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CHILDREN AND ADOLESCENTS

Is there an association between dietary intake and academic achievement: a systematic review

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Abstract

Background: The majority of literature examining the effect of dietary behaviour on academic achievement has focused on breakfast consumption only. Here, we aim to systematically review the literature investigating the broader effects of dietary intake and behaviours on school-aged children's academic achievement.

Methods: A search was undertaken across seven databases using keywords. For studies to be included, they needed to be conducted in: school-aged children (5–18 years); assess and report: (i) a measure of academic performance; (ii) a measure of dietary intake/behaviour; and (iii) the association between dietary intake/behaviours and academic performance. Forty studies were included in the review.

Results: The majority of studies were cross-sectional in design (n = 33) and studied children aged >10 years, with very few reports in younger age groups. More than 30 different dietary assessment tools were used, with only 40% of those using a validated/standardised assessment method. Half the studies collected outcomes of academic achievement objectively from a recognised educational authority, whereas 10 studies used self-reported measures. The dietary outcomes most commonly reported to have positive associations with academic achievement were: breakfast consumption (n = 12) and global diet quality/meal patterns (n = 7), whereas negative associations reported with junk/fast food (n = 9).

Conclusions: This review highlights that moderate associations exist for dietary intakes characterised by regular breakfast consumption, lower intakes of energy-dense, nutrient-poor foods and overall diet quality with respect to outcomes of academic achievement. Future studies should consider the use of validated dietary assessment methods and standardised reporting of academic achievement.

Introduction

Children's academic achievement has a significant influence on their future health and social outcomes [1,2]. Academic achievement is described as an outcome of education and is associated with long-term educational attainment, which determines health and life opportunities by affecting employment prospects, socio-economic status, access to health care and psychosocial well being [2–5]. Academic achievement can be measured in a variety of ways and this often takes the form of examinations or continuous assessment. Given the demonstrated significance of children's academic achievement, a better understanding of modifiable factors that can affect achievement is important for parents, educational authorities and public health researchers [6].

A child's academic achievement is affected by several determinants, including gender and family characteristics, such as socio-economic status, parent education level and attitudes towards school [7]. Individual characteristics

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such as behaviour, motivation and aptitude, as well as the learning environment, including teacher quality and school resources, are also acknowledged to have interdependent effects on academic achievement [1,4,7,8]. The existing literature has examined the relationships between health and lifestyle behaviours, with studies demonstrating links between poor quality sleep, reduced learning capability and poorer academic achievement in both children and adolescents [9]; tobacco smoking and lower academic achievement in adolescents [10]; and positive associations between physical activity and academic achievement [11,12]. Within this context, dietary behaviours are of high interest because of the significant physical, mental and cognitive development that occurs during childhood and adolescence, resulting in the highest nutrient requirements at any time across the life cycle [13,14].

The majority of literature examining the effects of dietary behaviours on academic achievement has focused on breakfast consumption [15,16]. The effects of breakfast consumption on children's academic achievement have been well investigated and synthesised in two recent reviews available in the literature [17,18]. Current global trends in food environments, such as the increased availability of convenience foods, which are often energy-dense and nutrient-poor, as well as rising rates of children who are overweight and obese, have resulted in an increased research focus directed towards examining the effects of the broader diet on academic achievement [19]. To date, no review has synthesised the literature investigating the effects of dietary behaviours and overall diet quality on children's academic achievement. The aim of the present study is to systematically review literature investigating the effects of dietary intakes and behaviours on the academic achievement of school-aged children (5–18 years). The results of this review endeavour to synthesise current understanding regarding the effects of the broader diet on children's academic achievement and to identify gaps in the current literature to inform further research.

Materials and methods

A three-step search strategy was undertaken to identify published studies in the English language between 1985 and April 2016. This search protocol was registered and available through Prospero CRD42015030189. To identify relevant key words, we undertook a staged approach. Stage 1 included an initial search of Medline and Cumulative Index to Nursing and Allied Health Literature (Cinahl) followed by an analysis of terms contained within the title, abstract and index terms used to describe the article. Stage 2 included a comprehensive search using keywords and index terms identified in Stage 1 across seven electronic databases: Premedline/Medline, The Cochrane Library, EMBASE (Excerpta Medica Database), Cinahl, Web of Science, Scopus and PsycInfo. The full search strategy is provided in the Supporting information (Data S1). For Stage 3, the reference lists of all identified studies were hand searched by the reviewers for additional studies, and were subsequently checked for eligibility. The key words used included: children, adolescent, paediatric (pediatric), dietary intake, food intake, dietary behaviour/behavior, nutrition, diet quality, academic performance, academic achievement, educational achievement and school performance. For studies to be included, they needed to include children and adolescents of school age, defined as aged 5–18 years (a majority of study participants had to be within this age range or report outcomes separately for this age group) and participants had to be from a healthy population (i.e. not a disease-specific population such as cystic fibrosis or malnutrition). Studies needed to assess and report: (i) a measure of academic performance; (ii) a measure of dietary intake/behaviour; and (iii) a measure of the association between dietary intake/behaviours and academic performance, which could include but was not limited to: correlation, regression and odds ratio analyses. Measures of academic performance had to measure the extent to which students had reached educational targets. This could include but was not limited to: grade point averages (GPA), school grades or reports, standardised educational assessment tests and self-reported academic performance. Measures of school attendance, behaviour at school and cognitive performance/skills were not included. Measures of dietary intake and behaviour could include but were not limited to: intake of specific food groups, meal consumption behaviours, diet quality, and macro- and micronutrient intakes. Studies focused on dietary supplementation (e.g. iron, omega 3) or fortification was excluded. All observational and experimental study types were included. Letters to the editor, thesis papers, dissertations and conference proceedings/abstracts were excluded.

Selection process

All studies identified via the database search were screened for eligibility in the review based on the information contained in the title, abstract and description/Medical Subject Heading (MESH) headings by two independent reviewers. The process is outlined in Fig. 1. In any cases of uncertainty concerning the inclusion of a study, a third independent reviewer was consulted until a consensus was reached. For all studies that meet the inclusion criteria, the full articles were retrieved. If eligibility was unclear based on the title, abstract and

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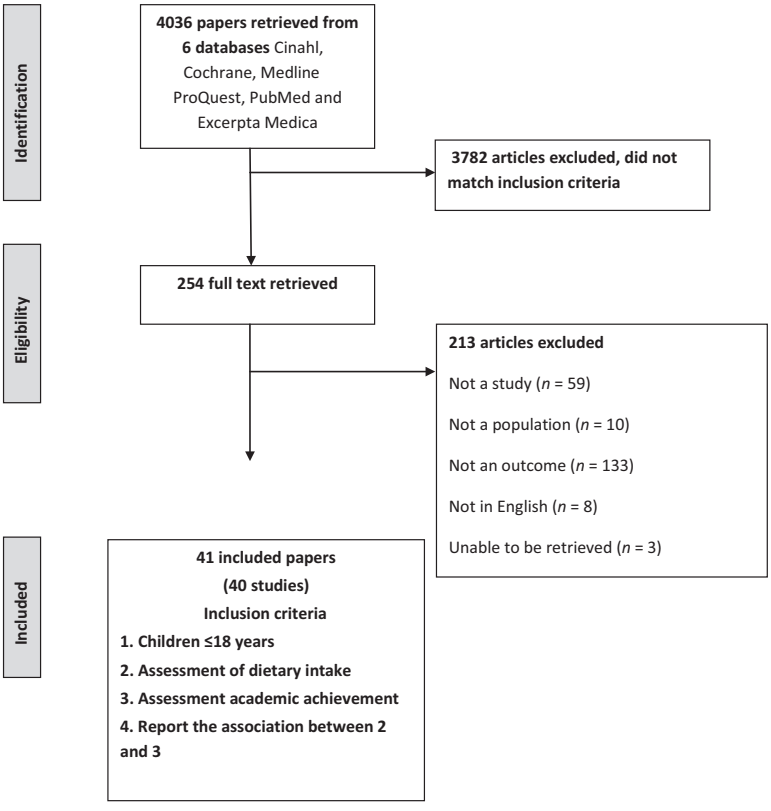

Figure 1 Flow diagram of article identification retrieval and inclusion in the systematic review.

description/MESH headings, the study was retrieved for further clarification.

All identified studies that meet the inclusion criteria were assessed for methodological quality by two independent reviewers. A third independent reviewer assessed studies when there was disagreement about the quality of the data extracted. Data extraction was carried out using standardised spreadsheets developed for the present review by two reviewers. Where there was disagreement between reviewers, a third opinion was sought.

Critical appraisal/risk of bias

Retrieved studies were assessed by two independent reviewers using a standardised specific tool from the American Dietetic Association [20]. The quality criteria assessed nine items, of which four relate to study validity. The items assessed included: the method of sample selection, methods of controlling for confounding factors, reliability of outcome measures and statistical analysis. Each item was classified as present 'Yes', absent 'No' or 'Unclear' for each included study and then each response was recoded as +1, 0 and −1, respectively. Studies were classified as positive quality where studies obtained a 'yes' to validity questions and had a score of eight or above, and neutral or negative if most of the answers were 'no' specifically for the validation questions. No studies were excluded based on quality ratings.

Results

A total of 41 papers reporting on 40 studies was included in the present study. There was a great deal of variation in the country of origin, with studies arising from 18 countries (Table 1). The countries of origin were spread between both developing (n = 7) and developed countries (n = 11) based on economy, with nine articles originating in the USA. The majority of reports were cross-sectional in study design (n = 33), one pre-post [21], two randomised controlled trials [22,23] and four longitudinal [16,24–26]. The majority of studies included both genders, with two studies in males only [27] [28] and one in females exclusively [29]. The total number of participants across studies was 166 148, with a mean of 4153 per study (range 36–75 643). The majority of studies assessed children aged 10–18 years, with only four studies investigating outcomes in children <10 years [19,22,24,30,31]. Ten studies reported the inclusion of an ethnicity other than Caucasian; however, none of these were the sole population group [21,31–33]. A total of 27 studies (65%) described the weight status of children, predominantly reported as the body mass index.

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Table 1 Description of included studies (n = 41)

Reference, first author (year)	Country	Study design	n	Gender	Age (year)	SES/ethnicity	Weight status (if reported)	Study quality*
Abudayya 2011	Palestine	Cross-sectional	932	M F (53.9%)	12–15	Range of SES	5.4% underweight 18.5% overweight/obese	+
Acham 2012	Uganda	Cross-sectional	645	M F (54%)	9–16	Nil	BMI for age: 89.9% normal, Weight-for-age: 87.0% normal, 13% underweight	+
Aquilani 2011	Italy	Cross-sectional	40	F	14–15	High SES	Mean BMI 20.6 (2.7) kg m^{-2} (healthy weight)	+
Boschloo 2012	Netherlands	Cross-sectional	605	M F (56%)	11–18 mean 14.8	NR	NR	+
Correa-Burrows 2016	Chile	Cross Sectional	395	F 52% M	16	NR	BMI *Z*-score −0.63	+
Correa-Burrows 2014	Chile	Cross Sectional	1073	M 52% F	13.1 (2.3)	NR	45% overweight/obese	+
DeGroot 2011	Netherlands	Cross-sectional	700	M F (56%)	12–18	NR	NR	+
Edwards 2011	USA	Cross-sectional	800	M F (51.7)%)	Mean 11.76	NR	Overweight/obese: 28.9% (0.8%) obese 12.5% (0.6%); overweight 16.4% (0.7%)	+
Esteban- Cornejo 2015	Spain	Cross Sectional	1371	M 50%	10–14	29% University education	Mean BMI 20.39 (3.55)	+
Feinstein 2007	UK	Longitudinal	5741	M (49.9%) F	3–11	NR	NR	+
Florence 2008	Canada	Cross-sectional	4589	M F (52.1%)	10–11	Public school	UC	+
Gjare 2008	India	Cross-sectional	379	M (55%) F	11–13	Middle class families	Approximately 7% underweight	NEU
Hulett 2013	Kenya	Randomised control trial	360	M (54%) F	7.1 (0.8)	NR	Only report weight for age *Z*-scores	+
Bagher 2015	Iran	Cross Sectional	300	UC	16.2 (0.9)	NR	Mean BMI 21.2 (3.7) kg m^{-2}, 10.9% overweight	NEU
Ickovics 2014	USA	Cross-sectional	940	M F (56.1%)	9–13 mean 10.8 (0.73)	46% Hispanic, 40.4% AA, 14.3% white	17.9% overweight and 26.6% obese	+
Ivanovic 1991, 1992	Chile	Cross-sectional	550	M (51%) F	13–19	NR	NR	+
Jafari 2013	Iran	Cross-sectional	300	M	Mean 16.2 (0.9); range 14–18	NR	Mean BMI 21.1 (3.7) kg m^{-2}. 66.8% healthy weight, 10.9% overweight, 2.4% obese	-
Kim 2003	Korea	Cross-sectional	6463	M (51% F	10–17 yr	NR	NR	+

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Table 1. Continued

Reference, first author (year)	Country	Study design	*n*	Gender	Age (year)	SES/ethnicity	Weight status (if reported)	Study quality*
Kim 2010	Sweden	Cross-sectional	9448	M (49%) F	NR	NR	69.6% healthy weight, 5.8% overweight, 1.8% obese	+
Kristjansson 2010	Iceland	Cross-sectional	5810	M F (52%)	14–15	NR	Mean BMI 21.05 (3.33) kg m^{-2}	+
Li 2012	USA	Longitudinal	6178	F M 50%	5.7	NR	% children >95th centile in grade 3 20.3%	NEU
Lien 2007	Norway	Cross-sectional	7305	M 50% F	15–16	NR	NR	NEU
Maclannan 2008	Canada	Cross-sectional	280	M F (54%)	13–15 yr	84% Caucasian	NR	NEU
McIsaac 2015	Canada	Cross-sectional	535	M 47% F	9–12 yr	NR	56% not overweight, 27% overweight, 17% obese	+
Martinez-Gomez 2012	Spain	Cross-sectional	1825	M 53% F	14.9	Hispanic	11% girls overweight/ obese and 26% of boys	NEU
Meyers 1989	USA	Pre-post test. 12 month	1023	50.9% M F	Grades 3–6	Low-income. Caucasian: 28.4%; Hispanic 65.1%;	NR	NEU
Nayardi 2015	Australia	Cross-sectional	779 maths, 741 reading, 470 writing	50% M F	14	92% white 6.9% other	69.8% normal 16.6 overweight, 7.7% obese	+
Nayardi 2016	Australia	Longitudinal	2247 grade 5 2287 grade 7	51% M F	10–12	88.3% Caucasian 2.4% indigenous	NR	+
Nigg 2014	USA	Longitudinal	334	53% F M	4–6th grade	53% Asian 15% white 20% native Hawaiian + pacific	NR BMI at 5-yr follow-up	+
Nilsson *et al.* 2011	Sweden	Cross-sectional	386	49% M F	15	Caucasian	Mean BMI girls = 20.9 (2.38) kg m^{-2} & boys = 20.6 (2.64)	+
Ptomey 2016	USA	Cross-sectional	698	F (50.5%) M	7.5 (0.6)	86% non-Hispanic 10.2% Hispanic/Latino	BMI non breakfast consumers 17.74 (3.32) kg m^{-2} breakfast consumers 17.26 (3.0) kg m^{-2}	+
Purtell 2015	USA	Longitudinal	8544	M (51%) F	5th and 8th grade	43% white, 11% Black, 19% Hispanic	NR	+
Ogunsile 2012	Nigeria	Cross-sectional	128	M F (53%)	10–19	NR	NR	+
Sigusssfotodir 2007	Iceland	Cross-sectional	5810	M F (52%)	15	NR	BMI 21.05 kg m^{-2} (9.42–57.1)	+
Snelling 2014	USA	Cross-sectional	1034	M (45%) F	12–18	708 AA, 62 white, 79 Hispanic	NR	+
So 2013	Korea	Cross-sectional	75 643	M F (49%)	15.10 (1.75)	NR	BMI 20.54 (2.95) kg m^{-2}	+

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Table 1. Continued

Reference, first author (year)	Country	Study design	*n*	Gender	Age (year)	SES/ethnicity	Weight status (if reported)	Study quality*
Sorenson 2015	Denmark	Crossover trial	726	M (51%) F	10 (0.6)	51% M	11.4% overweight 1.9% obese. BMI Girls 17.0 kg m^{-2}, Boys 17.3 kg m^{-2}	+
Stea 2014	Norway	Cross-sectional	2432	M F	15–17	NR	BMI 21.7 (4.2) kg m^{-2} girls and boys 22.2 (3.7) kg m^{-2}	+
Tayebi 2014	Iran	Cross-sectional	36	M	16–17	NR	NR	NEU
Tobin 2011	USA	Cross-sectional	5571	UC	Kindergarten	NR	UC	NEU

*American Dietetic Association critical appraisal tool; AA, African American; BMI, body mass index; F, female; M, male; NEU, neutral; NR, not reported; SES, socio-economic status; UC, unclear; yr, years; +, positive, −, negative.
Unless otherwise specified, data are presented as the mean (SD). BMI (kg m^{-2}).

Risk of bias

The majority of studies in this review were rated as positive quality (n = 28 studies) (Table 1). Those which rated as neutral (n = 10 studies) or negative (n = 1) tended to not provide adequate detail to confirm a positive assessment; specifically, the lack of an adequate description of study groups, and the use of self-reported/nonvalidated or reliable outcome measures (see Supporting information, Table S1). It is noted that more recent published studies (i.e. from 2015 onward) tend to more reliably assess and report diet and academic achievement outcomes.

Dietary assessment method

More than 30 different dietary assessment methods were used across the 40 studies (Table 2). Only 18 of the included studies reported the dietary method to be a validated assessment tool in a child population group [34] or used a recognised/standardised dietary assessment method such as a food diary [29], 24-h recall [22,35,36,37] or a validated food frequency questionnaire (FFQ) [15,16,36–39]. Four studies reported the internal reliability of the dietary measure or Cronbach α within the same study [10,40,41]. In the majority of studies (n = 32), children self-reported their own dietary intakes. Parent reporting was used exclusively in four studies [21,22,24,42], with two of those studies being in children aged less than 11 years and the remaining in children aged 12–18 years [42], 3–11 years [24] and 7 years [22]. One study used school staff to report diet outcomes and, in one study, the reporter was unclear. Fourteen studies reported more than seven aspects of diet, 16 studies assessed only one aspect of diet (breakfast, n = 6; fish, n = 2; fruit, n = 1; folate/supplements, n = 2; fast food, n = 2; meal patterns, n = 3) and 12 studies assessed two to five aspects of diet. Although some studies reported the frequency categories of dietary intake such as per week or per day, the majority of studies did not specify a reporting period for dietary data collection (n = 23 studies). Ten studies had a reporting period of 7 days, and seven studies were during a 24-h period. This made it difficult to determine whether the reporting period for diet aligned with the reporting period for academic achievement.

Academic assessment

Aspects of academic achievement were obtained from national standardised tests/sources in 14 studies [15,21,22,24,31,43–45] (Table 2). However, the majority of studies obtained measures of academic achievement from non-standardised sources, or sources where the measure of academic achievement was considered unclear

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and, in several studies, the source was not stated. Twenty-three studies obtained results of academic achievement from school administration staff or national registers, whereas the results were self-reported via study questionnaires in 10 studies and unclear in the remaining studies (n = 8 studies).

A large range of academic subjects were assessed with a significant variation in how they were reported. These included GPA for six studies (15%) with the majority being total academic scores or study specified/arbitrary cut-points of 'good' achievement (n = 33 studies) (80%). Across the included studies, the five most common reported subjects were arithmetic/maths (n = 28 studies), English (n = 11 studies), reading (n = 10 studies), language (n = 7 studies) and writing (n = 4 studies). The most common reporting period for academic achievement was 12 months (n = 14 studies), with end of term/semester being the next most common in seven studies. In the majority of studies, however, the reporting period was not stated or was unclear (n = 14 studies).

There were a number of factors that precluded meta-analysis from being performed. These factors contribute to significant heterogeneity between studies and include the range of countries in which studies were carried out; variable outcome measures for academic achievement and dietary behaviours; and differences in cut-off points which were used as definitive measures of 'good' or 'satisfactory' achievement.

Associations between diet and academic achievement

Breakfast consumption

The most common association between an aspect of dietary behaviour/intake and academic achievement was breakfast consumption, which was reported to be significantly associated in 12 studies (Table 3). Three studies reported associations as a correlation value, which varied between r = 0.12 and 0.278 [36,46–49]. Other studies reported the associations between breakfast and academic achievement as an odds ratio. The different reporting outcomes make it difficult to quantify the association and compare across studies as a result of the differences in cut-off points used for satisfactory/good achievement. However, if studies were considered collectively, regular intake/higher consumption was associated with increased scores of academic achievement in 12 studies. In one study by Acham *et al.* [50] , breakfast consumption was considered with collective meal intake, where cumulative meals (i.e. breakfast and a lunch meal) were shown to increase academic achievement.

Junk food/fast food

The next most common dietary association was that of energy-dense, nutrient-poor 'junk food(s)' consumption/intake, which was assessed in nine studies [19,24,31,34,40,43,45,51]. Lower intakes of fast food were associated with higher academic achievement. Specifically, in one study in kindergarten children, fast food [19] once per day was associated with reading scores of −18.25 points, whereas consumption at three times per day was associated with reading scores of −33.88 [19]. In another study by Li *et al.* [45], a one unit increase in fast food intake was associated with a 2.6 lower point score in math and a 2.87 lower score in reading. Other studies that reported this dietary variable more broadly as 'junk' [24] or termed 'bad food' and were negatively associated with collective scores (the sum of several subjects) of academic achievement with correlations of r = −0.14 to −0.15 [34,40]. Conversely, Purtell *et al.* [26] reported that any fast food consumption was associated with small gains in academic growth when kindergarten children were followed up at eighth grade. Lower intakes of sugar sweetened beverages (SSB) were associated in four studies with higher academic achievement [43] [51]. Specifically, Ickovics *et al.* [31] reported that a SSB intake <2 times per week was correlated with higher academic achievement and and Snelling *et al.* [52] described students who achieved higher grades who reported a lower consumption of soda.

Fruit and vegetables

A total of six studies reported significant associations for fruit and vegetables with academic achievement. For those studies that reported a correlation, a consistent positive relationship was demonstrated between fruit and vegetable consumption and academic achievement, with values ranging from r = 0.195 to 0.23 [53] [odds ratio = 1.61 (1.11–2.32)] [34] (r = 0.23) [25,32,33,40,47] for adequate amounts of fruit and vegetables [40,53]. Two studies reported on fruit only with increases in academic achievement[32,47].

Mirconutrients

Four studies reported on micronutrients as outcomes, with the most commonly reported to be associated with academic achievement being folate [54] and iron [22,29], as assessed in three studies. Other studies reported nutrients including energy, protein, B group vitamins [55] and omega 3, which also assessed supplement use [56], and these were found to be positively associated with academic achievement.

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Table 2 Outcome of included studies

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
Abudayya 2011	42 item FFQ	C	25 items were taken from validated survey	Animal Foods, fruit, veg, milk, traditional foods, cookies, soft drink, rice	School grades were collected from the school. Good performance classified as >70% overall avg grade	12 months	UC
Acham 2012	Household questionnaire and child questionnaire	C P	No	Meal patterns.	Unstandardised tests in Engl, Maths, life skills and oral comprehension. Total score was 120 points (30 points × 4 subjects), <30 defined as 'poor' performance	UC	N
Aquilani 2011	Food diary for 7 days in the 4 months after starting school	C	Standardised method	Macro/micro nutrients	Written mathematics (WM, oral mathematics (OM) and written Italian (WI), UC if reported or collected by researchers	4 months	UC
Boschlo 2012	One question only: b/fast consumption	C	No	B/fast consumption freq	End of term grades were obtained from staff - Dutch; Engl; maths. Ranging from 1 (bad) to 10 (outstanding)	End of term	UC
Correa-Burrows 2016	FFQ 'usual diet'	C	Y	Meal pattern categorised as unhealthy, fair or healthy	Language, maths, science and Soc Sci collected GPA	UC	Y
Correa-Burrows 2014	FFQ: snacking 25 items	C + P (5th grade) C only (9th grade)	Y	Meal patterns considered unhealthy, poor to fair, and healthy	Maths and Language test collected National Standardised system for the assessment of Educational quality (SIMCE)	UC	Y
DeGroot 2011	Self-report questionnaire fish consumption	P	Y	Freq of three types of fish consumption rated as low, intermediate or high EPA/DHA mg/ day	End of term grades: Dutch; Engl; maths Ranging from 1 (bad) to 10 (outstanding), standardised *Z*-scores calculated due to differences in grading	End of term	UC

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Table 2. Continued

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
Edwards 2011	20 question survey: adapted from Youth Risk Behaviour Surveillance Survey (YRBSS)	C	No YRBSS valid for questions relating to television viewing; not valid for nutrition question	Milk, sweetened bev (SB), 100% fruit juice (FJ), fruits, veg, b/fast, meals with family	Measures of Academic Progress (MAP) standardised tests: maths and reading	UC	Yes
Esteban-Cornejo 2015	Diet adherence to Mediterranean Diet assessed through the KIDMED diet quality index	C	Y	Mediterranean diet (MED)	An avg collected score of math and language combined GPA collected	Past yr	UC
Feinstein 2007	FFQ completed by mothers/female, # of items varied depending on age range 43–54 foods At 81 months: report if child ate meals at school or packed lunch and freq which they did	P	UC	'Health conscious': vegetarian food, nuts, salad, rice, pasta, fruit, cheese, fish, cereal, water, fruit juice. 'Traditional': meat and cooked veg. School meals	Key stage (KS) scores from National Pupil database were collected: (1) Entry assessment: maths, reading, language, writing (age 4–5). (2) KS 1 scores: maths, reading, writing (age 6–7). (3) KS2 scores: maths, Engl, science (age 10–11; used to assess school attainment)	UC	Y
Florence 2008	Harvard Youth/ Adolescent FFQ (YAQ); Diet Quality Index-International (DQI- I); Healthy Eating Index (HEI)	C	YAQ, DQI-I and HEI: validated	Food groups, diet quality: fruit, veg, grains, dietary fibre, protein, iron, calcium, vit C. Caloric intake from fat independently	The Elementary Literacy Assessment (Reading and writing) was collected from the Nova Scotia Department of education (Pass/Fail) pass is passing both assessments	UC	Y
Gjare 2008	Study specific questionnaire for b/fast consumption	C	N	B/fast freq, type of b/fast	Annual examination mark for math science and Engl	12 months	UC
Hadavand 2015	Questionnaire: amount of consumed b/fast and snack	C	Test retest stated but values not reported. Questionnaire developed by researcher	B/fast and snack consumption	GPA in Relig selfR via questionnaire	Prev semester	N

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Table 2. Continued

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
Hulett 2013	24-h semi quantitative food recall. 3 recalls over 3 weeks to calculate baseline intakes and every 2 months thereafter for 16 months. Trained data collectors	P	Y	Macro/micronutrient intakes.	Test scores using standardised Kenyan tests (ministry of education) Arithmetic, Engl, Kiswahili, Mother tongue, Science/Agriculture, Geo/ Relig, Arts, Music. Collected from school records. Total test score out of 350	End of term	Y
Ickovics 2014	Student survey questions adapted from WHO Health Behaviour in School Aged Children (HBSC). Single data collection point about intake and behaviours p/ w. No indication of reporting period	C	UC	Meets recommended fruit and veg intake, sugar sweet bev, freq of family meals, FF consumption	Standardised test scores on the Connecticut Mastery Test (CMT) and Connecticut Academic Performance Test (CAPT) for reading, writing and maths collected through school databases. Tested for validity and reliability. Categorised as below basic, basic, proficient, goal, advanced. Academic achiev defined as goal or higher on all three tests	12 months	Y
Ivanovic 1991, 1992	24-h dietary recall interviews	C	UC	1991: Nutrient intake, 1992: food groups (dairy, meat and eggs, fruit and veg, breads and cereals)	Achiev Evaluation Program (AEP) in elementary school, National achiev test of language and mathematics in high school (grade 8). Achiev expressed as high (>40%), medium (30–40%), low (<30%). Academic Aptitude Test for high school graduates for university entrance, scores established as high (>600), medium (450–600) or low (<450). Valid and reliable	12 months	Y
Jafari 2013	General b/fast and snack consumption	C	UC	B/fast and snack consumption	Researcher made questionnaire including math grade avg	UC	No

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Table 2. Continued

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
Kim 2003	86 item FFQ. FFQ compared to student's 24-hour recall. 3 portion-sizes	C	Yes in same study	Dietary behaviours b/fast, lunch and dinner. Energy intake (MJ), protein	Grade point avg from the prev semester ranging from 1–5 Subjects: Korean, mathematics, social studies, natural science, physical education, music, art, ethics, Engl and practical course	Semester	UC
Kim 2010	Self-administered questionnaire about weekly fish consumption.	C	UC	1 question: Fish consumption: '	Total school grade for 16 subjects via national register was collected. Score out of 320	9 yr	Y
Kristjansson 2010	SR poor dietary habits and consumption of fruits and veg. Reporting period not stated	C	Y	Poor Dietary Habits. (i) potato chips; (ii) French fries; or (iii) a hamburger or a hot dog. Consumption of fruits and veg	SR grades in Icelandic, Maths, Danish, Engl, Swedish, Norwegian. Grade range 0–10 with below 5 fail and above 5 pass	12 months	UC
Li 2012	Child food consumption questionnaire. 19 SR items. Reporting period prev 7 days	C	UC	Sweets, salty snacks and soda drinks, fruits and veg	Data for reading and maths achiev were obtained from the ECLS-K, a large-scale database collected by the National Center for Education Statistics (NCES) Data collected in 2004	UC	Y
Lien 2007	SelfRed b/fast consumption, dieting and soft drink consumption p/w as part of larger health survey. Reporting period not stated	C	UC	B/fast, dieting and soft drinks	SR grades from maths, Norwegian, Engl and Soc Sci scored out of 6. Avg grade dichotomised into fail ≤3 or pass >3	UC	N
Maclannan 2008	Food freq section of the 2003 Youth Risk behaviour Survey. 7 questions regarding the prev 7 days	UC	UC as not total survey used	Milk, veg and fruit (fruit juice, fruit, carrots, potatoes, green salad and 'other' veg)	Avg grades in the preceding 12 months were selfRed. Six categories ranging from >90% to <49% were converted to three categories (>90%, 80–89% and below 80%)	12 months	UC

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Table 2. Continued

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
McIsaac, 2015	Youth Adolescent Questionnaire, diet quality index (DQI)	C	Y	Diet quality (Youth Healthy Eating Index YHEI), fruit and veg intake, milk/dairy, sugar sweetened bev, b/ fast skipping	Math, Engl language arts/obtained from school board Grades A–D	Past yr	UC
Martinez-Gomez. 2012	FFQ	C	N	Fruit intake, grouped into ≥2 servings day^{-1} and <2 servings day^{-1}	School grades selfRed in 2 subjects: Language and Literature, and Maths. Grades reported on a 5-point scale (A, B, C, D, E). D grade minimum requirement for pass	Semester	N
Meyers (1989	School staff monitored and recorded attendance to school b/ fast for 1 week.	Staff	N	Participation in school b/fast. Children were classedas participating if they attended at least 60% of the time	Test scores in a battery of standardised school achiev tests: The Comprehensive Test of Basic Skills (CTBS). Language, reading, mathematics and overall score	Semester	Y
Nyardi 2015	FFQ prev 12 months	P with adolescent consultation	Y	Dietary patterns 'healthy' (high in fruit, wholegrains, legumes. Fish) 'western' (high intake of takeaway, red/ processed meat, soft drinks refined food	Mathematics, reading and writing obtained WALNA	Past year	Y
Nyardi 2016	24-h diet recall when children 1,2,3, 5, and 8	P with adolescent consultation	Y	Dietary score	Mathematics, reading, writing and spelling obtained Western Australian Literacy and Numeracy assessment (WALNA) grade 5 (10 yr) and 7 (12 yr)	Past year	Y

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Table 2. Continued

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
Nigg 2014	Report the number of servings of fruit and veg daily combined into one variable	C	Y	Fruit and veg	SelfR school grades reported as (A.b, c d, e, f)	UC	UC
Nilsson et al. 2011	Interviewer mediated 24-h recall and qualitative food record used as checklist for recall	C	Y	Level of folate intake in μg day^{-1}	Yr 9 school grades for 10 core subjects: Engl, Bio, Chem, Physics, Math, Soc Sci, Hist, Geo, Relig. Performance across all subjects represented by score out of 200	Semester	UC
Ptomey 2016	B/fast recall of all foods and drinks consumed in the morning before test, energy and macronutrients estimates collected from 10sample recalls	C	Y	B/fast consumption	WIAT III collected- sub tests of reading comprehension oral reading, spelling, maths, problems solving and numerical operations	UC	Y
Purtell 2015	One question 'During the past 7 days how many times did you eat a meal from a FF restaurant?'. 7 freq responses, Data were collapsed to 4 categories	C	Adapted from the Youth Risk Behaviour Surveillance System	FF consumption prev incl fruit and veg, milk, 100% juice bev, soda, other sugary bev	Reading/literacy, mathematics and science in 5th and 8th grade assessed with an 'academic growth' variable created	Past year	UC
Ogunsile 2012	Dietary Behaviour Questionnaire (8 items)	C	Internal reliability reported	Dietary patterns; healthy (milk, fruit, veg, b/fast consumption and three meals or unhealthy eating pattern (sweets, chewing gum and SSB)	Examination scores in 5 subjects (science, Soc Sci, arts, commercial) for three consecutive terms and the avg score for each participant was obtained. These were then classified as poor 40–49, fair 50–59, above avg 60–69 good and >70 excellent	3 terms	UC

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Table 2. Continued

	Diet				Academic measures		
Reference	Method + reporting period	Reporter (C = Child P = parent)	Method referenced/ validated	Outcome measures	Outcome measures	Reporting period	Standardised testing
Sigusssfotodir 2007	Questionnaire - reporting period freq of response/ day or/week	C	Cronbach alpha assessed within study	Poor Dietary Habits. Potato chips, French fries, or a hamburger or a hot dog. Consumption of fruits and veg	SelfRed grades of core subjects: Icelandic, mathematics, Engl and Danish, scores ranged on a category from 0–7 but were then combined to a score of 28 for the four subjects	UC	UC
Snelling 2014	Eight variables Questionnaire re; the past 7 days	C	No 2010 Youth Risk Behaviour Survey	Fruit juices, fruit, green salad, potatoes, carrots, other veg, soda FF	SelfR: no specific subjects mentioned	Past year	UC
So 2013	Questionnaire days p/w consuming b/fast	C	N	B/fast consumption	SelfRed academic performance for prev 12 months rates on a 5 point scale from very high to very low	Past yr	N
Sorenson 2015	7 day web diet assessment	C	Y	*n*-3 PUFA, iron + supplement use	Maths and reading collected	UC	UC
Stea 2014	Part of a general broader study questionnaire	C	Reliability tested in same study	Meal patterns, healthy food items (fruits, veg, un healthy foods (SSB, diet drinks candy and salty snacks	Assessed using grades from 3 mandatory academic classes, Norwegian, Engl, mathematics, based on SR mean school grades were calculated. Low scores 0–3 and high scores 4–6	UC	UC
Tayebi 2014	Dietary Habits questionnaire	C	N	Food meals, eating junk food, eating fruit, using supplements	GPA	12 months	N
Tobin 2011	Food consumption questionnaire, prev 7 days	C	UC	FF consumption	5th Grade math and reading item response their scale scores	UC	UC

Achiev, achievement; Assoc, associated; Avg, average; B/fast, breakfast; bev, beverage; Bio, biology; Chem, chemistry; DHA, docosahexaenoic acid; Engl, English; EPA, eicosapentaenoic acid; Fe, iron; FF, fast food; Freq, frequency; Geo, geography; GPA, grade point average; Hist, history; N, no; OR, odds ratio; p/w, per week; Prev, previous; Relig, religion; Sco Sci, social science; SD, standard deviation; selfR, self report; SR, school report; SSB, sugar sweetened beverages; UC, unclear; veg, vegetable; Y, yes; Yr, year; Zn, zinc.

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Table 3 Outcome of included studies

Reference	Associations Dietary outcomes	Academic outcomes	Relationships between diet and academic outcomes
Abudayya 2011	>2/3 of children had intakes <3/week of the five categories of food (animal foods, F&V, milk, cookies, traditional foods)	63% of children had 'good' school performance	Fruit and veg >3 p/w associated with 'good' school performance, compared with <3 p/w (72.6% versus 59.9% achieving 'good' ($P = 0.001$). Unadjusted model only higher freq of soft drink and rice associated with school performance OR 1.69 & 1.57
Acham 2012	74% report no b/fast, 44% report no midday meal	Avg score was 100 (47.1) [99.0 (45.6) for girls and 101.1 (48.9) for boys]. Gender diff in maths only: boys had significantly higher score than girls ($P < 0.001$) More than half (68.4%) of the children scored below 120 points	B/fast and midday meal consumers more likely to score higher than those who had only one type of meal. Child who consumed both meals had increased chances of scoring above the threshold [OR = 1.3 (95% CI 0.9–2.0)] compared to <1 meal
Aquilani 2011	Fe intake significantly higher in better performing students ($P = 0.08$ for each micronutrient) than in lower academic performing students ($P < 0.003$). Znintake higher in well performing students than lower performing student ($P = 0.1$)	Mean scores Maths WM 5.5 (0.9), WI 5.5 (0.4) (un satisfactory) OM 5.4 (0.7)	Fe significantly related to WM ($r = 0.43$), OM ($r = 0.40$) and WI ($r = 0.37$). Zn linked to WM ($r = 0.35$) and OM ($r = 0.33$)
Boschloo 2012	16.5% b/fast skippers (2.5% skipped b/fast on all school days). 83.5% b/fast eaters (5 school days)	Mean grade *Z*-scores for school performance for b/fast skippers −0.32 (1.04) and b/fast eaters 0.06 (0.98) ($P < 0.001$)	School performance significantly correlated with b/fast consumption ($r = 0.14$, $P < 0.01$). B/fast skipping associated with 0.15 SD lower school grades compared to b/fast eaters
Correa-Burrows, 2016	17% had an unhealthy diet, 50% had fair 33% had healthy	Language test 460.3, math 462.8, GPA 502.4	Avg scores in language and maths increased with higher quality diet (GPA unhealthy diet 459.7, fair 507.1 healthy 515.8). Students with healthy diet, academic achiev twice that of unhealthy group
Correa-Burrows 2014	56% had unhealthy snacking, 36% poor to fair 8% healthy	Maths: unhealthy snacks 272.7, healthy 290.0. Language unhealthy snacks 268.7, healthy snacks 281.3	Maths score: OR 0.46 unhealthy snacking, 0.82 poor to fair snacking. Language score unhealthy snacking 0.52 and poor to fair snacking 0.76.
DeGroot 2011	13.7% never ate fish; 6.4% met national recommended DHA and EPA intake guidelines (450 mg day^{-1}); 16.9% reached half the recommended amount; 63.0% ate fish but irregularly	*Z*-scores for academic performance: no fish intake −0.10 (0.86), <1575 mg DHA/EPA intake 0.02 (0.77), 1575–3150 mg DHA/EPA intake 0.14 (0.78), >3150 mg DHA/EPA intake −0.15 (0.75)	Higher fish consumption associated with better academic achiev, but not the highest intake group which was >3150 mg day^{-1}. The model explained 12% of variance
Edwards 2011	1/2 drank ≥3 glasses milk day^{-1}; 1/4 drank >12 oz SB./day; 1/5 drank ≥2 serves of 100% FJ; 1/3 met ≥5 serves fruit and veg day^{-1}. 2/3 ate both b/fast (≥5 days $week^{-1}$) and a meal with their family on the prev day. Female's significantly lower proportion drinking both milk and SB, eating fruits, veg and b/fast	MAP mean maths scores females 225.13, males 228.27 ($P = 0.002$), MAP mean reading score females 218.86, males 216.20 ($P = 0.004$)	Higher mean MAP math scores associated with students who drank more milk, less SB, less 100% FJ, ate b/fast frequently. Higher mean MAP reading scores associated with students who drank less SB

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Table 3. Continued

Reference	Associations Dietary outcomes	Academic outcomes	Relationships between diet and academic outcomes
Esteban-Cornejo 2015	The level of adherence to MED was higher in boys than girls Good adherence to MeD: significantly higher scores in all academic indicators	Girls had higher GPA scores for both subjects than boys ($P < 0.01$)	Higher diet adherence to MED better academic achiev math (β 0.119), Language (0.115), Math and Language (0.126), GPA 0.157)
Feinstein 2007	UC	UC	Positive association between higher 'junk' dietary pattern at 3, 4, 7 yr and lower avg KS2 scores. Positive association between 'health conscious' and KS2 scores. Negative association between eating school meals and attainment, whereas a positive association is seen for packed lunches
Florence 2008	Diet quality scores ranged from 26 to 86, avg score 62.4	19.1% failed assessments: 1 or both components of literacy assessment. Boys were twice as likely to fail literacy assessments	Increased diet quality less likely to fail the literacy assessment. Diets with variety and adequacy, rather than moderation and balance, most significantly associated with academic performance. Increased fruit and veg intake and lower caloric intake fat significantly less likely to fail assessment
Gjare 2008	62% regularly consumed b/fast, 4% did not consume b/fast. Common foods: cereal and pulse items with milk, eggs and fruit	% total (avg math, science, Engl) regular b/fast consumers 63.3 (18.5%), irregular 60% (14.5) and no b/fast 52.2% (15.2)	Regular b/fast significantly associated percentage of marks explaining 1.9% of the variation
Hadavand 2015	Mean number of b/fasts was 5.9 (1.9) days week^{-1}, 59.1% mentioned sugar intake, mean number of snacks week^{-1} 5.4 (2.4), 14.8% mentioned sandwich, 64.4% had snack at school everyday	Mean GPA 17.3 (1.8). Grades in relig G1 3.4 (16.9); G2 2.5 (16.9); G3 2.2 (18.7)	Relig grade had significant correlation 12.7% with b/fast consumption
Hulett 2013	Energy intake increased in all groups at the end of intervention. All nutrients increased significantly in meat and milk groups (protein, Fe, Zn, B12, Folate, B6, Riboflavin). Milk, plain stew and control groups below recommended intakes of Fe and Zn	Combined score plain stew 245·2 (74·7), Stew + milk 229·4 (67·9), stew + meat 192·5 (88·2), control 215·6 (79·7)	Intakes of energy kg^{-1} (20.71points), Fe (27.67 points), and folate (25.38 points) significantly associated with increases in total test scores ($P < 0.05$)
Ickovics 2014	3.2% students met fruit and veg intake recommendations, over half ate family meals ≥5weekly, and FF ≥1	Over half of students achieved goal or above in at least one of the tests but only 29.3% achieved goal or above on all three CMT and CAPT (below avg of other schools). Below statewide avg for achiev	Sugar sweetened bev <2 times p/w increased odds ratio for scoring goal or above (academic achiev) OR = 1.41 (0.18). Family meal ≥5 days week^{-1} not associated with academic achiev. FF meal ≤1 day week^{-1} increased odds ratio of academic achiev 2.65 (0.71)

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Table 3. Continued

Reference	Associations Dietary outcomes	Academic outcomes	Relationships between diet and academic outcomes
Ivanovic 1991, 1992	1991: 49% inadequate intake of energy, 62% inadequate protein, deficiencies in Vit A, riboflavin, niacin, calcium, iron	**AEP**: n = 75 students had low achiev (score ≤30%), n = 75 had medium achiev (score 30–40 points%), n = 101 had high achiev (score >40%). **AAT:** Low achiev n = 105 (score <450 out of 900), n = 93 medium achiev (score 450–600) and n = 69 high achiev (score >600)	**1991: AEP:** Achiev significantly correlated with energy, protein, riboflavin, vitamin C, calcium, vitamin A (multiple r = 0.456, P < 0.01). Calcium explained most variance (7.2%). **AAT:** Sig correlation between achiev and protein, riboflavin, calcium, and iron (multiple r = 0.438, P < 0.01, r^2 = 19.2). Protein explained most variance (8.22%) **1992 AEP:** Educational achievement significantly positively correlated with freq of consumption of dairy (r = 0.453 P < 0.01) and meat and eggs (r = 0.172, P < 0.01) and inversely correlated with fruit and veg (r = 0.134 P < 0.05) and misc (r = 0.134, P < 0.05). **AAT:** achiev significantly positively correlated with freq of consumption of dairy (r = 0.342, P < 0.01), meat and eggs (r = 0.244, P < 0.01), and inversely correlated with fruit and veg (r = 0.129, P < 0.05)
Jafari 2013	B/fast consumption: 72.6% everyday; 13.2%, 2.1% never. Avg b/fast consumption was 5.9 (1.9) times/w. Snack consumption: 64.6% every day, 10.1% never. Avg freq of snack consumption 5.4 (2.4) times week^{-1}	Avg scholastic score 17.3 (1.8). Avg math score 15.6 (3.8).	Sig relationship maths score and eating b/fast (r = 107, P = 045)
Kim 2003	Grade 5: 83.5% regular lunch, 63.6% regular b/fast, and 55.2% regular dinner. Grade 8, 79.0% regular lunch, 56.6% regular b/fast and 40.2% regular dinner. Grade 11, 60.1% regular lunch, 58.6% regular b/fast, and 45.6% regular dinner. Energy intake Grade 5 boys	Mean GPA Grade 5 boys 3.93 + 0.70, girls 4.18 + 0.64. Grade 8 boys 3.06 + 1.11, girls 3.37 + 1.00. Grade 11 boys 2.96 + 0.83, girls 3.17 + 0.81	B/fast and lunch associated with higher GPA (P < 0.05). Energy intake had negative relationships on GPA in all grades and genders (P ≤ 0.003) Micronutrient density associated with performance in year eight boys (coefficient 0.168, P = 0.004)
Kim 2010	Fish consumption <1 week 24.2%, Fish once p/w 56.5%, fish >1 week 19.3%	Mean total grade 213 out of 320. Boys had lower grades than girls (P < 0.01)	Higher fish intake comparedto no fish: fish once p/w 14.5 95% CI (11.8–17.1) P < 0.0001, fish >1 p/w 19.9 95% CI (16.5–23.3) P < 0.001
Kristjansson 2010	The mean scores consumption of fruits and veg and bad food, were 3.64 (1.74) and 7.07 (2.58)	Most students reported score of 7 or more out of 10 for each of the four subjects. Mean total score 16.60 (5.73)	Correlation academic achiev and bad food (r = −0.15 P < 0.01), fruit and veg (r = 0.23 P < 0.01). Positively related to fruit and veg (ß = 0.08, P < 0.01)

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Table 3. Continued

Reference	Associations Dietary outcomes	Academic outcomes	Relationships between diet and academic outcomes
Li 2012	Weekly freq of soda 1.97 (1.68), FF 1.11 (1.19). Weekly freq consumption at school of sweets 0.35 (0.81), snacks 0.23 (0.67), soda 0.19 (0.64)	Maths score grade 5 115.28 (20.81), reading score 141.31 (22.64)	FF consumption in Grade 5 significantly related to children's mathematics achiev (1 unit increase in freq of FF consumption = 2.60 points lower in maths) and reading achiev (1 unit increase in freq of FF consumption = 2.87 points lower in reading) ($P < 0.01$)
Lien 2007	B/fast consumption ≤2 times p/w 19% boys and 27% girls, 66% boys and 55% girls ate b/fast every day. Soft drink consumption ≥2 per day 20.3%. 65.3% had never dieted. 14.6% dieting now or always.	Fail grade (<3) 13% boys and 8% girls	Eating b/fast associated with improved academic performance. Adjusted OR (95% CI) for b/fast consumption on grades: Seldom/never boys 2.0. Every day boys 1.0, girls 1.0
Maclellan 2008	63% reported consuming milk at least once daily, 46% consumed fruit daily, veg were least likely to be consumed. Mean daily intakes of veg and fruitwas 4.3 (2.9) serves and milk 1.7 (1.4), 31% had adequate veg and fruit intakes, 35% had adequate milk.	A higher proportion of participants had academic scores above 90% in lower compared to higher grades ($\chi^2 = 23.3$, $P < 0.001$)	Students who reported higher academic grades were significant and consumed adequate amounts of veg and fruit than lower scores more likely to consume milk and VF daily
McIsaac 2015	UC	UC	Unhealthy lifestyle behaviours positively associated with poor academic performance, diet quality (OR 4.26) and consumption of SSB >1 day^{-1} (OR 2.42) and b/fast skipping (OR 0.87) YHEI (OR 3.22)
Martinez-Gomez 2012	22% of students consumed ≥2 serves fruit day^{-1}. 88% of students consumed <2 serves day^{-1}	40% had poor school attitude, 18% absenteeism	Consumption of ≥2 serves of fruit day^{-1} was significantly associated with an increased likelihood of achieving a pass grade in Language and Literature (OR = 3.10); Maths (OR = 1.70) or both subjects (OR = 1.96); $P \leq 0.05$ in girls only
Meyers 1989	Children participating in school b/fast $n = 335$ and non-participating $n = 688$	Change in CTBS battery score for school b/fast participants = +48.13 and non-participants = +40.78, $P \leq 0.5$	Participation in school b/fast programme significantly associated with a greater increase in school test scores compared to non-participation. Estimated test score increase for participation = 5.44, $P \leq 0.5$
Nayardi 2015	Mean energy intake 9298 (2792) kJ diet quality score 42.52 (9.97)	Mean score maths 541.14 (86.75) m reading 497.75 (78.46), writing 574.77 (104.91)	1 SD higher *Z*-score in 'western' diet pattern' associated with lower tests scores for mathematics reading and writing. There was an estimated 46 decrease in maths, 59 in reading and 57 decrease in writing compared between the lowest and highest quartile
Nayardi 2016	Diet score (grade5/grade 7): 1Y 42.1 (10)/42 (1.0) 2 yr 38.6 (10.3)/38.7 (10.4), 3 yr 37.6 (10.3)/ 37.4 (10.4)	UC	Fruit and dairy increased academic scores at grade 5 and 7. 6.2 points in maths, 7.2 points in reading, 11.1 writing and 9.8 in spelling grade 7

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Table 3. Continued

Reference	Associations Dietary outcomes	Academic outcomes	Relationships between diet and academic outcomes
Nigg 2014	6.96 (4.54) servings day^{-1}	Year 5 marks 4.26 0.84	Correlation Fruit and veg with academic marks −0.19 ($P < 0.01$)
Nilsson et al. 2011	Tertile 1: Females ≤ 173 μg day $^{-1}$; Males ≤ 227 μg day^{-1}. Tertile 2: Females = 173–253 μg d^{-1}; Males = 227–335 μg day^{-1}. Tertile 3: Females => 253 μg day^{-1}; Males => 335 μg day^{-1}	Mean school grade score out of 200: Females = 137.9; Males = 124.6	Higher levels of folate intake significantly associated with higher school grade scores
Ptomey 2016	617 b/fast consumers 81 non b/fast consumers	Non b/fast consumers/b/fast consumers: spelling 95.85/100.49 reading 95.16/100.06, maths 98.19/103.25	B/fast consumers had significantly higher scores in all academic domains compared to non b/fast eaters. % CHO positively correlated with spelling, servings fruit negatively correlated with reading, juice negatively associated with reading and maths
Purtell 2015	29% reported no FF, 1–3/week 52%, 4–6/week 10%, daily 10%	Theory Achievement scores 8th grade: reading 143.33 (21.65) Math 172.45 (27.06), science 85.63 (15.68)	Any FF consumption experienced significantly smaller gains than children who reported no FF consumption for all 3 subjects
Ogunsile 2012	7% took milk at least once per day, 16.4% reported daily b/fast consumption, 14% reported 3 regular meals, 16.4% ate fruit daily, 10.2% ate veg, 50% reported intakes of sweets, and 45% reported daily intake of soft drinks	50% of respondents had a score >60%	B/fast consumption and fruit intake had significant correlation with academic achiev (0.194) and 0.195 ($P < 0.05$). A health dietary pattern had a positive impact on academic performance
Sigusssfotodir 2007	Bad food combined mean 7.07 (3.33), Fruits 2.91 (1.0), Veg 2.72 (1.0)	Icelandic 5.2 (1.5), Engl 5.5 (1.7), Danish 5 (1.84), Mathematics 4.85 (1.94), Combined 16.60 (5.73)	Grades and Fruit and veg −0.23 and Bad foods −0.14 ($P < 0.05$) using regression Fruit and veg 0.11 and bad foods −0.06 with total grades
Snelling 2014		SelfR grades A, B, C, D, F	For students reporting 'a's and 'b's they consumed significantly less soda and FF. Grade increase associated with an increase in veg consumption
So 2013	No b/fast n = 9349 every day n = 32 090	School performance avg n = 20 375 low n = 19 335 very low n = 9367	The OR for achieving avg or higher academic achiev Males 1/week 1.004, everyday 1.70. Females everyday 1.922
Sorenson 2015	9 mg Fe, mean intake of fish 20 g day^{-1} EPA +DHA 0.12 g day^{-1}	Concentration performance 130.6 (22.9) processing speed 331.4 (57.7), senetence reading speed 55.9 (16.2), maths test (# correct) 31.9 (11.2)	The intervention resulted in intakes of 0.5 mg day^{-1} Fe, EPA+DHA 0.10 g day^{-1}. The intervention improved 'school performance' ($P = 0.015$), 'reading comprehension' and positively associated with 'school performance', Effect size 0.7 sentences for reading speed and 1.8 sentences or 0.1 SD for the number of correct sentences. No effect on maths scores

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Table 3. Continued

Reference	Associations Dietary outcomes	Academic outcomes	Relationships between diet and academic outcomes
Stea 2014	UC	Girls low academic achievement and: regular breakfast (40%) boys (55%), girls high academic achievement regular breakfast 66% boys 70%, vegetables >7 days per week low academic achievement 24% boys 18% versus high achievement girls 34% and boys 22%	Regular intake of breakfast and lunch strongly associated with increased odds of high academic achievement
Tayebi 2014	Mean score 26.3 (10.04), scores ranged from −1 to 46	GPA 0.472 (1.01). Groups were divided into positive and negative scores according to standardised GPA	Those with a positive GPA score attained a total diet score than those with negative GPA 26.3 (10.4) versus 21.04 (8.77)
Tobin 2011	FF consumption freq none in last 7 days 28.5%, 1–3 times 50%, 4–6 times 10%, once day^{-1} 6%, twice day^{-1} 2%, 3 times day^{-1} 1% >4/day 2%	Reading mean 141.52 (23.15) Math 115.24 (21.22)	FF consumption >1 p/w had lower test scores in math and reading

Achiev, achievement; Assoc, associated; Avg, average; B/fast, breakfast; bev, beverage; Bio, biology; Chem, chemistry; DHA, docosahexaenoic acid; Engl, English; EPA, eicosapentaenoic acid; Fe, iron; FF, fast food; Freq, frequency; Geo, geography; GPA, grade point average; Hist, history; N, no; OR, odds ratio; p/w, per week; Prev, previous; Relig, religion; Sco Sci, social science; SD, standard deviation; selfR, self report; SR, school report; SSB, sugar sweetened beverages; UC, unclear; Veg, vegetable; Y, yes; Yr, year; Zn, zinc.

