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Systematic Review

Effects of protein intake on blood pressure, insulin sensitivity and blood lipids in children: a systematic review

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Abstract

High protein intake in early childhood is associated with obesity, suggesting possible adverse effects on other cardiometabolic outcomes. However, studies in adults have suggested beneficial effects of protein intake on blood pressure (BP) and lipid profile. Whether dietary protein intake is associated with cardiovascular and metabolic health in children is unclear. Therefore, we aimed to systematically review the evidence on the associations of protein intake with BP, insulin sensitivity and blood lipids in children. We searched the databases Medline, Embase, Cochrane Central and PubMed for interventional and observational studies in healthy children up to the age of 18 years, in which associations of total, animal and/or vegetable protein intake with one or more of the following outcomes were reported: BP; measures of insulin sensitivity; cholesterol levels; or TAG levels. In the search, we identified 6636 abstracts, of which fifty-six studies met all selection criteria. In general, the quality of the included studies was low. Most studies were cross-sectional, and many did not control for potential confounders. No overall associations were observed between protein intake and insulin sensitivity or blood lipids. A few studies suggested an inverse association between dietary protein intake and BP, but evidence was inconclusive. Only four studies examined the effects of vegetable or animal protein intake, but with inconsistent results. In conclusion, the literature, to date provides insufficient evidence for effects of protein intake on BP, insulin sensitivity or blood lipids in children. Future studies could be improved by adequately adjusting for key confounders such as energy intake and obesity.

Key words: **Protein intake: Children: Cardiometabolic health: Systematic review**

Protein is an important component of infant and child nutrition, as it provides essential amino acids that are required for growth[1,2]. However, a high intake of protein in early childhood has also been associated with the development of obesity[3–5]. Already in childhood, obesity can lead to adverse cardiometabolic health outcomes such as hypertension, high cholesterol levels and insulin resistance[6,7]. This suggests that high protein intake in children may lead to unfavourable effects on these outcomes. However, studies in adults have reported beneficial effects of protein intake on blood pressure (BP), and insulin and TAG levels[8–11].

It is unclear whether dietary protein intake has an effect on BP, insulin sensitivity or blood lipids in children. Since cardiometabolic risk factors in childhood continue into later life and have been shown to predict CVD and type 2 diabetes in adulthood[12,13], it is important to study the determinants of cardiometabolic risk already in childhood. Therefore, we aimed to conduct a systematic review on the associations of protein intake with BP, insulin sensitivity and blood lipids in children. In addition, we aimed to explore whether the reported effects differ between vegetable and animal protein intakes.

Methods

The present systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[14]. Ethical approval was not required as this was a secondary data analysis.

Abbreviations: BP, blood pressure; E%, percentage of energy; HOMA-IR, homeostatic model assessment of insulin resistance.

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Literature search

An extensive literature search was conducted with the help of a medical librarian in the databases Medline (via OvidSP), Embase (via Embase.com) and the Cochrane Library. In addition, we searched PubMed for articles that were not yet available via Medline. All databases were searched from their inception until 31 May 2013. The search strategy consisted of three elements: infants, children or adolescents; protein intake; cardiovascular or metabolic health outcomes. To capture studies that did not explicitly mention protein in the title or abstract, we also included general terms referring to diet and nutrient intake. All elements were searched using both controlled vocabulary terms (Medical Subject Headings and/or Emtree) and free text words in the title or abstract. Limits were applied to include only human studies and to exclude conference papers, editorials and letters. No limits were set on language or year of publication. The full search strategies for all the four databases are provided in online Supplementary Table S1. In addition to the systematic search, we contacted authors and searched reference lists for the most recent 10 % of articles in our review.

Study eligibility criteria

Studies identified from the literature search were selected on the basis of the predefined selection criteria presented below.

Inclusion criteria were as follows:

1. Cross-sectional studies, case–control studies, cohort studies and intervention studies
2. Studies conducted among children ≤18 years old
3. Studies reporting total, animal and/or vegetable protein intake, either in absolute amounts (e.g. g/d or kJ/d) and/or relative to total energy intake (e.g. percentage of energy (E%))
4. Studies investigating the associations between protein intake and one or more of the following outcomes:
    a. BP: systolic or diastolic BP; mean arterial pressure; hypertension
    b. Insulin sensitivity: insulin levels; glucose levels; glucose tolerance; homeostatic model assessment of insulin resistance (HOMA-IR); type 2 diabetes mellitus
    c. Blood lipids: total cholesterol levels; HDL-cholesterol levels; LDL-cholesterol levels; TAG levels

Exclusion criteria were as follows:

1. Studies in children with congenital diseases, phenylketonuria, type 1 diabetes or kidney disease
2. Studies from which the exclusive effects of protein intake cannot be extracted (e.g. when protein supplements were combined with other nutrients without proper control)
3. Letters, conference abstracts, reviews or editorials
4. Studies not conducted in human subjects

Study selection

Working in pairs, two authors independently reviewed the titles and abstracts to determine whether the studies satisfied the selection criteria. Any disagreement with article selection was resolved through discussion or with the help of a third reviewer. Full-text articles were retrieved for the selected titles and were assessed again by two independent reviewers. For articles in languages other than English, colleagues fluent in the language assisted with translating.

Data extraction

Data were extracted using a structured data extraction form designed before data collection. Detailed study-level characteristics were collected including study design, study size, study duration, details on exposure and outcome assessment, and characteristics of the study population. We also derived information on the statistical analyses and covariate adjustments. All types of summary statistics were extracted, both for the entire study population and for subgroups; and both for crude models and for adjusted models where applicable. Authors were contacted if insufficient data were published (e.g. if effect estimates were not stated in the paper). A second reviewer checked the data extraction for a random 20 % of the studies.

Quality assessment

Using a predefined scoring system, two reviewers independently evaluated the quality of the included studies. The quality score was developed on the basis of previously used scoring systems and was modified to assess the quality of studies with different study designs[15,16]. A score of 0, 1 or 2 points was allocated to each of the following five items: study design; study size; exposure assessment; outcome assessment; and adjustment for potential confounders or randomisation. This resulted in a total score ranging from 0 to 10 points, with a score of 10 representing the highest quality. Details on the quality score are presented in online Supplementary Table S2.

