45)	0.600
Insulin (μUI mL^{-1})	6.97 (3.56)	11.7 (6.23)	0.039
C-peptide (ng mL^{-1})	1.95 (0.87)	3.19 (1.16)	0.007
HOMA	1.58 (0.90)	3.09 (2.14)	0.045
Dietary fat (g)			
Daily intake	73.0 (6.65)	78.5 (14.2)	0.254
Lunch	31.3 (3.18)	36.8 (5.81)	0.012

Women were classified as low or high risk of suffering P-HTG if they were below or above, respectively, 165 mg/dl for their 3-h post-lunch capillary triglyceride (DC-TG) concentrations.
HDL, high-density lipoprotein; HOMA, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.

self-assessed post-lunch TG levels was able to detect insulin resistance and hypo-HDL cholesterolaemia in women adhering to a Mediterranean eating pattern in which the greatest proportion of their daily energy intake was at lunch time.

Three large scale prospective epidemiological studies found evidence that post-prandial TG levels were better predictors of CVD than fasting levels. The Copenhagen City Heart Study showed that elevated nonfasting TG levels were associated with an increased risk of myocardial infarction and early death, especially in women [1,2]. Healthy American women were followed-up for 11 years and it was found that both fasting and nonfasting triglycerides were associated with future CVD. However, adjustments of fasting TG for HDLc and insulin resistance weakened this association [3]. The Norwegian Counties Study again showed an association between nonfasting TG and risk of cardiovascular mortality [4]. Interestingly, these

three studies agreed that nonfasting TG predicted future cardiovascular events better in women than in men.

Different associations and societies have proposed a number of cut-off points for P-HTG [13,15,16]. In the present study, we took a different approach and employed OFLT as a 'gold standard' to detect P-HTG. A recent meta-analysis of studies evaluating nonfasting TG levels in healthy subjects found that the most representative measurement of post-prandial metabolism was the 4-h time-point after a meal containing 70–79 g of fat [17]. Based on this methodology, an expert consensus concluded that a desirable post-prandial TG response should be considered when TG <220 mg dL^{-1} after the OFLT [13]. In the present study, we chose a standardised meal containing 50 g of fat as a trade-off between the minimal amount of fat capable of producing a significant TG response [18,19] and our intention to maintain the fat load close to a physiological intake. We also reduced the OFLT to 3 h in line with the observation that Southern Europeans had an early post-prandial TG peak and an increased clearance [20,21]. Analogous time-to-peak values were obtained in a similar cohort of patients with coronary artery disease [22].

Once we were able to define the incidence of P-HTG in our cohort, we aimed to translate this information for the development of a simple tool feasible to be used under daily life conditions. Values from pre- and post-meal self-assessments of diurnal DC-TG strongly correlated with post-prandial TG, as assessed by OFLT [7]. Yet, the DC-TG method still requires six measures a day, making it rather unpractical in nonclinical settings. We suggested that perhaps one of the main meals might be more representative of the post-prandial lipid metabolism. Recent studies, such as SENECA and EPIC, demonstrated different distributions of energy intake within the main meals [11,23]. Citizens of Mediterranean countries consume a greater proportion of their daily energy intake at lunch time compared to central and northern countries [11,23]. In the present study, we noted that TG levels increased throughout the day, reaching their highest levels post-lunch and then plateaued. We hence hypothesised that post-lunch TG will best reflect post-prandial metabolism in a Mediterranean context. The superior predictive value of lunch over breakfast and dinner was confirmed in our ROC analysis. We then determined that the optimal cut-off point for the diagnosis of P-HTG was 165 mg dL^{-1} when TG was measured 3 h post-lunch. To our knowledge, the present study is the first report to provide a threshold for the diagnosis of P-HTG in normotriglyceridaemic menopausal women adhering to a Mediterranean-style eating pattern. A similar cut-off point (175 mg dL^{-1}) has recently been provided for the detection of hypertriglyceridaemia in nonfasting women (approximately 50% menopausal) from the Women's Health Study [24].

Evidence from human studies supports the hypothesis that fasting hypertriglyceridaemia and low HDL-C may be causal factors of insulin resistance [25]. Because hypo-HDL cholesterolaemia is more prevalent in patients with high fasting TG, it might be assumed that having normal fasting TG levels and low HDLc precludes TG as cause of this reduction in HDLc. Rather, in our analysis, we clearly show an effect of the post-prandial TG levels on hypo-HDL cholesterolaemia. Notably, these observations might have gone unnoticed had we not measured post-prandial TG. The main limitation of the present study is the low number of subjects studied, warranting further investigations conducted in larger cohorts. Furthermore, although noncompliance (nibbling) with the study protocol may exist in some subjects, this cannot be generalised because it is not supported by the pre-lunch glucose measurements made on these subjects: 89 (15) mg dL^{-1} [mean (SD)] and 25 out of 29 women had pre-lunch glucose values <100 mg dL^{-1}. Whether or not this tool will be useful beyond menopausal women also remains to be tested. As strengths, our results provide potentially important clinical information concerning the link between P-HTG, hypo-HDL cholesterolaemia and insulin resistance in apparently healthy menopausal women in a Southern European Mediterranean context. Because an altered lipid metabolism may partially underlie these metabolic disturbances, which are preventable and even reversible by early detection, characterising an individual's post-prandial response may help menopausal women to reduce their CVD risk.

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.

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Conflict of interests, source of funding, authorship

The authors declare that they have no conflicts of interest.

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AS-P, AR-V, MAN, JP-F and JMA-M designed the study and collected the data. AS-P and JMA-M analysed the data. AS-P and JMA-M wrote the manuscript. All authors approved the final manuscript and take public responsibility for the content of the article.

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GASTROINTESTINAL DISORDERS

What are the dietary treatment research priorities for inflammatory bowel disease? A short report based on a priority setting partnership with the James Lind Alliance

M. C. Lomer,[1,2] A. L. Hart,[3] A. Verjee,[4] A. Daly,[5] J. Solomon[6] & J. Mclaughlin[7]

[1]Department of Nutrition and Dietetics, Guy's and St Thomas' NHS Foundation Trust, London, UK
[2]Diabetes & Nutritional Sciences Division, Kings College London, London, UK
[3]IBD Unit, St Mark's Hospital, Harrow, Middlesex, UK
[4]Bowel Disease Research Foundation, Royal College of Surgeons of England, London, UK
[5]Birmingham Women's Hospital, Birmingham Women's NHS Foundation Trust, Birmingham, UK
[6]British Society of Gastroenterology, London, UK
[7]Division of Endocrinology, Diabetes & Gastroenterology, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester and Manchester Academic Health Sciences Centre, Salford, UK

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Correspondence
M. C. Lomer, 4.104 Franklin-Wilkins Building, Diabetes and Nutritional Sciences Division, King's College London, 150 Stamford Street, London SE1 9NH, UK.
Tel.: +44 20 7848 4350
E-mail: miranda.lomer@kcl.ac.uk

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Abstract

Background: Treatment of inflammatory bowel disease (IBD) involves a multidisciplinary approach comprising medical management and sometimes surgery. Although diet is central to IBD management, the optimal diet for patients with IBD is uncertain. A UK collaborative partnership within the James Lind Alliance was set up between patients, clinicians and other stakeholders to develop research priorities in IBD. The aim of this short report is to provide a comprehensive summary of the research priority findings relating to diet in the treatment of IBD.
Methods: The James Lind Alliance Priority Setting Partnership process was used to develop research priorities in IBD. In brief, patients, clinicians and other stakeholders were invited to provide up to five treatment uncertainties in IBD. These uncertainties were collated, revised and ranked, leading to a final top 10 research questions in IBD.
Results: A total of 1671 uncertainties from 531 participants were collected and refined to exclude duplicates leaving 1253 uncertainties. Of these, 348 were categorised as diet-related and grouped according to topic. There were 206 uncertainties related to how diet can be used to treat IBD or alleviate symptoms. Seventy-two percent of diet-related questions came from patients. One broadly diet-related and two diet-specific treatment uncertainties were included in the top 10 research priorities for IBD.
Conclusions: Dietary treatment options in the management of IBD are important research priorities. Almost three-quarters of diet related questions came from patients, who were particularly interested in how diet can impact disease activity and symptom control.

Introduction

Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) caused by inflammation of the gastrointestinal tract with an increasing prevalence in the Western world. The aetiology is unknown but environmental factors (including diet and the gut microbiota), a genetic predisposition and immune dysfunction all have a crucial role in the relapsing and remitting nature of IBD. Symptoms include abdominal pain,

diarrhoea, fatigue, malabsorption and weight loss, all of which are debilitating and have a huge negative effect on quality of life. Treatment of IBD involves a multidisciplinary approach comprising medical management and sometimes surgery. Diet is central to IBD management [1,2]; however, there are many uncertainties for optimal dietary provision for different types and stages of IBD. A UK collaborative partnership within the James Lind Alliance was set up between patients, clinicians and other stakeholders to develop research priorities in IBD [3]. The James Lind Alliance priority setting partnership provides a framework based on a transparent, democratic and reproducible process for developing research priorities and this project was the first to be carried out in gastroenterology. The purpose of the overall project was to identify unanswered questions about IBD treatment from the patient and clinical perspectives and to prioritise those that patients and clinicians agreed were the most important. The aim of this short report is to provide a summary of the research priority findings relating to diet in the treatment of IBD to demonstrate what details are important to the IBD community.

Materials and methods

A detailed methodology for the development of research priorities following the James Lind Alliance process has been described elsewhere [3,4]. Ethical approval was not required.

In brief, a steering committee was set up in November 2013 comprising two patients with IBD; two gastroenterologists; two IBD specialist nurses; two colorectal surgeons; two dietitians; a representative from the UK inflammatory bowel disease charity organisation, Crohn's and Colitis UK; a representative of the James Lind Alliance; and an administrator.

A questionnaire survey was developed by the steering committee and invited participants to provide up to five treatment uncertainties (defined as 'known unknowns') anonymously. It was piloted amongst IBD patients and IBD specialist nurses for ease of use. The survey was available in electronic or paper format and was advertised widely in IBD patient and healthcare professional networks between March and May 2014. Target participants were IBD patients, carers, IBD specialist nurses, gastroenterologists, surgeons and dietitians. Completion of the survey implied consent to take part. Any uncertainties not represented from the survey results but registered on the UK Database of Uncertainties about the Effects of Treatments; Research Recommendations from Cochrane Systematic Reviews; and the National Institute for Health and Care Excellence were added.

The initial list of uncertainties was revised noting original participant group representation (i.e. patient/carer or healthcare professional). Similar treatment uncertainties were combined to create 'indicative uncertainties'. Non-questions (e.g. statements or comments); uncertainties unrelated to IBD or IBD treatment (e.g. aetiology that was outside scope); indicative uncertainties mentioned by only one respondent; and uncertainties that could already be resolved by published systematic reviews were removed. Remaining uncertainties were reviewed and refined into a standard format by members of the steering committee using the Health Research Classification Scheme and, where there were differences in opinion regarding the meaning of the uncertainty, agreement was sought by consensus.

After the list of 70 indicative uncertainties determined by the process above, steering group members and the wider partnership participated in a survey to vote for their top five research questions and rank them in order of priority for research. The wider partnership comprised British Society of Gastroenterology members, patients, carers and members of other societies: Royal College of Nursing, Core Charity, Crohn's and Colitis UK, Royal College of General Practitioners, Association of Coloproctology of Great Britain and Ireland, British Dietetic Association. A priority list of 25 questions was established by the steering group based on frequency of votes and rank order.

The top 10 research questions for IBD were developed during a final workshop of steering group members and partner organisations to ensure a balance of patients and clinicians. Members of the National Institute for Health Research (NIHR) were also included for discussion purposes but only patients and clinicians had voting rights. Participants were divided into four groups made up of a mixture of patients, clinicians and researchers. Each group discussed several treatment uncertainties and ranked then in order of priority and were led by an independent facilitator to ensure that all participants had an opportunity to express their opinion. The whole group ranked the overall set of uncertainties through active debate until consensus was reached regarding the uncertainties to be removed and the order of the final 10 research priorities. Finally, several focused research questions, based on 'PICO' (Participants, Intervention, Comparator, Outcome) were developed.

Results

A total of 1671 uncertainties from 531 participants and known uncertainties were collected in the initial survey. Following validation and refinement, 418 uncertainties were removed. Of the remaining 1253, 348 were categorised as diet-related and grouped according to topic (Table 1). Seventy-two percent of diet-related questions came from patients, followed by 16% from healthcare professionals in secondary care, then 6% from carers and 3% from healthcare professionals in primary care, and

Table 1 Diet-related treatment uncertainties

Topic and type of question	*n*
Diet	206
What is the role of diet in IBD treatment?	55
What is the role of diet in IBD to prevent relapse?	46
Does diet help manage symptoms in IBD?	45
What is the role of diet in Crohn's disease treatment?	20
What is the role of sugar/refined carbohydrate in IBD onset and/or relapse?	13
What is the role of diet in UC treatment?	12
What is the role of dietary fibre in IBD onset and/or relapse?	6
What is the role of dairy in IBD onset and/or relapse?	5
What is the role of gluten in IBD onset and/or relapse?	4
Probiotics and microbiota	36
What is the role of probiotics or prebiotics in IBD to induce remission and prevent relapse?	24
What is the role of probiotics in ulcerative colitis to induce remission and prevent relapse?	8
Does the gut microbiota have a role in managing IBD	3
Can probiotics be used to reset the gut microbiota in ulcerative colitis?	1
Enteral nutrition	27
What is the optimal formula of enteral nutrition to induce remission in Crohn's disease (polymeric, elemental, fat content, immunomodulatory components; e.g. transforming growth factor β)?	18
What is the optimal duration of enteral nutrition to induce remission and prevent relapse of Crohn's disease?	3
Why is enteral nutrition not offered to all Crohn's disease patients with a relapse?	2
What is the optimal reintroduction diet after enteral nutrition?	2
Can enteral nutrition benefit UC or indeterminate colitis?	1
Should enteral nutrition be used in pre-surgical Crohn's disease affecting the large bowel to optimise outcome?	1
Vitamins, minerals and supplements	26
Can vitamin, mineral, omega-3 and other supplements be helpful to prevent flare ups?	17
Do vitamin D supplements help joint pain or prevent flare ups?	9
Specific foods or food components	25
Are bodybuilding/protein supplements useful?	10
Does drinking more/less water affect IBD?	5
Does alcohol affect IBD?	3
Does bottled or tap water affect IBD?	3
Do acidic foods affect IBD?	2
Does cooking method affect IBD?	1
Does turmeric or ginger affect IBD?	1
Specific diets	14
Low FODMAP	4
Paleo	4
Specific carbohydrate diet	3
Low fat fibre limited exclusion diet (LOFFLEX)	2
Ketogenic	1
Complimentary therapy	9
Does aloe vera or other alternative therapies prevent flare ups or treat active IBD?	8
Does acupuncture help IBD?	1
Monitoring	5
Can better monitoring and avoidance of vitamin and mineral deficiency improve symptoms?	2
How useful is keeping a food diary to monitor diet related symptoms?	2
How does weight (e.g. obesity) affect IBD risk and outcomes?	1

FODMAP, fermentable, oligo-, di-, mono-saccharides and polyols; IBD, inflammatory bowel disease; UC, ulcerative colitis

the remaining 3% of responders did not indicate. There were 206 uncertainties related to how diet can be used to treat IBD or alleviate symptoms.

When the diet-related uncertainties were collated and revised, seven diet-related indicative uncertainties were created (Table 2). These indicative uncertainties were ranked as part of 70 indicative uncertainties which formed the second survey and are reported elsewhere [3]. All seven diet-related indicative uncertainties from Table 2 were within the 25 highest ranked questions. These top 25 questions were subsequently voted on at the final meeting for inclusion in the final top 10 research priorities. Two diet-specific (positions 3 and 7) and one broadly diet-related (position 10) treatment uncertainties for IBD were included in the final top 10 research priorities (Table 3).

Discussion

The findings of the present study confirm that dietary treatment options in the management of IBD are important research priorities for both clinicians and patients. The role of diet in IBD was strongly supported by patients, with almost three-quarters of diet-related questions coming from patients. The majority of topics related directly to how diet can impact disease activity and symptom control. This agrees with three previous research studies that have assessed the importance of diet from the patients' perspective [5–7]. These studies showed that 51–82% of patients reported problems in relation to food and nutrition. Two other recent research priority setting initiatives did not prioritise diet in the top 10, possibly as a result of a lack of patient representation. [8,9] The first study included 14 patients from the USA and 258 gastroenterologists from around the world. [8] They asked for up to three important comparative effectiveness research topics. Seven of the top 10 were directly related

Table 2 Diet-related indicative uncertainties from 70 grouped uncertainties before final distillation

Topic	Uncertainty
Bacteria and IBD	Are probiotics useful in the management of active or inactive? IBD to achieve symptom control and normal daily activities?
Diet and food	What role does diet have in the management of active or inactive Ulcerative colitis or Crohn's disease to achieve normal daily activities and symptom control? Is dietary therapy as effective as conventional treatment for maintaining remission in IBD and what role does dietary modification have on symptom control? What is the optimal dietary therapy (liquid enteral diet and/or reintroduction diet) and duration to achieve mucosal healing in active IBD and/or maintain remission either as a primary or adjunctive treatment? Is there a difference between adults and children?
Other therapies	Do complementary therapies (e.g. acupuncture, hypnotherapy or aloe vera) have a role in the management of IBD to achieve symptom control and normal daily activities (active/remission/maintenance)?
Self-management	Do expert patients who look after themselves well including diet and exercise have a better outcome than those who simply take their medication as prescribed?
Supplements	Are vitamin or mineral supplements effective, for example vitamin D, in inducing or maintaining remission in IBD?

Taken from main research findings paper [1].
IBD, inflammatory bowel disease.

Table 3 Top 10 research priorities for inflammatory bowel disease

1	What is the optimal treatment strategy considering efficacy, safety and cost-effectiveness (immunomodulators, biologics, surgery, combinations) in IBD management: selecting the right patient group, right stage of disease, and assessing potential for withdrawal?
2	What are the optimal markers/combinations of markers (clinical, endoscopic, imaging, genetics, other biomarkers) for stratification of patients with regards to (a) disease course, (b) monitoring disease activity and (c) treatment response?
3	What role does diet have in the management of mildly active or inactive ulcerative colitis or Crohn's disease to achieve normal daily activities and symptom control?
4	How can pain be most effectively managed in people with IBD?
5	What is an optimal treatment strategy for perianal Crohn's disease and what individual factors determine this?
6	What is the best treatment for controlling diarrhoea and/or incontinence symptoms in people with IBD, including novel pharmacological and nonpharmacological options? Is high-dose loperamide safe and effective in the treatment of diarrhoea in IBD?
7	What is the optimal dietary therapy (liquid enteral diet and/or reintroduction diet) and duration to achieve mucosal healing in active IBD and/or remission either as a primary or adjunctive treatment? Is there a difference between adults and children?
8	What is the association between IBD and fatigue and how should it be managed?
9	Does early surgery or later surgery for terminal ileal Crohn's disease result in better outcomes (quality of life, cost-effectiveness)?
10	Does influencing the gut microbiota influence the course of IBD?

Taken from main research findings paper [1].
IBD, inflammatory bowel disease.

to pharmaceutical treatments; the investigators report that comparing the effectiveness of competing dietary interventions was only in the top 15 proposed by patients who comprised only 5% of all respondents. The second study invited non-medical professionals, predominantly nurses from European Crohn's and Colitis Organisation (ECCO), to suggest research questions from five themes. [9] Their study's final top 10 did not include any diet-related questions and the investigators indicated the under-prioritising of diet was likely to be related to the low number of dietitians participating in the survey; however, diet topics did reach their final 44 questions.

The relationship between diet, the gut microbiota and interactions with the immune system are of paramount importance [10] and this research demonstrates that these are important to the IBD community by the wide range of diet-related topics. Furthermore, the current evidence [1,11] and guidelines [2,12,13] for the dietary management of IBD do not cover these treatment uncertainties; therefore, publication of this comprehensive list of diet-related topics is justified. Furthermore, addressing these research priorities will provide support for developing more IBD specific dietetic posts that are urgently needed to meet the needs of patients. [1,6,7,14]

The robust methodology demonstrated by The James Lind Alliance Priority Setting Partnership shows that engaging with a diverse mix of stakeholders from various roles, ages and ethnic groups, including patients and all members involved in multidisciplinary teams in IBD, is philosophically correct and gives the resultant priorities legitimacy. Thus, it is imperative to report the complete set of diet and nutrition research priorities to ensure that research funding bodies are aware of this research need for future commissioned calls.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the research 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 James Lind Alliance provides a framework with a transparent, democratic and reproducible process, and a panel of advisers who guide the process. It is recognised as the gold standard in setting research priorities.

Acknowledgments

The stakeholders included the British Society of Gastroenterology (BSG), Association of Coloproctology of Great Britain (ACPGBI), Royal College of Nursing (RCN), British Dietetic Association (BDA), British Society of Paediatric Gastroenterology Hepatology and Nutrition (BSPGHAN), the UK inflammatory bowel disease charity organisation called Crohn's and Colitis UK (CCUK), the Crohn's in Childhood Research Association (CICRA), Primary Care Society for Gastroenterology, the charity committed to fighting all diseases that affect the gastrointestinal tract (CORE), the National Institute for Health Research Gastroenterology Speciality Group (NIHR GI SG), the National Institute for Health Research Children Speciality Group (NIHR CSG) and the James Lind Alliance (JLA). Details of the members of the steering committee are listed at the end of the paper.

Conflicts of interest, source of funding and authorship

The James Lind Alliance and the steering group assessed organisations for any potential conflict of interest that might have led to unacceptable bias if they were to participate, in keeping with the ethos of the James Lind Alliance.

The BSG and CCUK provided core funding for the project in equal shares, with other members of the committee contributing time and expertise or facilities at no cost.

All authors were involved in the James Lind Alliance Partnership. MCL wrote the manuscript and all authors were involved in the revision and final wording of the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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GASTROINTESTINAL DISORDERS

Dietary glycaemic index and glycaemic load and upper gastrointestinal disorders: results from the SEPAHAN study

A.H. Keshteli,[1,2] F. Haghighatdoost,[3,4] L. Azadbakht,[3,4,5,6] H. Daghaghzadeh,[2] C. Feinle-Bisset,[7] H. Afshar,[8] A. Feizi,[8,9] A. Esmaillzadeh[3,4,5] & P. Adibi[2]

[1]Department of Medicine, University of Alberta, Edmonton, Canada
[2]Integrative Functional Gastroenterology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran
[5]Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
[6]Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[7]Discipline of Medicine, NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, University of Adelaide, Adelaide, SA, Australia
[8]Psychosomatic Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[9]Biostatistics and Epidemiology Department, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran

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Correspondence
L. Azadbakht, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran.
Tel: +98 313 7922776
Fax: +98 313 6681378
E-mail: azadbakht@hlth.mui.ac.ir

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Abstract

Background: Little is known about the effects of carbohydrate, particularly any association between dietary glycaemic index or glycaemic load and uninvestigated heartburn or uninvestigated chronic dyspepsia in the community. The present study aimed to determine associations between dietary glycaemic index or glycaemic load and uninvestigated heartburn or uninvestigated chronic dyspepsia.
Methods: This cross-sectional study was conducted in 2987 adults. Dietary glycaemic index and glycaemic load were estimated using a validated food-frequency questionnaire. Uninvestigated heartburn and uninvestigated chronic dyspepsia were determined using a modified and validated version of the Rome III questionnaire.
Results: After controlling for various confounders, high glycaemic load was associated with an increased risk of uninvestigated heartburn [odds ration (OR) = 1.75; 95% confidence interval CI = 1.03, 2.97; $P = 0.04$] and uninvestigated chronic dyspepsia (OR = 2.14; 95% CI: 1.04, 4.37; $P = 0.04$) in men but not in women. In normal-weight individuals, high glycaemic index was related to an increased risk of uninvestigated heartburn (OR = 1.52; 95% CI: 1.07, 2.15; $P = 0.02$) and high glycaemic load to an increased risk of uninvestigated chronic dyspepsia (OR=1.78; 95% CI: 1.05, 3.01; $P = 0.03$). No significant associations were observed in subjects with excess body weight.
Conclusions: Our data suggest that there are body mass index- and sex-specific associations between dietary carbohydrate quality with uninvestigated heartburn and uninvestigated chronic dyspepsia.

Introduction

Although upper gastrointestinal symptoms are highly prevalent in Western countries, the prevalence of gastrointestinal symptoms in middle-Eastern countries appears to be low [1,2], with inconsistent findings being reported in Iran [3,4], ranging from approximately 2–52% for gastroesophageal reflux disease (GERD) [3] and

approximately 3–30% for functional dyspepsia (FD) [4]. Gastrointestinal symptoms may be induced by pathophysiological, psychological, socio-demographic and genetic factors [5].

The potential role of lifestyle factors, particularly dietary intakes, has largely been ignored until recently [6]. Because gastrointestinal symptoms frequently occur after meal ingestion [7], gastrointestinal dysmotility [8] or visceral hypersensitivity to nutrient and mechanical stimuli [9] have been implicated. Increasing evidence suggests that high-fat foods or meals induce gastrointestinal symptoms [6,10,11]. Information regarding dietary fibre is controversial [12–16]. Some studies have reported beneficial effects of fibre on gastrointestinal symptoms [12–14], whereas others have not [15,16]. Carbohydrate type might be important in relation to gastrointestinal symptoms. A diet low in 'FODMAPs' (Fermentable, Oligosaccharides, Disaccharides and Monosaccharides and Polyols) has been suggested as an effective approach to the management of symptoms in irritable bowel syndrome [17]. A diet high in monosaccharides and short-chain carbohydrates, on the one hand, or fibre, on the other, may also influence factors related to carbohydrate quality, including dietary glycaemic index (GI) and glycaemic load (GL) [18]. Thus, dietary glycaemic index and glycaemic load may affect gastrointestinal symptoms, including via an effect on gastrointestinal functions. For example, high GI and GL diets increase cortisol [19] and insulin [20] secretion, which may compromise normal gastric motility [21,22]. Moreover, a high intake of simple carbohydrates may stimulate the growth of harmful microbiota [23]. Such potential associations are highly relevant in middle-Eastern countries as a result of the substantial contribution of carbohydrates to total energy intake. Therefore, the present study aimed to examine the association between dietary GI and GL with upper gastrointestinal disorders, specifically uninvestigated heart burn (UH) and uninvestigated chronic dyspepsia (UCD), in an Iranian cohort.

Materials and methods

Participants

The data analysed in the present study originated from the Study of the Epidemiology of Psychological, Alimentary Health and Nutrition (SEPAHAN). This study was conducted to evaluate relationships between gastrointestinal disorders and lifestyle factors, including dietary intake and psychological disorders. Details about SEPAHAN have been reported previously [24]. Briefly, SEPAHAN was conducted among non-academic members of staff of Isfahan University of Medical Sciences (IUMS), Isfahan, Iran, who were working in hospitals, university campus and health centres affiliated with IUMS, in two phases, aiming to enhance the accuracy of data collection. Accordingly, we prevented tiring participants and they also answered questions with more accuracy in two sessions. In the first phase, we distributed questionnaires to collect information regarding demographic characteristics and dietary intakes. In the second phase, questionnaires were concerned with common gastrointestinal symptoms and psychological profiles. All of the questionnaires were self-administered, and participants were first instructed on how to complete the questionnaires. Participants who had provided complete information ($n = 2987$) were included in the analysis. The study protocol was approved by the Regional Bioethics Committee of IUMS. Written informed consent was provided by each subject before participation.

Gastrointestinal assessment

Functional gastrointestinal symptoms were determined using a reliable and valid version of the modified Rome III questionnaire [24,25], which diagnoses functional gastrointestinal disorders and consists of six major domains, with functional oesophageal disorders and functional gastrointestinal disorders being two domains in the questionnaire for adults. Each domain contains several questions to aid the diagnosis of these disorders based on Rome III criteria. As such, we defined UH as the presence of heartburn at least sometimes, often or always during the last 3 months, and UCD as the presence of bothersome postprandial fullness, early satiation, and/or epigastric pain or epigastric burning, often or always. Participants who often or always reported uncomfortable fullness after normal-sized meals were considered to have 'bothersome postprandial fullness'; if they were often or always unable to finish a normal-sized meal, they were considered to have 'early satiation'; and if they often or always felt pain or burning in the upper abdomen, they were considered to have 'gastric pain'.

Dietary intake assessment

Dietary intakes were assessed using a 106-item dish-based, semi-quantitative food-frequency questionnaire, designed based on the Willett-format food-frequency questionnaire, as described in detail previously [26]. Briefly, the dish-based, semi-quantitative food-frequency questionnaire contained five main components, including mixed dishes, all grain-based foods and potato, dairy products, fruits and vegetables, and miscellaneous food items (e.g. sweets, fast foods, nuts, desserts) and beverages. Common portion sizes were used to determine the amount of food consumed. Food items were converted to grams per day

using household measures [27]. Daily nutrient intakes of participants were estimated according to the United States Department of Agriculture food composition database.

Glycaemic index and glycaemic load estimation

To calculate dietary GI and GL, we extracted dietary GI values of the food items from Iranian and international glycaemic index tables, as available [28,29]. Total carbohydrate and fibre content of food items were derived from United States Department of Agriculture food composition table and used to estimate available carbohydrate$_a$ as total carbohydrate$_a$ minus fibre$_a$ content. Total GI was calculated by the formula: $\sum$ (GI$_a$ × available carbohydrate$_a$)/total available carbohydrate [30]. Dietary GL was estimated as (total GI × total available carbohydrate)/100 [30]. All derived GI values were relative to glucose as the reference food. Details on extracting GI values, as well as the validity of the dish-based, semi-quantitative food-frequency questionnaire to estimate GI values, have been published recently [31].

Statistical analysis

Subjects were grouped into two categories based on the medians for GI and GL: below and above medians. To identify significant differences in general characteristics, we used independent-samples *t*-test and a chi-squared test for continuous and categorical variables, respectively. Age-and sex-adjusted means for energy intake, and age-, sex- and energy-adjusted means for other dietary variables, were estimated by analysis of covariance (ANCOVA). We calculated odds ratios of having UH or UCD using logistic regression in crude and adjusted models. Model 1 was adjusted for age, sex and energy. Additional adjustments for marital status, educational level, physical activity, smoking, liquid consumption after meals (yes, no), short interval between eating and sleeping, medication use (yes, no), regular meals (yes, no), frequency of spice consumption, eating rate (slow, fast), breakfast skipping (yes, no) and thorough mastication (yes, no) were made in model 2. Model 3 was additionally adjusted for fat, fibre, fructose, cocoa, coffee, tea and sugar-sweetened beverages. Adjustment for body mass index (BMI) was made in model 4. All analyses were also performed in models stratified by sex and BMI. Accordingly, we grouped participants into two categories: normal weight (BMI < 25 kg m^{-2}) and overweight or obese (BMI ≥ 25 kg m^{-2}). Crude and multivariable-adjusted odds ratios and 95% confidence intervals (CIs) for the frequency and severity of gastrointestinal symptoms (postprandial fullness, early satiation, epigastric pain) were calculated by ordinal logistic regression. Proportional odds assumption as a perquisite condition for conducting ordinal logistic regression was examined using a chi-squared test. All models were the same as described above for binary logistic regression. $P < 0.05$ (two-sided) was considered statistically significant.

Results

Median (range) participant ages and BMI were 36 (19–70) years and 24.7 (16.0–42.8) kg m^{-2}, respectively. Mean (SD) GI and GL were 62 (5) and 179 (93), respectively. The medians of GI and GL were 62 and 158, respectively. General characteristics of participants by median of GI and GL are shown in Table 1. Individuals in category 1 (lower than median) for GI were significantly older [36.7 (0.2) versus 35.9 (0.2) years; $P = 0.01$] and more physically active than those in category 2 (higher than median; 49.6% versus 42.8%; $P < 0.0001$). Participants in category 2 for GL were less likely to be smokers than those in category 1 (12.1% versus 15.5%; $P = 0.007$).

Table 1 General characteristics of participants in two levels (less and higher than median) of dietary glycaemic index and glycaemic load*

	Glycaemic index			Glycaemic load		
Variables	Low	High	*P* value†	Low	High	*P* value†
Age (years)	36.7 (0.2)	35.9 (0.2)	0.01	36.3 (0.2)	36.3 (0.2)	NS
BMI (kg m^{-2})	25.2 (0.1)	24.8 (0.1)	NS	25.3 (0.1)	24.8 (0.1)	0.05
WC (cm)	83.3 (0.5)	84.2 (0.5)	0.06	83.8 (0.5)	83.6 (0.5)	0.06
Male (%)	40.6	42.9	NS	39.2	44.3	0.003
Physical activity [active or moderate active (%)]	49.6	42.8	<0.0001	45.8	46.6	NS
Medication use (%)	5.5	4.2	0.08	5.9	3.8	0.004
Academic education (%)	58.1	64.0	<0.0001	57.6	64.4	<0.0001
Current smokers (%)	14.0	13.6	NS	15.5	12.1	0.007

*Values are the mean (SE), unless indicated otherwise.
†Independent-samples *t*-test for continuous and chi-squared test for categorical variables.
BMI, body mass index; NS, not significant; WC, waist circumference.

Participants in category 1 for GI consumed greater amounts of fructose and fibre than those in category 2. Conversely, participants in category 1 for GL had lower fructose and fibre intakes. Macronutrient consumption differed significantly between the two categories of GI and GL. Carbohydrate intake was lower, whereas fat and protein intakes were higher, in individuals in categories 1 for GI and GL. Individuals in category 1 for GI were less likely to drink liquids after a meal, consume fried foods and chew food little. Subjects in category 2 for GL consumed more liquids after meals, fried and spicy foods, although they were less likely to skip breakfast, eat quickly and have irregular meals (Table 2).

Crude and multivariable-adjusted odds ratios (OR) for associations of GI or GL with UH or UCD in the entire study population, and each sex, are illustrated in Table 3. In the entire study population, higher GI was associated with an increased risk for UH in the crude model (OR = 1.2, 95% CI = 1.0–1.4; $P = 0.05$). However, controlling for various confounders eliminated this association (OR = 1.14, 95% CI = 0.90–1.45; $P = 0.3$). An increased risk for UCD in category 2 of GL was significant after adjustment for potential confounding variables (OR = 1.56, 95% CI = 1.04–2.33; $P = 0.03$). Stratified analysis by sex indicated direct and independent associations between GL and UH (OR = 1.75, 95% CI = 1.03, 2.97; $P = 0.04$) and UCD (OR = 2.14, 95% CI = 1.04–4.37; $P = 0.04$) in men but not women.

Stratified analysis by BMI revealed no association between GI or GL with UH or UCD in subjects with excess body weight. However, in individuals with normal weight, a higher GI was associated with a greater odds of UH (OR = 1.52, 95% CI = 1.07–2.15; $P = 0.02$), whereas a higher GL was associated with a greater risk of UCD (OR = 1.78, 95% CI = 1.05–3.01; $P = 0.03$) (Table 4).