Fish consumption

Self-reported fish intake was associated with academic achievement in two studies [42,44]. Both studies report increased intake/or higher consumption of fish is associated with higher academic achievement. Specifically, one study reported broad classifications of consumption of no fish compared to fish once per week showed an increase in scores of academic achievement of 14.5 (11.8–17.1) points and >1 per week of 19.9 (16.5–23.3) points [44]. This relationship was not observed at the highest consumption level of 3150 mg day^{-1}.

Diet quality

Seven studies showed an association of academic achievement with more global measures of diet including: diet quality [15,27,39] (n = 3 studies), meal quality [37], adherence to Mediterranean diet, and dietary patterns [16]. Two studies using validated diet quality scores from the Diet Quality Index/Healthy Eating Index and the KIDMED index, which showed that variety and adequacy, rather than moderation and balance, were most associated with academic achievement [57]. However, the other study showed those with a positive GPA [15] attained a higher diet quality score compared to those with a negative GPA [mean (SD) diet quality score: 26.3 (10.4) versus 21.04 (8.77)]. Higher adherence to the Mediterranean diet and less of a 'western' dietary intake were also associated with higher outcomes of academic achievement.

Discussion

The aim of the present review was to evaluate the existing literature and determine the associations between dietary intake and behaviours on academic achievement in children and adolescents. This is the first review to critically appraise the literature regarding the relationship between broader dietary behaviours and nutritional intake and academic achievement. It was identified that moderate associations exist between several aspects of dietary intakes/profiles and academic achievement. These were characterised by regular breakfast consumption, lower intake of energy dense, nutrient poor foods assessed in studies as 'junk/fast foods', including sweetened drinks, and overall better diet quality and better outcomes of academic achievement.

Because the studies reviewed were from widespread international regions, it is clear the relationship between diet and academic achievement is of international interest. Given that the range of countries in the included studies is broad, it needs to be recognised that there are likely to be different food systems between developed or developing countries. The children residing in more developed countries may have better baseline nutrition,

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and this may affect the comparability across studies. Access to different foods/cultural foods is also likely, and so results should be considered accordingly.

The majority of studies reported statistically significant outcomes between diet and academic achievement, which is likely a result of the relatively large numbers of participants in each of the included studies. The reporting period of diet was not provided in the majority of studies, and a significant proportion of studies did not use a validated or standardised approach, such as 24-h recall or weighed food record dietary assessment method. This may be reflective of a variety of reasons, including fewer dietary assessment tools existing in paediatric populations compared to adult populations; budget; dietary outcomes not being the primary outcomes of the study; or the absence of a dietary or nutrition expert to assess diet. It was noted, however, that there was a tendency for studies published after 2015 to be of better study quality, with more studies achieving high quality ratings, most often as a result of more recognised or validated dietary assessment methods being used, making outcomes measures more reliable.

The majority of studies included in this review assessed only a single dietary behaviour/aspect of diet, such as breakfast consumption, and did not consider the broader concept of dietary intake, which is often a highly complex process of many meals, foods and nutrients. Future studies should investigate multiple dietary behaviours and assess using tools that capture usual/habitual intake to better capture the 'whole diet' and diet quality rather than single one-off meal occasions, single nutrients or one meal type such as fast food.

The highly varied reporting time period of academic outcomes (days to years) contributed to the difficulties of this review with respect to determining the true associations/relationships between diet and academic achievement in addition to the variable reporting period of diet (days to year). Dietary intakes have a high variability day to day, month to month, and season to season, and this should be reflected in the assessment tool and cover the same period of academic assessment as that being assessed. Future studies should ensure the dietary reporting period or the chosen dietary method aligns with the reporting time period of academic outcomes. For example, FFQs reflecting a 6–12-month-reporting period would better capture academic achievement over the same semester/12-month time period of academic achievement or multiple diet recalls over the specified academic period. This chosen dietary assessment tool should be validated and population-specific.

In the majority of studies, dietary intake was self-reported, which is likely the result of a majority of children in the included studies being aged >10 years. It has previously been acknowledged that children aged >8 years can accurately report their own dietary intakes [58]. Future studies in younger children < 8 years should consider using the parent as a proxy reporter. More studies need to be conducted in younger age groups (<10 years) because dietary intakes and behaviours formed in childhood track well to adulthood[59].

Many studies used self-reported measures of academic achievement, which introduces a considerable level of bias to those studies because of social desirability. Several studies used academic measures from standardised national testing, which are often calibrated and of a particular standard; however, other studies used non-standardised testing. Non-standardised testing also introduces some bias with regard to validity and reliability and likely has more variability compared to standardised tests. The addition of studies using categorical data with different set points of what is deemed 'good' academic outcomes also makes it difficult to interpret the relationships between diet and academic achievement.

There are several limitations to the present review. We only reviewed studies published in English and did not include research theses. The majority of studies were cross-sectional in design and thus preclude any inferences that can be made about cause and effect. This would be important to consider when designing future studies. In many studies, it was difficult to determine the sample characteristics (e.g gender, ethnicity, socio-economic status) and how each was assessed, which limits generalisability and applicability to broader populations. However, the present review used a standardised approach, which included a published methodology, assessed study quality using recognised tools and was completed in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.

This review synthesises the current evidence base of diet and academic achievement. The results demonstrate that the research area is still developing and highlights the current limitations of the published research investigating the relationship between diet and academic achievement. Future studies should consider the use of validated dietary assessment methods and the standardised assessment and reporting of academic outcomes of children.

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/STROBE/PRISMA 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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All authors contributed to this paper. TB and SG led and guided the search. KP, SG and RL completed extractions and checks. All authors reviewed and collated the data. 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:
Data S1. Full search strategy.
Table S1. Quality Appraisals of the included studies.

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CHILDREN AND ADOLESCENTS

Development and validation of a quantitative snack and beverage food frequency questionnaire for adolescents

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Abstract

Background: A short, reliable and valid tool to measure snack and beverage consumption in adolescents, taking into account the correct definitions, would benefit both epidemiological and intervention research. The present study aimed to develop a short quantitative beverage and snack food frequency questionnaire (FFQ) and to assess the reliability and validity of this FFQ against three 24-h recalls.
Methods: Reliability was assessed by comparing estimates of the FFQ administered 14 days apart (FFQ1 and FFQ2) in a convenience sample of 179 adolescents [60.3% male; mean (SD) 14.7 (0.9) years]. Validity was assessed by comparing FFQ1 with three telephone-administered 24-h recalls in a convenience sample of 99 adolescents [52.5% male, mean (SD) 14.8 (0.9) years]. Reliability and validity were assessed using Bland–Altman plots, classification agreements and correlation coefficients for the amount and frequency of consumption of unhealthy snacks, healthy snacks, unhealthy beverages, healthy beverages, and for the healthy snack and beverage ratios.
Results: Small mean differences (FFQ1 versus FFQ2) were observed for reliability, ranking ability ranged from fair to substantial, and Spearman coefficients fell within normal ranges. For the validity, mean differences (FFQ1 versus recalls) were small for beverage intake but large for snack intake, except for the healthy snack ratio. Ranking ability ranged from slightly to moderate, and Spearman coefficients fell within normal ranges.
Conclusions: Reliability and validity of the FFQ for all outcomes were found to be acceptable at a group level for epidemiological purposes, whereas for intervention purposes only the healthy snack and beverage ratios were found to be acceptable at a group level.

Introduction

Adolescents typically adopt unhealthy eating habits, such as snacking, low consumption of dairy products, fruit, vegetables, and high intake of energy-dense snacks, sugar-sweetened beverages (SSBs) and other high-caloric beverages [1,2]. The overconsumption of high energy-dense beverages, such as sodas, sweetened milk beverages, fruit-based drinks and alcohol, has already been associated with excess sugar and energy intake and obesity in

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adolescence and adulthood [3–5]. However, snacking, or eating in between meals, has been associated with both excess energy intake and being overweight and also an improved diet quality and reduced obesity [6–10]. The consequences are dependent on snacking frequency, food types eaten as snacks and portion sizes consumed [6–10]. If mainly energy-dense foods such as cookies, chocolate, chips or fast-food are consumed, energy, sugar and fat intakes from snacks are substantial and nutrient intakes are lower [7,8]. When more healthy foods, such as fruits and milk products, are eaten, energy intakes from snacks are lower and the overall nutritional quality of the diet is higher [7,8]. Not only do unhealthy snack and beverage intake both contribute independently to excess energy intake, but also their intakes are also related [11,12]. High SSB drinking children and adolescents were found to consume more sweet and salty snack foods than non SSB drinkers [11,12]. Effective evaluation of both habitual snack and beverage consumption is needed to determine important correlates of snack and beverage consumption, as well as to analyse interventions aimed at improving snack and/or beverage consumption.

Existing tools such as dietary records, 24-h recalls or large food frequency questionnaires (FFQs) assessing the total diet of adolescents are time-consuming, burdensome for the respondents and provide unnecessary details [13–16]. Especially when evaluating dietary intake in adolescents, rapidly administrable tools are necessary because adolescents are less interested in giving accurate reports [14]. Short assessments tools, focusing on specific behaviours, have been used before in adolescent populations to assess nutrient or food group intakes and were found to be easy to administer, reliable and valid [13–15,17,18]. To date, only a brief questionnaire exists that measures snack and beverage intake in adolescents [15]; however, this questionnaire was developed specifically for evaluating school policies and does not contain all possible snack foods or high-calorie beverages. Nor does it contain portion size estimation or evaluate the intake of snack foods at snack times. The latter is of crucial importance because the effects of snacking are determined by the type and portion size of snacks. Snack intake should be measured as the consumption of typical snack foods, both healthy and unhealthy, at snack times (e.g. any food eaten in between the main meals) [19,20].

The aim of the present study was to develop and validate a short quantitative FFQ to measure both habitual snack and beverage intake, using the correct definition of snacking (e.g. snack foods eaten at snack times) [21]. The reliability and validity of this FFQ was assessed in a sample of Flemish adolescents aged 14–16 years for both epidemiological and intervention purposes. Reliability and validity were assessed for the variables: frequency and quantity (g or mL) of unhealthy snacks, healthy snacks, unhealthy beverages and healthy beverages, and the healthy snack and beverage ratios.

Materials and methods

The present study is part of the REWARD project (www.rewardstudy.be), a multidisciplinary project that aims to increase healthy food choices in children and adolescents using reward-based mechanisms. In adolescents, the overall goal was to study and/or improve adolescents' snack and beverage choices. The first step was the development of a quantitative snack and beverage FFQ for adolescents, of which the present study reports the development and the validation and reliability analyses. This FFQ will be used in a subsequent cross-sectional study to research adolescents' snacking and drinking behaviours, and a smartphone-based intervention study to increase adolescents' healthy snack choices.

Development of the quantitative snack and beverage food frequency questionnaire

The selection of surveyed food and beverages items consisted of two steps. In step 1, a review of survey items from existing research examining food intake in children and adolescents was conducted [22–25]. From this review, one FFQ was selected to be used as the basis for our FFQ [22,25]. Step 2 assessed whether the items from this FFQ were commonly consumed as snacks or beverages by adolescents in Flanders. The frequent consumption of a food as snack or beverage was assessed based on the 24-h recall data of Flemish adolescents from the HELENA study [26]. The latter study evaluated the food intake and eating patterns of European adolescents aged 12.5–17.5 years from 10 European countries, including Belgium (Flanders) [26]. Items that were not commonly consumed as snacks or beverages by adolescents in the HELENA study were removed; and snacks or beverages that were commonly consumed, but were not present, were added. In total, the FFQ consists of 14 beverage items and 28 snack items. The FFQ is provided as Supporting information (Appendix S1).

Frequencies of consumption, portion sizes and examples of typical portions were adapted from the same quantitative FFQ that was used as basis for the selection of the items [22,25].

The snack and beverage FFQ was pretested by 40 adolescents (±2 classes) on clearness and appropriateness of the items and examples. Wording of the items and examples were revised based on their feedback.

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Validation and reliability study

Design

Reliability and validity of the FFQ were examined in a convenience sample of Flemish adolescents. Reliability was assessed by comparing measurement agreement of a repeated administration [FFQ at time 1 (FFQ1) versus FFQ at time 2 (FFQ2)]. Validity was evaluated by comparing measurement agreement between the FFQ1 and the average of three 24-h dietary recalls. Executing the 24-h dietary recall three times is considered sufficient to obtain an estimation of the habitual intake of adolescents for the purpose of validation studies in adolescents [24,27,28]. Administering the 24-h dietary recalls by telephone is common and convenient in research with adolescents [27,29–31]. Main outcomes were the consumption frequency of unhealthy snacks, healthy snacks, unhealthy beverages and healthy beverages; the intake of unhealthy snacks (g), healthy snacks (g), unhealthy beverages (mL) and healthy beverages (mL); and the healthy snack and beverage ratios.

Recruitment of participants

Data were collected from February to March 2014 using a convenience sample of 14–16-year-old Flemish adolescents. These adolescents were recruited from three secondary schools, in each school three classes (±60 students per school) were selected by the principals to participate in the study. Adolescents were asked separately if they also wanted to participate in the validation study, because this required more effort. Incentives were raffled among adolescents that participated in both studies. Parents or legal guardians of the selected adolescents received a letter explaining the study purpose and were asked for passive consent for the participation of their adolescent. Adolescents were also informed that they could withdraw from the study at any time without explanations. No inclusion or exclusion criteria were applied. The study protocol was approved by the Ethics Committees of the Ghent University Hospital.

Study procedure

A team of researchers visited the schools on a previously agreed time during school hours. Adolescents completed the FFQ (FFQ1) in the presence of a research assistant. Adolescents were instructed to carefully read the instructions (see Supporting information, Appendix S1) given with the FFQ and were informed that they could ask questions at any time. Adolescents also completed a short demographic questionnaire at the same time. Completing the FFQ took the adolescents approximately 20 min. Adolescents who agreed to participate in the validation study also provided a telephone number and the hours they were available for the 24-h recalls at this time point.

For the purpose of the reliability study, the FFQ was administered a second time (FFQ2), 14 days after the first administration (FFQ1) following the same procedures.

For the purpose of the validation study, three 24-h recalls were administered between FFQ1 and FFQ2 in such a way that all participants provided data for two weekdays and one weekend day. At a group level, a balanced representation of each week day was obtained. Participants were called in between the agreed hours and were asked about food consumption of the previous day. Participants were unaware at which days they would be called. The administration of the 24-h recall took approximately 15 min each time. The 24-h recalls were conducted by dieticians, who were trained to administer these recalls in a standardised way [22,25]. No specific automatised procedure, such as the multiple-pass method [32], was used. Adolescents were called three times on different days before being regarded as having dropped out.

Instruments

The quantitative beverage and snack food frequency questionnaire

The FFQ assessed usual food intake with a reference period of 1 month. The six frequency categories used were: never or seldom; 1–3 days month^{-1}; 1 day week^{-1}; 2–4 days week^{-1}; 5–6 days week^{-1}; every day. Depending on the item, four to six portion size categories were provided together with a list of common standard portion measures as examples.

The FFQ comprised of two sections: beverages (14 items) and snacks (28 items). The intake of beverages was evaluated over the whole day because beverages such as soft and fruit drinks provide additional calories and sugars throughout the whole day and not only at snack times [33]. The intake of snacks was evaluated in terms of all food items consumed outside (>30 min) of breakfast, lunch and dinner, in accordance with Rodriguez and Moreno's definition of snacking [21]. Items in both sections were presented in such a way that closely related items were presented on the same page with the more specific items presented before the general ones [13].

Snacks and beverages were classified as either healthy or unhealthy using the UK Ofcom Nutrient Profiling model [34]. This model provides a score as a proxy for 'unhealthiness' of a beverage or food product. Food items that scored 4 points or more and beverage items that scored 1 point or more were considered to be unhealthy [34]. Following this scoring system, the snack and beverage items, sport drinks, energy drinks, soft drinks, sweetened milk drinks, cocktails, aperitif drinks, liquor, crisps, other salty snacks, sausage/cheese rolls and pizza, other fried snacks, fries, hamburgers, cheese or meat cubes, sandwich

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with sweet or savoury spread, ice-cream, popsicles, breakfast cereals, pudding, mousses, chocolate, candy bars, candy, dry cookies, other cookies, breakfast rolls and pastries were considered to be unhealthy. The items water, fruit juice, coffee, milk substitutes, milk, beer, wine, fruit, dried fruit, nuts, raw vegetables, pitta, pasta cups, unsweetened and sweetened yoghurt were considered healthy.

The daily intake of each snack and beverage item of the FFQ was obtained by multiplying frequency of consumption with quantity of consumption per week (g) divided by 7. These daily estimates were then summed to obtain the daily intake of healthy snacks (g), unhealthy snacks (g) unhealthy beverages (mL) and healthy beverages (mL). The consumption frequency of unhealthy and healthy snacks or beverages was calculated by summing the frequencies of the different food or beverage items and dividing this sum by 7. Finally, healthy snack and beverage ratios were calculated. These ratios represent how much percentage of the total snack or beverage intake was healthy. The higher these ratios, the more healthy the snack or beverage intake of the adolescents.

$$\text{Healthy snack ratio} = \left(\frac{\text{daily intake of healthy snacks}}{\text{daily intake healthy and unhealthy snacks}}\right) \times 100$$

$$\text{Healthy beverage ratio} = \left(\frac{\text{daily intake of healthy beverages}}{\text{daily intake healthy and unhealthy beverages}}\right) \times 100$$

The 24-h recalls

All information obtained during the telephone administered 24-h recalls was noted in a document subdivided into six eating occasions: breakfast, morning snacks, lunch, afternoon snacks, dinner and evening snacks. For each of these occasions, detailed information was requested from the adolescent by the researcher concerning the type of food consumed, the brand (with description) and the quantity consumed. For each of these occasions, product categories were also provided depending on the type of meal; for example, for breakfast, these are: cereal, bread, spreads/meat/cheese/etc., margarine/butter/etc., drinks and others.

Because the focus of our FFQ was only on snacks (all food items consumed outside the three main meals) and beverages (evaluated over the whole day), only the 24-h recall data regarding food items obtained in the sections morning, afternoon and evening snacks and beverage items from all sections were used and imported into LUCILLE, version 0.1 [35]. Lucille is a software package designed to process food intake developed by our own research group [35]. The present study opted to question all eating occasions and not only snack occasions because beverage intake was evaluated over the whole day. This would also not interfere with the normal way of performing a telephone-administered 24-h recall.

All foods and beverages consumed by the adolescents in the 24-h recalls were summed to obtain the intakes per snack and beverage item from the FFQ and per recall day. These intakes per item were then summed to obtain again the daily intake of healthy snacks (g), unhealthy snacks (g) unhealthy beverages (mL) and healthy beverages (mL) per recall day. The latter were then averaged to obtain an average of the three recall days to represent the habitual intake of healthy snacks (g), unhealthy snacks (g), unhealthy beverages (mL) and healthy beverages (mL) comparable with the data obtained via the FFQ. Also, the consumption frequencies of unhealthy or healthy beverage or snack items were calculated by summing the different snack or beverage items consumed each recall day and then again averaging these numbers over the three recall days to obtain the usual consumption frequencies of unhealthy or healthy snack and beverage items consumed per day. Finally, the healthy snack and beverage ratios were calculated in the same manner as stated above.

Statistical analysis

All analyses were performed in STATA, version 13.1 (StataCorp, College Station, TX, USA). Although correlation coefficients are a poor estimate of measurement agreement, they are provided in the present paper to allow comparison with other studies [36,37].

Reliability analysis

Only participants who completed both FFQ1 and FFQ2 were retained for the reliability analysis. Descriptive analyses were used to evaluate the characteristics of the participants (mean age and sex) in the reliability study and to describe the mean intakes and frequencies obtained via FFQ1 and FFQ2.

Reliability was assessed first by determining the correlation coefficients, Spearman's rho, between the outcomes derived from FFQ1 and FFQ2. Second, agreement between the repeated administration for each of the outcomes was evaluated using Bland–Altman plots [38]. The same procedure to determine mean difference, its confidence interval (CI) and the 95% limits of agreement (LOA) was followed as previously proposed by Ambrosini *et al.* [39], including the transformation of all outcomes to their natural logarithms before analyses because of the usual skewness in intake distributions. Mean differences (FFQ1 – FFQ2) and LOAs were thus back transformed

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by taking the antilog, and values are presented as percentages. For example, a mean agreement of 100% for energy intake would suggest exact agreement, whereas a mean agreement of 120% indicates that the FFQ1 overestimated unhealthy snack intake by 20% compared to FFQ2, on average. Furthermore, 95% LOAs of 55–184% for unhealthy snack intake would suggest that 95% of all subjects' FFQ1 estimates are between 55% and 184% of their FFQ2 unhealthy snack intake estimate [38,39]. Third, the classification agreement between FFQ1 and FFQ2 was assessed using weighted kappa statistics and its SD by comparing classifications of the outcomes into low, medium and high tertiles [40] using the standards as proposed by Landis and Koch (1977) [41]. These standards are less than 0 'less than chance agreement', 0.01–0.20 'slight agreement', 0.21–0.40 'fair agreement', 0.41–0.60 'moderate agreement', 0.61–0.80 'substantial agreement' and 0.81–0.99 'almost perfect agreement'. To account for prevalence and bias effects, the prevalence-adjusted and bias-adjusted kappa (PABAK) was presented alongside the kappa statistics [42].

Validation analysis

Only participants who completed at least two 24-h recalls and FFQ1 were retained for the validation analysis. Descriptive analyses were used to evaluate the characteristics of the participants (mean age and sex) in the validation study and to describe their mean intakes and frequencies obtained via FFQ1 and the 24-h recalls (average of the three evaluated days).

Validity was assessed by first determining correlation coefficients (Spearman's rho) between the outcomes derived from FFQ1 and the average of the three 24-h recalls. Second, this was followed by a comparison of the agreement for all outcomes between FFQ1 and the average of the recalls by means of Bland–Altman plots, in the same manner as explained above for the reliability study. The third and final assessment determined the classification agreement for all outcomes between FFQ1 and the recalls by means of kappa statistics, as explained above.

Results

Reliability study

Participants and descriptives

In total, 179 adolescents [60.3% male; mean (SD) age 14.7 (0.9) years], or 97% of 184 adolescents sampled in the reliability study, provided valid data for both administrations of the FFQ.

Table 1 shows the estimates for the outcomes obtained from FFQ1 and FFQ2. FFQ1 had higher estimates for all outcomes except for the healthy snack ratio.

Reliability

Mean differences for all outcomes were small (less than 30% difference). The largest mean difference observed was +28.8% for the quantity of healthy beverages consumed. The smallest difference observed was +3.8% for the healthy beverage ratio (Table 2). FFQ1 thus overestimated the quantity of healthy beverages by 28.8% or FFQ1 measured 128.8 mL and FFQ2 100 mL. Except for the healthy snack ratio, all mean differences were positive and different from 100%, indicating that FFQ1 overestimated intakes compared to FFQ2. The 95% CIs included 100% agreement except for the frequency of unhealthy and healthy snacks, the quantity of unhealthy and healthy snacks and the quantity of unhealthy beverages, indicating nonsignificant differences between FFQ1 and FFQ2. LOAs were wide for all outcomes (Table 2).

Table 1 Mean snack and beverage intakes for the reliability (n = 179) and validation study (n = 99)

	Reliability (n = 179)				Validity (n = 99)			
	FFQ1		FFQ2		FFQ1		Average of the recalls	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Frequency of unhealthy snacks per day	2.8	(1.9)	2.4	(1.8)	2.4	(1.5)	0.8	(0.7)
Frequency of healthy snacks per day	1.1	(0.7)	0.9	(0.7)	1.1	(0.7)	0.5	(0.7)
Quantity of unhealthy snacks consumed per day (g)	225.8	(237.1)	220.9	(308.9)	180.0	(154.4)	44.7	(41.5)
Quantity of healthy snacks consumed per day (g)	195.6	(173.0)	181.0	(190.8)	201.6	(160.7)	65.0	(104.3)
Healthy snack ratio (%)	45.5	(27.8)	46.3	(27.7)	51.5	(26.8)	26.6	(32.7)
Frequency of unhealthy beverages per day	0.8	(0.7)	0.7	(0.7)	0.7	(0.7)	0.8	(0.8)
Frequency of healthy beverages per day	2.0	(0.8)	1.8	(0.8)	2.0	0.8)	2.7	(1.1)
Quantity of unhealthy beverages consumed per day (mL)	295.2	(390.2)	269.3	(388.1)	286.0	(436.9)	185.2	(260.6)
Quantity of healthy beverages consumed per day (mL)	987.0	(542.5)	841.2	(559.8)	988.9	(504.3)	921.8	(481.2)
Healthy beverage ratio (%)	77.3	(24.0)	76.5	(24.3)	79.1	(24.1)	76.7	(24.3)

FFQ, food frequency questionnaire.

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Table 2 Mean differences and confidence intervals (CIs), as well as limit of agreement (LOA), kappa, prevalence-adjusted and bias-adjusted (PABAK) and Spearman's rho values, for the reliability study (n = 179)

	Mean agreement (%)*	95% CI (%)*	LOA (%)*	Kappa	PABAK	Spearman's rho
Frequency of unhealthy snacks per day	118.9	109.1, 129.4	37.7, 375.0	0.51	0.56	0.69
Frequency of healthy snacks per day	119.1	106.2, 133.4	26.4, 537.0	0.49	0.55	0.69
Quantity of unhealthy snacks consumed per day (g)	119.1	106.7, 133.1	16.0, 843.3	0.57	0.62	0.75
Quantity of healthy snacks consumed per day (g)	115.1	99.1, 133.4	16.3, 812.8	0.49	0.55	0.62
Healthy snack ratio (%)	95.3	85.1, 106.7	21.8, 415.9	0.54	0.59	0.73
Frequency of unhealthy beverages per day	107.4	93.3, 123.6	18.3, 629.5	0.56	0.61	0.68
Frequency of healthy beverages per day	113.2	105.7, 121.1	45.1, 283.8	1.00	1.00	0.71
Quantity of unhealthy beverages consumed per day (mL)	107.9	91.2, 127.7	13.2, 885.1	0.55	0.60	0.70
Quantity of healthy beverages consumed per day (mL)	128.8	117.2, 141.9	35.2, 472.1	0.53	0.59	0.67
Healthy beverage ratio (%)	103.8	96.8, 111.2	41.1, 261.8	0.52	0.58	0.69

*Antilogs in percentages: mean agreement of 100% for quantity of unhealthy snacks would suggest exact agreement, whereas mean agreement of 119.1% indicates that the FFQ1 overestimates the quantity of unhealthy snacks by 20%, on average. FFQ, food frequency questionnaire.

Moderate classification agreement (kappa in Table 2) was observed for all outcomes except for the frequency of healthy beverages, where substantial agreement was observed. The kappa coefficient improved for all outcomes when it was adjusted for prevalence and bias (PABAK) (Table 2).

Spearman's rho's (Table 2) ranged from 0.62 (healthy snacks g day^{-1}) to 0.75 (unhealthy snacks g day^{-1}).

Validation study

Participants and descriptives

In total, 99 adolescents [52.5% male, mean (SD) age 14.8 (0.9) years], or 82% of 121 adolescents sampled in the validation study, provided valid data for at least two 24-h recalls and FFQ1. Of these 99 participants, 88 (88.9%) completed three recalls and 11 (11.1%) complete only two.

Table 1 indicates that the FFQ provided higher estimates than the 24-h recalls for snack intake in terms of frequencies and quantities consumed and the healthy snack ratio. For beverage intake, the FFQ provided lower estimates for the frequencies of unhealthy and healthy beverages, although higher estimates for the quantities consumed and the healthy beverage ratio.

Validity

Small mean differences (less than 30%) were observed for unhealthy and healthy beverages (frequencies and quantities), ranging from −24.7% to +7.6% (Table 3). The FFQ overestimated the quantities consumed by 9 or 4 mL, whereas it underestimated the frequency of unhealthy and healthy beverages by 0.25- or 0.17-fold. The FFQ and the 24-h recalls showed almost perfect agreement for the healthy beverage ratio (mean difference=100.5%). Large mean differences, however, were observed (Table 3) for the intake of healthy and unhealthy snacks, especially the quantity and the frequency of unhealthy snacks was overestimated by the FFQ (+152.9% and 225.8%, respectively). The FFQ overestimated the frequency of eating unhealthy snacks by 1.5-fold and the quantity consumed by 226 g. For the healthy snack ratio, the difference between both methods was small (+11.2%). The 95% CIs did not include 100% agreement, except for the healthy snack ratio, the frequency of unhealthy beverages, the quantity of unhealthy and healthy beverages, and the healthy beverage ratio, indicating significant differences between both methods. LOAs were wide for all outcomes (Table 3). Figure 1 illustrates the differences in mean agreement and LOAs by presenting the Bland–Altman plots for the intake of unhealthy snacks, healthy snacks, unhealthy beverages and healthy beverages, and the healthy snack and beverage ratios.

Slight to moderate classification agreement was observed between the FFQ and the recalls. Classification agreement improved for all outcomes when adjusted for prevalence and bias (Table 3).

Spearman's rho's (Table 3) ranged from 0.17 (healthy beverages frequency day^{-1}) to 0.69 (unhealthy beverages g day^{-1}).

Discussion

The present study reports on the reliability and validity of a newly developed quantitative snack and beverage FFQ for adolescents.

The reliability of the FFQ was adequate at a group level for snack and beverage intake because mean differences were small and kappa values and correlation coefficients fell within the common range [13]. Significant mean differences between both administrations of the FFQ were observed for the frequency of unhealthy and healthy

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Table 3 Mean difference and confidence intervals (CIs), as well as limit of agreement (LOA), kappa, prevalence-adjusted and bias-adjusted (PABAK) and Spearman's rho values, for the validation study

	Mean agreement (%)*	95% CI (%)*	LOA (%)*	Kappa	PABAK	Spearman's rho
Frequency of unhealthy snacks per day	252.9	214.8, 297.9	58.9, 1088.9	0.13	0.24	0.27
Frequency of healthy snacks per day	142.9	112.2, 182.4	26.4, 776.3	0.16	0.25	0.39
Quantity of unhealthy snacks consumed per day (g)	325.8	261.8, 404.6	47.4, 2238.7	0.18	0.27	0.31
Quantity of healthy snacks consumed per day (g)	214.3	161.1, 285.8	29.7, 1548.8	0.28	0.32	0.42
Healthy snack ratio (%)	111.2	89.5, 138.4	24.8, 500.0	0.25	0.30	0.35
Frequency of unhealthy beverages per day	82.6	67.1, 101.9	14.8, 460.3	0.33	0.39	0.63
Frequency of healthy beverages per day	75.3	66.5, 85.3	21.6, 262.4	0.13	0.27	0.17
Quantity of unhealthy beverages consumed per day (mL)	108.9	84.3, 140.6	15.0, 790.7	0.49	0.55	0.69
Quantity of healthy beverages consumed per day (mL)	104.0	90.0, 120.0	24.4, 441.6	0.31	0.38	0.44
Healthy beverage ratio (%)	100.5	91.4, 110.7	38.7, 261.2	0.43	0.48	0.68

*Antilogs in percentages: mean agreement of 100% for quantity of unhealthy snacks would suggest exact agreement, whereas mean agreement of 325.8% indicates that the FFQ overestimates the quantity of unhealthy snacks by 285%, on average. FFQ, food frequency questionnaire.

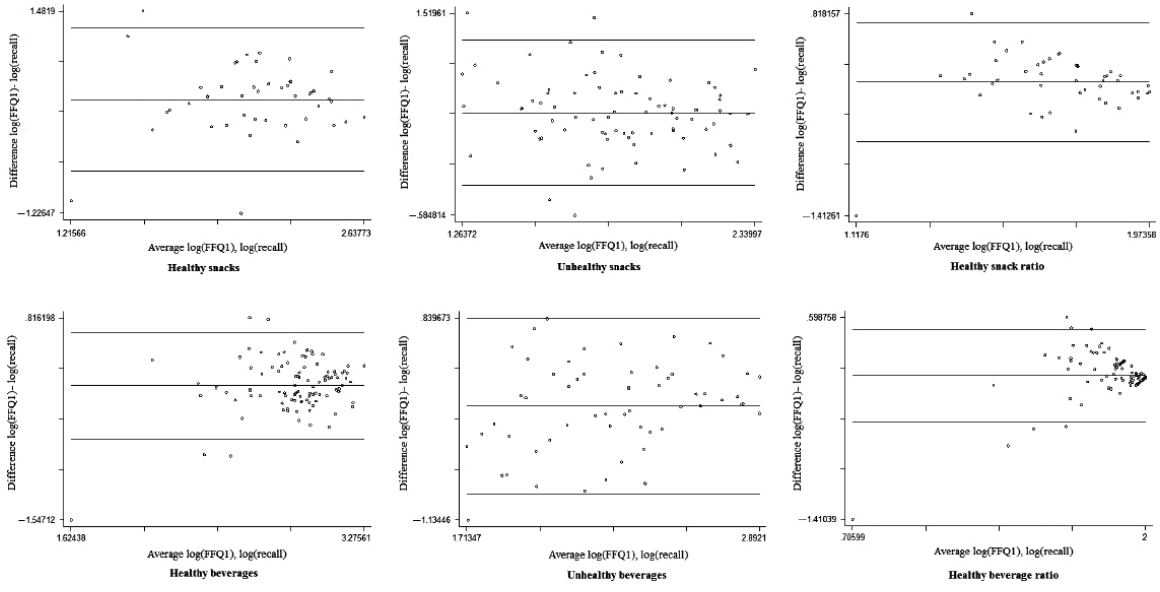

Figure 1 Bland–Altman plots for snack and beverage intake in the validation study. FFQ, food frequency questionnaire.

snacks, the quantity of unhealthy and healthy snacks, and the quantity of unhealthy beverages. These differences, however, were small and not higher than 30%, indicating a discrepancy of only 0.3 snacks or 30 g eaten more per day. LOAs, on the other hand, were large, indicating that reliability is inadequate at an individual level. Because the present study is the first to specifically measure snack and beverage intakes, no comparable reliability studies could be found. Findings were therefore compared with reliability studies that capture total food intake in adolescents. The study by Watson *et al.* [43] also used Bland–Altman plots to test the reliability of a FFQ in adolescents and reported similar results: small mean differences but large LOAs. Other reliability studies of FFQs in adolescents, reported similar ranges of kappa values and correlation coefficients [24,37,39].

The results of the validation analyses showed that mean differences for beverage intake (frequencies, absolute intakes and healthy beverage ratio) were small. The FFQ underestimated the frequency of beverages consumed by 0.25-fold for healthy beverages and by 0.17-fold for unhealthy beverages and overestimated the quantity of unhealthy and healthy beverages consumed by respectively 8.9 or 4 mL. For the healthy beverage ratio, almost perfect agreement (mean difference = 100.5%) was observed. Mean differences for absolute snack intakes were large, whereas the mean difference for the healthy snack ratio was small. Differences in absolute snack

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consumption corresponded to an overestimation of 225 g of unhealthy snacks and 114 g for healthy snacks. Snack foods are abundant in our environment [44] and adolescents are thus presented with wide range of snack options each day, making it difficult for adolescents to estimate their snack consumption for the past month. Other studies also already reported that it is difficult to capture this highly variable food intake pattern of adolescents [18,45]. In addition, adolescents may have ticked several snacks for a small frequency in the FFQ, leading to a larger overall amount estimated in the FFQ than actually consumed. Twenty-eight different snack options were presented in the FFQ. To limit the ticking of too many snacks and the related overestimation of absolute snack intake, it might be better to offer less choice and to group some of the snack items. For all outcomes, however, Spearman's correlation coefficients and ranking ability were considered acceptable. Here, the findings were also compared with validation studies in adolescents that measured total food intakes with a FFQ by lack of comparable studies. Other FFQ validation studies also found rather large discrepancies between both methods of food intake estimation but found acceptable ranking ability [24,37,39,43]. LOAs, obtained via Bland–Altman plots, were wide for all outcomes of the validation study. This indicates that the FFQ is thus inadequate for estimating snack and beverage intake at an individual level. The latter is also in concordance with these other validation studies of FFQs in adolescents [27,38,40,44].

For means of intervention evaluation, a good test–retest reliability and precise estimates of intakes at a group level are necessary to detect changes [13,14]. Small mean differences were observed between the repeated administration of the FFQ for all outcomes; however, large differences were observed between the FFQ and the 24-h recalls except for the healthy snack and beverage ratio. Thus, only the healthy snack and beverage ratio are appropriate for evaluating dietary change in intervention studies. For means of cross-sectional research, mainly a moderate to good ranking ability [13,14] is needed, which was achieved for all outcomes.

The present study was the first to develop and report on the reliability and validity of quantitative snack and beverage FFQ, incorporating the evaluation of snack food at snack times, for the purpose of epidemiological or intervention studies. Other strengths of the present study are its use of standard portion sizes to help the portion size estimation, a sample that contained a balanced amount of boys and girls and the use of Bland–Altman plots alongside correlation coefficients to assess reliability and validity. Previous research already showed that correlation coefficients can be misleading indicators of agreement [37]. The present study, however, also had some limitations. First, the sample population was obtained via convenience sampling and therefore the results might not be generalisable to other populations. Second, the selection of the items of the snack-and-beverage centered FFQ was based on the frequentness of consumption by the general population of adolescents; thus, it could be possible that not every adolescent feels that he or she is able to fully describe his or hers snack and/or beverage intake. Third, the source of error of a 24-h recalls tends to be more correlated with the error in an FFQ as a result of reliance upon memory and conceptualisation of portion sizes [13]. For example, the use of biomarkers, with errors that are are uncorrelated with FFQs, would have greatly increased both respondent and researchers burden. Fourth, a possible memory effect could have occurred in the reliability study; some adolescents might have remembered their answers to the FFQ1 when completing the second FFQ. Cade *et al.* [13] stated that, when there is a very short interval between the repeated administration of the FFQ, participants could indeed remember their previous responses. Two weeks, however, is a not uncommon interval in reliability studies in adolescents [46]. A larger interval between both FFQs was also not possible because the Easter examination period was approaching. Fifth and finally, when using this FFQ to estimate the effect of interventions, the FFQ should be complemented with a 24-h recall or another instrument that captures the total diet to account for possible spillover effects on other eating behaviours.

Conclusions

The reliability and the validity of the snack and beverage FFQ were found to be acceptable at a group level for the purpose of analysing diet–disease relationships. Caution, however, should be exercised when presenting and researching absolute snack intakes, especially for unhealthy snack intake. The reliability and the validity of the snack and beverage FFQ was also found to be acceptable at a group level for the purpose of analysing intervention effects, although only for the healthy snack and beverage ratios.

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 the STROBE guidelines.

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Conflict of interests, source of funding and authorship

The authors declare that there were no conflicts of interest.
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NDC conducted research, conducted the analyses and wrote the paper. WVL and JV conducted research and helped revise the manuscript. KB, CL and LH designed research and helped revise the manuscript. RV and BD helped revise the manuscript. SE, LM, JVC and PK designed research. 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. the snack and beverage FFQ.

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CHILDREN AND ADOLESCENTS

Quantitative and qualitative analysis of breakfast nutritional composition in French schoolchildren aged 9–11 years

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Abstract

Background: The present study aimed to analyse the nutritional quality of childrens' breakfasts using data collected during a cross-sectional observational study on the prevalence of urinary osmolality in 529 French children aged 9–11 years.
Methods: Total nutrient intake, mean adequacy ratio (MAR), energy density and solid energy density were calculated from breakfast food and fluid nutritional composition. To identify the main qualitative breakfast patterns, each breakfast item was categorised into 15 solid and liquid food categories and a principal component analysis followed by a cluster analysis was performed.
Results: Only 9.8% included skipped breakfast. Breakfast provided, on average, 22.9% of the recommended daily energy intake and 24.7% of the mean adequacy ratio of 23 key nutrients. Four breakfast patterns were identified: 'Sweets breakfast' (40.0% of children), 'Traditional French breakfast' (27.2%), 'Ready-to-eat cereal (RTEC) + milk' (18.1%) and 'Dairy and juice breakfast' (9.5%). Nutritionally, the 'RTEC + milk' pattern was the most advantageous. Flavoured milk was the most frequently consumed food (50.5%) and the major component of the 'Traditional French breakfast'.
Conclusions: Although breakfast provided a substantial contribution to a range of nutrients, opportunity for improvement, particularly to less nutrient breakfast patterns, should not be overlooked.

Introduction

Eating breakfast is recommended and is considered to be the most important meal of the day because it is associated with good health behaviours, a reduced risk of chronic diseases, and improved cognitive and school performance [(1)]. In addition, eating breakfast, especially a breakfast rich in proteins and low in glycaemic index carbohydrates, correlates with a normal body mass index (BMI), whereas skipping breakfast is frequently associated with being overweight and obesity [(2,3)].

Defining precisely how much energy breakfast should provide is difficult and national recommendations vary across the world. The US Department of Agriculture established four definitions of breakfast composition [(4)]. The simplest definition is any food or beverage except water. The three other definitions constitute 'substantial breakfasts' and include foods from at least two of the five major food groups (breads/grains, fruits, vegetables, meat/meat alternate and milk). They provide, respectively, >10%, 15% and 25% of the Recommended Dietary Allowances for energy and one-fourth of the Recommended Daily Allowance of nutrients based on the latest Dietary Guidelines for Americans [(5)].

France does not appear to have a specific recommendation on the percentage of energy provided by breakfast,

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although current recommendations are to eat at least one food containing cereals, one fruit or freshly squeezed juice or juice with no added sugar, and one dairy product [6]. Quantities for each of these components are not provided in the recommendations but can be found in the revised version of the *Recommandation Nutrition* of the *Groupe d'étude des Marchés de Restauration Collective et Nutrition GEM-RCN* (Guide on Nutrition Recommendations for Mass Catering) [7] published by the French Ministry of Economic Affairs and Finance. Rates of breakfast consumption in French children 3–12 years of age were 91% in 2003 and 87% in 2010 [8] Little detailed information is available about breakfast habits, and the most recent nutritional studies are more than a decade old [9,10].

In the present study, we analysed breakfast composition in France using data extracted from a recent survey examining breakfast water intake by schoolchildren aged 9–11 years [11]. Our objectives were (i) to assess energy and nutritional quality of breakfast and (ii) to identify the main breakfast patterns (i.e. how foods and drinks were associated in the children's breakfasts). We also evaluated whether the children were meeting French national recommendations.

Materials and methods

Study design and dietary record

The current analysis was based on data collected as part of a prospective, cross-sectional study carried out in Rennes, France between 2010 and 2011 [11]. The protocol for the study was approved by AFSSAPS (French Health Products Safety Agency) and the Ethics Committee of Rennes Ouest V, France, and the study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from both parents and children.

Briefly, in the Rennes study, children aged 9–11 years were recruited on a voluntary basis via 14 primary schools from the city of Rennes and vicinity. This area includes both urban and rural populations from various socio-economic strata. All children were given a study pack to take home. The pack contained a study information document; a parent and child consent form; and a questionnaire covering demographic information, height and weight, and breakfast composition. Breakfast was defined as the first meal of the day consumed in the morning. Breakfast intake data were reported for 1 day. To address any potential difference between weekday and weekend breakfast composition, the clinical investigators' recommendation was to fill the questionnaire on a weekday only. Children were instructed to follow their usual morning routine and, if breakfast was eaten, to record their consumption in a diary, including the time of consumption, product brand name, cooking method and portion size. Children returned study materials, including the urine sample, to the teachers on designated days. Children were included if both parents had signed the informed consent and if they fulfilled the inclusion criteria, which included a usual intake of at least two main meals per day. Children were excluded if they currently or previously had a metabolic or digestive disease (with the exception of appendectomy) or renal disease (renal insufficiency, etc.), if they had fever, or if they were prescribed a local or systemic treatment likely to interfere with evaluation of the study parameters, including hydration state in particular (diuretic treatment or treatment interfering with metabolism and eating behaviour).

Overweight, obesity, and underweight were determined from the body mass index and age according to the 2012 International Obesity Task Force cut-off values [12].

Energy and nutrient intakes

Dietary intake was determined using information recorded in diaries by children and/or parents. Breakfast skippers included children having no food and no fluid and children who took their first meal at lunch time. Having only a drink was considered as breakfast. All food and drink consumed at breakfast, or the last meal of the previous day in case of children not consuming breakfast, was recorded, including brand name and portion size (household measures or package weight) [11]. Dietary data were entered in BILNUT, version 7.5 (Nutrisoft, Mesa, AZ, USA), which converts the amount of food eaten into individual energy, macronutrients and micronutrients and then assigns consumed foods to food groups and subgroups. For children consuming several foods, if the nutritional content of one or more foods was missing, no intake was assumed. If all foods were missing, the child's data were considered missing and therefore not included in the calculations. Foods were categorised as described previously [13] and included the food and drink categories: bread, ready-to-eat cereals (RTEC), sweets (biscuits, pastries, bakery goods, chocolate, jam, candy, honey, sugar, and syrup), fat/oil, fruits/vegetables, meat/fish/egg, dairy, water, milk, juices, flavoured milk, coffee, tea, soda/soft drink/sport drink and miscellaneous. The combination of milk and sweetened cocoa powder was considered as a single food (flavoured milk). For each individual, the total amount of food consumed, total energy intake, energy intake from liquids and energy intake from solid foods were calculated.