Synthesis of evidence

Because of the diversity in study designs, outcome measures and low methodological quality of the studies, a meta-analysis could not be performed. Instead, a qualitative analysis (best-evidence synthesis) was performed to synthesise the results and quality of the included studies[17]. In line with previous systematic reviews, we defined the following four levels of evidence[18,19]: (1) strong evidence is provided if at least two higher-quality studies are available and if these report consistent findings; (2) moderate evidence is provided if one higher-quality study and one or more lower-quality studies are available with generally consistent findings; (3) limited evidence is provided if only one higher-quality study is available or if multiple lower-quality studies report generally consistent findings; (4) insufficient evidence is provided if no higher-quality studies are available or if studies report inconsistent findings. Studies were considered as generally consistent if at least 75 % of the studies showed statistically significant results in the same direction[17]. Studies were considered as higher quality if they had a quality score of 6 or higher. If two or more studies on the same outcome were of higher quality, we disregarded lower-quality studies in the evidence synthesis[17].

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Results

Study selection

In the systematic search, 6636 unique references were identified (Fig. 1). Of these references, 6305 were excluded on the basis of the title and abstract. For the remaining 331 articles, the full text was retrieved and critically reviewed. After the selection process, sixty papers were included, reporting on unique fifty-six studies. Fig. 1 shows the flow chart of the selection process.

Characteristics of the included studies

Table 1 shows the characteristics of the included studies and study populations. The fifty-six studies included a total number of 22 040 participants (n 19–4508 subjects per study), with a variation in mean age from 0 to 17·5 years. We decided to include three studies (published in four papers) performed in subjects with an age range up to 19 or 20 years as their age ranges were very wide[39,55,65,73]. Most studies included both boys and girls, except for one study in boys only[48] and one study in girls only[42]. Most studies were performed in Europe[20-23,29,36,38,40,42,49,50,52,53,57,59,61,64,65,75], the USA or Canada[24,26,27,31,32,34,35,39,51,55,56,58,60,66,69–74,76–79], and Australia or New Zealand[25,37,43,46,62]. Other studies were performed in populations in South or Central America[28,41,63], South Africa[54,67,68], Korea[44], Russia[45] and Turkey[47], and one study included subjects from the Philippines, Ghana and three European countries[48]. Most studies examined general population-based samples of children. Some studies specifically included high-risk populations, i.e. children with high cholesterol levels[43,59,65,72,77,79,80] or overweight children[31–33,37,38,41,47,57,63,71,76].

Only four of the fifty-six studies were randomised controlled trials comparing high-protein (22·5–30 E%) with low-protein (10–15 E%) diets, for 1 or 6 months[30,33,37,38]. In three of the four trials, lower- and higher-protein diets were isoenergetic and energy-restricted, with energy from protein being replaced by carbohydrate[33,37,38]. In the fourth trial, protein was also replaced by carbohydrate, but without energy restriction[30]. The remaining fifty-two studies were observational, of which five had a longitudinal design (follow-up 1·1–7·5 years) and forty-seven were cross-sectional. The mean protein intake in the observational studies ranged from 7·7 to 19·2 E% (see online Supplementary Table S3). Of the included studies, twenty-three investigated the associations of protein intake with BP, fifteen with insulin sensitivity and forty-two with blood lipids. Details on exposure and outcome measurements are presented in online Supplementary Table S3.

The overall quality score of the included studies ranged from 1 to 9 (Table 1), with a mean score of 4·2. Of these studies, fifteen received a quality score of 6 or higher. Because of the large number of cross-sectional studies, most studies scored low on the item study design. Most studies did receive a high score on exposure and outcome assessment methods. The majority of the studies received a score of zero for the item on adjustments, since they did not control for important potential confounders such as age, sex, energy intake and body weight.

Protein intake and blood pressure

Overall, twenty-three studies reported on the associations between protein intake and BP in children, of which ten studies were considered higher quality (Table 2).

In four intervention studies, of which three were performed in overweight children, no significant effects of a high-protein diet compared with a low-protein diet on systolic BP, diastolic BP or mean arterial pressure were found[30,33,37,38].

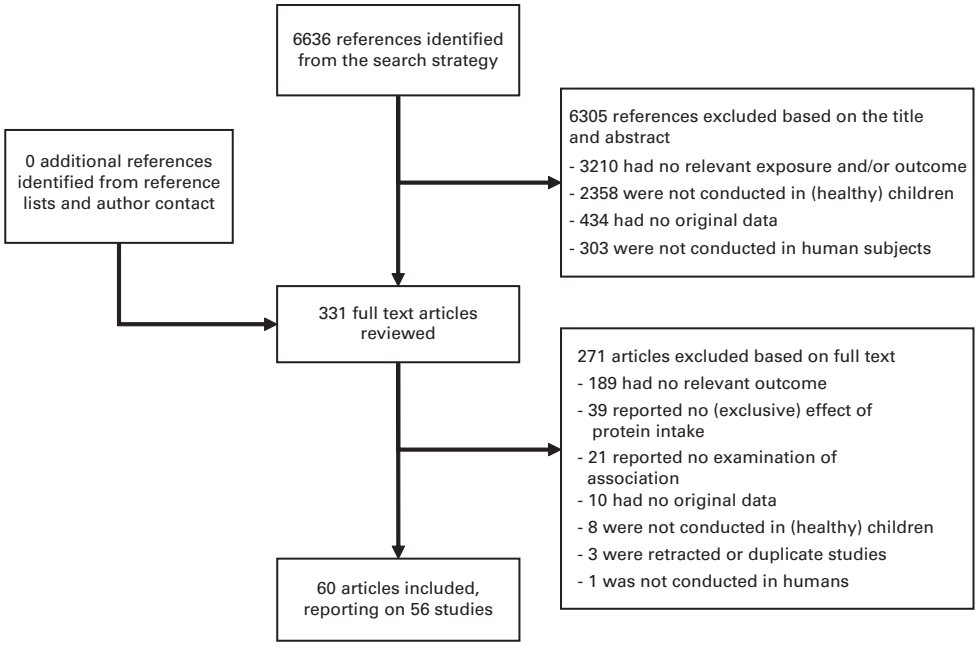

Fig. 1. Flow chart of the study selection.