Multivariable-adjusted ORs for the frequency and severity of various gastrointestinal symptoms are shown in Table 5. After statistically controlling for potential confounders, neither the frequency or severity of any of the gastrointestinal symptoms was related to GI or GL. There was a marginally significant trend for an association between GL and the severity of epigastric pain (OR = 0.82, 95% CI = 0.67–1.01; $P = 0.06$).

Discussion

As a result of the substantial contribution of carbohydrates to total energy intake in middle-Eastern countries [32], carbohydrate quality might be relevant in relation to UH or UCD. The major findings of the present study were that: (i) GL was directly linked to UCD in the whole population; (ii) GL was related to UH and UCD in men;

Table 2 Dietary intakes and other relevant factors in two levels (less and higher than median) of dietary glycaemic index and load*

	Glycaemic index			Glycaemic load		
Variables	Low (≤62.395) (n = 1497)	High (>62.395) (n = 1490)	P value†	Low (≤160.291) (n = 1479)	High (>160.291) (n = 1508)	P value†
Energy (kcal day^{-1})	2292.5 (21.0)	2473.1 (21.0)	<0.0001	1809.1 (15.3)	2945.0 (15.1)	<0.0001
Carbohydrate (g day^{-1})	278.8 (1.3)	309.3 (1.3)	<0.0001	263.9 (1.3)	323.6 (1.3)	<0.0001
Fat (g day^{-1})	104.8 (0.4)	92.3 (0.4)	<0.0001	110.0 (0.5)	87.3 (0.5)	<0.0001
Protein (g day^{-1})	92.1 (0.3)	84.4 (0.3)	<0.0001	93.3 (0.4)	83.3 (0.4)	<0.0001
Fibre (g day^{-1})	24.0 (0.1)	21.2 (0.1)	<0.0001	22.1 (0.2)	23.1 (0.2)	0.001
Fructose (g day^{-1})	18.3 (0.3)	15.5 (0.3)	<0.0001	14.7 (0.3)	19.1 (0.3)	<0.0001
Tea (g day^{-1})	354.8 (7.3)	392.8 (7.3)	<0.0001	363.6 (8.9)	383.7 (8.8)	0.2
Coffee	10.1 (0.7)	7.1 (0.7)	0.004	14.5 (0.9)	2.8 (0.9)	<0.0001
Liquid‡(yes %)	47.8	55.3	<0.0001	49.8	53.3	0.04
Spicy§ (%)	21.4	24.5	0.09	19.3	26.7	<0.0001
Eating rate (% quick)	26.9	27.4	0.8	30.3	24.0	<0.0001
Breakfast skipper (%)	7.7	7.0	0.4	9.0	5.8	<0.0001
Regular meals¶ (%)	59.4	61.2	0.3	56.1	64.5	<0.0001
Chewing little (%)	12.7	15.2	0.02	15.1	12.9	0.2
Milk intolerance (%)	14.6	15.9	0.3	15.9	14.6	0.3
Diabetes (%)	1.9	1.7	0.6	2.1	1.4	0.1
Colitis (%)	0.3	0.1	0.1	0.3	0.1	0.1

*Values are the mean (SE) unless indication. Dietary intakes were adjusted for age, sex and calories by a general linear model (ANCOVA).
†From ANCOVA for continuous variables and chi-squared for categorical variables.
‡Participants who consumed liquid after meal at often or always.
§Participants who consumed spicy more than seven times per week.
¶Participants who had regular meals at often or always.

Table 3 Multivariable-adjusted odds ratio (OR) and [95% confidence interval (CI) for OR] of the association of dietary glycaemic index and load levels with uninvestigated heartburn and uninvestigated chronic dyspepsia*

	Glycaemic index			Glycaemic load		
Variables	Low (n = 1497)	High (n = 1490)	P value†	Low (n = 1479)	High (n = 1508)	P value†
UH						
Crude model	1 (Ref)	1.2 (1.0–1.4)	0.05	1 (Ref)	0.9 (0.8–1.1)	0.3
Model 1	1 (Ref)	1.2 (1.02–1.4)	0.03	1 (Ref)	1.0 (0.8–1.3)	1.0
Model 2	1 (Ref)	1.18 (0.97–1.44)	0.096	1 (Ref)	1.21 (0.92–1.58)	0.2
Model 3	1 (Ref)	1.16 (0.92–1.47)	0.2	1 (Ref)	1.21 (0.88–1.66)	0.2
Model 4	1 (Ref)	1.14 (0.90–1.45)	0.3	1 (Ref)	1.21 (0.88–1.67)	0.2
UCD						
Crude model	1 (Ref)	1.0 (0.8–1.2)	0.8	1 (Ref)	0.9 (0.8–1.1)	0.6
Model 1	1 (Ref)	1.0 (0.8–1.3)	0.6	1 (Ref)	1.1 (0.8–1.5)	0.4
Model 2	1 (Ref)	1.03 (0.81–1.32)	0.8	1 (Ref)	1.26 (0.90–1.77)	0.2
Model 3	1 (Ref)	0.97 (0.72–1.29)	0.8	1 (Ref)	1.51 (1.01–2.24)	0.04
Model 4	1 (Ref)	0.92 (0.68–1.23)	0.6	1 (Ref)	1.56 (1.04–2.33)	0.03
Stratified by sex						
Men						
UH						
Crude model	1 (Ref)	1.2 (0.9–1.5)	0.2	1 (Ref)	1.0 (0.8–1.3)	0.8
Model 1	1 (Ref)	1.2 (0.9–1.6)	0.2	1 (Ref)	1.2 (0.8–1.7)	0.5
Model 2	1 (Ref)	1.21 (0.87–1.69)	0.3	1 (Ref)	1.39 (0.88–2.22)	0.2
Model 3	1 (Ref)	1.38 (0.93–2.06)	0.1	1 (Ref)	1.75 (1.04–2.96)	0.04
Model 4	1 (Ref)	1.38 (0.92–2.06)	0.1	1 (Ref)	1.75 (1.03–2.97)	0.04
UCD						
Crude model	1 (Ref)	1.0 (0.7–1.4)	1.0	1 (Ref)	1.1 (0.8–1.5)	0.6
Model 1	1 (Ref)	1.0 (0.7–1.4)	0.9	1 (Ref)	1.2 (0.7–2.1)	0.4
Model 2	1 (Ref)	0.95 (0.61–1.46)	0.8	1 (Ref)	1.47 (0.81–2.69)	0.2
Model 3	1 (Ref)	0.89 (0.53–1.50)	0.7	1 (Ref)	1.86 (0.93–3.71)	0.08
Model 4	1 (Ref)	0.73 (0.42–1.26)	0.3	1 (Ref)	2.14 (1.04–4.37)	0.04
Women						
UH						
Crude model	1 (Ref)	1.2 (1.0–1.4)	0.1	1 (Ref)	0.9 (0.7–1.1)	0.2
Model 1	1 (Ref)	1.2 (1.0–1.5)	0.07	1 (Ref)	0.9 (0.7–1.2)	0.6
Model 2	1 (Ref)	1.14 (0.89–1.47)	0.3	1 (Ref)	1.11 (0.79–1.57)	0.5
Model 3	1 (Ref)	1.04 (0.77–1.40)	0.8	1 (Ref)	0.96 (0.64–1.46)	0.9
Model 4	1 (Ref)	1.01 (0.75–1.37)	0.9	1 (Ref)	0.97 (0.64–1.48)	0.9
UCD						
Crude model	1 (Ref)	1.0 (0.8–1.3)	0.7	1 (Ref)	0.9 (0.7–1.1)	0.4
Model 1	1 (Ref)	1.1 (0.8–1.4)	0.5	1 (Ref)	1.1 (0.8–1.5)	0.7
Model 2	1 (Ref)	1.06 (0.78–1.44)	0.7	1 (Ref)	1.16 (0.76–1.75)	0.5
Model 3	1 (Ref)	0.98 (0.68–1.41)	0.9	1 (Ref)	1.35 (0.82–2.23)	0.2
Model 4	1 (Ref)	0.94 (0.65–1.37)	0.8	1 (Ref)	1.32 (0.79–2.18)	0.3

*Values are OR and 95% CI.

†Resulted from crude and multivariable binary logistic regression.

Model 1: Adjusted for age, energy intake (sex was included in the adjustments for whole population). Model 2: Further adjustment for, liquid, short interval between eating and sleeping, meal pattern, spicy, eating rate, breakfast skipping, chewing, medication, marital status, educational level, physical activity and smoking. Model 3: further control for fat, fructose, fibre, cocoa, sugar-sweetened beverage, tea, coffee. Model 4: Additional control for body mass index.

UH, uninvestigated heartburn; UCD, uninvestigated chronic dyspepsia.

(iii) higher dietary GI was related to an elevated risk of UH in normal-weight individuals; (iv) GL was associated with a trend towards lower epigastric pain severity; and (v) after controlling for potential confounders, GI and GL were not associated with UH and UCD, either in women or in individuals with excess body weight.

The association between GI or GL and GERD or FD symptoms has not been assessed previously. Previous studies have focused on the perception of meal-related fullness following low- or high-GI meals [33] or diets for 10–12 weeks [34–36] and found either no association [33,35,36] or that a low-GI diet for 10 weeks

Table 4 Multivariable-adjusted odds ratio (OR) and [95% confidence intarval (CI) for OR] of the association of dietary glycaemic index and load with uninvestigated heartburn and uninvestigated chronic dyspepsia in the medians of dietary glycaemic index and load, stratified by BMI*

	Glycaemic index			Glycaemic load		
Variables	Low (n = 1497)	High (n = 1490)	P value†	Low (n = 1479)	High (n = 1508)	P value†
BMI ≤ 24.9						
UH						
Crude model	1 (Ref)	1.4 (1.1–1.7)	0.01	1 (Ref)	0.9 (0.7–1.1)	0.4
Model 1	1 (Ref)	1.5 (1.2–1.9)	0.001	1 (Ref)	1.0 (0.7–1.3)	0.8
Model 2	1 (Ref)	1.45 (1.0.8–1.94)	0.01	1 (Ref)	1.17 (0.80–1.71)	0.4
Model 3	1 (Ref)	1.52 (1.07–2.15)	0.02	1 (Ref)	1.09 (0.69–1.72)	0.7
UCD						
Crude model	1 (Ref)	1.1 (0.8–1.4)	0.6	1 (Ref)	0.9 (0.7–1.2)	0.4
Model 1	1 (Ref)	1.2 (0.9–1.5)	0.3	1 (Ref)	1.1 (0.8–1.6)	0.5
Model 2	1 (Ref)	1.07 (0.77–1.48)	0.7	1 (Ref)	1.35 (0.87–2.09)	0.2
Model 3	1 (Ref)	1.03 (0.69–1.53)	0.9	1 (Ref)	1.78 (1.05–3.01)	0.03
BMI > 25						
UH						
Crude model	1 (Ref)	1.1 (0.8–1.3)	0.6	1 (Ref)	0.9 (0.8–1.2)	0.7
Model 1	1 (Ref)	1.0 (0.8–1.3)	0.8	1 (Ref)	1.1 (0.8–1.5)	0.7
Model 2	1 (Ref)	1.0 (0.75–1.33)	0.98	1 (Ref)	1.31 (0.88–1.96)	0.2
Model 3	1 (Ref)	0.91 (0.65–1.28)	0.6	1 (Ref)	1.37 (0.86–2.18)	0.2
UCD						
Crude model	1 (Ref)	1.0 (0.7–1.3)	0.8	1 (Ref)	1.0 (0.7–1.3)	1.0
Model 1	1 (Ref)	0.9 (0.7–1.2)	0.6	1 (Ref)	1.1 (0.7–1.7)	0.6
Model 2	1 (Ref)	0.96 (0.64–1.4)	0.8	1 (Ref)	1.25 (0.72–2.18)	0.4
Model 3	1 (Ref)	0.85 (0.53–1.36)	0.5	1 (Ref)	1.46 (0.77–2.78)	0.2

*Values are OR and 95% CI.

†Resulted from crude and multivariable binary logistic regression.

Model 1: Adjusted for age, sex, energy intake. Model 2: Further adjustment for, liquid, short interval between eating and sleeping, meal pattern, spicy, eating rate, breakfast skipping, chewing, medication, marital status, educational level, physical activity and smoking. Model 3: further control for fat, fructose, fibre, cocoa, sugar-sweetened beverage, tea, coffee.

Ref, reference; UH, uninvestigated heartburn; UCD, uninvestigated chronic dyspepsia.

increases fullness in healthy, overweight women [34]. We found no associations between gastrointestinal symptoms, except for a trend between GL and lower epigastric pain severity. The association of gastrointestinal symptoms and dietary GI or GL warrants further examination in prospective studies.

Our findings revealed a direct association between dietary GI and UH in individuals with normal body weight. In line with previous studies [37], we found a higher prevalence of heartburn among individuals with excess body weight. However, although the prevalence of UH in overweight participants was similar across GI categories, corresponding values were different in normal-weight subjects. This suggests that GI could be a relevant determinant of UH, as well as GL for UCD, in subjects with normal body weight. By contrast, in overweight subjects, this does not appear to be the case and thus other important determinants of UH and UCD may weaken the associations. Indeed, excess adipose tissue in overweight and obese subjects imposes an extrinsic gastric compression, increases intragastric pressure and decreases lower esophageal sphincter pressure [38,39]. Additionally, hormonal changes related to obesity might play an important role in the pathogenesis of GERD [40]. One study suggested that colonic fermentation of indigestible carbohydrates enhances plasma glucagon-like peptide (GLP)-1 concentrations and exacerbates GERD episodes and symptoms [16]. Likewise, our findings revealed that GL was an independent determinant of UCD and UH in men but not women, even when considering different statistical models. The reason(s) for sex-specific associations is (are) unclear, and more studies are needed to determine the associations of GI and GERD or FD in men and women.

One explanation underlying the positive link between UH and GI might be the effect of dietary GI on GLP-1, cholecystokinin (CCK) [34,41] and/or gastric emptying [42]. A higher dietary GI is associated with higher GLP-1 and CCK secretion and lower emptying index [42], as well as both CCK and GLP-1 slow gastric emptying [43]. Additionally, an excess release of GLP-1 has been linked to GERD symptoms and reflux episodes [16].

Table 5 Multivariable-adjusted odds ratio (OR) and [95% confidence interval (CI) for OR] of the association of dietary glycaemic index and load levels with frequency and severity of upper gastrointestinal symptoms in the medians of dietary glycaemic index and glycaemic load*

	Glycaemic index			Glycaemic load		
Variables	Low (n = 1497)	High (n = 1490)	P value*	Low (n = 1479)	High (n = 1508)	P value*
Frequency						
Early satiation						
Crude model	1 (Ref)	1.07 (0.9–1.2)	NS	1 (Ref)	0.8 (0.7–1.0)	0.04
Model 1†	1 (Ref)	1.1 (0.9–1.3)	NS	1 (Ref)	0.9 (0.7–1.1)	NS
Model 2†	1 (Ref)	1.09 (0.9–1.32)	NS	1 (Ref)	0.96 (0.74–1.25)	NS
Model 3†	1 (Ref)	1.15 (0.91–1.44)	NS	1 (Ref)	1.07 (0.80–1.45)	NS
Model 4†	1 (Ref)	1.09 (0.87–1.38)	NS	1 (Ref)	1.03 (0.76–1.40)	NS
Epigastric pain						
Crude model	1 (Ref)	1.2 (1.01–1.3)	0.03	1 (Ref)	0.9 (0.8–1.0)	NS
Model 1	1 (Ref)	1.2 (1.1–1.4)	0.006	1 (Ref)	1.05 (0.9–1.3)	NS
Model 2	1 (Ref)	1.11 (0.94–1.32)	NS	1 (Ref)	1.04 (0.82–1.33)	NS
Model 3	1 (Ref)	1.11 (0.91–1.37)	NS	1 (Ref)	1.08 (0.83–1.42)	NS
Model 4	1 (Ref)	1.09 (0.89–1.34)	NS	1 (Ref)	1.08 (0.82–1.41)	NS
Postprandial fullness						
Crude model	1 (Ref)	1.04 (0.9–1.2)	NS	1 (Ref)	0.8 (0.7–1.0)	0.02
Model 1	1 (Ref)	1.1 (1.0–1.3)	NS	1 (Ref)	1.1 (0.9–1.3)	NS
Model 2	1 (Ref)	1.05 (0.88–1.25)	NS	1 (Ref)	1.10 (0.86–1.40)	NS
Model 3	1 (Ref)	0.98 (0.80–1.21)	NS	1 (Ref)	1.18 (0.91–1.56)	NS
Model 4	1 (Ref)	0.98 (0.79–1.21)	NS	1 (Ref)	1.21 (0.92–1.59)	NS
Severity						
Early satiation						
Crude model	1 (Ref)	1.0 (0.8–1.4)	NS	1 (Ref)	1.3 (1.0–1.7)	0.04
Model 1	1 (Ref)	1.0 (0.8–1.4)	NS	1 (Ref)	1.4 (0.9–2.1)	NS
Model 2	1 (Ref)	1.17 (0.83–1.65)	NS	1 (Ref)	1.72 (1.06–2.77)	0.03
Model 3	1 (Ref)	0.98 (0.66–1.47)	NS	1 (Ref)	1.39 (0.81–2.39)	NS
Model 4	1 (Ref)	0.92 (0.61–1.38)	NS	1 (Ref)	1.35 (0.78–2.35)	NS
Epigastric pain						
Crude model	1 (Ref)	0.9 (0.7–1.0)	0.04	1 (Ref)	1.1 (1.0–1.3)	NS
Model 1	1 (Ref)	0.8 (0.7–0.9)	0.006	1 (Ref)	0.9 (0.7–1.1)	NS
Model 2	1 (Ref)	0.88 (0.74–1.05)	NS	1 (Ref)	0.97 (0.76–1.23)	NS
Model 3	1 (Ref)	0.82 (0.67–1.0)	0.06	1 (Ref)	0.92 (0.70–1.20)	NS
Model 4	1 (Ref)	0.82 (0.67–1.01)	0.06	1 (Ref)	0.90 (0.69–1.19)	NS
Postprandial fullness						
Crude model	1 (Ref)	1.1 (0.9–1.4)	NS	1 (Ref)	0.9 (0.8–1.2)	NS
Model 1	1 (Ref)	1.2 (0.9–1.5)	NS	1 (Ref)	0.9 (0.7–1.3)	NS
Model 2	1 (Ref)	1.18 (0.89–1.58)	NS	1 (Ref)	0.97 (0.66–1.44)	NS
Model 3	1 (Ref)	0.98 (0.70–1.39)	NS	1 (Ref)	0.69 (0.44–1.08)	NS
Model 4	1 (Ref)	0.95 (0.67–1.35)	NS	1 (Ref)	0.67 (0.42–1.06)	NS

*Values are OR and 95% CI and resulted from ordinal logistic regression.

†Model 1: Adjusted for age, sex, energy intake. Model 2: Further adjustment for liquid, short interval between eating and sleeping, meal pattern, spicy, eating rate, breakfast skipping, chewing, medication, marital status, educational level, physical activity and smoking. Model 3: further control for fat, fructose, fibre, cocoa, sugar-sweetened beverage, tea, coffee Model 4: Additional control for body mass index.

Ref, reference; NS, not significant.

The direct link between GL and UCD in the present study might be related to differences in dietary intakes. The individuals in category 2 consumed greater amounts of carbohydrate, whereas their fibre intake was only 1 g day^{-1} more than in those in category 1. This difference in digestible carbohydrate may adversely affect gut microbiota and consequently gastrointestinal disorders [12]. Moreover, we were only able to control for overall fat intake, whereas different fatty acid types may have different physiological effects in the gastrointestinal tract [44].

The differences between GI and GL in relation to UH and UCD might be related to their association with other dietary intakes. A recent study from the UK National Diet and Nutrition Survey indicated that lower carbohydrate consumption is associated with a higher consumption of

processed meat, butter, soft drink, vegetables, oily fish and pulses [45]. Therefore, it is possible that a higher dietary GL as achieved by a higher carbohydrate consumption is different from diets that are low in GI or carbohydrate content.

Some limitations to the present study should be considered when interpreting the results. The cross-sectional design of the present study does not allow causal inferences. Indeed, individuals with gastrointestinal disorders may have changed their dietary intakes in an attempt to alleviate symptoms. However, such changes would have attenuated the associations identified. Therefore, the true estimates are probably even stronger than those we found. Using self-administered questionnaires might lead to misclassifications of individuals. We did not assess any biochemical markers, which may explain the associations between dietary GI or GL and gastrointestinal diseases. It has been shown that higher dietary GI and GL are directly linked to the risk of insulin resistance and polycystic ovary syndrome [46], which are associated with gastrointestinal disorders. However, there is no information regarding these conditions in our data; therefore, the lack of association in women might not be generalisable to females with polycystic ovary syndrome. The strengths of the present study include the assessment of dietary intakes by a dish-based, semi-quantitative food-frequency questionnaire, which provides more precise and reliable information than a semi-quantitative food-frequency questionnaire. Additionally, we performed our analysis in a large sample size and considered various confounders.

In conclusion, we found BMI- and sex-specific associations between dietary carbohydrate quality and gastrointestinal disorders. Higher dietary GI and GL may be risk factors for uninvestigated heartburn and uninvestigated chronic dyspepsia in men, as well as normal-weight subjects, but not in women and overweight individuals. Consuming a low GL diet might ameliorate UCD, particularly in men, whereas a low-GI diet might be beneficial in normal-weight patients with UH. However, in women and overweight individuals, these strategies might not be appropriate. These findings warrant evaluation in prospective studies to establish the potential role of carbohydrate quality in the management of GERD and FD, particularly in relation to BMI and sex.

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 in accordance with STROBE guidelines.

Conflict of interests source of funding and authorship

The authors declare that they have no conflict of interests.

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AE, AHK, HD, HA and PA contributed to the conception and design of the study, data collection, statistical analysis and the drafting of the manuscript. AF, LA and FH contributed to statistical analysis, data interpretation and drafting of the manuscript. CFB contributed to the design of the study, data interpretation and the drafting and revision of the manuscript. LA, AE and PA supervised the study. All authors approved the final version of the manuscript submitted for publication.

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GASTROINTESTINAL DISORDERS

Disordered eating patterns in coeliac disease: a framework analysis

R.-M. Satherley, S. Higgs & R. Howard

University of Birmingham, Edgbaston, UK

Keywords
anorexia nervosa, binge eating, bulimia nervosa, coeliac disease, eating behaviours, food attitudes, framework analysis.

Correspondence
R.-M. Satherley, University of Birmingham, Edgbaston B15 2TT, UK.
Tel.: +44 (0)121 4142942
Fax: 0121 414 4897
E-mail: RXS027@bham.ac.uk

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Abstract

Objective: The need for dietary-management in coeliac disease may lead to the development of disordered eating patterns. A theoretical model of disordered eating has been proposed to explain disordered eating in coeliac disease. The aim of this study was to explore the experiences of typical and disordered eating in coeliac disease to gain a greater understanding of these processes and explore specific pathways within this model.
Methods: We interviewed 21 individuals with coeliac disease, recruited from a previous database, about their experiences with food and food environments. Information about disordered eating status was assessed via questionnaire. The interviews were analysed qualitatively using Framework analysis, which was underpinned by the theoretical model of disordered eating in coeliac disease.
Results: Experiences differed between participants scoring high on measures of disordered eating and those who scored low (typical eaters). Participants scoring high on measures of disordered eating were concerned about the consequences of their gluten-free diet on body image and they described eating patterns similar to binge/restrict cycles. Typical eaters reported being able to integrate their dietary self-management into their daily lives; however, general concerns around food and cross-contamination were associated with a restriction in food intake.
Conclusions: Coeliac disease has a varied impact on eating patterns. The need to follow a gluten-free diet and to be vigilant around food has to be balanced with concerns around food availability and cross-contamination which have the potential to contribute towards disordered eating attitudes and behaviours. The findings suggest that the theoretical model of disordered eating provides an adequate explanation of disordered eating patterns in coeliac disease.

Introduction

Coeliac disease (CD) is a life-long condition characterised by flattened villi (villous atrophy) and inflammation of the small intestine [(1)]. These intestinal changes occur in response to the ingestion of gluten, which is formed from two proteins: gliadin and glutenin [(2)]. Gliadjn is the toxic protein for individuals with CD, as are structurally similar proteins hordein and secalin that are found in barley and rye. The symptoms of CD can be both gastrointestinal and non-gastrointestinal and include nausea, bloating, cognitive impairments and weight changes [(1)]. There is no cure for CD but the condition is managed by following a life-long gluten-free diet (GFD), which requires the exclusion of wheat, rye, barley and sometimes oats from the diet. Management of the GFD also requires vigilance around cross-contamination of food products, as small amounts of gluten can cause symptoms in some individuals [(3)].

In the majority of people with CD, successful management of the GFD reverses damage to the gut and reduces symptoms. However, the GFD can be challenging to

follow and can create concerns around eating outside the home and cross-contamination of food products [3]. Although the GFD is physically beneficial for the individual, its restrictive nature may impact quality of life and result in maladaptive behaviours, including disordered eating patterns [4–6].

The majority of individuals with CD score in the healthy range on self-report measures of disordered eating [7,8]. However, for some, CD may act as a risk factor for the development of disordered eating via a number of mechanisms. Factors essential in managing the GFD, including food preoccupation and awareness, may harm relationships with food [9]. Additionally, factors relating to the diagnostic experience, including gastrointestinal symptoms and changes in weight, may affect body image and eating patterns [10]. Alternatively, the non-specific burden of chronic illness may account for the presence of disordered eating in this population. Satherley, Howard and Higgs [8] suggest that factors both unique to the CD diagnosis (gastrointestinal symptoms, dietary management) and nonspecific factors (psychological distress) are important factors in disordered eating and CD.

This study was theoretically informed by Satherley, Howard and Higgs' [6] model of disordered eating in gastrointestinal disease (Fig. 1). Central to this model are two pathways; the first pathway describes individuals who experience anxiety around food and cope with this by consuming a limited variety of gluten-free foods. The second pathway describes those who struggle with weight changes experienced after diagnosis (usually weight gain) and engage in poor dietary self-management to promote gastrointestinal symptoms and associated weight loss. In an evaluation of this model, dietary-management and gastrointestinal symptoms were associated with disordered eating scores, lending some support to pathway two [8]. However, the relationships between gastrointestinal symptoms, dietary-management and disordered eating were not clear. Furthermore, no evidence was found to support pathway one, the role of anxiety in disordered eating. This was attributed to a lack of appropriate tools to measure concerns around food in individuals with CD. Understanding these factors and their role in the development of disordered eating is essential if appropriate supportive strategies are to be adopted by healthcare professionals.

The present study aimed to gain a holistic view of the experiences of typical and disordered eating in CD. This was done by exploring the pathways of the Satherley, Howard and Higgs [6] model by using a structured framework. According to the model, the type of disordered eating pattern that develops will depend on beliefs about CD and the GFD, as well as the psychological response to weight changes after CD diagnosis. By using this model to create the framework for the interviews, we were able to assess how well this model was supported by qualitative data.

Materials and methods

Participants (18–69 years) with a self-reported biopsy-confirmed diagnosis of CD, for at least 2 years, without additional food allergies or health conditions, were eligible to participate. Purposive sampling was used to recruit both typical and disordered eaters from a previous database. Participants who scored above 20 on the EAT-26 or above 17 on the BES were categorised as disordered eaters (DE), participants scoring below were classified as typical eaters (non-DE) [11,12]. The EAT-26 is a screening tool that measures symptoms and concerns characteristic of eating disorders and the BES screens for the presence of binge eating behaviour, high scores are associated with more disordered eating symptoms and behaviours. These are not diagnostic tools but screen for the presence of disordered eating behaviours. The measures of disordered eating were taken between 18–63 days (mean = 41 days) prior to the interviews.

Sample size was based on data saturation, by repeatedly comparing data across participants, which occurred when no new information was obtained from the interviews [13]. Twenty-five participants were invited to take part in the interviews but three withdrew their data and one was removed from analysis, as the inclusion criteria were not met. Individuals who withdrew their data were all classified as disordered eaters, no other demographic differences were found in this group. Participants were informed that the interview would explore eating patterns in CD. Demographic information (gender, age, years since diagnosis, body mass index (BMI)) and health information (EAT-26 and BES scores) were taken from the existing database; these measures were all based on self-report.

A semi-structured interview schedule allowed us to frame questions to fit the theoretical model of disordered eating [6]. The interview explored key themes concerning the diagnosis of CD, the daily management of the GFD and how CD has affected participants' relationship with food and body image.

Procedure

Participants provided written consent before their interviews. The first author conducted and audiotaped the interviews. Each interview lasted between 30 and 45 min and was conducted in the participant's home. If any current or past disordered eating was reported, participants were asked to discuss this in more detail, and reflect on any links with their CD diagnosis. The interviewer encouraged participants to elaborate on relevant themes.

Figure 1 The Satherley, Howard and Higgs' model of disordered eating in gastrointestinal disease. Figure taken with permission from: Satherley *et al.* [6].

Data analysis

Ritchie and Spencer's [14] Framework methodology was used as it allows the use of a theoretically-driven framework to structure and explore the data. Framework analysis was beneficial for this study because it can include *a priori* themes drawn directly from the model of disordered eating [6], as well as emergent concepts.

Interviews were transcribed verbatim by the first author, and read repeatedly in order to identify key themes. These themes were developed into a framework for coding the entire dataset. Additional categories were created for data that did not fit into the framework. To enhance reliability, the coding process and emerging themes were discussed among the authors until consensus was achieved. Trustworthiness of the data was enhanced using a decision trail to ensure transparency [15].

Ethical approval

Ethical approval was granted by the Research Ethics Committee, University of Birmingham.

Results

Five males and 16 females took part in the interviews, (mean age = 39 years; mean time since diagnosis = 5 years). Of these, 10 participants scored above EAT-26 and BES cut-offs resulting in them being classified as 'disordered eaters'. Participant information can be found in Table 1. Illustrative quotes presented are annotated with pseudonyms and participants' disordered eating status (DE or non-DE). 'Disordered eaters' and 'typical eaters' displayed significantly different BES ($t(19) = -7.09$, $P = <0.001$) and EAT-26 ($t(19) = -61$,

$P < 0.001$) scores. There were no significant differences between participants for age, BMI or years since diagnosis. There were also no significant differences across gender for EAT-26 and BES scores.

The theoretical model of disordered eating in gastrointestinal disease [6], describes three stages in the development of disordered eating: adaptation to diagnosis, illness beliefs and dietary management. These stages provided the analytic framework for the hierarchical themes. Each hierarchical theme was coded in depth to identify subordinate themes. Each of these subordinate themes were explored, resulting in 17 sub-themes (see Table 2). All themes were reported across participants but experiences and opinions differed across individuals.

Thoughts, feelings and behaviours underlying disordered eating

Adaptation to diagnosis

The new self. The diagnostic process was related to physical changes in body image, which were important in the adoption of disordered eating patterns. Disordered eaters described distress around weight changes after diagnosis. This was linked with a desire to lose weight by restricting food intake.

> I liked being thin. I was over 30 and I wasn't putting on weight. I've definitely got a big belly now, I've put on weight and it's really bothering me. I really have lost a lot of confidence in terms of the way I look. So I go on more diets now, to try and get back to how I was. I'd like to be back to my pre-diagnosis weight. (Georgia, DE)

These weight changes were challenging for disordered eaters and Dan felt that more support could have been provided from healthcare professionals.

> Associating thinness with unhealthiness is strange. Putting on weight but being healthy, it goes against the things you read about. I think the dietician could have explained that once your stomach goes back to normal there will be a process where you start to gain weight. I don't remember her explaining that. That may have helped me feel better. (Dan, DE)

Some individuals did not experience post-diagnosis weight changes and others felt happier with their weight once they were following the GFD. Typical eaters felt better after diagnosis because of their increased energy, which was associated with an improved body image.

> The thing I've really noticed is that when I'm feeling ok, I've got so much more strength and energy. And that makes me perceive my body better. (Amy, non-DE)

Mourning gluten. Participants described the challenging process of mourning gluten-containing foods after diagnosis, which was accompanied by distress. Twelve participants described a ritualistic consumption of gluten 'for the last time'. These feelings of loss were still present in disordered eaters and were associated with a desire to consume gluten-containing foods.

> There's a certain food that I'd normally eat, I remember I cried when I ate that for the last time. I ate loads of it, to try and say goodbye. That was really upsetting. I still miss the food, it's really hard. I just want to eat it again. I get upset seeing friends eat it. (Paula, DE)

For typical eaters, this mourning process was brief and no longer occupied their thoughts.

> There is a sort of grieving process for maybe a few months. But now it's just a part of life. There's no reason to miss food that made me ill. (Colette, non-DE)

Table 1 Participant characteristics and disordered eating scores

	Pseudonym	Age (years)	Years since diagnosis	EAT-26 score	BES score
'Typical eaters'	Katy	19	3	6	8
	John	53	2	3	3
	Mel	26	2	0	3
	Louise	29	10	8	1
	Sue	49	5	0	11
	Colette	59	19	5	2
	Richard	49	4	4	5
	Anna	28	3	7	1
	Katherine	32	3	9	5
	George	36	7	0	2
	Andrea	29	6	3	3
	Mean	37.2	5.8	4.1	4
'Disordered eaters'	Caroline	48	3	12	23†
	Amy	48	3	26*	18†
	Paula	41	3	26*	8
	Georgia	48	2	26*	30†
	Dan	40	6	21*	25†
	Julie	22	4	30*	13
	Martha	35	4	27*	14
	Steve	38	6	19	22†
	Holly	29	2	26*	21†
	Lisa	54	8	27*	19†
	Mean	40.3	4.1	24	19.3

*>20 on EAT-26.
†>17 on BES.