Nutritional quality of breakfast

Energy intake from breakfast was compared with the daily recommended energy intake derived from the joint FAO/WHO/UNU Expert Consultation of 2001 [14]. Nutritional

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quality of breakfasts was estimated using the energy density [MJ 100 g^{-1} (kcal 100 g^{-1})] and the mean adequacy ratio (MAR). Energy density was the ratio between energy intake and amount of foods (g) and was calculated first for total foods and then for solid foods only. The MAR was calculated as the mean percentage of daily recommended intakes for 23 key nutrients [15,16] in relation to the French recommendations [17]. These 23 nutrients included protein, fibre, retinol, thiamine, riboflavin, niacin, vitamin B_6, folates, vitamin B_{12}, ascorbic acid, vitamin E, vitamin D, calcium, potassium, iron, magnesium, zinc, copper, iodine, docosahexaenoic acid (DHA), linoleic acid, linolenic acid and selenium. The ratio for each nutrient was truncated at 100 so that a high intake of one nutrient could not compensate for the low intake of others.

Dietary patterns

Based on the amounts consumed of the 15 food categories, a principal component analysis including a varimax rotation was used to extract latent variables explaining most variability in individual dietary habits. Only three subjects were considered as outliers and were removed from the PCA to obtain homogeneous and robust dietary patterns. The first four latent variables were retained and used to run a hierarchical ascendant classification based on Ward criteria.

Statistical analysis

Averages of energy intake (total, solid, and liquid) and amount of foods (total, solid, and liquid), energy density (total and solid), MAR and nutrients expressed as a percentage of recommendations were calculated in the whole sample and by sex. Differences by sex were compared using a general linear model. Additional calculations included the percentage of breakfast skippers, the number of consumers and the average amount consumed (g) by food category. The four most frequent breakfast patterns were determined by principal component analysis. *P*-values were calculated using a general linear model with food group as the dependant variable and the dietary pattern variable as the independent variable. A pairwise statistical *t*-test was performed to compare the MAR between dietary patterns. The consumption of the different food categories was compared between breakfast patterns using a general linear model with food group as the dependant variable and the dietary pattern variable as the independent variable.

Statistical analysis was performed using SAS, version 9.4 (SAS Institute, Cary, NC, USA). Missing data were not replaced and no imputation was made. $P < 0.05$ was considered statistically significant.

Results

Subjects

The analysis included 529 children aged 9–11 years. Just over half ($n = 269$; 50.8%) were boys. Using International Obesity Task Force cut-offs [12], according to the BMI, 70.1% ($n = 371$) had a normal weight, 5.9% ($n = 31$) were overweight, 12.9% ($n = 68$) were underweight and none were considered obese. BMI could not be calculated for 59 subjects because of missing height and/or weight data. Mean (SD) BMI was 16.7 (1.9) kg m^{-2} in boys and 16.2 (2.0) kg m^{-2} in girls. The mean body weight (31.6 kg) was slightly higher than the values previously observed in a French population [18], although the prevalence of both obesity and excessive weight observed in the present study was lower than that reported in Europe for children of the same age [19,20].

Breakfast intake

Overall, 9.8% of the children ($n = 52$) skipped breakfast. The frequency of breakfast skipping increased with age (8.2% at 9 years of age, 11.4% at 10 years of age and 16.7% at 11 years of age).

For breakfast consumers ($n = 477$), the mean (SD) quantity of food consumed at breakfast was 442 (167) g (Table 1) and the mean energy intake was 1.86 (0.66) MJ [445 (159) kcal] (Table 1). Apart from the chocolate milk, other drinks consumed in big quantities by the children (mainly water and tea) provided no calorie at all. Thus, the contribution of liquids to the total breakfast quantity was far greater than the contribution of liquids to the breakfast total energy: approximately three-quarters of the total quantity ingested was provided by drinks, whereas breakfast energy was mainly provided by food, reflecting the fact that children consumed drinks that had low calorie contents and that they consumed energy-rich foods. Total energy intake did not significantly differ between girls and boys: 1.81 (0.63) MJ [433 (151)] kcal versus 1.91 (0.69) MJ [457 (165) kcal] ($P = 0.0995$). Energy intake from foods and drinks also did not differ between girls and boys.

Sweets were the most frequently consumed food item, consumed by 67.7% of the subjects, and they accounted for 40.1% (25.3%) of the total energy consumed for those who ate them (Table 2). Fat (mainly butter) (41.5%) and bread (46.8%) were also frequently consumed. Flavoured milk accounted for the highest number of grammes of food consumed and was consumed by almost half (48.9%) of the children. RTECs, which are not a

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Table 1 Breakfast total quantity, energy and mean adequacy ratio (MAR)

Category	Mean (SD) amount						*P*-value (girls versus boys)
	N	Overall	*N*	Girls	*N*	Boys	
Total quantity (g)	477	442 (167)	230	440 (164)	247	443 (171)	0.8479
Quantity from foods (g)	477	100 (66)	230	98 (67)	247	102 (66)	0.4701
Quantity from drinks (g)	477	341 (169)	230	342 (170)	247	341 (168)	0.9256
Energy (kcal)	477	445 (159)	230	433 (151)	247	457 (165)	0.0995
Energy from foods (kcal)	477	303 (141)	230	293 (134)	247	312 (144)	0.1389
Energy from drinks (kcal)	477	142 (80)	230	140 (82)	247	145 (77)	0.5038
MAR (%)	424	24.7 (9.4)	213	24.1 (9.9)	211	25.4 (8.9)	0.1503
Energy density from solid foods (kcal 100 g^{-1})	477	350 (100)	230	347 (98)	247	352 (102)	0.6216
Total energy density (kcal 100 g^{-1})	477	111 (51)	230	109 (57)	247	112 (45)	0.6290

P-values for girls versus boys were calculated using a general linear model.

Table 2 Consumption by food category for breakfast consumers (*N* = 477)

Food category	Frequency, *n* (%)	Amount consumed (g), mean (SD)	Percentage of total amount consumed, mean (SD)	Percentage of total energy, mean (SD)	MAR provided (%)	Mean nutrient density, % 100 $kcal^{-1}$
Bread	223 (46.8)	41.75 (21.36)	10.14 (7.24)	25.54 (11.15	2.49	2.28
Fat/oil	198 (41.5)	12.20 (4.82)	3.17 (2.84)	19.41 (8.30	1.28	1.39
Fruits/vegetables	52 (10.9)	123.94 (47.27)	26.49 (14.02)	17.96 (15.40	8.52	6.84
Juice	249 (52.2)	150.24 (32.24)	37.98 (18.96)	17.32 (8.39	5.47	7.81
Milk	126 (26.4)	280.79 (124.54)	59.14 (20.85)	31.22 (14.88	11.15	8.66
Dairy	55 (11.5)	110.81 (36.50)	33.87 (17.67)	27.03 (15.97)	4.81	5.34
Flavoured milk	233 (48.9)	324.99 (119.19)	65.28 (18.19)	42.87 (15.53)	15.51	7.82
Sweets	323 (67.7)	49.18 (41.23)	13.67 (14.21)	40.10 (25.31)	5.83	2.12
RTEC	134 (28.1)	30.45 (16.09)	8.06 (5.35	32.14 (16.38)	11.27	10.13
Soda, soft drinks	7 (1.5)	172.86 (61.84)	37.50 (18.06)	18.09 (4.81)	0.45	0.64
Tea	22 (4.61)	246.82 (92.14)	47.11 (20.34)	0.13 (0.07)	0.00	0
Water	80 (16.8)	135.63 (25.65)	32.27 (14.79)	0.00 (0.00)	0.00	0
Meat/fish/egg	4 (0.8)	72.50 (55.60)	15.26 (11.18)	19.14 (14.04)	–	–
Miscellaneous	1 (0.2)	100	16.75	43.53	–	–

MAR, mean adequacy ratio; RTEC, ready-to-eat cereal.

traditional breakfast food in France, were consumed by 28.1% of children.

Nutrient intake at breakfast

Nutritional quality as assessed by MAR was similar for boys [25.4% (8.9%)] and girls [24.1% (9.9%)] and, overall, the MAR for breakfast was 24.7% (Table 1). Energy density from solid foods and total energy density was also similar between boys and girls. On average, breakfast provided 22.9% of the daily energy requirement according to international recommendations [14] (see Supporting information, Table S1). It also provided 49.2% of recommended protein (0.9 g kg^{-1}) [with the main contributors to protein intake being flavored milk (33%), sweets (20%) and milk (15%) categories], 18.5% of fat, 28.3% of carbohydrates and 15.9% of fibre. Breakfast contributed to more than half the daily requirement of vitamin B_2 (riboflavin; 51.2%) and more than 40% of the daily requirements for protein, phosphorus, pantothenic acid and vitamin B_{12}. However, breakfast provided less than 10% of linoleic acid, α-linolenic acid, DHA, selenium and vitamin D.

The contribution of breakfast to the daily energy requirement was significantly different between boys and girls: 21.74% (8.08%) versus 24.17% (9.49%) (P = 0.0026) (see Supporting information, Table S1). Breakfast contribution to daily requirements also differed between boys and girls for energy from solids: 14.85% (6.97%) versus 16.28% (8.08%) (P = 0.0374); energy from liquids: 6.89% (3.79%) versus 7.89% (4.93%) (P = 0.0134); fat: 17.48% (9.91%) versus 19.51% (10.63%) (P = 0.0313);

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carbohydrates: 26.89% (9.42%) versus 29.83% (12.24%) ($P = 0.0033$); saturated fatty acids: 28.71% (17.45%) versus 34.47% (21.50%) ($P = 0.0014$), and sodium: 14.50 (8.12%) versus 16.27% (9.86%) ($P = 0.0322$).

The main contributor to total energy at breakfast was flavoured milk, followed by sweets and RTEC, milk and dairy (Table 2). The highest MAR was provided by flavoured milk (15.5%), RTEC (11.3%) and milk (11.2%). Although sweets contributed substantially to energy intake, they contributed less to nutrient intake (MAR = 5.8%). Fruits and vegetables accounted for 8% of recommended values and 18% of total energy. After adjustment for energy, the food categories providing the most nutrients were (in decreasing order) RTEC, milk, flavoured milk and fruits.

Dietary patterns

Four main dietary patterns were identified: 'RTEC + milk breakfast' (18.1%), which included mainly RTEC, milk, and juice; 'Sweets breakfast' (40.0%), which included mainly flavoured (i.e. chocolate) milk and sweets, especially brioche and chocolate spread, plus water or juice; 'Traditional French breakfast' (27.2%), which included mainly flavoured milk, bread, fat (especially butter) and juice; and 'Dairy and juice breakfast' (9.5%), which included mainly dairy (especially whole milk yogurt or yogurt drink), sweets and tea or juice (Table 3).

On average, energy intake was slightly higher for the 'Traditional French breakfast' [2.08 MJ (496 kcal)] and the 'Sweets breakfast' [1.84 MJ (440 kcal)] than for the other two breakfast patterns [with approximately 1.67 MJ (400 kcal) each] (Table 3). When adjusted for total energy intake at breakfast, the mean MAR was higher for the 'RTEC + milk breakfast' (30.2%), followed by the 'Sweets breakfast' (24.6%) and the 'Traditional French breakfast' (22.6%) and lower for the 'Dairy and juice breakfast' (18.0%). Nutrient content was significantly different between dietary patterns, except for linoleic acid, poly-insaturated fatty acid, manganese and vitamin E (see Supporting information, Table S2). The 'RTEC + milk' breakfast provided more vitamins and minerals and lower

Table 3 Main breakfast patterns and compositions in breakfast consumers ($n = 474$)

Composition/category	RTEC + milk breakfast $N = 86$	Sweets breakfast $N = 214$	Traditional French breakfast $N = 129$	Dairy and juice breakfast $N = 45$	*P*-value
Nutritional quality, mean (SD)					
MAR (%)	30.2 (8.0)	24.6 (9.1)	22.6 (8.7)	18.0 (8.3)	0.001
Solid energy density (kcal 100 g^{-1})	359 (89)	380 (81)	344 (97)	207 (94)	0.001
Total energy density (kcal 100 g^{-1})	92 (28)	116 (58)	114 (47)	112 (51)	0.001
Total quantity (g)	456 (163)	426 (171)	471 (163)	391 (149)	0.013
Quantity from foods (g)	73 (51)	84 (55)	117 (69)	174 (61)	0.001
Quantity from drinks (g)	382 (150)	342 (169)	354 (160)	217 (175)	0.001
Energy (kcal)	395 (125)	440 (165)	496 (151)	399 (152)	0.001
Energy from foods (kcal)	227 (99)	294 (151)	351 (120)	332 (134)	0.001
Energy from drinks (kcal)	168 (65)	146 (81)	145 (79)	68 (55)	0.001
Amount consumed by food group (g), mean (SD)					
Bread	11 (18)	7 (13)	49 (24)	13 (21)	0.001
Fats	1.9 (4.4)	5.5 (4.9)	12.2 (6.0)	3.2 (5.3)	0.001
Fruits and vegetables	13 (40)	10 (37)	21 (52)	12 (34)	0.142
Juice	93 (76)	77 (76)	63 (82)	99 (77)	0.011
Milk	256 (163)	42 (110)	14 (52)	39 (82)	0.001
Dairy	5.3 (22.8)	0.4 (3.5)	8.5 (31.0)	98.4 (54.2)	0.001
Flavoured milk	8 (34)	203 (184)	240 (181)	11 (40)	0.001
Sweets	12 (19)	51 (48)	16 (22)	41 (38)	0.001
RTEC	29.2 (13.6)	4.6 (10.5)	1.71 (6.1)	3.3 (8.0)	0.001
Soda, soft drinks	6.5 (29.9)	0.7 (10.3)	3.9 (31.6)	0.0 (0.0)	0.146
Tea	1.4 (12.9)	5.3 (36.9)	18.1 (69.4)	40.9 (103.7)	0.001
Water	365 (143)	330 (151)	363 (141)	307 (136)	0.033
Meats, fish, eggs	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	3.1 (12.6)	NC

MAR, mean adequacy ratio; RTEC, ready-to-eat cereal.

The four most frequent breakfast patterns were determined by principal component analysis. Three subjects considered as outliers were removed from the principal component analysis to obtain homogeneous and robust dietary patterns. *P*-values were calculated using a general linear model with food group as the dependant variable and the dietary pattern variable as the independent variable. NC, *P*-value could not be calculated.

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cholesterol and saturated fatty acid compared to other dietary patterns. However, intakes of essential fatty acids were lower in the 'RTEC + milk' breakfast versus the others. Compared to the 'RTEC + milk' breakfast, the 'Traditional French breakfast' was especially higher in fats, fibres, saturated fatty acids, cholesterol, copper and vitamin A (retinol and β-carotene), and lower in folate, vitamin C and iron. Total energy density was lower for the 'RTEC + milk breakfast' but not significantly different from the 'Dairy and juice breakfast'.

Discussion

The present study provided a snapshot of a single breakfast in over 500 schoolchildren aged 9–11 years in France. Data were collected as part of a previous study examining fluid intake at breakfast [11]. The sample included a similar number of girls and boys, and the majority of children were 9 –10 years of age because they were recruited in primary schools. Only 10% of the children skipped breakfast, which is less than in most developed countries [13,21,22]. However, we observed a trend of declining breakfast with age. Skipping breakfast is becoming more and more prevalent in many developed countries, with 10–30% children and adolescents having lunch or even dinner as the first meal of the day [23].

Almost half of the children (48.6%) consumed a breakfast that included cereals, dairy and fruit/fruit juice in accordance with the recommendations of France's *Programme National Nutrition Santé* (National Health Nutrition Programme). All children consumed at least one beverage. Most breakfast consumers (83%) consumed milk, flavoured milk, a dairy product or a combination of them. As a beverage, flavoured milk has a lower energy density than solid foods. It contributes to several nutrient recommendations without providing much energy. More than half of breakfast consumers (58%) consumed fruit juice and 11% consumed fruits. Bread was consumed by 47% of breakfast consumers and RTEC by 28%. The amount of RTEC consumed (30.45 g) is very close to the portion recommended (30 g). This rather high percentage is interesting because RTEC are not traditionally consumed in France, and are not, as such, a recommendation of the *Programme National Nutrition Santé*.

The present study showed that breakfast provided, on average, 22.9% of the recommended daily energy intake. This is slightly higher than in the Stanislas family study [9] but similar to the 15–25% medium interval observed in France in 1999 [10]. Flavoured milk was the main contributor to energy intake, followed by sweets, RTEC, milk, dairy, bread, fat/oil, fruits/vegetables and juice, which is consistent with them being amongst the most consumed breakfast items in terms of grammes and energy.

The study also showed a MAR of breakfast of 24.7%, indicating that, on average, breakfast provided a substantial amount of the daily recommended intakes for 23 key nutrients. When adjusted for energy, the foods contributing most to total nutrient intake were (in decreasing order) RTEC, milk, flavoured milk and fruits. Average intake of protein, carbohydrates, most minerals (e.g. calcium, magnesium, phosphorus and potassium) and vitamins (e.g. vitamin B_2 and pantothenic acid) were sufficient. Intake was below national recommendations for vitamin E, vitamin B_{12}, vitamin D, eicosapentaenoic acid (EPA)/DHA, essential fatty acids, copper, zinc, selenium and fibre, whereas intake of saturated fatty acids was in excess of recommendations. Because other meals may not provide sufficient nutrients to reach adequate intake, substituting foods currently consumed at breakfast with others of nutritional content may be necessary. For example, consuming bakery products or RTEC containing whole grains or whole wheat flour may help to provide adequate fibre.

In terms of total grammes consumed, the largest contributor to breakfast was flavoured milk, which was consumed by just over half (50.5%) of the children. For these children, milk provided 43% of their energy intake. In addition, the highest MAR was provided by flavoured milk (15.5%) and the third highest was from plain milk (11.2%). This agrees with the French Individual National Food Consumption Survey 2 in 2005–2007, which found that, in children, milk is a strong contributor of several nutrients, especially calcium, iodine, phosphorus, potassium, zinc, retinol and vitamins B_2, B_{12}, B_5 and D [24].

We identified two dominant breakfast patterns: the 'Sweets breakfast', eaten by 45% of children and included mainly flavoured (i.e. chocolate) milk, fruit juice and a Danish biscuit or pastry; and the 'Traditional French breakfast', eaten by 27% of children and included bread, butter or margarine, and flavoured milk. The 'RTEC + milk' breakfast pattern, which consisted of RTEC, milk and fruit juice, was the most nutrient-dense but was eaten by only 18% of children. This suggests that, although the traditional French breakfast (bread and butter) is still common, new patterns are emerging, including the consumption of RTEC.

Nutritionally, the 'RTEC + milk' pattern was the most advantageous pattern because it was low in total fat, saturated fatty acids and cholesterol, and was rich in fluids, vitamins B, vitamin C, calcium and iron. The 'Dairy and juice breakfast' did not provide sufficient calcium, protein or water. This may be a result of the fact that, in France, by portion, dairy products have lower contents of these nutrients than milk.

The present study has some limitations: fluid and food intake were estimated from a self-reported questionnaire,

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which could have led to inaccuracies in portion size, as well as height and weight. Second, the classification we adopted was aimed essentially at defining the various breakfast patterns. To obtain even more information on the nutritional quality of foods eaten at breakfast, a classification with further groups would be useful. For example, the sweets category could be subdivided into pastries (bringing complex and simple carbohydrates, as well as fats), sugar/jam (bringing simple carbohydrates) and chocolate spreads (bringing simple carbohydrates and fats).

The present study is the first report on breakfast composition in French children. Based on a large sample size, it provides insights about the breakfast habits in school children and opens new perspectives in terms of nutritional education and public policies. Based on our results, some recommendations can be made to optimise the breakfast composition and the formula of breakfast food products and drinks. For example, the breakfast composition of French children included all food and drink categories (one portion of cereal, one dairy product and one drink) recommended in the *Programme National Nutrition Santé* except the fruit/100% fruit juice category. In all dietary patterns identified in the present study, the consumption of fresh fruit was low. Children preferred fruit juices as observed in the 'RTEC + milk' and 'Dairy and juice' patterns. However, this trend was not observed in all dietary patterns: fruit juice consumption was low in the 'Sweets' and 'Traditional French' patterns. In addition, insufficient intake of some micronutrients including copper, zinc, selenium and vitamin B_{12} and vitamin D, as well as EPA/DHA, has been observed. Fortification of food and drink products may provide a solution to compensate for these deficiencies. Fibre intake is also below recommendations. This could be improved by encouraging the consumption of RTEC (with an adequate amount of salt and sugar) or breakfast bakery products containing cereals and/or whole flour. Finally, the consumption of margarine with omega 6/omega 3/vitamin E and partially skimmed milk could be encouraged to reduce the intake of saturated fatty acids.

Conclusions

The present study investigating breakfast consumption by French schoolchildren produced globally encouraging results: only 10% of children skipped breakfast, breakfast composition and energy intake were close to national recommendations, and breakfast provided a substantial amount of the daily recommended intake for 23 key nutrients. To optimise the breakfast composition further for children, an increase in the intake of some micronutrients (copper, zinc, selenium, vitamin B_{12}, vitamin D, EPA/DHA) is recommended, possibly via food and drink fortification, as well as an increase in the intake of fibres by encouraging the consumption of fruits and whole grain products.

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Conflict of interest, source of funding and authorship

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FB, EML, MM and FV conceived or designed the study. FB, EML, MM and FV collected or analysed data. FB, MV, EML, MM and FV interpreted the results of the study. FB, MV, EML, MM and FV contributed to writing the manuscript. FB, MV, EML, MM and FV approved the final version of 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:
Table S1. Intake of nutrients as a percentage of the daily recommended values for breakfast consumers.
Table S2. Mean (SD) nutrient intakes by dietary pattern.

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MOLECULAR NUTRITION

Polymorphism of neuropeptide Y gene rs16147 modifies the response to a hypocaloric diet on cardiovascular risk biomarkers and adipokines

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Keywords
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Abstract

Background: The main genetic variant described in *NPY* gene is rs16147 (G-399A) and it is located within the promoter region upstream of the gene for neropeptide Y (NPY). We evaluate the effects of the *rs16147 NPY* gene polymorphism on metabolic changes secondary to weight loss after 3 months of a hypocaloric diet in adult obese patients.
Methods: A population of 82 obese patients was analysed in an interventional design of one arm. Before and after 3 months on a hypocaloric diet, an anthropometric evaluation, an assessment of nutritional intake and a biochemical analysis were performed. The statistical analysis was performed for combined *GA* and *AA* as a group (minor allele group) and *GG* as second group (major allele group) (dominant model).
Results: In *A* allele carriers, the mean (SD) decrease in weight was −2.8 (2.2) kg [decrease in non *A* allele carriers −2.6 (1.1) kg, $P > 0.05$), body mass index was −1.2 (0.6) kg m^{-2} [decrease in non *A* allele carriers −1.1 (0.8) kg m^{-2}, $P > 0.05$], fat mass was −1.7 (1.4) kg [decrease in non *A* allele carriers −1.9 (1.3) kg, $P > 0.05$], waist circumference was −5.5 (3.4) cm [decrease in non *A* allele carriers −3.7 (4.1) cm, $P = 0.006$], C-reactive protein (CRP) was −0.7 (0.6) mg dL^{-1} [decrease in non *A* allele carriers −0.1 (0.3) mg dL^{-1}, $P = 0.02$], insulin was −1.5 (0.4) mUI L^{-1} [decrease in non A allele carriers −0.8 (2.0) mUI L^{-1}, $P = 0.001$] and homeostasis model assessment-insulin resistance (HOMA-IR) was −0.4 (0.5) [decrease in non *A* allele carriers −0.2 (0.1), $P = 0.005$]. interleukin (IL)-6 changes were significant in *A* allele carriers [−0.7 (0.2) pg mL^{-1}] versus non *A* allele carriers [−0.1 (0.3) pg mL^{-1}] ($P = 0.01$).
Conclusions: We found that the rs164147 genotype affected the reduction of waist circumference, HOMA-IR, insulin, CRP and IL-6 levels in response to weight loss diet in obese subjects.

Introduction

Neuropeptide Y (NPY) is expressed in the nervous system (central and peripheral) and is a 36-amino acid peptide neurotransmitter [(1)]. Numerous functions have been attributed to NPY, including those related to circadian rhythms, appetite, vascular resistance and sexual function [(2)]. Additionally, increased NPY signalling in the central nervous system (hypothalamus) contributes to the development of obesity, diabetes mellitus type 2 and cardiovascular disease [(3–5)].

The human *NPY* gene is located on chromosome 7p15.1 and it has four exons. Single nucleotide polymorphisms (SNP) are genetic variants of one nucleotide and these changes could have functional implications. The main genetic variant described in this gene is rs16147

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(G-399A) and it is located within the promoter region upstream of the NPY gene [6]. This genetic variant has been shown to be related to serum levels of NPY [7] and is responsible for more than half of the variation in the expression of NPY *in vivo* [8]. The association between NPY rs16147 SNP and obesity and its related phenotypes has not been studied extensively and few studies have been conducted [9]. Moreover, adipose tissue is recognised as an endocrine organ that is associated with the extensive production of several proteins, called adipokines, which are produced by adipose tissue (e.g. leptin, adiponectin, resistin and so on).

The effect of diet on human health and disease is affected by different genetic backgrounds [10,11]. As far as we know, few studies have been designed aiming to explore the effect of rs16147 on metabolic response and weight change after a dietary intervention. Crescenti *et al.* [12] reported that polymorphisms in *NPY* genes potentiate the response to *plantago ovata* husk in C-reactive protein (CRP) plasma concentration and systolic blood pressure. In another study [13], *NPY rs16147* genotypes were found to affect the change in abdominal adiposity in response to dietary intervention.

Given the limited information about the relationship of this SNP with metabolic response after dietary intervention, in the present study, we evaluate the effects of the *rs16147 NPY* gene polymorphism on metabolic changes secondary to weight loss after 3 months of a hypocaloric low-fat diet in adult obese patients without diabetes mellitus.

Materials and methods

Subjects

The study population (Caucasian) included 82 adult, obese, nondiabetic outpatients, who were aged more than 30 years. All subjects were enrolled in a prospective way in an interventional design comprising one arm. Exclusion criteria included a history of cardiovascular disease during the previous 24 months, total cholesterol >200 mg dL^{-1}, triglycerides >250 mg dL^{-1}, blood pressure >140/90 mmHg and fasting plasma glucose >126 mg dL^{-1}, as well as the use of metformin, sulphonilurea, glifozins, dypeptidil type IV inhibitors drugs, thiazolidinedions, insulin, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs.

All participants provided their informed consent with respect to a protocol that was approved by the local ethical review boards and were recruited in the Department of Endocrinology and Nutrition.

Procedures

A fasting blood sample was taken at baseline and at week 12 to measure all parameters. The biomarkers measured were: basal fasting glucose, CRP, insulin, homeostasis model assessment-insulin resistance (HOMA-IR), total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, plasma triglycerides concentration and adipokines levels (leptin, adiponectin, resistin). The genotype of the *NPY* gene was studied using rs16147 from DNA extracted from blood cells.

A bioimpedance analysis was performed to measure fat mass. Weight, height, and blood pressure measures were measured within the start of the trail and repeated 3 months of intervention. These measures were performed at same time of the day (morning).

Analytical procedures

Peripheral blood was obtained from the subjects in fasting state of 12 h. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0–7 mg dL^{-1}) and analytical sensivity 0.5 mg dL^{-1}. Insulin was determined by a radioimmunoassay (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI L^{-1} (normal range 0.5–30 mUI L^{-1}) [14] and plasma glucose levels were determined using an automated glucose oxidase method (Glucose Analyser 2; Beckman Instruments, Fullerton, CA, USA). The HOMA-IR was calculated using these values [15]. Triglyceride and serum total cholesterol were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd, New York, NY, USA), whereas HDL-cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL-cholesterol was calculated using the Friedewald formula [16].

Adiponectin was measured by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.246 ng mL^{-1} and a normal range of 8.65–21.43 ng mL^{-1} [17]. Resistin was measured by an ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng mL^{-1} and a normal range of 4–12 ng mL^{-1} [18]. Leptin was measured by an ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng mL^{-1} and a normal range of 10–100 ng mL^{-1} [19]. Interleukin (IL)-6 and tumou necrosis factor (TNF)-α were measured by an ELISA (R&D Systems, Inc.) with a sensitivity of 0.7 pg mL^{-1} and 0.5 pg mL^{-1}, respectively. The normal value for IL-6 was in the range 1.12–12.5 pg mL^{-1} and that for TNF-α was in the range 0.5–15.6 pg mL^{-1}.

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Genotyping of NPY gene polymorphism

Genomic DNA from each subject was isolated from peripheral blood leucocytes using a commercial kit extraction (Bio-Rad, Hercules, CA, USA). Primers were designed with the SEQUENOM ASSAY DESIGN, version 4 (Sequenom, Inc., San Diego, CA, USA). Genotyping for the rs16147 polymorphism was performed by chain reaction real-time analysis. This polymerase chain reaction (PCR) was carried out with 20–25 ng of genomic DNA and 0.1–0.15 μL each of oligonucleotide primer for rs16147 (primer forward: 5′- ACGTTGGATGCACAAAGA GGATTCAGGTGC -3′ and reverse 5′- ACGTTGGATGAG CCCAGACGATTCTTGTC -3′ in a 2-μL final volume (Termociclador Lifetecnologies, Los Angeles, CA, USA). DNA was denatured at 85 °C for 5 min; this was followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing at 58.1 °C for 45 s). The PCR was run in a 2-μL final volume containing 0.1 μL of iPLEx Termination mix (Bio-Rad) with hot start Taq DNA polymerase. Hardy–Weinberg equilibrium was calculated via a chi-squared statistical test.

Anthropometric procedures and blood pressure

Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences were measured to derive the waist-to-hip ratio (WHR). Body weight was measured to an accuracy of 0.1 kg and body mass index (BMI) was computed as body weight height^{-2}. Bioimpedance was used to determine the body composition with an accuracy of 5 g [20] (EFG, Akern, Italy). Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer (Omrom, Scarborough, ON, Canada), and the readings were averaged.

Dietary intervention

The dietary program during 3 months consisted of a hypocaloric low-fat diet (1520 calories per day), the distribution of macronutrient was 52% of carbohydrates, 25% of lipids and 23% of proteins. The distribution of fats was 50.7% of monounsaturated fats, 38.5% of saturated fats and 11.8% of polyunsaturated fats. All enrolled subjects received instruction to record their daily dietary intake for 3 days, including a weekend day, and all participants were screened for potential causes of noncompliance. This adherence was assessed each 14 days with a telephone call by a dietitian to improve compliance with the energy restriction and macronutrient distribution. Records were reviewed by a dietitian and analysed with a computer-based data evaluation system. National composition food tables were used as reference [21]. The exercise activity allowed was aerobic exercise at least three times per week (60 min each) and this was recorded by the patient using a self-reported questionnaire.

Statistical analysis

All data were analysed using SPSS, version 15.0 (SPSS Inc. Chicago, IL, USA). The sample size was calculated to detect differences of more than 3 kg in body weight loss with 90% power and 5% significance ($n = 80$). There were no dropouts in the study. The Kolmogorov–Smirnov test was used to determine variable distribution. The results are expressed as the mean (SD). The statistical differences in genotype distribution and allele frequencies between groups and analysis of deviation from the Hardy–Weinberg equilibrium were assess using chi-squared or Fisher's exact tests. Other variables were analysed by analysis of variance (for normally-distributed variable) or a Kruskal–Wallis test (for non-normally-distributed variable). The statistical analysis was performed for a dominant model. $P < 0.05$ was considered statistically significant.

Results

Eighty-two patients were enrolled in the present study. All patients completed the 3-month follow-up period without dropouts. The variant of *NPY* gene was in Hardy–Weinberg equilibrium ($P = 0.23$). The mean (SD) age was 48.3 (10.2) years and the mean BMI was 36.7 (5.4), with 20 males (24.4%) and 62 females (75.6%). Twenty-three patients (28%) had the genotype *GG* (major allele group) and 59 (72%) patients had the next genotypes; *GA* (46 patients; 56.1%) or *AA* (13 patients; 15.9%) (minor allele group).

After 3 months of intervention, all patients achieved dietary recommendations in both allele groups without statistical differences: calorie intake (major allele group: 62.27 (12.46) MJ day^{-1} [1488.1 (297.9) kcal day^{-1}] versus minor allele group: 59.81 (10.13) MJ day^{-1} [1429.4 (242.1) kcal day^{-1}]). The distribution of macronutrient intakes was: (major allele group: 49.9% from carbohydrates versus 51.0% minor allele group), [major allele group: 25.0% from fats (50.3% of monounsaturated fats, 38.1% of saturated fats and 12.6% of polyunsaturated fats) versus 24.7% minor allele group (50.2% of monounsaturated fats, 39.1% of saturated fats and 11.7% of polyunsaturated fats)] and (major allele group: 24.1% from proteins versus 24.3% minor allele group). Exercise was also similar in both groups [166.1 (39.2) min week^{-1} versus 164.6 (33.2) min week^{-1}].

The sex and age distribution was similar in both allele groups: age [major allele group: 48.9 (9.8) years versus

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minor allele group: 48.6 (9.0) years: not significant]. Sex distribution was similar in both groups (major versus minor allele), males (30.4% versus 23.9%) and females (69.6% versus 76.1%). Anthropometric characteristics of participants at baseline and at month 3 of the intervention are shown in Table 1. After dietary treatment and in both allele groups, weight, BMI, fat mass and waist circumference decreased with statistical differences. In *A* allele carriers, the mean (SD) decrease in weight was −2.6 (2.2) kg [decrease in non *A* allele carriers −2.8 (1.1) kg, $P > 0.05$], BMI −1.1 (0.6) kg m^{-2} [decrease in non *A* allele carriers −1.2 (0.8) kg m^{-2}, $P > 0.05$], fat mass −1.7 (1.4) kg [decrease in non *A* allele carriers −1.9 (1.3) kg, $P > 0.05$] and waist circumference −5.5 (3.4) cm [decrease in non *A* allele carriers −3.7 (4.1) cm, $P = 0.006$]. No differences were detected among other variables. No differences were detected among basal and post-treatment values of variables between both genotypes.

Table 2 shows parameters of glucose metabolism and lipid profile. No differences were detected among basal and post-treatment values of variables between both allele groups. In *A* allele carriers, insulin, HOMA-IR and CRP levels decreased significantly. In non *A* allele carriers and after dietary treatment, biochemical changes did not reached statistical differences. In *A* allele carriers, the improvement in metabolic parameters was statistically significant as : CRP −0.7 (0.6) mg dL^{-1} [decrease in non *A* allele carriers −0.1 (0.3) mg dL^{-1}, $P = 0.02$], insulin −1.5 (0.4) mUI L^{-1} [decrease in no *A* allele carriers −0.8 (2.0) mUI L^{-1}, $P = 0.001$] and HOMA-IR −0.4 (0.5) [decrease in non *A* allele carriers −0.2 (0.1), $P = 0.005$].

Table 3 shows levels of adipokines and cytokines. Leptin levels decreased in both genotypes after dietary treatment [−11.1 (1.5) ng dL^{-1} in non *A* allele carriers versus −15.2 (1.2) ng dL^{-1} in *A* allele carriers, $P > 0.05$]. IL-6 changes were significant in *A* allele carriers [−0.7 (0.2) pg mL^{-1}] versus non *A* allele carriers [−0.1 (0.3) pg mL^{-1}] ($P = 0.01$). Other adipokines (resistin and adiponectin) and TNF-α remained unchanged in both groups. No differences were detected among basal and post-treatment values of adipokines between both genotypes.

Discussion

In the present study, we found that the *NPY* gene rs16147 SNP was related to the change in insulin levels, HOMA-IR, CRP, IL-6 and waist circumference at 3 months after a hypocaloric low-fat diet.

The *NPY* gene is highly polymorphic and some SNPs of this gene have been associated with obesity [9,22,23]. It has been reported that this SNP (rs16147) is a result of the loss of a trascriptional factor (Sp1) binding consensus by substitution G to A [24]. However, other studies [25] reported that the effect of the rs16147 polymorphism on NPY expression might follow the interaction of G/A allele with other regulatory genomic DNA regions or the involvement of transcriptional factors other than Sp1.

The relationship detected between the *NPY* rs16147 polymorphism and the effect of a low fat hypocaloric diet on CRP and IL-6 levels is reported by Crescenti *et al.* [12], who demonstrated a reduction of CRP in subjects with the AA genotype after 14 g day^{-1} of soluble fibre in the form of *plantago ovata* husk. This intervention with fibre added a low-fat diet for 8 weeks. The present study showed the same reduction of CRP levels after a low fat hypocaloric diet during 12 weeks and, as an added result, we observed the decrease of serum levels of IL-6. CRP and IL-6 are two sensitive markers for systemic inflammation, and the evidence suggests that NPY, via

Table 1 Changes in anthropometric variables [mean (SD)]

	rs16147			
	GG		GA+AA	
Characteristics	Basal	3 months	Basal	3 months
BMI	36.7 (5.9)	35.5 (5.1)*	36.8 (6.0)	35.6 (5.0)*
Weight (kg)	90.3 (14.6)	87.7 (13.7)*	90.1 (15.1)	87.3 (12.1)*
Fat mass (kg)	40.7 (12.1)	38.8 (9.2)*	39.8 (10.1)	38.1 (12.0)*
WC (cm)	107.9 (8.1)	104.2 (7.1)*	107.7 (10.1)	102.2 (9.6)*
Waist to hip ratio	1.01 (0.7)	1.00 (0.6)	0.99 (0.7)	0.98 (0.5)
SBP (mmHg)	131.5 (11.8)	127.8 (9.1)	132.4 (14.6)	128.6 (9.9)
DBP (mmHg)	84.4 (4.2)	82.1 (4.9)	83.9 (6.0)	82.1 (5.3)

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.
*$P < 0.05$ in each genotype group.
No statistical differences between genotype groups.

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Table 2 Biochemical parameters [mean (SD)]

	Rs16174			
	GG		GA+AA	
Characteristics	Basal	3 months	Basal	3 months
Glucose (mg dL^{-1})	100.1 (12.1)	99.9 (8.1)	99.1 (11.1)	101.3 (10.9)
Total cholesterol (mg dL^{-1})	200.5 (21.8)	193.7 (22.1)	201.1 (20.7)	195.9 (23.2)
LDL-cholesterol (mg dL^{-1})	126.7 (20.1)	124.8 (27.1)	123.2 (20.1)	120.1 (19.4)
HDL-cholesterol (mg dL^{-1})	45.6 (8.1)	46.2 (4.4)	47.1 (11.0)	48.5 (10.8)
Triglycerides (mg dL^{-1})	153.7 (28.1)	150.4 (21.4)	148.2 (31.6)	137.4 (41.1)
CRP (ng dL^{-1})	4.8 (1.2)	4.7 (1.5)	5.3 (1.7)	4.6 (1.1)*
Insulin (mUI L^{-1})	13.6 (10.1)	12.8 (8.6)	13.5 (6.8)	12.0 (7.2)*
HOMA-IR	3.4 (1.5)	3.2 (1.6)	3.5 (1.9)	3.1 (1.4)*

CRP, C-resctive protein; HOMA-IR, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*$P < 0.05$, in each genotype group.
No statistical differences between genotype groups.

Table 3 Adipokines and cytokine levels [mean (SD)]

	Rs16174			
	GG		GA+AA	
Characteristics	Basal	3 months	Basal	3 months
Resistin (ng dL^{-1})	4.2 (1.1)	4.1 (1.2)	4.1 (1.5)	4.0 (1.9)
Adiponectin (ng dL^{-1})	24.2 (9.4)	23.4 (6.5)	27.8 (12.1)	25.1 (10.3)
Leptin (ng dL^{-1})	83.9 (21.4)	72.5 (20.5)*	78.1 (19.4)	63.5 (20.1)*
IL-6 (pg dL^{-1})	1.3 (2.1)	1.4 (0.7)	1.3 (0.9)	0.6 (0.9)*
TNF-α (pg dL^{-1})	6.4 (1.4)	6.7 (1.5)	6.9 (3.1)	6.8 (2.1)

HOMA-IR, homeostasis model assessment; IL, interleukin; TNF, tumour nnecrosis factor.
*$P < 0.05$, in each genotype group.
No statistical differences between genotype groups.

regulation of Y1-receptor expression, plays an important role in inflammation [26]. Other studies suggest that NPY can influence the release and synthesis of pro-inflammatory cytokines [27].

In the present study, we identified an association of rs16147 SNP with insulin and HOMA-IR changes after weight loss. Our results show that the *A* allele influence insulin levels and HOMA-IR after weight loss. Some studies [9] have reported a relationship of rs14167 SNP and the risk of the metabolic syndrome. The metabolic syndrome is characterised by a group of biochemical and clinical features that include central adiposity, insulin resistance, impaired glucose tolerance, dyslipaemia and hypertension. Perhaps the presence of the *A* allele produces an alteration in the release and synthesis of insulin levels and also insulin resistance. Interaction with other genetic variants of this gene and environmental factors may also explain this relationship.

The relationship between this SNP and obesity parameters has been found in other studies but with contradictory results. Mutschler *et al.* [28] showed that the SNP rs16147 is significantly associated with the WHR. Zain *et al.* [29] reported that minor alleles of rs16147 and rs16139 are associated with an increased risk of obesity, whereas a minor allele of rs5574 is associated with a reduced risk of obesity in a meta-analysis of 942 children. Olza *et al.* [30] showed similar results with rs16147 in a paediatric population. Moreover, no significant associations were reported by other studies conducted in adult populations [31]. Our results indicate that low-fat hypocaloric diets might be of more benefit in those with the *A* allele. This difference in response to a low-fat hypocaloric diet has also been described by Zang *et al.* [32] but, in this case, with respect to lowering systolic blood pressure in prehypertensive patients. It has been postulated that NPY can regulate white adipose tissue metabolism via the nerve endings located in adipose tissue [33]. Leptin, produced from white adipose tissue, then forms a feedback loop with NPY and informs the brain of body fat levels [34]. In a previous study [13], subjects on a high-fat weight

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loss diet showed more effectiveness in the reduction of central adiposity compared to those on a low-fat diet. Finally, there is evidence that dietary fat may regulate NPY gene expression [35], supporting the potential interactions between NPY genotypes and dietary fat in modifying body adiposity.

Our study has some limitations. First, the data should be interpreted and generalised with caution because the diet was calorically restricted and all participants were obese. Second, we did not investigate whether r16147 influences NPY levels. Third, we only analysed one SNP of the *NPY* gene, and so other low frequency genetic variants could also be related to adipose tissue. Finally, the analysis of the measures of fat distribution with waist circumference is a central indirect parameter distribution of fat and further studies with imaging techniques are required.

In conclusion, we report that the rs164147 genotype affected the reduction of waist circumference, HOMA-IR, insulin, CRP and IL-6 levels in response to weight loss diet in obese subjects. Further studies are needed to clarify this area, as well as its the clinical and therapeutic implications.

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 guidelines.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interests.
No funding declared.
DA de L write the article and performed the statistical analysis. RA, OI and B de la F carried out the anthropometric evaluation. DP performed the biochemical evaluation. ER wrote the article. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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MOLECULAR NUTRITION

Green tea extract and catechol-O-methyltransferase genotype modify the post-prandial serum insulin response in a randomised trial of overweight and obese post-menopausal women

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Keywords
green tea, insulin sensitivity, obesity, post-menopausal women, post-prandial response, satiety.

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Abstract

Background: Green tea extract (GTE) may be involved in a favourable post-prandial response to high-carbohydrate meals. The catechol-O-methyltransferase (COMT) genotype may modify these effects. We examined the acute effects of GTE supplementation on the post-prandial response to a high-carbohydrate meal by assessing appetite-associated hormones and glucose homeostasis marker concentrations in women who consumed 843 mg of (−)-epigallocatechin-3-gallate (EGCG) or placebo capsules for 11–12 months.
Methods: Sixty Caucasian post-menopausal women (body mass index $\geq$ 25.0 kg m^{-2}) were included in a randomised, double-blind feeding study. GTE was consumed with a breakfast meal [2784.0 kJ (665.4 kcal); 67.2% carbohydrate]. Blood samples were drawn pre-meal, post-meal, and every 30 min for 4 h. Participants completed six satiety questionnaires.
Results: Plasma leptin, ghrelin and adiponectin did not differ between GTE and placebo at any time point; *COMT* genotype did not modify these results. Participants randomised to GTE with the high-activity form of COMT (GTE-high COMT) had higher insulin concentrations at time 0, 0.5 and 1.0 h post-meal compared to all COMT groups randomised to placebo. Insulin remained higher in the GTE-high COMT group at 1.5, 2.0 and 2.5 h compared to Placebo-low COMT ($P < 0.02$). GTE-high COMT had higher insulin concentrations at times 0, 0.5, 1.0, 1.5 and 2.0 h compared to the GTE-low COMT ($P \leq 0.04$). Area under the curve measurements of satiety did not differ between GTE and placebo.
Conclusions: GTE supplementation and *COMT* genotype did not alter acute post-prandial responses of leptin, ghrelin, adiponectin or satiety, although it may be involved in post-meal insulinaemic response of overweight and obese post-menopausal women.

Introduction

Being overweight and obesity are major public health concerns, with worldwide obesity rates having doubled since 1980 [(1)]. Obesity is associated with several serious health conditions, including cardiovascular disease, diabetes and select cancers, such as post-menopausal breast cancer [(2–4)]. Studies have suggested that green tea and its high content of polyphenolic catechins may reduce risk for these diseases in part via beneficial effects on body

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weight because green tea consumption has been shown to modestly reduce body weight and adiposity in many randomised controlled trials (RCTs) and one meta-analysis [5–8]. Among the catechins present in green tea, the four most prominent types are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and (−)-epigallocatechin-3-gallate (EGCG). EGCG has been the most widely studied and is considered to be the most bioactive catechin [9]. However, these results have not been consistent across varying study designs and populations because several RCTs and one Cochrane review have shown no clinically meaningful weight loss or weight maintenance effects with green tea supplementation [7,10,11].

Numerous mechanisms have been proposed for the anti-obesity effects of green tea catechins (GTC), including increases in β-oxidation and thermogenesis [12–14], as well as reductions in adipocyte differentiation and proliferation, lipogenesis and nutrient absorption [9,15–17], as demonstrated in *in vitro*, animal and human experiments. Another mechanism by which GTC may reduce body weight or promote weight maintenance is by modifying several hormones associated with energy balance, the post-prandial glycaemic response and satiety. In humans, the gut-derived hormone ghrelin, as well as adipose-derived adiponectin and leptin, have been shown to be involved in appetite cues and satiety after the ingestion of a meal, respectively. Insulin has also been associated with energy balance [18], and several RCTs have demonstrated increased insulin sensitivity with the consumption of GTC [19–21]. However, few human studies examining the immediate post-prandial effects of GTC on these hormones have been conducted [22,23]. To date, the results are inconclusive, with one study finding no differences in the area under the curve (AUC) for glucose and insulin after meal consumption, although higher satiety was reported among participants [22], whereas another single-arm study did not provide a control group to enable a comparison of the results [23].

The proposed beneficial effects of GTC on body weight and adiposity may be further modulated by the catechol-O-methyltransferase (COMT) enzyme, which is one of the main enzymes responsible for catechin degradation as well as metabolism of catecholamines, including norepinephrine. The gene encoding COMT is polymorphic and its alleles correspond to different activity levels of the enzyme: A/A = homozygous low-activity; A/G = heterozygous intermediate-activity; and G/G = homozygous high-activity. An amino acid change from valine to methionine at codon 108/158 reduces the thermostability of the enzyme and lowers its enzymatic activity by 66–75% [24,25]. These differences in activity are considered to affect individual variation in metabolism of GTC, thus potentially influencing the biological effects of green tea consumption on weight loss and weight control. In addition, GTC have been shown to inhibit the action of COMT *in vitro*, which is significant given that COMT is also responsible for the metabolism of norepinephrine, a potent sympathetic nervous system stimulant. Reduced activity of COMT may prolong the effects of norepinephrine with respect to increasing thermogenesis and satiety [26,27].

The primary aim of the present study was to determine the effect of a decaffeinated green tea extract (GTE) containing 1315 mg total catechins day^{-1} (843 mg as EGCG) on post-prandial concentrations of appetite-related hormones and blood glucose in overweight and obese post-menopausal women. The secondary aims were to assess the effects of GTE on satiety and to evaluate the modification of these effects by *COMT* genotype. We hypothesised that GTE supplementation would cause favourable changes in hormone concentrations and increase post-prandial satiety, and that women with the low-activity genotype (A/A) would have a more favourable response to GTE consumption compared to those with the intermediate- (A/G) or high-activity (G/G) *COMT* genotypes, as a result of greater exposure to GTC.

Materials and methods

Study design

This ancillary study was conducted in a subset of overweight and obese women who were enrolled in the Minnesota Green Tea Trial (MGTT), a phase II, randomised, double blind, placebo-controlled, intervention study that has been described in detail elsewhere [28]. In the parent study, healthy post-menopausal women at high-risk of breast cancer as a result of increased mammographic density were randomised by *COMT* genotype to consume either four decaffeinated (<16 mg caffeine day^{-1}) GTE capsules containing a mean (SD) total of 1315 (116) mg GTC [843 (44) mg as EGCG] or placebo capsules daily for 12 months to determine the effects of GTE exposure on several breast cancer biomarkers, including mammographic density, reproductive hormones, oxidative stress and insulin-like growth factor axis proteins.

To evaluate the acute post-prandial effects of GTE consumption following a standardised meal, 60 subjects (10 from each treatment/genotype group) were invited to participate during months 11 or 12 of the 12-month parent study. This research took place during a half-day clinic visit at the University of Minnesota's Delaware Clinical Research Unit. For the half-day visit, participants were instructed to adhere to their normal energy intake and to refrain from exercise and alcohol on the day before the test day. They arrived at the research unit after a 10-h fast. Baseline satiety questionnaires were completed and fasting blood was drawn for assessment of the energy-related hormones. Participants were then instructed to consume two

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GTE capsules and a standardised high-carbohydrate breakfast meal (consisting of a bagel with cream cheese, orange juice and low-fat, fruit-flavored yogurt or 2% milk). Blood was drawn immediately post-meal and every 30 min over a period of 4 h for evaluation of change in energy- and obesity-related hormones. To minimise external influencers of satiety, each test meal session was conducted independently and the participant and test meal administrator were the only individuals in the room. Participants were allowed to bring in outside materials to read during their session, although instructions were not given to avoid food-related content. Satiety questionnaires were completed before the meal, immediately after the consumption of the meal, and every hour thereafter for a total of six questionnaires.