Table 1. Characteristics of the sixty included papers (fifty-six studies) on protein intake and cardiometabolic health outcomes in children

First author, year	Country	Study design (follow-up)	*n*	Female (%)	Age (years) at baseline		Population characteristics	Quality score
					Range	Mean		
Aeberli *et al.*, 2007[20]*a	Switzerland	Cross-sectional	74	45·9	6–14	10·1	Normal-weight (mean BMI 15·9 kg/m^2) and overweight (mean BMI 23·4 kg/m^2) children	5
Aeberli *et al.*, 2009[21]*a	Switzerland	Cross-sectional	79	46·8	6–14	10·2	Normal-weight (mean BMI 15·9 kg/m^2) and overweight (mean BMI 23·6 kg/m^2) children	5
Akerblom *et al.*, 1984[22]	Finland	Cross-sectional	237	45·6	NR	12	General population	3
Andersen *et al.*, 1979[23]	Denmark	Cross-sectional	95	46·3	Two groups: 0·7 and 4	NR	Healthy children participating in a screening programme	4
Berenson *et al.*, 1979[24]	USA	Cross-sectional	224	Approximately 50	NR	0·5	General population	3
Boulton *et al.*, 1995[25]	New Zealand	Cross-sectional	232	47·8	Four moments: 8, 11, 13 and 15	NR	General population	3
Casazza *et al.*, 2009[26]*b	USA	Cross-sectional	202	47·0	7–12	9·6	Healthy children	4
Casazza *et al.*, 2009[27]*b	USA	Cross-sectional	250	53·6	7–12	9·6	Healthy children	6
Colin-Ramirez *et al.*, 2009[28]	Mexico	Cross-sectional	1239	49·5	8–10	9·4	General population, low-SES area	4
Cowin *et al.*, 2001[29]	UK	Longitudinal (13 months)	389	45·0	1·5	NR	General population	3
Damsgaard *et al.*, 2013[30]	Eight European countries‡	Interventional (6 months)	253	50·1	5–18	13·0	Children with at least one overweight parent (BMI >27 kg/m^2), from Denmark, the Netherlands, UK, Greece, Germany, Spain, Bulgaria and the Czech Republic	9
Davis *et al.*, 2005[31]	USA	Cross-sectional	63	49·2	9–13	11·5	Overweight, otherwise healthy children (BMI >85th percentile, based on age- and sex-specific population data)	6
Davis *et al.*, 2009[32]	USA	Longitudinal (18 months)	85	43·5	11–17	14·2	Overweight, otherwise healthy children (BMI >85th percentile, based on age- and sex-specific population data)	6
Duckworth *et al.*, 2009[33]	UK	Interventional (1 month)	95	64·2	9–18	14·4	Overweight children (mean BMI 33·9 kg/m^2)	6
Frank *et al.*, 1977[34]	USA	Cross-sectional	68	NR	10–16	NR	General population	1
Frank *et al.*, 1978[35]	USA	Cross-sectional	185	NR	9–11	10·5	General population	1
Garemo *et al.*, 2006[36]	Sweden	Cross-sectional	95	44·2	3·9–4·6	4·3	General population	4
Garnett *et al.*, 2013[37]	Australia	Interventional (6 months)	111	59·5	10–17	13·1	Overweight children with pre-T2DM and/or features of insulin resistance, but without T2DM	8
Gately *et al.*, 2007[38]	UK	Interventional (1 month)	98	61·2	11–17	14·2	Overweight children (mean BMI 33·1 kg/m^2), without medication use	7
Glueck *et al.*, 1982[39]*c	USA	Cross-sectional	1234	46·4	6–19	13·5	General population	5
Gonzalez-Requejo *et al.*, 1995[40]	Spain	Cross-sectional	1682	46·1	2–12	6·3	General population	5
Hermelo *et al.*, 1995[41]	Cuba	Case–control†	80	50·0	10·7–12·7	11·7	Overweight and matched normal-weight children	4
Heyman *et al.*, 2012[42]	France	Cross-sectional	19	100	Post-menarche <18·5	16·6	Healthy controls from a case–control study on T1DM	4
Hitchcock *et al.*, 1977[43]	Australia	Cross-sectional	58	NR	6–17	NR	Low (<3·9 mmol/l), median (3·9–5·2 mmol/l) and high (>6·2 mmol/l) cholesterol in both the mother and child, selected from a larger population-based study (*n* 929)	1

Table 1. *Continued*

First author, year	Country	Study design (follow-up)	*n*	Female (%)	Age (years) at baseline		Population characteristics	Quality score
					Range	Mean		
Hong *et al.*, 2009[44]	Korea	Cross-sectional	246	47·6	12–13	12·6	General population	4
Il'chenko *et al.*, 1989[45]	Russia	Cross-sectional	250	NR	11–14	NR	General population	1
Jenner *et al.*, 1988[46]	Australia	Cross-sectional	884	50·9	7·5–10·6	9·0	General population	6
Keser *et al.*, 2010[47]	Turkey	Cross-sectional	308	NR	11–18	NR	Overweight, otherwise healthy children	2
Knuiman *et al.*, 1983[48]	The Netherlands; Finland; Italy; the Philippines; Ghana	Cross-sectional	589	0	7·6–10·2	9·0	General population	3
Kouvalainen *et al.*, 1982[49]	Finland	Cross-sectional	415	46·4	Two groups: 3 and 12	NR	General population	4
Larsen *et al.*, 1989[50]	Denmark	Cross-sectional	42	52·4	7–11	NR	Low (3·1–4·1 mmol/l), average (4·3–4·8 mmol/l) and high (5·0–5·9 mmol/l) cholesterol, selected from a larger population-based study (*n* 947)	1
Lindquist *et al.*, 2000[51]	USA	Cross-sectional	95	NR	6·5–13	9·6	General population	4
Lucas *et al.*, 1994[52]	UK	Longitudinal (7·5 years)	758	50·4	Birth	NR	Low birth weight (<1850 g)	5
Menghetti *et al.*, 2004[53]	Italy	Cross-sectional	293	46·1	11–14	NR	General population	3
Mia *et al.*, 2000[54]	South Africa	Cross-sectional	321	50·5	16–18	NR	South African Indian children	4
Morrison *et al.*, 1980[55]*c	USA	Cross-sectional	949	48·2	6–19	NR	General population	4
Nicklas *et al.*, 1993[56]	USA	Cross-sectional	1281	56·5	10–11	10·6	General population	5
Obuchowicz *et al.*, 1997[57]	Poland	Cross-sectional	191	50·8	5·8–9·6	7·6	Normal-weight (mean BMI 16·1 kg/m^2) and overweight (mean BMI 23·8 kg/m^2) children	4
Perry *et al.*, 1997[58]	USA	Cross-sectional	22	54·6	11·0–12·6	11·7	General population	4
Pistulkova *et al.*, 1992[59]	Czech Republic	Case–control†	200	52·2	11–12	NR	High *v.* low cholesterol (>95th and 5–10th percentiles, selected from a larger population-based cohort (*n* 2000)	3
Potter *et al.*, 1989[60]	USA	Cross-sectional	53	56·6	9–16	12·4	General population	2
Räsänen *et al.*, 1978[61]	Finland	Cross-sectional	1496	49·1	Three groups: 5, 9 and 13	NR	General population	2
Regan *et al.*, 2006[62]	New Zealand	Longitudinal (6·6 years)	37	64·9	Birth	NR	Healthy, developmentally normal children, who were born preterm (≤32 weeks of gestation)	5
Rinaldi *et al.*, 2012[63]	Brazil	Cross-sectional	147	51·7	NR	7·9	Overweight (BMI >85th percentile, based on age- and sex-specific population data)	6
Sanchez-Bayle *et al.*, 2008[64]	Spain	Cross-sectional	673	47·7	NR	6	General population	5
Sarría Chueca *et al.*, 1997[65]	Spain	Cross-sectional	89	48·3	4–20	NR	High cholesterol levels (≥5·2 mmol/l), or a family history of CHD or hyperlipidaemia	2
Schachter *et al.*, 1979[66]	USA	Cross-sectional	150	NR	NR	0·5	Healthy, full-term infants, born in a hospital	4
Schutte *et al.*, 2003[67]*d	South Africa	Cross-sectional	631	53·1	10–15	12·5	General population	6
Schutte *et al.*, 2003[68]*d	South Africa	Cross-sectional	694	53·7	10–15	12·5	General population	6