Table 2 Final thematic structure and example quotes from disordered and typical eaters

Superordinate theme	Sub-theme	Example quotes from disordered eaters	Example quotes from typical eaters
Adaptation	The new self	Caroline: I've lost a lot of confidence in the way I look. Julie: My stomach is a lot bigger now, it's hard to accept that that's healthier. Dan: My weight kept going up and down, I found that very difficult.	Sue: I have more strength and energy, so I perceive myself as better. John: My weight hasn't changed much at all. Richard: I've lost some weight, I think that's one of the benefits of being a Coeliac.
	Mourning of gluten	Julie: Viennese whirls. I miss them, they were my favourite and I get sad thinking about them. Dan: I ate a lot of gluten, to say goodbye to the foods I wouldn't be able to eat anymore. Caroline: My diet is so restrictive, it's impossible not to miss old foods.	Richard: I don't really miss any foods because they made me so ill. Louise: It felt like a mourning for what you couldn't have, I was angry but made peace with it in the end. Sue: I feel so much better now, I don't think I could miss gluten.
Illness beliefs	The dangers of cross contamination	Amy: I clean the surfaces before cooking and reduce the contamination risk. Georgia: I don't let cross-contamination control me, I just do a quick check before eating. Julie: It doesn't overly concern me, I might get ill but a small bit of gluten won't kill me.	Sue: I'm worried about the crumbs, if my husband's bread is in my kitchen, I won't eat. Louise: Sometimes it's safer not to eat because cross-contamination is everywhere. Mel: I have a gluten radar on at all times, if that radar is activated, it's best not to eat.
	My GFD makes me fat	Georgia: Gluten-free foods are full of calories, they make me feel fat. Caroline: Gluten-free food is full of rubbish, it definitely contributed to my weight gain.	Katy: Gluten-free cakes are unhealthy but I limit them like anyone else would limit cakes. Richard: I knew that I would gain weight as my body healed.
Dietary management	Risk-taking	Paula: Sometimes I'll take a very small risk. Georgia: I think I should probably be more careful than I am. Caroline: It's hard outside the home, I may take some risks then.	Mel: Gluten is poison, I would never cheat. Richard: I'm very ill when I make mistakes, I can't let it happen. Louise: I haven't had gluten. I just don't allow it.
Eating knowledge and practices	Eating for pleasure	Georgia: Food is my enemy at the moment. Paula: Food makes me upset. It makes me scared. It makes me jealous. Amy: Eating isn't enjoyable anymore, it causes a lot of stress, particularly outside the home	John: Food is just a tool for my body now. Sue: I've gone off food, it causes me a lot of anxiety. Richard: Eating is a lot more difficult than it used to be, it can be done but it involves a lot more planning and isn't as relaxed.
	Food preoccupation	Caroline: I'm a lot more aware of the calories in food now and more careful about what I eat. Julie: The gluten-free foods are full of fat and calories, I just avoid them. Georgia: Food is always on my mind, I think I'm a little bit obsessive about food.	Katy: You're always thinking about food. You're always cooking food. Mel: It does make you a bit conscious about how you are with foods. Richard: Food is always on my mind but it motivates me to cook and I now want to make a gluten free cake shop.
	New eating patterns	Julie: I overcompensate with cakes and cookies. Caroline: I eat a limited range of foods but it works for me. Dan: I always search for the new gluten-free treats. They're hard to find, so I feel like I deserve them when I can have them.	Colette: I will eat anything, as long as it's gluten-free. Richard: I cook a lot more now and I'm more interested in cooking, which makes sourcing food a lot easier. Sue: I don't eat out as much now, but in my home it's just the same as it used to be.

After the adjustment process and acceptance of their diagnosis, participants began to develop beliefs about their CD and the GFD.

Illness beliefs

The dangers of cross contamination. Cross-contamination was frequently referred to during the interviews. However, disordered eaters were less concerned about cross-contamination than were typical eaters, and believed that accidental gluten ingestion would not impact their long-term health.

> I'm rarely ill from cross-contamination, so I take risks and deal with the consequences. A tiny amount of gluten every so often won't have adverse effects on your long-term health; it just might make you feel sick. (Julie, DE)

Typical eaters had greater concerns around cross-contamination and went to greater lengths to avoid cross-contamination than did the disordered eaters. Louise coped with these concerns by limiting her food intake when outside of her home environment.

> Sitting in the staff room with everyone else eating food, that's scary. Um, I know they're not going to touch me or make me eat it or anything but I won't eat anything. There's just too much risk. I only eat my own foods in my own home... if I'm out shopping all day, I won't eat but I'll eat my own safe food when I get home. (Louise, non-DE)

For three individuals, these cross-contamination concerns extended into their own home: the kitchen was viewed as an unsafe environment and resulted in a restricted food intake.

> The kitchen isn't safe. It's gluten-free, but it's more that food in general isn't safe. I get worried around food. I have a few safe things that I do eat but food has become the enemy now. It's just safer not to eat. (Mel, non-DE)

Response to weight changes due to GFD. Participants were asked about the causes of any weight changes experienced after commencing the GFD. Seventeen participants experienced weight gain after starting the GFD whereas the remainder experienced no change or weight loss. Disordered eaters attributed weight changes to the GFD and the poor nutritional quality of gluten-free foods; they responded by restricting their food intake.

> And the gluten-free foods, if it's not super fatty, it's super sugary. Eating gluten-free food made me really fat. It's hard to stay slim on a gluten-free diet. I've had to go on diets to lose the weight but it's hard. (Paula, DE)

For typical eaters, weight changes were attributed to the recovery of the intestine and improved health.

> My weight has been quite stable, I put on a bit at first but I was really underweight. I read all the books and they said that when your body recovers your weight should be normal. And that's what happened. (Mel, non-DE)

Dietary management

Risk taking. The majority of participants managed their GFD well. However, five disordered eaters reported consuming small amounts of gluten.

> There was this really good sauce and I did take a really small piece of crusty bread. Because crusty bread is the thing I miss the most. And I very gingerly sort of scooped up all the sauces and ate it. It would be a small piece that hopefully I'm going to sort of eat without my stomach noticing. (Dan, DE)

Not all individuals with disordered eating reported deliberate gluten ingestion and this was not recognised as a technique to promote weight loss.

For typical eaters, their concerns around cross-contamination and the fear of re-experiencing unpleasant symptoms meant that risk taking was not tolerated.

> I don't take risks. I can't take risks. Gluten poisons me, why would you risk being poisoned? (Sue, non-DE)

Patterns of disordered eating

Eating knowledge and practices

All participants felt that their eating patterns and the way they thought about food had changed since their diagnosis. Their thoughts and feelings about their CD affected both their attitudes towards food and the way they consumed food. Three sub-themes emerged related to these changes in eating patterns and beliefs: food preoccupation, eating for pleasure and new eating patterns.

Food preoccupation. All participants reported that their diagnosis of CD had made them more aware of the foods they were consuming and more aware of the nutritional content of food. This awareness arose from the need to manage the GFD and the preparation and planning that this involved. Participants were always thinking about food, what meal they were having next and where this food was coming from. For disordered eaters, this food preoccupation dominated their thoughts.

> You've got to think about the range of colours you're eating, the nutrients and about the quantity, you're thinking about a whole range of stuff. I'm a bit obsessive about food. It does change your relationship with food. You're always thinking about food. (Paula, DE)

This awareness of food often led to an increased awareness of the calorific content of food. Seven individuals became dissatisfied with the amount of calories they were consuming and became dissatisfied with their body image.

> Since becoming coeliac I'm also a lot more calorie conscious as well. And the gluten free foods. They're full of calories and fat, and that has made me, well, fat. Now I'm much more conscious, about everything I eat. (Georgia, DE)

Typical eaters described an awareness of food, but they were able to integrate these thoughts around food into their life.

> I'm a lot more aware of food now, it's on my mind a lot but that doesn't bother me. I might see a Chinese recipe but I'd just wonder how I could make it gluten-free. It's just a part of life. (Richard, non-DE)

Eating for pleasure. After CD diagnosis, emotional relationships with food had changed. Meal times were described as challenging and eating was no longer enjoyable. For disordered eaters, a loss of pleasure around eating was common and was strongly interlinked with emotions: food became a source of distress.

> Initially I was anxious. Finding out all these foods you couldn't have and thinking why the hell does that have gluten in it, was upsetting. Food is now my enemy, food kills me, food attacks me. I know that sounds really melodramatic but that's how it feels. (Dan, DE)

A lack of enjoyment in the eating process resulted in typical eaters simply viewing food as fuel for the body.

> I've gone off food really. Food is the baddie in my life at the moment. I just eat what I have to; I've lost the enjoyment of sitting down and going out for a meal. (Amy, non-DE)

In comparison, the majority of typical eaters enjoyed eating outside the home, whilst managing their GFD.

> It's harder to eat out but you can't let that dictate your life. I still enjoy going out with friends for a meal, I just have to be careful. (Richard, non-DE)

New eating patterns. Some participants reported an improvement in their diet since diagnosis; however, others reported eating patterns that appeared disordered in nature.

For eight disordered eaters, overconsumption of food was reported and this was linked with emotional distress. The restrictive nature of the GFD made participants long for certain foods. When these foods were available, they would be bought in bulk and consumed in a short space of time, indicating a binge-type eating pattern. However, the consumption of this food was not associated with guilt.

> When you're unable to eat certain foods, you then overcompensate with other things like wine, chocolate, biscuits. It's depressing not getting these foods, so when you do, you just enjoy it. And eat loads of it. I don't feel guilty, when I eat it, I feel happy again. The cakes aren't going to be there tomorrow, so eat it while you can. (Paula, DE)

Some disordered eaters felt that because of the restrictive nature of their GFD they deserved to indulge in certain foods. Some participants hoarded gluten-free foods and ate them at a fast rate.

> When the gluten-free Kit Kat bars first came out, I hoarded those because they were delicious. If it's good, I'll be hoarding. Sometimes I eat them all myself. I think that's probably my way of dealing with it. And I eat faster than I used to, I just eat it quickly before someone's like – no you can't eat that. (Julie, DE)

Other disordered eaters felt a need to limit their food intake due to concerns around weight increase since their CD diagnosis.

> It's like being on several diets at once. I can't eat gluten, I eat naturally gluten-free because of all the calories in gluten-free breads and pasta, and I'm on a Slimming World diet because of all the weight I put on after my diagnosis. I just want to lose the weight. (Martha, DE)

Typical eaters used strategies to improve food availability. This included cooking large quantities of food and storing them to consume during the week.

> I kind of, I think I make up for the fact that I can't eat gluten by baking a lot of gluten free cookies and meals. I portion them and freeze them for later in the week. (Katy, non-DE)

Five typical eaters developed a fear of trying new foods or trying foods in new environments. This stemmed from concerns around cross-contamination and the belief that it was dangerous to eat foods outside the home. Some typical eaters reported going for long periods of time without eating outside the home. These participants no longer enjoyed eating in general and felt more at ease when they were not around food, which resulted in restricted food intake.

> If I'm out shopping all day, I prefer not to eat. It's just not safe to eat. Eating has become scary because of my coeliac. I only eat if I'm desperate. Food is too dangerous now, when I'm not eating I feel safe. (Richard, non-DE)

Others felt that their eating patterns were not affected by their CD diagnosis. They were still able to maintain a nutritionally balanced diet. These participants were able to consume a range of foods both inside and outside the home, despite sticking to their GFD.

> As long as I know it is gluten free, I'll try anything. I'm not a fussy eater at all. I've always been that way. The only restriction to that is whether it's gluten free or not. (Katy, non-DE)

Discussion

This study investigated the experiences of disordered eating in CD, in order to test a theoretical model [6]. Disordered eaters reported eating patterns suggestive of a

binge/restrict cycle, which was associated with psychological distress, poor dietary-management and a preoccupation with food.

Disordered eaters

Disordered eaters, as determined by the EAT-26 and the BES, developed eating beliefs that stemmed from concerns around weight changes associated with commencing the GFD. These weight changes caused distress and participants found it challenging to adapt to their new body image. They described a desire to reach their pre-diagnostic weight and responded by restricting their dietary intake. Weight increase is a known trigger for disordered eating behaviours that may be viewed positively by those who are underweight at diagnosis but may be unwelcome in those who begin at a normal or higher weight [16]. These findings are in line with Leffler *et al.* [4] who described three cases where concerns around weight increased after starting the GFD which led to disordered eating behaviours.

Distress and mourning the loss of gluten-containing foods were associated with disordered eating status. All participants experienced a mourning period, but for disordered eaters, there was an extended period of distress surrounding the loss of gluten-containing foods, that lasted for years after diagnosis. Participants coped with these feelings by overcompensating with high energy-dense, gluten-free foods such as cakes and biscuits. Consumption of high-energy dense foods has frequently been reported in those with CD [17], but our results indicate that this may occur to help manage distress. Participants reported no guilt around the consumption of these foods because they felt they 'deserved' to eat them. This resulted in the hoarding of foods and fast food consumption. This could be an indication of binge-eating type behaviour in a sub-group of participants, all of whom were classified as disordered eaters according to the BES [18].

Disordered eaters reported that overconsumption occurred in combination with restrictive eating: weight loss was promoted by restricting food intake but this resulted in a preoccupation with food and psychological distress, which resulted in binge eating. These findings are in line with Herman and Polivy's [19] Boundary Model, which suggests that those who restrict their intake are more responsive to external stimuli and at risk for both under and overconsumption of food. Similar patterns of eating have been described in people with Type Two Diabetics who also follow a prescribed dietary regimen [20]. These findings highlight the complex interplay of emotions and food, which may alter eating patterns and beliefs in CD. An increased intake of high-density gluten free foods may be used to cope with feelings of distress that arise from the restrictive nature of the GFD. Mazzeo and Bulik [21] suggested that disordered eating arises after a stressful event as a way to manage emotions and acts as a coping mechanism.

Intentional gluten consumption to promote weight loss was not reported. When asked about gluten-consumption in an anonymised web-meditated survey, poor dietary management was associated with disordered eating [8]. In addition, case studies have documented the interaction between intentional gluten consumption and a desire to promote weight loss through villous atrophy [4,7]. However, only four participants, categorised as 'disordered eaters', described occasional gluten ingestion or risk-taking behaviours. However, participants may not have been willing to talk about intentional gluten consumption as a way of losing weight with the interviewer due to perceived lack of anonymity.

Low risk disordered eaters

Typical eaters differed from disordered eaters in thoughts, feelings and behaviours. Despite experiencing weight changes after diagnosis, typical eaters felt healthy and energetic with increased confidence. This is in line with findings suggesting that quality of life increases after initiation of the GFD [22,23]. Typical eaters also experienced a mourning period after diagnosis but these feelings of loss were no longer present at the time of interview. Typical eaters associated gluten-containing foods with the symptoms they had experienced prior to commencing the GFD and had no desire to consume these items again.

Caution around cross-contamination is essential for those with CD but may contribute to limited food consumption, both inside and outside the home. Some typical eaters reported going for long periods of time without consuming food because they believed that limiting food consumption was keeping them safe, particularly when outside the home. Neither the EAT-26 nor the BES captured the consequences of these cross-contamination beliefs on eating patterns. However, this form of dietary self-management may result in eating behaviours that could be considered 'disordered' (i.e. restricting and bingeing behaviours) as they deviate from the norm [24].

Importantly, not all participants displayed high levels of concern around food. Twelve individuals were happy to try new foods that they believed were gluten-free. These individuals described a healthy eating style and adaptive beliefs about food, with the caveat that their diet was gluten-free.

The theoretical model of disordered eating

These findings provide support for the two-pathway theoretical model of disordered eating in CD [6]. The first

pathway of the model suggests that an unwanted increase in weight after diagnosis results in the belief that the GFD is responsible for this weight gain, which results in poor dietary self-management to lose weight. Although our data suggests that distress around weight change is associated with disordered eating attitudes and behaviours, there was no evidence for the role of intentional gluten ingestion to promote weight loss. In addition, the mourning and distress around the loss of gluten-containing foods was associated with a desire to consume gluten. These findings are closely in line with the CD grief process described by Rose and Howard, whereby the benefits of following a GFD were not always viewed as beneficial, resulting in problems with dietary management [25]. Future revisions of the theoretical model should consider the role of distress and feelings of loss in relation to gluten-containing foods.

The second pathway describes those who adapt well to their CD diagnosis and have good dietary self-management but overly extreme concerns around cross-contamination may develop. Our findings suggest that some participants developed an extreme vigilance around food, which was associated with limited food intake and concerns around food preparation and consumption. However, these individuals did not score above clinical cut-offs on measures of disordered eating. Vigilance around cross-contamination is essential for GFD management but it is unclear from the current data whether these extreme concerns around cross-contamination are maladaptive. Future revisions of the theoretical model need to consider the types of concerns around food in those with CD to identify factors that may promote maladaptive concerns.

Strengths and limitations

All participants were diagnosed at 16 years of age or older; however, age of diagnosis may have an impact on interactions with food, and this is often associated with the development of disordered eating in chronic health conditions [26]. Childhood diagnosis may differ from adolescent and adult diagnosis in the risk for disordered eating patterns, as diagnosis under four years has been associated with better dietary-management and better psychological well-being whereas those diagnosed in adolescence show more problems with social interactions and more physical health problems [27,28]. Furthermore, disordered eating attitudes and behaviours tend to be more common in healthy females [29]. Although our sample contained both males and females, the samples were too small to explore the influence of gender on disordered eating attitudes and behaviours. Future research should explore the relationship between gender and disordered eating in CD.

The assessment of disordered eating and BMI were all based on self-report. This may be unreliable when assessing individuals who are motivated to keep their eating patterns and weight secret, as is the case in disordered eating [30]. Furthermore, we recognise that the EAT-26 and the BES allow screening of disordered eating but cannot be used as diagnostic tools. Future research could focus on looking at those who display clinically significant disordered eating patterns, assessed through clinical interview and the use of diagnostic tools. Furthermore, EAT-26 and BES scores were assessed between 18–63 days prior to the interview. Given the unstable nature of disordered eating attitudes and behaviours and their tendency to change over time [31], it would be beneficial to verify disordered eating status immediately before the interview. Additionally, the use of the EAT-26 and BES has not been validated in individuals with CD. The scales contain items such as 'I find myself preoccupied with food' and 'Sometimes I do not eat what I want around others because I am aware of my problem with food'. As a result, there is potential for individuals with CD to be misclassified as disordered eaters on these tools, particularly as management of CD requires a focus on food intake [32]. However, these behaviours may represent a skill used to manage the GFD as opposed to a disordered eating attitude or behaviour. At present, there is no validated tool to assess disordered eating attitudes and behaviours in CD. There is a need to consider the development of tools that may be more appropriate individuals with CD and other dietary controlled health conditions.

Despite these limitations, this qualitative study was guided by the model of disordered eating in gastrointestinal disease and allowed us to gain in-depth understanding into the application of this model to CD [6]. The study provides insight into the types of disordered eating attitudes and behaviours and motivations behind these behaviours in CD. The BES and EAT-26 appear to be effective in screening individuals who display binge/restrict-eating patterns. However, these tools were not able to select individuals who limited their food intake due to concerns around cross-contamination. Directions for future research should focus on tools to assess concerns around food and cross-contamination in CD.

Clinical implications

Individuals expressed a desire for more information regarding potential weight change after commencing the GFD. This is in line with previous research, which highlights the value of dietician-led services and the desire for more dietetic support in individuals with CD [33,34]. Furthermore, disordered eaters discussed distress surrounding weight changes at CD diagnosis. The current NICE guidelines do not recommend that individuals newly diagnosed with CD are consulted about the benefits of a nutritionally balanced GFD and how the initiation of

the GFD may influence weight change and body shape, despite individuals with CD explaining the benefits of this type of support [34]. It is recommended that research informing clinical guidelines should focus on the role of educating all newly diagnosed individuals with CD about the nutritional content of gluten-free foods and possible weight changes after starting the GFD, as well as how to manage these weight changes.

Conclusions

This study has provided insight into the factors that may contribute to the development of disordered eating patterns in CD. The results suggest that experiences of disordered eating differ across individuals with CD but relate closely to the CD diagnosis and management of the GFD. Greater understanding is still needed, especially in regards to atypical eating patterns, which are not detected by current measures of disordered eating.

Transparency statement

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

The authors declare that we have no competing interests.
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RS is the guarantor of this paper, collected and analysed data and wrote the initial draft; SH and RH reviewed analyses, advised on interpretation, and contributed to the final version of this article. All authors approved the final version of the manuscript.

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Appendix A
STROBE assessment

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract – see study title 'A framework analysis'
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found - see Abstract
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported – see lines 54–74
Objectives	3	State specific objectives, including any prespecified hypotheses – see lines 68–74
Methods		
Study design	4	Present key elements of study design early in the paper – see lines 72–74; 97–100
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection – see lines 86–87; 103
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants – see lines 77–82
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable – see lines 80–85; 94–96; 98–100

Appendix A. Continued

	Item No	Recommendation
Data sources/measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group –see lines 80–85; 94–96; 98–100
Bias	9	Describe any efforts to address potential sources of bias – N/A
Study size	10	Explain how the study size was arrived at – see lines 88–90
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why – see table one; see lines MIGHT NEED TO ADDRESS THIS
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding – see lines 112–121
		(b) Describe any methods used to examine subgroups and interactions N/A?
		(c) Explain how missing data were addressed – see lines 90–93
		(d) If applicable, describe analytical methods taking account of sampling strategy – see lines 88–90
		(e) Describe any sensitivity analyses N/A
Results		
Participants	13	(a) Report numbers of individuals at each stage of see lines 88–90
		(b) Give reasons for non-participation at each stage – see lines 88–90
		(c) Consider use of a flow diagram
Descriptive data	14	(a) Give characteristics of study participants (e.g demographic, clinical, social) and information on exposures and potential confounders – see lines 125–128 and Table One
		(b) Indicate number of participants with missing data for each variable of interest – see line 88–90
Outcome data	15	Report numbers of outcome events or summary measures – missing data was not analysed so numbers are the same throughout
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g, 95% confidence interval). Make clear which confounders were adjusted for and why they were included N/A
		(b) Report category boundaries when continuous variables were categorized – lines 80–82
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N/A
17 Other analyses	17	Report other analyses done—e.g analyses of subgroups and interactions, and sensitivity analyses N/A
Discussion		
Key results	18	Summarise key results with reference to study objectives – see lines 312–315

Appendix A. Continued

	Item No	Recommendation
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias – see lines 402–428
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – see lines 438–442.
Generalisability	21	Discuss the generalisability (external validity) of the study results – see line 399
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based x- see lines 443–444

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GASTROINTESTINAL DISORDERS

An evaluation of the feasibility and validity of a patient-administered malnutrition universal screening tool ('MUST') compared to healthcare professional screening in an inflammatory bowel disease (IBD) outpatient clinic

K. Keetarut,[1] S. Zacharopoulou-Otapasidou,[2] S. Bloom,[1] A. Majumdar[3]* & P. S. Patel[1]*

[1]University College London Hospitals NHS Foundation Trust, London, UK
[2]London Metropolitan University, London, UK
[3]St Mary's University, Twickenham, London, UK

Keywords
inflammatory bowel disease, malnutrition universal screening tool, nutritional screening, outpatients.

Correspondence
K. Keetarut, Department of Nutrition and Dietetics, Third Floor East, 250 Euston Road, University College London Hospital NHS Foundation Trust, London NW1 2PG, UK.
Tel.: +44 02034479289
Fax: +44 02034479811
E-mail: katie.keetarut@uclh.nhs.uk

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*These authors contributed equally to the present study.

Abstract

Background: Malnutrition is common in inflammatory bowel disease (IBD) and is associated with poor health outcomes. Despite this, screening for malnutrition in the outpatient-setting is not routine and research in the area is limited. The present study aimed to evaluate whether agreement between malnutrition screening completed by patients and healthcare professionals (HCPs) could be achieved by comparing patient self-administered 'MUST' ('MUST'-P) with HCP administered 'MUST' ('MUST'-HCP) in a single tertiary IBD outpatient clinic.

Methods: We conducted a feasibility and validity study on adult outpatients with IBD. We collected anthropometric, nutritional and clinical data from patients. All patients completed 'MUST'-P using a self-administered questionnaire, followed by 'MUST'-HCP. 'MUST'-P was timed and feedback on ease-of-use was obtained. The risk of malnutrition was classified as low (score = 0), medium (score = 1) and high (score ≥ 2) and agreement was tested using kappa statistics (κ).

Results: Eighty patients were recruited (Crohn's disease: $n = 49$, ulcerative colitis: $n = 29$, unclassified: $n = 2$), with a mean (SD) age of 39.9 (15.1) years (51.2% were males). Seventy-one (92%) of patients found 'MUST'-P either easy or very easy. The mean (SD) time to complete 'MUST'-P was 3.1 (1.8) min (range 1–10 min). Sixty-eight (85%) of patients were at low risk of malnutrition when screened by the HCP. There was moderate agreement ($\kappa = 0.486$, $P < 0.001$) between 'MUST'-P and 'MUST'-HCP, with 100% agreement in scoring for medium- and high-risk categories.

Conclusions: The results of the present study suggests that self-screening using 'MUST' could be effectively used in an IBD outpatient clinic to identify those at medium and high risk of malnutrition. The patient friendly version of 'MUST' ('MUST'-P) was considered quick and easy to use by patients. Implementation of self-screening with 'MUST' could improve the nutritional management of IBD patients.

Introduction

Malnutrition can be defined as 'a state of nutrition in which deficiency, excess or imbalance of energy, protein, and other nutrients causes measurable adverse effects on tissue and body form (body shape, size, composition), function and clinical outcome' [1,2]. It is a serious and common condition associated with significant morbidity and mortality, affecting adults and children with all types of diseases in all healthcare settings. The prevention, identification and treatment of malnutrition at an early stage could reduce potential health risks, dependency on others, hospital admissions and costs [3,4]. The economic impact of a risk of malnutrition as a result of an increased use of health and social care resources, hospitalisation and length of hospital stay, as identified using tools including 'MUST', is well documented [5,6]. A study conducted in Portugal on 637 inpatients found that a high risk of malnutrition in 21–29% patients, as identified using malnutrition screening tools, was an independent predictor of increased hospitalisation costs [7]. The National Institute for Health and Care Excellence recommend that all outpatients should be screened for malnutrition at their first appointment and screening should be repeated when there is clinical concern [8].

Crohn's disease (CD) and ulcerative colitis (UC) are the main types of inflammatory bowel diseases (IBD), with a rarer type (unclassified IBD-U) accounting for approximately 10% of all cases [9]. In a northern English population, the prevalence of IBD has been estimated at approximately 387 per 100 000 population (243 per 100 000 with UC and 144 per 100 000 with CD) in 1995, with the prevalence of CD increasing faster than UC [10]. IBD is associated with substantial morbidity; one aspect includes nutritional status where malnutrition and weight loss are common [11,12]. Up to 75% of adults with active IBD are malnourished [13–15] and up to 33% of adults in remission have been found to be malnourished ([16]). IBD patients often alter their eating habits to alleviate their symptoms, potentially leading to malnutrition and weight loss [17]. In addition to protein-energy malnutrition, deficiencies in trace elements and vitamins such as magnesium, iron and vitamin B_{12} are common [18,19]. Prolonged symptoms, as well as disease management either by drug treatment or surgery, may further impact on the nutritional status of patients.

Food and nutrition is viewed as a high priority for IBD patients [20] and yet dietetic service provision remains poor, with approximately 60% of inpatients receiving no dietetic contact [21]. Malnutrition can be under-recognised in IBD patients because routine screening is not common practice, resulting in under-detection and thus under-treatment of malnutrition [22,23]. Factors contributing to this include: a lack of recognition of the detrimental effects of malnutrition in IBD, difficulties implementing nutritional plans, a lack of staffing in busy outpatient clinics and a lack of guidance on the management of those identified at risk of malnutrition [21]. A systematic review looking at barriers and facilitators of adoption of nutritional screening by nurses concluded that it was unlikely, unless it was considered as an integral part of the nursing assessment and was appropriately resourced [24]. The use of patient self-administered malnutrition screening tools has been shown to be beneficial in the hospital outpatient setting [25].

The UK IBD Audit [21] advises that all IBD inpatients are screened for malnutrition and recommend 'MUST' as an appropriate tool. In addition, although nutritional screening guidelines exist for a variety of healthcare settings [26], no specific screening tool has been developed for IBD outpatients. Patient-administered self-screening has recently been investigated in different studies and has demonstrated benefits in various disease states [1,22,25,27].

The 'MUST' tool is considered an appropriate malnutrition screening tool because it has face, content, concurrent and predictive validity with a range of other screening tools. It is also internally consistent and reliable and has very good to excellent reproducibility when used with different assessors in a variety of settings. Guerra *et al.* [7] found agreement between 'MUST' and the ESPEN (European Society of Parenteral and Enteral Nutrition) recommended Nutrition Risk Screening tool [26] as a predictor for increased hospitalisation costs. The 'MUST' tool has been found to be easy, quick to use and acceptable to patients, research participants and healthcare workers [28,29]. Previous research examining self-screening in outpatients is either not IBD specific [1,27,28] or has not been conducted in the UK population [22].

The present study aimed to assess feasibility (completion time and ease of use) and validity of 'MUST'-P compared to risk classification obtained by healthcare professional administered 'MUST' ('MUST'-HCP) in IBD outpatients. This research has the potential to improve patient care by contributing to the identification of a risk of malnutrition, which has an impact not only on the disease related complications, but also on healthcare costs [30]. Nutritional support to treat malnutrition may improve symptoms and allow deficiencies in calories as well as macro- and micronutrients to be rectified [18].

Materials and methods

Study design and population

The present study comprised a feasibility and validity study [31]. Eighty-three patients in the adult IBD outpatient clinic at University College London Hospital (UCLH) were approached from the waiting area using

convenience sampling over an 8-week period between May 2015 and July 2015. The inclusion criteria were patients with a confirmed IBD diagnosis and who were aged ≥18 years. Exclusion criteria were unwillingness or inability to provide informed consent and an inability to communicate in the English language. Patients accompanied by a relative able to translate or act as an interpreter were recruited. Every effort was made to recruit all eligible patients to minimise selection bias. However, three patients declined the invitation to participate, making the sample size 80 patients.

Ethical approval was sought from London Metropolitan University Ethics Committee and by the UCLH research and development committee. Full ethical approval was not required because the study was deemed part of service evaluation. Written informed consent was obtained from all study participants and patients were assured of confidentiality and anonymity.

Data collection

The tools utilised for the data collection were the patient-administered screening tool ('MUST'-P) followed by the 'MUST' tool completed by the researcher ('MUST'-HCP) to screen the participants for malnutrition. Using routinely collected data from electronic databases and paper medical records, information was collected on the characteristics of the patient group, including demographics (date of birth, sex); anthropometry (height, weight and weight changes); and IBD type and date of diagnosis obtained from medical records. Well-being was taken from validated tools to measure disease activity in IBD: the Harvey Bradshaw Index [32] for CD and the Simple Clinical Colitis Activity Index [33] for UC, which measures wellbeing on a five-point likert scale from 'very well' (0) to 'terrible' [4]. Referral to a dietitian subsequent to diagnosis was also obtained. Area deprivation was based on national specific data of multiple deprivation rank from 2015, a composite score including income; employment; education, training and skills; health deprivation and disability; crime, barriers to housing and services; and living environment deprivation, with one missing value because one patient's postcode could not be assigned a deprivation score [34]. The research team consisted of two qualified dietitians.

Malnutrition tools

'MUST'-P

Patients were provided with a simple instruction sheet, body mass index (BMI) chart and weight loss tables. The HCP recorded the length of time that the patient took to complete the tool. The patients were asked initially to complete the 'MUST'-P independently. The 'MUST'-P was the 'MUST' tool developed by Cawood *et al.* [27] who adapted 'MUST' for patient use in a hospital outpatient setting. The BMI and weight loss charts were used from the British Association for Parenteral and Enteral Nutrition (BAPEN) tool kit [35]. Following completion of the 'MUST'-P, the patient was asked to rate the ease-of-use of the 'MUST'-P tool on a Likert scale (very difficult to very easy) and time for completion (in minutes) was estimated by the patient.

'MUST'-HCP

The screening was completed by a trained HCP researcher using the BAPEN resources [35]. Weighing scales and a stadiometer were both available in the clinic. Patients' height and weight was measured by a trained HCP and documented in the medical notes. The patients were informed of their weight and height.

Statistical analysis

Frequencies and percentages (%) were used to describe categorical variables. Mean, SD and range (minimum and maximum) were used to describe continuous variables. Area deprivation was categorised as 'least' and 'most' by using the median of the national index of multiple deprivation rank. Risk scores from both administrations of 'MUST' were classified as low (score = 0), medium (score = 1) and high (score ≥ 2) risk, from which sensitivity and specificity was calculated. Agreement between the two tools was assessed using kappa statistics. The kappa coefficient (κ) was interpreted using the grading system of Landis and Koch (<0 = no agreement; 0–0.20 = slight; 0.21–0.40 = fair; 0.41–0.60 = moderate; 0.61–0.80 = substantial; 0.81–1 = almost perfect agreement) [36]. In sensitivity analyses, we examined whether patient characteristics; age (young versus old); sex (men versus women); and IBD duration (short versus long) would influence agreement between 'MUST'-P and 'MUST'-HCP.

Differences in demographic variables by IBD status (CD versus UC) are presented as the mean (SD) for normal continuous data and *n* (%) for categorical data, and were tested using *t*-tests and chi-squared tests, respectively. $P < 0.05$ (two-tailed) was considered statistically significant. Statistical analysis was conducted using STATA, version 14 (StataCorp, College Station, TX, USA).

Results

Study population

Table 1 shows the demographic and clinical characteristics of the 80 IBD patients who participated in the present study. Overall, the study sample consisted of 51.2%

Table 1 Demographic and clinical characteristics of the study participants (total $n = 80$)

Characteristic	UC, % (*n*) 36.2 (29)	CD, % (*n*) 61.3 (49)*	Comparison of UC and CD, *P* value	IBD-U 2.5 (2)	Total IBD cohort ($n = 80$)
Age: mean (SD) years	43.1 (16.2)	37.8 (14.6)	0.14	45 (5.7)	39.9 (15.1)
Sex (*n*,%)			0.91		
Female	14 (48.3)	23 (46.9)		2 (100.0)	39 (48.8)
Male	15 (51.7)	26 (53.1)		0 (0.0)	41 (51.2)
Time subsequen to diagnosis (*n*, %)			0.90		
≤ 10 years	17 (58.6)	28 (57.1)		2 (100.0)	47 (58.8)
>10 years	12 (41.4)	21 (42.9)		0 (0.0)	33 (41.2)
Well-being (*n*, %)†			0.80		
0 (very well)	11 (37.9)	20 (40.8)		1 (50.0)	32 (40.0)
1 (slightly below average)	17 (58.6)	22 (44.9)		0 (0.0)	39 (48.8)
2 (poor)	1 (3.5)	4 (8.2)		0 (0.0)	5 (6.2)
3 (very poor)	0 (0.0)	2 (4.1)		1 (50.0)	3 (3.8)
4 (terrible)	0 (0.0)	1 (2.0)			1 (1.2)
Height (m) mean (SD)	1.71 (0.09)	1.71 (0.08)	0.75	1.54 (0.11)	1.71 (0.09)
Weight (kg) mean (SD)	81.7 (20.9)	74.2 (19.5)	0.12	50.1 (9.3)	76.3 (20.4)
BMI (kg m^{-2}) mean (SD)	27.6 (6.0)	25.3 (5.8)	0.10	20.9 (1.0)	26 (5.89)
Area (*n*, %) Deprivation‡			0.01		
Most deprived	23 (79.3)	24 (50.0)		1 (50.0)	48 (60.8)
Least deprived	6 (20.7)	24 (50.0)		1 (50.0)	31 (39.2)

Data are presented as the mean (SD), *n* (%), using an unpaired *t*-test and a chi-squared test to test for differences by IBD group. P-values represent differences between subgroups ulcerative colitis (UC) and Crohn's disease (CD) only.

*Including Crohn's colitis.

†Well-being variable was categorised as very well (score 0) versus all other scores (1–4) when compared by IBD group using the chi-squared test.

‡Area deprivation variable includes $n = 1$ missing value.