Participant recruitment and eligibility criteria

Participant eligibility, screening, recruitment and randomisation followed the same protocol as the parent study [28]. Primary inclusion criteria included healthy, nonsmoking post-menopausal women aged 50–70 years classified as having 'heterogeneously dense' or 'extremely dense' breast tissue by a trained radiologist after a routine screening mammogram. Of 1075 women randomised into the parent study, 230 women classified as overweight (body mass index = 25.0–29.9 kg m^{-2}) or obese (body mass index = 30.0–40.0 kg m^{-2}) at the screening clinic visit were invited to participate in this acute feeding study from 2012 to 2014. Of these, 149 participants responded to the invitation, 50 were excluded because of the need for equal numbers in each *COMT* genotype group. An additional 38 women did not participate for other reasons (Fig. 1). Sixty-one participants were randomised into the study (GTE: n = 30; placebo: n = 31). One participant in the placebo group was excluded as a result of not consuming the intervention product with the breakfast meal, and so the final sample size was 30 participants per treatment group.

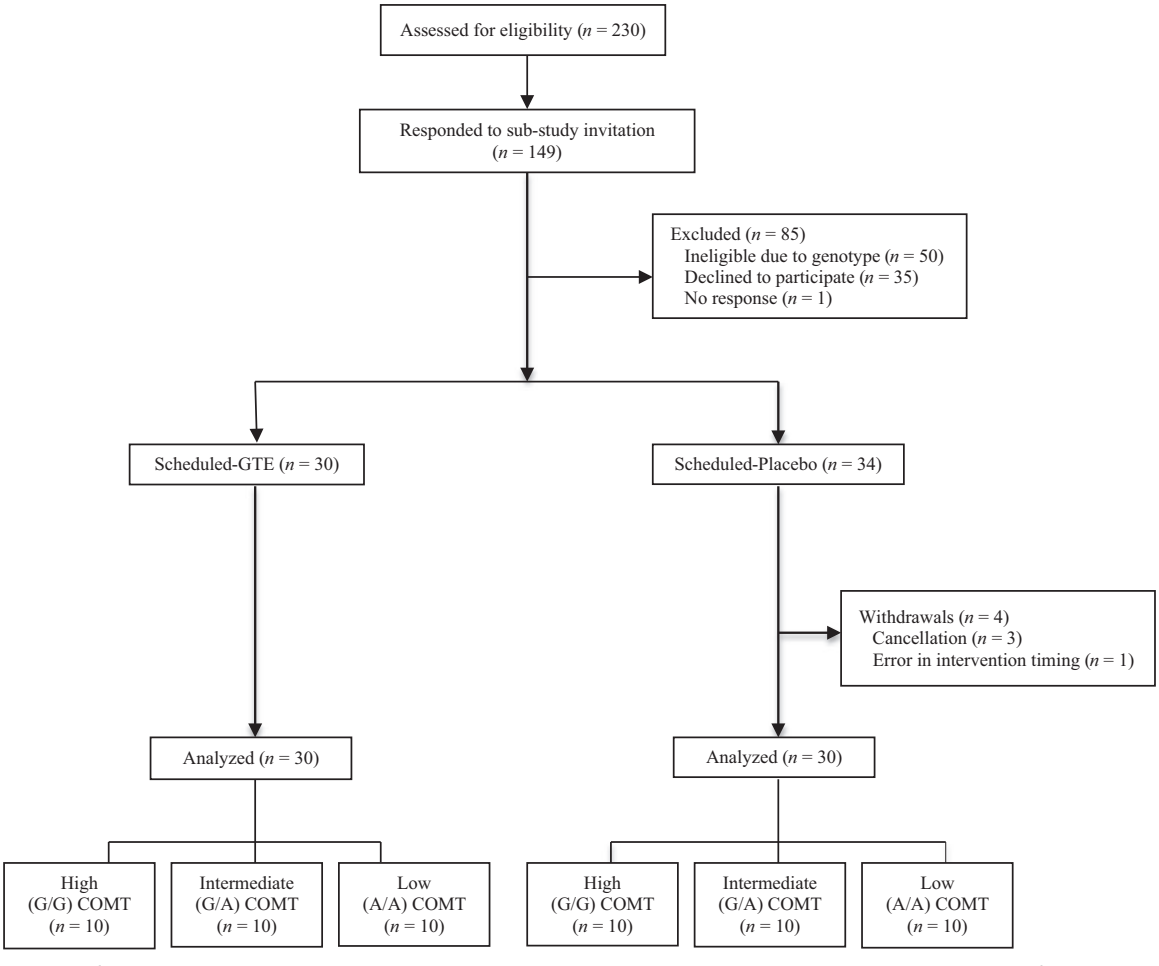

Figure 1 Flow of overweight obese postmenopausal Minnesota Green Tea Trial participants randomized to GTE or Placebo for 12 months. This figure depicts the flow of Minnesota Green Tea Trial (MGTT) participants through the sub-study of the postprandial response to a high-carbohydrate meal.

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Randomisation, blinding and participant consent

Randomisation of subjects was performed by the University of Minnesota Medical Center-Fairview's Investigational Drug Service pharmacy using a computer-generated permuted block randomisation scheme. Both participants and investigators were blinded to the treatment of subjects. Institutional Review Board approval was obtained at each clinical centre. All participants provided their additional written informed consent for this ancillary study. This trial was registered at clinicaltrials.gov as NCT00917735.

Determination of catechol-O-methyltransferase genotype

DNA was purified from buffy coats of peripheral blood samples using a PureGene Blood kit (Gentra Systems, Minneapolis, MN, USA). A TaqMan assay was developed for determining the *COMT* G/A polymorphism using a TaqMan polymerase chain reaction (PCR) Core Reagent kit (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. PCR amplification using approximately 10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, Inc., High Point, NC, USA) with an initial step of 95 °C for 10 min followed by 50 cycles of 95 °C for 25 s and 62 °C for 1 min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System (Applied Biosystems) and the results were analysed using Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the three genotypes at each locus (G/G, G/A, and A/A).

Green tea extract composition

The present study used decaffeinated Green Tea Extract Catechin Complex (referred to as GTE in this publication) in capsule form, as provided by Corban Laboratories (Eniva Nutraceutics, Plymouth, MN, USA). Mean (SD) daily catechin content was 1315 (116) mg day^{-1} [843 (44) mg as EGCG]. Placebo capsules were identical in appearance to GTE and contained 816 mg of maltodextrin, 808 mg of cellulose and 8 mg of magnesium stearate (flow agent). Participants were required to consume four capsules daily and were advised to ingest two capsules in the morning hours and two in the evening to maintain circulating catechin concentrations throughout each day, and to take the capsules with meals to reduce any potential gastrointestinal discomfort associated with consuming GTE in the fasted state. For this sub-study, participants were asked to consume the morning dose of GTE with the breakfast meal.

Demographics and data collection

All participants completed a health history questionnaire upon entry into the parent study that included comprehensive data of demographics, lifestyle factors (physical activity, smoking history and alcohol intake), as well as information about medical history, medication use (current and former) and full reproductive history.

Dietary intake

To evaluate dietary intake, participants were asked to record food intake on two assigned weekdays and one weekend day in the week prior to the half-day visit. Recording errors were minimised by providing the subjects detailed instructions on how to keep accurate diet records. Subjects were encouraged to measure foods eaten using measuring spoons and cups whenever possible. Diet records were then reviewed for accuracy and completeness during the clinic visit by a registered dietitian nutritionist and later analysed for nutrient content using the FOOD PROCESSOR DIET NUTRITION ANALYSIS AND FITNESS software, version 10.10 (ESHA Research, Salem, OR, USA). The average of the 3 days was used to represent typical food and nutrient intake.

Test meal composition

The high-carbohydrate test meal consisted of a plain bagel [1255.2 kJ (300 kcal), 59.4 g carbohydrate, 11.6 g protein, 1.8 g fat], 28.5 g of cream cheese spread [351.5 kJ (84 kcal), 0.1 g carbohydrate, 2 g protein, 8.1 g fat], 8 ounces (240 mL) of orange juice [510.5 kJ (122 kcal), 28.7 g carbohydrate, 1.7 g protein, 0.3 g fat] and the choice of 6 ounces (170 g) of low-fat fruit yogurt [765.7 kJ (183 kcal), 34.5 g carbohydrate, 7.4 g protein, 2.1 g fat] or 240 mL of 2% milk [577.4 kJ (138 kcal), 13.5 g carbohydrate, 9.7 g protein, 4.9 g fat]. Mean total energy content was 2784.0 kJ (665.4 kcal); macronutrient content was: carbohydrate = 113.3 g (67.2%); protein = 23.9 g (14.3%); and fat = 10.0 g (18.5%). Participants were instructed to take two GTE or placebo capsules with the test meal.

Biological sample analysis

Plasma adiponectin, leptin and ghrelin were measured using radioimmunoassay kits manufactured by EMD Millipore (Billerica, MA, USA) [inter-assay % coefficient of variation (% CV): leptin = 9.8%, adiponectin = 8.6%, ghrelin = 6.2%; intra-assay % CV: leptin = 10.1%; adiponectin = 7.7%, ghrelin = 8.1%]. Serum insulin was measured using a simultaneous one-step

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immunoenzymatic, chemiluminescent assay (Access Ultrasensitive Insulin assay; Quest Diagnostics, Wood Dale, IL, USA, intra-assay % CV: 3–5.0%; inter-assay % CV = 3.9%). Serum glucose concentrations were measured using a hexokinase enzymatic reference method (Quest Diagnostics, monthly % CV = 1.4%).

Assessment of satiety

To test satiety, a set of nine visual analogue scale (VAS) ratings associated with the standardised high-carbohydrate test meal was used. Questionnaires were completed pre-meal, post-meal (time 0), and hourly thereafter and were administered after blood draws at these time points. Participants were asked to answer each separate VAS question relating to hunger, fullness, desire to eat, prospective consumption, perceived satiety, contentedness, irritability, sleepiness and mental alertness in a continuous linear scale from 0 to 10 cm, where 0 represented 'not/none at all' and 10 represented 'extremely/very'. The VAS is considered to be a valid and reliable assessment tool for monitoring the effects of energy, palatability and macronutrient manipulations on subjective ratings in appetite studies [29,30].

Statistical analysis

Power calculations were unable to be performed for the half-day post-prandial sub-study because there were no data available on these outcomes at that time. We chose 60 participants as a feasible number that was comparable to similar studies examining energy-related hormones in response to a specific dietary component. We aimed to achieve equal numbers of participants with each *COMT* genotype in each treatment group and, although this reduced the power for between-genotype comparisons, it should be noted that these results were intended to be exploratory in nature.

Demographic characteristics of participants at baseline were compared between treatments using a one-sample *t*-test for continuous variables. Natural logarithmic transformation was considered to normalise the distribution of these variables. Chi-squared and Fisher's exact tests were used to compare the distribution of categorical variables between treatments. Change in hormones (leptin, ghrelin, adiponectin and insulin) and blood glucose over time were evaluated using linear regression with repeated measurements. Hormones and blood glucose were transformed using natural logarithms to normalise their distribution. The model included time, treatment, *COMT* genotype and the two- and three-way interactions as explanatory variables. Interactions were excluded from the model if nonsignificant at $P < 0.05$, using backward elimination. When statistically significant differences were detected, a *post hoc* pairwise comparison across treatment and *COMT* genotype groups was performed. An unstructured working correlation matrix was fitted to model the correlation between time points within participants; this was chosen based on Akaike's information criterion. The AUC of the VAS at different time points before and after the meal was calculated for each participant and each VAS question using the trapezoid method. The AUC values of each question and participant were used as dependent variables in a linear mixed model to evaluate the effect of treatment on the AUC of each question. Baseline VAS (pre-meal) was used as a random effect in the model. Model assumptions were evaluated using residual plots and Cook's distance greater than 0.5 was used to evaluate possible influential outliers for both hormones and VAS measures. $P \leq 0.05$ was considered statistically significant for all comparisons. All analyses were conducted using SAS, version 9.4 (SAS Institute, Inc., Cary NC, USA).

Results

Baseline characteristics

Participants randomised to GTE and placebo were similar with respect to baseline demographics and characteristics (Table 1). Groups differed in the distribution of level of education, in that the GTE group had a greater number of participants who had obtained education beyond high school ($P = 0.001$). Mean intake of macro- and micronutrients as assessed by 3-day diet records did not differ by treatment group.

Post-prandial hormone and glucose response

Post-prandial concentrations of leptin, ghrelin and adiponectin are presented in Fig. 2. Baseline fasting concentrations of all variables were similar between treatment groups. These hormone concentrations were not different between GTE and placebo at any time point, and *COMT* genotype did not modify these results.

There was a statistically significant interaction between treatment and *COMT* genotype for insulin and glucose that varied across time (Fig. 3). Participants randomised to GTE with the high-activity form of the COMT enzyme (GTE-high COMT) had significantly higher insulin concentrations at time 0 (post-meal), 0.5 and 1.0 h post-meal compared to all *COMT* genotype groups randomised to placebo. Insulin concentrations remained significantly higher in the GTE-high COMT group 1.5, 2.0 and 2.5 h compared to the Placebo-low COMT group ($P < 0.02$). The GTE-high COMT group also had higher insulin

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Table 1 Baseline characteristics and mean 3-day dietary intake of study participants, by treatment group

	GTE (n = 30)	Placebo (n = 30)	P-value
Age (years)	61.0 (59.2, 62.8)	60.8 (59.0, 62.6)	0.88
White, non-Hispanic, *n* (%)	29 (96.7)	30 (100)	1.00
COMT genotype, *n* (%)			
A/A	10 (33.3)	10 (33.3)	1.00
G/A	10 (33.3)	10 (33.3)	1.00
G/G	10 (33.3)	10 (33.3)	1.00
Weight (kg)	74.2 (71.1, 77.4)	75.3 (72.1, 78.4)	0.64
Body mass index (kg m^{-2})	28.2 (27.1, 29.4)	28.3 (27.2, 29.5)	0.91
Waist-to-hip ratio	0.87 (0.83, 0.89)	0.85 (0.82, 0.87)	0.31
Years post-menopausal	8.8 (6.3, 12.2)	7.8 (5.6, 10.8)	0.60
Type of menopause, *n* (%)			
Natural	23 (76.7)	24 (80.0)	0.75
Surgical	7 (23.3)	6 (20.0)	
Education, *n* (%)			
Masters/PhD/Professional	10 (33.3)	8 (26.7)	0.001*
College degree	8 (26.7)	15 (50.0)	
Some college	12 (40.0)	2 (6.7)	
High school or below	0	5 (16.7)	
Physical activity (MET-h $week^{-1}$)	39.5 (26.2, 52.7)	43.8 (30.6, 57.1)	0.64
Dietary variables			
Total energy (kJ day^{-1}) [(kcal day^{-1})]	7360 (6650, 8075) [1760 (1590, 1930)]	7740 (7030, 8450) [1850 (1680, 2020)]	0.48
Protein (g day^{-1})	73.4 (65.7, 81.0)	78.6 (70.9, 86.2)	0.35
Total fat (g day^{-1})	67.5 (57.6, 77.4)	73.9 (64.0, 83.8)	0.37
Dietary cholesterol (mg)	230 (190, 271)	238 (197, 278)	0.79
Saturated fat (g)	23.4 (19.9, 26.9)	24.5 (21.0, 28.0)	0.66
MUFA (g)	11.9 (9.7, 14.6)	13.4 (10.9, 16.4)	0.44
PUFA (g)	7.0 (5.0, 9.0)	8.5 (6.5, 10.5)	0.31
Omega-3 FA (g)	0.5 (0.4, 0.7)	0.6 (0.5, 0.8)	0.65
Omega-6 FA (g)	1.2 (1.0, 1.5)	1.3 (1.1, 1.7)	0.38
Total carbohydrate (g day^{-1})	217 (192, 241)	216 (191, 240)	0.95
Dietary fibre (g day^{-1})	19.0 (16.8, 21.2)	20.6 (18.4, 22.9)	0.31
Soluble fibre (g)	1.8 (1.4, 2.2)	1.6 (1.2, 2.0)	0.42
Alcohol (g day^{-1})†	0.1 (0.0, 0.3)	0.1 (0.0, 0.4)	0.75
Caffeine (mg day^{-1})†	36.8 (13.6, 99.9)	46.2 (17.0, 125.3)	0.75

COMT, catechol-O-methyltransferase; FA, fatty acid; GTE, green tea extract; MET-h, metabolic equivalent hours; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Continuous data expressed as arithmetic mean (95% confidence interval) unless otherwise indicated by superscript. Categorical variables compared using chi-squared test and expressed as *n* (%).

*Fisher's exact test used for comparison.

†Data expressed as geometric mean (95% confidence interval).

concentrations at time 0, 0.5, 1.0, 1.5 and 2.0 h after the meal compared to the GTE-low COMT group ($P \leq 0.04$). No differences between treatment groups and *COMT* genotypes existed 3.0, 3.5 or 4.0 h after the meal. With respect to glucose, the GTE-high COMT group had significantly higher glucose concentrations at time 0 compared to Placebo-high COMT ($P = 0.05$) and the GTE-low COMT group ($P = 0.004$). The GTE-low COMT group had higher serum glucose 3.5 h post-meal compared to the GTE-high COMT and Placebo-low COMT groups ($P = 0.01$ and $P = 0.05$, respectively). Insulin AUC comparisons within genotypes between treatment groups are shown in Table 2. The insulin AUC for GTE-high COMT was significantly higher compared to Placebo-high COMT at all time points, although the mean AUC comparison failed to reach significance ($P = 0.06$). Mean insulin AUCs between intermediate- and low-COMT groups did not differ at any time point, and glucose AUCs did not differ within any *COMT* genotype between treatment groups (data not shown).

Appetite sensations

AUC measurements of satiety-related variables did not show any significant difference between treatments at baseline or over the 4-h time period between GTE and

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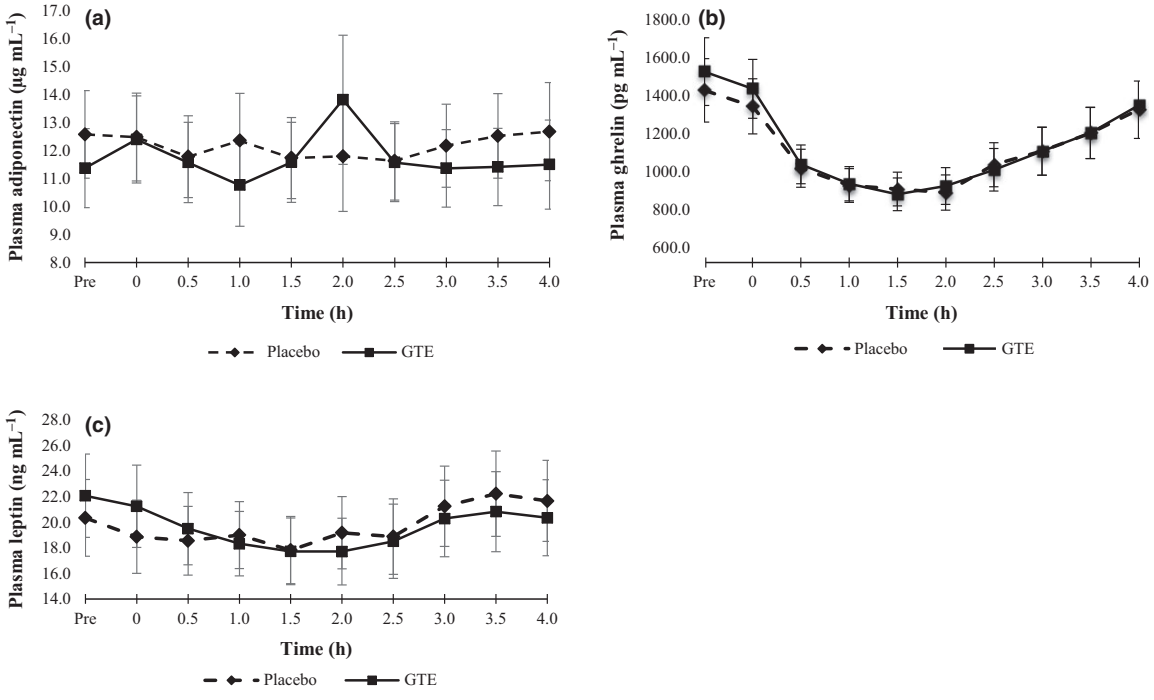

Figure 2 Mean postprandial adiponectin (A), ghrelin (B), and leptin (C) concentrations in overweight or obese postmenopausal women randomized to GTE or Placebo for 12 months. The x-axis is labeled in units of hours, with "Pre" indicating the pre-meal (fasting) blood draw. The placebo group (n=30) is indicated by a dashed line and the green tea extract (GTE) group (n=30) is indicated by solid black line.

placebo groups (See Supporting information, Table S1). *COMT* genotype did not modify these results.

Discussion

The results of the present study indicate that GTE supplementation does not alter the acute post-prandial response of leptin, ghrelin or adiponectin after the consumption of a high-carbohydrate meal, and that *COMT* genotype does not modify this relationship. However, GTE supplementation and *COMT* genotype may be involved in the post-meal glycaemic response because significant interactions were observed between *COMT* genotype and glucose and insulin concentrations over the 4-h test period. No effect of GTE or *COMT* genotype was seen on measures of post-prandial satiety.

To our knowledge, this is the first study to examine the effect of GTE supplementation on acute post-prandial concentrations of the appetite-associated hormones leptin, ghrelin and adiponectin. We did not find significant differences between treatment groups in these hormones prior to the meal or at any time point thereafter, indicating that GTE did not modify fasting concentrations of ghrelin, leptin and adiponectin in these participants. This is consistent with our previous work, which demonstrated that GTE did not modify concentrations of these hormones over the 12-month intervention period of the MGTT in a larger sample of overweight and obese study participants [11]. We did observe a somewhat unexpected leptin response, in which plasma leptin concentrations decreased moderately from pre-meal to 1.5 h in both groups before increasing to the end of the 4-h period. Yet, these results correlate with other studies that have examined leptin concentrations in overweight and obese women after a high-carbohydrate meal [31,32], affirming that obesity is associated with an impaired post-prandial leptin response. When correlated with the AUC analysis of satiety-related endpoints, satiety and prospective food intake were not differentially influenced by the effect of GTE on energy-related hormones. Taken together, the results of these studies indicate that any effect of GTE supplementation is not mediated through alteration of leptin, ghrelin or adiponectin concentrations.

We observed a significant interaction between GTE, *COMT* genotype and time, in which participants randomised to GTE with the high-activity (G/G) form of the COMT enzyme demonstrated increased post-prandial insulin concentrations compared to the Placebo-high COMT and GTE-low (A/A) COMT groups, despite similar glucose profiles at most time points. The significance of an increased insulin response to a high-carbohydrate meal in participants with the G/G genotype taking GTE is unclear, although it could be indicative of the need to secrete additional insulin to manage blood glucose

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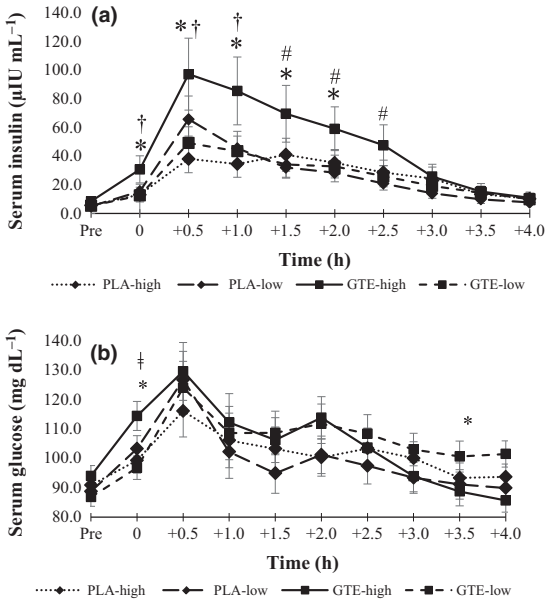

*$P < 0.05$, GTE-high ($n = 10$) versus GTE-low COMT ($n = 10$);
#$P < 0.05$, GTE-high ($n = 10$) versus PLA-low COMT ($n = 10$);
†$P < 0.05$, GTE-high ($n = 10$) versus all PLA COMT ($n = 10$);
‡$P < 0.05$, GTE-high ($n = 10$) and PLA-high COMT ($n = 10$).
Abbreviations: GTE, green tea extract; PLA, placebo

Figure 3 Mean postprandial serum insulin (A) and glucose (B) concentrations in overweight or obese postmenopausal women randomized to GTE or Placebo for 12 months. Figure 3 details the mean change in postprandial insulin and glucose concentrations, by treatment group and COMT genotype. Participants in the Placebo group with high (G/G) catechol-O-methyltransferase (COMT) genotype (PLA-high, n=10) are indicated by a small-dashed line and diamond-shaped markers. Participants in the Placebo group with low (A/A) COMT genotype (PLA-low, n=10) are indicated by a wide-dashed line and diamond-shaped markers. In the GTE group, participants with high COMT genotype (GTE-high, n=10) are indicated with a solid black line and squared-shaped marker, while those with the low COMT genotype (GTE-low, n=10) are indicated by a dashed line with a squared-shaped marker. Participants with the intermediate (G/A) COMT genotype (n=10 in both Placebo and GTE groups) have been omitted from this figure for the purposes of simplification, since their postprandial insulin and glucose responses did not significantly differ from participants with high or low COMT genotypes or between treatment groups. An asterisk indicates a significant difference ($P < 0.05$) between the GTE-high and GTE-low groups. A "#" indicates a significant difference between GTE-high and PLA-low groups and "†" indicates a significant difference between GTE-high and all Placebo-COMT genotype groups. "‡" indicates a significant difference between GTE-high and PLA-high groups. *$P < 0.05$, GTE-high ($n = 10$) versus GTE-low COMT ($n = 10$); #$P < 0.05$, GTE-high ($n = 10$) versus PLA-low COMT ($n = 10$); †$P < 0.05$, GTE-high ($n = 10$) versus all PLA COMT ($n = 10$); ‡$P < 0.05$, GTE-high ($n = 10$) and PLA-high COMT ($n = 10$). COMT, catechol-O-methyltransferase; GTE, green tea extract; PLA, placebo.

concentrations over time compared to individuals with other forms of the *COMT* genotype and those not taking GTE; yet, the sample size of this group was small, making it difficult to draw definitive conclusions. Our results are in agreement with those of another study [23] that reported finding greater post-prandial insulin concentrations after the consumption of GTE (836 mg catechins) in individuals with the G/G genotype compared to those with the G/A or A/A *COMT* genotypes. Similarly, Kring, *et al.* [33] determined that there was an 11.6% increased frequency of the G/G *COMT* genotype in individuals with impaired glucose tolerance or type 2 diabetes. These studies and our results appear to suggest that the high-activity form of the COMT enzyme is associated with increased risk for glycaemia-related health conditions, and that GTE consumption may potentiate an exaggerated insulin response after a meal. These results coincide with our previous findings in a larger sample of post-menopausal overweight and obese women ($n = 237$) [11], in which participants with the G/G form of COMT showed significantly higher insulin concentrations at month 12 compared to those with the A/A genotype irrespective of treatment group (GTE and placebo groups combined); yet, among participants with baseline fasting insulin ≥ 10 μIU mL^{-1}, reductions in fasting insulin concentrations were seen in the GTE group over 12 months compared to both the placebo group and all participants with baseline insulin < 10 μIU mL^{-1}. It is plausible that GTE consumption may elicit a higher immediate post-prandial insulin response, particularly in those with the G/G *COMT* genotype, at the same time acting to reduce fasting insulin concentrations over time. Additional research is needed to determine the specific mechanisms of the COMT enzyme on insulin concentrations after the administration of GTE and how this may affect the short- and long-term glycaemic response.

Early animal studies demonstrated reduced food intake with administration of green tea catechins [9,34], indicating that green tea might increase satiety. However, this effect has not been confirmed in human subjects. One study found that the inclusion of 167 mg of green tea catechins and 100 mg of caffeine in a beverage containing 10 g of soluble fibre created lower hunger and higher fullness ratings and was associated with the lowest energy intake in the next meal compared to fibre-only (46 mg caffeine), isocaloric control beverage (no caffeine) and no beverage conditions [35]. By contrast, Diepvens, *et al.* [26] showed that women randomised to receive green tea (1125 mg catechins + 225 mg caffeine day^{-1}) for almost 3 months with a low-energy diet became hungrier over time and showed increased prospective food consumption compared to placebo. It was suggested that this could be a result of down-regulation of the leptin response through stimulation of the sympathetic nervous system by green tea because leptin is known to reduce appetite. However, leptin concentrations were not measured in their study, and so this conclusion could not be

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Table 2 Mean insulin area under the curves in in overweight or obese post-menopausal women after the ingestion of a meal with or without GTE, by *COMT* genotype [μIU (mL h^{-1})$^{-1}$]

	Placebo (n = 30)			GTE (n = 30)		
Time (h)	High (G/G) (n = 10)	Intermediate (G/A) (n = 10)	Low (A/A) (n = 10)	High (G/G) (n = 10)	Intermediate (G/A) (n = 10)	Low (A/A) (n = 10)
0–0.5[†]	14.0 (4.9)	14.6 (4.9)	21.2 (4.9)	35.4 (4.9)*	25.4 (5.1)	16.4 (4.6)
0–1	31.9 (11.4)	37.6 (11.4)	51.1 (11.4)	81.1 (11.4)*	55.1 (12.0)	45.0 (10.9)
0–1.5	48.1 (18.5)	58.9 (18.5)	70.5 (18.5)	120 (18.5)*	83.9 (19.5)	73.3 (17.6)
0–2	65.8 (25.8)	79.0 (25.8)	84.1 (25.8)	156 (25.8)*	120 (27.2)	99.3 (24.6)
0–2.5	82.9 (32.9)	95.0 (32.9)	96.1 (32.9)	190 (32.9)*	148 (34.7)	124 (31.4)
0–3	98.7 (38.7)	106 (38.7)	106 (38.7)	216 (38.7)*	168 (40.8)	143 (36.9)
0–3.5	109 (42.8)	114 (42.8)	113 (42.8)	231 (42.8)*	184 (45.1)	156 (40.8)
0–4	115 (45.7)	120 (45.7)	118 (45.7)	241 (45.7)	196 (48.2)	166 (43.6)

GTE, green tea extract; COMT, catechol-O-methyltransferase.
Data presented as mean (SEM).
*Statistically significant difference between *COMT* genotypes, GTE versus placebo ($P \leq 0.05$).
[†]Time 0 indicates post-meal.

confirmed. Several other RCTs examining the effect of GTC on acute measures of satiety [36,37] or long-term changes in energy intake [5,11,38] have shown null results, including the present study, in which we did not observe differences between treatment groups in any of the nine questions related to hunger, satiation and prospective food intake in the 4 h following a high-carbohydrate breakfast meal.

The results of this research contribute depth not only to the growing body of research on the effects of GTC on insulin sensitivity, but also to the association between *COMT* genotype and risk for glycaemia-related health conditions. Our results also indicate that GTE does not induce appetite inhibition or satiety, thus weakening the argument for these effects as mechanisms behind the association of green tea with reductions in body weight and adiposity. However, our research has several limitations. We conducted this analysis after just one meal and were unable to compare each participant's response to that of a reference meal. There was no measure of hedonic liking of the meal, which may have influenced satiety and prospective food intake. Standardisation of the diets of participants 24 h prior to the breakfast meal could have increased the similarity of the glycaemic response. Participants were not required to follow a low-catechin diet for the duration of the study, and so the ingestion of catechins from foods and beverages other than green tea likely contributed to total catechin intake. However, green tea consumption was restricted throughout the duration of the study, and the amount of GTC given in the intervention was far greater than could be ingested in a typical daily diet. Therefore, any risk of confounding was minimal. Lastly, because this was a small ancillary study with only 10 participants in each treatment/genotype group, we may have been underpowered to detect significant results and cannot rule out the possibility of type 2 error. Despite these limitations, these results indicate that supplementation of 1315 mg GTE per day for 12 months did not influence pre- or post-prandial concentrations of appetite-associated hormones or measures of satiety after a high-carbohydrate breakfast meal in overweight or obese post-menopausal women. Yet, GTE and *COMT* genotype may have specific influences on post-meal insulin and glucose concentrations, and these findings warrant additional research in larger study populations aiming to determine the specific impact of GTE on the post-prandial glycaemic response.

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.

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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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AMD, MSK and NRS contributed to the conception, design and implementation of the project. AMD, SB, AA and NRS contributed to data collection and analytical procedures. AA and LE conducted the statistical analysis. AMD, AA, LE and MSK interpreted data. AMD and NRS wrote the manuscript and had primary responsibility for final content. All authors read and approved the final version of 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. Mean satiety and fullness AUCs after ingestion of a high-carbohydrate meal (expressed in cm h^{-1}) in overweight or obese post-menopausal women randomised to GTE or placebo for 12 months.

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OBESITY AND RELATED DISORDERS

Modified body adiposity index for body fat estimation in severe obesity

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Keywords
bioelectrical impedance analysis, body adiposity index, body composition, severe obesity, waist–hip ratio.

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Abstract

Background: The body adiposity index (BAI) comprises a simple method for estimating body fat (BF) that needs to be validated in patients with severe obesity. The present study aimed to determine BAI accuracy with respect to the determination BF in patients with severe obesity.
Methods: A cross-sectional prospective study comparing two methods for BF estimation was conducted in 433 patients with severe obesity between August 2012 to December 2014. BF was estimated by bioelectrical impedance analysis (BIA) with specific equations developed for BF estimation in patients with severe obesity and BAI. The BF estimation in 240 patients with severe obesity (Group 1: G1) was used to evaluate BAI limitations and to develop a specific equation in this population. The new equation proposed was validated in another 158 patients with severe obesity (Group 2: G2).
Results: There was a significant difference between BF determination by BIA and BAI ($P = 0.039$). The mean (SD) BF in G1 was 52.3% (6.1%) determined by BIA and 51.6% (8.1%) determined by BAI. Sex, waist–hip ratio (WHR) and obesity grade determined significant errors on BF estimation by BAI. A new equation (modified body adiposity index; MBAI) was developed by linear regression to minimise these errors [MBAI % = 23.6 + 0.5 × (BAI); add 2.2 if body mass index ≥ 50 kg m^{-2} and 2.4 if WHR ≥ 1.05]. The new equation reduced the difference [1.2% (5.9%), $P < 0.001$ to 0.4% (4.12%), $P = 0.315$] and improved the correlation (0.6–0.7) between methods.
Conclusions: BAI present significant limitations in severe obesity and MBAI was effective for BF estimation in this population.

Introduction

Obesity rates are growing worldwide [1] and, in the USA, approximately 35.7% of the adult population is considered to be obese. Obesity is directly responsible for 112 000–365 000 deaths annually, with an estimated cost of 139 billion dollars [2–4]. Body fat (BF) assessment in patients with severe obesity is important with respect to treatment (clinical and surgical) evaluation because a loss of lean mass may be associated with deleterious health effects [5]. The maintenance of lean mass contributes to improving quality of life by maintaining the functional capacity [6].

Severe obesity is characterised by excessive body fat deposition, increased total body water and a reduction of lean mass [7–9]. The hydration state could affect the values of fat free mass in body composition assessments [9]. These changes determine several limitations related to methods commonly used for evaluating body composition in normal weight, overweight or moderately obese subjects [8]. Furthermore, there are also physical limitations with respect to the equipment, as well as the ability to perform the manoeuvres required for body composition evaluation [7,8].

Bioelectrical impedance analysis (BIA) significantly underestimates BF in morbid obese subjects [7,10–15]

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because the equations developed to calculate BF using BIA resistance are population specific [16] and are considered inappropriate for patients with severe obesity [7,10,13,15,17]. Nevertheless, specific equations for BF estimation in severe obesity were developed and validated by Horie-Waitzberg using BIA data, with a high specificity and sensitivity compared with plethysmography [18].

Anthropometry data also presents several limitations in patients with severe obesity [19]. However, Bergman *et al.* [20] developed the body adiposity index (BAI) using only two anthropometric measurements (hip circumference and height) and suggested that it could be an effective method for BF estimation even in patients with severe obesity. It was developed and validated in non-Caucasian subjects and might be adequate in populations of Central and South America [20].

The present study aimed to evaluate BAI for BF determination in patients with severe obesity.

Materials and methods

Sampling procedure

A cross-sectional study comparing two methods for BF estimation was conducted in 433 patients with severe obesity undergoing bariatric surgery between August 2012 and December 2014 in the Bariatric and Metabolic Surgical Unit, Hospital das Clínicas, University of São Paulo Medical School. Patients with a pacemaker ($n = 1$), acute or chronic disease (congestive heart failure, chronic renal failure, liver failure) associated with excessive water retention ($n = 25$) or subjects who did not agree to participate ($n = 9$) were excluded, leaving 398 patients in the study.

The present study was performed in accordance with the ethical recommendations of the Declaration of Helsinki and was approved by the Hospital das Clínicas Ethical Committee. All persons involved provided their informed consent prior to study enrollment. The sample was calculated using GPOWER, version 3.1 (http://www.gpower.hhu.de), with 5% of significance and 95% statistical power. Data were collected during the first nutritional interview in our ambulatory unit. Body weight was measured to the nearest 0.1 kg with a high precision electronic balance (model W 300A; Welmy, Santa Bárbara D'Oeste, Brazil) and height was measured with a stadiometer coupled to the balance to the nearest 0.5 cm. The circumferences were measured by using a flexible plastic tape with graduated scale. Waist circumference (WC) was measured between the last rib and the iliac crest (at the umbilical level) and hip circumference (HC) was measured in the largest circumference of the buttocks. Body fat was estimated by BAI and BIA.

Measures

Body adiposity index

Body fat was determined by BAI as described by Bergmann *et al.* [20]. This index comprises the use of two anthropometrics measurements to estimate body fat:

$$\mathrm{BF}(\%) = \left[\frac{\text{hip circumference (cm)}}{\text{height (m)}^{1.5}}\right] - 18.$$

Bioelectrical impedance analysis

A single-frequency BIA (Model 310; Biodynamics, Seattle, WA, USA) was used to perform the analysis. Patients were in supine position with arms and legs lying parallel and separated, so that the thighs were not touching. Two electrodes were placed on the hand and wrist, and two others were positioned on the foot and ankle of the right side of the body. An electrical current of 50 kHz was introduced into the subject, and resistance and reactance were measured. BF was calculated using the Horie-Waitzberg [18] equation: Body fat (kg) = 23.25+ (0.09 × resistancein ohms) + (1.00 × weight kg) − (0.08× height in meters) + (0.13 × age in years). The results were transformed into BF% using the formula:

$$\mathrm{BF}\% = \frac{\mathrm{BF}\ (\mathrm{kg})}{\text{weight}\ (\mathrm{kg}) \times 100}.$$

Statistical analysis

All statistical analyses were performed in R, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). The statistical methods of the present study were reviewed by of Epidemiology and Statistics Laboratory, Gastroenterology Department, University of São Paulo Medical School. Results are expressed as the mean (SD). A *t*-test and intraclass correlation were used to compare BAI with BIA. We used the Lausen [21] method to determine the cut-off points to waist–hip ratio (WHR) that might interfere with the BAI. Multiple linear regression analysis was used to develop a new equation for BF determination. In all of the analyses, the percentage of body fat estimated by BIA (Horie-Waitzberg equation) was used as the reference method. $P < 0.05$ was considered statistically significant.

Results

The BF determination in 240 patients with severe obesity (Group 1: G1) was used to determine the BAI limitations and to develop a specific correction equation (G1). The new equation proposed was validated in 158 subjects (Group 2: G2). There was no significant difference in subject characteristics between the two groups (Table 1).

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Inter- and intra-observer variability coefficients for BIA and BAI evaluations were calculated (Table 2). There was a significant difference ($P < 0.05$) between BF determination by BIA and BAI [0.6% (5.1%)]. The mean (SD) BF in G1 determined by BIA and BAI was 52.3% (6.1%) and 51.6% (8.1%), respectively. The two methods were similar according to the intraclass correlation (0.74; 95% confidence interval = 0.68–0.79) (Table 3). In patients with excessive abdominal fat accumulation (android obesity), BAI may underestimate BF. We determined a WHR cut-

Table 1 Characteristics of the 398 patients with severe obesity evaluated before bariatric surgery (Group 1 and Group 2)

	Group 1			Group 2		
Characteristics	Total (n = 240)	Female (n = 190)	Male (n = 50)	Total (n = 158)	Female (n = 136)	Male (n = 22)
Age (years)	44.1 ± 11.1	44.5 ± 10.8	42.8 ± 12.2	44.1 ± 12.1	43.6 ± 12.2	43.4 ± 12.7
Weight (kg)	128.8 ± 23.8	122.6 ± 18.8	149.3 ± 27.1	128.5 ± 25.5	122.6 ± 19.8	156.1 ± 29.9
Height (m)	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
BMI (kg m^{-2})	49.2 ± 7.4	48.7 ± 7.1	50.9 ± 8.0	49.1 ± 7.6	48.3 ± 7.2	52.8 ± 7.4
WC (cm)	139.6 ± 18.5	136.8 ± 18.7	148.9 ± 14.6	135.2 ± 17.0	131.3 ± 14.4	152.6 ± 16.7
HC (cm)	142.5 ± 14.0	141.5 ± 13.3	145.5 ± 15.9	142.1 ± 14.8	140.5 ± 14.3	149.1 ± 14.0
WHR	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1

Results are expressed as the mean (SD).
BAI, body adiposity index; BF, body fat; BIA, bioelectrical impedance analysis; BMI, body mass index; WHR, waist–hip ratio.

Table 2 Inter- and intra-observer variability coefficients for bioelectrical impedance analysis (BIA) and body adiposity index (BAI) evaluations according to body mass index (BMI) in patients with severe obesity (n = 240): Group 1 (G1)

Variable	BMI	Male (M)	Female (F)	Q1 (M/F)	Median (M/F)	Q3 (M/F)	Min (M/F)	Max (M/F)	CV(%) (M/F)
BF % BAI	40–45	43.9 ± 2.9	43.8 ± 4.0	43.5/42.0	43.3/44.1	42.0/44.2	41.6/42.1	44.9/44.8	15.1/9.1
	45–50	46.4 ± 4.7	47.5 ± 4.6	43.5/44.0	45.3/47.2	47.3/49.2	46.1/45.2	48.2/49.7	10.1/9.7
	50–55	49.4 ± 8.0	52.1 ± 5.0	43.7/48.8	48.2/51.8	51.2/55.3	50.1/50.2	54.6/54.1	16.2/9.7
	55–60	52.7 ± 4.3	55.9 ± 5.7	48.4/51.9	54.0/55.2	56.9/60.3	55.9/56.9	57.6/59.8	8.2/10.2
	60–65	61.5 ± 7.5	63.6 ± 3.5	60.6/61.6	64.9/63.8	65.8/65.5	60.3/60.0	64.8/65.0	12.3/5.5
BF % BIA	40–45	43.7 ± 0.5	43.1 ± 1.3	43.5/42.9	43.7/43.3	44.1/44.1	42.8/40.4	44.4/44.6	1.2/3.0
	45–50	47.4 ± 1.1	48.0 ± 1.3	46.5/47.2	47.4/48.2	48.5/49.2	46.0/45.1	48.8/49.9	2.4/2.7
	50–55	52.0 ± 1.5	52.4 ± 1.4	50.7/51.2	51.8/52.2	53.0/53.6	50.2/50.1	54.5/54.9	3.0/2.7
	55–60	56.2 ± 0.9	57.1 ± 1.4	55.6/55.8	55.9/57.3	56.6/58.2	55.2/55.3	57.7/59.8	1.6/2.4
	60–65	62.5 ± 1.1	61.7 ± 1.1	61.9/61.1	62.4/61.6	63.1/62.2	60.7/60.0	64.6/64.4	2.6/1.8

Results are expressed as the mean (SD).
BF, body fat; CV %, variability coefficients; F, female; M, male; Max, maximum value; Min, minimum value; Q1, quantile 1; Q3, quantile 3.

Table 3 Body fat determined by bioelectrical impedance analysis (BIA) and body adiposity index (BAI), percentage differences between methods and intraclass correlation in patients with severe obesity: Group 1 (n = 240)

Characteristics	BIA (BF%)	BAI (BF%)	Difference % (BIA-BAI)	P value	Minimum error	Maximum error	Intraclass correlation (95% CI)
Females	52.8 ± 5.8	52.7 ± 7.6	0.2 ± 4.8	0.664	−11.5	10.2	0.75 (0.68–0.81)
Males	50.1 ± 6.7	47.6 ± 8.9	2.5 ± 5.9	0.004*	−14.2	14.1	0.68 (0.50–0.80)
BMI 35–49.99 kg m^{-2}	49.4 ± 5.2	48.0 ± 6.1	1.4 ± 4.7	0.001*	−10.8	14.1	0.63 (0.52–0.72)
BMI ≥ 50 kg m^{-2}	56.5 ± 4.7	56.9 ± 7.9	−0.5 ± 5.5	0.569	−14.2	13.0	0.64 (0.50–0.74)
WHR < 1.05	52.2 ± 6.0	52.3 ± 7.7	0.0 ± 4.8	0.899	−14.2	12.1	0.76 (0.69–0.81)
WHR ≥ 1.05	52.3 ± 6.6	47.3 ± 9.2	5.0 ± 4.7	0.002*	−7.2	14.1	0.67 (0.43–0.82)
Total	52.3 ± 6.1	51.6 ± 8.1	0.6 ± 5.1	0.039*	−14.2	14.1	0.74 (0.68–0.79)

Results are expressed as the mean (SD).
BMI, body mass index; CI, confidence Interval; WHR, waist–hip ratio.
*$P < 0.05$.

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off point of 1.05 where the difference between the methods was ≥5% for the same subject. Therefore, when WHR ≥ 1.05, BAI underestimates BF in 5.0% (4.7%) ($P = 0.002$). BAI also underestimates BF in males [2.5% (5.9%); $P = 0.004$] and in patients with a body mass index (BMI) between 35 and 49.99 kg m^{-2} [1.4% (4.7%); $P = 0.001$] (Table 3). Multiple linear regression analysis (Table 4) with variable selection using the model reduction method (Backward) gave the following correction equation (modified body adiposity index; MBAI) with a coefficient of determination (r^2) of 0.65: MBAI $= 23.6 + 0.5 \times$ BAI% Add 2.2 if BMI ≥ 50 kg m^{-2} and 2.4 if WHR ≥ 1.05.

The final model was chosen when all variables were significant. In addition, the good performance of the model was ratified by a residual analysis. It was performed by the envelope method in generalised linear models.

The new equation was used for BF estimation on data collected from G2 and the results were compared with the BF provided by BIA (Horie-Waitzberg equation).

The same BAI limitations found in G1 were also observed in G2. The mean (SD) BF determined by BIA and BAI was 52.4% (5.7%) and 51.2% (7.9%), respectively [mean (SD) difference = 1.2% (5.9%), $P < 0.001$]. BAI also underestimated BF in males [3.1% (4.8%); $P = 0.007$], as well as in patients with a WHR > 1.05 [7.2 (6.1); $P < 0.001$] or with a BMI between 35 and 49.99 kg m^{-2} [2.3% (5.5%); $P < 0.001$] (Table 5). MBAI corrected these limitations (Figs 1 and 2) and improved the correlation between methods (from 0.61 to 0.70). BF determined by MBAI and BIA was 52.0% (4.8%) and 52.4% (5.7%) [mean (SD) difference of 0.4% (4.12%), $P = 0.315$], respectively (Table 5).

There was a positive and linear correlation between methods (BIA and BAI) with a significant improvement with MBAI (Fig 1).

Table 4 Multiple linear regression analysis in patients with severe obesity ($n = 240$): Group 1 (G1)

Variable	Estimate	*P*
(Intercept)	23.6 ± 1.8	<0.001
BAI	0.5 ± 0.0	<0.001
BMI ≥ 50 kg m^{-2}	2.2 ± 0.6	<0.001
WHR ≥ 1.05	2.4 ± 0.7	<0.001

Results are expressed as the mean (SD).
BAI, body adiposity index; BMI, body mass index; WHR, waist–hip ratio.

Discussion

Traditional methods present several limitations to estimating BF in patients with severe obesity [10–12]. BIA is a simple and non-invasive method [22] for estimating BF via specific equations in these population patients [23].

In recent studies, BAI presented positive correlations with other methods for body composition analysis with small significant differences compared to dual energy X-ray absorptiometry (DXA) [24–26]. The major advantage of the test is its low cost and simplicity [27].

In our population ($n = 398$), the mean differences between BAI and BIA were small [0.6% (5.1%) in G1 and 1.2% (5.9%) in G2] but significant ($P = 0.039$ in G1 and $P < 0.001$ in G2) and the maximum and minimum errors were high (−11.9% and 20.3%). The BF underestimation in our series was also observed in studies with BAI in women with severe obesity (1.7% [27] and 1.2% [28]) with similar maximum and minimum errors (−13.63 and 11.13) [29].

Table 5 Body fat determined by bioelectrical impedance analysis (BIA), body adiposity index (BAI) and modified body adiposity index (MBAI), differences between BIA and BAI and between BIA and MBAI, and intraclass correlation in patients with severe obesity: Group 2 ($n = 158$)

Characteristics	BIA (BF%)	BAI (BF%)	MBAI (BF%)	Difference % (BIA-BAI)	*P* value	Difference % (BIA-MBAI)	*P* value	Intraclass correlation (95% CI)	
								BIA x BAI	BIA x MBAI
Females	52.6 ± 5.6	51.6 ± 8.1	52.1 ± 4.9	0.9 ± 6.1	0.078	0.4 ± 4.1	0.246	0.6 (0.5–0.7)	0.7 (0.6–0.8)
Males	51.5 ± 6.2	48.5 ± 6.6	51.2 ± 4.2	3.1 ± 4.8	0.007*	0.3 ± 3.5	0.672	0.6 (0.3–0.8)	0.8 (0.6–0.9)
BMI 35–49.99 kg m^{-2}	49.8 ± 5.1	47.5 ± 6.0	49.0 ± 2.9	2.3 ± 5.5	<0.001*	0.7 ± 4.4	0.112	0.4 (0.3–0.6)	0.4 (0.2–0.6)
BMI ≥ 50 kg m^{-2}	56.4 ± 4.0	56.8 ± 7.2	56.5 ± 3.5	−0.4 ± 6.2	0.605	−0.1 ± 3.4	0.833	0.4 (0.2–0.6)	0.6 (0.4–0.7)
WHR < 1.05	52.4 ± 5.3	52.2 ± 7.2	52.1 ± 4.6	0.2 ± 5.3	0.664	0.3 ± 4.0	0.438	0.7 (0.6–0.7)	0.7 (0.6–0.8)
WHR ≥ 1.05	52.7 ± 7.5	45.5 ± 9.6	51.5 ± 5.9	7.2 ± 6.1	<0.001*	1.2 ± 4.4	0.211	0.5 (0.1–0.8)	0.8 (0.6–0.8)
Total	52.4 ± 5.7	51.2 ± 7.9	52.0 ± 4.8	1.2 ± 5.9	<0.001*	0.4 ± 4.0	0.315	0.6 (0.5–0.7)	0.7 (0.6–0.8)

Results are expressed as the mean (SD).
BF, Body Fat; BMI, body mass index; WHR, waist–hip ratio.
*$P < 0.05$.