Table 1. *Continued*

First author, year	Country	Study design (follow-up)	*n*	Female (%)	Age (years) at baseline		Population characteristics	Quality score
					Range	Mean		
Sharma *et al.*, 2009[69]	USA	Cross-sectional	80	55·0	9–11	NR	Overweight (BMI >85th percentile based on age- and sex-specific data)	6
Simons-Morton *et al.*, 1997[70]	USA	Longitudinal (3 years)	662	46·1	8–11	9·6	High LDL-C (80th–98th percentile, based on age- and sex-specific population data), participating in a trial, otherwise healthy	8
Smith *et al.*, 2003[71]	USA	Cross-sectional	102	47·1	9–18	NR	Normal-weight and overweight children	2
Starc *et al.*, 1998[72]	USA	Cross-sectional	67	65·7	2–10	5·8	Hyperlipidaemia, not taking lipid-lowering medications, without a congenital disease that might affect blood lipid levels	4
Sugiyama *et al.*, 2007[73]	USA	Cross-sectional	4508	49·1	12–19	16·0	General population	7
Suter *et al.*, 1993[74]	Canada	Cross-sectional	97	59·8	10–15	13·0	General population	4
Ulbak *et al.*, 2004[75]	Denmark	Cross-sectional	73	NR	NR	2·5	General population	6
Ventura *et al.*, 2008[76]	USA	Cross-sectional	109	43·1	10–17	NR	Overweight (mean BMI 31·2 kg/m^2) and a family history of T2DM, but without current T2DM	4
Vobecky *et al.*, 1979[77]	Canada	Case–control†	70	60·0	NR	0·5	High cholesterol (≥5·2 mmol/l) and matched controls	1
Ward *et al.*, 1980[78]	USA	Cross-sectional	74	NR	2·5–2·9	2·6	General population	2
Weidman *et al.*, 1978[79]	USA	Cross-sectional	93	NR	6–16	NR	High (>90th percentile), median (45–55th percentile) and low (<10th percentile) cholesterol, from a larger population-based cohort (*n* 4021)	2

NR, not reported; SES, socio-economic status; T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; LDL-C, LDL-cholesterol.
* Articles with the same superscript letter used data from the same study population.
† All case–control studies used cross-sectional data.
‡ Denmark, the Netherlands, UK, Greece, Germany, Spain, Bulgaria and the Czech Republic.

Table 2. Reported associations between protein intake and blood pressure (BP) in children

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Higher-quality studies								
Damsgaard *et al.*, 2013[30]	9	Linear mixed models	Effect of protein intake (control *v.* high *v.* low) on BP (mmHg) after 1 and 6 months of intervention	Age, sex, family, country, participant, baseline MAP, time since randomisation	SBP	Whole group	NR	NS
						Intensive group*	NR	NS
					DBP	Whole group	NR	NS
						Intensive group*	−1·0	<0·01
					MAP	Whole group	NR	0·66
						Intensive group*	−6·5	0·01
Duckworth *et al.*, 2009[33]	6	ANOVA	Comparison of BP between groups that received high *v.* low protein intervention diets	Randomised	SBP	–	NR	NS
					DBP		NR	NS
Garnett *et al.*, 2013[37]	8	Linear mixed models	Effect of protein intake (high *v.* low) on BP (mmHg) after 3 and 6 months of intervention	Randomised	SBP	–	NR	NS
					DBP		NR	NS
Gately *et al.*, 2007[38]	7	ANOVA	Comparison of BP between groups that received high *v.* low protein intervention diets	Randomised	SBP	–	NR	NS
					DBP		NR	NS
Jenner *et al.*, 1988[46]	6	Linear regression	Increase in BP (mmHg) per energy-adjusted (residual method) g increase in protein intake	Age, height, body weight, SES	SBP	M	−0·01	0·85
					DBP	M	−0·10	0·06
					SBP	F	−0·14	0·05
					DBP	F	−0·03	0·64
Schutte *et al.*, 2003[67,68]	6	Linear regression	Increase in BP (mmHg) per g increase in protein intake	Sex, pubertal stage, BMI, body fat, intake of macronutrients and several other nutrients	SBP	–	NR	NS
					DBP		NR	NS
					MAP		NR	NS
					Pulse pressure		NR	NS
Sharma *et al.*, 2009[69]	6	Linear regression	Standardised regression coefficient (protein intake in g/d)	Sex, pubertal stage, waist circumference, intake of carbohydrate and fat	SBP	–	0·00	NS
					DBP		0·02	NS
Simons-Morton *et al.*, 1997[70]	8	Linear regression	Increase in BP (mmHg) per g increase in protein intake	Sex, height, body weight, energy intake	SBP	Baseline, cross-sectional	−0·01	NS
					DBP		−0·01	NS
				Sex, height, body weight, and energy intake	SBP	3-year follow-up, model 1†	−0·01	<0·01
					DBP		−0·01	<0·01
				Sex, height, body weight, energy intake, intake of macronutrients and several micronutrients	SBP	3-year follow-up, model 2†	−0·02	NS
					DBP		−0·03	NS
Sugiyama *et al.*, 2007[73]	7	Linear regression	Increase in BP (mmHg) per 10 g increase in protein intake (energy-adjusted)	Sex, age, height, body weight, BMI, energy intake, SES, physical activity, intake of macro-nutrients and several micronutrients	SBP	–	−0·02	0·07
					DBP		NR	NS
Ulbak *et al.*, 2004[75]	6	Linear regression	Increase in BP (mmHg) per E% increase in protein intake	Sex, age, height, body weight, outside temperature	SBP	–	−0·56	0·035
					DBP		−1·86	0·028