BMI, body mass index; IBD, inflammatory bowel disease.

males and the mean (SD) age of participants was 39.9 (15.1) years (range 19–84 years). The majority of the participants $n = 49$ (61.3%) had CD. No demographic or clinical characteristics were significantly different by IBD status except area deprivation, where those with CD were least likely to live in a deprived area compared to UC patients ($P = 0.01$). However, there was a nonsignificant trend towards a lower BMI in the CD versus UC group. In total, one UC patient had active disease and three CD patients had active disease (two mild and one moderate).

Agreement between 'MUST'-P and 'MUST'-HCP screening

Of the eighty IBD patients included in the study, three patients (3.8%) refused to complete the 'MUST'-P for the following reasons: one because of eye sight difficulties, one considered that it should be conducted by a HCP and one did not state a reason. Thus, the total sample size included for agreement analysis of 'MUST'-P and 'MUST'-HCP is $n = 77$.

There was 100% sensitivity for patients who were at medium or high risk using the 'MUST'-P tool compared to the 'MUST'-HCP tool. However, specificity was somewhat lower in that two were scored as medium risk and 15 patients scored as high risk using 'MUST-P', whereas they were scored as low risk using 'MUST'-HCP. Overall, this meant that there was moderate agreement between the 'MUST'-P and 'MUST'-HCP scores as determined by the kappa statistic ($\kappa = 0.486$, $P < 0.001$). We found no evidence that agreement between 'MUST'-P and 'MUST'-HCP was affected by stratification by age, sex or IBD duration.

Ease of use and time to complete 'MUST'-P

Overall, 51.9% ($n = 40$) of patients' reported the completion of 'MUST'-P as easy, with 40.2% ($n = 31$) rating it as very easy, 6.5% ($n = 5$) as difficult and 1.3% ($n = 1$) as very difficult. The mean (SD) time for the completion of the questionnaire was 3.1 (1.8) min (range 1–10 min).

Prevalence of malnutrition assessed by 'MUST'-P

A comparison of the risks of malnutrition as identified by the patients themselves and the researcher is shown in Table 2. There was 100% agreement between 'MUST-P and 'MUST'-HCP for all patients with medium and high risk of malnutrition. However, this reduced to 74.3% agreement with the 'MUST'-HCP score in the low-risk

Table 2 Comparison of malnutrition risks as identified by the 'patient self-administered 'MUST' ('MUST'-P) and the healthcare professional administered 'MUST' ('MUST'-HCP) (total $n = 77$)

		Malnutrition risk by 'MUST'-P						
		Low		Medium		High		Total
		n	%	*n*	%	*n*	%	*n*
Malnutrition risk by MUST-HCP	Low	49	74.2	2	3.0	15	22.7	66
	Medium	0	0.0	6	100.0	0	0.0	6
	High	0	0.0	0	0.0	5	100.0	5
Total		49	63.6%	8	10.4%	20	26.0%	77

category. This was a result of 17 discrepancies with low-risk categories, mostly associated with difficulty reading the BMI chart 22.7% ($n = 15$) and 3% ($n = 2$) were related to the weight loss score.

The proportion of participants with medium- and high-risk scores of malnutrition was explored using the 'MUST'-HCP. The results show similar proportions of the sample in the medium- and high-risk malnutrition categories: 8.8% ($n = 7$ patients) at medium risk and 6.3% ($n = 5$ patients) at high risk of malnutrition when screened by the researcher. Of the patients in the study at high risk of malnutrition, two out of five had not been referred to a dietitian subsequent to diagnosis and one out of five had seen a dietitian but did not arrange a follow-up. In total, 50 patients (62.5%) had seen a dietitian subsequent to diagnosis. The majority of patients (91.3%) had a BMI score 0 in the initial part of the 'MUST'. Seventy-one patients (88.8%) had minimal weight loss (≤5%) in the past 6 months and all the patients (100%) were not acutely ill when completing the study.

Outcomes of the three steps of 'MUST' used by the researcher to identify malnutrition

For the 80 patients screened, the 'MUST'-HCP identified that 85% ($n = 68$) 8.8% ($n = 7$) and 6.3% ($n = 5$) had a low, medium and high risk of malnutrition, respectively. Some 91.3% ($n = 73$) of patients had a low-risk BMI, 3.8% ($n = 3$) had a medium-risk BMI and 5% ($n = 4$) had a high-risk BMI. In addition, 85% ($n = 68$) of patients had no weight loss. Of the 15% with weight loss, 88.8% ($n = 71$) had <5%, 8.8% ($n = 7$) had 5–10% and 2.5% ($n = 2$) had >10% weight loss. None of the patients were considered acutely unwell. One patient at medium risk and one patient at high risk using 'MUST'-HCP had moderately active disease.

Discussion

Overall, the results of the present study showed that 'MUST'-P can be used to capture medium and high risks of malnutrition in the IBD outpatient setting. If accurately implemented, this could be included in patients' nutritional assessments. This bridges a gap in knowledge because there is limited research to date exploring use of self-screening in IBD outpatients, particularly from UK-based studies.

Accuracy of tool and ease of use of 'MUST'-P

Patient self-screening has been found to be an easy and well accepted tool, generating precise measurements compared to those made by a HCP [25]. The present study found a moderate agreement between 'MUST'-P and 'MUST'-HCP ($\kappa = 0.486$, $P < 0.001$), such that 100% of IBD patients with medium and high risk of malnutrition were identified by the patient and the HCP, providing confidence in using a patient-administered tool.

However, 17 'MUST'-P related discrepancies were identified, mainly relating to difficulty reading the BMI chart. In addition, there was no influence of age, sex and IBD duration on agreement between 'MUST'-P and 'MUST'-HCP. Other studies have found the discrepancies between HCP and patient self-screening were mostly associated with the weight loss and BMI score [22,27]. The use of mobile technology for calculating 'MUST' scores could help facilitate the implementation of 'MUST'-P by improving its accuracy and ease of use for patients, thus improving compliance. McGurk *et al.* [25] investigated 'MUST' self-screening using digital technology to calculate BMI in a gastroenterology outpatient clinic. All patients were able to self-screen and there was perfect agreement in test–retest reliability between the patient and dietitian, suggesting that use of digital screening may produce more accurate results.

Based on previous published studies, with the exception of reports from McCurk *et al.* [25], the majority of IBD patients reported the completion of 'MUST'-P as being either easy or very easy. The present study is consistent with previous findings by Sandhu *et al.* [22] where 96% of IBD patients rated self 'MUST' screening as being either easy or very easy to understand and complete.

The present study used a patient friendly version of 'MUST' adapted from Cawood *et al.* [27]. In our study, the mean (SD) time for completion was 3.1 (1.8) min (range 1–10 min) and 100% completed the tool in 5 min or less. Cawood *et al.* [27] found that 75% of 205 outpatients were able to screen themselves in less than 5 min and rated the self-screening as easy or very easy. In a Canadian study [22] of 154 IBD adult outpatients, all patients were able to self-screen and 96% reported the tool as being either easy or very easy to use. Cawood *et al.* [27] observed that the overall prevalence of malnutrition (medium and high risk) was similar between self-screening (19.6%) and HCP screening (18.6%), which correlated well with our study findings.

Prevalence of malnutrition

The present study suggests that the prevalence of malnutrition in the IBD outpatient setting at UCLH is low compared to other published studies [13–16]. This is possibly enhanced via the close monitoring by an IBD multidisciplinary team. However, because of the small size in the present study, these results should be viewed with caution. When screened by the HCP, the majority of patients (85%) were at low risk of malnutrition, with 8.8% and 6.3% of the sample being at medium and high risk, respectively. Seventy-one patients reported less than 5% of weight loss in the last 6 months and had a low-risk BMI.

Few studies to date have specifically looked at prevalence of malnutrition in IBD outpatients. Vadan *et al.* [15] found that 59.3% of 30 patients attending a gastroenterology clinic in Bucharest were malnourished, whereas, in a UK-based study [29], there was a high prevalence of malnutrition identified in general gastroenterology outpatients using different tools including 'MUST'. Interestingly, in the present study, the mean BMI score indicated the UC patients were overweight (mean BMI = 27.6 kg m^{-2}) and CD patients were at the upper end of the healthy weight range (mean BMI = 25.3 kg m^{-2}). Obesity and increased fat mass are both associated with elevated inflammatory markers and a more severe disease course in CD patients [37,38]. Although 'MUST' is able to detect higher proportions of a risk of malnutrition compared to BMI alone, basic anthropometry is insufficient to differentiate fat mass and lean body mass. In a prospective controlled study among IBD patients, despite 74% of IBD patients having a normal BMI, handgrip strength and lean body mass was impaired in both CD and UC patients [12]. More than half of IBD patients were found to have muscle mass depletion despite a normal BMI [39] because IBD not only causes weight change, but also alters body composition. Assessment of body composition in addition to simple anthropometry would better indicate nutritional status in IBD patients.

Specific micronutrient deficits, loss of body cell mass and muscle strength often persist even in disease remission and would not be detected by standard malnutrition screening alone [12]. In the IBD cohort, it may not be possible to fully evaluate a risk of malnutrition based solely on malnutrition screening as a result of the complex nature of the disease.

Bioelectrical impedance analysis (BIA) is a measure of body composition that can be used to differentiate between fat and fat free mass and is also a predictor for nutritional status [39]. BIA is used in clinical settings because it is considered to be non-invasive, no technical skill is required and it is comfortable for patients compared to other methods. However, BIA is expensive and time consuming and, as a result of time and staffing constraints in a busy outpatient setting, a more economic and practical measurement of body composition is required.

Tricep skinfold thickness (TSF) is the most frequently used method for assessment of body composition because it is cheap and feasible. Body fat can be predicted by the sum of skinfold thickness from different parts because the total body fat correlates with subcutaneous fat [40]. TSF has been found to correlate well with BIA in a study that evaluated the body fat estimated by BIA and TSF on 348 undergraduate students and it was concluded that the anthropometric method can surrogate fat mass percentage and assess body fat when BIA is unavailable [41]. The addition of TSF may be useful to support 'MUST' in identifying a risk of malnutrition in the IBD patient cohort. However, the acceptability of this additional measure in the IBD patient group would require further testing in clinical practice.

Implications

Implementing 'MUST'-P could potentially reduce the workload demands on HCPs to screen patients for the identification of a risk of malnutrition with respect to patients in the outpatient setting. Furthermore, the use of self-screening has the capacity to promote patient involvement in their own care. However, as a result of the complex nature of IBD, there are concerns that using a generic malnutrition screening tool may not capture all patients at risk of malnutrition. It may be that screening in the community is a more appropriate setting for 'MUST' where rates of under-recognised and under-treated malnutrition are known to be high [35]. Patients could be advised to use the web-based malnutrition self-screening tool based on 'MUST developed and available on the BAPEN website [35], which is designed to help adults to identify their own risk of malnutrition in the community.

Recommendations for further research

To be able to generalise these findings to the wider IBD population, larger studies are required in different UK hospital outpatient settings.

The use of HCP led focus groups could be used to explore perceptions of 'MUST'-P and help to identify the potential barriers and facilitators of its use develop the tool further and improve its accuracy and validity. To enable successful implementation of 'MUST'-P in the outpatient setting, appropriate and practical malnutrition care pathways would need to be developed so that those identified as malnourished are appropriately managed and treated. However, dietetic resourcing available for those patients identified at high risk may be a limiting factor.

Limitations

Test–retest reliability was performed both by Cawood *et al.* [27] and McCurk *et al.* [25] aiming to compare the accuracy of two different self-screening scores. Similar to the work of Sandhu *et al.* [22], the present study did not perform test–retest reliability because there would be a short duration of time between baseline 'MUST'-P and repeat screening and it is highly likely the patients would recall their baseline score, potentially introducing reporting bias. Only three patients approached refused to complete 'MUST'-P, indicating a high response rate. The sample size of 80 compares favourably to other studies in IBD cohorts [20]. A limitation of the validity of the study was, that because of the low numbers of patients with active disease, it was not possible to assess whether there was a significant relationship between disease activity and 'MUST' score. The results of the present study correlate well with a previous larger study in a similar patient cohort [22]. However, the results of our study cannot be generalised to the wider population because of the small sample size, which was restricted to a single UK-based large tertiary hospital.

Conclusions

The findings of the present study confirm previous findings suggesting that 'MUST'-P is a quick and easy method of nutritional screening for use in a busy outpatient setting. Moderate agreement was found between 'MUST'-HCP and 'MUST'-P, with the strongest agreement for medium- and high-risk patients. Although the overall malnutrition rates were found to be low, not all patients recognised as being at high risk of malnutrition by 'MUST'-HCP were referred to the dietitian. Furthermore, as a result of the complexity of nutritional issues specific to IBD patients, the use of a generic tool may risk missing patients considered as low risk that may still require nutritional intervention. To ensure all nutritionally at risk patients are identified, it is recommended that this tool is combined with measurement of body composition and consideration of micronutrient serum levels. Frequent and regular nutritional screening in all healthcare settings will allow a risk of malnutrition to be identified early and be prevented or treated appropriately.

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 interest, source of funding and authorship

The authors declare that they have no conflicts of interest.

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KK, SB and AM were responsible for the study conception and design. SZ-O was responsible for data collection. KK, SZ-O and PSP were responsible for data analysis. AM and PSP were responsible guidance on interpretation of the data. KK was responsible for interpretation of data. KK, SZ-O and PSP were responsible for the drafting of the paper. SB, KK and AM were responsible for the editing the paper. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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GASTROINTESTINAL DISORDERS

The economic burden of gluten-free products and gluten-free diet: a cost estimation analysis in Greece

S. Panagiotou & M. D. Kontogianni

Department of Nutrition & Dietetics, School of Health Sciences and Education, Harokopio University, Athens, Greece

Keywords
coeliac disease, cost, economic burden, gluten-free diet, gluten-free products.

Correspondence
M. D. Kontogianni, Department of Nutrition & Dietetics, School of Health Sciences and Education, Harokopio University, El. Venizelou 70, 17671 Kallithea, Greece.
Tel.: +30 210 9549359
Fax: +30 210 9549141
E-mail: mkont@hua.gr

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Abstract

Background: Adherence to a gluten-free diet (GFD) demonsrates various difficulties, including the high cost of the diet. The present study aimed to (i) compare the cost of gluten-free products (GFP) from supermarkets and pharmacies with the cost of their conventional counterparts and (ii) estimate the weekly economic burden of a GFD.
Methods: The prices of all food products labelled as 'gluten-free' available at four supermarket chains in Athens, as well as the prices of all similar conventional food products, were recorded. The prices of the pharmacy GFP were recorded from the official list of the National Health Service Organisation. For every product, the price per 100 g was calculated. All products were classified into 24 categories, which consisted of three subcategories: conventional, supermarket GFP and pharmacy GFP. Three weekly menus were designed for children, adolescents and adults, selecting the upper levels of energy intake, to cover the majority of the patients. For all three weekly menus, the price difference between conventional and GFP, both from supermarkets and pharmacies, was calculated.
Results: Compared with conventional food products, all supermarket GFP, except for one, were more expensive by 22–334% ($P < 0.05$) and all pharmacy GFP were more expensive by 88–476% ($P < 0.05$). The weekly economic burden of a GFD ranged from €12 to €28 per week, depending on age and GFP place of purchase.
Conclusions: The present study confirms the higher cost of GFP compared to their conventional equivalents in Greece, leading to a weekly economic burden for people on a gluten-free diet.

Introduction

Coeliac disease is a common autoimmune enteropathy triggered by the ingestion of gluten that affects genetically predisposed individuals. Gluten is a generalised term that describes the storage proteins found in wheat, rye and barley. Coeliac disease is estimated to affect approximately 1% of the world population [(1)]. However, the vast majority of the patients remain undiagnosed as a result of variation in the range and severity of symptoms that a patient experiences. The only available treatment for coeliac disease is lifelong adherence to a gluten-free diet (GFD), which excludes all foods that contain wheat, rye and barley, as well as their derivatives. Such a diet includes foods that are naturally gluten-free (e.g. fruits, vegetables, unprocessed meats, dairy products) and specially manufactured gluten-free products (GFP) (e.g. bread, pasta, flour), in which gluten-containing grains are substituted by alternative gluten-free grains.

According to the existing literature, patients with coeliac disease encounter several barriers regarding compliance with the GFD and cost has been reported as one of these [(2)]. A recent study found that up to 77% of the patients are concerned with the cost of the GFD [(3)]. The high cost of such a diet is basically a result of the high cost of the gluten-free manufactured products. Moreover, a study conducted in the USA found that GFP were more expensive than their gluten-containing versions by two- to

three-fold [4]. A similar study conducted in Canada, estimated that GFP were 242% more expensive on average than regular products [5], whereas, in a study conducted in the UK, GFP cost 2–518% more than their standard versions [6]. Additionally, more recent data from the UK showed that GFP were at least four times more expensive than their regular alternatives [7]. Finally, a recent study from Austria found that the cost of all analysed GFP was 205–267% higher than that of the conventional foods [8].

In Greece, specially manufactured GFP were for many years almost exclusively provided by pharmacies, whereas their current availability in supermarkets is increasing constantly. Until now, no data have been published regarding the cost of GFP available in the Greek market. Therefore, the present study aimed to (i) compare the cost of GFP from Greek supermarkets and pharmacies with the cost of their standard versions and (ii) estimate the patient's economic burden caused by compliance with the GFD.

Materials and methods

Stores

The prices of all the GFP available in pharmacies were recorded from the official list of the National Health Service Organisation, according to which all pharmacies adapt the selling prices of the GFP and the appropriate reimbursement to patients is calculated. Regarding supermarkets, food prices were collected from three major supermarket chains in the region of Attica. Attica is the most populous region in Greece, including urban and semi-urban areas, and it represents a large part of the Greek population. The three supermarket chains were selected not only as a result of the wide variety of GFP that they merchandise, but also because they have a large branch network both within the region of Attica and all over Greece, which entails greater access for patients and competitive prices. In addition, the prices of the products for every supermarket chain are not differentiated from store to store, regardless of the area where each store is located, and thus this reflected the economic burden of the GFD for a significant proportion of patients with coeliac disease throughout the entire country. Finally, prices were recorded from a small delicatessen chain in the same region that sells a wide variety of GFP, many of which could not be found in the other supermarkets. The survey took place between January and July 2015.

Food items

The prices of all the products labelled as 'gluten-free', as well as the prices of all the similar conventional food products, were collected. More specifically, a nutritionist reviewed all ingredients labels for sources of gluten to classify the products in the appropriate category. In total, prices for 24 food categories were recorded. The main food categories included savoury pastries, cereals, flours and pasta, processed meat products and sweets. Each category consisted of three subcategories: conventional products, supermarket GFP and pharmacy GFP.

Diet cost calculation

Three weekly dietary menus were designed, one for a child, one for an adolescent and one for an adult, according to the principles of a healthy diet (50% carbohydrates, 20% protein, 30% fat), based on the dietary guidelines for Greeks [9]. For each age group, the upper level of energy intake was chosen to estimate the maximum economic burden of each group. The daily energy intake of each diet was defined as:

- Child's menu [8.368 MJ (2000 cal)]: according to the recommended daily energy intake for a 9–10-year-old boy, who has an average weight of 30 kg and a moderate physical activity level [10].
- Adolescent's menu [14.225 MJ (3400 cal)]: according to the recommended daily energy intake for a 17–18-year-old boy, who has an average weight of 68 kg and moderate physical activity level [10].
- Adult's menu [10.878 MJ (2600 cal)]: according to the daily energy intake, which was calculated by using the Schofield equation for a 30–59-year-old man, who has an average height of 1.75 m, a normal body mass index (body mass index = 24.5 kg m^{-2}) and moderate physical activity level (PAL = 1.5).

All three menus included foods that originally contain gluten. First, the weekly cost of the conventional versions of these gluten-containing foods was calculated for each menu separately. Subsequently, all gluten-containing products were replaced by their gluten-free versions, and the weekly cost was re-calculated, both for GFP purchased from supermarkets and from pharmacies. The extra cost of a GFD (which in the first case included pharmacy available GFP and in the other case supermarket GFP) per week was equal to the difference of the overall weekly cost of GFP from the overall weekly cost of their standard versions and was calculated in €/week.

Statistical analysis

For each product, the price per 100 g was calculated for purposes of comparison. For each one of the three subcategories of every food category, the median of the prices per 100 g was calculated. Because of the wide variety of products and food prices, the median was selected as a measure of central tendency because the median is not influenced

by extreme values. The three medians of each food category were compared in pairs by conducting a Mann–Whitney *U*-test in SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA). $P \leq 0.05$ was considered statistically significant.

Ethical standards disclosure

No ethical approval was required for the study given its design.

Results

Cost of gluten-free products

All supermarket GFP, except for one (pasta sauce), were from 22% up to 334% ($P < 0.05$) more expensive compared to their standard counterparts. Regarding the pharmacy GFP, all were more expensive compared to their standard counterparts by 88–476% ($P < 0.05$) (Tables 1 and 2).

Concerning cereal-based foods, the gluten-free version was more expensive than the conventional one in every single case, both in supermarkets and pharmacies. For the vast majority of the available supermarket cereal-based foods, the gluten-free version was significantly more expensive than the standard counterpart. For example, standard bread cost a median of €0.30 per 100 g compared to €1.30 per 100 g for gluten-free bread, representing a 334% higher cost. Regarding the pharmacy, almost all gluten-free foods that are originally cereal-based were found to be significantly more expensive than the conventional versions. Almost all of the statistically nonsignificant results, both from supermarkets and pharmacies, were a result of the small sample size of the gluten-free versions, which, in most cases, consisted of only one product (Table 1).

For all noncereal-based foods, except for one (pasta sauce), every gluten-free version had a higher cost than the conventional one. From a total of twelve noncereal-based gluten-free foods that were available at the supermarket, six of them differed significantly in price compared to their standard counterparts. In pharmacies, no noncereal-based GFP were found (Table 2).

When comparing the prices of GFP available at supermarkets with the prices of similar products available at pharmacies, all products, excluding two (crackers and pizza base), were more expensive at the pharmacy by 17–92%. Nevertheless, the difference in price was significant only for four products (bread, flour, pasta and chocolate wafer). These products were from 33% up to 92% more expensive at the pharmacy (Table 3).

Weekly economic burden of gluten-free diet

Table 4 refers to the weekly estimated amount of money that is spent on conventional foods containing gluten, as well as on GFP purchased at a supermarket and at a pharmacy, for children, adolescents and adults, accordingly. The maximum economic burden for children who follow a GFD was estimated at €15.38 per week for purchases from pharmacies. Similarly, the maximum economic burden of a GFD for adolescents and adults was estimated at €27.84 per week and €23.80 per week, respectively, for purchases of pharmacy GFP (Table 4).

Table 1 Cereal-based products cost per 100 g

	Conventional				Supermarket GFP						Pharmacy GFP					
Product	*N*	Median (€)	Q_1 (€)	Q_3 (€)	*N*	Median (€)	Q_1 (€)	Q_3 (€)	% diff	*P*	*N*	Median (€)	Q_1 (€)	Q_3 (€)	% diff	*P*
Bread	80	0.30	0.22	0.44	20	1.30	1.04	1.62	334	<0.001	8	1.72	1.63	2.07	476	<0.001
Sliced bread	45	0.40	0.35	0.45	5	1.25	1.24	1.68	212	<0.001	3	1.38	–	–	246	0.004
Crackers	115	0.72	0.52	1.01	6	2.25	1.69	3.96	213	<0.001	19	1.39	1.15	2.42	93	<0.001
Pizza base	3	0.41	0.30	-	5	1.60	0.97	2.12	286	0.025	1	1.60	–	–	286	0.180
Pizza	33	0.92	0.82	1.20	4	1.85	1.24	1.94	102	0.005	0	–	–	–	–	–
Flour	75	0.15	0.12	0.20	10	0.50	0.43	0.54	241	<0.001	10	0.75	0.59	1.07	408	<0.001
Pasta	191	0.21	0.17	0.26	37	0.63	0.42	0.73	198	<0.001	28	1.21	1.20	1.76	472	<0.001
Cereal	47	0.95	0.66	1.09	10	1.17	0.86	1.21	27	0.136	6	1.59	1.16	1.95	88	0.001
Cereal bars	46	2.06	1.79	2.38	34	2.80	2.08	3.14	36	<0.001	0	–	–	–	–	–
Chocolate wafer	21	1.03	0.81	1.17	2	1.80	–	–	75	0.101	3	2.48	–	–	141	0.006
Cookies	44	0.81	0.71	1.22	17	2.11	1.91	2.62	162	<0.001	19	2.48	1.73	3.12	208	<0.001
Cake	37	0.78	0.66	0.82	0	–	–	–	–	–	11	2.43	2.26	2.57	214	<0.001

% diff, price differential (%) compared to regular product; €, cost in euros; GFP, gluten-free products; *N*, sample size; *P*, *P*-value; Q_1, first quartile; Q_3, third quartile.

Table 2 Noncereal-based products cost per 100 g

	Conventional				Supermarket GFP					
Product	*N*	Median (€)	Q_1 (€)	Q_3 (€)	*N*	Median (€)	Q_1 (€)	Q_3 (€)	% diff	*P*
Oats	4	0.49	0.26	0.61	1	0.73			48	0.157
Ham	10	1.39	1.10	1.58	12	1.61	1.38	1.77	16	0.210
Turkey ham	21	1.54	1.32	1.73	22	1.74	1.49	1.85	13	0.076
Luncheon meat	5	0.76	0.63	0.77	11	0.93	0.86	1.18	22	0.017
Sausage	12	0.98	0.79	1.08	4	1.17	0.93	1.32	19	0.144
Chocolate, plain	45	1.53	1.21	2.08	11	2.28	2.09	2.33	49	0.001
Chocolate, with added ingredients	81	1.30	1.16	1.51	10	2.28	2.08	2.33	75	<0.001
Bouillon cubes	12	1.52	1.33	1.52	7	3.94	2.82	4.53	160	<0.001
Pasta sauce	30	0.63	0.46	1.28	27	0.56	0.47	1.05	−11	0.737
Soy sauce	15	1.86	1.33	2.10	2	3.17	–	–	71	0.037
Baking powder	3	0.55	–	–	1	3.02	–	–	453	0.180
Beer	13	0.30	0.26	0.40	2	0.81	–	–	170	0.027

% diff, price differential (%) compared to regular product; €, cost in euros; GFP, gluten-free products; *N*, sample size; *P*, *P*-value; Q_1, first quartile; Q_3, third quartile.

Table 3 Differences in the cost of gluten-free products available at pharmacies compared to those available at supermarkets

Product	% difference	*P*-value
Bread	33	0.003
Flour	49	0.001
Pasta	92	<0.001
Cereal	49	0.064
Cookies	17	0.136
Chocolate wafer	38	0.008
Crackers	−38	0.121
Pizza base	0	>0.999

Discussion

The present study is the first attempt to investigate the cost difference of the GFP that are sold in the Greek market compared to their conventional counterparts, as well as to evaluate the cost of complying with a GFD in Greece. According to present data, almost all GFP were more expensive than their conventional counterparts and, additionally, GFP available in pharmacies were generally more expensive than those available in supermarkets. Furthermore, the weekly economic burden of the GFD ranged from €12 to almost €28 depending on the age group and the place from where GFP are purchased (supermarkets versus pharmacies).

Concerning the cost of GFP, the results of the present study are consistent with the results of similar studies previously reported in the literature (Table 5). Specifically, a study conducted in the UK [6] demonstrated the cost of GFP being higher than that of regular foods by 2–518%. In another study conducted in Chile, the average price difference between GFP and conventional foods was estimated to be approximately 300%, with a considerable range, from 85% to 1530% [11]. These two studies were the only available ones that (similarly to the present study) included both cereal-based and noncereal-based

Table 4 Weekly economic burden of a gluten-free diet

Age group	Food products	Weekly cost (€/week)	Weekly economic burden of gluten-free diet compared to normal diet (€/week)
Children	Conventional products containing gluten	5.91	–
	Supermarket gluten-free products	18.03	+12.12
	Pharmacy gluten-free products	21.29	+15.38
Adolescents	Conventional products containing gluten	13.72	–
	Supermarket gluten-free products	37.28	+23.56
	Pharmacy gluten-free products	41.56	+27.84
Adults	Conventional products containing gluten	11.56	–
	Supermarket gluten-free products	29.77	+18.21
	Pharmacy gluten-free products	35.36	+23.80

€, cost in euros.

Table 5 Evidence table

Article	Citation	Country	Types of gluten-free products	Venues	Results
1	Lee *et al.* [4]	USA	Cereal-based	Regular grocery stores Upscale markets Health food stores	Generally, gluten-free products were more expensive than regular products by 240%
2	Stevens & Rashid [5]	Canada	Cereal-based	General grocery stores	On average, gluten-free products were 242% more expensive than regular products
3	Singh & Whelan [6]	UK	Cereal-based Noncereal-based	Quality supermarkets Regular supermarkets Budget supermarkets Health food shops Corner shops	Gluten-free products were more expensive than regular products by 2–518%
4	Missbach *et al.* [8]	Austria	Cereal-based	Supermarkets	Gluten-free products were more expensive than regular products by 207–267%
5	Lambert & Ficken [12]	Australia	Cereal-based	Supermarkets	Gluten-free products were more expensive than regular products by 316–574%
6	Panagiotou & Kontogianni [28]	Greece	Cereal-based Noncereal-based	Supermarkets Pharmacies	Gluten-free products were more expensive than regular products by 22–476%

goods. In the USA [4], it was shown that GFP were more expensive than their gluten-containing counterparts by 240% on average, whereas, in Canada [5], GFP were 242% more expensive on average than regular products. In the same study, GFP was show to cost up to 1000% more than their conventional equivalents. In Austria, a recent study reported that GFP cost 205–267% more than regular products [8]. Finally, in Australia, GFP were between 316% and 574% more expensive than their regular equivalents [12]. It should be noted that the aforementioned studies investigated the extra cost of GFP found in supermarkets. In the present study, GFP available at supermarkets were 22–334% more expensive than the regular products, whereas the cost was even higher (88–476%) for GFP available at pharmacies.

The place of the specially manufactured GFP in the diet of coeliac disease patients remains controversial given their high cost and low nutritional value. Previous studies have acknowledged these GFP as poor sources of minerals (such as iron), vitamins (such as folate, thiamine, niacin and riboflavin) and fibre [13], whereas scarce and less evidenced-based information suggests that calcium, vitamin D and magnesium deficiencies are less prevalent in patients who follow a GFD including specially manufactured GFP, compared to patients who follow a natural GFD that does not include GFP [14]. Nevertheless, GFP allow patients the opportunity to access to a wider range of reliable food choices, given that, as recently reviewed by White *et al.*[15], a reduced enjoyment of food and difficulty in identifying gluten-free foods are included among the burdens associated with following a GFD in individuals with coeliac disease. Given that some GFP are enriched/fortified with vitamins and/or minerals (e.g. iron, folic acid), the choice of these products is preferred with respect to preventing the deficiencies associated with GFD noted above, and this should be emphasised during nutrition counselling.

As far as the economic burden of a GFD is concerned, in a study conducted in adult coeliac patients who lived in the UK, participants reported (by answering a questionnaire) that the perceived extra cost of a GFD was approximately €15 per week[16]. In another study conducted recently in Chile, in which the monthly cost of a conventional and a gluten-free basic food basket was calculated, it was found that the excess cost of the gluten-free basket amounted to US$88.5 per month [11]. According to the results of the present study, the economic burden of a GFD was estimated at €12–28 per week, depending on the age of the patient and the place from which the GFP are purchased, with purchases from pharmacies imposing a greater economic burden on patients. In the context of the GFD, although cereals containing gluten and their products have to be avoided, there is a wide range of other foods that are naturally gluten-free, such as fish, poultry and meats, as well as fruits and vegetables, pulses, nuts, rice, corn and potatoes, and this should be emphasised during the education of patients. In the weekly menus designed in the present study, taking also into consideration the principles of the Mediterranean diet, meals were based on poultry (two times a week), fish (two times a week), red meat (one time a week), pulses and dishes made from cooked vegetables, as is typical of the Greek cuisine. Menus also included at least five servings of fruits and vegetables per day. In these menus, the only specially manufactured GFP that were included were bread/rusks/crackers, breakfast cereals

and pasta. Moreover, in the menus for children and adolescent, small amounts of biscuits (two times a week) and pizza base were also included, acknowledging that younger patients also need some other choices popular in these ages. Hence, menus emphasised mainly natural GFP, including only a small range of specially manufactured GFP that are very widely consumed and probably important for the convenience of patients. Regarding 'minor cereals' (fonio, teff and millet) and 'pseudo-cereals' (buckwheat, Tartarian buckwheat, quinoa and amaranth grain) that have been proposed as good alternatives as a result of their increased content of fibre, iron and folic acid [17] compared to specially manufactured GFP, the decision was made not to include them in our menus because they are not locally produced and therefore their availability in the Greek market is limited and their price consequently high.

Apart from the increased prices of the specially manufactured GFP, an increased consumption of the aforementioned naturally GFP [18] has been also linked to increased nutritional costs [19] and, hence, may indirectly increase the economic burden of a GFD. Furthermore, the supplements that are usually administered to patients with coeliac disease because of the nutritional deficiencies that they often develop also add to their nutrition related cost and, along with the constant increases in taxes on food products (as part of the policy for handling the current economic crisis in Greece), this contributes to unfavourable economic conditions for coeliac disease patients.

For all of the above reasons, and given that the high cost of the GFD has been associated with a lower compliance with a GFD both in children [20] and in adults [16,21,22], many countries try to support coeliac patients either by giving them a monthly allowance or by tax deductions. In Greece, the National Health Services Organisation provides a monthly allowance only for gluten-free cereal-based products. This allowance is €100 per month for adults and €150 per month for patients under the age of 18 years; however, it is given only for purchases from pharmacies because, until recently, these products were not found in supermarkets. In Italy, patients receive vouchers up to €140 per month to buy gluten-free foods [23]. In the UK, patients receive gluten-free foods as part of their prescription for the GFD, at a heavily discounted price [23,24], whereas, in Ireland, coeliac disease patients may claim tax deductions [25]. Ireland also used to have a programme that entitled some patients to specific gluten-free foods free of charge; however, this has been discontinued [23]. Canadian patients receive tax deductions for the extra cost of gluten-free foods versus their regular counterparts [26]. Finally, Argentinian healthcare providers cover the cost of alternative flours and gluten-free mixes for patients with coeliac disease [27]. All these policies aim to ensure equal access for all patients to a set of basic gluten-free goods, regardless of their income and their economic status, and such efforts are currently very important, especially for countries such as Greece (i.e. with respect to the current economic crisis) because, otherwise, patients' strict compliance with a GFD could be dramatically hampered.