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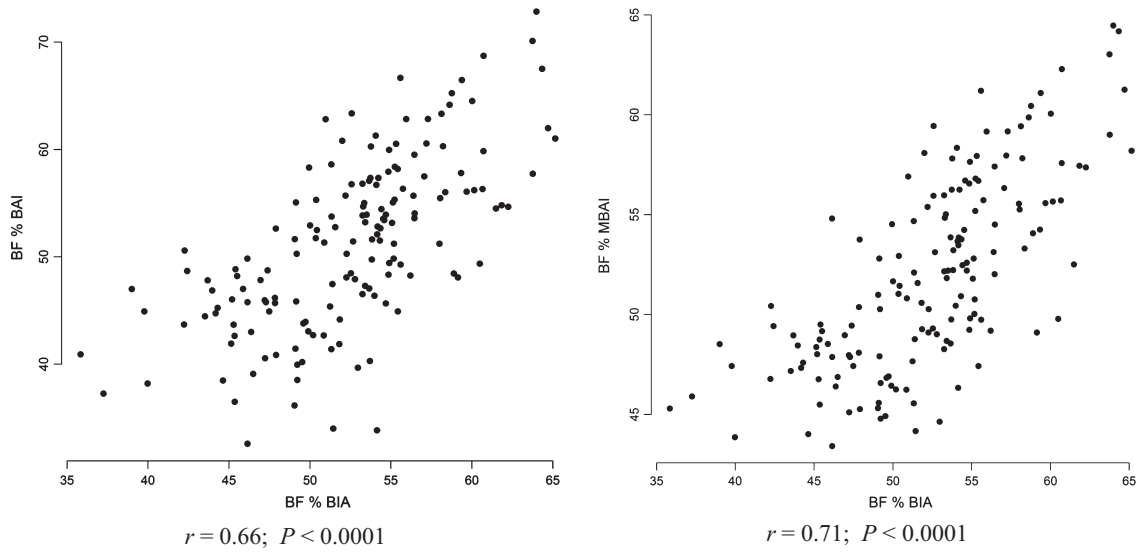

Figure 1 Relationship between bioelectrical impedance analysis (BIA) and body adiposity index (BAI) and between BIA and modified body adiposity index (MBAI) in patients with severe obesity: Group 2 (n = 158).

BAI underestimates BF in 8.2% and 10% of women and in 2.6–4.3% of patients with severe obesity [15,28,29] and in 7.6% of overweight post-menopausal women using DXA as the reference method [30]. BAI also underestimates BF in 5% and 5.5% of severely obese women compared to plethysmography [29,31].

In the present study, BAI has a positive linear relationship and a strong intraclass correlation (0.74) with BIA. Gibson *et al.* [28] and Geliebter *et al.* [29] also found significant correlations between BIA and BAI in women with severe obesity (0.86 [28] and 0.87 [29]). The correlation between methods was higher in females (0.75 versus 0.68), as observed in other studies (BMI 17–55 kg m^{-2}; correlation of 0.82 versus 0.74) [25]. BAI underestimated BF by 2.5% only in males (P = 0.04).

BAI also underestimated BF when the WHR was above 1.05. After linear regression analysis, sex lost significance, suggesting that fat distribution affects BAI accuracy. A recent Chinese study (BMI = 28 kg m^{-2}) demonstrated a higher correlation between DXA-BF and WC than with HC in men (0.85 versus 0.81) [25].

In the present study, we did not compare BAI with a gold-standard method of body fat estimation such as DXA. However, our results were closely similar to other studies [24–26,28–30] that used DXA and plethysmography to assess BAI accuracy in patients with severe obesity. BAI was a good method for population assessment, although it presents some limitations in these populations. We also did not consider the effects of nutraceuticals and diet in body composition analysis by BIA [32].

The new equation (MBAI) proposed for BF estimation in severe obesity corrects the limitations observed with BAI. The method remained simple (requiring only WC, HC, height and weight) and the variables included were able to explain 65% of the BF variation. The mean difference between BAI and BIA was reduced from 1.2% (P = 0.029) to 0.4% (P = 0.18) with MBAI.

MBAI eliminated limitations previously observed in BAI. The differences were reduced in males [from 3.1% (4.8%) to 0.3% (3.5%)], in individuals with WHR ≥ 1.05 [from 7.2% (6.15) to 1.2% (4.4%)] and in patients with a BMI between 35 and 49.99 kg m^{-2} [from 2.3 (5.5) to 0.7 (4.4)]. The correlations were strengthened in males (0.6–0.8), in the group with BMI > 50 kg m^{-2} (0.4–0.6) and when the WHR ≥ 1.05 (0.4–0.8).

We emphasise the importance of a simple and inexpensive method for BF estimation in countries where the availability of sophisticated equipment is not wide.

In conclusion, BAI had limitations with respect to estimating BF in patients with severe obesity, especially with respect to WHR and obesity grade. The new equation proposed (MBAI) correctly determines BF in this population and can be widely used in clinical practice.

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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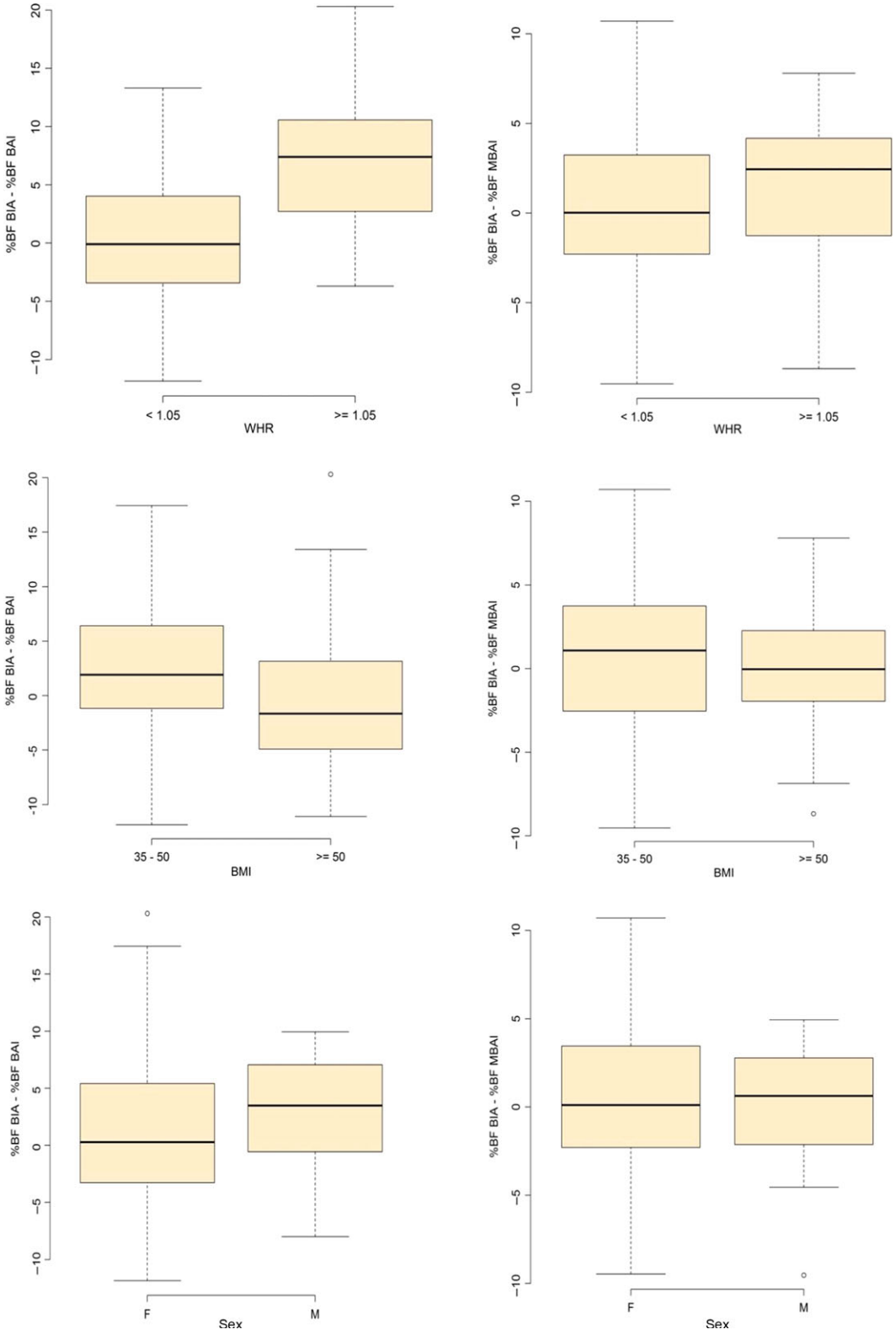

Figure 2 Differences between bioelectrical impedance analysis (BIA) and body adiposity index (BAI) and between BIA and modified body adiposity index (MBAI) in patients with severe obesity (Group 2) according to waist–hip ratio (WHR), body mass index (BMI) and sex (n = 158). BF, body fat; F, female; M, male.

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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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RC and MAS contributed equally to this work. RC and MAS were responsible for the study conception and design, and also drafted and critically revised the manuscript. ABB, VMS, MPS and DP were responsible for the measurement of body circumferences, body adiposity index calculations and bioelectrical impedance analysis in all patients. All authors participated in the analysis and interpretation of data. All authors contributed to and have approved the final manuscript submitted for publication. All authors take public responsibility for its content.

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OBESITY AND RELATED DISORDERS

Waist-to-height ratio is independently related to whole and central body fat, regardless of the waist circumference measurement protocol, in non-alcoholic fatty liver disease patients

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Abstract

Background: Waist-to-height ratio (WHtR) has been reported as a preferable risk related body fat (BF) marker, although no standardised waist circumference measurement protocol (WCmp) has been proposed. The present study aimed to investigate whether the use of a different WCmp affects the strength of relationship between WHtR and both whole and central BF in non-alcoholic fatty liver disease (NAFLD) patients.
Methods: BF was assessed with dual energy X-ray absorptiometry (DXA) in 28 NAFLD patients [19 males, mean (SD) 51 (13) years and nine females, 47 (13) years]. All subjects also underwent anthropometric evaluation including height and waist circumference (WC) measurement using four different WCmp (WC1, minimal waist; WC2, iliac crest; WC3, mid-distance between iliac crest and lowest rib; WC4, at the umbilicus) and WHtR was calculated using each WC measurements (WHtR1, WHtR2, WHtR3 and WHtR4, respectively). Partial correlations were conducted to assess the relation of WHtR and DXA assessed BF.
Results: All WHtR were particularly correlated with central BF, including abdominal BF ($r = 0.80$, $r = 0.84$, $r = 0.84$ and $r = 0.78$, respectively, for WHtR1, WHtR2, WHtR3 and WHtR4) and central abdominal BF ($r = 0.72$, $r = 0.77$, $r = 0.76$ and $r = 0.71$, respectively, for WHtR1, WHtR2, WHtR3 and WHtR4), after controlling for age, sex and body mass index. There were no differences between the correlation coefficients obtained between all studied WHtR and each whole and central BF variable.
Conclusions: Waist-to-height ratio was found a suitable BF marker in the present sample of NAFLD patients and the strength of the relationship between WHtR and both whole and central BF was not altered by using different WCmp in the present sample of NAFLD patients.

Introduction

Waist-to-height ratio (WHtR) is an index of abdominal obesity initially proposed by Hsieh and Yoshinaga in the 1990s [1,2]. At that time, WHtR was suggested to be a better predictor of multiple coronary heart disease risk factors than other obesity and fat distribution indices in both men [1] and women [2]. Despite not being accepted consensually [3,4], WHtR was further suggested to be preferable to other indices and clinical assessments, including body mass

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index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR), for predicting cardiovascular risk factors in different ethnic and age groups [5,6]. WHtR also appeears to be at least similarly associated with abdominal fat as is WC, and better than both BMI and WHR [7,8]. To our knowledge, few studies have focused on non-alcoholic fatty liver disease (NAFLD) patients using WHtR [9,10]. These studies have found rather high WHtR in NAFLD patients [9,10] which is concordant with the increased cardiovascular risk found in NAFLD patients [10–14]. It is therefore of utmost important to establish standardised clinical body composition (BC) surrogates, as well as potential therapy targets, particularly in higher risk subpopulations, such as patients with NAFLD.

Despite being a promising clinical marker of BC [8,15] and related cardiometabolic risk [5], there is still some inconsistency considering the WC measurement protocol (WCmp) used to calculate WHtR [16]. Several WCmp have been proposed by sound authorities, and used by prominent researchers, although scientific rational is lacking to recommend one single protocol [17–19]. The association of WC with cardiometabolic risk is independent of WCmp [19]. However, measurements using different WCmp have different magnitudes and therefore are not interchangeable [19]. Proposed protocols differ mainly on the anatomical landmarks and correspondent measuring sites. WHtR was initially proposed using WC measured at the umbilicus [1,2]. In subjects without diagnosed diseases WHtR calculated using WC measured at the umbilicus was suggested to be preferable for the estimation of both whole and trunk body fat (BF); however, only two WC measurement protocols were tested (narrowest point between the lower costal border and the top of the iliac crest and at the level of the umbilicus) [15]. In a recent review on WHtR [16], the WC as measured midpoint between the lowest rib and iliac crest was found to be used in 50% of the reviewed papers and, for that reason, its routine use was encouraged.

To our knowledge, it is unknown whether the use of different commonly used waist circumferences, with different measuring sites, affects the relationship between WHtR and both whole and central BF content in NAFLD patients. The independent magnitude of such a relationship is also unknown. Therefore, the present study aimed to determine which of the most used WCmp is better for calculating WHtR for use in clinical practice with NAFLD patients as a surrogate for whole and central BF.

Materials and methods

Subjects

The present study was conducted at Exercise and Health Laboratory, from the Interdisciplinary Centre for the Study of Human Performance (Faculty of Human Kinetics, Technical University of Lisbon, Portugal). To be selected for the study, subjects had to be aged >18 years of age without a history of hepatotoxic substances intake (e.g. steroids) and tobacco consumption. Exclusion criteria included alcohol consumption >20 g day^{-1}; the presence of other potential causes for fatty liver disease, including viral hepatitis, auto-immune disease and others; any physical and/or mental disabilities or any condition that constituted an absolute restriction to exercise; or other diagnosed diseases, except for metabolic and cardiovascular disease (insulin resistance, hypertension or dyslipidaemia), with mandatory specific pharmacological therapy. We studied 28 NAFLD patients [19 males, mean (SD) 51 (13) years and nine females, 47 (13) years] who were diagnosed via liver biopsy or ultrasound. Cardiorespiratory fitness was assessed as described previously [20] for characterisation purposes. Subjects were recruited from the outpatient medical departments in Santa Maria Hospital and Curry Cabral Hospital; 59 consecutive patients were selected based on selection criteria; 37 of the selected subjects accepted to participate and 28 were found eligible to enter the study after exclusion criteria were considered. Subjects were taking one or more of the following medications: platelet inhibitors, angiotensin-converting enzyme inhibitors, nitrates, statins, ezetimibe, nicotinic acid and biguanides, with similar use among both sexes. All participants provided their informed consent before being included in the present study and undergoing any study procedure. All methods used in the present study complied with ethics and Portuguese laws and were approved by Faculty of Human Kinetics institutional review board for human studies

Body composition

Body composition was assessed using dual energy X-ray absorptiometry (DXA) (Explorer W, Hologic; Waltham, MA, USA; Fan beam mode) whole body scans and anthropometric measurements. Repeated measurements with DXA in 18 young adults showed a coefficient of variation (CV) of 1.7% for total BF mass and 1.5% for total %BF. All scans were made in the morning after an overnight 12-h fast. Quality control with spine phantom was made every morning, and with step phantom every week. By default, DXA software (QDR, version 12.4; Hologic Inc., Marlborough, MA, USA) estimates the head, trunk, arms and legs, both left and right, and region BC, according to a three-compartment model (fat mass, lean tissue and bone mass). The trunk region of interest (ROI) (CV = 0.5%) includes chest, abdomen and pelvis regions from the scan [21]. All scans analysis were made by the same observer. All scans were submitted to additional

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analysis by ROI to assess fat content of the abdominal and central abdominal regions (CV = 1.0%) [21]. The upper and lower limits of the abdominal and central abdominal ROI were determined as the upper edge of the second lumbar vertebra to the lower edge of the fourth lumbar vertebra, respectively [22–24]. The lateral limits of the abdominal ROI were determined to include all trunk length but to exclude any upper limb scan area [23,24], whereas the lateral sides of central abdominal ROI were the vertical continuation of the lateral sides of the ribs cage to exclude lateral subcutaneous fat of the trunk, although including anterior and posterior subcutaneous abdominal fat, as well as intra-abdominal fat [22] (Fig. 1). Absolute and relative BF content results were registered to the nearest 0.01 kg and 0.1%, respectively.

Anthropometric measurements consisted of weight, height and BMI, as well as WC and WHtR. Some standardisation procedures were taken into account, as recommended previously [25], to avoid any bias in the measurements; therefore, all WC measurements were made with subjects in a standing comfortable position, in their underwear, in a 12-h fasting state. All WC measurements were made by the same technician, who was a trained level 2 technician, certified by the International Society for the Advancement of Kinanthropometry, using an inelastic flexible metallic tape (W606PM; Lufkin, Vancouver, BC, Canada) parallel to the floor after a tidal exhalation, to the nearest 0.1 cm. The WC measurement sites in the present study were the narrowest torso (WC1) [26,27], also called minimal waist [19], superior border of the iliac crest (WC2) [18,28], midpoint between the lowest rib and iliac crest (WC3) [29] and umbilicus (WC4) [1,2]. These are the most commonly used protocols endorsed by sound authorities in this field [17,19]. Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm, on a scale with an attached stadiometer (model 770; Seca, Hamburg, Germany), in accordance with a standard protocol [30]. Both weight and height were used to calculate the subjects' BMI (kg m^{-2}]. WHtR was calculated by dividing each WC by the subjects' height [WHtR = WC (cm)/height (cm)]. Because we used four different WCmp for each subject, we calculated four different WHtR using each measured WC. Therefore, WHtR1, WHtR2, WHtR3 and WHtR4 were calculated using WC1, WC2, WC3 and WC4, respectively. We considered a boundary value of 0.5 for the identification of high WHtR [9,31]. All anthropometric measurements were repeated two times and, if the second differed by more than 1 cm (for waist and height measurements) or 0.5 kg (for weight measurement) from the first measurement, a third measurement was carried out. We always considered the result obtained in the second measurement unless a third measurement was carried out. When a third measurement was taken, we considered the mode or, if mode was absent, the median value of all three measurements. By use of this procedure, we aimed to always use the most suitable value that was actually measured on the subjects (instead of mean values).

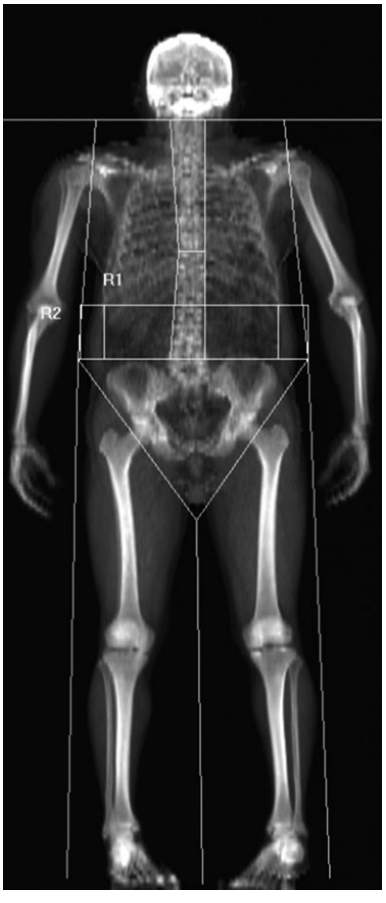

Figure 1 Image of a dual energy X-ray absorptiometry scan showing the abdominal region of interest (R2), defined as the area within the upper edge of the second lumbar vertebra, and the lower edge of the fourth lumbar vertebra and central abdominal region of interest (R1), defined as R2 but the vertical sides limited to the continuation of the lateral sides of the ribs cage.

Statistical analysis

Descriptive statistics are presented as the mean (SD) and range for all analysed variables. The Gaussian distribution of the data was assessed with the Shapiro–Wilk goodness-of-fit test. A paired samples *t*-test was used to compare different WHtR. The association of all WHtR with DXA measures was assessed using partial and semipartial correlations [32], controlling for age, sex and BMI. A statistical power of 80% ($\beta = 0.20$) at a significance level of 5% ($\alpha = 0.05$) was considered

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statistically significant. Consequently, only coefficients of correlation equal or superior to 0.5, corresponding to a large effect size, attained this criteria ($P \leq 0.05$ and $\beta \leq 0.20$) and could be considered significant [this was in accordance with Cohen *et al.* (1983) to ensure that results are unexposed to type I and II errors, despite a rather modest sample size]. Pairs of coefficients of correlation obtained using different WHtR for each DXA measure were compared using a *Z*-statistic to determine whether any WHtR, according to the WC used in its calculation, was more strongly associated with whole and central BF. Statistical calculations were performed using IBM SPSS, version 19 (IBM Corp., Armonk, NY, USA), except for the *Z*-statistic, which was calculated using MEDCALC, version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Mean values for all the studied variables are presented in Table 1. From among the 28 studied NAFLD patients, WHtR above the boundary value of 0.5 was present in almost 100% of the sample, depending on the WCmp used. Results for WC measurements were considered to be different between all studied WCmp (WC4 > WC2 > WC3 > WC1) and the magnitudes of WHtR mean values were also different according to the WC used. Obesity was present in nine subjects (three were female), according to BMI classification, with no differences between sexes in mean BMI ($P = 0.075$ via an independent samples *t*-test).

Table 2 shows the results for partial and semipartial correlations between each WHtR and each whole or

Table 1 Descriptive data fpr the study sample

Variables	NAFLD patients ($n = 28$)		
	Mean (SD)	Minimum – Maximum	
Age, year (median, year)	49.5 (12.8) (49)	25–68	
Sex, *n*, female (% female)	9 (32.1)		
$VO_{2\ max}$ (mL kg^{-1} min^{-1})	24.9 (6.4)	13.8–38.0	
Type 2 diabetes mellitus, *n* (%)	8 (28.6)		
Insulin resistance, *n* (%)	12 (42.9)		
Anthropometry			
Weight (kg) (CV, %)	87.6 (12.7) (0.07)	66.2–115.8	
Height (cm) (CV, %)	167.2 (9.2) (0.03)	149.5–183.7	
BMI (kg m^{-2}) (% obese)	29.1 (4.0) (32.1)	22.6–42.2	
WC 1 (cm) (CV, %)	100.7 (8.2)‡ (0.45)	86.0–119.8	
WC 2 (cm) (CV, %)	104.8 (10.6)‡ (0.49)	85.3–128.7	
WC 3 (cm) (CV, %)	103.7 (10.4)‡ (0.47)	85.7–129.3	
WC 4 (cm) (CV, %)	106.3 (11.7)‡ (0.73)	86.7–129.1	
WHtR 1 (≥0.5, %)	0.60 (0.07)† (96.4)	0.48–0.75	
WHtR 2 (≥0.5, %)	0.63 (0.08)† (100.0)	0.50–0.82	
WHtR 3 (≥0.5, %)	0.62 (0.08)† (96.4)	0.49–0.81	
WHtR 4 (≥0.5, %)	0.64 (0.09)† (100.0)	0.50–0.85	
Whole and regional body composition			
BF (kg) (%)	27.2 (9.3) [31.31 (8.20)]	13.7–51.2	(18.84–46.28)
FFM (kg) (%)	58.7 (9.1) [68.69 (8.20)]	39.6–77.7	(53.72–81.16)
Trunk BF (kg) (%)	15.2 (5.2) [33.15 (7.65)]	7.4–25.0	(20.87–48.01)
Trunk FFM (kg) (%)	29.9 (3.9) [66.85 (7.65)]	21.1–38.6	(51.99–79.13)
Appendicular BF (kg) (%)	10.8 (4.8) [30.42 (10.39)]	5.2–25.7	(13.63–50.40)
Appendicular FFM (kg) (%)	24.5 (5.1) [69.58 (10.39)]	14.9–34.8	(49.60–86.37)
Abdominal BF (kg) (%)	3.5 (1.2) [37.57 (6.59)]	1.7–6.3	(26.09–49.40)
Central abdominal BF (kg) (%)	2.9 (0.8) [35.82 (5.70)]	1.6–5.0	(24.28–44.64)

BF, body fat; BF, body fat; BMI, body mass index; CV, coefficient of variation; FFM, fat free mass; FFM, fat free mass; HRR1, heart rate recovery at 1 min; HRR2, heart rate recovery at 2 min.; Máx., highest observed value; Min., lowest observed value; WC1, waist circumference measured at narrowest torso; WC2, waist circumference measured at iliac crest; WC3, waist circumference measured at midpoint between lowest rib and iliac crest; WC4, waist circumference measured at the umbilicus; WHtR 1, waist-to-height ratio calculated using waist circumference measured at narrowest torso; WHtR 2, waist-to-height ratio calculated using waist circumference measured at iliac crest; WHtR 3, waist-to-height ratio calculated using waist circumference measured at midpoint between lowest rib and iliac crest; WHtR 4, waist-to-height ratio calculated using waist circumference measured at the umbilicus.

Results are presented as the mean (SD), unless otherwise noted.

†Different from all other WHtR mean values. $P < 0.05$ in paired samples *t*-test.

‡Different from all other WC mean values. $P < 0.05$ in paired samples *t*-test.

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Table 2 Partial and semipartial correlations between all studied waist-to-height ratios and body fat content variables

Variables		Whole BF	Trunk BF	Abd BF	C Abd BF	Whole %BF	Trunk %BF	Abd %BF	C Abd %BF
WHtR 1	†	0.49	0.63*	0.81*	0.72*	0.51*	0.56*	0.65*	0.63*
	‡	0.41	0.58*	0.80*	0.72*	0.45	0.51*	0.66*	0.63*
	§	0.22	0.38*	0.70*	0.69*	0.22	0.32	0.54*	0.55*
WHtR 2	†	0.61*	0.73*	0.82*	0.74*	0.56*	0.59*	0.61*	0.61*
	‡	0.48	0.64*	0.84*	0.77*	0.46	0.52*	0.66*	0.63*
	§	0.26	0.43	0.74*	0.74*	0.23	0.32	0.54*	0.55*
WHtR 3	†	0.60*	0.72*	0.83*	0.74*	0.55*	0.59*	0.62*	0.61*
	‡	0.48	0.64*	0.84*	0.76*	0.46	0.52*	0.66*	0.62*
	§	0.25	0.42	0.74*	0.73*	0.22	0.32	0.54*	0.54*
WHtR 4	†	0.59*	0.68*	0.76*	0.68*	0.51	0.53*	0.56*	0.57*
	‡	0.44	0.58*	0.78*	0.71*	0.42	0.45	0.62*	0.60*
	§	0.23	0.38	0.68*	0.67*	0.20	0.27	0.49	0.50*

Abd BF, Abdominal body fat; BF, body fat; C Abd BF, Central abdominal body fat; Trunk BF, Trunk body fat; WHtR 1, waist-to-height ratio calculated using waist circumference measured at narrowest torso; WHtR 2, waist-to-height ratio calculated using waist circumference measured at iliac crest; WHtR 3, waist-to-height ratio calculated using waist circumference measured at midpoint between lowest rib and iliac crest; WHtR 4, waist-to-height ratio calculated using waist circumference measured at the umbilicus.

*Significant for $P < 0.05$ and $\beta = 0.20$.

†Partial correlations between studied WHtR and dependent variables, controlled for age and sex.

‡Partial correlations between studied WHtR and dependent variables, controlled for age, sex and body mass index.

§Semipartial correlations between studied WHtR and dependent variables, adjusted for age, sex and body mass index.

central studied BF depot, controlling for sex, age and BMI. All WHtR were correlated with the studied BF depots, even after adjusting for age, sex and BMI, showing coefficients of correlation magnitudes above 0.5. Coefficients of correlation tended to decrease as control variables were added, particularly when the effect of age, sex and BMI was removed; however, the strength of association remained for abdominal fat depots.

Table 3 shows the results for the comparison (*P*-values) between pairs of competing WHtR coefficients of correlation with each dependent variable, as listed in Table 2. No differences were found between all compared coefficients of correlation.

Discussion

To our knowledge, this is the first report to focus on the strength of correlation between WHtR and BF in NAFLD patients, as well as its variation associated with the different WCmp used to calculate WHtR. Mean WHtR was reasonably high and the prevalence of elevated WHtR, considering the 0.5 boundary value, was very high in the present sample. This was expected because NAFLD patients have high values of WHtR [9,10]. The magnitudes of the WHtR mean values were different according to the WC (WHtR4 > WHtR2 > WHtR3 > WHtR1) used in its calculus, meaning that they are not interchangeable. This may have large implications in clinical practice and data collection and in the interpretation in longitudinal assessments (pre – post), as well as for between-group comparisons. Several previous studies have reported WC magnitudes (the changeable component of WHtR) to be influenced by WCmp [33–35]. It has been proposed that current WC thresholds, generalised using WHO protocol (at the midpoint between lowest rib and iliac crest), could be applied to National Institutes of Health measurements (at the superior border of the iliac crest) [19] because of the small or absent differences, particularly in men, found between measurements using these WCmp [34,35]. As noted, the present study does not confirm such interchangeability when absolute values were taken into account. However, when a dichotomous approach was applied based on the boundary value of 0.5, both WHtR1 and WHtR2 only misclassified one subject (3.6%) at elevated risk compared to WHtR2 and WHtR4, which diagnosed 100% of the sample above the boundary value, and may be considered as support for an interchangeable use of the protocols for WHtR assessment.

In the present sample of NAFLD patients, as expected, WHtR was highly associated with whole and central BF, adjusted for age, sex and BMI. Correlation coefficient magnitudes revealed a large effect size ($r > 0.5$) for central BF depots. The association of WHtR with BC, particularly with central BF, has been reported in diverse groups [7,8] but not in NAFLD patients until now. WHtR was also shown to predict higher cardiometabolic risk better than WC and BMI [5]. The present study showed consistent coefficients of correlation of WHtR and central fat depots, even when BMI was added to age and sex as control variables, meaning that WHtR explains the

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Table 3 *Z*-statistic *P*-values for the comparison between the coefficients of correlation obtained in partial and semipartial correlation between the studied waist-to-height ratios and all dependent variables.

		WHtR 1		WHtR 2		WHtR 3		WHtR 4			
		*P**	*P*†	*P**	*P*†	*P**	*P*†	*P**	*P*†		
				0.98	0.99	0.99	1.00	0.89	0.93	%BF	WHtR 1
				0.99	0.99	0.97	0.98	0.76	0.86	Trunk %BF	
				1.00	1.00	1.00	1.00	0.81	0.80	Abd %BF	
				0.98	0.99	0.99	0.99	0.86	0.84	C Abd %BF	
WHtR 2	BF	0.73	0.87			0.99	0.99	0.87	0.92	%BF	WHtR 2
	Trunk BF	0.72	0.86			0.98	0.99	0.75	0.85	Trunk %BF	
	Abd BF	0.66	0.80			0.99	1.00	0.80	0.80	Abd %BF	
	C Abd BF	0.71	0.74			0.97	0.98	0.84	0.83	C Abd %BF	
WHtR3	BF	0.79	0.90	0.94	0.97			0.88	0.93	%BF	WHtR 3
	Trunk BF	0.74	0.87	0.98	0.99			0.73	0.84	Trunk %BF	
	Abd BF	0.65	0.79	0.98	0.99			0.81	0.81	Abd %BF	
	C Abd BF	0.74	0.78	0.96	0.96			0.87	0.85	C Abd %BF	
WHtR4	BF	0.88	0.96	0.85	0.91	0.91	0.94				
	Trunk BF	0.98	0.98	0.70	0.84	0.72	0.85				
	Abd BF	0.72	0.87	0.54	0.68	0.52	0.67				
	C Abd BF	0.95	0.90	0.66	0.65	0.70	0.68				

Abd BF, abdominal body fat; BF, body fat; C Abd BF, central abdominal body fat; trunk BF, Trunk body fat; WHtR 1, waist-to-height ratio calculated using waist circumference measured at minimal waist; WHtR 2, waist-to-height ratio calculated using waist circumference measured at iliac crest; WHtR 3, waist-to-height ratio calculated using waist circumference measured at midpoint between lowest rib and iliac crest; WHtR 4, waist-to-height ratio calculated using waist circumference measured at the umbilicus.

*Comparison between correlation coefficients obtained in partial correlations between different WHtR and all dependent variables, controlled for age, sex and body mass index.

†Comparison between correlation coefficients obtained in semipartial correlations between different WHtR and all dependent variables, controlling for age, sex and body mass index.See bottom-left half for comparisons between coefficients of correlation obtained between WHtRs and obsolute values of body composition; see upper-right half for comparisons between coefficients of correlation obtained between WHtRs and relative values of body composition.

variation of abdominal fat far beyond BMI. This relationship was already found in subjects without NAFLD, although with no control variables included in the analysis [15]. This may explain the marginally lower correlation coefficients found in the present study.

Comparisons between pairs of competing WHtR correlation results with each dependent variable showed that all studied WHtR are similarly associated with the analysed BF depots, irrespective of the WC used for its calculation. Previous studies have already shown no differences in the association of WC alone, measured at different sites, with BF depots [33,35]. A recent review concluded that the use of different WCmp does not change the well-established relationships between WC and morbidity of cardiovascular disease and diabetes, as well as cardiovascular and all-cause mortality [19]. However, because WHtR have proven to be more sensitive in the prediction of cardiovascular risk, the absence of an influence of WCmp in risk prediction should be confirmed when WC is used to calculate WHtR.

There are several strengths and limitations to the present study. The WCmp investigated do not cover all the protocols existent in the literature, although the focus was on those most commonly employed and endorsed by prominent institutions for use in the clinical setting [17–19]. In addition, the assessment method (DXA) used for BC, comprising a gold standard instrument for assessing BC in a three-compartment model, is unable to determine visceral adiposity independent of subcutaneous fat. However, there is a strong correlation between abdominal fat estimated from selected DXA ROI and visceral fat assessed by magnetic resonance imaging [23] and computed tomography [36]. Patients' physical activity and diet were not assessed; however, patients' cardiorespiratory fitness was assessed, which was low (Table 1), reinforcing the importance of the study of cardiovascular risk related markers in this population [37]. Finally, we could not establish the usefulness of WHtR for assessing changes in BF depots based on the present results because we used a cross-sectional approach and therefore no follow-up data are available.

The present study confirms the strong association between WHtR and BF, especially for central BF, even after controlling for age, sex and BMI, in NAFLD patients, supporting WHtR as an independent central obesity index. Moreover, the relationship between WHtR and both whole and central BF was not altered by the choice of a particular

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WCmp in the present sample of NAFLD patients. Unlike previous studies in subjects without diagnosed NAFLD [15], we could not recommend the use of one specific WC measurement protocol over another for the calculation of WHtR as a whole and/or central BF surrogate. Thus, the results of the present study may endorse an interchangeable use of different WCmp for identifying a subject's WHtR above the boundary value. Additional research is needed to confirm the influence of different WCmp on the variation of WHtR in specific subpopulations, as well as on the relationship between WHtR and other NAFLD and cardiometabolic risk factors beyond BC alone.

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 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.

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NMP, HC-P and HS-C contributed equally to the conception and design of the research. NMP and XM contributed to the acquisition, analysis and interpretation of the data. HC-P and JS-N contributed to the acquisition of data. HS-C contributed to the analysis of data. LBS contributed to the interpretation of the data. NNP drafted the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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OBESITY AND RELATED DISORDERS

Body composition of obese adolescents: association between adiposity indicators and cardiometabolic risk factors

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anthropometry, adolescence, body composition, cardiovascular disease, obesity.

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Abstract

Background: The association between obesity during adolescence and the increased risk of cardiometabolic diseases indicates the need to identify reproducible and cost effective methods for identifying individuals who are at increased risk of developing diseases. The present cross-sectional study investigated the occurrence of metabolic consequences of obesity in adolescents and the use of adiposity indicators as predictors of cardiometabolic risk.
Methods: A fasting blood sample was taken in 93 pubertal obese adolescents aged 13–18 years old (39 males, 54 females) for the assessment of cardiometabolic risk markers (glucose, lipid profiles, insulin resistence, and inflammatory and endothelial dysfunction markers). Together with anthropometry, total fat mass and lean mass were determined by dual-energy X-ray absorptiometry (DXA).
Results: The prevalence of dyslipidaemia and disorders in glucose metabolism are noticeably higher in the present study. There was no correlation between the percentage of body fat according to DXA and most indicators of adiposity. For boys, the arm circumference values predicted the increase in fasting insulin ($r^2 = 0.200$), homeostasis model assessment of insulin resistance ($r^2 = 0.267$) and cardiometabolic risk score ($r^2 = 0.338$). The percentage of body fat according to DXA predicted the inflammation score ($r^2 = 0.172$). For girls, body mass index was the parameter that best described the variability of fasting insulin ($r^2 = 0.079$) and inflammation score ($r^2 = 0.263$). The waist-to-stature ratio was able to predict the triglyceride values ($r^2 = 0.090$).
Conclusions: Anthropometric measures of adiposity, such a body mass index, waist-to-stature ratio, arm circumference and waist circumference, should be considered in the clinical evaluation of obese adolescents.

Introduction

Obesity in children and adolescents, defined as a body mass index (BMI) ≥95% for children of the same age and sex, is characterised by an excessive accumulation of body fat that is often associated with health problems, such as hypertension, heart disease and diabetes, as well as an increased risk of emotional problems [1,2].

The occurrence of being overweight and obesity (overweight) has increased in recent decades among children and adolescents in various regions of the world. In Brazil, between 1974 and 2009, the occurrence of excess weight among adults almost tripled, resulting in 49% of these individuals being overweight and 14.6% being obese. A focal point was the prevalence in children (47.8%) and adolescents (21.5%), which showed percentage increases of three- to four-fold over the same period [3].

Adolescence is characterised by significant changes in body composition, especially during puberty. Monitoring these changes is important because many aspects of this

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composition, such as weight, body fat and lean tissue, are predictive characteristics of adulthood [4].

The association between obesity during childhood and adolescence and the increased risk of the development of cardiometabolic diseases indicates the need for reproducible and inexpensive methods that identify individuals who are at greatest risk [5]. Studies show that the BMI evaluation is essential, and the use of additional anthropometric indices is required to describe the distribution of body fat accurately [6]. Although there are more accurate methods of determining the body fat content such X-ray absorptiometry (DXA), these methods are not practical for epidemiological studies [7]. In turn, indirect measures such as waist circumference (WC), skinfold, arm circumference (AC), waist-to-hip ratio (WHR) and the waist-to-stature ratio (WSR), are methods that can be used to assess adiposity [8].

The present study aimed to examine the occurrence of inadequacy in biochemical levels in obese adolescents and to evaluate their relationship with anthropometric indicators of abdominal or generalised adiposity as a screening instrument for discriminating individuals at high cardiometabolic risk.

Materials and methods

Ethical aspects

This research was approved by the Ethics and Research Committee of the University of Pernambuco under number 739.695, meeting all the requirements of the National Health Council – Resolution 466/2012. All legal caregivers and teenage volunteers provided their written informed consent form after being informed of the possible risks and benefits of the study.

Study population

The study was a cross-sectional study with a sample defined by convenience and duration from January to April 2013. Adolescents were recruited through invitation via the media in the metropolitan area of Recife, Pernambuco, Brazil. An initial medical evaluation was conducted for inclusion in the study, for both health diagnosis and the classification of the stage of sexual maturation. Inclusion criteria were: age between 13 and 18 years of age, obesity (BMI >95th percentile for age and sex) according to the criteria of the Centers for Disease Control and Prevention [9] and a maturational puberty stage (stages 3 and 4) according to Tanner's criteria [10]. Each participant received drawings of the five stages of breast size, genital and pubic development [10]. In an isolated room, the teenagers were asked to look at the drawings, read the descriptive text, think about how they compared with the overall illustrations (i.e. drawings and descriptive text) and choose the one that resembled them. For boys, the images of genitalia and pubic hair were classified into five stages of development. The same classification applied to the images of breasts and pubic hair among girls.

Exclusion criteria were: having chronic diseases such as diabetes and hypertension; chronic use of alcohol; smoking; use of anti-inflammatory or other drugs that alter metabolism; and pregnancy.

Anthropometry and body composition

The weight was measured on a Filizola® (Filizola, São Paulo, Brazil) balance (accurate to 0.1 kg), whereas height was measured using a wooden stadiometer (accurate to 0.1 cm). After taking the measurements of weight and height, BMI (kg m^{-2}) was calculated.

The waist circumference (midpoint between the lower margin of the last rib and the iliac crest in the horizontal plane) [11] and hip circumference (HC) (measured at the gluteal region at the widest circumference between the waist and the knees) [11] were measured with a metric, flexible and inelastic tape, divided into centimetres and subdivided into millimetres, and the measurements were performed in triplicate and the average was used. The WHR and WSR were calculated. The AC was performed on the right arm, at the midpoint between the acromion and the olecranon, with the arm bent close to the body [11].

The triceps skinfold (TSF) and subscapularis (SSF) were measured with a Lange® (Power Systems, Inc., Tennessee, USA) caliper on the right side of the body. After three measures, the average was calculated. The percentage of fat according to the sum of skinfolds (% Fat SF) was estimated by equation of Slaughter *et al.* [12], which is based on triceps and subscapular skinfold values.

The DXA method was used for the total body fat and total lean mass. For the DXA examination, Hologic QDR WI equipment was used (Hologic Inc., Waltham, MA, USA) and a standard procedure for the placement of adolescents was adopted. The composition software provided the fat mass values and fat-free mass (lean body mass and bone mineral content) (g) to the legs and arms on both sides of the body, which together made up the references of the regional composition of upper and lower members. They were also considered as regional compositions for the trunk and head, completing the information to obtain the total fat mass, reported as a percentage (% Fat DXA) and total lean mass (kg). The subject remained lying supine on the table until the end of the scan, which lasted 6 min. The feet were kept together and the arms arranged along the side of the trunk.

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Professionals specifically trained for the study performed all of the evaluations.

Laboratory analysis

Blood samples were taken after an overnight fast of 12 h to test fasting glucose (FG), fasting insulin (FI), total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-chol) low-density lipoprotein-cholesterol (LDL-chol), leptin, interleukin (IL)-6, tumor necrosis factor (TNF)-α, intercellular adhesion molecule-1 (sICAM) and vascular cell adhesion molecule-1 (sVCAM).

FG, FI, TC, HDL-chol and TG were determined by the automated enzymatic method (Cobas Integra 400; Roche Diagnostics, Basel, Switzerland). The LDL-chol was calculated using the Friedewald formula [13]. IL-6, leptin, TNF-α, sICAM and sVCAM were determined by the enzyme-immunoassay technique, using commercial enzyme-linked immunosorbet assay kits (Phoenix Pharmaceuticals Inc., Belmont, CA, USA) in accordance with the manufacturer's instructions.

For dyslipidaemia and hyperinsulinaemia, the cut-off points recommended by the Brazilian Society of Cardiology [14] were used and, for impaired fasting glucose, those of the American Diabetes Association [15] ≥100 mg dL^{-1} were recommended. Insulin resistance was determined by the formula of the homeostasis model assessment of insulin resistance (HOMA-IR) = ([fasting insulin ($\mu U\ mL^{-1}$) × fasting glucose ($mmol\ L^{-1}$)]/ 22.5 ≥ 3.43) [16].

Combined risk factors

Aggregation of risk factors was expressed continuously by the sum of the *Z*-scores for each metabolic risk factor assessed. Four cardiometabolic risk profiles were created, in an adaption of the method described by Okosun *et al.* [17]. High scores indicate worse profiles.

Metabolic risk score (MRS): *Z*-scores were obtained for the variables WC, TC, LDL-chol, TG, FG, FI and HOMA-IR using the formula: *Z*-score = [(value-average)/SD]. The scores for the HDL-C were obtained by the inverted formula (*Z*-score = [(average-value)/SD]) because of its inverse relationship with cardiovascular risk [17].

Inflammation score (IS): *Z*-scores were obtained for the variables IL-6, TNF-α and leptin.

Endothelial dysfunction score (EDS): *Z*-scores were obtained for sICAM and sVCAM variables.

Cardiometabolic risk score (CRS): The results were from the sum of MRS, IS and EDS.

Statistical analysis

Data were analysed using SPSS, version 20.0 (IBM Corp., Armonk, NY, USA.). Initially, the continuous variables were tested for normal distribution using the Kolmogorov–Smirnov test. When the variables showed normal distribution, they were described as average, and the SD and Student's *t*-test was applied. When a non-normal distribution was presented, the variables were described as median and interquartile range, using the Mann-Whitney *U*-test for its analysis.

The association between categorical variables was evaluated by the chi-squared test, and Pearson correlation was used to analyse the correlation between adiposity indicators. Correlations above 0.6 were considered as strong; correlations between 0.4 and 0.6 were considered as moderate, and correlations below 0.4 were considered as considered weak.

Linear regression analysis was used to quantify the effect of adiposity indicators on cardiometabolic risk factors. Regression analyses were all corrected by age to isolate any influence of this variable on the results, and the coefficient of determination (r^2) was used to confirm the increase in variance explained in the model. $P < 0.05$ was considered statistically significant.

Results

One hundred and seven adolescents met the inclusion criteria of the present study. However, fourteen were excluded from the analysis because of a lack of biochemical data, resulting in a sample size of 93 pubescent teenagers.

Table 1 shows the anthropometric and biochemical characteristics of the study sample. Boys had a higher stature, WHR, % Fat SF and total lean mass by DXA compared to their female peers. In turn, the averages for BMI, HC, AC and % Fat DXA were significantly higher in girls. There was a significant difference between the mean values of body fat percentage obtained by DXA and by the sum of skinfolds, particularly among boys ($P < 0.001$).

When analysing biochemical indicators, boys had a greater average of TG, TNF-α and sVCAM. The mean serum leptin concentration was significantly higher among girls. For other biochemical variables, as well as for metabolic risk scores, inflammation, endothelial dysfunction and cardiometabolic risk, no significant differences were identified between the sexes.

As shown in Table 2, there was high prevalence of inadequacy in biochemical parameters indicative of metabolic risk among adolescents. Of particular note, there was a high level of HDL-chol <45 mg dL^{-1} (75.3%) and a prevalence of hypertriglyceridaemia in boys ($P = 0.007$).

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Table 1 General characteristics of obese adolescents, according to sex

Variables	Total (n = 93)	Male (n = 39)	Female (n = 54)	P-value
Age (years)*	14.39 (13.75–15.92)	14.38 (13.85–15.85)	14.54 (13.56–16.31)	0.686
Weight (kg)	96.12 (13.60)	96.94 (13.70)	95.52 (13.62)	0.622
Height (m)	1.65 (0.07)	1.69 (0.07)	1.62 (0.05)	<0.001
BMI (kg m^{-2})	34.99 (4.09)	33.43 (3.25)	36.11 (4.29)	0.001
WC (cm)	97.98 (9.60)	98.77 (8.94)	97.24 (10.08)	0.451
HC (cm)	97.88 (9.60)	113.46 (8.10)	118.28 (9.42)	0.012
AC (cm)	37.55 (3.61)	35.87 (2.74)	37.61 (3.54)	0.012
WHR	0.84 (0.07)	0.87 (0.06)	0.82 (0.07)	0.001
WSR	0.59 (0.05)	0.58 (0.05)	0.59 (0.06)	0.147
Fat DXA (%)	50.49 (4.95)	47.22 (4.64)	52.85 (3.69)	<0.001
Fat SF (%)	55.66 (5.24)	59.99 (4.66)	52.54 (4.66)	<0.001
Lean mass DXA (kg)	44.68 (7.29)	48.18 (7.73)	42.14 (5.81)	<0.001
FI (mU L^{-1})	24.72 (11.67)	24.10 (12.22)	25.17 (11.36)	0.664
HOMA-IR	5.09 (2.48)	4.96 (2.73)	5.19 (2.31)	0.669
TG (mg dL^{-1})	129.73 (65.32)	149.76 (71.70)	115.25 (56.68)	0.011
HDL-chol (mg dL^{-1})	40.15 (9.16)	39.02 (6.82)	40.96 (10.52)	0.316
LDL-chol (mg dL^{-1})	104.92 (31.32)	103.31 (32.51)	106.08 (30.69)	0.676
TC (mg dL^{-1})	171.02 (37.44)	172.22 (39.78)	170.14 (36.01)	0.793
IL-6 (pg mL^{-1})*	0.82 (0.82–0.99)	0.82 (0.82–0.82)	0.82 (0.82–1.01)	0.235
TNF-α (pg mL^{-1})	12.56 (6.28)	14.07 (6.23)	11.47 (6.14)	0.048
Leptin (μg dL^{-1})	3.41 (1.46)	2.76 (1.33)	3.87 (1.37)	<0.001
sICAM (ng mL^{-1})*	237 (178–284)	250 (196–285)	226.00 (163.50–284.25)	0.319
sVCAM (ng mL^{-1})	751.46 (177.99)	809.05 (172.95)	709.87 (171.29)	0.007
MRS	0.00 (3.27)	0.38 (3.50)	−0.27 (3.09)	0.336
IS	0.00 (1.97)	−0.32 (1.74)	0.23 (2.11)	0.175
EDS	0.00 (1.49)	0.33 (1.27)	−0.24 (1.59)	0.066
CRS	0.00 (4.32)	0.39 (4.55)	−0.28 (4.16)	0.460

*Values reported as the median (interquartile interval).