Table 2. *Continued*

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Lower-quality studies								
Aeberli *et al.*, 2009[21]	5	Linear regression	Standardised regression coefficient (protein intake in E%)	Sex, age, BMI	SBP	–	−0·13	0·23
Berenson *et al.*, 1979[24]	3	Pearson's correlation	Correlation between protein intake (g/4184 kJ (1000 kcal)) and BP	–	BP BP	0·5 years 1 year	NR NR	NS NS
Casazza *et al.*, 2009[26]	4	Linear regression	Standardised regression coefficient (protein intake in E%)	Sex, age, total body fat, SES	SBP	–	−0·15	NS
Colin-Ramirez *et al.*, 2009[28]	4	ANOVA	Comparison of protein intake (E%) between children with normal BP *v.* diastolic hypertension *v.* systolic hypertension	–	Hypertension Diastolic hypertension Systolic hypertension	–	NR NR NR	NS NS NS
Frank *et al.*, 1977[34]	1	Pearson's correlation	Correlation between protein intake (g/4184 kJ (1000 kcal)) and BP	–	BP	–	NR	NS
Frank *et al.*, 1978[35]	1	Pearson's correlation	Correlation between protein intake (g/4184 kJ (1000 kcal)) and SBP	–	SBP	–	NR	NS
Hong *et al.*, 2009[44]	4	Correlation	Partial correlation between protein intake (g/d) and BP	Sex, age, Tanner stage	SBP DBP	–	0·08 −0·02	NS NS
Il'chenko *et al.*, 1989[45]	1	*t* test‡	Comparison of protein intake (g) between children with elevated *v.* normal arterial pressure	–	Arterial pressure	–	NR	NS
Lucas *et al.*, 1994[52]	5	Linear regression	Increase in BP (mmHg) per g increase in protein intake	Height, BMI, BP-measuring device, mid-arm circumference	SBP DBP	–	NR NR	NS NS
Menghetti *et al.*, 2004[53]	3	*t* test	Comparison of protein intake (E%) between children with hypertension *v.* normal BP	–	Hypertension	–	Positive	<0·05
Schachter *et al.*, 1979[66]	4	Correlation	Correlation between protein intake (g) and BP	–	BP	–	NR	NS
Smith *et al.*, 2003[71]	2	Spearman's correlation	Partial correlation between protein intake (g) and BP	Age	SBP DBP	–	0·05 0·10	NS NS
Ventura *et al.*, 2008[76]	4	Pearson's correlation	Correlation between protein intake (E%) and BP	–	SBP DBP	–	NR NR	<0·05 <0·05

MAP, mean arterial pressure; SBP, systolic blood pressure; NR, not reported; DBP, diastolic blood pressure; SES, socio-economic status; M, male; F, female; E%, percentage of energy.
* The intensive intervention group received most of their foods for free and had a higher adherence to the intervention diets.
† See the column 'Covariate adjustments': model 2 additionally adjusted for intake of other nutrients.
‡ Statistical analysis used was not clearly reported.

All the four trials had a quality score of 6 or higher. In the trial by Damsgaard *et al.*[30], the authors did observe a significant beneficial effect of a high-protein diet in a subgroup of 5- to 18-year-olds who received a more intensive intervention. This subgroup received free foods in addition to dietary instructions and had a higher adherence to the intervention diet. In this subgroup, children who received the high-protein diet had a 1·0 mmHg (95 % CI 0·3, 1·7) lower diastolic BP and a 6·5 mmHg (95 % CI 1·5, 15·0) lower mean arterial pressure than those who received a low-protein diet[30].

Of the observational studies, six had a quality score of 6 or higher. One longitudinal and two cross-sectional studies of higher quality reported inverse associations between protein intake and BP in at least one of their subgroups[46,70,75]. The three remaining higher-quality studies did not find a significant association between protein intake and BP[26,69,73], but two did report non-significant inverse associations[26,73]. In contrast, one study with a quality score of 3 observed that children with high BP had a significantly higher intake of protein than those with normal BP[53]. The remaining lower-quality studies showed no significant associations[21,24,28,34,35,44,45,52,66,68,71,76].

Protein intake and insulin sensitivity

Of the included studies, fifteen (published in sixteen papers) examined the associations between protein intake and measures of insulin sensitivity in children (Table 3). The studies examined various measures of insulin sensitivity, including fasting insulin or glucose levels, HOMA-IR; or measures of insulin responses following an oral or intravenous glucose tolerance test, such as the insulin sensitivity index and acute insulin response. No studies were identified with type 2 diabetes mellitus in children as outcome.

Of the fifteen studies, six had a quality score of 6 or higher. In three of these higher-quality studies, a significant association was reported between protein intake and measures of insulin sensitivity. In the previously described trial by Damsgaard *et al.*[30], again, no effects were observed in the full study population. However, in the subgroup that underwent a more intensive intervention, insulin levels and HOMA-IR were significantly reduced in the high-protein group compared with the low-protein group[30]. In a cross-sectional study in 7- to 12-year-old children, Casazza *et al.*[26,27] observed that, after extensive adjustments, higher protein intake was significantly associated with lower fasting glucose levels, but not with fasting insulin levels or the insulin sensitivity index, and that it was inversely associated with acute insulin response. Data from another cross-sectional study showed that higher protein intake was associated with lower insulin resistance (HOMA-IR) in 9- to 11-year-old children[69]. In summary, all the three studies show a beneficial effect of protein intake on one or more measures of insulin sensitivity (i.e. lower glucose levels and lower insulin resistance), but one of the studies also reported a harmful effect of protein intake (lower insulin response). The remaining three higher-quality studies did not find significant associations between dietary protein intake and measures of insulin sensitivity[31,32,37], neither did any of the lower-quality studies[21,36,42,44,45,47,51,57,62,76].