Some limitations of the present study should be taken into account. Our analysis was limited to foods labelled as 'gluten-free'. This sampling method may have excluded several foods that do not contain gluten but, unfortunately, are not labelled as such. A second limitation of the study is the small sample size of certain food categories. In some cases, only a few GFP were available (e.g. supermarket gluten-free baking powder or pharmacy pizza base, only one item was available), not allowing for a proper statistical analysis to reveal statistical significances. In addition, although the supermarket chains chosen have the largest branch network throughout the entire country, in certain remote rural areas or islands, we cannot rule out the possibility of GFP being even more expensive because of transportation costs. Finally, the weekly economic burden of patients was calculated based on a theoretical healthy dietary pattern and not on patients' actual intakes. Therefore, the data provided by the present study are a solid starting point for future analyses, which should explore the actual contribution of the GFP in patients' diet, as well as the place from where patients prefer to buy them, with the aim of achieving a reliable estimation of the actual GFD cost.

Conclusions

The present study demonstrates the higher cost of the GFP available in the Greek market compared to their conventional counterparts and highlights the economic burden imposed on coeliac disease patients. The monthly allowance given to these patients from the National Health Services Organisation is therefore necessary to cover the additional cost of a GFD and may contribute to a better compliance of the patients with the diet, as well as equal access to a set of basic GFP.

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 (see Appendix 1).

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
This research received no specific grant from any funding agency, commercial or not-for-profit sectors.
SP collected and analysed data and drafted the manuscript. MDK conceived and designed the study, interpreted the data and critically revised the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Appendix S1. STROBE Statement: checklist of items that should be included in reports of cross-sectional studies

BDA The Association of UK Dietitians
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Journal of Human Nutrition and Dietetics

PREGNANCY AND INFANT NUTRITION

Dietary epigallocatechin 3-gallate supplement improves maternal and neonatal treatment outcome of gestational diabetes mellitus: a double-blind randomised controlled trial

H. Zhang, S. Su, X. Yu & Y. Li

Department of Gynecology, The Central Hospital of Zibo, Zibo, Shandong, China

Keywords
epigallocatechin 3-gallate, gestational diabetes mellitus, glucose, hypoglycaemia, insulin, macrosomia.

Correspondence
H. Zhang, Department of Gynecology, The Central Hospital of Zibo, 54 Gongqingtuan West Road, Zibo 255000, Shandong, China.
Tel: +86-18678186036
Fax: +86-18678186036
E-mail: 15053102459@163.com

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Abstract

Background: Gestational diabetes mellitus (GDM) is an increasing prevalent health risk in pregnant women. Epigallocatechin 3-gallate (EGCG) is known to benefit the insulin secretory machinery. We aimed to investigate the effect of daily dietary EGCG supplementation on both the maternal and neonatal treatment outcomes in GDM-affected pregnancies.
Methods: In total, 472 pregnant women during their third trimester of pregnancy were diagnosed with GDM and subsequently enrolled into this trial. After exclusion, 404 patients were randomly assigned into EGCG and placebo study groups and subsequently administered either 500 mg of EGCG or placebo, respectively, on a daily basis until full term. The daily nutritional intake of all patients was monitored throughout the study. Maternal diabetic parameters at baseline and full term, including metabolism of glucose and insulin, as well as neonatal symptoms at birth, including birth weight, macrosomia, hypoglycaemia, respiratory distress and Apgar scores, were analysed.
Results: In total, 176 and 150 patients from the EGCG and placebo study groups, respectively, completed the trial. Patients from the EGCG group displayed significantly improved maternal diabetic parameters, and fewer cases of neonatal complications, compared to the placebo group.
Conclusions: Daily dietary EGCG supplement improves both maternal and neonatal treatment outcomes of GDM.

Introduction

Gestational diabetes mellitus (GDM) has become a worldwide health risk for pregnant women, with an increasing incidence every year.[1] The maternal symptoms of GDM patients are similar to the diabetic complications commonly manifested in type 2 diabetes mellitus (T2DM), including glucose intolerance and insulin resistance. Pregnant women who are diagnosed with GDM often do not shown any diabetic symptoms before pregnancy, and these symptoms only start from the second trimester of pregnancy. Even after pregnancy, approximately 5% of the GDM patients develop an increased long-term risk of diabetes.[2] The clinical symptoms of GDM are highly similar to those in T2DM, including hyperglycaemia and hyperinsulinaemia. On the other hand, besides maternal symptoms, GDM also displays an increased risk of abnormal foetal development, as well as neonatal complications, including low birth weight, hypoglycaemia, respiratory distress and macrosomia.[3,4]

Epigallocatechin 3-gallate (EGCG) is a natural compound mainly found in green tea extract and it exhibits beneficial effects in health and disease.[5] EGCG was reported to enhance the insulin secretory machinery by stimulating the activation of pancreatic β-cells in a rat model of insulinoma and chronic hyperglycaemia.[6] In

addition, EGCG could improve the insulin-stimulated glucose uptake in skeletal muscle cells.[7] Importantly, EGCG was found to improve diabetes-related symptoms, including obesity and residual albuminuria in diabetic nephropathy.[8,9] Recently, in a clinical study among adults with obesity, EGCG was even demonstrated to exert therapeutic potential in reducing risk of T2DM.[10]

To the best of our knowledge, no study has been conducted that has evaluated the effects of EGCG on pregnant GDM patients. Given the similar diabetic symptoms between GDM and T2DM, the present study aimed to investigate the use of EGCG as a dietary supplement, in addition to evaluating its effect on maternal diabetic symptoms among GDM patients, as well as their neonatal complications. We therefore designed the present randomised, placebo-controlled, double-blind clinical study in an effort to determine whether daily dietary EGCG supplement is able to improve maternal diabetic symptoms and neonatal outcomes of GDM-affected women.

Materials and methods

Ethics

The present study is an intend-to-treat analysis. The study was designed in accordance with the Declaration of Helsinki guidelines and was approved by the Ethical Committee of Central Hospital of Zibo. All participants provided their written informed consent before entering the study. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation.

Patients

From February 2013 to September 2016, a total of 472 pregnant women carrying a singleton pregnancy, aged between 25–34 years and with a diagnosis of GDM at the beginning of the third trimester (29 weeks), were recruited for the study. Patients were diagnosed with GDM using criteria in accordance with the American Diabetes Association guidelines:[11] (i) fasting plasma glucose (FPG) $\geq$ 92 mg dL^{-1}; (ii) 1-h oral glucose tolerance test (OGTT) with 75 g glucose $\geq$180 mg dL^{-1}; and (iii) 2-h OGTT $\geq$153 mg dL^{-1}.

Exclusion criteria

Sixty-eight patients were excluded who met the following exclusion criteria:(i) pre-eclampsia or eclampsia; (ii) maternal hypertension; (iii) multiparity; (iv) urinary tract infection; (v) hypo- and hyperthyroidism; (vi) liver, kidney or renal diseases; (vii) history of diabetes and/or requiring insulin therapy during the study; and (viii) habitual tea drinker.

Randomisation and treatment

After exclusion, 404 GDM patients were eligible to participate in the trial. Using a permuted-block design stratified according to the baseline 1-h OGTT results, the 404 eligible patients were randomly assigned into two study groups: (i) an EGCG group, who were instructed to consume one capsule containing 500 mg of EGCG (≥95%; Sigma Aldrich, St Louis, MO, USA) every day, and (ii) a placebo group, who were instructed to consume one capsule containing 500 mg of starch powder as placebo every day. The two types of capsules were prepared by investigators who were blind to the group assignment, and were identical in appearance to mask their contents to the patients.

Daily nutritional intake records

All patients were instructed by study investigators that: (i) they should not consume any tea drinks or any other tea-derived products, except those issued capsules; (ii) they should comply with the therapeutic healthy diet prescribed for their GDM diagnosis; and (iii) they should keep records of their daily nutritional intakes. The daily nutritional intake records were analysed using NUTRITIONIST software (Axxya, Woodinville, WA, USA), with ingredient modifications regarding Chinese food. All patients were revisited every month to assess compliance and to issue new capsule supplies for the next month.

Glucose and insulin metabolisms

A 20-mL blood sample was collected, from each patient following an overnight fasting, into a tube with 0.1% ethylenediaminetetraacetic acid. The blood sample was immediately centrifuged to separate the plasma, which was then stored at −80 °C for further analysis. Measurement of plasma glucose levels was performed using a glucometer (LifeScan Surestep; LifeScan Inc., Milpitas, CA, USA) and measurement of plasma insulin levels was performed using an enzyme-linked immunosorbent assay kit (Biovision, Inc., Milpitas, CA, USA). The quantitative insulin check index (QUICKI), homeostasis model of assessment of insulin resistance (HOMA-IR) and homeostasis model of assessment of beta cell function (HOMA-β) were performed to evaluate insulin resistance as described previously.[12] All assessments were conducted by investigators who were blind to the group assignment.

Anthropometrics

Body weight was measured with patients in light clothing and without shoes on a digital scale with an accuracy of 0.1 kg. Body height was measured with patients without

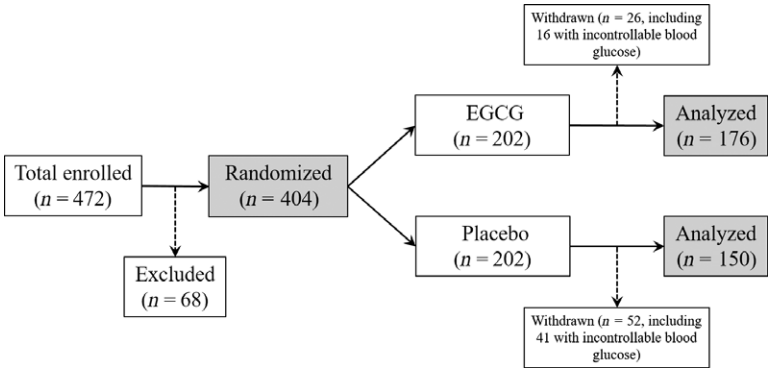

Figure 1 Flow chart illustrating the study design.

shoes standing straight on a stadiometer with the accuracy of 0.1 cm. Body mass index (BMI) was calculated as weight/square of height in (kg m^{-2}). All measurements were conducted by investigators who were blind to the group assignment.

Statistical analysis

Statistical analysis in this study was conducted using SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA). Values are calculated as the mean (SD). A two-tailed Student's *t* test was used to analyse the normally distributed data, whereas a Mann–Whitney *U*-test was used to analyse the non-normally distributed data. $P < 0.05$ was considered statistically significant.

Results

General characteristics of the gestational diabetes mellitus patients

In total, 472 pregnant women were diagnosed with GDM in the beginning of the third trimester (baseline) and were enrolled into our current trial, in which 68 patients were excluded (Fig. 1). The remaining 404 eligible GDM patients were randomly assigned to EGCG or placebo study groups, with 202 patients in each group. During the course of the study, 26 patients were withdrawn from the EGCG group: four for personal reasons, six because of noncompliance and 16 as a result of incontrollable blood glucose levels (1-h postprandial whole blood level > 140 mg dL^{-1}). On the other hand, 52 patients were withdrawn from the placebo group, with five for personal reasons, six because of noncompliance and 41 as a result of incontrollable blood glucose levels. At the end of study (full term), 176 patients from the EGCG group and 150 patients from the placebo group completed the trial and their data were analysed.

The characteristics of the GDM women from both of the two study groups are listed in Table 1. At baseline, no significant differences were observed between the two groups, in terms of age at pregnancy [29.6 (3.4) years in EGCG, 28.7 (4.5) years in placebo, $P = 0.15$] and body height [162.4 (9.8) cm in EGCG, 164.1 (8.1) cm in placebo, $P = 0.27$], or baseline body weight [62.1 (6.2) kg in EGCG, 63.2 (7.6) kg in placebo, $P = 0.39$] and BMI [25.9 (4.2) kg m^{-2} in EGCG, 26.2 (3.9) kg m^{-2} in placebo, $P = 0.52$]. At the end of study, gestational age [38.9 (1.2) weeks in EGCG, 39.6 (1.1) weeks in placebo, $P = 0.35$], body weight [66.7 (5.6) kg in EGCG, 67.2 (7.5) kg in placebo, $P = 0.41$] and BMI [26.5 (4.1) kg m^{-2} in EGCG, 27.1 (4.4) kg m^{-2} in placebo, $P = 0.38$] were also essentially the same between all patients of the two study groups.

Table 1 General characteristics of the gestational diabetes mellitus patients in the two study groups at baseline

Characteristics	EGCG (n = 176)	Placebo (n = 150)	P value
Age at pregnancy (years)	29.6 (3.4)	28.7 (4.5)	0.15
Body height (cm)	162.4 (9.8)	164.1 (8.1)	0.27
Body weight at baseline (kg)	62.1 (6.2)	63.2 (7.6)	0.39
BMI at week 0 (kg m^{-2})	25.9 (4.2)	26.2 (3.9)	0.52
Full term (weeks)	38.9 (1.2)	39.6 (1.1)	0.35
Body weight at full term (kg)	66.7 (5.6)	67.2 (7.5)	0.41
BMI at full term (kg m^{-2})	26.5 (4.1)	27.1 (4.4)	0.38

Data are the mean (SD). A two-tailed Student's *t*-test was used to analyse the normally distributed data, whereas a Mann–Whitney *U*-test was used to analyse the non-normally distributed data.
BMI, body mass index.

Daily nutritional intake of the gestational diabetes mellitus patients

Throughout the study, we also analysed the daily nutritional intakes of all patients in the two study groups (Table 2). There were no statistically significant differences in daily dietary intakes between the two groups, with regard to energy [9.03 (0.61) MJ [2158 (146)] in

Table 2 Daily nutritional intake of the gestational diabetes mellitus patients in the two study groups

Daily nutritional intake	EGCG (n = 176)	Placebo (n = 150)	P value
Energy (kcal)	2158 (146)	2231 (123)	0.31
Protein (g)	86.3 (18.6)	87.4 (19.0)	0.40
Fat (g)	77.3 (10.3)	80.3 (11.6)	0.26
Carbohydrate (g)	252.6 (35.7)	268.7 (32.9)	0.18
Dietary fibre (g)	22.6 (3.9)	24.1 (4.0)	0.36
Soluble fibre (g)	1.5 (0.2)	1.6 (0.3)	0.59

Data are the mean (SD). A two-tailed Student's t-test was used to analyse the normally distributed data, whereas a Mann–Whitney U-test was used to analyse the non-normally distributed data.

EGCG, 9.33 (0.51) MJ [2231 (123) kcal] in placebo, $P = 0.31$], protein [86.3 (18.6) g in EGCG, 87.4 (19.0) g in placebo, $P = 0.40$], fat [77.3 (10.3) g in EGCG, 80.3 (11.6) g in placebo, $P = 0.26$], carbohydrate [252.6 (35.7) g in EGCG, 268.7 (32.9) g in placebo, $P = 0.18$], dietary fibre [22.6 (3.9) g in EGCG, 24.1 (4.0) g in placebo, $P = 0.36$] or soluble fibre [1.5 (0.2) g in EGCG, 1.6 (0.3) g in placebo, $P = 0.59$].

Glucose and insulin metabolism of the gestational diabetes mellitus patients

Blood samples were collected from patients of both study groups, at both baseline and full term, to evaluate their glucose and insulin metabolisms (Table 3). From baseline to full term, FPG level of patients in the EGCG group was significantly decreased [104.6 (8.7) mg dL^{-1} at baseline, 89.3 (6.5) mg dL^{-1} at full term, $P = 0.04$], whereas it remained unchanged [104.5 (6.9) mg dL^{-1} at baseline, 105.7 (6.4) mg dL^{-1} at full term, $P = 0.31$] in the placebo group. Comparing FPG at full term between the two study groups, the EGCG group also displayed a significantly lower FPG compared to the placebo group [89.3 (6.5) mg dL^{-1} in EGCG, 105.7 (6.4) mg dL^{-1} in placebo, $P = 0.02$]. On the other hand, insulin levels exhibited very different changes from baseline to full term between the two groups, being significantly reduced in the EGCG group [15.7 (4.3) μIU mL^{-1} at baseline, 8.8 (4.9) μIU mL^{-1} at full term, $P = 0.03$], whereas it was significantly increased in the placebo group [14.9 (6.3) μIU mL^{-1} at baseline, 16.7 (4.8) μIU mL^{-1} at full term, $P = 0.04$]. Similar to FPG, at full term, the insulin level of the EGCG group was significantly lower compared to the placebo group [8.8 (4.9) μIU mL^{-1} in EGCG, 16.7 (4.8) μIU mL^{-1} in placebo, $P = 0.01$].

Next, the QUICKI score was used as an index of diabetic symptoms, which was found to be significantly increased from baseline to full term in the EGCG group [0.45 (0.16) at baseline, 0.62 (0.14) at full term, $P = 0.03$], whereas it remained largely the same in the placebo group [0.42 (0.15) at baseline, 0.31 (0.18) at full term, $P = 0.08$]. At full term, the QUICKI score in the EGCG group was also much higher compared to the placebo group [0.62 (0.14) in EGCG, 0.31 (0.18) in placebo, $P = 0.03$]. As expected, the HOMA-IR and HOMA-β scores of the EGCG group were both significantly decreased from baseline to full term [HOMA-IR: 3.8 (1.4) at baseline, 2.0 (1.6) at full term, $P = 0.02$; HOMA-β: 56.5 (19.6) at baseline, 45.4 (18.5) at full term, $P = 0.01$]; however, in the placebo group, they were both unchanged [HOMA-IR: 4.0 (1.5) at baseline, 3.8 (1.6) at full term, $P = 0.29$; HOMA-β: 55.3 (17.2) at baseline, 58.3 (21.2) at full term, $P = 0.34$]. In addition, at full term, both HOMA-IR and HOMA-β of the EGCG group were also significantly lower compared to the placebo group [HOMA-IR: 2.0 (1.6) in EGCG, 3.8 (1.6) in placebo, $P = 0.04$; HOMA-β: 45.4 (18.5) in EGCG, 58.3 (21.2) in placebo, $P = 0.02$].

Neonatal complications at birth

Besides maternal diabetic symptoms, we also analysed cases of neonatal complications (Table 4). The numbers

Table 3 Glucose and insulin metabolism of the gestational diabetes mellitus patients in the two study groups at baseline and full term

Glucose metabolism	EGCG (n = 176)			Placebo (n = 150)			Intergroup P value at full term
	Baseline	Full term	P value*	Baseline	Full term	P value*	
FPG (mg dL^{-1})	104.6 (8.7)	89.3 (6.5)	0.04	104.5 (6.9)	105.7 (6.4)	0.31	0.02
Insulin (μIU mL^{-1})	15.7 (4.3)	8.8 (4.9)	0.03	14.9 (6.3)	16.7 (4.8)	0.04	0.01
QUICKI	0.45 (0.16)	0.62 (0.14)	0.03	0.42 (0.15)	0.31 (0.18)	0.08	0.03
HOMA-IR	3.8 (1.4)	2.0 (1.6)	0.02	4.0 (1.5)	3.8 (1.6)	0.29	0.04
HOMA-β	56.5 (19.6)	45.4 (18.5)	0.01	55.3 (17.2)	58.3 (21.2)	0.34	0.02

Data are the mean (SD). A two-tailed Student's t-test was used to analyse the normally distributed data, whereas a Mann–Whitney U-test was used to analyse the non-normally distributed data.

*Comparison between baseline and full term in the same study group.

FPG, fasting plasma glucose; HOMA-IR and HOMA-β, homeostasis model of assessment of insulin resistance and beta cell function; QUICKI, quantitative insulin check index.

Table 4 Neonatal complications at birth in the two study groups

Cases of neonatal complications	EGCG (n = 176)	Placebo (n = 150)	P value
Low birth weight (< 2600 g) (n)	10	32	0.03
Hypoglycaemia (n)	4	11	0.01
Respiratory distress (n)	3	5	0.21
Macrosomia (n)	3	8	0.07
1-min Apgar	9.7 (0.4)	9.4 (0.3)	0.04
5-min Apgar	10.7 (0.6)	9.3 (0.5)	0.02

Data are the mean (SD). A two-tailed Student's t-test was used to analyse the normally distributed data, whereas a Mann–Whitney U-test was used to analyse the non-normally distributed data.

of new born with low birth weight (<2600 g) and hypoglycaemia were significantly reduced in the EGCG group compared to the placebo group (low birth weight: 10 in EGCG, 32 in placebo, $P = 0.03$; hypoglycaemia: four in EGCG, 11 in placebo, $P = 0.01$). On the other hand, we only observed a slight reduction in respiratory distress and macrosomia (respiratory distress: three in EGCG, five in placebo, $P = 0.21$; hypoglycaemia: three in EGCG, eight in placebo, $P = 0.07$). However, both 1- and 5-min Apgar scores were significantly higher in the EGCG group compared to the placebo group [1-min Apgar: 9.7 (0.4) in EGCG, 9.4 (0.3) in placebo, $P = 0.04$; 5-min Apgar: 10.7 (0.6) in EGCG, 9.3 (0.5) in placebo, $P = 0.02$].

Discussion

There have been increasing reports suggesting that dietary intervention could exert beneficial effects in GDM women. For example, 1 month of DASH (Dietary Approaches to Stop Hypertension) diet consumption, which is enriched in vegetables, fruits, whole grains and lower in saturated fatty acids, was reported to improve the pregnancy outcomes of GDM women.[13] Adherence to a healthy diet before pregnancy, such as the Mediterranean-style diet,[14] was found to improve glucose tolerance and lower the risk of GDM.[15,16] Despite the fact that diabetic symptoms such as glucose intolerance and insulin resistance are similar in both T2DM and GDM, GDM patients often face the clinical problem that pharmaceutical drugs effective for T2DM may not be safe during pregnancy. As a result, conservative therapies are usually prescribed for GDM patients, such as dietary intervention, which involves healthier food ingredients that are natural and safe. Surprisingly, even conservative dietary intervention, such as vitamin D, has been reported to be very effective in the clinical treatment of T2DM.[17] We therefore reasoned that dietary intervention might be effective against GDM, given the fact that it has been proven safe and, more importantly, successful in treating more severe diabetic symptoms in T2DM.

In the present clinical study, we have reported for the first time that the administration of 500 mg of EGCG on a daily basis for GDM patients, starting from the beginning of their third trimester of pregnancy, could greatly improve treatment outcomes of both maternal symptoms and neonatal complications. The daily dose of 500 mg of EGCG was well within the safety limit of consumption.[18] We have found that circulating glucose and insulin metabolisms of GDM-affected women are markedly improved by dietary EGCG intervention, as indicated by significantly reduced levels of plasma FPG and insulin levels. Furthermore, scores of the diagnostic index QUICKI were found to be increased, whereas HOMA-IR and HOMA-β were decreased and QUICKI was increased, in patients of the EGCG group, which clearly demonstrated a significantly improved insulin response. Taken together, these data strongly support the beneficial therapeutic effect of dietary EGCG intervention in alleviating maternal the diabetic symptoms of GDM patients.

Another interesting observation of the present study lies not in the patients who have completed trial, but rather in those who have not. Throughout the study, 57 patients had to withdraw from the conservative dietary intervention and start insulin treatment, as a result of incontrollable blood glucose levels (1-h postprandial whole blood level >140 mg dL^{-1}). Of these 57 patients, 16 (or 7.9% of 202 initial participants) were from EGCG group, whereas 41 (or 20.3% of 202 initial participants) were from placebo group. This marked difference in numbers of patients presenting severe maternal GDM symptoms indicates that EGCG may be more effective than we expected in improving glucose and insulin metabolisms.

Besides maternal outcomes, the present study also assessed the incidence rate of neonatal complications in pregnant women who were diagnosed with GDM, such as low birth weight, hypoglycaemia, respiratory distress and macrosomia. GDM patients in the EGCG group gave birth to a significantly lower number of infants with low birth weight and hypoglycaemia compared to GDM patients in the placebo group, whereas incidences of respiratory distress and macrosomia were also reduced slightly. These observations suggest that dietary EGCG supplemention for GDM women could also improve neonatal complications, at least in terms of low birth weight and hypoglycaemia.

To summarise, our current clinical trial is the first to report the potential therapeutic value of the natural compound EGCG in GDM, which is able to both alleviate maternal diabetic symptoms and reduce the incidence of neonatal complications. Studies investigating the long-

term effects of EGCG, for example from pre-pregnancy till full term, could further validate our current understanding on the beneficial effects of EGCG among GDM patients because pre-pregnancy adherence to a healthy diet has been associated with a lower incidence of GDM.[19] In addition, one limitation of the present study is that green tea is a common and popular drink among Chinese population; hence, it would be interesting and informative to perform a study involving large cohorts of pregnant women and compare the incidence of GDM between tea drinkers and those who are not.

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 conflicts of interest. No funding is declared.
HZ designed the study and wrote the manuscript. HZ, SS, XY and YL performed the experiments and analysed the data. All authors approved the final version submitted for publication.

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PREGNANCY AND INFANT NUTRITION

Breastfeeding as a public health responsibility: a review of the evidence

A. Brown

Department of Public Health, Policy and Social Sciences, Swansea University, Swansea, UK

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Correspondence
A. Brown, Department of Public Health, Policy and Social Sciences, Swansea University, Room 136 Haldane Building, Singleton Park, Swansea SA2 8PP, UK.
Tel.: +44 1792 518672
E-mail: a.e.brown@swansea.ac.uk

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Abstract

Background: Although intention to breastfeed in Western culture is high, many women stop breastfeeding before they are ready. From a physiological perspective, rates of primary milk insufficiency or contraindications to breastfeed should be low. However, numerous women encounter numerous barriers to breastfeeding, many of which occur at the social, cultural and political level and are therefore outside of maternal control. This review identifies and examines the impact of these barriers and considers how public health services should play a central role in creating a supportive breastfeeding environment.
Methods: A narrative review to synthesise themes in the literature was conducted, using Web of Science, PubMed and Science Direct. Barriers to breastfeeding at the societal rather than individual level were identified (e.g. in relation to health services, policies and economic factors). Only English language papers were included.
Results: Many barriers to breastfeeding exist at the societal rather than individual level. These influences are typically outside mothers' control. Five core themes were identified; the need for investment in (i) health services; (ii) population level health promotion; (iii) supporting maternal legal rights; (iv) protection of maternal wellbeing; and (v) reducing the reach of the breast milk substitute industry.
Conclusions: Although individual support is important, breastfeeding must be considered a public health issue that requires investment at a societal level. Focusing solely on solving individual issues will not lead to the cultural changes needed to normalise breastfeeding. Countries that have adopted a multicomponent public heath strategy to increase breastfeeding levels have had significant success. These strategies must be emulated more widely.

Introduction

Breastfeeding is established as protecting infant and maternal health [1,2] thus reducing healthcare costs through decreased use of services [3,4]. Global public health policy therefore recommends exclusive breastfeeding for the first 6 months of life, continued alongside solid foods for as long as mother and infant desire [5]. However, despite this, breastfeeding rates in many Western countries remain low. Only half of mothers in the USA and Australia are giving any breastmilk at all by 6 months, with only one-third doing so in the UK [6].

These figures cannot be explained by weak intentions. In the UK, over 80% of women want to breastfeed [7]. Moreover, early breastfeeding cessation can be associated with feelings of guilt and regret [8] and even post-natal depression [9]. Furthermore, low rates cannot be explained by a widespread primary physiological inability to breastfeed. Although some health issues, such as polycystic ovary syndrome and hypoplastic breasts, can impede milk production, partial or even full milk production is possible for some. A limited number of medications are contraindicated with breastfeeding, although there is often an alternative. These issues should, however, be statistically rare across populations [10].

Instead, it is well established that maternal experiences heavily influence breastfeeding intention, initiation and duration. Aside from physical pain and difficulties, issues with stretched professional care, negative social attitudes, body image, conflicting responsibilities and a lack of familial support have all been highlighted as barriers to breastfeeding [11–13]. Unfortunately, many of these societal factors can lead to women not breastfeeding responsively, which in turn can negatively impact upon milk supply, leading to cessation [14].

These influences are notably based on social and cultural attitudes and on values wider than the individual mother, yet many interventions aiming to improve breastfeeding focus primarily on supporting women at the medical and individual level. Although these are vital, effective and valued services [15–17], tackling issues such as pain and physical difficulty once they have arisen is only part of the solution. Instead, as in many areas of health, a preventative, public health approach to enable women to breastfeed is also needed.

It is widely recognised that our behaviour as individuals is affected by the systems and structures of the environment and society in which we live [18]. Social, economic and political factors all influence our knowledge, attitudes and ability to make healthy choices [19]. Public health recognises this and puts in place systems that give individuals the best possible chance of health, seeking to promote healthier choices and reduce the risk of illness occurring [20]. Examples of this include the prohibition of smoking in public places, adding fluoride to the water supply and removing value added tax from fresh produce. A key aspect of public health is using health promotion campaigns to raise awareness and change behaviour not just for those who issues might affect, but for all [21].

Recently, attention at a policy level has turned to the importance of taking a public health approach to support breastfeeding women. In 2016, a seminal series in the *Lancet* highlighted the importance of public health to breastfeeding success emphasising:

> The reasons why women avoid or stop breastfeeding range from the medical, cultural and psychological, to physical discomfort and inconvenience. These matters are not trivial and many mothers without support turn to a bottle of formula.[22]
>
> and
>
> The success or failure of breastfeeding should not be seen solely as the responsibility of the woman. Her ability to breastfeed is very much shaped by the support and the environment in which she lives. There is a broader responsibility of governments and society to support women through policies and programmes in the community.[23]

These quotes highlight how essential a wider public health approach is to supporting breastfeeding mothers. Although it is the mother herself who ultimately breastfeeds, her ability to do so is affected by the culture and context she lives in. Rather than considering breastfeeding solely as an individual issue that the mother is responsible for solving, public health must take broader ownership by identifying how wider issues at the societal level can be changed to also enable breastfeeding. The aim of the present review is to highlight which aspects policies and programmes need to target to affect breastfeeding at the public health level, as well as the potential mechanisms to do this.

Methods

A narrative review was conducted to synthesise themes in the literature. A literature search was conducted using Web of Science, PubMed and Science Direct, specifying dates from 1997 to 2016. Search terms included individual and combinations of milk type/process (breastfeeding; breast milk; formula feeding; formula milk; artificial feeding; infant feeding; bottle-feeding), behaviour (initiation; continuation; cessation; stopping; duration; decision; choice; reason) and influence (social; cultural; economic; political; industry; environmental; health professionals; public; public health). Studies examining wider social, cultural and economic influences upon breastfeeding that could be targeted at a public health level were included, whereas research that examined physiological properties of breast milk, health impacts and physiological complications/impediments was excluded. Studies were limited to the English language. Both quantitative and qualitative studies were included because women's experiences are an important body of work in this area.

Results

Five core themes were identified; the need for investment in (i) health services provision; (ii) population level health promotion; (iii) supporting maternal legal rights; (iv) protection of maternal wellbeing; and (v) reducing the reach of the breast milk substitute industry. The importance of these within wider social, economic and cultural communities is considered.

1. Investment in health services provision

The health system in which a woman gives birth in affects her ability to breastfeed. This is not simply related to services supporting breastfeeding; maternal experiences during pregnancy, birth and post-natal care can all impact upon breastfeeding intentions and behaviour at both a physiological and psychological level.

Antenatal education

Good quality antenatal breastfeeding education is associated with a longer breastfeeding duration [24]; however, many women report that their experience of antenatal education in relation to breastfeeding is lacking [25]. Often breastfeeding education does not prepare women to breastfeed; it highlights benefits but does not discuss the process of breastfeeding and how it may differ from formula feeding. This can lead to mothers questioning whether the behaviour of their breastfed infant in comparison to formula fed infants is wrong [26].

At the heart of providing good quality antenatal education should be an emphasis on the potential differences between breast and formula feeding. Breastfed infants feed more frequently and irregularly than formula fed infants [27], partly as a result of the faster digestion of breast milk [28]. Breastfed babies also take less milk per feed from the first day of life, leading to more frequent feeds [29]. In the early days, breastfed babies will feed more frequently at night [30], although this difference disappears by 6 months [31].

This information is not always covered in antenatal education [26], leading to concerns that breastfed infants are not receiving sufficient milk [32]. This can lead to unecessary supplementation [33] or attempts to manipulate feeding patterns, both of which can reduce milk supply [34]. Breastfed infants often lose more weight after birth and regain this weight more slowly than formula fed infants which can exacerbate concerns [35]. This difference is a common concern for breastfeeding mothers [36].

Labour and delivery

Critics have long challenged the impact of increasing medicalisation of childbirth in Western culture on maternal and infant health. Unnecessary monitoring, interventions and a lack of continuous support can lead to an increased risk of complications [37]. However, interventions during childbirth can also negatively impact on breastfeeding success. Infants born by caesarean section are less likely to be breastfed [38]. Aside from associated pain, the release of oxytocin and prolactin is weaker after a caesarean, delaying milk production [39]. Babies born by assisted delivery are less likely to be breastfed at 2 weeks, potentially as a result of bruising affecting latch [40]. Medications during labour, particularly pethidine, can also affect breastfeeding outcomes because of sub optimal rooting and latch [41]. Epidurals have also been associated with reduced breastfeeding continuation [42].

Post-natal care

High-quality post-natal care plays a critical role in breastfeeding success. Hospitals that are accredited as Baby Friendly, following the 10 steps to successful breastfeeding have higher breastfeeding rates, with the more steps a hospital follows, the better their breastfeeding outcomes [43]. Pre- and post-adoption Baby Friendly studies suggest an increase in breastfeeding rates attributed to these steps [44].

For example, infants who have skin-to-skin, placed on their mother's chest after the birth, are more likely to breastfeed, which may be explained through better latch and stronger sucking skills [45]. Keeping mother and baby together after birth is another important step. In one randomised controlled trial, only 45% of babies allocated to a hospital nursery after birth were exclusively breastfed on discharge, whereas 86% of those kept with their mother were [46]. Formula supplementation can also damage breastfeeding because it can decrease supply [47]. In one study, infants who received formula supplements in hospital were twice as likely not to be breastfeeding at 1 month compared to those who had been exclusively breastfed [48].

Change needed

Many breastfeeding issues may be preventable if women had better knowledge and enhanced support during the birth and in the post-natal period. These services may be particularly vital in neonatal care units, where infants obtain the greatest benefit from breastmilk, yet complications with breastfeeding from an infant prematurity perspective or as a result of maternal birth complications make breastfeeding more difficult [49].

Support specifically in relation to breastfeeding during the antenatal and post-natal period from well-qualified, knowledgeable staff is critical [50]. However, many women fail to receive high-quality care from the professionals who support them [51]. For many, this is not because of a lack of professional interest. Increasing demands on midwives and health visitors to care for more women, with fewer resources, leads to many professionals feeling angry and frustrated that they do not have the time to offer support. Consequently, formula can sometimes be offered as a quick solution [52]. However, not all professionals are supportive of breastfeeding; some do not value giving breastfeeding support, perceive little benefit or are reluctant to discuss breastfeeding in case it makes mums feel guilty [53].

These issues indicate the need for healthcare systems to support breastfeeding from the start. On a policy level, this involves prioritising breastfeeding, with increased resources needed for staff to spend time with new mothers during pregnancy, labour and after the birth. High-quality detailed antenatal breastfeeding education is needed that focuses on the realities of normal breastfeeding rather than aiming to increase intention alone. Investing support during the birth may impact upon breastfeeding success because fewer complications will arise [54]. More intensive breastfeeding support is needed for those mothers who have been through a complicated delivery.