AC, arm circunference; BMI, body mass index; CRS, cardiometabolic risk score; EDS, endothelial dysfunction score; Fat DXA, body fat according to dual beam X-ray absorptiometry; Fat SF, body fat according to the sum of skinfolds; FG, fasting glucose; FI, fasting insulin; HC, hip circunference; HDL-chol, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistence; IL-6, interleukin 6; IS, inflammation score; LDL-chol, LDL cholesterol; Lean mass DXA, lean mass total according to dual beam X-ray absorptiometry; MRS, metabolic risk score; sICAM, intercellular adhesion molecule-1; sVCAM, vascular-1 cell adhesion molecule; TC, total cholesterol; TG, triglycerides; TNF, tumor necrosis factor; WC, waist circunference; WHR, waist-to-hip ratio; WSR, waist-to-stature ratio.

Table 2 Prevalence of inadequacy in variables indicative of metabolic risk in obese adolescents

Variables	Both sexes, n (%)	Male, n (%)	Female, n (%)	P-value
FG ≥ 100 mg dL^{-1}	7 (7.5)	4 (10.3)	3 (5.6)	0.448
FI ≥ 15 mU L^{-1}	71 (76.3)	30 (76.6)	41 (75.9)	0.911
HOMA-IR ≥ 3.43	62 (66.7)	25 (64.1)	37 (68.5)	0.656
TG ≥ 100 mg dL^{-1}	62 (66.7)	32 (82.1)	30 (55.6)	0.007
TC ≥ 150 mg dL^{-1}	64 (68.8)	27 (69.2)	37 (68.5)	0.942
HDL-chol < 45 mg dL^{-1}	70 (75.3)	31 (79.5)	39 (72.2)	0.423
LDL-chol ≥ 100 mg dL^{-1}	51 (54.8)	21 (53.8)	30 (55.6)	0.870

FG, fasting glucose; FI, fasting insulin; HDL-chol, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistence; LDL-chol, LDL cholesterol; TC, total cholesterol; TG, triglycerides.

In most cases, the correlation coefficients between the different measures of adiposity showed a strong correlation (Table 3). There was no correlation between the percentage of body fat according to DXA and most indicators of adiposity. A similar result was seen in the relationship between WHR and most other variables. For boys, only the % Fat SF correlated with % Fat DXA (r = 0.36), whereas, for girls, it was correlated with BMI (r = 0.50), % Fat SF (r = 0.27) and AC (r = 0.51).

Table 4 reports the regression analysis according to sex. For boys, the AC values predicted the increase in fasting insulin and HOMA-IR. When risk scores were evaluated, WSR was found to be the best predictor of MRS and % Fat DXA predicted IS. The AC was also the best predictor of the CRS values.

For girls, other indices of body adiposity were shown to be able to predict cardiometabolic risk variables. The BMI was able to predict the variability of fasting insulin, whereas WSR was able to predict TG values. HOMA-IR values could be best provided by % Fat SF. Similar to boys, WSR was the best predictor of MRS in girls.

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Table 3 Correlation coefficients between the indicators of adiposity in obese adolescents according to sex

	BMI	WC	WHR	WSR	Fat DXA	Fat DC	AC
Male (n = 39)							
BMI	1.00						
WC	0.77***	1.00					
WHR	0.20	0.61***	1.00				
WSR	0.71***	0.61***	0.69***	1.00			
Fat DXA	0.12	0.19	0.03	0.28	1.00		
Fat SF	0.51**	0.44**	0.16	0.43**	0.36*	1.00	
AC	0.74***	0.65***	0.21	0.46**	0.07	0.41**	1.00
Female (n = 54)							
BMI	1.00						
WC	0.84***	1.00					
WHR	0.27*	0.61***	1.00				
WSR	0.82***	0.94***	0.64***	1.00			
Fat DXA	0.50***	0.22	−0.24	0.26	1.00		
Fat SF	0.47***	0.39***	0.20	0.29*	0.27*	1.00	
AC	0.84***	0.73***	0.21	0.67***	0.51***	0.50***	1.00

*P < 0.05; **P < 0.01; ***P < 0.001.

AC, arm circumference; BMI, body mass index; Fat DXA, body fat according to dual beam X-ray absorptiometry; Fat SF, body fat according to the sum of skinfolds; WC, waist circumference; WHR, waist-to-hip ratio; WSR, waist-to-stature ratio.

However, BMI was the parameter that best described the variability of IS, whereas the WC better predicted the increase in CRS.No body composition parameter was able to predict the variability of HDL-chol and EDS in both sexes.

Discussion

The present study reports the prevalence of variables indicative of metabolic risk in obese adolescents and determines the relationship between adiposity indicators and multiple cardiometabolic risk markers, alone or grouped.

Similar to the findings of other studies [18,19], girls had higher levels of body fat, which can be explained by hormonal fluctuations and changes in body composition resulting from puberty. During this stage, the boys gain a greater amount of bone mass and lean body mass, tending to deposit fat in the central region, and assuming an android form, whereas girls have significantly more fat deposited preferentially in the thighs and hip, assuming a gynaecoid form [20].

Although girls have higher average levels of body fat than boys, the lipid profile of women appears to be less affected by obesity, considering the average obtained for the concentration of TG and the prevalence of significant increase in plasma TG among male teenagers. This could be partly explained by the differences in body fat distribution of boys and girls during puberty discussed above. This location of the fat in most visceral regions in boys is closely related to changes in the metabolic profile and the liberation of various molecules with pro-inflammatory action, such as TNF-α, IL-6 and sICAM [21–24].

Serum leptin levels were shown to be higher in girls compared to those observed in boys. This result is already reported in the literature [25,26]. Koester-Weber *et al.*[27] evaluated leptin levels in adolescents in the same age group as those in the present study and observed a positive correlation between the concentration of the hormone and BMI in both sexes. However, leptin levels among boys did not suffer significant variation with age, unlike in girls, who had a higher leptin concentration with increasing age. Demerath *et al.*[28] already noted this difference in leptin levels between the sexes during puberty, suggesting an interaction with gonadal steroids because oestradiol has a stimulatory effect on the leptin concentration, in contrast to the suppressive effect of testosterone [21].

Regarding the correlation between obesity indicator variables, we found that no measure was strongly correlated with body fat percentage determined by DXA, which many considered to be the gold standard for field research [29]. It is important to highlight the difference between the body fat percentage measured by DXA and the sum of skinfolds between the sexes, as supported by the low correlation between these variables (r = 0.36 and 0.27 for boys and girls, respectively). It is likely that this discrepancy between % Fat DXA and % Fat SF in the study results is derived from the location of the skinfolds used in the equation of Slaughter *et al.*[12]. One should also consider ethnic differences because the sample for validation of the equation of Slaughter *et al.*[12] included

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Table 4 Association between cardiometabolic risk factors and indicators of adiposity in obese adolescents

Male (n = 39)	Fasting insulin			Triglycerides			HDL-cholesterol			HOMA-IR		
	β	r^2	P	β	r^2	P	β	r^2	P	β	r^2	P
BMI	0.485	0.108	0.080	3.977	0.047	0.275	−0.515	0.067	0.137	0.068	0.175	0.599
WSR	51.810	0.171	0.072	284.381	0.084	0.167	−26.382	0.073	0.118	9.909	0.227	0.109
WHR	39.372	0.118	0.307	215.813	0.038	0.360	−31.489	0.061	0.159	5.711	0.180	0.491
Fat DXA	0.094	0.093	0.825	−0.295	0.015	0.909	0.010	0.007	0.967	0.037	0.173	0.686
Fat SF	0.236	0.099	0.575	3.877	0.078	0.126	−0.141	0.016	0.566	0.058	0.178	0.522
AC	1.495	0.200	0.034	6.533	0.075	0.134	−0.119	0.026	0.402	0.318	0.267	0.035
WC	0.341	0.153	0.114	1.359	0.044	0.306	−0.234	0.100	0.061	0.057	0.203	0.220
	MRS			IS			EDS			CRS		
	β	r^2	P	β	r^2	P	β	r^2	P	β	r^2	P
BMI	0.302	0.137	0.079	0.096	0.055	0.275	0.016	0.204	0.786	0.414	0.224	0.052
WSR	22.490	0.290	0.006	−0.323	0.022	0.941	1.272	0.207	0.659	23.439	0.254	0.023
WHR	23.333	0.171	0.034	6.138	0.054	0.281	2.108	0.209	0.579	31.579	0.259	0.020
Fat DXA	0.173	0.110	0.156	0.146	0.172	0.015	0.036	0.219	0.386	0.354	0.266	0.016
Fat SF	0.238	0.158	0.047	0.100	0.094	0.100	0.013	0.204	0.756	0.351	0.265	0.017
AC	0.600	0.270	0.003	0.136	0.067	0.200	0.023	0.204	0.749	0.758	0.338	0.002
WC	0.164	0.232	0.007	0.037	0.059	0.246	0.015	0.015	0.480	0.216	0.216	0.004
Female (n = 54)												
	Fasting insulin			Triglycerides			HDL-cholesterol			HOMA-IR		
	β	r^2	P	β	r^2	P	β	r^2	P	β	r^2	P
BMI	0.743	0.079	0.043	-0.140	0.005	0.940	0.099	0.003	0.775	0.148	0.078	0.049
WSR	33.601	0.045	0.132	233.132	0.090	0.034	−35.621	0.059	0.084	5.095	0.028	0.263
WHR	37.748	0.042	0.146	99.914	0.017	0.444	−12.235	0.006	0.615	6.101	0.030	0.250
Fat DXA	−0.524	0.030	0.225	−3.625	0.060	0.090	0.639	0.051	0.108	−0.094	0.026	0.283
Fat SF	1.054	0.074	0.051	−0.311	0.005	0.910	0.258	0.060	0.612	0.247	0.100	0.024
AC	0.829	0.067	0.063	−1.229	0.011	0.586	0.117	0.012	0.461	0.201	0.098	0.025
WC	0.285	0.065	0.068	0.580	0.016	0.462	−0.123	0.015	0.400	0.049	0.049	0.124
	MRS			IS			EDS			CRS		
	β	r^2	P	β	r^2	P	β	r^2	P	β	r^2	P
BMI	0.165	0.057	0.101	0.241	0.263	<0.001	0.038	0.017	0.473	0.443	0.234	0.001
WSR	19.536	0.206	0.001	−0.786	0.029	0.849	1.102	0.009	0.727	19.851	0.144	0.012
WHR	16.055	0.105	0.021	11.773	0.143	0.012	1.026	0.008	0.780	28.854	0.207	0.001
Fat DXA	−0.109	0.023	0.352	0.168	0.113	0.032	−0.048	0.019	0.431	0.010	0.030	0.948
Fat SF	0.242	0.057	0.100	0.038	0.031	0.708	0.062	0.020	0.418	0.342	0.087	0.080
AC	0.196	0.056	0.107	0.176	0.114	0.031	0.080	0.037	0.208	0.452	0.175	0.004
WC	0.110	0.135	0.008	0.063	0.120	0.026	0.019	0.022	0.383	0.193	0.248	<0.001

Model adjusted by age.

AC, arm circumference; BMI, body mass index; CRS, cardiometabolic risk score; EDS, endothelial dysfunction score; Fat DXA, body fat according to dual beam X-ray absorptiometry; Fat SF, body fat according to the sum of skinfolds; HOMA-IR, homeostasis model assessment of insulin resistence; IS, inflammation score; MRS, metabolic risk score; WC, waist circumference; WHR, waist-to-hip ratio; WSR, waist-to-stature ratio.

white and black teenagers, whereas the present study included a mixed group, and there is the possibility that individuals in the sample used in the study by Slaughter *et al.*[12] have thinner skinfolds than those in the current analysis. It is unlikely that an equation developed among slimmer children and adolescents can accurately estimate body fatness in the much heavier individuals.

Previous studies [6,30,31] have shown that BMI can be used as an alternative measure to DXA for assessing body fat in female and male adolescents, which is is contrast to our findings. Lindsay *et al.* [32] showed that the relationship between BMI and % Fat DXA is likely to change with age, especially in the age range 15–19 years. Although both were classified as obese according to BMI, girls had a higher percentage of body fat and the boys had greatest amount of lean body mass, similar to that described previously [33]. This indicates that BMI, despite showing good correlation with measures of adiposity in

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adolescents as described in previous studies[30,31], does not reflect adequately the major changes in body composition that occur in this age group and that are different between the sexes [34]. However, given the difficulty of using more sophisticated methods to measure body fat in epidemiological studies, BMI remains a viable alternative. The recognition of best cut-offs and more appropriate references to identify adolescents with high body fat remains a challenge.

The correlation between WHR and BMI was weak in females and absent in males, and similar results were found in other studies [35,36]. When comparing the diagnostic quality of BMI, WC and WHR in screening for obesity in children, Hubert *et al.* [37] concluded that WHR was not very effective in classifying childhood obesity. In the study by Perez *et al.* [38], which was conducted in Venezuelan children and adolescents, the WHR also did not effectively identify fat distribution because it did not provide adequate sensitivity and specificity. In adolescents, it appears that WHR is not appropriate as an anthropometric measure to assess the distribution of body fat because the pelvic width undergoes rapid changes during sexual maturation, which may mean that WHR is more related to this variation than to the distribution of body fat [36,39].

Thus, some studies suggest that the WSR and WC are anthropometric indices that can be evaluated with the BMI to diagnose obesity and the location of body fat [40]. The results of the present study revealed that BMI, WSR and WC are strongly correlated, as described in previous studies [40,41]. Pelegrini *et al.* [42] evaluated 1197 Brazilian adolescents aged between 15 and 17 years, and observed that BMI, WSR and WC have high predictive power with respect to high body fat in both sexes. The AC is not an anthropometric measure that is usually searched for, although it can act as a measure of physical growth, reflecting adiposity and improving the diagnosis given by the BMI [43]. Therefore, these variables would be useful for the identification of body fat in adolescents because they are simple, have a low cost and are non-invasive.

The definition of cardiometabolic risk in young people is key to developing strategies for the prevention of chronic diseases. It becomes even more relevant when we consider the high prevalence of inadequacy in biochemical parameters indicative of metabolic risk that might be identified in obese adolescents in the present study. Compared to other studies that evaluated non-obese and obese individuals in the sample, the prevalences of dyslipidaemia and disorders in glucose metabolism in the present study are noticeably higher [44,45]. Although it was not the purpose of the present study, it is known that these teenagers belonged to a disadvantaged economic group from the urban area of Recife, Pernambuco. The socio-economic level interferes with the availability of food and access to information, and may be associated with certain patterns of physical activity, constituting a major determinant of the prevalence of obesity and changes in the risk variables assessed in the present study.

In the face of differences in body composition between sexes and magnitude of correlations between adiposity indicators, we evaluated the predictive power of these indicators on cardiometabolic risk factors, singly or grouped in scores, by sex. The AC was shown to be a predictor of insulin and HOMA-IR in boys. Already in girls, BMI predicted the variability of insulin and % Fat DC and AC were the best predictors of HOMA-IR. There are few studies evaluating this association separated by sex, especially in adolescents. Gómez-García *et al.* [46] evaluated the presence of insulin resistance by HOMA-IR index in Mexican adults who were overweight and obese and found that insulin-resistant individuals had higher BMI and AC, and that these measures were good predictors of insulin resistance, regardless of sex. In studies with Greek children, BMI was correlated with fasting insulin and HOMA-IR, and was considered useful for identifying children at risk of developing insulin resistance[47]. The amount and difference in the distribution of body fat between the sexes and the increased oestrogen levels in women, resulting in an improvement in insulin sensitivity, are factors that explain, at least in part, the difference between the sexes observed for associations between indicators of adiposity and insulin resistance[48].

Conventional measures, such as BMI, are better predictors of diabetes markers than the measure of adiposity according to DXA[49]. A possible explanation is proposed by Wells [29], who suggests that BMI is a result of the variability of components such as fat, lean body mass and height, and each of these components may independently contribute to cardiometabolic risk. Thus, the BMI is no longer a simplistic method of the assessment of nutritional status, making it a risk index, whereas an analysis measuring body composition helps to explain how this risk arises through the stages of life.

It is known that the WSR can identify individuals with an atherogenic lipid profile precisely because it is an index used as a central indicator of adiposity [50]. We find an association between WSR and TG values only among girls, although the literature shows such an association in both sexes [50]. The calculation of waist–height is simple, does not require percentiles by sex or age, and can be easily understood. Furthermore, the possibility of using a single cut-off (0.5) to identify cardiovascular risk factors results in a simple public health message 'Hold your waist circumference to less than half its height.' [40].

It is noteworthy that, in this analysis, we have established the relationship between adiposity indicators and risk factors, alone or grouped into profiles, rather than

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diagnosing the metabolic syndrome. This is because there is no consensus about its definition and clinical use in children and adolescents. Another advantage of establishing profiles by aggregating risk factors is the use of new markers, such as IL-6, TNF-α and adhesion molecules, therefore extending the concept of the metabolic syndrome, and eliminating the need for the dichotomised variables [17].

In the present study, the various adiposity indicators analysed were shown to predict the variability of MRS, depending on sex. We suggest that especially the indicators of central and peripheral adiposity WSR, WC and AC are used in obese adolescents to estimate the presence of metabolic risk. This observation is supported by the studies of Sardinha *et al.*[51] and Freedman *et al.*[52], which state that anthropometric variables are able to provide a set of cardiometabolic risk factors.

The adiposity variables investigated in the present study also proved to be able to predict the variability of IS, highlighting % Fat DXA for boys and BMI for girls. Given the difficulty of assessing the % Fat DXA in clinical practice, as well as that described by Samouda *et al.*[53], we suggest that a study with a larger sample size is accomplished by combining the BMI evaluation with an anthropometric measurement of regional adiposity, such as WC or WSR. In such a study, inflammation, as assessed by C-reactive protein, improved its prediction when the WSR *Z*-score was added to the BMI *Z*-score [53].

The EDS score was the only variable that could not be predicted by the adiposity indicators. A recent review reports the exploration of the association between anthropometric markers and the concentration of sICAM and sVCAM, but with controversial results [24]. It appears that there is no relationship between the concentration of sICAM and subcutaneous and visceral fat after adjustment for BMI and WC, indicating the possibility of other mechanisms to explain the changes in the concentration of sICAM, such as the presence of low HDL-chol levels and subsequent oxidation of LDL-chol and damage to the endothelium [24].

When all risk scores were grouped in the CRS, anthropometric variables AC and WC showed a greater predictive power of this in boys and girls, respectively. This justifies the importance of integrating these anthropometric measurements in clinical evaluation of obese adolescents.

The selected nature and relatively small size of our sample, including only obese subjects, might be a limitation of the present study in that it does not allow the extrapolation of our findings to the general population. Also, it cannot establish a cause and effect relationship between adiposity and metabolic effects, as in cross-sectional studies. The possible determinants and outcome are seen at the same time, preventing the use of temporality as a causal criterion. However, the present study has relevant aspects such as the maturational stage control of adolescents, as well as the use of inflammatory markers and endothelial dysfunction described in this small sample. We recommend conducting studies with larger sample size to identify cut-off points for these anthropometric variables, as well as prospective studies to follow the obese and identify future health risk factors.

In conclusion, obese adolescents have a high prevalence of alterations in cardiometabolic risk variables. The correlation between anthropometric indices and the risk variables considered in the present study allow us to recommend the measurement of BMI, WC, AC and WSR when evaluating obese adolescents, allowing intervention before health problems arise.

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.

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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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OBESITY AND RELATED DISORDERS

Low socio-economic status is a newly identified independent risk factor for poor vitamin D status in severely obese adults

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Abstract

Background: Hypovitaminosis D is very prevalent, especially in the obese population. However, the degree of severity and the parameters involved in vitamin D deficiency in this population are still unclear. The present study aimed to identify, from among the factors known to influence vitamin D status in a healthy population, those impacting the same parameter in obese population.

Methods: Serum 25-OH-D concentration was measured in 564 patients with class III obesity [i.e. severe and morbid obesity; mean (SD) body mass index (BMI) 42.04 (6.92) kg m^{-2}] and their demographic, clinical, biological, anthropometric, dietary and socio-economic data were collected.

Results: We observed that 96% of the obese patients had serum 25-OH-D lower than 30 ng mL^{-1}. Severe vitamin D deficiency (serum 25-OH-D concentration <10 ng mL^{-1}) affected 35% of this population. We found an inverse relationship between 25-OH-D levels and BMI ($P = 0.012$), fat mass ($P = 0.041$), metabolic syndrome ($P < 0.0001$), fasting blood glucose ($P = 0.023$), homeostasis model assessment for insulin resistance ($P = 0.008$), waist circumference ($P = 0.001$), and fasting blood triglycerides ($P = 0.002$) and C-reactive protein ($P = 0.005$). Low socio-economic status independently increased the risk of severe vitamin D deficiency [odds ratio (OR) = 1.98; 95% confidence interval (CI) 1.25–3.13], especially in the autumn–winter season (OR = 2.94; 95% CI 1.98–4.36), morbid obesity (OR = 3.19; 95% CI 1.49–6.82), metabolic syndrome (OR = 1.6; 95% CI 1.06–2.42) and inflammation (OR = 1.03; 95% CI 1.01–1.06).

Conclusions: Vitamin D deficiency is extremely common among obese patients, and the prevalence of severe deficiency is high. The association of adiposity, high body mass index, metabolic syndrome and inflammation with vitamin D status is marked, whereas low socio-economic status appears to be a major risk factor for severe vitamin D deficiency, suggesting that vitamin D deficiency may at least in part be responsible for the greater health vulnerability of populations with low socio-economic status.

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Introduction

Vitamin D has recently aroused much interest because of the high prevalence of its deficiency throughout the world [1] and also because of the advancement of fundamental and clinical knowledge on this steroid and its ubiquitous expressed receptor, vitamin D receptor (VDR) [2]. Furthermore, the enzyme 1-α-hydroxylase, which is expressed and functionnal mainly in the kidney and enables the synthesis of the biologically active form of vitamin D, is also found in other tissues (aortic endothelium, epidermis, growth cartilage, osteoblasts, brain, skeletal muscle, cells of the immune system, and adipose tissue) [3]. The broad expression of VDR, and the extrarenal expression of 1-α-hydroxylase activity, could explain the extra-skeletal effects of vitamin D. Hypovitaminosis D has been suggested to be a potential factor in many illnesses [4–8]. Interestingly, subjects with hypovitaminosis D (<20 ng mL^{-1}) were demonstrated to be at higher risk of insulin resistance and the metabolic syndrome [9]. In obese people, vitamin D status has been significantly correlated with insulin-sensitivity and body mass index (BMI) [10]. Furthermore, hypovitaminosis D was also associated with metabolic syndrome (MetS) in obese patients [11], as well as with the degree of obesity [12]. Finally, it has been suggested that vitamin D deficiency was associated with an increased risk of developing obesity in subjects who were non-obese [13]. Inversely, obesity appeared to be a risk factor for hypovitaminosis D in nondialysed patients with chronic kidney disease and in renal transplant patients [14,15]. Taken together, these studies have highlighted a strong link between MetS, obesity and hypovitaminosis D.

Many factors can modify the plasma level of vitamin D. First, ultraviolet (UV)-B radiation enables the skin synthesis of cholecalciferol, provitamin D_3, from the 7-dehydrocholesterol present in the skin. Hence, endogenous vitamin D production can be modulated by atmospheric pollution, the ozone layer, cloudiness, and the solar zenithal angle, which varies according to the latitude (i.e. the country) and the seasons. Skin melanin content, topical sunscreen, skin temperature and clothing habits are also factors influencing the skin synthesis of vitamin D [16]. A sedentary lifestyle or an inadequate dietary intake of foods and supplements containing vitamin D are also causes of vitamin D deficiencies [16]. In addition, vitamin D metabolism can be impaired by chronic kidney disease [17], intestinal malabsorption of fat [18], liver failure [19], certain cancers, especially those of mesenchymal origin such as breast and prostate carcinomas [20], or by the use of medical drugs such as phenobarbital, phenytoin, carbamazepine, rifampin and antiretroviral agents [21].

In respect of obesity, studies have shown decreased levels of serum vitamin D in obese compared to lean individuals, showing an inverse relationship between BMI or body fatness and serum vitamin D [22–25]. Several studies have elaborated the hypothesis that obesity-associated vitamin D insufficiency could be a result of the storage of vitamin D in adipose tissue because the vitamin is a lipophilic compound [22,26,27]. Other factors as previously described may lead to deficiency, especially reduced sun exposure because of an indoor lifestyle, few outdoor activities, clothing habits, and an inadequate dietary intake of vitamin D-rich foods and supplements [28].

Vitamin D deficiency is more prevalent in the obese population than in non-obese subjects (between 21% and 90% according to published studies) [28,29]. The average plasma level of 25-OH-D in obese subjects is half that observed in the non-obese population [28]. However, the degree of severity of hypovitaminosis D in the obese population, as well as the parameters involved in severe vitamin D deficit, still remains unclear.

Taking these results as a whole, we hypothesised that the factors known to influence vitamin D status in healthy population may also impact the vitamin D status in obese population.

We therefore assessed those factors already known to influence vitamin D status in non-obese populations, and the factors that may be more specific to them, such as anthropometric, biological, lifestyle, demographic, environmental and socioieconomic parameters, aiming to determine the specific risk factors for severe vitamin D deficiency (<10 ng mL^{-1}) in severe and morbid obesity.

Materials and methods

Subjects

This retrospective study involved 673 obese patients hospitalised in the department of Clinical Nutrition of the university hospital, Clermont-Ferrand, France, from 1 January 2010 to 30 September 2011. Participants were hospitalised for 1 day in the Clinical Nutrition Service of Gabriel Montpied University Hospital Center (Clemont-Ferrand, France). During this day, patients were subjected to a normal health check (i.e. body composition, biological check-up) and they met a dietician and a nutritionist. The ethical committee of Auvergne University approved the study protocol, and written informed consent to partake in the study was provided by each participant. All the participants had to be older than 18 years and had a BMI $\geq$ 30 kg m^{-2}. Exclusion criteria were: any form of bariatric surgery (n = 58 adjustable gastric band, n = 10 sleeve gastrectomy, n = 2 gastric by-pass), total colectomy (n = 1), evidence of renal insufficiency with glomerular filtration rate <60 mL min^{-1} (n = 28), type 1 diabetes (n = 1), evolutive malignancy (n = 4), pancreatic failure (n = 2), history of post-operative hypoparathyroidism

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($n = 1$), pregnancy ($n = 1$) and use of orlistat ($n = 1$). None of the patients were taking anticonvulsant drugs or corticosteroids. After applying the exclusion criteria, data from 564 patients were collected for analysis and interpretation. All of the data were taken from available medical records.

Physical examination and data collection

Data on season, age, sex, smoking, regular consumption of alcohol, use of vitamin D supplements (in the last 3 months), medical history of type 2 diabetes and hypertension were collected.

Patients enrolled in our protocol noted their total dietary intakes during 5 days. Then, their dietary consumption was collected by dietitians during a face-to-face interview at the day of their hospitalisation. Dietitians determined hidden and additional fats using the CIQUAL (Centre d'Information sur la Qualité des Aliments – Information Center for Food Quality) table. The CIQUAL project aims to collect, evaluate and make available the nutritional composition data of generic food consumed in France. The collected data are then aggregated using the CIQUAL to produce reference mean values for generic foods. The site presents a table of nutritional composition of foods.

Weight and height were measured in patients wearing light clothing and the BMI was calculated (kg m^{-2}). Subjects with a BMI between 30.0 and 34.9 were classified in the obese class I category. Subjects with a BMI between 35.0 and 39.9 were classified in the obese class II category. Finally, subjects with a BMI superior or equal to 40.0 were classified in the obese class III category [30]. Waist circumference was measured midway between the lowest rib margin and the iliac crest. Validated equations for body composition analysis using bioelectrical impedance (Body Stat QuadScan 4000, multifrequency) in morbidly obese individuals were used [31].

Laboratory analysis

The following parameters were assayed: fasting blood glucose (g L^{-1}), serum insulin (mUI L^{-1}), glycosylated haemoglobin (%), triglycerides (g L^{-1}), high-density lipoprotein (HDL)-cholesterol (g L^{-1}), alanine aminotransferase (ALAT, UI L^{-1}), aspartate aminotransferase (ASAT, UI L^{-1}), γ-glutamyl transpeptidase (GGT; UI L^{-1}), alkaline phosphatase (UI L^{-1}), serum creatinine (μmol L^{-1}), calcium (mmol L^{-1}), phosphate, C-reactive protein (CRP, mg L^{-1}), intact parathyroid hormone (PTH) and 25-OH-D (ng mL^{-1}).

Serum 25-OH-D was measured using the LIAISON® 25 OH Vitamin D TOTAL assay (DiaSorin, Dartford, UK), which is a chemiluminescent immunoassay technology, in accordance with the manufacturer's instructions and with intra- and inter-assay coefficients of variation of 0.1–3.8% and 6.0–9.8%, respectively. Internal and external quality controls were used to validate the laboratory's 25-OH-D methodology and in-house control materials. Internal quality controls were used before and after sample analysis. External quality controls were used to be comparable with international laboratories using the same methodology for serum 25-OH-D measurement. Furthermore, we used quality controls from the French National Security Agency of Medicines and Health Products, which are regulatory controls imposed by the French Ministry of Health.

The studied population was then divided into three subgroups: patients with severe vitamin D deficiency (25-OH-D < 10 ng mL^{-1}); patients with vitamin D deficiency (10 ng mL^{-1} ≤ 25-OH-D < 20 ng mL^{-1}); and patients with insufficient vitamin D (20 ng mL^{-1} ≤ 25-OH-D < 30 ng mL^{-1}) [32,33].

Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the formula: fasting blood glucose [mmol L^{-1}] × serum insulin [mUI L^{-1}]/22.5) [34]. Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease formula [35].

Measuring low socio-economic status

A French economic and social scale for the calculation of socio-economic predictors, *the EPICES score (Évaluation de la précarité et des inégalités de santé dans les centres d'examens de santé – Evaluation of low socio-economic status and inequalities in Health Examination Centers*), was used as the studied population is French (Table 1) [36,37]. This score is calculated according to an algorithm based on the replies to 11 questions, and ranges from 0 (least deprived) to 100 (most deprived) [38]. A low socio-economic status is defined by a score ≥30.17, a threshold established in a large cohort study carried out by the French Technical Center of Support and Training for Health Centers. Although the EPICES score can be considered as a continuous variable, most of the studies of health conditions assessed by this score use this threshold to define low socio-economic status. In this context, the EPICES score becomes a dichotomous variable. If the EPICES score is equal or superior to 30.17, the subject is considered as being of a low socio-economic status. If the EPICES score is inferior to 30.17, the subject is considered as being of a normal socio-economic status.

Covariates

Season of measurement was divided into 'spring–summer' from April to September and 'autumn–winter' from October to March.

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Table 1 Calculation of EPICES (French evaluation of low socio-economic status and inequalities in Health Examination Centers) score

	Initial score for each patient	75.14	
		Answer	
Number	Questions	Yes	No
1	Do you sometimes see a social worker?	+10.06	0
2	Do you have extra health insurance?	−11.83	0
3	Are you in a relationship?	−8.28	0
4	Do you own your home?	−8.28	0
5	Are there any times of the month when you have real financial problems meeting your needs (food, rent, electricity, etc.)?	+14.80	0
6	Have you practiced any sport in the last 12 months?	−6.51	0
7	Have you gone to a show in the last 12 months?	−7.10	0
8	Have you gone on holiday in the last 12 months?	−7.10	0
9	In the last 6 months, have you had any contact with relatives other than your parents or your children?	−9.47	0
10	If you were in trouble, are there people around you who you could rely on to put you up for a few days if needed?	−9.47	0
11	If you were in trouble, are there people around you who you could count on to give you practical help?	−7.10	0
	Total score		

Score calculation: each coefficient is added to the constant if the answer is 'yes'. If total score is ≥30.17, the subject is considered as being of a low socio-economic status. If total score is <30.17, the subject is considered as being of a normal socio-economic status. Adapted with permission [23]. Participants were hospitalised for 1 day in the Clinical Nutrition Service of Gabriel Montpied University Hospital Center (Clemont-Ferrand, France). During this day, patients were subjected to a normal health check (i.e. body composition, biological check-up) and they met a dietician and a nutritionist.

Metabolic syndrome was defined according to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP/ATPIII), which required at least three of the following five criteria [39]:

1. Elevated waist circumference (≥102 cm in men; ≥88 cm in women)

2. Elevated fasting triglycerides (≥1.50 g L^{-1} and/or use of medication for elevated triglycerides)

3. History of hypertension and/or use of antihypertensive drug treatment

4. Elevated fasting plasma glucose (≥1.10 g L^{-1} and/or known diabetes)

5. Reduced HDL-cholesterol (<0.4 g L^{-1} in men and <0.5 g L^{-1} in women)

Statistical analysis

All statistical calculations were performed using SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). Associations were considered statistically significant at a two-tailed α value of 0.05. Data are expressed as the mean (SD) for continuous variables, and as percentages for categorical and dichotomous variables. Because there were only 21 patients with sufficient vitamin D (25-OH-D > 30 ng mL^{-1}), we did not consider this subgroup for bivariate and multivariate analysis. Comparison between the three subgroups was performed through bivariate analysis with the chi-squared test for categorical and dichotomous variables or Fisher's exact test when necessary. Continuous data were analysed using one-way analysis of variance, or a Kruskal–Wallis test when appropriate (normality studied using the Shapiro–Wilk test and homoscedasticity by Bartlett's test). When appropriate, an appropriate post-hoc test was performed for multiple comparisons: a Tukey–Kramer post-hoc analysis of variance and Dunn's test after a Kruskal–Wallis test. Multivariate analyses were performed using binary logistic regression to identify predictors of severe vitamin D deficiency. Covariates were chosen using a stepwise approach according to univariate results (variables significant at $P \leq 0.20$) and clinical relevance with respect to age, sex, BMI and season [40,41]. Because the EPICES score was missing for some patients, we could not determine the socio-economic status of 144 patients (i.e. 26% of the study population). To take these patients into account in multivariate analysis, we created another category for the socio-economic status that corresponds to the missing value. Finally, a sensitivity analysis was carried out to measure the impact of the missing data on the results.

Results

Description of the population

The clinical and laboratory characteristics of the 564 patients are summarised in Tables 2 and 3. All of the biological parameters of the studied population were inside the reference limits, except for the GGT parameter (Table 3). Elevated GGT is commonly observed in obese

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Table 2 Clinical characteristics of studied population

Variable	Value*
Sex ratio (women/men)	3/1
Age (years)	43 (14)
BMI (kg m^{-2})	42.0 (6.9)
Population distribution	
Obese class I (BMI 30–34.9)	12% (n = 69)
Obese class II (BMI 35–39.9)	34% (n = 189)
Obese class III (BMI > 40)	54% (n = 306)
Fat mass (%)	42.7
Fat mass (kg)	48.5 (13.8)
Season	
Spring–Summer	56%
Autumn–Winter	44%
Low socio-economic status	49%†
Smoking	29%
Alcohol	6%
Type 2 diabetes	16%
Metabolic syndrome	49%
Elements of the metabolic syndrome	
Elevated blood pressure	38%
Elevated waist circumference	100%
Elevated triglycerides	32%
Reduced HDL-C	60%
Elevated fasting glucose	30%
Insulin resistance (HOMA-IR > 2.5)	62%
Excessive consumption of hidden fats	76%
Excessive consumption of additional fats	29%

*Data are the mean (SD) for continous variables and percentages for categorical variables.
†n = 420 persons. Socio-economic status could not be determined for 144 patients. BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance.

population certainly as a result of non-alcoholic fatty liver disease [42].

In total, 96% of the studied population (n = 543) was considered to be in a hypovitaminosis D state because measurement of the serum 25-OH-D concentration in patients revealed a concentration lower than 30 ng mL^{-1} (Table 4). Among them, 77% had values lower than 20 ng mL^{-1}. Severe vitamin D deficiency (<10 ng mL^{-1}) affected 35% of patients. Only 21 individuals (4%) had a normal vitamin status.

The results of multivariate analysis using binary regression are summarised in Table 5.

Vitamin D and adiposity

We noted a relationship between BMI and vitamin D status. The distribution of patients in accordance with their degree of obesity was significantly different between the three groups of vitamin D status (P = 0.0121) (Table 6). Among the group with severe vitamin D deficiency, 64% of patients were classified in the obese class III category, 29% in the obese class II category and only 7% in the obese class I category (Table 6). Furthermore, we noted that subjects with class II obesity had a more than threefold higher risk of severe vitamin D deficiency than subjects with class I obesity, after adjusting for the other parameters [odds ratio (OR) = 3.19; 95% confidence interval (CI) 1.49–6.82; P = 0.0066] (Table 5). We also identified a relationship between body composition and severity of vitamin D deficiency. Indeed, fat mass was significantly different between the three vitamin D groups, being highest for the lowest values of 25-OH-D (P = 0.0007) (Table 6).

Vitamin D and metabolic parameters

Metabolic syndrome (MetS) was significantly related to 25-OH-D status (P < 0.0001) (Table 6). The prevalence of MetS was greater in the severe vitamin D deficient group (61%) than in the vitamin D deficient group (46%) and also in the vitamin D insufficiency group (36%). We also noted that MetS increased the risk of developing a severe vitamin D deficiency by 2.23-fold, as well as the risk of developing a vitamin D deficiency by 1.35-fold (OR = 2.23; 95% CI 1.3–3.84; P = 0.0078) (Table 7).

Hypertension and diabetes did not appear to be associated with vitamin D status (Table 6). However, we noted that median fasting blood glucose was significantly linked to vitamin D status (P = 0.0225) (Table 6). In addition, the percentage of patients with normal insulin sensitivity tended to be greater in the vitamin D insufficient group (27%) than in the vitamin D deficient group (21%) or in the severe vitamin D deficient group (17%) (Table 6). Furthermore, HOMA-IR was associated with serum levels of vitamin D (P = 0.0084) (Table 6). Indeed, we noted a higher HOMA-IR index in the case of severe vitamin D deficiency (HOMA-IR = 4.23) than in the case of vitamin D deficiency (HOMA-IR = 3.97) or insufficiency (HOMA-IR = 3.33).

Waist circumference, another marker of MetS, was associated with vitamin D status, being higher in the subjects with low levels of 25-OH-D (P = 0.0008) (Table 6).

For lipid parameters, no link between level of HDL-cholesterol and vitamin D status was found (Table 6), although we did find evidence of an inverse relationship between triglycerides and 25-OH-D levels (P = 0.0022). The median triglyceride concentration was increased more in the case of severe deficiency of vitamin D than in case of vitamin D deficiency or insufficiency.

Vitamin D and inflammation

Among the 564 patients in the present study with no history of chronic inflammatory disorder, median CRP was

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Table 3 Laboratory characteristics of the studied population

Assay	Biological parameter	Value*	Reference
Calcium	25-OH-D (ng mL^{-1})	14.34 (7.78)	30–80
	Calcium (mmol L^{-1})	2.25 (0.10)	2.20–2.50
	Phosphate (mmol L^{-1})	0.98 (0.16)	0.8–1.5
	PTH (ng L^{-1})	53 (21)	15–65
Liver	ALAT (UI L^{-1})	35 (22)	10–50
	ASAT (UI L^{-1})	28 (11)	10–40
	GGT (UI L^{-1})	47 (61)	7–32
	Alkaline phosphatases (UI L^{-1})	71 (27)	40–120
Lipids	Triglycerides (g L^{-1})	1.31 (0.73)	<1.50
	HDL-cholesterol (g L^{-1})	0.46 (0.13)	Men: >0.40
			Women: >0.50
Inflammatory	CRP (mg L^{-1})	7.83 (7.53)	<5
Glucose	Fasting glucose (g L^{-1})	1.05 (0.30)	<1.26
	HbA1C (%)	6.09 (0.96)	4–6
	HOMA-IR	6.13 (12.29)	<2.5

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CRP, C-reactive; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; PTH, parathyroid hormone.
*Data are the mean (SD). The assays were performed for all patients in the studied population.

Table 4 Description of vitamin D status in the studied population

Normal vitamin D status (vitamin D ≥ 30 μg L^{-1})		Vitamin D insufficiency (20 μg L^{-1} ≤ vitamin D < 30 μg L^{-1})		Vitamin D deficiency (10 μg L^{-1} ≤ vitamin D < 20 μg L^{-1})		Severe vitamin D deficiency (vitamin D < 10 μg L^{-1})	
n	Percentage of total studied population	*n*	Percentage of total studied population	*n*	Percentage of total studied population	*n*	Percentage of total studied population
21	4	106	19	238	42	199	35

Vitamin D status was evaluated in the 564 patients included in the present study.

Table 5 Predictors for severe vitamin D deficiency and vitamin D deficiency.

	Severe vitamin D deficiency		Vitamin D deficiency	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex		0.7091		0.8796
Women versus men	1.09 (0.68–1.75)		0.96 (0.58–1.6)	
Age	1.01 (0.99–1.02)	0.3063	1 (0.99–1.02)	0.7579
BMI		0.0066		0.1831
Obese class II versus Obese class I	2.19 (0.99–4.79)		1.1 (0.58–2.08)	
Obese class III versus Obese class I	3.19 (1.49–6.82)		1.57 (0.85–2.93)	
Low socio-economic status	1.98 (1.25–3.13)	0.0119	2.14 (1.31–3.49)	0.0085
Season		<0.0001		<0.0001
Autumn–winter versus Spring–summer	2.94 (1.98–4.36)		2.63 (1.67–4.13)	
MetS	1.6 (1.06–2.42)	0.0268	1.66 (1.06–2.61)	0.0268
Excessive intake of hidden fat	0.58 (0.36–0.93)	0.0249		
CRP	1.03 (1.01–1.06)	0.0157		

Results of multivariate analysis using binary logistic regression as used to find predictors for severe vitamin D deficiency. Only clinically relevant parameters and variables significant at $P \leq 0.20$ in the bivariate analysis were considered eligible for inclusion in the logistic regression. The level of significance for a factor to remain in the final model was set at 5%. BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; MetS, metabolic syndrome; OR, odds ratio.

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Table 6 Association between vitamin D groups and adiposity, metabolic parameters or inflammation

	Vitamin D insufficiency (20 μg L^{-1} ≤ vitamin D < 30 μg L^{-1})	Vitamin D deficiency (10 μg L^{-1} ≤ vitamin D < 20 μg L^{-1})	Severe vitamin D deficiency (vitamin D < 10 μg L^{-1})	*P*-value
BMI				
Obese class I (% of vitamin D group)	14	15	7	0.0121
Obese class II (% of vitamin D group)	39	34	29	
Obese class III (% of vitamin D group)	47	51	64	
Fat mass (kg), mean (SD)	45.92 (10.38)	47.7 (13.44)	51.71 (15.49)	0.0007
MetS				
Absence of MetS (% of vitamin D group)	64	54	39	<0.0001
Presence of MetS (% of vitamin D group)	36	46	61	
Hypertension				
Absence of hypertension (% of vitamin D group)	67	62	58	0.2622
Presence of hypertension (% of vitamin D group)	33	38	42	
Diabetes				
Absence of diabetes (% of vitamin D group)	83	88	79	0.0574
Presence of diabetes (% of vitamin D group)	17	12	21	
Glycaemia (g L^{-1})				
Median (quantile 1 – quantile 3)	0.96 (0.87–1.09)	0.96 (0.87–1.07)	1 (0.91–1.15)	0.0225
Insulin				
Sensitivity (% of vitamin D group)	27	21	17	0.0613
Resistance (% of vitamin D group)	56	67	62	
Diabetic (% of vitamin D group)	17	13	21	
HOMA-IR index Median (quantile 1 – quantile 3)	3.33 (1.91–5.99)	3.97 (2.32–6.36)	4.23 (2.73–7.37)	0.0084
Waist circumference (cm), mean (SD)	123.75 (12.62)	125.46 (14.63)	129.96 (16.76)	0.0008
HDL-cholesterol (g L^{-1}) Median (quantile 1 – quantile 3)	0.46 (0.4–0.54)	0.44 (0.38–0.51)	0.44 (0.37–0.54)	0.2118
Triglycerides (g L^{-1}) Median (quantile 1 – quantile 3)	1.05 (0.7–1.31)	1.14 (0.79–1.66)	1.23 (0.88–1.66)	0.0022
CRP (mg L^{-1}), median (quantile 1 – quantile 3)	5.7 (2.7–9.9)	5.2 (3–8.5)	6.95 (3.5–11.2)	0.0053

Comparison between the three subgroups was made through bivariate analysis with the chi-squared test for categorical and dichotomous variables or Fisher's exact test when necessary. Continuous data were analysed using one-way analysis of variance, or a Kruskal–Wallis test when necessary. Associations were considered statistically significant at a two-tailed α value of 0.05. BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; MetS, metabolic syndrome.

greater in subjects presenting the lowest values of 25-OH-D [i.e. 6.95 mg L^{-1} (3.5–11.2) for the severe vitamin D deficiency group, 5.2 (3–8.5) and 5.7 mg L^{-1} (2.7–9.9) for the vitamin D deficiency and vitamin D insufficiency groups, respectively] (Table 6). These differences were considered statistically significant ($P = 0.0053$). Finally, we estimated that, for every increase of 1 mg L^{-1} of CRP, the risk of developing a severe vitamin D deficiency was multiplied by 1.03 (95% CI 1.01–1.06; $P = 0.0157$) (Table 5).

Vitamin D, dietary habits and demographic data

No link was identified between 25-OH-D and age, sex, smoking habits or regular consumption of alcohol (Table 8). However, regarding dietary habits, we noted that an excessive intake of hidden fat was a protective factor against hypovitaminosis D. Furthermore, we noted that dietary habits with excess hidden fat were associated with a reduced risk of severe vitamin D deficiency (by 1.72-fold) (OR = 0.58; 95% CI 0.36–0.93; $P = 0.0249$) (Table 5). Nevertheless, the excessive intake of hidden fat appearing to comprise a protective factor is questionable from a biological stand point.

We noted a marked seasonal variation in serum vitamin D levels, with some subjects presenting a severe vitamin D deficiency over the autumn–winter period. Also, the proportion of subjects with a vitamin D insufficiency was greater during the spring–summer season ($P < 0.0001$) (Table 8). The risk of severe vitamin D deficiency was almost tripled during the autumn–winter season compared to spring–summer (OR = 2.94; 95% CI 1.98–4.36; $P < 0.0001$) (Table 5).

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Table 7 Metabolic syndrome is a predictor for severe vitamin D deficiency and vitamin D deficiency

	Severe vitamin D deficiency (vitamin D < 10 μg L^{-1}) OR (95% CI)	Vitamin D deficiency (10 μg L^{-1} ≤ vitamin D < 20 μg L^{-1}) OR (95% CI)	*P*-value
MetS	2.23 (1.3–3.84)	1.35 (0.81–2.24)	0.0078

Vitamin D insufficiency group was the reference group for the multinomial logistic regression. Thus, the indicated odds ratios (OR) have to be interpreted with respect to the vitamin D insufficient group. Multinomial logistic regression was used to quantify the association between vitamin D levels and each variable after adjusting for the other variables. CI, confidence interval; MetS, metabolic syndrome.

Table 8 Association between vitamin D groups and demographic data, life habits or season

	Vitamin D insufficiency (20 μg L^{-1} ≤ vitamin D < 30 μg L^{-1})	Vitamin D deficiency (10 μg L^{-1} ≤ vitamin D < 20 μg L^{-1})	Severe vitamin D deficiency (vitamin D < 10 μg L^{-1})	*P*-value
Age (years), mean (SD)	42.35 (13.49)	42.88 (14.2)	44.45 (13.94)	0.3587
Sex				0.6556
Men (% of vitamin D group)	21	25	23	
Women (% of vitamin D group)	79	75	77	
Smoking habits				0.3642
Absence of smoking habits (% of vitamin D group)	76	69	71	
Presence of smoking habits (% of vitamin D group)	24	31	29	
Alcohol consumption				0.6751
Absence of alcohol consumption (% of vitamin D group)	93	95	93	
Presence of alcohol consumption (% of vitamin D group)	7	5	7	
Season				<0.0001
Spring–Summer (% of vitamin D group)	74	61	39	
Autumn–Winter (% of vitamin D group)	26	39	61	
Socio-economic status				0.0013
Normal socio-economic status (% of vitamin D group)	65	52	40	
Low socio-economic status (% of vitamin D group)	35	48	60	

Comparison between the three subgroups was made through bivariate analysis with the chi-squared test for categorical and dichotomous variables or Fisher's exact test when necessary. Continuous data were analysed using one-way analysis of variance, or a Kruskal–Wallis test when necessary. Associations were considered statistically significant at a two-tailed α value of 0.05.

Vitamin D and socio-economic status

The present study revealed a link between socio-economic level and vitamin D status (Table 8). The low socio-economic status was associated with hypovitaminosis D ($P = 0.0013$). The prevalence of a low socio-economic status was 60% in the severe vitamin D deficiency group versus 48% and 35% in the vitamin D deficiency and insufficiency groups, respectively. Finally, we noted that a low socio-economic status doubled the risk of severe vitamin D deficiency (OR = 1.98; 95% CI 1.25–3.13; $P = 0.0119$) or vitamin D deficiency (OR = 2.14; 95% CI 1.31–3.49; $P = 0.0085$) in the obese population (Table 5).

Discussion

The present study highlights the high prevalence of vitamin D deficiency in severely obese patients. Although it has been widely reported (Table 4), we noted that 96% of our studied population presented with hypovitaminosis D compared to 21–90% of the studied population in the literature [28]. In particular, 35% of our studied population presented a severe vitamin D deficiency compared to a previous study by Stein *et al.* [29] in which it was reported that 20% of their studied population was severely vitamin D deficient. In France, the national study on nutrition and health conducted in 2006–2007 reported that the overall rate of vitamin D insufficiency was 80.1% in the general non-obese population, and only 4.8% presented a severe vitamin D deficiency [43].