Protein intake and blood lipids

Of the fifty-six included studies, forty-two reported on associations between protein intake and levels of one or more blood lipid parameters in children (Table 4). Among these, twenty-two studies (published in twenty-three papers) investigated effects on TAG levels, of which five were of higher quality. None of these higher-quality studies reported a significant effect[30,37,38,63,69]. One lower-quality study reported that higher protein intake was correlated with higher TAG levels[47]. However, this correlation was not adjusted for potential confounders and the study had a quality score of only 2. The remaining sixteen lower-quality studies did not find significant associations[24,34–36,39,41,44,45,51,54,55,58,60,64,74,76].

The relationship between protein intake and HDL-cholesterol levels was reported in twenty-four studies (published in twenty-five papers; Table 4). Statistically significant associations were observed in three studies: two positive[48,72] and one negative[47]. However, all the three studies reported only simple correlations without adjustment for potential confounders and had quality scores ranging from 2 to 4. In the remaining studies, including four higher-quality studies, no significant associations were observed[22,25,27,29,30,37,39–42,44,45,49,55,56,60,63,65,69,72,74,76].

Associations between protein intake and total and/or LDL-cholesterol levels were investigated in thirty-eight studies (Table 4). Of these, five studies reported significant associations between protein intake and cholesterol levels; however, they all had a quality score of 5 or lower and there was no consistency in the direction of the effect[24,25,54,59,77]. The remaining studies, including five higher-quality studies, did not find significant associations[20,22,23,29,34–37,39–45,47–50,54,55,60,64,65,69,74,78,79].

Vegetable and animal protein

Only four of all the fifty-six studies included investigated the associations between vegetable or animal protein intake and cardiometabolic factors in children. One higher-quality cross-sectional study reported no associations between animal or vegetable protein intake and BP[68]. Furthermore, three lower-quality studies reported inconsistent results. The first study found no effect of animal or vegetable protein intake on blood lipid levels in overweight children[47]. The second study reported that animal protein intake was positively correlated with serum LDL-cholesterol levels (r 0·20, P=0·0002), whereas vegetable protein intake was not[54]. In contrast, the last study reported an inverse correlation between the ratios of animal:vegetable protein intake and levels of LDL-cholesterol (r −0·28, P=0·05)[60].

Discussion

To our knowledge, the present systematic review was the first to summarise the published literature on the effects of protein intake on BP, insulin sensitivity and blood lipids in children. In

the present review, fifty-six studies (published in sixty papers) on the association between protein intake and one or more of these cardiometabolic outcomes in children were identified. Overall, the literature shows insufficient evidence for an effect of animal, vegetable or total protein intake on BP, insulin sensitivity or blood lipids, due to a lack of high-quality studies and inconsistency in the results.

Protein and cardiometabolic outcomes

Of the ten higher-quality studies that investigated the relationship between protein intake and BP in children, four reported inverse associations in one or more of their subgroups[30,46,70,75], while the other studies found no significant effects. Although these results suggest a possible inverse association, there is insufficient evidence to draw meaningful conclusions. Also, the observed effect estimates for reductions in BP are small and not clinically relevant at an individual level. Nevertheless, they may be relevant at a population level. More studies are needed to verify whether protein intake is associated with BP already in childhood, and whether this effect tracks into adulthood. A possible inverse association between protein intake and BP is in line with meta-analyses of studies in adults[8–10]. The mechanisms underlying a beneficial effect of protein intake on BP have not yet been clarified[81]. Proposed pathways include the increased synthesis of cellular ion channels in response to protein intake; increased renal plasma flow and increased glomerular filtration rate; or the vasodilating effects of certain amino acids[82].

For the association between protein intake and measures of insulin sensitivity in children, three of the six higher-quality studies reported significant results, but in different directions. Therefore, we conclude that there is insufficient evidence for an association between protein intake and insulin sensitivity in children. Two systematic reviews of intervention studies in adults showed inverse associations between protein intake and fasting insulin levels, but no significant effect on fasting glucose levels[9,83]. The effects of protein intake on insulin sensitivity are suggested to act through the stimulation of insulin secretion[84].

Many studies assessed the associations between protein intake and blood lipids in children, but only five had a quality score of 6 or higher, and none of these higher-quality studies reported a significant effect. Meta-analyses of trials in adults also reported no significant effects of protein intake on LDL- or HDL-cholesterol levels[9,83]. For TAG levels, one meta-analysis reported that subjects following a higher-protein diet had lower TAG levels than those consuming a diet lower in protein[9], while another meta-analysis, which included only trials with a duration of more than a year, observed no effect[83].

In children, endpoint measures of cardiometabolic health can usually not be observed, since they occur later in life. Several studies in adults investigated the relationship of protein intake with CVD and type 2 diabetes. Findings from three cohorts were that total and animal, but not vegetable, protein intakes were associated with an increased risk of type 2 diabetes[85–87]. In three large cohort studies in women, total protein intake was found to be inversely associated with the incidence of CHD[88], stroke[89] and IHD[90]. In contrast, data from two other large cohorts showed that diets high in protein were associated with an increased risk of CVD[91,92]. Finally, in a Dutch prospective cohort, a U-shaped association was observed between protein intake and cardiovascular events after 6·4 years of follow-up, with incidence rates being higher in subjects with low or high protein intake than in those with median protein intake levels[93].

Types of protein

Studies on the effects of vegetable or animal protein intake on BP, insulin sensitivity and blood lipids in children are scarce, and the results are inconsistent. Studies in adults also reported inconsistent effects of either animal or vegetable protein intake on cardiometabolic risk factors. Meta-analyses of trials and prospective studies reported no differences between the effects of vegetable and animal protein intake on BP[8,10]. In contrast, prospective cohort studies reported that animal, but not vegetable, protein intake decreases the risk of stroke[89,94], but increases the risk of diabetes[85,87]. More studies, both in adult and child populations, are needed to elucidate the differential effects of animal and vegetable protein intake on cardiometabolic health.

Some of the inconsistencies in the results of the studies included in the present review may be explained by the type of dietary protein intake. If vegetable and animal protein intakes differently affect cardiometabolic risk factors and the ratio of vegetable:animal protein intake varies between populations, this might explain some of the discrepancies in the results for total protein intake. Furthermore, if vegetable and animal protein intakes affect outcomes in opposite directions, their effects might cancel each other out when studying total protein intake.

Not only the ratio of vegetable:animal protein intake, but also the main food sources and therefore the amino acid composition of protein might vary between populations. For example, a study in children reported a positive association of dairy protein intake with BMI and body fat, but no associations of meat or cereal protein intake with these outcomes[95].