Post-natally, an increase in staffing on hospital wards and in the community is vital for ensuring that women receive the one-to-one support needed after the birth. Peer support should offer an additional level to this. Mothers value the knowledgeable and empathetic relationship that peer supporters can offer [55], particularly in areas where breastfeeding levels are low, because it provides a community that is supportive and normalising of breastfeeding [56]. However, government cuts to services have seen many of these groups close.

In addition to increasing staffing, investment must be made into increasing the training of professionals with respect to understanding breastfeeding and valuing supporting new mothers [57]. This should be implemented across the spectrum of those who interact with breastfeeding mothers, rather than simply midwives and health visitors alone. For example, providing doctors with educational training increases breastfeeding rates particularly if solutions on how to effectively manage difficulties are focused on [58].

2. Investment in societal public health messages

Numerous models of human behaviour show that affected individuals should not be the sole target of health promotion because the attitudes and behaviours of those around them affect their decisions [59]. This is the case for breastfeeding; mothers who feel supported by those around them are more likely to initiate and continue breastfeeding [60]. Unfortunately, societal understanding, value and support of breastfeeding in many Western countries is poor. Although mothers may be told 'breast is best', many think formula is sufficient, with little tangible difference between the two methods [61]. Societal experience, knowledge and understanding of infant feeding is also often heavily weighted towards bottle-feeding [62].

The attitudes and experiences of those close to the mother do matter. A strong predictor of breastfeeding is whether a woman was herself breastfed [63]. A grandmother who has experience of breastfeeding is more likely to be supportive and able to offer practical advice [64]. However, many grandmothers today fed their infants in a time when breastfeeding rates were very low and routines for feeding common [65]. This may mean that grandmothers, although supportive, may not be able to offer up to date advice [66]. Conversely, some grandmothers may try to dissuade their daughter from breastfeeding [67] and the more frequent contact a mother has with her mother in this circumstance the less likely she is to breastfeed, particularly for younger mothers [68].

Additionally, partner attitude matters. When a partner is supportive, mothers are more likely to breastfeed, particularly if they act as her advocate if she experiences difficulties [69]. Although most fathers are supportive, many feel helpless if she experiences difficulties [70], wanting more information about how to support breastfeeding, yet many are excluded from antenatal breastfeeding education sessions [71]. Conversely, when a father feels excluded, wants his partner to return to 'normal' or feels embarrassed, women are less likely to breastfeed [72].

Finally, wider public attitudes can affect maternal decisions. Around a third of the public in the UK, USA and Australia consider that a baby should not be breastfed in public [73]. This is not simply about physical encounters; many consider it inappropriate to show breastfeeding on the television or in print [74]. These attitudes are strongly tied into perceptions of the sexualisation of the female body and an assumption that breastfeeding should be a private act [75]. This is further tied to male sexist attitudes towards women. Men who score highly on sexist traits are more likely to react angrily to breastfeeding in public [76]. Breastfeeding may imply to some that a woman's priority is motherhood rather than for the purpose of being sexually available, which can incite anger in those who consider women should be sexually available to them [77].

Change needed

Interventions to improve breastfeeding should not be targeted solely at the mother. Those around her have considerable influence and need education about the importance of breastfeeding and their support role, particularly for older generations whose breastfeeding knowledge may have been learnt when infant feeding advice was markedly different [26].

Interventions that target the knowledge of fathers have been successful at increasing breastfeeding rates, particularly those that teach fathers to identify and solve breastfeeding issues [78]. Caution must be taken about the messages given because a potentially increased involvement of fathers in infant care can lead to lower breastfeeding rates [79]. Some mothers may also be uncomfortable with learning about breastfeeding in front of other men. Sensitivity is key, particularly in relation to cultural differences [80]. Likewise, involving grandmothers in education, particularly to update them on current guidelines, reduces the amount of unhelpful advice [81]. This education should be more widespread.

Additionally, given the influence of societal attitudes upon maternal confidence [7], wider public health campaigns to improve public perceptions of breastfeeding (and challenge the notion of breastfeeding as sexual) are vital. Australia, for example, has developed a series of breastfeeding adverts to promote acceptance and knowledge of breastfeeding [82]. Images of breastfeeding, and the inclusion of breastfeeding in television and other

media could be beneficial with respect to portraying breastfeeding as normal and part of life rather than current media stereotypes of being difficult, sexual or comical [83]. Viewing images of breastfeeding mothers increases positive attitudes towards breastfeeding [84]. Social media could be a useful vehicle for accessing a wide audience, particularly amongst younger demographics [85].

These interventions should ideally start young as part of biology and social education [26]. Many teens have never been exposed to a woman breastfeeding [86] and yet hold some of the strongest negative views towards breastfeeding, or doing so in public [87]. However, teens who have witnessed breastfeeding are more likely to plan to breastfeed in the future [88]. Creating a positive cycle is needed, although breastfeeding is often missing from the school curriculum. In one study, only half of teachers considered it appropriate for primary school age to learn about breastfeeding [89]. Again, challenging unhealthy societal notions of breastfeeding as sexual is central. The key way to do this is to enhance exposure of it.

3. Policy and law to protect breastfeeding mothers

In many Western countries, various laws are in place to help protect breastfeeding mothers. However, media stories frequently illustrate how often these laws are broken, either deliberately or as a result of poor understanding, or are not sufficiently detailed to support new mothers fully.

The right to breastfeed in public

Linked to the discussion above regarding public attitudes towards breastfeeding, the maternal right to breastfeed in public must be more strongly reinforced. Mothers in the UK, Australia and many areas of the USA are protected by law to breastfeed their infants in public places. However, despite this, many breastfeeding women report receiving negative comments or even being asked to stop breastfeeding [90]. Members of the public also feel they are entitled to negative attitudes regarding breastfeeding in public, despite laws put in place. In one US study, fewer than 60% of respondents agreed that women should have the right to breastfeed in public [91]. Because of this, despite legal protection, many women naturally feel uncomfortable at breastfeeding in public places [92].

Maternity leave

Increased paid maternity leave is associated with a longer breastfeeding duration [93]. However, the amount of paid leave varies widely. In the USA, most women receive no paid leave. Conversely in the UK, there is paid leave until 9 months, although it drops to a lower amount after 6 weeks. Many women, particularly if they are the main wage earner, return to work for financial reasons when they are still breastfeeding frequently and need to make a decision to stop, or express milk during their working day [94]. Concerns around inflexibility and balancing both can lead to mothers stopping before they return [95]. This particularly affects women in less senior positions, those who are lower paid, and with inflexible jobs [96].

Return to work

Linked to the duration of maternity leave, needing to return to work is a common reason given for stopping breastfeeding, or not initiating it at all [97]. A fifth of women in the UK report this as a barrier [7] and similar patterns are seen in many Western countries [98].

Workplace policies can significantly affect whether mothers are able to breastfeed on return to work. In the USA, for example, the break time for nursing mothers law requires that employers provide a time and place for employees to express milk [99]. Conversely, in the UK, the law simply requires that organisations provide a room for breastfeeding mothers to be able to rest and lie down, although there is no requirement for women to be given paid breaks [100]. This means that many women do not have breaks, let alone paid breaks, to express, making breastfeeding maintenance a challenge [101].

If women do choose to express at work, their colleagues can react negatively, damaging maternal confidence to do so. Reactions can include embarrassment, jealousy and even offence [102]. Subconsciously, bodily fluids can be associated with inconvenience and illness and some view it as leaking sexual fluids in the work place [103]. Many women feel too intimidated to complain.

Change needed

Interventions to increase breastfeeding rates must consider how wider policy can be used to protect women from a legal standpoint to breastfeed for longer. In terms of the law supporting women to breastfeed in public, a greater awareness and understanding of why these guidelines are in place is needed. Approaches such as the Breastfeeding Welcome Scheme, where public places sign up to support breastfeeding in their venue, are important. The scheme works not only by reassuring mothers, but also sending a message to others that breastfeeding is acceptable. Large scale roll out of this in Australia had a positive impact upon maternal feelings of acceptance, alongside increased public awareness [104]. Potentially, sanctions are needed for public places (or individuals) who break this law.

In terms of maternity leave, governments must invest in extended leave that is paid at a feasible level for women to use. Countries such as Sweden, where maternity leave is

paid at a high rate for an extended period of time, have some of the highest breastfeeding rates in the West [6]. Given the economic benefits of breastfeeding for mother, infant and society [3,4], this investment would likely pay a significant return.

Supporting mothers to return gradually to work and ensuring that work place regulations such as paid breaks are in place to support continued breastfeeding across all types of employment is important and also brings a financial return for the organisation. In the USA, employers that are deemed breastfeeding friendly (e.g. allowing breaks and providing a suitable location to express milk) have increased morale, decreased turnover and increased retention [105]. These improved outcomes, combined with lower health costs for staff health insurance, mean that investing in breastfeeding directly saves the employer money, which is estimated at a $3 saving for every $1 invested [106]. These interventions work; states that have written the right for breastfeeding breaks and private space into law have higher initiation and continuation rates [107].

4. Protection of maternal wellbeing

Infant feeding does not happen in isolation; it is part of mothering. However, the transition to motherhood can be a challenge; many mothers are not prepared for the intensity of caring for a newborn, reporting feelings of anxiety and shock and even grief [108]. Professional, educated mothers feel this shift most harshly [109]. This is exacerbated by isolation for many. In non-Western cultures, it is traditional for family to care for the mother after birth, letting her rest and recover [110], whereas new mothers in Western culture are often almost solely responsible for infant care, leaving them exhausted.

It is unsurprising that maternal identity issues, exhaustion and isolation are risk factors for post-natal depression, which in turn is associated with a decreased breastfeeding duration [111]. Confidence and self efficacy play key underlying roles here. Although wider maternal parenting confidence is associated with increased breastfeeding duration [63], post-natal depression can make the concept of breastfeeding, or infant care in general, feel difficult and overwhelming [112]. Low maternal self efficacy is associated with increased perception of insufficient milk [113].

Breastfeeding difficulties, particularly with pain and perceived insufficient milk, are associated with an increased risk of both stopping breastfeeding and post-natal depression [9]. However, feelings of depression and anxiety can interfere with the ability to breastfeed. Mothers with post-natal depression are more likely to have poorer interactions with their newborn, such as being less sensitive in their touch and positioning on the breast, which can lead to breastfeeding difficulties [114].

Change needed

Alongside enhanced investment in breastfeeding support to prevent difficulties arising, greater investment is needed more widely into supporting new mothers. Mothers need to feel socially supported in their new role, which can also help give mothers more confidence to breastfeed [115]. Professional support will play a vital role in this. Listening visits from health visitors are valued by new mothers and are associated with a clinical reduction in symptoms of post-natal depression and a rise in life satisfaction [116]. However, a lack of funding in the area means that services are stretched. Investment is needed.

Awareness of the importance of maternal mental health needs to extend to everyone, not simply those with a professional role to care. Enhancing community support for new mothers would likely reduce post-natal illness and enhance breastfeeding rates. Raymond discusses the concept of mothers building a safety net around them of community contacts that could support them when their mood is low [117]. Likewise, peer support can reduce symptoms of depression and anxiety [118], which may in turn help women to breastfeed for longer.

5. Reducing the reach of the breast milk substitute industry

Finally, despite the global promotion of breastfeeding, sales of formula milk reach over 40 billion US dollars per year [119]. Although the International Code of Marketing of Breast milk Substitutes [120] sets out a code banning the promotion of breast milk substitutes for babies under 6 months old, this only has to be followed if written into the law of individual countries. Even in countries where it becomes legislation, this regulation is often broken or there is variation regarding to what extent countries follow the code [121]. For example, although banned in the UK, free infant formula is commonly included in hospital discharge packs in the USA [122].

Companies also circumvent regulations with brand advertising. Advertisements focus on toddler milks, although brand recognition increases sales for other products in that range [123]. Many cannot tell the difference between adverts for infant and follow-on formula [124]. Mothers who recall seeing formula adverts on television are more likely to formula feed [125].

Change needed

Governments must take responsibility for ensuring that the code is met. Simple interventions such as preventing hospitals from advertising products in discharge bags increase breastfeeding [126]. Fuller implementation is also needed to close loopholes such as companies offering

education to practitioners or pregnancy support lines for women would. Fines for breaking laws, or exaggerating claims, should be more widely used.

Simultaneously, a more active approach is needed in using similar tactics to promote breastfeeding. As noted above, this might comprise using government funded adverts that adopt the same strategies as those used by formula companies (rather than simple breast is best messages) and ensuring a greater visibility of breastfeeding in the public sphere [26].

Discussion and conclusions

The complexity of infant feeding decisions is seen in the number of systems level factors that affect maternal behaviour. Although biological impediments exist, environmental influences, particularly at the societal level, are pervasive. Maternal decisions and the ability to breastfeed are affected by the knowledge, attitudes and expectations of the society around her. To change breastfeeding, we must therefore change how breastfeeding and mothering is perceived in our society by removing structural barriers rather than targeting the individual alone [23]. We must create an environment where breastfeeding is normal, accepted and protected.

Governments must invest financially into protecting new mothers, not least because of the potential financial return. However, although some aspects such as laws and policy can easily be universal, given limited economies, interventions must focus on the most vulnerable in our society. Mothers who live in areas of economic deprivation are the least likely to breastfeed [127], yet their infants stand to benefit the most from being breastfed [128]. Poverty itself does not damage breastfeeding, but formula use is normative amongst more deprived communities [56]. Lower incomes and job insecurity may also affect ability to breastfeed dictating an earlier return to work [129] In addition, an increase in mental health issues and poorer social support can make breastfeeding more challenging [130].

A complex relationship is also seen with ethnicity. In the UK, breastfeeding rates amongst mothers from non-white backgrounds, particularly immigrants to the UK, are significantly higher than those from white backgrounds [7]. This is attributed to social norms around motherhood and feeding. However, over time, this relationship diminishes, particularly if families adopt Western norms and values surround infant feeding [131]. Conversely, in the USA, women from Black American backgrounds are significantly less likely to breastfeed. Poverty, community norms, a history of oppression and a lack of imagery of black women are key influences [132]. Black women in the USA are statistically less likely to live in an area attached to a hospital that follows the Baby Friendly steps [133] and less likely to receive breastfeeding advice from their health professionals [134]. Investment to narrow the gap between the richest and the poorest is therefore vital.

Investment can and does work. Brazil, for example, is an excellent example of how implementing such a society wide approach significantly increases breastfeeding rates. In 1986, the median duration of breastfeeding was 2.5 months but, by 2006, it had risen to 14 months. Exclusive breastfeeding rates to 4 months also increased from 4% to 48% [135]. To undertake this, the government invested heavily in promoting breastfeeding at the societal level, including multi-organisation working, media campaigns, training for health workers and the development of mother-to-mother support groups. Policywise, a strict enforcement of the International Code was introduced, maternity leave was extended from to 6 months and more than 300 maternity hospitals gained Baby Friendly Hospital Initiative certification. Investment in over 200 human milk banks led to Brazil having the highest number in the world. These interventions were successful as a result of their combination, as well as the fact that they did not focus solely on maternal knowledge, instead focusing on a mother's wider environment and support system, enabling her to breastfeed her baby [136].

Tackling our low breastfeeding rates should therefore not only focus on fixing the physical issues that women eventually present with, but also should systematically break down the environmental factors that negatively impact upon breastfeeding attitudes, intentions and ability. Given that infant feeding decisions are not made in isolation, strategies to raise breastfeeding rates should not purely be individualistic. Although good quality, individualised one-to-one support is vital, it is unfair to hold women responsible for behaviour that is affected by structural factors. Morally, given the negative mental health impacts that a failed breastfeeding experience can bring, should we really be promoting breastfeeding unless we also provide an environment that is conducive to its success? We must hold public health services accountable to raising and sustaining breastfeeding rates rather than placing responsibility and blame in the laps of individual mothers[137].

Conflict of interests, source of funding and authorship

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AB was solely responsible for all aspects of this work.

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PREGNANCY AND INFANT NUTRITION

Effect of *RRR*-α-tocopherol supplementation on serum of breastfeeding women up to 60 days after delivery: a randomised controlled trial

L. Q. Lira,[1] C. S. S. Gurgel,[1] A. L. de Sousa,[2] A. G. A. da Silva,[2] J. C. Padovam,[2] M. N. Moia,[2] R. L. M. Silva[2] & R. Dimenstein[1]

[1]Department of Biochemistry, Federal University of Rio Grande do Norte, Natal, RN, Brazil
[2]Department of Nutrition, Federal University of Rio Grande do Norte, Natal, RN, Brazil

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Correspondence
L. Q. Lira, Department of Biochemistry, Federal University of Rio Grande do Norte, Rua Cícero Pinto, 304, Edifício Cristo Redentor II, apto 102, Bairro: Lagoa Nova, Natal/RN, CEP: 59054-460, Brazil.
Tel.: +55 (84) 3215-3416, ext 205
Fax: +55 (84) 3342-2812
E-mail: liralq@gmail.com

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Abstract

Background: Maternal supplementation is a viable strategy to combat vitamin E deficiency in newborns, although a protocol for maternal vitamin E supplementation has not been defined. The present study assessed the effect of maternal supplementation in a single dose on the serum of postpartum women up to 60 days after delivery.
Methodology: Fifty healthy breastfeeding women were recruited at two maternity hospitals both located in Natal, RN, Brazil. The participants were randomly allocated to a control group and a treatment group in a 1 : 1 ratio. Serum was collected 1, 20, 30 and 60 days after delivery. Immediately after the first collection, the treatment group received a single dose of 400 IU of *RRR*-α-tocopherol. α-Tocopherol was quantified by high-performance liquid chromatography. The usual dietary vitamin E intake was determined using four 24-h recalls, and intake adequacy was assessed based on the estimated average requirements for lactating women (16 mg day^{-1}).
Results: The mean dietary vitamin E intakes of the both groups were similar ($P > 0.05$) and inadequate. The serum levels of α-tocopherol assessed at 1, 20, 30 and 60 days indicated adequate vitamin E status in both the control group (1194.6, 907.7, 910 and 748.6 μg dL^{-1}, respectively) and treatment group (1183.7, 956.0, 935.9 and 766.4 μg dL^{-1}, respectively). The comparison at each day showed no difference between treatments ($P > 0.05$).
Conclusions: A single vitamin E supplement did not change the mean serum level of α-tocopherol in breastfeeding women; thus, it does not improve their vitamin E status in the first 60 days after delivery.

Introduction

Vitamin E plays important roles in health maintenance and disease prevention, especially because of its antioxidant capacity [(1)]. Therefore, the consequences of vitamin E deficiency have prompted considerable interest. In humans, the symptoms of clinical vitamin E deficiency have been well characterised and include progressive peripheral neuropathy, ataxia, muscular degeneration, retinal lesions, infertility and dementia [(2)]. In general, plasma α-tocopherol levels below 12 μmol L^{-1} (516 μg dL^{-1}) are associated with negative outcomes during pregnancy for the mother and child [(3)].

Establishing a daily vitamin E intake aims to promote optimal serum level of vitamin E, although, according to Dror & Allen [(2)], it aims to prevent symptomatic deficiency and not promote health or prevent diseases. The Institute of Medicine recommends a mean vitamin E intake of 16 mg day^{-1} for lactating women (which is 4 mg more than the daily requirement of nonlactating

women) to support the provision of vitamin E in breast milk [4]. However, the recommended vitamin E intake has been contested because it may have been overestimated [3,5]. Dietary vitamin E intake is hardly capable of achieving the established requirement.

Vitamin E deficiency is more frequently found in children and newborns, especially preterm infants who are particularly more vulnerable to its onset and serious consequences. Consequently, strategies have been proposed aiming to prevent and treat vitamin E deficiency in this group, such as newborn and maternal supplementation.

Nevertheless, nutrient and oligo-element supplementation in newborns still faces many challenges not only because their basic requirements, proper administration route and proper dosage have not been well defined, but also because supplementation is still associated with potentially life-threatening effects, such as sepsis [6–8].

Regarding maternal supplementation, studies have suggested that vitamin E supplementation during pregnancy is not capable of increasing foetal α-tocopherol in detriment of its metabolite, α-carboxyethyl hydroxychroman, which may not effectively increase foetal reserves and thereby reduce the incidence of complications secondary to vitamin E deficiency in newborns [9,10]. Hence, by improving maternal nutritional status and, consequently, increasing the duration of vitamin E in breastmilk, maternal supplementation becomes a viable and safer strategy to meet the vitamin E requirements of newborns.

However, along with the few conclusions about the dose–response effect of supplementation in the scope of various interventions, studies that focus on the impact of supplementation on human nutritional status are scarce because studies in animal models are more frequent [11,12].

Our research group has developed a research line on the influence of vitamin E supplementation on maternal and newborn nutritional status during breastfeeding, and, accordingly, the present study aimed to improve the understanding of the biochemical behaviour of α-tocopherol and to provide data for the development of efficient programmes for groups at greater risk of vitamin E deficiency [13–16].

Hence, the importance of the present study rests in clarifying the behaviour of serum α-tocopherol after the mother takes a single supplement immediately after delivery to improve her vitamin E status.

Materials and methods

Participants and intervention

The present study comprised a prospective, randomised, controlled, and parallel study. The participants were recruited at delivery at the Maternity Hospital Escola Januário Cicco and Unidade Mista das Quintas, both located in Natal, RN, Brazil. These two healthcare establishments were chosen because both provide free obstetric care to women from many neighborhoods of Natal and other municipalities in the state of Rio Grande do Norte and also because they are representative of Natal's annual number of deliveries. Sampling occurred from January 2015 to February 2016 and the participants were followed for the first 60 days after delivery.

The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte under protocol number 1.093.344 CAAE 43894615.4.0000.5537 and complied with the Declaration of Helsinki. The study was also registered at ReBEC – Brazilian Registry of Clinical Trials under code RBR-9wch5 m. It is available at: http://www.ensaiosclinicos.gov.br/rg/?q=RBR-9wch5m.

The minimum sample size was calculated using GPOWER, version 3.1.7 (http://www.gpower.hhu.de) using the parameters: α of 5%, power of 0.8 and an expected effect of 0.25. Cohen defines *F* values of 0.1, 0.25 and 0.4 as small, medium and large effects, respectively. Each group should have at least 10 individuals [17]. The minimum sample size was exceeded during the recruitment process, giving a total of 25 individuals in each group.

The inclusion criteria were: age between 18 and 40 years, low obstetric risk, term delivery (gestational age ≥37 weeks) and breastfeeding practice. The exclusion criteria were: use of multivitamin supplements during pregnancy and lactation, presence of acute or chronic diseases, mental disorders, use of licit or illicit drugs, use of antithrombotic agents, preterm delivery (gestational age <37 weeks), multiple foetuses, and foetuses with malformations and/or cerebral palsy.

All eligible participants were informed about the objectives of the study and those who accepted to participate in the study provided their written informed consent before joining the study. The participants were randomly allocated to a control group (CG) and a treatment group (TG) in a 1 : 1 ratio according to the order of delivery in each maternity hospital. In other words, the first woman to deliver was allocated to the CG, the second, to the TG, and so on, comprising a simple randomisation. The study was compiled in compliance with the Consolidated Standards of Reporting Trials – CONSORT (Fig. 1).

The CG did not receive any supplements until the end of the study. The treatment group received a single oral dose of 400 IU of natural vitamin E in the form of *RRR*-α-tocopherol acetate (CVS Health, Woonsocket, RI, USA) immediately after the first maternal blood collection. The supplement was taken with water at breakfast.

A 400 IU dose of *RRR*-α-tocopherol corresponds to 294 mg of α-tocopherol. This dose was chosen because it

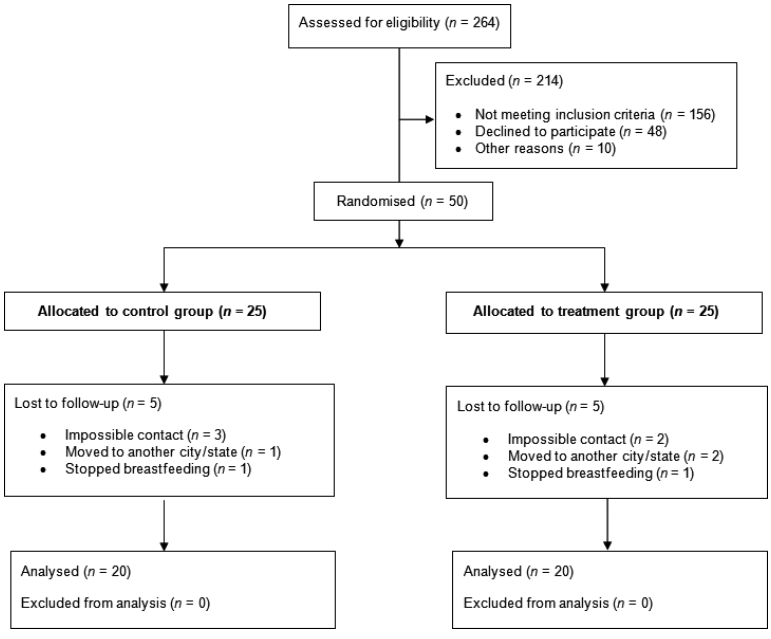

Figure 1 Consolidated standards of reporting trials (CONSORT) flow diagram.

has been considered as safe by the Institute of Medicine (2000) [4] after analysis of long-term studies, which reported no deleterious effects associated with this dose.

Outcome assessment

The primary outcome of the present study was confirmation of the effect of supplementation on maternal vitamin E status during lactation. This outcome was assessed in accordance with the serum level of α-tocopherol up to 60 days after delivery in the control and treatment groups. The secondary outcome of the present study was to evaluate the usual dietary intake of vitamin E during lactation. This outcome was assessed by 24-h recalls administered up to 60 days after delivery in both groups.

Data collection

Socio-economic, prenatal, delivery, medical history data and biochemical test results were collected from the medical records and by a structured interview conducted by the researcher. Maternal nutritional status was given by the relationship between body mass index and gestational age and classified as recommended by Atalah *et al.* (1997) [18]. Usual dietary vitamin E intake was determined by 24-h recalls (24HR) administered 7, 20, 30 and 60 days after delivery. These 24HRs were administered by a dietitian and filled based on the information provided by the participants.

Dietary data were converted into nutrient intakes using Virtual Nutri Plus (http://www.virtualnutriplus.com.br) and a hybrid table was constructed using food composition data from the United States Department of Agriculture (USDA) food and nutrient database [19]. The usual dietary intake of vitamin E was given by the total intake adjusted for intrapersonal intake and corrected for total energy intake. The resulting values were obtained using SPSS, version 21.0 (IBM Corp., Armonk, NY, USA) employing the residual method.

The prevalence of inadequate vitamin E intake was assessed in accordance with the estimated average requirements cut-off method [20] and is given by the area under the curve, which corresponds to the proportion of individuals with inadequate intake, and was assessed further in a normal distribution table (*Z*-score) using 16 mg day^{-1} as the mean estimated requirement of vitamin E for the lactation period [4].

Dietary assessment characterised the usual intake of vitamin E by the study population in the first 60 days after delivery to eliminate the influence of diet on the supplementation effect.

The first blood sample (5 mL) was collected on day 1 after delivery by brachial venipuncture. The women in the TG (but not the women in the CG) then received a single capsule of 400 IU of *RRR*-α-tocopherol. The second and third blood samples (5 mL) were collected 20 and 60 days, respectively, after the first collection. All samples were collected in the morning, after an overnight fast. Forty of the fifty recruited women finished the study (20 in the CG and 20 in the TG).

The blood samples of days 20 and 60 after delivery were collected at the homes of participants by a trained

team. Serum collected on the first day after delivery was defined as baseline.

Blood was collected in completely opaque polypropylene tubes and immediately transported under refrigeration to the Laboratory of Food and Nutrition Biochemistry of the Federal University of Rio Grande do Norte. The blood aliquots were centrifuged at 4000 × ***g*** for 10 min to separate the serum. The serum was stored at −20 °C for 0–4 days until the analyses.

Chemical analyses

Serum α-tocopherol was extracted as recommended by Lira *et al.* [21] and quantified using an LC-20AT chomatograph (Shimadzu, Kyoto, Japan), which has an injector loop of 20 μL connected to the CBM 20A communicator and SPD-20A UV-VIS detector (Shimadzu). A reversed-phase chromatographic column C18 was used for chromatographic separation (LiChroCART 250-4; Merck, Darmstadt, Germany). The isocratic mobile phase consisted of 100% methanol flowing at 1.0 mL min^{-1} at a wavelength of 292 nm.

The α-tocopherol level of the samples was quantified by comparing the area under the chromatographic curve with the area of the standard (Sigma-Aldrich, St Louis, MO, USA). The concentration of the standard was confirmed by the specific extinction coefficient for α-tocopherol (ε 1%, 1 cm = 75.8 at 292 nm) in absolute ethanol (Merck) [22].

The sensitivity method resulted in 0.03 mmol L^{-1} of α-tocopherol; the long-term impression was 0.01% at a concentration of 0.29 mmol L^{-1} for the standard and 0.05% in 41 mmol L^{-1} for the serum samples. The vitamin added to the serum was fully recovered (100%).

The *RRR*-α-tocopherol content of the supplement capsule was confirmed by comparison with the standard (Sigma-Aldrich), reaching an agreement of 98% with the information provided by the manufacturer.

Women with serum α-tocopherol below 12 μmol L^{-1} (516 μg dL^{-1}) were considered to be vitamin E, as defined for healthy adults [4].

Statistical analyses

Statistical analyses were performed using SPSS, version 21.0 (IBM Corp.). Serum α-tocopherol was expressed as the mean (SD).

The Shapiro–Wilk test confirmed the normal distribution of the variables. The chi-squared test confirmed the homogeneity of the groups. Student's *t*-test for independent samples confirmed the difference of dietary levels between groups and Levene's test confirmed the homogeneity of intergroup variance. A paired Student's *t*-test measured the intragroup difference.

The mixed-design (split-plot) analysis of variance (ANOVA) with a post-hoc Bonferroni test was used to check serum α-tocopherol levels over time and between groups. The effect size was assessed by the interpretation of partial eta squared coefficient (η^2) proposed by Cohen, where values between 0.01 and 0.06 indicate little effect; values in the range indicate 0.07–0.14 moderate effect; and values higher value than 0.14 indicate a great effect on α-tocopherol levels [17]. The one-way ANOVA with Tukey's post-hoc test compared the usual intakes of vitamin E and energy between the groups. $P < 0.05$ was considered statistically significant.

Results

The entire sample had similar characteristics, indicating intergroup homogeneity. The women were young adults, with a mean of two children, per capita income below one minimum salary and a low education level (no more than elementary school). Most participants gained too little or too much weight at the end of pregnancy and received no nutritional care during gestational and postpartum periods (Table 1).

The mean (SD) usual intake of vitamin E in the first 60 days postpartum was 5.12 (1.57) mg day^{-1} in the control group and 5.03 (1.32) mg day^{-1} in the treatment group. Thus, during that period, 100% of the participants had inadequate vitamin E intake (<16 mg day^{-1}). The usual intake of vitamin E did not differ between the groups ($P = 0.070$). The mean (SD) energy intake was 9581.3 (9133.6) kJ day^{-1} [2290 (2183) kcal day^{-1}] in the control group and 8556.2 (4623.3) kJ day^{-1} [2045 (1105) kcal day^{-1}] in the treatment group. The usual energy intake of the groups did not differ ($P = 0.110$).

The mean (SD) of serum α-tocopherol levels (μg dL^{-1}) at baseline, 20, 30 and 60 days were: 1194.6 (327.7), 907.7 (439.0), 910 (405.2) and 748.6 (302.2) for the control group, respectively, and 1183.7 (410.8), 956.0 (381.1), 935.9 (205.0) and 766.4 (301.7) for the treated group, respectively. All means indicated an appropriate vitamin E status.

A significant main effect was found when comparing the α-tocopherol levels along the postpartum period ($P < 0.01$, $\eta^2 = 0.403$, power = 1.00); however, no significant effect was found when comparing treatments ($P = 0.497$, $\eta^2 = 0.018$, power = 0.102), nor when analysing the interaction between the control and treatments groups ($P = 0.746$, $\eta^2 = 0.013$, power = 0.104). Thereby, the supplementation did not affect the serum α-tocopherol levels along the postpartum period. Compared with baseline, serum α-tocopherol had decreased by approximately 22% by day 20, and by 64% by day 60 in both groups (Fig. 2).

Table 1 Characterization of 50 breastfeeding women and their infants randomized in the control and treatment groups of this controlled trial

Characteristics	Control group (n = 25)	Treatment group (n = 25)	P-value
Maternal age (years), mean	25.4 (6.1) (22.8–27.9)	25.9 (5.7) (23.6–28.1)	0.944*
Per capita income (%), n[†]			
≤1/2 salary	61 (15)	44 (11)	0.093[‡]
>1/2 to ≤1 salary	33 (8)	56 (14)	
>1 to ≤2 salaries	6 (2)	0 (0)	
>2 salaries	0 (0)	0 (0)	
Education level[§] (%), n			
Never attended school	0 (0)	0 (0)	0.541[‡]
Illiterate	0 (0)	0 (0)	
Elementary education	31 (8)	35 (9)	
Incomplete high school	30 (7)	25 (6)	
High school education	35 (9)	30 (7)	
Higher education	5 (1)	10 (3)	
Gestational age (weeks), mean	40.5 (1.5) (39.9–41.2)	40.4 (1.6) (39.8–41.0)	0.855[‡]
Type of delivery (%), n			
Vaginal	51 (13)	65 (16)	0.337[‡]
Caesarian	49 (12)	35 (9)	
Number of children (n), mean	1.8 (1.1) (1.4–2.2)	2.1 (1.4) (1.6–2.7)	0.863*
Maternal weight gain (%), n[¶]			
Inadequate	32 (8)	36 (9)	0.220[‡]
Appropriate	36 (9)	32 (8)	
Excessive	32 (8)	32 (8)	
Nutritional care (%), n**			
Yes	24 (6)	28 (7)	0.732[‡]
No	76 (19)	72 (18)	
Birth weight (g), mean	3.244 (0.721) (2.958–3.529)	3.367 (0.385) (3.217–3.517)	0.564*
Birth length (cm), mean	49.3 (2.4) (48.3–50.2)	48.8 (2.2) (47.9–49.6)	0.507*

*Student's t-test for independent samples. Data are the mean (SD) (95% confidence interval).
[†]Brazilian minimum salary: R$ 880.00.
[‡]Chi-squared test.
[§]Semi-literate.
[¶]Body mass index/gestational week [18].
**Nutritional care assistance received during gestational and postpartum periods.

Discussion

Sample homogeneity determines the accuracy of the results of studies that assess supplementation protocols. The two groups in the present study had homogeneous socio-economic, health and obstetric characteristics. In addition, the present study ensured that the maternal diet did not influence the supplementation effect.