Our results revealed an inverse relationship between vitamin D status, BMI and fat mass ($P = 0.0121$ and 0.0007, respectively) (Table 6). These results are in line with those of the literature [29]. The specific impact of obesity and adipose tissue on vitamin D metabolism is one of the proposed hypotheses for this finding. Indeed, the marked sequestration of 25-OH-D within the adipose

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tissue may limit its bioavailability [26,42,44]. Furthermore, the skin synthesis of vitamin D in response to sun exposure is altered in obese individuals [26]. In addition, it was previously reported that the catabolism of vitamin D was increased as a result of the presence of the 24-hydroxylase enzyme in human adipose tissue [45].

Interestingly, it has been suggested that vitamin D could have a role in the regulation of the adipose mass and the prevention of obesity [46]. Indeed, adipose cells express vitamin D and PTH receptors and have 1-α-hydroxylase activity, suggesting that adipose tissue regulates and is regulated by vitamin D [45,47]. Notably, 1α,25-dihydroxyvitamin D increases lipolysis of adipose tissue, and inhibits lipogenesis *in vitro* [48]. Furthermore, a clinical investigation demonstrated that postprandial fatty acid oxidation was improved and the thermogenesis rate was favoured following a hypocaloric diet in obese women presenting a normal vitamin D status [49]. However, the few interventional studies that have estimated the impact of a vitamin D substitution on weight loss in obese individuals remain somewhat inconclusive [50]. Taken as a whole, these results demonstrate that BMI and/or fat mass influence vitamin D status and, inversely, vitamin D status may influence body composition.

Among other factors studied, MetS appears to be closely related to hypovitaminosis D ($P < 0.0001$) (Table 6). Specifically, waist circumference ($P = 0.0008$), triglyceridaemia ($P = 0.0022$), fasting blood glucose ($P = 0.0225$) and HOMA-IR ($P = 0.0084$), which are criteria defining MetS, have an inverse relationship with the serum vitamin D level (Table 6). Our results are consistent with the literature [9,51–53]. However, among the components of the MetS that we have studied, only hypertension and HDL-cholesterol were not associated with 25-OH-D concentrations. Of note, the prevalence of hypertension in our population is likely underestimated because we considered only those cases with known high blood pressure. In the same way, the level of HDL-cholesterol could also have been modified by a widespread use of statins in our population, slightly masking the reduced HDL-cholesterol.

Our results do not highlight any cause-and-effect relationship between vitamin D status and altered metabolic parameters. We cannot tell whether the MetS is the origin or a consequence of the hypovitaminosis D. Although the literature is rich in epidemiological data on this subject, cohort or interventional studies are scant. Two cohort studies estimated the association between circulating vitamin D level and the incidence of MetS [54,55]. The first study, conducted in 524 individuals, revealed an inverse relationship between initial status of 25-OH-D and MetS appearance after a 10-year follow-up [54]. The second study concerned a cohort of 6537 individuals. Lower serum 25-OH-D concentrations were associated with increased MetS risk and higher waist circumference, serum triglyceride, fasting glucose and insulin resistance in the next 5 years [55].

The results of interventional studies converge to an improvement of insulin sensitivity and a reduced insulin resistance following vitamin D supplementation [56]. However, these trials are rare and use different protocols [57]. For example, a cohort study conducted in an obese population for 1 year demonstrated a reduction in the prevalence of MetS of approximately 50% simply by following lifestyle advice (i.e. sun exposure of between 5 and 30 min per day, and vitamin D-enriched food consumption) [58]. Our results, together with the data available in the literature, appear to indicate that vitamin D modulates the development of MetS and that vitamin D supplementation may limit MetS development.

We noted an inverse relationship between serum vitamin D and CRP levels ($P = 0.0053$) (Table 6). This result suggests that vitamin D could have an anti-inflammatory effect. It is well known that inflammation in obesity depends on visceral adiposity, and that abdominal adiposity is associated with hypovitaminosis D. However, there is little clinical evidence of any direct relationship between vitamin D status and inflammation. In a cohort of more than 1700 asymptomatic subjects, a link was identified between circulating vitamin D level (<21 ng mL^{-1}) and CRP, independently of cardiovascular risk factors [59]. The hypothesis of a potential pro-inflammatory effect of hypovitaminosis D is supported by the fact that the vitamin D receptor is expressed in lymphocytes, macrophages and dendritic cells. These cells express also the 1-α-hydroxylase enabling the production of the active form of vitamin D locally. *In vitro*, the 1α,25-dihydroxyvitamin D inhibited the production of pro-inflammatory cytokines, which modulated any immunological reaction, and decreased inflammation [60]. However, clinical results on the effect of vitamin D on inflammation status are conflicting, and further research is needed to assess the anti-inflammatory effect of vitamin D [3].

As a retrospective study, the present study has some limits. Even by proceeding to a dietary intake survey, we were unable to evaluate customary vitamin D intakes or the daily sun exposure time of patients outside the 5-day period of dietary consumption collection. Nor did we obtain any details about the ethnicity of the subjects, another factor influencing vitamin status. However, concerning 5-day food intake, we found that a high consumption of hidden fat was a protective factor for vitamin D status, decreasing the risk of severe vitamin D deficit by 1.72 (OR = 0.58; 95% CI 0.36–0.93; $P = 0.0249$). To our knowledge, no

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similar observation has yet been reported elsewhere. However, this finding has to be interpreted with caution because the estimation of the intake of hidden fat was based on a subjective analysis of dietary habits, and was not made by precise intake calculations.

The strong seasonal impact on vitamin D status was confirmed in our results (Table 5). The risk of severe deficiency was multiplied by 3 during the autumn and winter period compared to the spring–summer season (OR = 2.94; 95% CI 1.98–4.36; $P < 0.0001$) (Table 5). The influence of the season on vitamin D status has been widely described as a result of increased vitamin D skin synthesis under the effect of the UV-B radiation. It has been shown that vitamin D deficiency (<20 ng mL^{-1}) was 3.8-fold more prevalent during winter than during summer [23]. Thus, seasons influence vitamin D status in both non-obese and obese population.

Finally, the present study reveals that being of a low socio-economic status is a major independent risk factor for severe vitamin D deficiency (Table 5). Evaluation of socio-economic status was assessed using the EPICES score, which takes into account the multidimensional aspects of socio-economic status, including its material, psychological and social aspects. We found that low socio-economic status doubled the risk of severe vitamin D deficiency, after adjusting for BMI and all the other factors studied (OR = 1.98; 95% CI 1.25–3.13; $P = 0.0119$) (Table 5). To our knowledge, this is the first time that the EPICES score has been used to investigate the link between socio-economic and vitamin D status. The data in the literature on this subject are rare. Two recent British studies found a strong association between vitamin D status and the socio-economic conditions in a population of obese and non-obese individuals. In these studies, the prevalence of hypovitaminosis D was more pronounced in individuals of low socio-economic status [61,62]. The association between vitamin D deficiency and socio-economic status appears to be supported by analogies of geography, seasonality and ethnicity [63]. Behavioural factors could be another explanation for the link between low socio-economic status and hypovitaminosis D. For example, dwellings are more frequently located in inner city areas with more atmospheric pollution, a factor decreasing the efficiency of UV-B radiation on endogenous vitamin D production. Furthermore, they likely have no garden, which does not allow the opportunity for outdoor leisure. People with low income may not have ready access to the countryside or holidays in the sun. Social isolation could further contribute to lower sun exposure. Also, there is a lack of vitamin D supplementation within a population that has lower access to healthcare. Dietary habits may also be incriminated; lack of vitamin D-enriched food consumption as a result of its high cost (especially fish, seafood and dairy products) could also favour this dietary deficiency. A study of dietary behaviour and nutritional status in individuals with low socio-economic status receiving food aid showed that fewer than half of the subjects met the French recommendations for 'meat, fish and eggs'; 27.3% met the requirements for seafood; and only a very small proportion of participants met the recommendations for dairy products (9.2%). In addition, 16.7% of subjects were obese, 14.8% were anaemic, 67.9% were at risk of folate deficiency and 85.6% had vitamin D deficiency [64]. Thus, it appears that obese subjects of a low socio-economic position consume a less healthy diet, increasing the risk of demonstrating a low vitamin D status. Although food may be very energy-dense, balance and nutritional quality is poorer, and this contributes to diverse nutritional deficiencies, including hypovitaminosis D. Indeed, individuals with a low socio-economic position guide their food choices to cheaper products, which are generally very energy-dense and have a poor balance and nutritional quality. This contributes to the development of diseases related to obesity and diverse nutritional deficiencies, including hypovitaminosis D. Public health experts should consider social inequalities in health and nutrition when aiming to improve public health, or at least to limit the impact of social status in this respect. Regarding nutrition, they should develop a strategy to provide better information about the nutritional quality of products on the market and their value. Thus, the population may have the possibility of accessing quality products at the same time as controlling their budget. Therefore, a better quality diet may improve health and limit the development of diseases such as obesity, or deficiencies such as hypovitaminosis D.

In conclusion, we noted that factors such as season that are known to influence vitamin D status in a healthy population also impact the vitamin D status in an obese population. The present study, conducted in a large population of severely obese patients, revealed a strong prevalence of hypovitaminosis D, with a considerable proportion of severe vitamin D deficiency. The serum vitamin D concentrations of patients were associated with anthropometric, metabolic, biological, dietary, seasonal and socio-economic parameters. These results highlight the consequential or causal role of adiposity, BMI, MetS and inflammation with respect to vitamin D status. In addition, it also appears that a low socio-economic status is a major independent risk factor for vitamin D deficiency in obese individuals. These new findings highlight the need to pay particular attention to the health impact of vitamin D deficiency in socio-economically disadvantaged populations.

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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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JL-G was involved in the design of the study, subject recruitment, the running of the study, the interpretation of the results and the drafting of the article. CD-F was involved in the collection of data, the interpretation of the results and the drafting of the article. MM was involved in subject recruitment and the drafting of the article. FP, LG and BP were involved in the statistical analysis. RM-Q and VS were involved in the biological analysis. SW was involved in the collection of data, the interpretation of the results and the drafting of the article. YB was involved in the design of the study and the interpretation of the results, and was the principal investigator. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

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

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OBESITY AND RELATED DISORDERS

Adherence to the Healthy Eating Index and Alternative Healthy Eating Index dietary patterns and mortality from all causes, cardiovascular disease and cancer: a meta-analysis of observational studies

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Abstract

Background: This meta-analysis investigated the association of diet quality indices, as assessed by HEI and AHEI, and the risk of all-cause, cardiovascular and cancer mortality.
Methods: We used PubMed, ISI Web of Science and Google Scholar to search for eligible articles published before July 2015. A total of 12 cohort studies (38 reports) and one cross-sectional study (three reports) met the inclusion criteria and were included in our meta-analysis.
Results: The highest level of adherence to the Healthy Eating Index (HEI) and Alternative Healthy Eating Index (AHEI) was significantly associated with a reduced risk of all-cause mortality [relative risk (RR) = 0.77, 95% confidence intterval (CI) = 0.76–0.78], cardiovascular mortality (RR = 0.77, 95% CI = 0.74–0.80) and cancer mortality (RR = 0.83, 95% CI = 0.81–0.86). Egger regression tests provided no evidence of publication bias.
Conclusions: The present study indicates that high adherence to HEI and AHEI dietary patterns, indicating high diet quality, are associated with reduced risk of all-cause mortality (as well as cardiovascular mortality and cancer mortality).

Introduction

During recent decades, the consumption of different dietary components has attracted increased attention as a result of their synergistic and inter-related effects [1–3]. Although an assessment of nutrients, single foods or food groups is useful for detecting relationships between diet and disease, dietary components are not consumed in isolation [2]. To assess adherence to dietary patterns, different countries have independently created dietary guidelines. In the USA, the Healthy Eating Index (HEI) was developed to promote health and prevent chronic disease and is updated every 5 years [4,5]. The most recent version focused on food quality, including healthy choices such as whole grains, seafood and plant proteins, and placed more attention on the ratio of unsaturated to saturated fatty acids [6]. The Alternative Healthy Eating Index (AHEI), based on the original HEI, focused on absolute food intake rather than nutrient density [7].

HEI has been associated with reduced risk of different chronic diseases, including cardiovascular diseases and cancers, which could decrease mortality risk [8–10]. Beneficial

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influences of this healthy dietary pattern have been evaluated across a wide range of different ages and by sex [11–13]. Reedy *et al.* [8] reported that HEI and AHEI were related to lower risk of mortality both in men and women. However, Yu *et al.* [14] found a stronger association among men compared to women and especially lower mortality for cardiovascular compared to cancer outcomes. In another study, reduced cancer deaths were related to HEI but not to AHEI scores [9]. Furthermore, higher HEI conformity has been linked to lower mortality risk in men, whereas a higher AHEI score has been associated with risk reduction in women [14].

Although a meta-analysis assessed the relationship of HEI, AHEI and Dietary Approaches to Stop Hypertension with health outcomes was published in 2014 [15], only fifteen cohort studies assessing all-cause mortality, cardiovascular mortality or incidence, cancer mortality or incidence, type 2 diabetes mellitus and neurodegenerative diseases were included in this meta-analysis. In our review, we only assess HEI and AHEI on all causes, cardiovascular and cancer mortalities; however, a comprehensive search updated the analysis by including articles published in 2015 and also by adding more reports published before 2014 that had not been included in the prior study.

The present meta-analysis aimed to investigate how diet quality indices as assessed by HEI and AHEI relate to all-cause and specific causes of mortality (cardiovascular mortality, cancer mortality).

Materials and methods

Data sources and searches

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [16]. A literature search was conducted using the electronic databases PubMed, ISI Web of Science and Google Scholar (up to 14 December 2015) with no restriction on publication date or language. Search terms included 'Healthy Eating Index' or 'HEI' or 'Alternative Healthy Eating Index' or 'AHEI' in combination with 'mortality' or 'case fatality rate' or 'death rate'. Additionally, we complemented the database by manually searching the reference lists from retrieved articles. This systematic review was planned, conducted and reported with adherence to quality standards for reporting meta-analyses of observational studies in epidemiology [17]. A literature search was conducted independently by both authors, with disagreements resolved by consensus.

Study selection

Articles included in the meta-analysis met the criteria: (i) cohort or cross-sectional studies evaluating the association of diet quality by HEI and/or AHEI on all-cause mortality; (ii) reporting of relative risks (RRs), hazard radios (HRs) or odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs); (iii) including outcomes of interest (i.e. all-cause mortality, cardiovascular mortality and cancer mortality); and (iv) when a study was published in duplicate, the most comprehensive one was selected.

Data extraction and quality assessment

The data extracted from each study were: the first author's last name, year of publication, study origin,

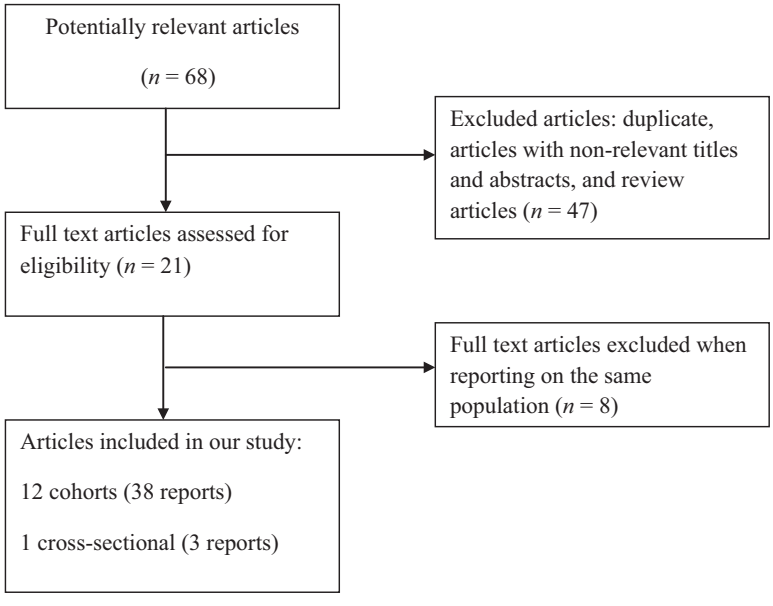

Figure 1 Summary of search strategy and study selection.

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Table 1 Healthy Eating Index (HEI) and Alternative Healthy Eating Index (AHEI) dietary patterns and all-cause, cardiovascular and cancer mortalities: overview of studiesselected for the meta-analysis

Author, year	Country	Cohort	Outcome	Population (*n*) Follow-up	Age at entry (years)	Sex	Diet quality index	Adjustment	RR/HR (95% CI) Multivariate adjusted*
Yu (2015)[14]	USA	SCCS	All-cause mortality Vascular mortality Cancer mortality	77 572 6.2 years	40–79	M/F	HEI-2010	Socio-demographic, cigarette smoking, BMI, physical activity, total energy intake	HR: 0.80 (0.73–0.86) HR: 0.81 (0.70–0.94) HR: 0.81 (0.69–0.95)
Harmon (2015)[25]	USA	MEC	All-cause mortality Vascular mortality Cancer mortality	156 804 13–18 years	45–75	M/F	HEI-2010	Age, BMI, energy, socio-demographic, smoking, physical activity	HR: 0.77 (0.74–0.80) HR: 0.76 (0.72–0.81) HR: 0.79 (0.73–0.86)
Harmon (2015)[25]	USA	MEC	All-cause mortality Vascular mortality Cancer mortality	156 804 13–18 years	45–75	M/F	AHEI-2010	Age, BMI, energy, socio-demographic, smoking, physical activity	HR: 0.79 (0.76–0.82) HR: 0.76 (0.72–0.81) HR: 0.87 (0.82–0.92)
Yu (2014)[26]	China	SMHS & SWHS	All-cause mortality Vascular mortality Cancer mortality	61 239 6.5 years	40–47	M	Modified AHEI-2010	Age, SES, smoking, physical activity, BMI, waist-to-hip ratio, history of diseases energy intake	HR: 0.68 (0.61–0.76) HR: 0.56 (0.46–0.68) HR: 0.87 (0.74–1.02)
Yu (2014)[26]	China	SMHS & SWHS	All-cause mortality Vascular mortality Cancer mortality	73 216 12 years	40–47	F	Modified AHEI-2010	Age, SES, smoking, physical activity, BMI, waist-to-hip ratio, history of diseases energy intake	HR: 0.80 (0.73–0.87) HR: 0.73 (0.62–0.87) HR: 0.92 (0.80–1.06)
Thomson (2014)[27]	USA	WHI	All-cause mortality Cancer mortality	636 17 years	62.9	F	HEI-2005	Age, stage at diagnosis	HR: 0.73 (0.55–0.97) HR: 0.75 (0.55–1.01)
Reedy (2014)[8]	USA	NIH-AARP	All-cause mortality Vascular mortality Cancer mortality	424 663 15 years	50–71	M/F	HEI-2010	Age, SES, physical activity, smoking, energy intake, BMI	HR: 0.77 (0.75–0.79) HR: 0.79 (0.69–0.91) HR: 0.79 (0.73–0.85)
Reedy (2014)[8]	USA	NIH-AARP	All-cause mortality Vascular mortality Cancer mortality	424 663 15 years	50–71	M/F	AHEI-2010	Age, SES, physical activity, smoking, energy intake, BMI	HR: 0.76 (0.75–0.78) HR: 0.76 (0.69–0.83) HR: 0.85 (0.80–0.90)
George (2014)[9]	USA	WHI	All-cause mortality Vascular mortality Cancer mortality	63 805 12.9 years	50–79	F	HEI-2010	Age, energy intake, SES, smoking, physical activity, BMI	HR: 0.76 (0.70–0.83) HR: 0.78 (0.65–0.93) HR: 0.77 (0.68–0.89)
Fung (2014)[28]	USA	NHS	All-cause mortality Cancer mortality	1201 11.2 years	61–72.2	F	AHEI-2010	Age, physical activity, BMI, weight change, smoking status, energy intake	HR: 0.71 (0.52–0.98) HR: 0.72 (0.43–1.21)
Mursu (2013)[29]	US	IWHS	All-cause mortality Vascular mortality Cancer mortality	29 634 20.3 years	61.4 + 4.2	M/F	AHEI-2010	Age, energy intake, SES, BMI, waist-to-hip ratio, physical activity, and smoking	HR: 0.82 (0.77–0.87) HR: 0.79 (0.72–0.88) HR: 0.88 (0.79–0.98)
Li (2013)[30]	USA	NHS	All-cause mortality Vascular mortality	4098 –	30–75	M/F	AHEI-2010	Age at diagnosis, calendar year (questionnaire cycle, continuous, 2-year period)	HR: 0.76 (0.60–0.96) HR: 0.73 (0.51–1.04)
Kappeler (2013)[34]	US	NHANES III	All-cause mortality Vascular mortality Cancer mortality	17611 22 years	>18	M/F	HEI	Age, cigarette smoking, physical activity, socioeconomic status, BMI	HR: 0.77 (0.63–0.94) HR: 0.85 (0.65–1.11) HR: 0.75 (0.51–1.11)

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Table 1. Continued

Author, year	Country	Cohort	Outcome	Population (*n*) Follow-up	Age at entry (years)	Sex	Diet quality index	Adjustment	RR/HR (95% CI) Multivariate adjusted*
Izano (2013)[31]	USA	NHS	Cancer mortality	4103 9.33 years	30–55	F	AHEI-2010	Age at diagnosis, energy intake, BMI, smoking, and physical activity	RR: 1.07 (0.77–1.49)
Akbaraly (2011)[32]	UK	Whitehall II	All-cause mortality Vascular mortality Cancer mortality	7319 18 years	39–63	M/F	AHEI	Age, smoking status, total energy intake, physical activity, BMI	HR: 0.76 (0.61–0.95) HR: 0.58 (0.37–0.91) HR: 0.80 (0.58–1.11)
Shahar (2009)[33]	USA	Health ABC	All-cause mortality	298 9 years	70–82	M/F	HEI	Age, smoking status, energy intake	HR: 1.90 (0.70–5.20)

*In case of multiple HR/RR values, the order in which data are presented corresponds to the respective order of outcomes listed in the 'Outcome' column.
RR, relative risk; HR, hazard ratio; SCSS, Southern community cohort study; M, Male; F, Female; MEC, Multi-ethnic cohort; SMHS & SWHS, Shanghai men's health study & Shanghai women's health study; WHI, Women's health initiative; NIH, AARP, National Institutes of Health-AARP; NHS, Nurse's health study; IWHS, Iowa women's health study; NHANES III, National health and nutrition examination survey III.
Health ABC, The health, aging, and body composition; BMI, body mass index; SES, socio-economic status.

number of participants, age, sex, study design, study duration (years of follow-up), number of deaths occurring in the study period, adjustment factors and risk estimates (HR or RR or highest versus lowest category) with their corresponding 95% CIs. If separate risk estimates were reported for males and females, they were included as two separate studies. The multivariate adjusted model was selected for studies with several risk estimates. The Newcastle–Ottawa Quality Assessment Scale was used to perform the quality assessment for the included studies [18].

Healthy Eating Index and Alternative Healthy Eating Index components and scoring

Healthy Eating Index

Ten components included grains, vegetables, fruits, milk, meat, total fat, saturated fat, cholesterol, sodium and variety. Overall scores ranged between 0 and 100 [19].

Healthy Eating Index-2005

Twelve components included total fruits, whole fruits, total vegetables, dark green and orange vegetables and legumes, total grains, whole grains, milk, meat and beans, oils, saturated fat, sodium, and calories from solid fats, alcoholic beverages (i.e. beer, wine, and distilled spirits), and added sugars. Overall scores ranged between 0 and 100 [4].

Healthy Eating Index-2010

Twelve components included total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids (polyunsaturated fatty acid monounsaturated fatty acid-to-saturated fatty acid ratio), refined grains, sodium, and empty calories from solid fats, alcoholic beverages (i.e. beer, wine and distilled spirits), and added sugars. Overall scores ranged between 0 and 100 [5].

Alternative Healthy Eating Index

Nine components included vegetables, fruits, nuts and soy protein, ratio of white to red meat, cereal fibre, trans-fat, polyunsaturated-to-saturated fat ratio, duration of multivitamin use, and alcohol. Overall scores ranged between 2.5 and 87.5 [7].

Alternative Healthy Eating Index-2010

Eleven components included whole grains, vegetables (excluding potatoes), fruits, nuts and legumes, trans-fat, eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) (*n*–3 FAs), polyunsaturated fatty acids (PUFAs), alcohol, red and processed meat, sugar-sweetened beverages and fruit juices, and sodium. Overall scores ranged between 0 and 110 [20].

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Statistical analysis

The meta-analysis was conducted using random effects at the same time as combining the multivariable adjusted RRs, HR or ORs from the highest compared to the lowest HEI and AHEI adherence categories for various outcomes [21]. Subgroup analyses were performed for different clinical outcomes (i.e. all-cause mortality, cardiovascular mortality and cancer mortality). The Cochrane Q test together with the I^2 statistic was used to specify possible sources of heterogeneity. I^2 >50% indicated substantial heterogeneity across studies [22]. Between-subgroup heterogeneity was assessed with fixed effect models. Publication bias was assessed by examining funnel plots [23]. Egger's regression asymmetry and Begg's adjusted rank correlation tests were carried out for formal statistical assessment of funnel plot asymmetry [24]. Sensitivity analyses were conducted to investigate the extent to which conclusions might rely on a particular study or studies. All statistical analyses were conducted in STATA, version 11.2 (Stata Corp., College Station, TX, USA).

Results

Literature search and study characteristics

A total of 12 cohort studies (38 reports) [8,9,14,25–33] and one cross-sectional study (three reports) [34] extracted from 68 articles met the inclusion criteria and were included in our meta-analysis. Detailed steps of the article selection process are shown in Fig. 1.

The general study characteristics are provided in Table 1. The sample size of studies included in the meta-analysis ranged from 298 to 242 321, with a total number of 922 199 adults (aged 18–79 years). Follow-up time ranged from 6 to 22 years. All dietary intake assessment was perfomed using food frequency questionnaires, except

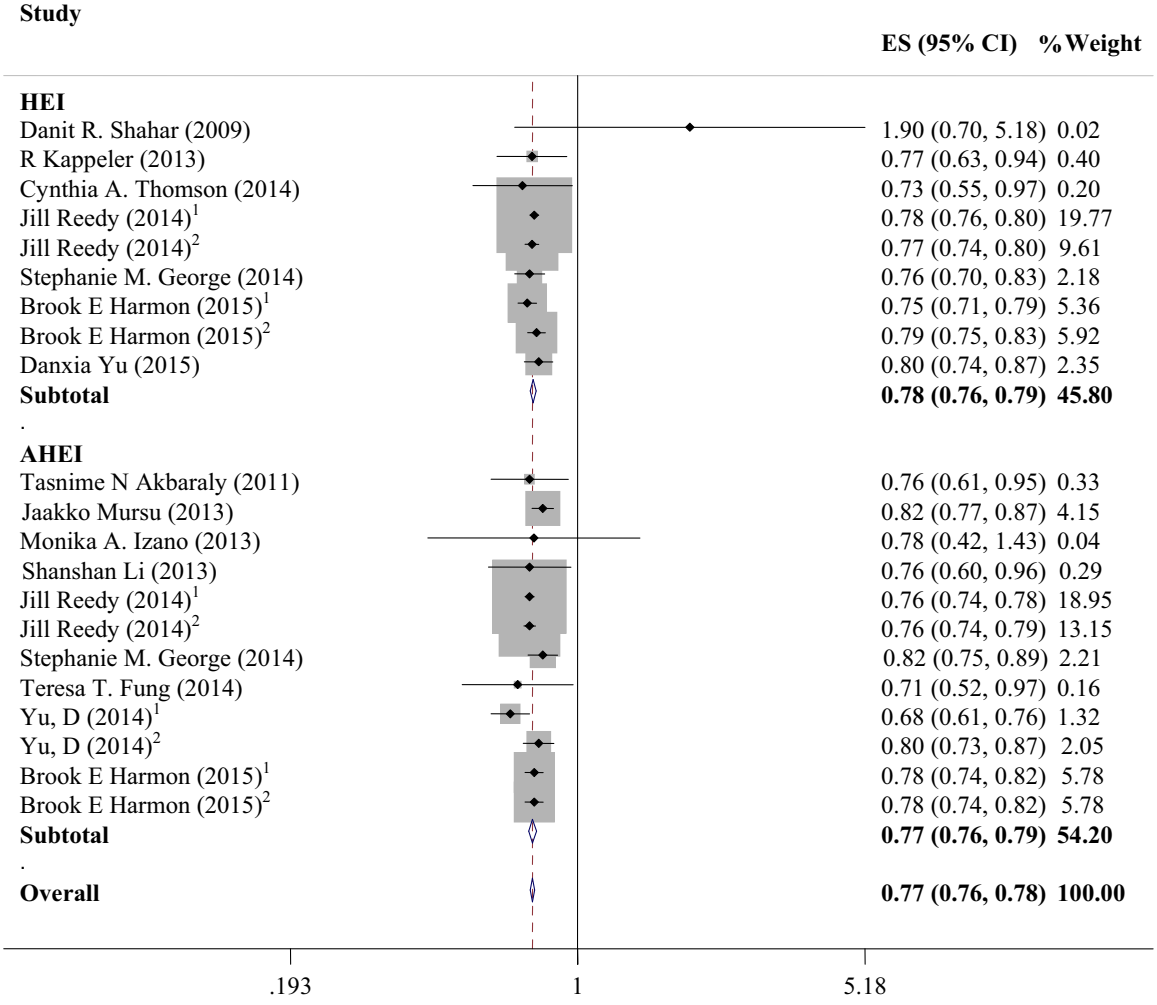

Figure 2 Forest plot of the Healthy Eating Index (HEI) and Alternative Healthy Eating Index (AHEI) dietary patterns in relation to all-cause mortality. [1]Men; [2]Women. ES, Effect size; CI, confidence interval.

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for the study by Kappler *et al.* [34], which used a single 24-h dietary recall.

Main outcomes

All-cause mortality was assessed in twelve cohorts studies (15 reports including eight AHEI and seven HEI) [8,9,14,25–30,32–34], cardiovascular mortality in eight cohort studies and one cross-sectional study (12 reports including seven AHEI and five HEI) [8,9,14,25,26,29,30,32,34], and cancer mortality in 10 cohort studies and one cross-sectional study (14 reports, eight and six including AHEI and HEI, respectively) (colorectal, lung, liver, stomach, breast, ovarian, endometrial, recto sigmoid, uterine and other/unknown cancers) [8,9,14,25–29,31,32,34].

Using a random effect model, we found that high adherence to HEI and AHEI was significantly associated with a reduced risk of all-cause mortality (RR = 0.77, 95% CI = 0.76–0.78), cardiovascular mortality (RR = 0.77, 95% CI = 0.74–0.80) and cancer mortality (RR = 0.83, 95% CI = 0.81–0.86). To determine whether HEI or AHEI was a stronger predictive index, a subgroup analysis was performed based on the method of evaluating diet quality.

Subgroup analysis based on dietary quality indices showed significant inverse associations between all-cause mortality and HEI (RR = 0.78, 95% CI = 0.76–0.79) and AHEI (RR = 0.77, 95% CI = 0.76–0.79). However, heterogeneity between subgroups was not significant (I^2 = 4.6%, P = 0.399) (Fig. 2).

Subgroup analyses demonstrated that higher scores for both the HEI and AHEI dietary patterns were associated with reduced risk of cardiovascular and cancer mortalities. The HEI pattern was associated with a 20% reduction in cancer mortality (RR = 0.80, 95% CI = 0.76–0.83), which was more effective than the

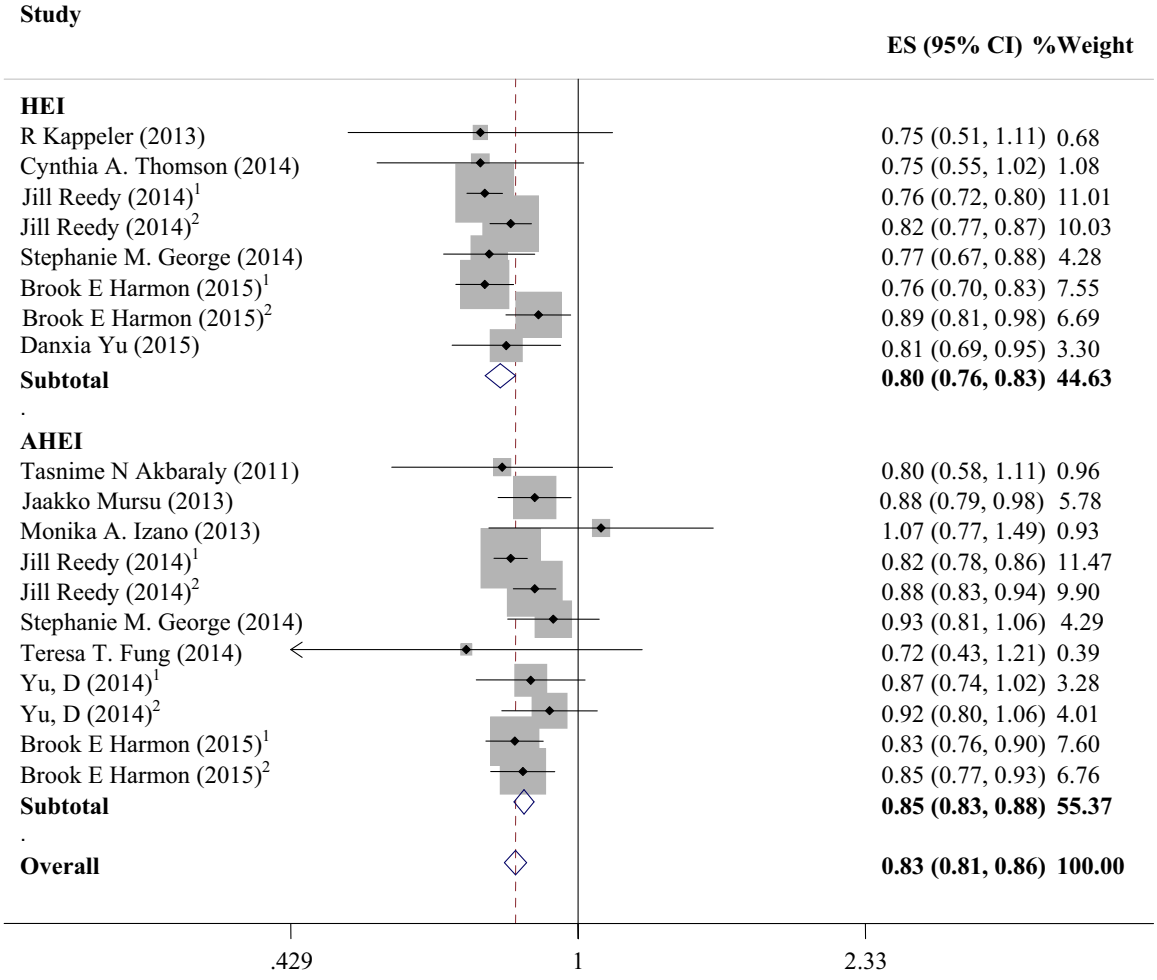

Figure 3 Forest plot of the Healthy Eating Index (HEI) and Alternative Healthy Eating Index (AHEI) dietary patterns in relation to cancer mortality. [1]Men; [2]Women. ES, Effect size; CI, confidence interval.

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AHEI pattern (RR = 0.85, 95% CI = 0.83–0.88) (Fig. 3). By contrast, the AHEI pattern reduced cardiovascular mortality (RR = 0.74, 95% CI = 0.71–0.78) more than the HEI pattern (RR = 0.79, 95% CI = 0.76–0.83) (Fig. 4).

Additional subgroup analyses based on sex and region for cause of mortality and diet quality indices were conducted to identify sources of heterogeneity (Table 2). Neither sex, nor region were sources of heterogeneity. However, mortality between-study heterogeneity was only significant for cancer when subgroup analyses based on sex were conducted ($P < 0.01$). Moreover, cardiovascular mortality significantly decreased as the adherence to HEI in different regions increased ($P = 0.01$).

Publication bias

Egger linear regression tests provided no evidence of potential publication bias for all-cause ($P = 0.57$ and 0.74), cardiovascular ($P = 0.58$ and 0.39) and cancer ($P = 0.89$ and 0.30) mortalities following comparisons of the highest versus lowest quintiles of HEI and AHEI scores, respectively. Begg's funnel plots for all-cause mortality ($P = 0.53$ and 0.95), cardiovascular mortality ($P = 0.65$ and 0.42) and cancer mortality ($P = 0.46$ and 0.48) indicated no asymmetry for HEI and AHEI scores, also suggesting that publication bias was not present.

Sensitivity analysis

Removal of individual studies from the meta-analysis in each subgroup (according to the HEI/AHEI calculations and cause of mortality) had little effect on the results of the meta-analysis and did not change their significance (data not shown).

Discussion

This meta-analysis of prospective studies suggests that there is a significant relationship between higher

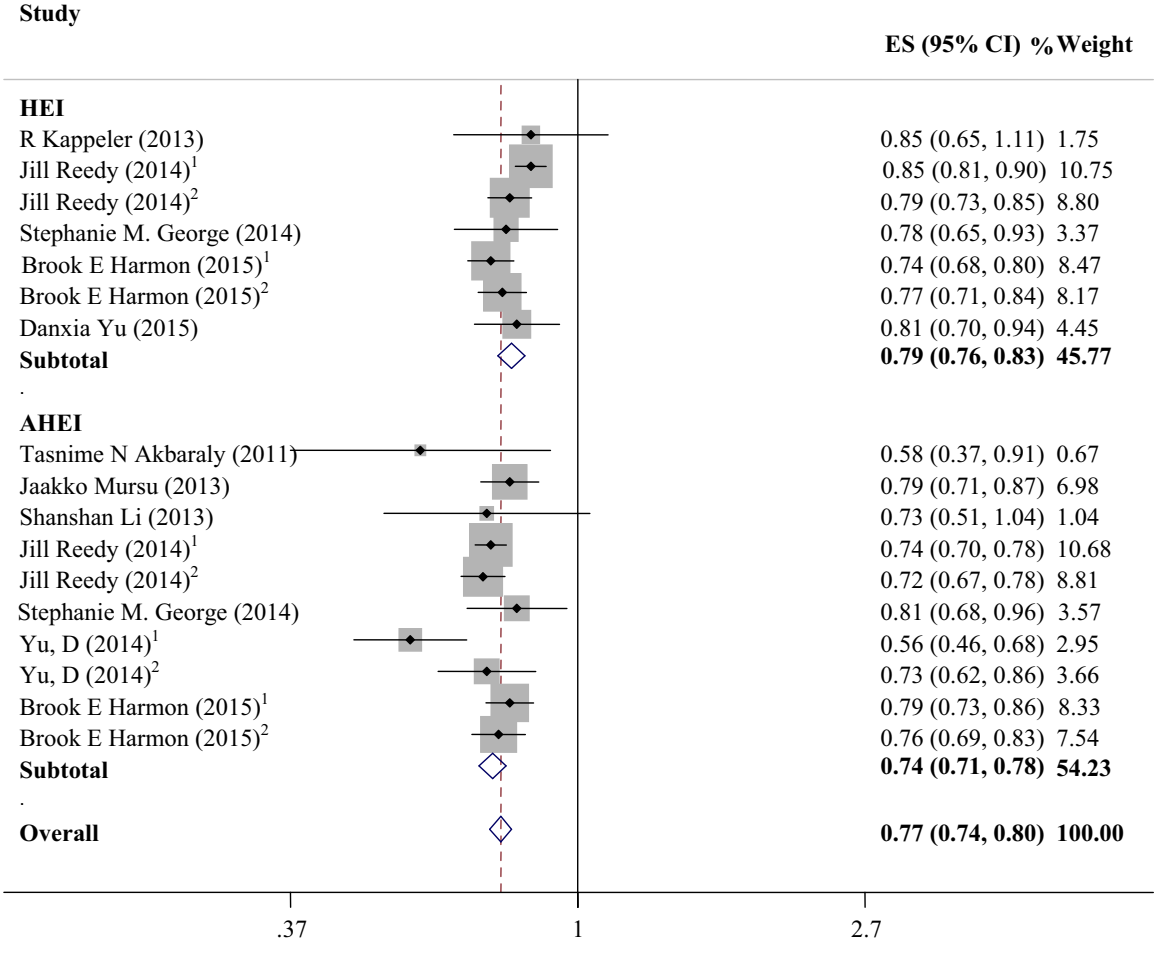

Figure 4 Forest plot of the Healthy Eating Index (HEI) and Alternative Healthy Eating Index (AHEI) dietary patterns in relation to cardiovascular mortality. [1]Men; [2]Women. ES, Effect size; CI, confidence interval.

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Table 2 Subgroup analysis for Healthy Eating Index (HEI) and Alternative Healthy Eating Index (AHEI) dietary patterns and all-cause, cardiovascular and cancer mortalities

	Effect size	Confidence interval	I^2	*P* for heterogeneity	*P* subgroup heterogeneity
HEI and all-cause mortality					
Region					
USA	0.78	0.76, 0.79	0.0	0.638	0.7
Non-USA	0.77	0.73, 0.81	47.8	0.166	
Sex					
Male	0.77	0.74, 0.80	40.6	0.194	0.7
Female	0.77	0.75, 0.80	0.0	0.794	
HEI and cardiovascular mortality					
Region					
USA	0.83	0.79, 0.86	0.0	0.569	0.01
Non-USA	0.75	0.71, 0.80	0.0	0.503	
Sex					
Male	0.80	0.69, 0.91	87.4	0.005	0.5
Female	0.78	0.74, 0.82	0.0	0.906	
HEI and cancer mortality					
Region					
USA	0.78	0.76, 0.81	0.0	0.582	0.3
Non-USA	0.82	0.70, 0.96	83.0	0.015	
Sex					
Male	0.76	0.73, 0.79	0.0	1.000	0.03
Female	0.83	0.78, 0.88	21.7	0.280	
AHEI and all-cause mortality					
Region					
USA	0.77	0.75, 0.79	24.8	0.240	0.7
Non-USA	0.77	0.74, 0.80	34.1	0.194	
Sex					
Male	0.75	0.72, 0.79	59.3	0.086	0.1
Female	0.77	0.75, 0.79	0.0	0.565	
AHEI and cardiovascular mortality					
Region					
USA	0.75	0.72, 0.77	0.0	0.547	0.9
Non-USA	0.71	0.64, 0.80	65.4	0.021	
Sex					
Male	0.72	0.63, 0.81	80.4	0.006	0.7
Female	0.74	0.70, 0.78	0.0	0.596	
AHEI and cancer mortality					
Region					
USA	0.87	0.82, 0.91	34.2	0.179	0.97
Non-USA	0.85	0.81, 0.90	0.0	0.786	
Sex					
Male	0.83	0.79, 0.86	0.0	0.779	0.08
Female	0.88	0.85, 0.93	0.0	0.634	

adherence to the HEI and AHEI dietary patterns and a decreased risk of all-cause mortality, as well as cardiovascular and cancer mortalities. The HEI pattern was more effective in decreasing cancer mortalities, whereas the AHEI pattern more effectively reduced cardiovascular mortality.

Despite major similarities between the HEI and AHEI patterns, there were some differences between them, which may consequently lead to their also having distinct associations with health outcomes. The similarities are that both diets emphasise vegetables, fruits and plant-based proteins. Therefore, it is expected that both patterns would have favourable effects on health status; nonetheless, it is not clear which pattern is more effective. The relationships between high fruit and vegetable consumption and lower risks of cardiovascular diseases [35], different types of cancers [36,37] and all-cause mortality [38] have been shown in earlier meta-analyses. This association may be a result of antioxidant activity, inflammation inhibition of flavonoids, fibre, magnesium and potassium contents [39].

Differences between the two diets may be a result of HEI not counting red and processed meat, whereas they are AHEI components. Findings from previous meta-analyses have indicated that red (and especially processed) meats are associated with increased risk of all-cause, CVD and cancer mortalities [40–43]. Moreover, the AHEI pattern recommends moderate alcohol consumption [32], whereas the HEI pattern has no recommendation regarding alcoholic beverages [19] and the HEI-2005 grouped it with calories from solid fats and added sugars [4,44]. In the HEI-2010, an excessive intake of alcohol energy is penalised. In a meta-analysis by Wang *et al.* [45] heavy drinkers, especially female drinkers, had an increased risk of all-cause mortality. However, a recent cohort study showed a direct association between healthy alcohol-drinking pattern and a reduction of mortality [46]. Another point worthy of note is the recommendation of low-fat dairy in HEI-2010 as a beneficial component, whereas the AHEI pattern does not include low-fat dairy in its score. Low-fat dairy, as a healthy dietary pattern component, could decrease all-cause mortality [47,48].

Our findings indicated that the AHEI pattern led to a larger decrement in cardiovascular mortality. This is not unexpected because all components of AHEI (e.g. fruits, vegetables, plants proteins, trans-fats, polyunsaturated-to-saturated fat ratio and multivitamins) have protective roles against cardiovascular diseases [7]. Additionally, the focus of HEI on the diversity of vegetable consumption could justify its inverse effect on cancer mortality [19].

Meta-analyses of different cohort studies typically result in high levels of heterogeneity and I^2 values decrease considerably after stratification [49]. Subgroup analyses were conducted to identify sources of heterogeneity. However, neither sex, nor region was a source of heterogeneity. Nonetheless, men who followed both HEI or AHEI dietary patterns had greater decreased in cancer mortality than women. Moreover, for the HEI index, populations from various geographical regions suggested greater decreases in cardiovascular mortalities compared to studies from the USA.

To interpret our findings, several limitations must be considered. Several potential sources of heterogeneity exist, including differences in definitions and scoring of

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dietary quality indices, heterogeneous risk estimates, population/age/sex, sample size and follow-up duration. The HEI, AHEI and their updates are heterogeneous in terms of scoring methods (i.e. the HEI is based on absolute values, whereas the HEI-2005/2010 is based on quantities per 1000 kcal). The association between diet and mortality is complex, requiring adjustment and stratification by multiple confounding variables and careful interpretation. Multivariate adjustments for potential confounders in each study were different. Almost all studies were adjusted for age, race, sex, cigarette smoking, physical activity, socio-economic status, body mass index and total energy intake. Nevertheless, a history of cardiovascular disease, diabetes, hypertension, dyslipidaemia or postmenopausal hormone use were not adjusted for in most studies. Another limitation is related to the design of cohort studies compared to randomised controlled trials. Cohort studies have caveats, including a reliance on nutritional assessment methods, with validity and reliability being lower compared to randomised controlled trials. Case–control studies are still prone to error (e.g. relative to cohort studies) and possible measurement errors and recall bias should be taken into account

Our meta-analysis has several strengths as well. Many of the studies, especially cohort studies, were of high methodological quality. Overall, more than 900 000 subjects were included in this meta-analysis, providing adequate power to detect statistically significant RRs, as well as assess publication bias.

In conclusion, the present meta-analysis shows that high adherence to the HEI and the AHEI dietary patterns, as high diet quality indices, are associated with reduced risk of all-cause mortality (as well as cardiovascular mortality and cancer mortality).

Conflict of interests, source of funding and authorship

The authors declare that there are no conflicts of interest.

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SHO and FH were involved in study conception, searches, study selection, data extract, statistical analysis and data interpretation. LA was involved in study conception and design. BL commented on the study and also on the paper. PS edited the English and scientific writing in the manuscript submitted for publication and also commented on the paper. All authors contributed to the drafting of the manuscript and approved the final version for submission.

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CRITICAL ILLNESS AND AGEING

Does body mass index impact on muscle wasting and recovery following critical illness? A pilot feasibility observational study

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Abstract

Background: Critical illness is associated with muscle loss, weakness and poor recovery. The impact that illness and the ensuing metabolic response has on obese patients is not known. Objectives were to test if obese patients lose less muscle depth compared to non-obese patients; if a reduction in muscle depth was associated with reduced strength and recovery; and to assess the feasibility of these methods with a range of body mass index's (BMI).
Methods: A prospective observational pilot study of muscle depth in critically ill patients categorised by BMI was performed. Muscle depth changes were assessed by ultrasound on study days 1, 3, 5, 7, 12 and 14. Strength was measured via handgrip dynamometry and Medical Research Council (MRC) sum score on waking and at discharge from the intensive care unit. Level of dependency was measured with the Barthel index.
Results: 44 critically ill patients; 17 had normal BMI, 10 were overweight and 17 were obese. The three groups did not differ in baseline characteristics, except obese patients had significantly greater initial muscle depth. Muscle depth loss was similar between the BMI groups at each of the time points. Handgrip and MRC sum score were only possible in a small number of patients because of reduced alertness and weakness. Majority were deemed fully dependent based on the Barthel index.
Conclusions: Obese patients lost muscle depth in a comparable manner to non-obese patients, suggesting that BMI may not prevent muscle depth loss. It was not possible to determine the effect on strength because the clinical condition of patients precluded reliable measurements.

Introduction

Critical illness is associated with hypermetabolism and catabolism, which results in a dramatic loss of muscle mass [1,2]. Patients who are mechanically ventilated for longer than 7 days frequently experience muscle wasting syndromes or profound weakness [referred to as intensive care unit (ICU) acquired weakness] [3,4]. This is unequivocally associated with decreased survival, increased rates of infections, longer hospital stays and delayed recovery, and increase in healthcare costs [5,6].

While many different, potentially interacting pathophysiological mechanisms have been proposed for ICU acquired weakness, [7] no previous work explored the possible role of patients body composition or body mass index (BMI). The prevalence of obesity is increasing in ICUs, with large retrospective databases suggesting prevalence rates of 26–31% [8,9].

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There is a lack of research detailing the nutritional and metabolic processes seen in obese critically ill patients. It is not clear what impact critical illness and the ensuing metabolic response has on obese patients, nor how this compares with respect to to non-obese patients. For example, patients with obesity may be able to metabolise their excess adipose stores as the dominant fuel source and preserve muscle mass [10–12]. One study of only 17 patients (seven obese) investigated whether the metabolic response to trauma is different between obese and non-obese critically ill patients. Obese patients experienced increased nitrogen losses and reduced protein synthesis compared to non-obese patients [13], suggesting that critically ill obese patients may lose the ability to conserve protein stores.

Our study objectives were to: (i) test if obese patients lose less muscle depth compared to non-obese patients; (ii) detect if a reduction in muscle depth was associated with reduced muscle strength and delayed functional recovery; and (iii) as a pilot study, assess the feasibility of performing these methods in patients with a range of BMIs to inform a larger trial.