Besides the source and type of protein, also the total amount of protein consumed in the population might have affected the results. Mean protein intake in the studies included in the present review ranged from 8 to 19 E% (see online Supplementary Table S3). A potential relationship between total protein intake and cardiometabolic risk factors might not be linear[93]. The effects could therefore be different for various levels of protein intake and might not be identified when statistical approaches are used that assume a linear relationship.

Quality of the included studies

We applied a scoring system with a theoretical range from 0 to 10 to assess the quality of the included studies. Only fifteen studies were regarded as having a relatively high quality (score ≥6).

Table 3. Reported associations between protein intake and measures of insulin sensitivity in children

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Higher-quality studies								
Damsgaard *et al.*, 2013[30]	9	Linear mixed models	Effect of protein intake (control *v.* high *v.* low) on insulin levels (pmol/l) and HOMA-IR (points) after 1 and 6 months of intervention	Age, sex, family, country, participant, baseline insulin or HOMA-IR, time since randomisation	Fasting insulin, HOMA-IR	Whole group Intensive group*	NR −6·0	0·63 0·01
						Whole group Intensive group*	NR −0·8	0·66 0·02
Casazza *et al.*, 2009[26,27]	4/6	Linear regression	Standardised regression coefficient (protein intake in E%)	Sex, age, total body fat, SES	Fasting glucose	–	−0·43	<0·05
				Sex, Tanner stage, fast mass, lean mass, SES, ethnicity (and Si for AIR)	Fasting insulin Si AIR	– –	0·03 0·01 −0·13	0·61 0·83 0·02
Davis *et al.*, 2005[31]	6	Linear regression	Standardised regression coefficient (protein intake in g/d)	Sex, age, Tanner stage, lean tissue mass, fat mass, (and Si for AIR)	Si AIR Disposition index (β-cell function)	–	NR NR NR	NS NS NS
Davis *et al.*, 2009[32]	6	Correlation	Partial correlation between a 2-year change in protein intake and a 2-year change in Si, AIR or disposition index	Sex, Tanner stage, time, baseline protein intake, baseline insulin measures, body composition	Si AIR Disposition index (β-cell function)	–	NR NR NR	>0·20 >0·20 >0·20
Garnett *et al.*, 2013[37]	8	Linear mixed models	Effect of protein intake (high *v.* low) on Si after 3 and 6 months of intervention	–	Si	–	NR	NS
Sharma *et al.*, 2009[69]	6	Linear regression	Standardised regression coefficient (protein intake in g/d)	Sex, pubertal stage, waist circumference, intake of carbohydrate and fat	HOMA-IR	–	−0·341	<0·05
Lower-quality studies								
Aeberli *et al.*, 2009[21]	5	Linear regression	Standardised regression coefficient (protein intake in E%)	BMI-SDS	Log fasting insulin QUICKI	Normal weight Overweight Normal weight Overweight	−0·03 0·05 NR NR	0·87 0·79 NS NS
Garemo *et al.*, 2006[36]	4	Pearson's correlation	Correlation between protein intake (E%) and glucose, insulin, or HOMA	–	Fasting glucose, insulin, HOMA-IR, HOMA-β	–	NR	NS
Heyman *et al.*, 2012[42]	4	Pearson's/Spearman's correlation	Correlation between protein intake (E%) and glucose and insulin	–	Fasting glucose, insulin	–	NR	NS
Hong *et al.*, 2009[44]	4	Correlation	Partial correlation between protein intake (g/d) and glucose	Sex, age, Tanner stage	Fasting glucose	–	−0·08	NS

Table 3. *Continued*

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Keser *et al.*, 2010[47]	2	Correlation	Correlation between protein intake (g) and measures of insulin sensitivity	–	HOMA-IR, QUICKI, insulin, glucose	–	NR	NS
Lindquist *et al.*, 2000[51]	4	Linear regression	Log increase in insulin sensitivity (10^{-4} litres/min per pmol) per g increase in protein intake	Fat mass, ethnicity, SES, intake of carbohydrate and fat	Si AIR	–	0·00 0·00	0·45 0·76
Obuchowicz *et al.*, 1997[57]	4	Correlation	Correlation between protein intake (E%) and insulin levels	–	Fasting insulin Fasting insulin	Obese Normal weight	−0·03 −0·00	0·76 0·99
Regan *et al.*, 2006[62]	5	Linear regression	Increase in Si per g increase in protein intake	Sex, age, weight SDS, height SDS, energy intake, birth weight, intake carbohydrate and fat	Si	–	NR	NS
Ventura *et al.*, 2008[76]	4	Pearson's correlation	Correlation between protein intake (E%) and post-challenge glucose levels	–	2 h post-challenge glucose levels	–	NR	NS

HOMA-IR, homeostatic model assessment of insulin resistance; NR, not reported; E%, percentage of energy; SDS, standard deviation score; SES, socio-economic status; Si, insulin sensitivity index; AIR, acute insulin response; QUICKI, quantitative insulin sensitivity check index; HOMA-β, homeostatic model assessment of β-cell function.
*The intensive intervention group received most of their foods for free and had a higher adherence to the intervention diets.

Table 4. Reported associations between protein intake and blood lipid levels in children