Nutritional requirements are physiologically higher during pregnancy and lactation to support foetal growth and development, as well as maternal metabolic changes. Maternal diet during lactation correlates with desirable advantages with respect to a child's health, including long-term health [23]. However, the characteristics of the study population, such as low socio-economic status, low maternal education level and limited access to efficient and quality dietary advice, are factors that have been related to insufficient nutrient intakes [24].

Accordingly, the usual dietary energy intake of the postpartum women in both study groups was lower than the estimated energy requirement for the first 6 months of lactation [108.19–104.34 kJ day^{-1} (2586–2733 kcal day^{-1})] [25]. Both groups also had monotonous diets and a low intake of dietary sources of vitamin E, meaning that the women were unable to meet their vitamin E requirements. Consequently, all postpartum women had an inadequate vitamin E intake because they consumed only approximately 37% of their daily requirement.

Even though the regular diets consumed in most developing countries are low in vitamin E [2], an inadequate vitamin E intake may also be a consequence of the overestimation in the current nutritional recommendations for vitamin E. The need to review those cut-off points has been suggested once the majority of the world population cannot meet them [3]. In agreement with the reasons supporting this argument, we found that serum

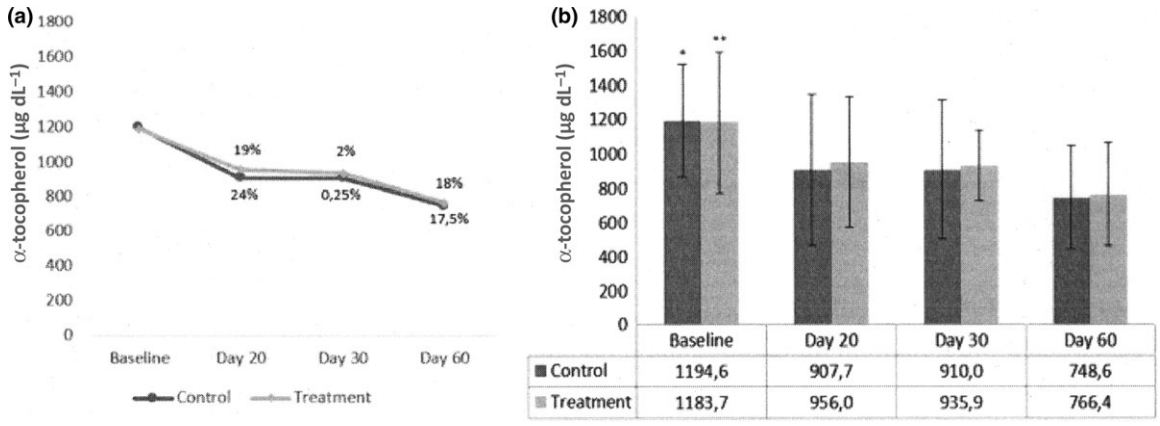

Figure 2 Tocopherol profile in maternal serum up to 60 days after delivery. (a) postpartum effect on the levels of serum α-tocopherol. Split-plot ANOVA: $P < 0.01$, $\eta^2 = 0.403$, power = 1.00. (b) *difference between the α-tocopherol baseline serum compared to the days 20 ($P = 0.044$), 30 ($P = 0.03$), and 60 ($P = 0.01$); **difference between α-tocopherol baseline serum compared to day 60 ($P = 0.003$).

vitamin E levels remained adequate in our study population, even in insufficient dietary intake situations.

The mean serum α-tocopherol levels of both groups on each study occasion indicated appropriate nutritional status and agreed with previously reported data [13,16,21]. Sixty days after delivery, in agreement with the physiological behaviour of vitamin E [26], the mean serum α-tocopherol levels of both groups decreased similarly.

According to Novotny *et al.* [27] and Dimitrov *et al.* [28], serum vitamin E returns to pretreatment levels within 10–20 days after supplementation, regardless of dosage. Consequently, because the present study measured serum vitamin E level only 20 days after supplementation, it was not possible to determine when the α-tocopherol peak occurred, which can be considered as a limiting factor of the study design. Nonetheless, given that the importance of supplementation is not only restricted to an increasing serum level, but also to maintaining it within the normal range, the present study precisely demonstrates that the protocol of maternal supplementation with a single 400 IU dose of RRR-α-tocopherol administered immediately after delivery was not effective for ensuring the improvement of maternal vitamin E status at 60 days postpartum.

Furthermore, Novotny *et al.* [27] reporrted that serum vitamin E decreased to baseline levels as α-tocopherol was absorbed by peripheral tissues for use and storage. Hence, the gradual reduction in α-tocopherol levels in both study groups may be a consequence of vitamin E transport to the mammary gland. This metabolic pathway was not addressed in the present study; yet, this approach likely characterises the metabolic priority of ensuring proper nutrient contents in breast milk rather than restoring or increasing maternal reserves, thereby promoting adequate infant growth and development.

Considering the above, we suggest that maternal serum assessed during lactation is possibly a more accurate biochemical indicator of maternal nutritional status compared to its measurement in the immediate postpartum period. The resulting measurement performed in the immediate postpartum period may reflect the physiological increase of α-tocopherol at the end of pregnancy, in response to an accumulation that occurs in the third trimester, which is associated with increased maternal adipose tissue [1].

Thus, future studies should investigate the influence of maternal α-tocopherol supplementation on breastmilk throughout the lactation period aiming to confirm not only how vitamin E content of breastmilk varies over time, but also how adequate vitamin levels are maintained and whether they meet the infant's requirement. Additionally, we consider that investigating different supplementation protocols, taking into consideration its period of decline in human serum, may provide better results with respect to the adequacy and maintenance of vitamin E status in the long run. In populations with a diagnosis of vitamin E deficiency, maternal supplementation with α-tocopherol may present different effects and higher efficacy.

The main outcome of the present study was that, despite the decrease in maternal vitamin E reserves, a single supplement of 400 IU of *RRR*-α-tocopherol immediately after delivery did not affect the serum α-tocopherol levels of breastfeeding women.

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 at Brazilian Registry of Clinical Trials have been explained. The reporting of this work is compliant with CONSORT guideline in the Supporting

information (Appendix S1). This study was registered at Brazilian Registry of Clinical Trials under the code RBR-9wch5m. It is available at: http://www.ensaiosclinicos.gov.br/rg/?q=9wch5m.

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Conflict of interest, source of funding and authorship

The authors declare that they have no conflict of interests.
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LQL and RD designed the research. LQL, CSSG, ALDS, AGADS, JCP, MNM and RLMS conducted the research and analysed data. LQL and RD wrote the paper. LQL has primary responsibility for the final content. All authors read and approved the final manuscript submitted for publication.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Appendix S1. CONSORT 2010 checklist of information to include when reporting a randomised trial.

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PREGNANCY AND INFANT NUTRITION

Birthweight, HIV exposure and infant feeding as predictors of malnutrition in Botswanan infants

P. Chalashika,[1] C. Essex,[1] D. Mellor,[2] J. A. Swift[1] & S. Langley-Evans[1]

[1]Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Loughborough, UK
[2]School of Life Sciences, Coventry University, Coventry, UK

Keywords
1000 days, Botswana, child undernutrition, HIV, infant feeding practices, malnutrition.

Correspondence
P. Chalashika, Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK.
Tel.: +44 (0) 115 951 6400
Fax: +44 (0) 115 951 6122
E-mail: paphani.chalashika@nottingham.ac.uk

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Abstract

Background: A better understanding of the nutritional status of infants who are HIV-Exposed-Uninfected (HEU) and HIV-Unexposed-Uninfected (HUU) during their first 1000 days is key to improving population health, particularly in sub-Saharan Africa.
Methods: A cross-sectional study compared the nutritional status, feeding practices and determinants of nutritional status of HEU and HUU infants residing in representative selected districts in Botswana during their first 1000 days of life. Four hundred and thirteen infants (37.3% HIV-exposed), aged 6–24 months, attending routine child health clinics, were recruited. Anthropometric, 24-h dietary intake and socio-demographic data was collected. Anthropometric *Z*-scores were calculated using 2006 World Health Organization growth standards. Modelling of the determinants of malnutrition was undertaken using logistic regression.
Results: Overall, the prevalences of stunting, wasting and being underweight were 10.4%, 11.9% and 10.2%, respectively. HEU infants were more likely to be underweight (15.6% versus 6.9%), ($P < 0.01$) and stunted (15.6% versus 7.3%), ($P < 0.05$) but not wasted ($P = 0.14$) than HUU infants. HEU infants tended to be formula fed (82.5%), whereas HUU infants tended to breastfeed (94%) for the first 6 months ($P < 0.001$). Significant predictors of nutritional status were HIV exposure, birthweight, birth length, APGAR (appearance, pulse, grimace, activity and respiration) score and mother/caregiver's education with little influence of socio-economic status.
Conclusions: HEU infants aged 6–24 months had worse nutritional status compared to HUU infants. Low birthweight was the main predictor of undernutrition in this population. Optimisation of infant nutritional status should focus on improving birthweight. In addition, specific interventions should target HEU infants aiming to eliminate growth disparity between HEU and HUU infants.

Introduction

Globally, mortality of children aged <5 years has declined from 90 to 43 deaths per 1000 live births between 1990 and 2015 [(1)]. However, in sub-Saharan Africa, mortality of children aged <5 years still remains higher at 86 deaths per 1000 live births [(1)]. Mortality in children aged <5 years is mainly attributed to undernutrition, with 45% of these deaths being preventable through optimal nutrition, especially in the first 1000 days (the period from conception to the child's second birthday) [(2–4)].

HIV/AIDS is still a major health challenge in Botswana [(5,6)]. Strategies including the prevention of mother-to-child transmission (PMTCT) of HIV have been highly successful in Botswana, reducing mother-to-child transmission rates to approximately 2.6% [(7,8)]. Without PMTCT strategies, HIV transmission from mother to child could be as high as 25% [(8)]. However, this success has resulted in the

increase in the population of HIV-exposed but uninfected (HEU) infants [9,10]. Health and/or nutritional issues unique to HEU infants will have major population health implications as their numbers increase [11,12]. Currently, the health and nutritional consequences of HIV-exposure are largely under investigation [10,12]. However, a higher risk for mortality in HEU compared to HIV-unexposed uninfected (HUU) infants has been reported previously [13–16]. The risk of mortality can be modified by optimising the nutritional status of infants, although this requires a good understanding of context specific patterns and determinants of undernutrition in this group [17].

Studies conducted in other African countries comparing the nutritional status of HEU and HUU infants show large variations in the levels of undernutrition [12,18–20]. The majority of these studies were conducted before antiretroviral therapy (ART) was widely available to mothers and infants [12,18–20]. By contrast, ART is available to approximately 92% of pregnant women in Botswana [8]. Monitoring and management of infant health and nutrition is intensive and widely accessible [21]. The same conditions are often not present in other sub-Saharan African countries with high HIV prevalence. However, the level of mortality in HEU infants in Botswana is comparable or higher than in other sub-Saharan African countries [13]. Furthermore, the feeding policy adopted for HEU infants in Botswana is unique because it has inadvertently undermined breastfeeding levels via the provision of free formula [22]. Currently, the nutritional status of HEU and HUU in Botswana have not been well documented. Therefore, understanding nutritional status and its determinants between HEU and HUU infants in Botswana is important for informing policies and interventions that can be used to achieve comparable growth between these infants, if such differences exist, thus helping reduce the risk of mortality in HEU infants. The present study aimed to investigate the patterns of undernutrition per HIV-exposure within context of feeding practices in infants aged 6–24 months in selected districts in Botswana. In addition, the study also aimed to identify determinants of nutritional status in these infants.

Materials and methods

Study participants and population

The present study was conducted in Botswana using a comparative cross-sectional study design between December 2014 and February 2015 in 19 different government health facilities of varying sizes (hospital, primary hospital, clinics and/or health posts) located across the four districts (Kweneng-East, Kgatleng, Selebi Phikwe and Francistown). Health facilities in districts with high HIV prevalence in the adult population were selected to obtain an adequate number of HEU infants. The prevalence of HIV in these districts ranged from 26.3% in Kweneng East to 39.6% in Selebi Phikwe. Participants were selected on the basis of having a higher HIV prevalence than the national average, aiming to ensure an appropriate sample of HEU infants. These four districts were selected to represent urban, semi-urban and rural areas. Kweneng-East is mainly rural with some semi-urban locations. Kgatleng is mainly rural, Selebi Phikwe is semi-urban, whereas Francistown is mostly urban. These locations span the eastern hardveld where at least 80% of the population of Botswana live [23,24]. All caregivers from the general population with infants aged 6–24 months, attending their monthly growth monitoring in a health facility, were invited to participate in the study. Eligible caregivers had to be citizens of Botswana, aged over 18 years and were the parent and/or legal guardian of the infant. There were no other exclusion criteria. Participants were approached as they arrived at the health facility. Children in Botswana, aged 0–59 months attend routine monthly growth monitoring in government health facilities across the country. When more participants than required showed interest in the study, simple randomisation was used to select participants by allocating each participant a number.

Sample size

A representative sample of infants in selected districts was stratified in accordance with the population of the infants aged under 5 years in each district based upon data supplied by the Ministry of Health and Wellness in Botswana (Nutrition and Food Control division). Therefore, a district with a higher number of children aged <5 years had a larger representation within the sample. In addition, the composition of the sample within each district was selected such that it represented the proportions of infants attending each type of health facility (hospital, primary hospital, clinics and/or health posts) within that district. Accordingly, a type of health facility receiving a higher number of infants would have a higher share of the sample within each district.

To facilitate a logistic regression analysis, an adequate sample size assuming a medium size relationship between the dependant variables (underweight, stunting and wasting) and independent variables and, $\alpha = 0.05$ and $\beta = 0.20$ was taken to be $n \geq 50 + 8\ m$ (where m is the number of independent variables) [25]. In total, 44 potential independent variables were identified *a priori* to the data collection; resulting in a minimum sample size of 402 caregiver–infant pairs (see Supporting information, Table S1). In addition, oversampling by 10% was also

employed to counter missing data. Independent variables identified *a priori* and known to affect undernutrition in infants such as birthweight, sex and maternal age, caregiver education level and socio-economic factors were included ([26–28]). These variables were derived from data collection (anthropometry, dietary recall, interview of caregivers) and review of the child health card. However, as a result of the cross-sectional nature of the study, maternal nutrition and health variables prior to the study, such as during pregnancy, were not available. HIV-exposure was maintained in all analysis because it was a variable of interest.

Procedures

Participants were recruited during the free monthly routine health check-up of their infant at a health facility. In total, 419 participants were approached to take part in the study. Five infants with an undocumented HIV status and/or missing polymerase chain reaction (PCR) DNA/rapid HIV tests were not enrolled into the study. Of all the participants approached, only one declined to take part in the study. The final sample size was 413 infants.

Data were collected by the lead investigator and two trained assistants using a structured interview with the caregiver and review of each child's health card. All caregivers in Botswana are given and keep a health card for their infant at birth. This card contains details such as birthweight and length, vaccinations, monthly weight and feeding practices. HIV-exposure was determined from the child's health card in accordance with the latest DNA/PCR or rapid test result. HIV negative mothers were tested every 3 months for HIV during antenatal care, with the latest test at 36 weeks documented in the child's health card. Socio-demographic characteristics, feeding practices and health history as potential independent variables were collected from the caregiver and the health card. Anthropometric measures of length/height and weight were measured in duplicate from all the infants in accordance with a World Health Organization (WHO) standard procedure [29] using standardised equipment. Weight was measured to the nearest 0.05 g using calibrated scales (Seca 385 and 875; Seca GmbH & Co., Hamburg, Germany) and length/height was measured to the nearest 1 mm using a measuring board 417 (Seca gmBH & Co.) and stadiometer (Seca 217; Seca GmbH & Co.). Length for age *Z*-scores (*LAZ*), weight for age *Z*-scores (*WAZ*) and weight for length *Z*-scores (*WLZ*) were calculated in accordance with the 2006 WHO child growth standards using the WHO Anthro 2005 programme (beta version) [30]. Stunting, being underweight and wasting was determined at a *Z*-score <-2 SD based on LAZ, WAZ and WLZ, respectively.

A modified United States Department of Agriculture five step multiple Pass 24-h dietary recall protocol [31] was used to measure infant's current nutritional intake as recalled by the caregiver. A similar multiple pass 24-h dietary recall was validated in Ugandan children and was found to be valid with respect to assessing dietary intake of infants residing in communities with similar diets [32]. Dietary diversity was calculated by allocating a score for consumption of food from one of the seven food groups (grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A rich fruits and vegetables; other fruits and vegetables) in the preceding 24 h [33]. Therefore, with a maximum possible score of 7, an infant's diet that scores ≥ 4 is considered to be diverse [33]. In addition, to dietary diversity [33], NUTRITICS software [34] was used to derive the energy and protein intake of each infant. Nutritional information of foods consumed was derived from packaging, data from the South African Composition Database [35] and McCance and Widdowson's Composition of Foods databases [36]. Cereals such as sorghum and fortified sorghum were consumed by majority of infants but nutritional content was not available. Therefore, cooked samples of these were weighed, frozen then freeze dried and analysed in the laboratory for protein per 100 g using the Flash EA1112 nitrogen elemental analyser (Soeks, Oakland Park, FL, USA). Energy per 100 g was analysed using a Parr 6300 Oxygen bomb calorimetre (Parr Instrument Co., Moline, IL, USA).

Data were entered into SPSS, version 22 [37] for analysis and 10% of the data was randomly selected using a computer number generator and then screened for accuracy.

Ethics

Ethical approval was received both from the University of Nottingham's Medical School Research Ethics Committee and the Health Research and Development Committee in Botswana. Informed consent was obtained from all caregivers. The two assistants were trained in seeking informed consent. When inappropriate feeding and/or malnutrition were identified, the caregiver was briefly counselled by the lead investigator, who is also a registered dietitian. The caregiver was then referred to the health facility for further follow-up and this was documented in the child's health card to ensure continuity of care.

Statistical analysis

Data were analysed using SPSS, version 22 [37]. A case–control analysis approach was employed where HEU and HUU infants were compared for outcomes of interest.

Baseline data are described as per HIV exposure. A chi-squared test was used to test for proportions between the two groups (HEU and HUU infants) to determine the prevalence of underweight, wasting and stunting. Continuous variables were analysed using Kolgorov–Smirnov test to determine whether the distribution was Gaussian or not. An independent samples *t*-test or a Mann–Whitney *U*-test was used to test for differences between the two groups for parametric and nonparametric variables, respectively. Variation of the mean is presented as the SD. Forward logistic regression was performed to determine predictors of stunting, underweight and wasting. The threshold for introducing the variables into the logistic regression model was set at $P < 0.1$. Cases with missing values for some of the independent variables were excluded. On this basis, 86.2% of cases with no missing values were included in the analysis for each of the three dependent variables (stunting, underweight and wasting). Variables with missing data included feeding method at <6 months (2.6%), feeding method at 6–12 months (6.1%), birthweight (4.1%), APGAR (appearance, pulse, grimace, activity and respiration) score (2.9%) and age at which complementary feeds were introduced (2.4%). One of the investigators (JAS) had the overall oversight of the statistical methods and analysis. $P < 0.05$ was considered statistically significant.

Results

Characteristics of participants

A total of 413 participants were recruited of whom 154 were HEU (37.3%) and 259 were HUU (62.7%). Table 1 shows the characteristics of participants by HIV exposure. No significant differences were found between HEU and HUU infants in terms of age, proportions of sex, birthweight or length, or birthweight classification. However, HEU infants had significantly more siblings compared to HUU infants ($P < 0.001$). In addition, HEU infants were more likely to have had a sibling who died compared to HUU infants ($P < 0.05$).

As shown in Table 1, HIV positive mothers tended to be older at the time of the infant's birth ($P < 0.001$). In addition, the primary caregivers of HEU infants had significantly lower education levels ($P < 0.001$). No significant differences were found in other mother/caregiver and household characteristics between the two groups.

Feeding practices

Table 2 shows feeding practices of infants per HIV-exposure from birth to age at time of data collection. These feeding practices were self-reported by the caregiver and corroborated using data from each child's health card, where possible. HEU infants were more likely to be formula fed from birth and at 6–12 months compared to HUU infants ($P < 0.001$). The remainder of the infants ($n = 11$) not breastfeeding or formula feeding in the first 12 months were taking cow's milk. Of those infants aged more than 12 months, it was found that HUU infants were more likely to be breastfed compared to their HEU counterparts ($P < 0.001$). Overall, the energy and protein intakes for male and female HEU and HUU infants were higher than recommended nutrient intakes (i.e. recommended nutrient intake for infants aged 1–3 years). Average energy and protein intake was found to be higher in HEU compared to HUU infants for females and vice versa for males. However, both these differences did not reach statistical significance. In addition, there were no significant differences between HEU and HUU infants in age at which the infant was introduced to complementary feeds. Dietary diversity was low for all infants and there was no significant difference between HEU and HUU infants.

Nutritional outcomes

The prevalence of being underweight was higher in HEU infants ($P < 0.01$) (Table 3). In addition, HEU infants also had significantly higher prevalence of stunting compared to HUU infants (15.6% versus 7.3%, $P < 0.05$). Wasting prevalence was higher in HEU infants; however, this did not reach statistical significance ($P = 0.14$).

Determinants of nutritional status

The results of logistic regression to identify the determinants of being underweight, stunting and wasting are shown in Tables 4–6. Table 4 shows the determinants of being underweight. The analysis revealed that infants living in homes where a child had previously died were over three times more likely to be underweight [adjusted odds ratio (OR) = 3.205, 95% confidence interval (CI) = 1.097–9.362]. However, a higher birthweight or birth length was negatively associated with being underweight ($P < 0.001$ and $P = 0.03$ respectively). Each 1 kg increase in weight reduced the risk of being underweight by 82% (OR = 0.182, 95% CI = 0.073–0.450). Similarly, a 1-cm increase in birth length reduced risk by 10% (OR = 0.899, 95% CI = 0.818–0.988). Importantly, HIV exposure, infant nutrient intakes, and maternal and household factors were not associated with the risk of being underweight. Predictors for stunting, as shown in Table 5, were consistent with the simple chi-squared

analysis of prevalence. HEU infants were found to be more than twice as likely to be stunted compared to HUU infants (adjusted OR = 2.361, 95% CI = 1.105–5.046). In addition, a lower level of mother/caregiver's education and a lower birthweight were associated with stunting. Again, nutrient intakes and other maternal and household factors were not significantly associated with the risk of stunting. Wasting was more likely in infants with a high APGAR score; however, residing in Kweneng East district (rural/semi urban) and having a higher birthweight was negatively associated with wasting. Each 1 kg of extra weight at birth reduced the risk of wasting by 58% (adjusted OR = 0.423, 95% CI = 0.205–0.872). HIV exposure, infant nutrient intake and other household and maternal factors were not significantly associated with the risk of wasting.

Table 1 Characteristics of HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants from selected districts in Botswana (n = 413)

Characteristic	Total (n = 413)	HEU infants (n = 154)	HUU infants (n = 259)	P-value
Infant's characteristics				
Age in months, median, (IQR)*†	14.00 (9.00)	14.00 (9.00)	14.00 (9.00)	0.96
Sex (%)‡				
Females	52.3	50.6	53.3	0.68
Males	47.7	49.4	46.7	
Birthweight (kg), mean (SD)†	3.01 (0.47)	2.96 (0.50)	3.03 (0.46)	0.15
Birth length (cm), mean (SD)†	50.01 (3.87)	49.71 (3.96)	50.19 (3.80)	0.23
Birthweight classification (%)‡				
Low: <2.5 kg	2.1	12.3	12.1	1.00
Normal: ≥2.5 kg	87.4	87.7	87.9	
Number of siblings, median (IQR)†	1.00 (2.00)	2.00 (2.00)	1.00 (1.00)	**<0.001**
Siblings who have died? (%)‡				
Yes	7.7	11.7	5.4	**0.03**
No	92.3	88.3	94.6	
Mother/caregiver's characteristics				
Mother's age at birth, median (IQR)†	26.00 (9.00)	30.00 (8.00)	25.00 (7.00)	**<0.001**
Primary caregiver's education level (%)‡				
0–7 years	9.9	17.5	5.4	**<0.001**
≥8 years	90.1	82.5	94.6	
Primary caregiver's marital status (%)‡				
Single/widowed/divorced/other	79.2	76.0%	81.1	0.27
Married/lives with partner	20.8	24.0%	18.9	
Primary caregiver's employment status (%)‡				
Not employed	75.8	77.3	74.9	0.63
Self-employed	3.9	4.5	3.5	
Formally employed	20.3	18.2	21.6	
Primary caregiver's monthly income (%)‡				
0–599 BWP§	69.0	68.8	69	0.86
600–999 BWP	5.1	5.8	5.1	
1000 + BWP	25.9	25.3	25.9	
Characteristics of the household				
Number of people in the household, median (IQR)†	6.00 (4.00)	6.00 (4.00)	6.00 (4.00)	0.88
Primary water source (%)‡				
Piped	99.5	99.4	99.6	0.71
Not piped	0.5	0.6	0.4	
Toilet type in homestead (%)‡				
Flush	25.6	21.6	28	0.18
Pit latrine	74.4	78.4	72	

*Age at the time of data collection

†Mann–Whitney U-test/ t-test was used to test for differences between HEU and HUU infants for nonparametric and parametric variables respectively.

‡Chi-squared was used to test difference in proportions (%) between HEU and HUU infants for various variables.

§$1 = 10.30 BWP (Botswana Pula) at the time of data collection.

IQR, interquartile range.

Significant values are shown in bold.

Table 2 Feeding practices of infants by HIV exposure from selected districts in Botswana.

Feeding practice	Total	HEU infants (n = 154)	HUU infants (n = 259)	P-value
Feeding method at <6 months (%)*				
Breastfeeding (BF)	n = 260 (63.0)	n = 27 (17.5)	n = 233 (94.0)	**<0.001**
Formula feeding (FF)	n = 142 (34.3)	n = 127 (82.5)	n = 15 (6.0)	
Feeding method between 6–12 months (%)*				
Breastfeeding (BF)	n = 186 (45.0)	n = 0 (0)	n = 186 (82.3)	**<0.001**
Formula feeding (FF)	n = 188 (45.5)	n = 148 (100)	n = 40 (17.7)	
Feeding method at 12+ months (%)*				
Breastfeeding (BF)	n = 29 (19.0)	n = 0 (0)	n = 29 (30.2)	**<0.001**
Formula feeding (FF)	n = 12 (7.9)	n = 7 (12.5)	n = 5 (5.2)	
Cow's milk	n = 111 (73.0)	n = 49 (87.5)	n = 62 (64.6)	
Age introduced complementary feeds in months†, median (IQR)	n = 413 6.00 (0.00)	n = 154 6.00 (0.00)	n = 259 6.00 (0.00)	0.92
Average energy intake (kcal), mean (SD)‡				
Females	n = 216 1684.9 (867.7)	n = 78 1778.5 (855.4)	n = 138 1632.0 (878.7)	0.23
Males	n = 197 1810.3 (830.6)	n = 76 1747.7 (716.8)	n = 121 1849.6 (895.4)	0.38
Average protein intake (g), mean (SD)‡				
Females	n = 216 53.2 (27.5)	n = 78 56.5 (26.9)	n = 138 51.3 (27.7)	0.18
Males	n = 197 57.5 (28.0)	n = 76 56.4 (26.8)	n = 121 58.2 (28.9)	0.65
Dietary diversity (%)§	n = 413	n = 154	n = 259	
Diet diverse	(16.8)	(20.1)	(14.8)	0.17
Diet not diverse	(83.2)	(79.9)	(85.2)	

*Chi-squared was used to test for differences in proportions of feeding method between HEU and HUU infants.
†Mann–Whitney U-test was used to test for differences in age introduced complementary feeds.
‡Independent-samples t-test was used to test for differences in energy and protein intake between HEU and HUU infants per sex.
§Dietary diversity: proportion of children 6–23 months of age who receive foods from 4 or more food groups (food groups: grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A rich fruits and vegetables; other fruits and vegetables).
HEU, HIV-exposed-uninfected; HUU, HIV-unexposed-uninfected; IQR, interquartile range.
Significant values are shown in bold.

Table 3 Prevalence of undernutrition in HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants from selected districts in Botswana.

Nutritional status	Total (n = 413)	HEU infants (n = 154)	HUU infants (n = 259)	P-value
Underweight (%)*				
Yes	10.2	15.6	6.9	**<0.01**
No	89.8	84.4	93.1	
Stunting (%)*				
Yes	10.4	15.6	7.3	**<0.05**
No	89.6	84.4	92.7	
Wasting (%)*				
Yes	11.9	14.9	10	0.14
No	88.1	85.1	90	

*Chi-squared was used to test for difference in proportions (%) of nutritional status between HEU and HUU infants.
Significant values are shown in bold.

Discussion

The present study has demonstrated that HEU infants aged 6–24 months have poor nutritional outcomes compared to HUU infants. This has implications for policy and programming because, currently, the prevention of mother-to-child transmission of HIV in HEU infants is prioritised over achieving optimal nutritional status. This has inadvertently resulted in inequitable growth between HEU and HUU infants. Data from 154 HEU infants and 259 HUU infants living in selected districts in Botswana demonstrated that HEU infants had a higher prevalence of being underweight and stunted. HEU infants were also more likely to formula feed in their first 12 months of life, whereas HUU infants were more likely to breastfeed. Low birthweight was the strongest predictor of undernutrition in addition to HIV exposure, birth length, mother/caregiver's education level, high APGAR score and residing in Kweneng East.

The prevalence of undernutrition in the present study was higher in HEU infants compared to HUU infants during their first 1000 days. This is consistent with findings from a number of studies conducted in Zambia, Kenya, South-Africa, Uganda and Tanzania, which have demonstrated that HEU infants have poor growth compared to HUU infants [9,12,20,38,39]. A study in Kenyan infants found that HEU infants had poor nutritional

Table 4 Logistic regression model of predictors of being underweight in infants aged 6–24 months in selected districts in Botswana (n = 356)

						95% CI for odds ratio	
	B	SE	Wald	*P*-value	Odds ratio	Lower	Upper
Step 1							
Birthweight	−2.091	0.410	25.981	0.000	0.124	0.055	0.276
Constant	3.836	1.127	11.589	0.001	46.346		
Step 2							
Primary water source	−2.696	1.468	3.375	0.066	0.067	0.004	1.198
Birthweight	−2.144	0.416	26.510	0.000	0.117	0.052	0.265
Constant	6.646	1.939	11.749	0.001	769.770		
Step 3							
Primary water source	−2.689	1.456	3.412	0.065	0.068	0.004	1.179
Birthweight	−1.694	0.460	13.567	0.000	0.184	0.075	0.453
Birth length	−0.104	0.048	4.811	0.028	0.901	0.821	0.989
Constant	10.475	2.661	15.498	0.000	35411.123		
Step 4							
Primary water source	−2.821	1.459	3.738	0.053	0.060	0.003	1.040
Infant lives in home where a child has died	1.165	0.547	4.537	0.033	3.205	1.097	9.362
Birthweight	−1.706	0.463	13.550	0.000	0.182	0.073	0.450
Birth length	−0.106	0.048	4.902	0.027	0.899	0.818	0.988
Constant	10.610	2.665	15.852	0.000	40537.131		

Model 1: r^2 = 16.3%, χ^2 = 30.25, d.f. = 1, $P < 0.001$: Model 2: r^2 = 17.8%, χ^2 = 33.03, d.f. = 2, $P < 0.001$: Model 3: r^2 = 20.0%, χ^2 = 37.46 d.f. = 3, $P < 0.001$: Model 4: r^2 = 22.0%, χ^2 = 41.48, d.f. = 4, $P < 0.001$.

Other independent variables entered into the model are: HIV exposure, sex, feeding method at <6 months, feeding method at 6–12 months, Infant primary caregiver, is mother alive?, mother/caregivers education, mother/caregiver's marital status, mother/caregiver's employment status, mother/caregiver's income per month, toilet type in homestead, health facility type, district, consumption of at least one source of iron rich food?, dietary diversity, age in months, APGAR score, age introduced complementary feeds, number of servings of tsabana per week, number of consultations with diarrhoea, number of feeds given yesterday, energy intake, protein intake, mother's age at birth, number of people in household and number of siblings/relatives living with infant aged <5 years.

APGAR, appearance, pulse, grimace, activity and respiration. CI, confidence interval; The APGAR test is usually given to a baby twice, 1 min after birth and then 5 min after birth.

outcomes, especially very high levels of stunting, by 24 months [12]. The prevalence of stunting in the present study between HEU and HUU infants was similar to that found in a study of Ugandan infants enrolled in the PMTCT programme [20]. Our bivariate analysis of the prevalence of stunting and being underweight between HEU and HUU infants is therefore consistent with the larger body of literature. However, other studies conducted in sub-Saharan Africa did not find any differences in nutritional outcomes between HEU and HUU infants [19,40,41]. It was found that HEU infants, although born slightly smaller compared to HUU infants, were able to quickly catch up in weight and length [19,41,42]. This lack of difference in growth patterns was attributed to higher levels of breastfeeding and/or effective counselling for feeding choices in HEU infants [19,41]. In the present study, HEU infants were more likely to be formula fed than breastfed compared to HUU infants. This may have contributed to their poor growth compared to HUU infants because poor growth is linked to no or sub-optimal breastfeeding [38,43]. Importantly, our regression modelling indicated that the mode of feeding in the first year of life was not a statistically significant predictor of undernutrition. However, these studies were conducted before ART was widely available to HIV positive women; therefore, this may have resulted in no difference in growth between HEU and HUU infants [19,41,42]. Other feeding practices, such as age of introduction of complimentary feeding (weaning), average energy and protein intake, and dietary diversity, were not significantly different between HEU and HUU infants. Dietary diversity was poor in both groups of infants because the majority of infants did not consume a variety of foods in the 24 h preceding the study. Dietary diversity is an important indicator of the quality of the diet as opposed to the quantity of the food served [26,33].

HEU infants in the present study were vulnerable to poorer nutritional outcomes, especially stunting because, even after adjusting for other variables, HIV-exposure remained a strong predictor for stunting. This finding is consistent with results from a number of studies [18,44,45]. A study, conducted in Tanzania found a lower length for age in HEU compared to HUU infants at 3 and 6 months [44]. A higher risk of stunting in HEU compared to HUU

Table 5 Logistic regression model of predictors of stunting in infants aged 6–24 months in selected districts in Botswana (n = 356)

						95% CI for odds ratio	
	B	SE	Wald	*P*-value	Odds ratio	Lower	Upper
Step 1							
Birthweight	−1.519	0.393	14.945	0.000	0.219	0.101	0.473
Constant	2.142	1.099	3.800	0.051	8.516		
Step 2							
Mother/caregiver education	−1.429	0.478	8.954	0.003	0.239	0.094	0.611
Birthweight	−1.588	0.400	15.740	0.000	0.204	0.093	0.448
Constant	3.563	1.228	8.417	0.004	35.276		
Step 3							
Mother/caregiver education	−1.475	0.481	9.410	0.002	0.229	0.089	0.587
Primary water source	−2.845	1.457	3.814	0.051	0.058	0.003	1.010
Birthweight	−1.637	0.406	16.295	0.000	0.194	0.088	0.431
Constant	6.552	2.006	10.661	0.001	700.311		
Step 4							
HIV exposure	0.859	0.388	4.913	0.027	2.361	1.105	5.046
Mother/caregiver education	−1.257	0.498	6.376	0.012	0.284	0.107	0.755
Primary water source	−2.797	1.441	3.769	0.052	0.061	0.004	1.027
Birthweight	−1.637	0.413	15.728	0.000	0.195	0.087	0.437
Constant	5.902	2.025	8.499	0.004	365.789		

Model 1: r^2 = 9.1%, χ^2 = 15.64, d.f. = 1, $P < 0.001$: Model 2: r^2 = 13.4%, χ^2 = 23.40, d.f. = 2, $P < 0.001$: Model 3: r^2 = 15.1%, χ^2 = 26.45 d.f. = 3, $P < 0.001$: Model 4: r^2 = 17.8%, χ^2 = 31.44 d.f. = 4, $P < 0.001$.