Materials and methods

Study design

The present study comprised a pilot feasibility, prospective observational study of critically ill patients over a 1-year period (2010–2011). Patients were recruited from the general ICUs of three tertiary UK teaching hospitals. Ethical approval was gained from the North West London Research Ethics Committee (NRES reference: 10/H0722/40). Written informed consent was obtained from patients or agreement was sought from their designated representative, with retrospective patient consent obtained when full mental capacity was re-gained.

Inclusion criteria were those patients who were aged > 18 years, had a BMI > 19 kg m^{-2}, were expected to be mechanically ventilated for longer than 48 h, and were being artificially fed. Pregnant patients were excluded.

Patients were entered into the trial within 72 h of ICU admission. Baseline demographics included admission reason/diagnosis, number of comorbidities [14], severity of illness defined by Acute Physiology And Chronic Health Evaluation score (APACHE II) and anthropometric measurements (weight, height and BMI). Data on mortality, ICU length of stay and days on mechanical ventilation were collected on conclusion of the study.

Muscle depth

Peripheral muscle depth changes at three sites (bicep, forearm and thigh) were assessed by ultrasound in every participant on study days 1, 3, 5, 7, 12 and 14 to detect muscle depth change over time according to protocols described previously [15–17]. Three measurements were performed at each site and the mean was calculated [16]. For each patient, all measurements were performed by the same investigator. Standardised training protocols were followed.

Physical assessment

Two measurements of strength were used: hand grip strength and the Medical Research Council (MRC) sum score for muscle power [3]. These were measured at waking (sedation off and patient alert) and ICU discharge. To determine whether the patient was sufficiently awake to participate, a four battery test was conducted, including the instructions: open your eyes, follow my finger, stick your tongue out, and squeeze my hand.

The MRC sum score was carried out on six different bilateral muscle groups: shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension and foot dorsiflexion. Strength in each muscle group was scored according to the six-point MRC system, in which a score of 0 was no contraction, 1 was a flicker of contraction, 2 was active movement with gravity eliminated, 3 was active movement against gravity, 4 was active movement against gravity and resistance and 5 was normal power. The maximum score is 60. A score < 48 was used as a cut-off to indicate ICU acquired weakness or muscle wasting [18]. Two investigators (ES and LW) performed the MRC assessment after receiving training from an experienced physiotherapist.

Handgrip strength was measured to detect changes in lower arm and hand strength of the dominant hand using a hydraulic handgrip dynamometer (JAMAR; Lafayette Instruments, Lafayette, IN, USA). The patient was positioned in a sitting position with elbow flexion of 90°. An average of the three readings was used. A strength value < 11 kg in males and 7 kg in females corresponds with ICU acquired weakness or muscle wasting [19].

The Barthel index was used to assess the ability to carry out activities of daily living. It measures the capacity to perform 10 basic activities and gives a quantitative estimate of the patient's level of dependency and range from 0 (totally dependent) to 100 (totally independent). It has been used to assess functional recovery following critical illness [20,21].

Nutritional intake

Patients were fed according to the units' feeding protocol, which stated that the enteral route should be the first choice, and also that nutrition should be commenced

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within 48 h of admission. If enteral nutrition failed or was contraindicated, parenteral nutrition was commenced. The treating dietitian set the energy and protein targets. Energy requirements were calculated using Schofield equation, adding factors for stress and activity to make it clinically relevant [22], or the Penn State Equations [23,24] (currently recommended as the most accurate way of predicting energy requirements for both obese and non-obese critically ill patients) [25,26]. We had no access to indirect calorimetry. Protein requirements were calculated as 1.2–1.3 g kg^{-1} actual body weight for normal weight patients, [27,28] 0.8–1.2 g kg^{-1} actual body weight for obese patients [27], and were adjusted to reflect clinical parameters such as renal and liver impairment, filtration and excess losses. Nutritional intake data were collected on a daily basis for the days that the patient remained in the study. For the analysis, the energy and protein prescription and received are expressed as kcal kg^{-1} and g kg^{-1}, respectively. This is the overall intake divided by body weight, allowing for direct comparison with other studies.

Anthropometric measurements

BMI was calculated using the most recent accurate body weight and height obtained from the patient's medical notes. If unavailable, the patient was either weighed or estimated on the ICU bed, or weights sought from family members. When height was not available, this was estimated from ulnar length measurement, taken between the point of the acromion and the ulnar styloid. The resulting value was then converted into an estimated height [29].

Statistical analysis

Descriptive statistics are presented as median and interquartile range or the mean (SD). Patients were categorised into three independent groups according to BMI:

Group 1 (Normal weight) BMI 19–24.9 kg m^{-2}
Group 2 (Overweight) BMI 25–29.9 kg m^{-2}
Group 3 (Obese) BMI $\geq$ 30 kg m^{-2}

Comparisons between these groups were made using *t*-tests or Mann–Whitney tests. Missing data were assumed to be missing at random. Missing day 7 values for the primary outcome were imputed by carrying forward day 5 values.

Regression analysis was undertaken to quantify the association between muscle loss (loss as a percentage from the initial value at day 7) and the variables; baseline BMI, age, sex, comorbidities, APACHE II and thickest muscle depth. Results are presented as coefficients, *P*-values and 95% confidence intervals. All statistical analyses were performed using STATA, version 13 (Statcorp LP, College Station, TX, USA). $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics are shown in Table 1. Figure 1 presents the patient flow diagram. Thirty-three (75%) patients were male, the median age was 58 years and the admission APACHE II score was 20. There were no differences in baseline characteristics on admission. Six (14%) patients were recruited within 24 h of admission, 50% (22/44) within 48 h of admission and 36% (16/44) within 72 h of admission to the ICU.

There was considerable loss to follow up observed in all three groups (Fig. 1). From day 3, there were missing data (i.e. patients were unable to perform tests for clinical reasons) and most patients had been discharged or had died by day 12. For this reason, no results are presented for day 14 because only seven out of 44 patients remained in the ICU.

The obese group had statistically significantly greater initial combined muscle depth compared to the normal weight group, with an average greater muscle depth of 3 cm (Table 2). No significant difference in the combined

Table 1 Baseline characteristics of patients

Characteristics	Normal (BMI 19–24.9)	Overweight (BMI 25–29.9)	Obese (BMI ≥30)
Number	17	10	17
Median (IQR) age (years)	53 (29)	65 (16)	55 (15)
Sex: male (%)	13 (77%)	6 (60%)	14 (82)
Median (IQR) BMI	22 (4)	28 (2.5)	33 (1)
Diagnosis			
Surgical	4	2	4
Medical	7	5	8
Trauma/head injury	6	3	5
Comorbidities			
Limited nil or 1 (%)	9 (53)	3 (30)	8 (47)
Multiple >2 (%)	8 (47)	7 (70)	9 (53)
Median (IQR) APACHE II score	20 (7)	22 (7)	20 (10)
Median (IQR) Length of ICU stay (days)	16 (17)	7 (6)	11 (9)
Median (IQR) Duration of mechanical ventilation (days)	7 (9)	4 (4)	7 (10)
% Mortality (deaths/total number)	11.7% (2/17)	40% (4/10)	35% (6/17)

APACHE II, acute physiology and chronic health evaluation; BMI, body mass index (kg m^{-2}); IQR, interquartile range.
No statistically significant differences between any of the groups. All $P > 0.05$.

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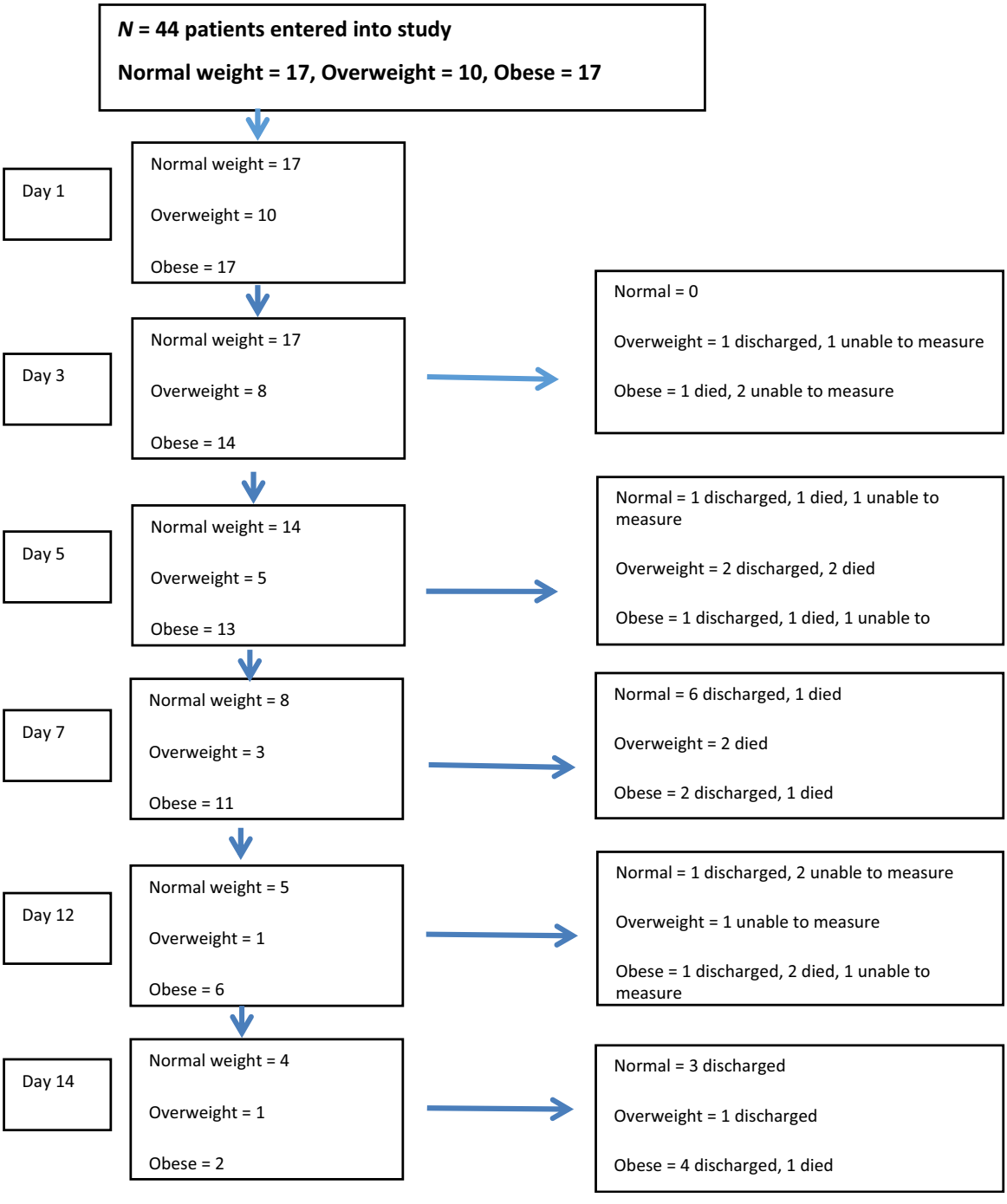

Figure 1 Patient flow diagram illustrating the available data at different time points.

muscle depth loss expressed as loss (cm) or percentage loss between the groups was seen over days 5, 7 or 12.

The loss for each site (bicep, forearm and thigh) is reported in the Supporting information (Table S1). Obese patients had significantly more thigh depth compared to the non-obese patients (0.05). The percentage loss from initial over day 3, 5, 7 and 12 for each site was not different between the groups. The normal patients lost significantly more muscle depth in biceps compared to thighs at day 5 ($P = 0.003$). However, this upper and lower limb difference was not observed in the other groups. Regression analysis of the relationship between day 7 percentage loss and various independent variables (Table 3) found that only sex was significant. When

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Table 2 Measurements of muscle depth changes during critical illness

Outcome	Normal weight (n = 17)	Overweight (n = 10)	Obese (n = 17)
Median (IQR) initial muscle depth (cm)	8.19 (2.8)*	8.90 (3.8)	11.15 (3)*
Muscle loss as percentage (IQR) from initial at			
Day 5	10.1 (11.4)	11.0 (9)	8.5 (12.8)
Day 7	14.9 (19.5)	17.9 (19.8)	19.7 (18.5)
Imputed day 7	11.6 (14.4)	17.9 (19.8)	15.6 (16.6)
Day 12	15.9 (11.8)	32.2 (0)	26.8 (3.6)
Median (IQR) muscle loss (cm) at			
Day 5	0.88 (1)	1.2 (0.8)	1.13 (1.6)
Day 7	1.49 (1.6)	2.08 (1.7)	2.39 (2.2)
Imputed day 7	1.11 (1.7)	1.62 (1.5)	2.04 (2.2)
Day 12	1.44 (1.4)	4.14 (0)	2.85 (0.7)
% loss (IQR) per day at			
Day 5	1.7 (1.9)	1.8 (1.5)	1.4 (2.1)
Day 7	2.5 (3.3)	3.0 (3.3)	3.3 (3.1)
Imputed day 7	1.9 (2.4)	3.0 (3.3)	2.6 (2.7)
Day 12	2.6 (2.0)	5.4 (0)	4.5 (0.6)

IQR, interquartile range.For the number of participants in each group at each time point, see Fig. 1.
Imputed day 7: if day 7 values were missing, day 5 values were assumed to be equal and therefore multiple imputation was applied.
The day is the study day (not time from admission) (i.e. days 5, 7 and 12 from first data collection).
*Initial muscle depth: $P = 0.006$ between normal and obese.
For other values, no statistically significant differences between any of the groups. All $P > 0.05$.

explored further (see Supporting information, Table S2), women had significantly less initial muscle depth and lost significantly more at day 7 compared to men.

Nutritional data are also shown in the Supporting information (Table S3). Most patients (84%) were fed via the enteral route, with the rest receiving parenteral, a combination of enteral and parenteral or oral nutrition. The mean energy prescription was significantly less for the obese group compared to the other groups. Irrespective of BMI group, all patients received inadequate energy and protein compared to their nutrition prescription.

Functional recovery was difficult to assess as a result of an inability of the patient to perform the tasks because of a lack of alertness, weakness and poly-trauma. As a result of the small numbers, no analysis was reported. It was only possible to undertake the MRC sum score and handgrip strength tests in 12 of 44 patients (27%; normal weight = 4/17; overweight = 2/10; obese = 6/17). The majority of patients were deemed fully dependent by the Barthel index at days 1 (98%), 3 (90%) and 7 (85%), with only four patients out of 27 gaining a score > 5 at day 7 of ICU admission.

Discussion

To our knowledge, the present study is the first to investigate whether muscle depth loss observed during critical illness differs between obese, overweight and normal weight patients using a muscle ultrasound technique. The muscle depth loss was comparable and not statistically different between the BMI groups at each of the time points. Increased muscle breakdown in obese patients was seen in the study by Jeevanadam *et al.* [13], although patients were not fed for the duration of this study and this may have influenced the rate of muscle breakdown. Direct comparisons cannot be made because that study used the more precise method of whole body turnover to determine muscle loss compared to the ultrasound technique in the present study.

A trial using ultrasound muscle depth to measure muscle wasting in general ICU patients [16] found a decrease of 1.6% per day over 7 days and those with the greatest amount of muscle at the start lost significantly more muscle. This was not observed in the present study, where women had less muscle to start and lost significantly more. A low admission muscle mass has been

Table 3 Regression analysis of relationship between D7% muscle depth loss and various independent variables

	Univariate			Multivariate		
Variable	Coefficient	P	CI	Coefficient	P	CI
Baseline BMI						
Overweight	0.29	0.965	−13.0 to 13.5	0.29	0.96	−11.1 to 11.7
Obese	1.8	0.700	−7.7 to 11.4	1.59	0.70	−6.6 to 9.8
Age	3.94	0.357	−4.6 to 12.5			
Sex	−16.0	0.001	−25.1 to 6.9	−15.9	0.002	−25.3 to −6.6
Comorbidities	0.85	0.843	−9.6 to 7.89			
APACHE II	2.67	0.597	−6.4 to 10.9			
Thickest muscle depth at day 1	0.87	0.841	−9.6 to 7.88			

APACHE II, acute physiology and chronic health evaluation; BMI, body mass index (kg m^{-2}).

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shown to be a risk factor for mortality in critically ill patients [30]. Campbell *et al.* [15] studied nine patients with multi-organ failure (MOF) and found a decrease in muscle depth of 6% per day. The muscle loss was considerably higher than that in the present study and that of Reid *et al.* [16]. This could be explained by the illness severity, although Campbell *et al.* [15] provided no APACHE II or organ failure scores. Assumptions can only be made based on the fact that they all experienced MOF.

The idea that there is a relationship between the degree of organ failure and the rates of muscle loss is developed further in the largest published muscle ultrasound study of ICU patients [31]. Muscle mass decreased significantly at day 7 and was significantly greater in patients who experienced MOF by day 7 (losing 15.7%) compared to a 3% loss in those with single organ failure. Our findings are comparable with that of the MOF patients.

We present our muscle depth loss as a combined score of three sites and this may give the impression that all muscle groups waste at the same rate. This may not be the case. The different quadriceps muscles have been shown to waste at differing rates [32] and the diaphragmatic muscles wasted faster than quadriceps [33]. In a study that used limb circumference, muscle atrophy was observed more in the lower limbs compared to the upper limbs [34]. However, this method has been found to be inferior compared to ultrasound in a recent systematic review [35]. When we examined loss at each site individually, the opposite was found, with the normal weight patients experiencing a larger proportionate loss in the biceps compared to thighs. There was no difference between the groups in terms of the wasting seen at each of the three sites. This warrants further investigation in larger patient numbers.

The ultrasound technique was shown to be feasible and time efficient tool for use at the bedside in a range of BMI categories. It was not possible to undertake measurements on nine occasions for a variety of reasons, including the patient being too agitated, spastic flexion in the legs, skin breakdown, dressings, peripheral cannula and limbs in casts. There was no difference between the groups with respect to the reasons why measurements could not be performed. It was more difficult to obtain accurate images in the obese patients but, by increasing the scanning depth, it was found to be possible in all patients.

As a result of the low number of patients able to undertake the functional recovery measures, the results are of limited value. Despite the MRC score being advocated as a predictor of ICU acquired weakness [18], there are limitations to its clinical usefulness [36]. As in other trials, a significant proportion of patients in the present study were unable to perform the test [36,37]. The same was observed with the hand grip test, where many patients were too weak to even lift the tool. The Barthel score did not appear to be sufficiently sensitive to detect subtle changes in recovery for this group of patients when they were in the ICU. From our experience, we recommend that the use of the MRC score and grip strength are clinically limited in the ICU and are best used once the patient has left the ICU.

The majority of patients were fed enterally, which can be challenging to deliver [38]. Common reasons given for inadequate delivery are gastrointestinal intolerance and fasting for a variety of ICU-related procedures. The poor nutritional delivery in the present study may have influenced the rates of muscle depth lost, although we did not set out to measure this. In a previous study [16] where energy targets were determined by indirect calorimeter, achieving energy balance made no difference to muscle depth loss. Our findings and the results of other studies [13] do not support the notion that obese patients are able to metabolise their excess fat stores and preserve muscle mass. Therefore, based on the findings of our pilot study, the practice of underfeeding obese critically ill patients warrants further investigation.

Although the study participants are broadly representative of the general ICU population that they were recruited from, it is not possible to make any concrete inferences about the generalisability of the results as a result of the small sample size. A larger study with more power is needed.

Limitations

Because the present study was a feasibility pilot study, no power calculation was undertaken, and the number of patients recruited was small. We experienced considerable loss to follow-up as a result of the relatively short ICU stays, although we had intended to follow patients for 14 days. The high attrition rate limits the validity and reliability of our results. To facilitate recruitment, patients could be recruited at up to day 3 of admission to the ICU, with the majority being recruited on day 2. Substantial muscle loss can occur between days 1 and 3, especially in those in multiple organ failure [31]. In the present study, the majority of patients were not recruited on day 1 and so considerable muscle loss may already have occurred before our baseline measurements were taken. As such, our results maybe an under-estimation of the amount and rate of muscle depth loss during the ICU admission. As seen in the study by Puthucheary *et al.* [31], muscle wasting is significantly influenced by the level of organ failure. We did not collect data on the numbers of organs failing, which would have aided the interpretation of muscle depth loss in the present study.

The present study used ultrasound, which is a practical nonvolitional and effect-independent approach of

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monitoring muscle depth changes. Intra- and inter-rater reliability was not performed. However, previous work within our group showed good correlation and agreement, with an intra- and inter-rater reliability of 0.984 and 0.965, respectively. Reliability testing was not performed for the MRC scoring. We recommend following a training programme with extensive practice to ensure competency in ultrasound and MRC score techniques, in addition to undertaking reliability testing.

The present study categorised patients according to their BMI; this relied on weight and heights, which can be hard to determine accurately in critically ill patients [39,40]. A variety of methods were employed to achieve the most accurate measure possible. Experienced ICU dietitians were making the decisions and thus we feel confident that they are unlikely to place the patient in the wrong category. The limitations of BMI should also be acknowledged. Because it is a measure of excess weight rather than excess fat, other factors such as age, sex, ethnicity and muscle mass can influence the BMI [41]. These factors were considered when allocating patients to the appropriate BMI group. A patient was not recruited to the study if they had a raised BMI as a result of an increased muscle mass as opposed to increased adipose stores (as seen with some ethnic groups and those with increased musculature from excess physical training) [41].

Recommendations for future research

The results of the present pilot study suggest there may not be any difference in the rates of muscle wasting between BMI groups. It is also suggested that women may lose proportionately more muscle mass than men, although the study was underpowered to detect a meaningful difference. These finding need to be confirmed in further, adequately powered studies.

Future studies need to adjust for confounding factors such as age, sex, illness severity score and degree of organ failure, prior nutritional status and the adequacy of nutritional support received. The amount of nutrition support received (energy and protein intake) may influence the rate of muscle depth lost and needs to be investigated further. We recommend employing in-trial strategies to enhance enteral feeding delivery, such as guidance on reduced fasting times and management of gastrointestinal intolerance. Finally, to ensure that functional measures of strength and recovery can be performed, patients should be followed up on discharge from the ICU.

To conclude, there were no differences in the rates of muscle depth lost between ICU patients in different BMI categories in the setting of comparable nutrition support, suggesting that a high BMI may not prevent muscle depth loss.

Transparency declaration

The lead author (Ella Segaran) confirms that the 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 and authorship

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ES conceived and designed the study, conducted the study, performed the statistical analysis and prepared the manuscript. LW assisted with data acquisition. MH, MS and LW informed the study design, provided supervision and critically revised the manuscript for publication. MT assisted with statistical analysis and manuscript revision. 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:

Table S1. Measurements of upper and lower limb percentage weight loss from initial value.

Table S2. Measurements of muscle depth changes per sex.

Table S3. Nutrition support of the study patients during critical illness.

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CRITICAL ILLNESS AND AGEING

Longitudinal associations between micronutrient consumption and leukocyte telomere length

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Abstract

Background: There are few studies on the association between nutrient intake and telomere length, which may reflect cumulative oxidative stress and indicate biological ageing. In the present study, we evaluated longitudinal associations between the consumption of micronutrients, including antioxidant nutrients and B vitamins involved in one-carbon transfer pathways, and leukocyte telomere length (LTL).
Methods: The study included 1958 middle-aged and older Korean men and women (age range at baseline: 40–69 years) from a population-based cohort. We collected dietary information at baseline using a semiquantitative food frequency questionnaire (June 2001 to January 2003) and assessed the consumption of micronutrients, including vitamins A, B_1, B_2, B_3, B_6, B_9 (folate), C and E, as well as calcium, phosphorus, potassium, iron and zinc. We measured LTL using a real-time polymerase chain reaction at the 10-year follow-up examination (February 2011 to November 2012).
Results: In the multiple regression model adjusted for potential confounders, LTL was positively associated with the consumption of vitamin C ($P < 0.05$), folate ($P = 0.05$) and potassium ($P = 0.05$) in all participants. In the age-stratified analysis, the association between the consumption of vitamin C ($P < 0.01$), folate ($P < 0.05$) and potassium ($P < 0.05$) with LTL was significant only among participants aged <50 years.
Conclusions: Our findings suggest that the earlier consumption of vitamin C, folate and potassium, which are abundant in fruits and vegetables, can delay biological ageing in middle-aged and older adults.

Introduction

Telomeres are regions of repetitive DNA sequences (TTAGGG) protecting the ends of chromosomes. Telomere length may reflect cellular ageing because the loss of telomeric repeats possibly proceeds with successive DNA replication [1,2]. The attrition rate of telomeres varies with age, sex and ethnicity, as well as with external factors such as cigarette smoking, physical activity, diet and environmental pollution [3–7]. Telomere length is also associated with ageing-related diseases, such as hypertension, diabetes, coronary heart disease and dementia [8–11].

The hypothesis regarding the influence of dietary intake on the maintenance of telomere length has been raised. [12]. Some studies have reported that dietary patterns and the consumption of specific food items are associated with leukocyte telomere length (LTL) [6,7,13,14]. Leukocytes are commonly used for the measurement of telomere length because they are easily obtainable, and LTL reflects the attrition rate of telomeres in somatic cells [15]. It was reported that LTL was positively associated with a high consumption of fruits and vegetables [7,14] and inversely associated with meat consumption [6]. Because oxidative stress is considered to be one of factors that causes the attrition of telomeres, it has thus been hypothesised that antioxidant defence potential can delay telomere attrition [16]. A cross-sectional study found that a higher intake of nutrients with antioxidative properties, such as vitamins C

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and E, was associated with longer LTL [17]. The hypothesis that a deficiency of folate and vitamin B_{12}, which are involved in one-carbon transfer pathways for nucleic acid synthesis, influences telomere attrition has been suggested [18], although only folate intake was significantly associated with LTL [17]. These data were not confirmed in other epidemiological studies, and no data on the association between nutrient intake and telomere length are currently available for Asian populations.

In the present study, we evaluated the longitudinal associations between the earlier consumption of micronutrients, including antioxidant nutrients and B vitamins involved in one-carbon transfer pathways, and LTL in middle-aged and older Korean men and women. We used dietary data collected 10 years before LTL was measured to evaluate the earlier consumption of micronutrients on LTL given the assumption that dietary habits had been maintained during the period. We included micronutrients such as vitamins A, B_1, B_2, B_3, B_6, B_9 (folate), C and E, as well as calcium, phosphorus, potassium, iron and zinc, in the present study.

Materials and methods

Study design and participants

We investigated the association between micronutrient intake and LTL in a longitudinal data analysis. The study participants belonged to a population-based cohort from the Korean Genome Epidemiology Study, which is an ongoing prospective study. Detailed information on this study is available elsewhere [19,20]. Briefly, the cohort members are residents of Ansan City, Republic of Korea and were aged 40–69 years during the baseline period (June 2001 to January 2003). To enroll cohort members, we utilised a list of telephone numbers obtained from local telephone companies because the community has a high penetration rate of telephone subscribership. We conducted a two-stage cluster sampling on the basis of governing districts from the telephone directory and information regarding age and sex distribution from the 2000 Census. We received verbal agreement to participate from 5792 of 10 957 eligible subjects (a response rate of 53%). A total of 5012 participants visited the Korea University Ansan Hospital and completed a baseline health examination and a questionnaire-based interview to become cohort members. The distributions of age and sex, percentage of alcohol drinking, and prevalence of hypertension and diabetes mellitus were similar between participants and nonparticipants, although smokers were more likely to refuse participation in the cohort study. During the health examination, trained healthcare professionals measured the blood pressure (BP) and anthropometric parameters of the participants and collected biological specimens for biochemical analysis. The interview included a discussion of demographic and medical information, health condition, history of family disease, lifestyle, and dietary habits. This standardised health examination and interview were performed biennially. During a 10-year period, 64.1% of cohort members were followed up. At each follow-up visit, participants provided their written informed consent, as approved by the Human Subjects Review Committee of Korea University Ansan Hospital (ED0624).

Because only 2314 cohort members agreed to provide blood samples for LTL assays during the period from February 2011 to November 2012, they were included in the present study. They reported baseline information regarding demographics, medical status, lifestyle and diet, which we utilised in the present study. When we compared the baseline characteristics between 2698 nonparticipants and 2314 participants, nonsmokers and those with lower income were more likely to participate in the study, whereas dietary intake was similar between the two groups. Participants who were excluded included those considered to have outlying LTL values (LTL > 4; $n = 2$), those diagnosed with cancer or cardiovascular disease ($n = 277$), those with incomplete information on confounding variables ($n = 55$) and those with inappropriate total energy intake [<2.09 or ≥20.92 MJ day^{-1} (<500 or ≥5000 kcal day^{-1})] ($n = 22$). Thus, data for 1958 participants were entered into the analysis.

Dietary information

Information on dietary intake was collected using the semiquantitative food frequency questionnaire (FFQ), which was developed and validated by the Korea Centers for Disease Control and Prevention (Seoul, Korea) [21]. The FFQ collects information on the average consumption frequency and serving size for 103 food items and beverages consumed in the previous year using nine categories of consumption frequency (almost never, once a month, 2–3 times a month, 1–2 times a week, 3–4 times a week, 5–6 times a week, once a day, twice a day or three times a day) and three categories of serving size (larger than, equal to or smaller than a standard serving size). During the interview using the FFQ, trained interviewers showed pictures of foods to the participants to help them estimate the serving size. To calculate the daily average consumption frequency of each food item, the frequency was multiplied by 1.5 for larger amounts, by 1 for equal amounts and by 0.5 for smaller amounts compared to the standard serving size. The average daily consumption of total energy and other nutrients was calculated using the FFQ data and the food composition data published by the Rural Development Administration of Korea [22].

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Measurement of leukocyte telomere length

LTL was measured using the quantitative real-time polymerase chain reaction method [23]. Leukocyte genomic DNA was extracted from peripheral blood samples, which were collected at the end of the 10-year follow-up period, using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Purified DNA samples were diluted and quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The ratio of the telomere repeat copy number to the single-copy gene (36B4 gene, which encodes acidic ribosomal phosphoprotein) copy number (T/S) was determined for relative LTL using the iQ Multi-Color Real-Time Polymerase Chain Reaction Detection System (Bio-Rad, Hercules, CA, USA). More information on the assay procedure is available in a previous study [24]. A validity test showed that the Pearson correlation coefficients were 0.78 (intra-assay) and 0.69 (inter-assay) when 25 samples were run in triplicate.

Potential confounding variables

Information on potential confounding variables including age, sex, family monthly income, body mass index (BMI), smoking status, alcohol consumption status, physical activity, total energy intake and the presence of metabolic diseases was collected at baseline using the health examination and questionnaire-based interview. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, and BMI (kg m^{-2}) was calculated. For physical activity, metabolic equivalents of scores were calculated as reported in a previous study [19]. The presence of diabetes mellitus, hypertension or hypercholesterolaemia was confirmed if one of the following criteria was met: use of hypoglycaemic medications, fasting plasma glucose level $\geq$ 120 mg dL^{-1} or postprandial glucose level $\geq$ 200 mg dL^{-1} for diabetes mellitus, use of antihypertensive medications or systolic BP $\geq$140 mmHg or diastolic BP $\geq$ 90 mmHg for hypertension, and use of hypolipidaemic medications or serum total cholesterol level $\geq$ 240 mg dL^{-1} for hypercholesterolaemia. BP was measured using a mercury sphygmomanometer with each subject in a sitting position and was recorded to the nearest 2 mmHg. The average of two measurements in the left and right arms was calculated.

Statistical analysis

Descriptive statistics for data were obtained and the chi-squared test and analysis of variance were used to analyse data where appropriate. To test the association between nutrient intake and LTL, multiple linear regression models were constructed. In the models, LTL was natural logarithm-transformed to minimise the effect of outliers and was fitted as a dependent variable. The potential confounding variables adjusted for in the models were: age (continuous variable), sex, income status (five categories: $<10^6$, 10^6 to 1.9×10^6, 2×10^6 to 2.9×10^6, 3×10^6 to 3.9×10^6 and $\geq 4 \times 10^6$ won), BMI (continuous variable), smoking status (four categories: nonsmoking, smoking $\leq$10 cigarettes day^{-1}, smoking 11–20 cigarettes day^{-1} or smoking > 20 cigarettes day^{-1}), alcohol consumption status (three categories: abstainer, moderate drinker and heavy drinker), physical activity (continuous variable), total energy intake (continuous variable) and the presence of diabetes, hypertension or hypercholesterolaemia (binary variables). Furthermore, multiple regression analysis was stratified by age at baseline (age <50 years or $\geq$50 years). All testing was based on a two-sided level of significance. SAS, version 9.3 (SAS Institute, Cary, NC, USA) was used for the statistical analyses.

Results

Means for relative LTL (T/S) were 1.09 (range 0.16–2.88) in women and 1.05 (range 0.07–2.89) in men. The baseline characteristics and nutrient intake of the study participants were compared across LTL tertiles (Table 1). Age was significantly younger in the longest LTL tertile, and alcohol drinkers and participants with diabetes mellitus were likely to have shorter LTL tertile ($P < 0.05$). In addition, vitamin C consumption was significantly higher among those in the longest LTL tertile ($P < 0.05$). However, the consumption of total energy and other nutrients was not significantly different across the tertiles (Table 1).

Table 2 shows the regression coefficient estimates and their variations for the association between nutrient intake and LTL. The multiple linear regression analysis adjusted for total energy intake and other potential confounders indicates that a higher consumption of vitamin C and potassium was positively associated with longer LTL ($P < 0.05$). A borderline statistical significance was observed for the association between the consumption of folate and retinol and LTL ($P = 0.05$ and 0.07, respectively) (Table 2).

The results of multiple regression analyses stratified by age groups are shown in Table 3. Significant associations were observed for the consumption of folate, vitamin C, and potassium among participants <50 years of age. But, there was only a nonsignificant trend observed for the association between vitamin B_2 consumption and LTL among those $\geq$50 years of age. In the multiple linear regression models, however, the interaction term of the age groups and micronutrient intake was not significant (Table 3).

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Table 1 Characteristics according to the leukocyte telomere length tertiles among 1958 participants

Characteristics	LTL tertiles			*P*-value for trend*
	First tertile	Second tertile	Third tertile	
Relative LTL	0.73 (0.13)	0.98 (0.06)	1.49 (0.39)	
Age (years)	48.8 (7.4)	48.2 (7.5)	47.8 (6.9)	<0.01
Men (%)	51.7	55.9	49.3	0.39
Low income (%)†	39.4	39.4	43.3	0.15
Body mass index (kg m^{-2})	24.8 (2.9)	24.7 (3.0)	24.7 (3.0)	0.29
Current smoker (%)	15.6	18.5	12.6	0.12
Current alcohol drinker (%)	54.7	55.7	49.0	<0.05
Daily physical activity (MET-h)	24.8 (9.3)	24.6 (8.7)	23.7 (9.3)	<0.05
Presence of metabolic diseases				
Hypertension (%)	22.8	19.7	19.6	0.15
Diabetes mellitus (%)	12.7	11.2	8.9	<0.05
Hypercholesterolaemia (%)	16.7	17.0	13.9	0.17
Daily average consumption				
Total energy (MJ [kcal])	7.73 (1.85) [1848.4 (441.3)]	7.74 (1.81) [1849.8 (431.9)]	7.76 (1.98) [1855.6 (472.1)]	0.77
Vitamin A (μg RE)	538.1 (322.2)	544.2 (341.6)	551.1 (332.9)	0.48
Retinol (μg)	79.8 (55.6)	81.1 (55.4)	82.7 (57.2)	0.35
Carotene (μg)	2704.5 (1940.6)	2762.5 (2137.2)	2750.8 (1904.2)	0.68
Vitamin B_1 (mg)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	0.81
Vitamin B_2 (mg)	1.0 (0.4)	1.0 (0.4)	1.0 (0.4)	0.38
Niacin (mg)	15.7 (5.2)	15.8 (5.1)	15.6 (5.1)	0.79
Vitamin B_6 (mg)	1.7 (0.6)	1.7 (0.5)	1.7 (0.6)	0.51
Folate (μg)	232.5 (94.4)	229.5 (86.1)	239.6 (99.2)	0.17
Vitamin C (mg)	111.8 (62.0)	112.4 (58.5)	120.5 (75.9)	<0.05
Vitamin E (mg α-TE)	8.5 (3.5)	8.5 (3.4)	8.6 (3.8)	0.50
Calcium (mg)	495.9 (213.7)	488.5 (216.9)	503.6 (223.7)	0.53
Phosphorus (mg)	1003.9 (300.0)	995.8 (287.3)	1009.8 (312.5)	0.72
Potassium (mg)	2407.3 (836.5)	2377.1 (806.3)	2473.6 (897.3)	0.16
Iron (mg)	10.7 (3.8)	10.6 (3.7)	10.8 (4.0)	0.65
Zinc (mg)	8.7 (4.7)	8.5 (3.1)	8.5 (4.5)	0.49

LTL, leukocyte telomere length; MET-h, metabolic equivalent; RE, retinol equivalent; α-TE, α-tocopherol equivalent.
Values are the mean (SD) or proportions.
*Comparison by the tertiles was tested using analysis of variance or a chi-squared test.
†Monthly income < 2×10^6 won.
n = 652 for the first tertile group; n = 653 for the second tertile group and the third tertile group.

Discussion

In the present study, we observed longitudinal positive associations between the consumption of vitamin C, folate and potassium and LTL, and these associations were more apparent in middle-aged adults compared to older participants. Although the associations were moderate because dietary information was collected 10 years before LTL was measured, our findings are supportive, to a certain degree, of the hypothesis that earlier consumption of antioxidant nutrients and B-vitamins involved in one-carbon transfer pathways is associated with longer LTL, suggesting the effects of nutrient intake on biological ageing.

The data regarding the association between nutrient intake and telomere length are still limited [7]. However, some biological mechanisms uphold this association; antioxidant nutrients can modify the balance between oxidative stress and antioxidative response, which influences telomere attrition [16,25]; B vitamins involved in one-carbon transfer pathways may play a role in several cellular functions, including DNA methylation, chromosome stability and telomere maintenance [18,26]. The antioxidative properties of vitamins have been widely reported and discussed. In particular, a previous study found that a higher consumption of vitamins C and E from the diet or multivitamins is associated with longer telomere length in women [17]. Therefore, telomere attrition, which has been postulated to be caused by reactive oxygen species and hydrogen peroxides, may be suppressed by the consumption of vitamin C [25]. It was also

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Table 2 Associations between micronutrient intake and leukocyte telomere length among 1958 participants

Daily intake of nutrients	Model 1*			Model 2†			Model 3‡		
	β	SE	*P*-value	β	SE	*P*-value	β	SE	*P*-value
Vitamin A (μg RE)	1.04×10^{-5}	2.80×10^{-5}	0.71	1.53×10^{-5}	2.80×10^{-5}	0.59	1.71×10^{-5}	2.80×10^{-5}	0.54
Retinol (μg)	2.89×10^{-4}	1.76×10^{-4}	0.11	3.12×10^{-4}	1.77×10^{-4}	0.08	3.21×10^{-4}	1.77×10^{-4}	0.07
Carotene (μg)	-6.09×10^{-7}	4.38×10^{-6}	0.89	-1.08×10^{-8}	4.38×10^{-6}	0.99	2.19×10^{-7}	4.38×10^{-6}	0.96
Vitamin B_1, mg	-5.24×10^{-2}	3.77×10^{-2}	0.16	-3.75×10^{-2}	3.80×10^{-2}	0.32	-3.51×10^{-2}	3.80×10^{-2}	0.36
Vitamin B_2 (mg)	4.91×10^{-2}	3.88×10^{-2}	0.21	6.32×10^{-2}	3.90×10^{-2}	0.11	6.65×10^{-2}	3.91×10^{-2}	0.09
Niacin (mg)	-3.70×10^{-3}	2.98×10^{-3}	0.22	-2.09×10^{-3}	3.04×10^{-3}	0.49	-1.89×10^{-3}	3.05×10^{-3}	0.53
Vitamin B_6 (mg)	2.35×10^{-2}	2.57×10^{-2}	0.36	3.24×10^{-2}	2.58×10^{-2}	0.21	3.40×10^{-2}	2.59×10^{-2}	0.19
Folate (μg)	2.06×10^{-4}	1.16×10^{-4}	0.08	2.19×10^{-4}	1.16×10^{-4}	0.06	2.26×10^{-4}	1.16×10^{-4}	0.05
Vitamin C, mg	3.02×10^{-4}	1.43×10^{-4}	0.04	2.91×10^{-4}	1.43×10^{-4}	0.04	3.03×10^{-4}	1.43×10^{-4}	0.04
Vitamin E (mg α-TE)	2.51×10^{-3}	3.77×10^{-3}	0.51	2.97×10^{-3}	3.78×10^{-3}	0.43	3.26×10^{-3}	3.78×10^{-3}	0.39
Calcium (mg)	2.99×10^{-5}	5.04×10^{-5}	0.55	4.16×10^{-5}	5.05×10^{-5}	0.41	4.53×10^{-5}	5.06×10^{-5}	0.37
Phosphorus (mg)	2.63×10^{-5}	5.89×10^{-5}	0.65	5.01×10^{-5}	5.93×10^{-5}	0.40	5.49×10^{-5}	5.95×10^{-5}	0.36
Potassium (mg)	2.45×10^{-5}	1.47×10^{-5}	0.10	2.83×10^{-5}	1.47×10^{-5}	0.05	2.97×10^{-5}	1.48×10^{-5}	0.04
Iron (mg)	1.11×10^{-3}	3.48×10^{-3}	0.75	2.27×10^{-3}	3.51×10^{-3}	0.52	2.67×10^{-3}	3.52×10^{-3}	0.45
Zinc (mg)	-4.05×10^{-3}	2.45×10^{-3}	0.10	-3.84×10^{-3}	2.45×10^{-3}	0.12	-3.83×10^{-3}	2.46×10^{-3}	0.12

β, regression coefficient estimate; RE, retinol equivalent; SE, standard error of β; α-TE, α-tocopherol equivalent.
Leukocyte telomere length was natural logarithm-transformed and fitted as a dependent variable in linear regression models.
*Data are adjusted for total energy intake, age and sex.
†Data are adjusted for total energy intake, age, sex, family monthly income, body mass index, smoking status, alcohol drinking status and physical activity.
‡Data are adjusted for total energy intake, age, sex, family monthly income, body mass index, smoking status, alcohol drinking status, physical activity and presence of hypertension, diabetes, or hypercholesterolaemia.

Table 3 Associations between micronutrient intake and leukocyte telomere length by age groups at baseline

Daily intake of nutrients	Age <50 years (*n* = 1318)			Age ≥50 years (*n* = 640)			*P*-value for interaction*
	β	SE	*P*-value	B	SE	*P*-value	
Vitamin A (μg RE)	3.18×10^{-5}	3.18×10^{-5}	0.32	1.37E-06	5.73×10^{-5}	0.98	0.37
Retinol (μg)	3.11×10^{-4}	2.11×10^{-4}	0.14	4.99×10^{-4}	3.30×10^{-4}	0.13	0.11
Carotene (μg)	2.31×10^{-6}	4.91×10^{-6}	0.64	-2.67×10^{-6}	9.22×10^{-6}	0.77	0.49
Vitamin B_1 (mg)	-7.99×10^{-2}	4.43×10^{-2}	0.07	7.31×10^{-2}	7.28×10^{-2}	0.32	0.06
Vitamin B_2 (mg)	4.49×10^{-2}	4.59×10^{-2}	0.33	1.43×10^{-1}	7.38×10^{-2}	0.05	0.07
Niacin (mg)	-5.86×10^{-3}	3.46×10^{-3}	0.09	8.67×10^{-3}	6.13×10^{-3}	0.16	0.06
Vitamin B_6 (mg)	2.40×10^{-2}	3.02×10^{-2}	0.43	6.13×10^{-2}	4.90×10^{-2}	0.21	0.12
Folate (μg)	3.06×10^{-4}	1.39×10^{-4}	0.03	9.24×10^{-5}	2.12×10^{-4}	0.66	0.34
Vitamin C (mg)	4.40×10^{-4}	1.64×10^{-4}	0.007	-3.54×10^{-5}	2.85×10^{-4}	0.90	0.64
Vitamin E (mg α-TE)	4.40×10^{-3}	4.33×10^{-3}	0.31	2.80×10^{-3}	7.60×10^{-3}	0.71	0.13
Calcium (mg)	5.50×10^{-5}	5.93×10^{-5}	0.35	4.77×10^{-5}	9.53×10^{-5}	0.62	0.20
Phosphorus (mg)	2.27×10^{-7}	7.07×10^{-5}	1.00	1.79×10^{-4}	1.09×10^{-4}	0.10	0.10
Potassium (mg)	3.69×10^{-5}	1.71×10^{-5}	0.03	2.12×10^{-5}	2.84×10^{-5}	0.45	0.23
Iron (mg)	2.85×10^{-3}	4.09×10^{-3}	0.49	3.14×10^{-3}	6.74×10^{-3}	0.64	0.16
Zinc (mg)	-3.45×10^{-3}	2.52×10^{-3}	0.17	-2.61×10^{-3}	7.63×10^{-3}	0.73	0.21

β, regression coefficient estimate in multiple models; RE, retinol equivalent; SE, standard error of β; α-TE, α-tocopherol equivalent.
Leukocyte telomere length was natural logarithm-transformed and fitted as a dependent variable in linear regression models adjusted for total energy intake, age, sex, family monthly income, body mass index, smoking status, alcohol drinking status, physical activity and presence of hypertension, diabetes or hypercholesterolaemia.
**P*-value for the interaction term of age groups and micronutrient intake in the multiple linear regression models.

reported that higher blood levels of vitamin C, lutein and zeaxanthin are associated with longer LTL in normal elderly individuals [27]. Consequently, these data support the hypothesis that nutrients with antioxidative properties play a protective role in telomere maintenance. In particular, a recent study has proposed that vitamin C may

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enhance the activity of telomerase, which prevents telomere attrition [28].

Folate and vitamins B_2, B_6 and B_{12} participate in DNA repair, methylation and chromosome maintenance in one-carbon metabolism pathways [18,26]. Tetrahydrofolate (THF) plays a role in DNA synthesis; methylene-THF is required to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate. In folate deficiency, dUMP is accumulated and uracil is incorporated into DNA in place of thymine. Uracil may generate DNA mutations, leading to DNA breakage because excision repair enzymes remove uracil bases. Indeed, such uracil misincorporation was found along with telomere dysfunction and attrition in an *in vitro* model of folate deficiency [29]. Vitamins B_6 and B_2 are cofactors in the conversion of THF to methylene-THF and methyl-THF. Methyl-THF along with vitamin B_{12} provides methyl groups for the generation of methionine from homocysteine. Methionine is converted to *S*-adenosyl methionine, a methyl donor for the maintenance of DNA methylation. Therefore, deficiencies in folate and vitamins B_6 and B_{12} result in homocysteine accumulation. Indeed, previous studies reported an inverse association between blood homocysteine levels and telomere length [30,31]. The present study showed the potential protective effects of folate and vitamin B_2 on LTL, although the associations between these nutrients and LTL were not consistent in both age groups.

There have been no data available on the association between potassium intake and telomere length. Although biological mechanisms underlying this association are unclear, potassium is abundant in fruits and vegetables, which were reported to be associated with longer LTL in previous studies [7,14,24], along with vitamin C and folate. Because we observed strong correlations in the consumption of nutrients, we did not perform multiple testing or adjustment of them in the model. Thus, it is unclear whether potassium intake is associated with LTL independent of other nutrients.

In the present study, the association of vitamin C and folate, as well as potassium intake, with LTL was significant in younger participants but not among those aged ≥50 years at baseline (or ≥60 years when LTL was measured). These results may partly be a result of the lower consumption of vitamin C, folate and potassium or a smaller sample size in older participants than in younger participants. A previous cross-sectional study showed significant associations for vitamin C and folate intake among women aged 35–74 years but did not show the results stratified by age groups [17].

We observed no association between LTL and consumption of vitamins A, B_1, B_2, B_3, B_6 and E, calcium, phosphorus, iron and zinc. In another study, dietary intake of vitamins A and E was significantly associated with LTL, whereas that of vitamin B_6, calcium, iron and zinc was not in women who did not use supplemental vitamins [17]. We obtained information from participants regarding supplemental use, although we could not measure amounts of specific nutrients, which may account for the lack of an association for vitamins A and E in the present study.

The strengths of the present study include the large sample size, the use of data from a population-based cohort, and the examination of the temporal relationship between diet and telomere length. However, some limitations need to be addressed when interpreting our findings. We were unable to assess a broader range of micronutrients, which would include vitamin B_{12}, selenium, magnesium and other trace minerals. In particular, biological mechanisms regarding the role of vitamin B_{12} in nucleic acid synthesis are well understood [18], although epidemiological data on the association between vitamin B_{12} consumption and LTL are still unclear [17]. Because our nutrient data did not include vitamin B_{12}, we were unable to analyse the association between vitamin B_{12} intake and LTL. Alternatively, biomarkers of vitamin B_{12} can be investigated for an association with LTL in future studies. In addition, we were unable to take into account vitamin supplements use because this information was unavailable. Data on whether they used dietary supplements were available; 20.3% of the participants (17.2% and 26.7% for those aged <50 years and ≥50 years, respectively) had reported the use of dietary supplements, although LTL did not differ between users and non-users of supplements. On the basis of data from the Korea National Health and Nutrition Examination Survey for 2001, users of vitamin C supplements were more likely to have higher fruit consumption than non-users among participants aged between 40 and 70 years. Lastly, we were unable to measure changes in LTL during the 10-year period between data collections and, thus, a causal relationship between nutrient intake and LTL attrition remains unresolved. However, assuming that dietary habits did not change during the 10-year period, our study suggests cumulative dietary effects on LTL.

In summary, we found longitudinal associations of vitamin C and folate, as well as potassium intake, with LTL among middle-aged and older adults. However, further studies are necessary to determine the causal relationship between the consumption of these nutrients and changes in telomere length. In addition, whether the use of vitamin C and folate supplements can help maintain telomeres is not known and warrants further study. Nonetheless, because fruits and vegetables are rich in vitamin C, folate, and potassium, they are recommended as important components of a healthy diet.

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Conflict of interests, source of funding and authorship

The lead author declares that the manuscript submitted is a complete, honest, accurate and transparent manner of the study being reported, that no important aspects have been omitted and that any discrepancies from the study as planned have been in the manuscript. All authors declare that they have no conflicts of interest.

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JYL and IB provided the study inference and wrote the manuscript. JYL and CS conducted the study. JYL and IB conducted the statistical analysis. All authors contributed to interpreting result and finalising the manuscript. CS and IB had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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