Other independent variables entered into the model are: sex, feeding method at <6 months, feeding method at 6–12 months, Infant primary caregiver, is mother alive?, mother/caregiver's marital status, mother/caregiver's employment status, mother/caregiver's income per month, toilet type in homestead, health facility type, district, does infant live in environment where a child has died?, consumption of at least one source of iron rich food?, dietary diversity, age in months, birth length, APGAR score, age introduced complementary feeds, number of servings of tsabana per week, number of consultations with diarrhoea, number of feeds given yesterday, energy intake, protein intake, mother's age at birth, number of people in household and number of siblings/relatives living with infant aged <5 years.

APGAR, appearance, pulse, grimace, activity and respiration; CI, confidence interval; The APGAR test is usually given to a baby twice, 1 min after birth and then 5 min after birth.

infants has serious implications because stunting is associated with poorer psychomotor and mental development in HEU infants [45]. This may affect the future potential development of these infants, especially if stunting is not reversed within the first 1000 days [46–48]. Factors such as exposure to ART during pregnancy, poor sanitation and infections in infants, especially diarrhoea, may account for the increased risk of stunting in HEU compared to HUU infants [26,46]. In studies where poor growth was associated with HIV-exposure, it was found that HEU infants had lower birthweight compared to HUU infants [14,18,40]. In the present study, HEU infants had lower birthweight compared to HUU infants; however, this did not reach statistical significance. This is in contrast to a number of studies where HEU infants are more likely to be smaller at birth compared to HUU infants [11,44,46,49]. Interestingly, low birthweight was a strong and consistent predictor for poor nutritional status (underweight, stunting and wasting). Infants with a low birthweight tend to be more vulnerable to poor nutrition and/or diseases effect [14,18]. The findings of the present study show that birthweight is a more powerful predictor of later nutritional status than nutrient intakes from complementary feeds, breastmilk versus formula feeding, and household and environmental factors, including number of people living in a household, primary water source and income level. Even though birth length was not significantly lower in HEU compared to HUU infants, birth length remained a predictor for being underweight, indicating that a lower birth length increased the risk of being underweight in these infants. This is consistent with findings from some studies where birth length is a significant intermediary of growth in infants [44,49,50].

Consistent with a number of studies, it was found that mother/caregiver's education level was a predictor for stunting after adjustment for other variables [12,18,26,51]. In addition, HIV positive mothers were significantly older than HIV negative mothers. Younger age and a higher education level are associated with better nutritional outcomes because these caregivers tend to have more knowledge about optimal feeding, hygiene and child caring practices [12,18,51,52]. These caring practices may especially be relevant in settings where HEU infants tend to formula feed [18]. It was also found that HEU infants had significantly more

Table 6 Logistic regression model of predictors of wasting in infants aged 6–24 months in selected districts in Botswana (n = 356)

						95% CI for odds ratio	
	B	SE	Wald	*P*-value	Odds ratio	Lower	Upper
Step 1							
Residing in Kweneng East district	−0.908	0.349	6.750	0.009	0.404	0.203	0.800
Constant	−1.596	0.197	65.697	0.000	0.203		
Step 2							
Residing in Kweneng East district	−0.924	0.352	6.890	0.009	0.397	0.199	0.791
Birthweight	−0.807	0.354	5.188	0.023	0.446	0.223	0.894
Constant	0.775	1.043	0.553	0.457	2.172		
Step 3							
Primary water source	−2.734	1.454	3.536	0.060	0.065	0.004	1.123
Residing in Kweneng East district	−1.002	0.361	7.679	0.006	0.367	0.181	0.746
Birthweight	−0.834	0.358	5.435	0.020	0.434	0.215	0.876
Constant	3.588	1.848	3.768	0.052	36.152		
Step 4							
Primary water source	−2.517	1.456	2.989	0.084	0.081	0.005	1.400
Residing in Kweneng East district	−1.053	0.365	8.332	0.004	0.349	0.171	0.713
Birthweight	−0.860	0.369	5.437	0.020	0.423	0.205	0.872
Apgar score at birth	0.582	0.283	4.247	0.039	1.790	1.029	3.115
Constant	−2.106	3.330	0.400	0.527	0.122		

Model 1: r^2 = 3.9%, χ^2 = 7.30, d.f. = 1, $P < 0.001$: Model 2: r^2 = 6.6%, χ^2 = 12.50, d.f. = 2, $P < 0.001$: Model 3: r^2 = 8.0%, χ^2 = 15.36 d.f. = 3, $P < 0.001$: Model 4: r^2 = 10.7%, χ^2 = 20.61, d.f. = 4, $P < 0.001$.

Other independent variables entered into the model are: HIV exposure, sex, feeding method at <6 months, feeding method at 6–12 months, Infant primary caregiver, is mother alive?, mother/caregivers education, mother/caregiver's marital status, mother/caregiver's employment status, mother/caregiver's income per month, toilet type in homestead, health facility type, district, does child live in environment where a child has died?, consumption of at least one source of iron rich food?, dietary diversity, age in months, birthweight, birth length, age introduced complementary feeds, number of servings of tsabana per week, number of consultations with diarrhoea, number of feeds given yesterday, energy intake, protein intake, mother's age at birth, number of people in household and number of siblings/relatives living with infant aged <5 years.

APGAR, appearance, pulse, grimace, activity and respiration; CI, confidence interval; The APGAR test is usually given to a baby twice, 1 min after birth and then 5 min after birth.

siblings than HUU infants. A higher number of siblings is associated with poor nutritional outcomes in children [53]. Although growing up in a household where another child had died was a significant predictor of the risk of being underweight in univariate analysis, after adjusting for potential confounding factors, there was no relationship between the number of deceased siblings and risk of stunting, wasting or being underweight.

Other determinants of nutritional outcomes in these infants included residing in Kweneng East district and APGAR score. Infants who resided in Kweneng East had a lower risk of wasting compared to those in other districts. It is should be noted that Kweneng East district was the only district where growth and health monitoring services were still offered in the main and primary hospital. Other districts have moved these services to smaller clinics and/or health posts. Therefore, infants in Kweneng East district may have benefited from having close access to a multidisciplinary team of health professionals such as paediatricians and dietitians. These healthcare workers are not typically accessible in smaller clinics. Accessibility to specialised care is highly relevant to wasting because wasting is an acute form of undernutrition, characterised by rapid weight loss as a result of an acute inadequate intake and/or disease [54]. Therefore, infants in Kweneng East district were more likely to have accessed swift and specialised care upon being diagnosed with wasting compared to other districts. A higher APGAR score increased the risk of wasting in these infants by almost two-fold. This was not expected because a higher APGAR score is associated with better nutritional outcomes [55]. However, a study in Asian Indian infants found that APGAR is a poor prognosis for growth and development in infants [56].

It is important to note certain limitations about the present study. We have only considered the impact of HIV exposure, infant feeding, maternal and household factors upon nutritional status using the extreme outcome measures of stunting, wasting and being underweight, as determined by anthropometry and reference to WHO cut-offs for *Z*-scores. Indices such as micronutrient deficiencies were not included and, in addition, we did not focus on lower variance from cut-offs in terms of growth. Contribution of HIV-exposure may be greater at these subclinical levels and thus the *Z*-scores may be lower in HEU

compared to HUU infants. As a result of the cross-sectional study design, we did not have access to maternal nutrition and health indicators variables such as weight, height, CD4 count and use of ART pre- and post-natally. There is also a possibility, albeit a limited one, that some of the infants who were classified as HEU may have been HIV-infected after 6 weeks because testing of HIV in these infants in Botswana is carried out at 6 weeks, post-weaning, if the mother was breastfeeding (6 months) and at 18 months. Some of the infants in the present study were not yet aged 18 months at the time of data collection. However, a majority of these infants were formula feeding; therefore, it was highly unlikely that they would have seroconverted. The parity of the mother was not considered in logistic regression. In addition, we have to acknowledge the cross-sectional nature of the present study, especially with respect to HIV-exposure and nutritional outcomes. Longitudinal studies are therefore required to elicit more data that will allow us to disentangle feeding modalities from HIV-exposure and also to derive more information on maternal nutrition and health during pregnancy.

PMTCT strategies in Botswana need to be refined so that optimal nutritional outcomes in HEU infants are prioritised in addition to prevention of MTCT of HIV. This can be achieved by integrating nutrition-specific and nutrition-sensitive interventions into this programme. This will ensure equitable and optimal growth in HEU and HUU infants during their first 1000 days. Botswana as a country in terms of its healthcare system infrastructure, PMTCT strategies and growth surveillance for infants is in a good position to effect these significant changes, and thus improve population health.

In Botswana, HEU infants aged 6–24 months have poor nutritional status compared to HUU infants. Although the mode of feeding was not a statistically significant factor determining risk of undernutrition, HEU infants tended to formula feed, whereas HUU infants tended to breastfeed, for the first 12 months of life. Therefore, HEU infants are missing out on the well documented benefits of breastfeeding. To increase breastfeeding levels in HEU infants, there is need to review the current Botswana government's infant feeding policy to align with the new 2016 recommendations by WHO. Furthermore, the present study has demonstrated that the strongest predictor of nutritional outcomes is birthweight; therefore, strategies designed to optimise the nutritional status of infants in the first 1000 days should aim to improve birthweight.

Transparency declaration

The lead author confirms that the manuscript is an honest, accurate and transparent account of the study being reported and 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.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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PC, SLE, JAS and DM were responsible for the study design and protocol, as well as the tools used in the study. PC was responsible for data collection. CE was responsible for the nutritional analysis of the cereal samples consumed by the study participants. Data analysis and review was conducted by PC, JAS and SLE. All authors were responsible for completing the manuscript and approved the final version submitted for publication.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Table S1 Independent variables used in logistic regression model.

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NUTRITION IN DEPRIVATION

Deprivation and healthy food access, cost and availability: a cross-sectional study

S. Williamson, M. McGregor-Shenton, B. Brumble, B. Wright & C. Pettinger

School of Health Professions, University of Plymouth, Plymouth, UK

Keywords
food deserts, food retail mapping.
healthy food access, healthy food basket survey,
socio-economic inequalities in food retail.

Correspondence
C. Pettinger, School of Health Professions,
Plymouth University, Drake Circus, Plymouth, UK.
Tel.: 01752 588822
Fax: 01752 588874
E-mail: clare.pettinger@plymouth.ac.uk

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Abstract

Background: Food access, cost and availability have been identified as determinants of dietary choice. It has been suggested that these are socio-economically patterned; however, the evidence is inconclusive. The present study investigated whether differences exist with respect to healthy food access, cost and availability between areas of contrasting deprivation.
Methods: An ecological, cross-sectional study was conducted in two of the most and two of the least deprived wards in Plymouth. Food retail outlets (FROs) (n = 38) were identified and mapped using Geographic Information Systems to assess 'physical access', by foot, to food retail provision. Healthy food basket (HFB) surveys were conducted (n = 32) to compare the cost and availability of 28 healthy food items between the more and less deprived areas.
Results: Areas of poor access to food retail provision were identified in both study areas, with a higher number of households in the more-deprived areas being affected than in the less-deprived areas, after accounting for car ownership levels. Median [IQR] HFB availability was lower in more-deprived than the less-deprived areas (48%, [39-71%] vs. 75%, [68-82%]; P=0.003), and in convenience stores than supermarkets (54%, [43-72%] vs. 78%, [72-96%]; P=0.001). Descriptive summaries revealed negligible differences in total median HFB cost between the more-deprived and less-deprived areas (£55.97 versus £55.94) and a larger cost difference between convenience stores and supermarkets (£62.39 versus £44.25).
Conclusions: Differences were found with respect to healthy food access, cost and availability in areas of contrasting deprivation. These appeared to be related to FRO type rather than deprivation alone.

Introduction

The 'food environment' has been implicated as a critical determinant of food choice [1]. If UK diets matched nutritional guidelines, almost 70 000 premature deaths from chronic noncommunicable diseases could be prevented annually [2]. This is particularly pertinent to low socio-economic groups (LSGs) as a result of the documented social gradient in the nutritional quality of the diet, with studies reporting that those on the lowest incomes consume more salt, sugar and saturated fat, and less fruit and vegetables [3]. However, dietary choice is multifaceted and complex because of influences from a range of biological and societal factors [4]. Increasingly, research has focused upon the influence of the food environment on dietary choice, suggesting that food access, cost and availability may be important determinants of the nutritional quality of the diet [5].

Food access refers to physical access to food retail provision [5] and is dependent upon geographical location and resources such as transport accessibility [4]. The Geographic Information System (GIS) is considered useful for

assessing food retail access [6] as a result of its capacity to map and spatially analyse data [7]. Availability refers to the types of food retail outlets (FROs) in a geographical area, as well as the foods that they sell [8]. Previous research has measured the availability and cost of healthy food items using Healthy Food Basket (HFB) surveys [9,10], which have been found to have sufficient sensitivity to discriminate well between stores [9].

It has been suggested that food access, cost and availability are socio-economically patterned, with research from the USA finding that lower income areas have lower access to healthy foods [11]. Specifically, it was observed that the FROs in these areas offered lower healthy food availability, at the same time as also charging higher prices [12,13]. Areas where it is difficult to purchase healthy food items at a reasonable price are referred to as 'Food Deserts'[13]. The existence of Food Deserts is widely accepted in the USA [14], however, is vigorously debated in the literature elsewhere [13,15].

In the UK, a comprehensive review of the evidence concluded that 'Food Deserts do exist in the UK, although only for individuals who do not or cannot shop outside of their immediate locality, and when the locality itself has poor retail provision of healthy foods' [13]. It has previously been shown that deprived areas have reduced access to shopping facilities [16], which has been attributed to the rise of large, out-of-town superstores that tend to favour car owners [17]. Because those individuals from LSGs are less likely to own a car [18], this supports the existence of a social gradient regarding healthy food retail provision. However, a more recent systematic review contradicted this finding, concluding that unsubstantial evidence exists to suggest that food access is socio-economically-patterned in the UK [14]. Research into the relationship between the food retail environment and dietary intake is still underdeveloped in the UK [5] and therefore the evidence remains inconclusive.

It is clear that more UK-specific research is needed regarding healthy food provision in the food retail environment. Therefore, the present study aimed to explore whether the level of deprivation affects the access to, as well as the cost and availability of, foods representative of a healthy diet.

Materials and methods

Study design

This exploratory ecological cross-sectional study investigated healthy food retail access in areas of contrasting deprivation in Plymouth; a South West UK coastal city. FROs were identified using primary and secondary data sources, and were mapped using GIS to determine areas of poor physical access, by foot, to food retail provision. Healthy food availability and cost were assessed and compared using a HFB survey. All data were collected during 1 week in May 2016, aiming to minimise seasonable variations in food availability and cost.

Food retail outlets

In line with previous research, the food retail environment was investigated and compared at the electoral ward level [19–21]. The Indices of Multiple Deprivation Electoral Wards Rank [22] was used to identify two of the most and two of the least deprived of the 20 wards in Plymouth, and these were grouped to form two areas of contrasting deprivation. Electoral wards are aggregations of Lower Super Output Areas (LSOAs), which vary in size to maintain an average population of 1500 residents [23]. Identified wards in the present study included St Budeaux and Honicknowle, ranked the third and fourth most deprived wards in Plymouth, respectively; and Plymstock Dunstone and Plympton St Mary, ranked the two least deprived wards. The more-deprived area comprises 24 LSOAs and has a total population size of 28,173 [24], whereas the less-deprived area, comprising 21 LSOAs, has a population size of 25,173 [24].

Food retail outlets were consecutively sampled from an extensive list of all identified FROs in the four wards, generated using secondary data sources including Local Authority databases, Google Maps and Yell.com, as well as websites of major food retailers and symbol groups (e.g. Premier). In line with other studies, 500 m was considered to be a reasonable distance to travel to FROs by foot [21] and thus FROs within 500 m of the ward boundaries were included in the study because residents on ward boundary edges would still have access to these FROs[19]. Included FROs were superstores (25–60 000 square feet), supermarkets (3–25 000 square feet) and convenience stores (<3000 square feet), as defined in the UK by the Institute of Grocery Distribution (IGD) [25]. All other FROs were excluded as a result of the observation that food shopping in England is most commonly completed 'under one roof' [20].

To validate the secondary data sources used, all identified FROs were verified visually or by telephone contact because primary data collection in the form of field work has been identified as the 'gold standard' for verifying the food environment [26]. As a result of some identified discrepancies between the classification of FROs on Google and the retailers' own websites, the researchers re-classified FROs in accordance with the IGD definitions. The definition of a convenience store is well-established [27]; however, because of practical limitations, store managers were relied upon to verify the classification between supermarket and superstore. From this, the 39 verified

FROs were identified and invited to participate in the research. Consent to conduct in-store data collection was sought by postal letter and nonrespondents were followed-up in person.

ARCGIS, version 10.4 [28] was used to map the spatial coordinates of all 39 verified FROs, and to create 500-m geographical buffer zones around each. Areas within the ward which fell outside of these zones were considered to have poor physical access, by foot, to food retail provision. Census datasets relating to car ownership were also incorporated at the LSOA level [29]. This was to enable a visual appraisal of the percentage of households without car availability, which are located in areas identified to have poor physical access, by foot, to food retail provision.

Healthy food basket survey

Cost and availability of 28 healthy foods were measured using a HFB survey (Table 1); an adaptation of the previously validated Healthy Eating Indicator Shopping Basket [30] (HEISB). The intention was to use a range of products representing a healthy, balanced diet and therefore the adaptations were designed to better reflect the composition of the Eatwell Guide [31] and the South West UK locality of the study. An adapted version of food item descriptions and a list of acceptable substitutions [9] were used to reduce the risk of systematic error during data collection. The costs of food items were recorded according to the cheapest own-brand product available in the sizes specified [9]. If this information was unavailable, the price-per-kilogram of product was recorded, along with the product weight, to enable the price-per-unit to be calculated. In line with previous research, promotional prices were not recorded [10]. Informed, signed consent was sought from FRO managers prior to conducting the surveys.

Table 1 Differences in availability of healthy food basket items (%) by deprivation level and food retail outlet type

	Deprivation level		Food retail outlet type	
Food item (n = 28)	High (n = 20) Stocked, n (%)*	Low (n = 12) Stocked, n (%)*	Convenience store (n = 25) Stocked, n (%)*	Supermarket (n = 7) Stocked, n (%)*
Brown rolls	13 (65)	13 (65)	18 (72)	7 (100)
Potatoes	19 (95)	19 (95)	24 (96)	7 (100)
Brown rice	4 (20)	4 (20)	5 (20)	3 (57)
White rice	20 (100)	20 (100)	25 (100)	7 (100)
Pasta	20 (100)	20 (100)	25 (100)	7 (100)
Weetabix	18 (90)	18 (90)	22 (88)	7 (100)
Wholemeal bread	15 (75)	15 (75)	20 (80)	7 (100)
Apples	16 (80)	16 (80)	21 (84)	7 (100)
Bananas	14 (70)	14 (70)	19 (76)	7 (100)
Grapes	12 (60)	12 (60)	16 (64)	7 (100)
Orange	10 (50)	10 (50)	14 (56)	7 (100)
Orange juice	19 (95)	19 (95)	24 (96)	7 (100)
Broccoli	10 (50)	10 (50)	14 (56)	7 (100)
Carrots	12 (60)	12 (60)	17 (68)	7 (100)
Cucumber	14 (70)	14 (70)	19 (76)	7 (100)
Lettuce	13 (65)	13 (65)	17 (68)	7 (100)
Onions	20 (100)	20 (100)	25 (100)	7 (100)
Peas	18 (90)	18 (90)	23 (92)	7 (100)
Peppers	13 (65)	13 (65)	18 (72)	7 (100)
Tomatoes	19 (95)	19 (95)	24 (96)	7 (100)
Semi-skimmed milk	20 (100)	20 (100)	25 (100)	7 (100)
Skimmed milk	14 (70)	14 (70)	19 (76)	7 (100)
Low-fat yoghurt	12 (60)	12 (60)	16 (64)	7 (100)
Lean beef mince	3 (15)	3 (15)	2 (8)	6 (86)
Chicken breast	13 (65)	13 (65)	16 (64)	7 (100)
Salmon	6 (30)	6 (30)	8 (32)	7 (100)
Baked beans	20 (100)	20 (100)	25 (100)	7 (100)
Low-fat spread	10 (50)	10 (50)	14 (56)	7 (100)

*Category consists of groups: 'in-stock', 'out of stock, awaiting delivery', not stocked but first substitute available', not stocked, but second substitute available'.

Statistical analysis

Data were inputted into EXCEL (Microsoft Corp., Redmond, WA, USA) in duplicate, and cross-checked for consistency by another member of the research team to improve the inter-rater reliability. All data analysis was conducted by deprivation level (more-deprived, less-deprived), by FRO type (convenience store, supermarket) and by FRO subtype (more-deprived convenience stores, more-deprived supermarkets, less-deprived convenience stores, less-deprived supermarkets) categories. No superstores were identified in the study areas.

Consistent with methodology from similar studies [9], to enable price comparisons between the HFB items across the FROs, varying product sizes were standardised to the specified unit in the substitution list. For those items without a weight, average weights for these items were determined, using values from three supermarket websites. As a result of the small number of stores that stocked the full HFB, a full HFB cost was calculated by deprivation level and FRO type using median prices-per-item.

A Mann–Whitney U-test was conducted to determine differences in percentage HFB availability between deprivation level and FRO type. A Kruskal–Wallis analysis of variance was also conducted to determine differences in percentage HFB availability between FRO subtype. Dunn's pairwise comparison with Bonferroni adjustment provided post-hoc analysis [32]. Statistical analysis was

conducted using EXCEL (Microsoft Corp.) and SPSS, version 22.0 (IBM Corp., Armonk, NY, USA) [33]. $P \leq 0.05$ was considered statistically significant.

Ethical considerations

Ethical approval was granted by the School of Health Professions Bachelor's Degree Ethics Subcommittee. To minimise the risk of reputational harm, FRO data remained anonymous throughout the study process.

Results

Food retail outlets

Thirty-eight FROs were confirmed within the study areas. Of these, 32 consented to participate in the HFB survey, five declined and one was closed for refurbishment at the time of surveying. The proportion of the total number of FROs is higher in the more-deprived areas than the less deprived areas [$n = 23$ (61%) versus $n = 15$ (39%), respectively], with a higher proportion of convenience stores to supermarkets, both in the more-deprived areas [$n = 19$ (83%) versus $n = 4$ (17%), respectively] and less-deprived areas [$n = 10$ (67%) versus $n = 5$ (33%), respectively]. The six nonparticipants of the survey were equally matched in terms of deprivation level and FRO type.

Access

All identified FROs are shown in Fig. 1, including 500-m geographical buffer zones. Areas outside of these buffer zones were considered to have poor physical access, by foot, to food retail provision. The percentage of households without car availability in these identified areas of poor access ranged from 13% to 46% in the more-deprived areas and from 4% to 22% in the less-deprived areas.

Healthy food basket survey

Descriptive summaries revealed negligible differences in median HFB cost between the more-deprived and the less-deprived areas (£55.97 versus £55.44). However, a larger cost difference was found between convenience stores and supermarkets (£62.39 versus £44.25). Subgroup

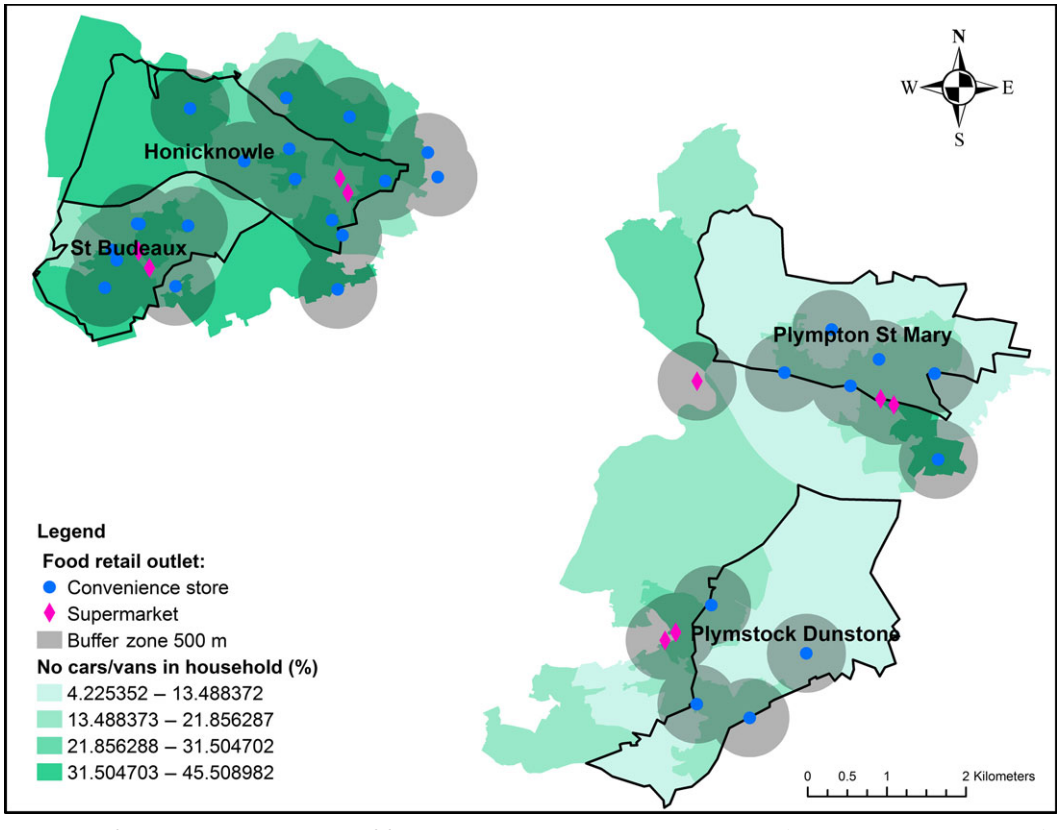

Figure 1 Geographic Information Systems mapping of food retail outlets in the more-deprived areas (Honicknowle and St Budeaux) and the less-deprived areas (Plympton St Mary and Plymstock Dunstone). Areas outside of the geographical buffer zones indicate poor physical access, by foot, to food retail provision, and car ownership data showing the percentage of households without car availability by Lower Super Output Area.

analysis found that the median HFB cost was lower in both convenience stores and supermarkets in the more-deprived areas than in convenience stores and supermarkets in the less-deprived areas (£60.15 and £42.30 versus £63.60 and £45.48, respectively).

Across the 32 FROs surveyed, four (13%) stocked all 28 HFB items, whereas 21 (66%) stocked at least half of the HFB. Median [IQR] HFB availability was lower in the more-deprived areas compared to the less-deprived (48% [39-71%] vs. 75% [68-82%]; U=195.000, P=0.003), and in convenience stores compared to supermarkets (54% [43-72%] vs. 78% [72-96%]; U=153.500, P=0.001). These data are reported in Table 1. Median HFB availability differed by FRO subtype ($H^2 = 16.272$, $P = 0.001$), with the largest difference identified between convenience stores in the more-deprived areas and supermarkets in the less-deprived areas ($P = 0.018$). Differences in availability were also found between convenience stores in the more-deprived areas and convenience stores in the less-deprived areas ($P = 0.044$), as well as between convenience stores in the more-deprived areas and supermarkets in the less-deprived areas ($P = 0.047$).

Discussion

The present exploratory study investigated whether deprivation level affects healthy food access, cost and availability. Areas of poor physical access, by foot, to food retail provision were identified in both study areas. However, within these areas of poor access, local data show that more households in the more-deprived areas did not have access to a car or van compared households to in the less-deprived areas [29] (Fig. 1). Previous research has failed to demonstrate socio-economic patterning regarding the access to healthy food retail provision[34]; however, those living in the more-deprived areas are less likely to have access to a car [27]. Despite their use of taxis [13] and online food shopping [35], individuals without car access are significantly more likely to travel home from food shopping by foot [36]. Therefore, they are likely to be particularly susceptible to changes in the local food retail environment regarding the provision of healthy food. Interestingly, the more-deprived areas contained more convenience stores and fewer supermarkets than the less-deprived areas [9]. Because less individuals in the more-deprived areas had access to a car or van [29], this suggests a heavier reliance upon convenience stores for those living in more-deprived areas.

In terms of the cost of healthy food, it was expected that convenience stores would charge more on average for the full HFB, and this is supported by the existing literature [13]. Therefore, it was surprising that negligible differences were found in the cost of healthy food between the more and the less-deprived areas. Although this aligns with findings obtained in the study by White *et al.* [13], it contrasts with other studies reported in the literature. Dawson *et al.* [9] found that healthy food cost less in less deprived areas, whereas Cummins and McIntyre [12] found that it cost more. An explanation for this finding is that cost data were only obtainable for in-stock items, therefore causing a bias towards the FROs that had higher availability and corresponding lower costs. Previous studies have also encountered difficulties in comparing the cost of food baskets [9,13,21], with Beaulac *et al.* [14] attributing the mixed findings to the low methodological quality of the studies cost comparisons. As such, findings relating to HFB cost in the present study, and indeed other food basket surveys, should be interpreted with caution. Despite this, the findings from the present study suggest that the average cost of healthy food is comparable between areas of contrasting deprivation; however, it clearly identifies considerable differences in the cost of healthy food between convenience stores and supermarkets. Considering the higher proportion of convenience stores in more-deprived areas, this suggests a social gradient in the cost of healthy food.

The differences found in HFB availability between ward deprivation level were expected. On average, availability was lower in the more-deprived areas compared to the less-deprived areas. Specifically, wholegrain carbohydrates, fruit and vegetables, low fat dairy products, lean meats, oily fish and low fat spread were less frequently stocked in the more-deprived areas (Table 1). This finding is in accordance with previous research [9] and is important because it suggests that residents of deprived areas could struggle to eat healthily [37], thereby increasing their risk of noncommunicable diseases [38]. However, findings from a larger study by White *et al.*[15] contradict this, countering that healthy food availability is not socio-economically patterned but, instead, is associated with store type. It is plausible that the findings from this small scale local research are a result of the high prevalence of convenience stores in the most-deprived area, which were found to have a lower availability of healthy foods compared to supermarkets. This finding is undisputed in the literature [39] and, in previous research, has been attributed to the lower demand for healthier and more perishable foods in deprived areas [15].

It was interesting to find that the more-deprived areas contained more convenience stores and fewer supermarkets than the less-deprived areas. This indicates that there is the potential for convenience stores to influence the food retail environment in deprived communities, where it is suggested that larger retailers avoid trading as a result of lower levels of disposable income in these areas [40]. Despite finding that convenience stores offered a lower

provision of healthy foods, anecdotal evidence collected found that some convenience store retailers were willing to stock healthier food items. One store ordered wholemeal bread upon customer request, whereas another stocked competitively priced, fresh produce variety packs suitable for single household customers. These observations highlight the potentially pivotal role that convenience store retailers could play in enhancing healthy food provision in deprived areas, although they also indicate that some stores could benefit from additional education and support to replicate this. Because households in the more-deprived areas appeared most likely to depend upon these stores, these promising anecdotal findings warrant further investigation. However, it should be recognised that there is little incentive for improving the availability of healthy foods if there is no demand [41] and so this recommendation would need to be considered within the wider determinants of food choice [42]. Community and public health dietitians promote the importance of a healthy diet within their local communities, and so they would be appropriately placed to lead this partnership with convenience store owners.

The present study provides a unique insight into the food retail environment in areas of contrasting deprivation in a South West UK coastal city. However, because of the specific locality of the four study areas, the generalisability of the findings to other areas may be limited. Strengths include the thorough identification and mapping of FROs, in addition to the comprehensive assessment of HFB availability, which further validates the previously developed HEISB tool [30]. However, methodological limitations are inherent in all research, and the present study was no exception. First, the ecological and cross-sectional design of the study was unable to differentiate cause and effect from simple association [43]. Second, the linear ARCGIS assessment of distance is somewhat oversimplistic. Mapping of the walking, driving and public transport routes would have generated the most comprehensive depiction of the food retail environment, although this was beyond the scope of the present study. Finally, the approach taken to compare the cost of HFB items has resulted in some being disproportionately adjusted, consequently reducing the validity of these findings. Despite the highlighted limitations, the findings from the present study will help to inform research regarding the physical and social determinants of food choice, which is an area of key importance for public health professionals.

Recommendations and future work

This exploratory research provides a better understanding of inequalities in healthy food provision, and offer insight into why individuals from LSGs can fail to adhere to nutritional recommendations [44]. The largest scope to make a difference lies in areas where individuals are most reliant upon their local food retail environment, which itself offers poor healthy food provision [13]. This highlights an area where public health specialists, public health dietitians and policy makers may have the largest impact. Interventions to increase healthy food provision could be achieved through partnership-working with convenience store retailers, building on the previous successes of Change4Life [45]. Such initiatives could include the redesign of store layouts to ensure prominent positioning of healthier foods and the introduction of legislation to increase the display of healthier foods at the point of sale and on in-store communications. Additionally, store owners could be encouraged to increase their provision of less-perishable healthier food items [46]. It would be interesting to develop this research further, to explore the extent to which the access to, as well as the cost and availability of, healthy food influences consumer dietary choice. This could complement research investigating both the influence of the retail provision of unhealthy food [47], and the density and location of fast food outlets, on dietary choice [48,49].

Conclusions

Differences were found in healthy food access, cost and availability in areas of contrasting deprivation. These appeared related to FRO type rather than deprivation alone, with convenience stores consistently demonstrating lower healthy food availability than supermarkets, and at a higher cost. Future interventions to improve the access to, as well as the cost and availability of, healthy food should concentrate upon the more-deprived communities. Partnership-working between public health professionals and convenience stores could be pivotal in this process.

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 [50] guidelines.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts ofinterest.
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SW, MMcG-S, BB and BW conducted the data collection. SW, MMcG-S, BB and BW conducted the statistical analysis. SW led the journal write up. MMcG-S, BB and BW conducted the proofreading. MMcG-S and CP assisted with the journal write up. CP provided supervision and feedback throughout the study.

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