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Higher-quality studies								
Damsgaard *et al.*, 2013[30]	9	Linear mixed models	Effect of protein intake (control *v.* high *v.* low) on blood lipid levels (mmol/l) after 1 and 6 months of intervention	Age, sex, family, country, participant, baseline TAG, time since randomisation	TAG	Whole group	NR	0·59
					TAG	Intensive group*	NR	NS
					TC	Whole group	NR	0·58
					TC	Intensive group*	NR	NS
					LDL-C	Whole group	NR	0·62
					LDL-C	Intensive group*	NR	NS
					HDL-C	Whole group	NR	0·84
					HDL-C	Intensive group*	NR	NS
Garnett *et al.*, 2013[37]	8	Linear mixed models	Effect of protein intake (high *v.* low) on blood lipid levels (mmol/l) after 3 and 6 months of intervention	–	TAG	–	NR	NS
					LDL-C		NR	NS
					HDL-C		NR	NS
Gately *et al.*, 2007[38]	7	ANOVA	Comparison of lipid levels between children who received high *v.* low protein intervention diets	Randomised	TAG	–	NR	NS
					TC		NR	NS
					LDL-C		NR	NS
					HDL-C		NR	NS
Rinaldi *et al.*, 2012[63]	6	Linear regression	Increase in TAG levels (mmol/l) per E% increase in protein intake	Sex, age, BMI, total energy intake, intake of other nutrients	TAG	–	−0·01	0·95
					TC		0·08	0·56
					LDL-C		0·20	0·26
					HDL-C		0·12	0·43
Sharma *et al.*, 2009[69]	6	Linear regression	Standardised regression coefficient (protein intake in g/d)	Sex, pubertal stage, waist circumference, intake of carbohydrate and fat	TAG	–	−0·09	NS
					TC		0·29	NS
					LDL-C		0·30	NS
					HDL-C		0·04	NS
Lower-quality studies								
Aeberli *et al.*, 2007[20]	5	Linear regression	Standardised regression coefficient (protein intake in E%)	Waist:hip ratio	LDL-C particle size	–	−0·18	0·10
Andersen *et al.*,	4	Spearman's rank correlation	Correlation between protein intake (g/d) and cholesterol levels	–	TC	Infants	−0·29	NS
Akerblom *et al.*, 1984[22]	3	*t* test	Comparison of protein intake (g/4184 kJ (1000 kcal)) between children in the lowest *v.* highest quartile of cholesterol levels	–	TC	–	NR	NS
					HDL-C		NR	NS
Berenson *et al.*, 1979[24]	3	Pearson's correlation	Correlation between protein intake (g/4184 kJ (1000 kcal)) and cholesterol levels	–	TAG	0·5 years	NR	NS
					TAG	1 year	NR	NS
					TC	0·5 years	NR	NS
					TC	1 year	0·30	<0·01
Boulton *et al.*, 1995[25]	3	Correlation	Correlation between protein intake (g/d) and cholesterol levels	–	TC	8 years	−0·08	NS
					TC	11 years	−0·13	NS
					TC	13 years	−0·19	<0·01
					TC	15 years	−0·16	<0·05
					LDL-C	–	NR	NR
					HDL-C	–	NR	NS

Table 4. *Continued*

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Cowin *et al.*, 2001[29]	3	Spearman's correlation	Correlation between protein intake (energy-adjusted, residual method) and cholesterol levels	–	TC	M	0·00	0·98
					TC	F	−0·07	0·38
					LDL-C	M	−0·04	0·68
					LDL-C	F	−0·17	0·08
					HDL-C	M	−0·07	0·36
					HDL-C	F	0·06	0·49
Frank *et al.*, 1977[34]	1	Pearson's correlation	Correlation between protein intake (g/4184 kJ (1000 kcal)) and blood lipid levels	–	TAG	–	NR	NS
					TC		NR	NS
Frank *et al.*, 1978[35]	1	Pearson's correlation	Correlation between protein intake (g/4184 kJ (1000 kcal)) and blood lipid levels	–	TAG	–	NR	NS
					TC		NR	NS
Glueck *et al.*, 1982[39]†	5	Pearson's correlation	Correlation between protein intake (g/d) and blood lipid levels	–	TAG	M, 6–12 years	−0·04	NS
					TAG	F, 6–12 years	−0·03	NS
					TAG	M, 13–19 years	0·02	NS
					TAG	F, 13–19 years	−0·06	NS
					TC	M, 6–12 years	0·04	NS
					TC	F, 6–12 years	−0·08	NS
					TC	M, 13–19 years	0·01	NS
					TC	F, 13–19 years	0·02	NS
					LDL-C	M, 6–12 years	0·00	NS
					LDL-C	F, 6–12 years	−0·04	NS
					LDL-C	M, 13–19 years	−0·03	NS
					LDL-C	F, 13–19 years	−0·04	NS
					HDL-C	M, 6–12 years	0·07	NS
					HDL-C	F, 6–12 years	−0·02	NS
					HDL-C	M, 13–19 years	0·02	NS
					HDL-C	F, 13–19 years	0·11	NS
Gonzalez-Requejo *et al.*, 1995[40]	5	*t* test	Comparison of cholesterol levels between children in the highest *v.* lowest tertile of protein intake (E%)	–	TC	–	NR	NS
					LDL-C		NR	NS
					HDL-C		NR	NS
Hermelo *et al.*, 1995[41]	4	Pearson's correlation	Correlation between protein intake (E%) and cholesterol levels	–	TC	M, obese	0·07	NS
					TC	M, not obese	0·06	NS
					TC	F, obese	0·11	NS
					TC	F, not obese	0·11	NS
					HDL-C	M, obese	−0·09	NS
					HDL-C	M, not obese	−0·1	NS
					HDL-C	F, obese	0·16	NS
					HDL-C	F, not obese	0·14	NS
					non-HDL-C	–	NR	NS
Heyman *et al.*, 2012[42]	4	Correlation	Correlation between protein intake (E%) and blood lipid levels	–	TAG	–	NR	NS
					TC		NR	NS
					LDL-C		NR	NS
					HDL-C		NR	NS

Table 4. *Continued*

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Hitchcock *et al.*, 1977[43]	1	ANOVA‡	Comparison of protein intake (E%) between children in tertiles of cholesterol levels	–	TC	–	NR	NS
Hong *et al.*, 2009[44]	4	Correlation	Partial correlation between protein intake (g/d) and blood lipid levels	Sex, age, Tanner stage	TAG	–	0·04	NS
					TC		−0·05	NS
					HDL-C		−0·04	NS
Il'chenko *et al.*, 1989[45]	1	*t* test‡	Comparison of protein intake between children with high *v.* normal TAG levels	–	TAG	–	NR	NS
					TC		NR	NS
					HDL-C		NR	NS
Keser *et al.*, 2010[47]	2	Correlation	Correlation between protein intake and blood lipid levels	–	TAG	–	0·24	<0·05
					TC		NR	NS
					LDL-C		NR	NS
					HDL-C		−0·18	<0·05
Knuiman *et al.*, 1983[48]	3	Pearson's correlation	Correlation between protein intake (E%) and cholesterol levels	–	TC	Finland	0·00	NS
					TC	The Netherlands	0·01	NS
					TC	Italy	0·08	NS
					TC	Philippines	0·14	NS
					TC	Ghana	−0·08	NS
					HDL-C	Finland	−0·01	NS
					HDL-C	The Netherlands	−0·02	NS
					HDL-C	Italy	0·04	NS
					HDL-C	Philippines	0·32	<0·001
					HDL-C	Ghana	−0·01	NS
Kouvalainen *et al.*, 1982[49]	4	*t* test	Comparison of protein intake (g/4184 kJ (1000 kcal)) between children in the highest *v.* lowest quartile of cholesterol levels		TC	3 years	NR	NS
					TC	12 years	NR	NS
					HDL-C	3 years	NR	NS
					HDL-C	12 years	NR	NS
Larsen *et al.*, 1989[50]	1	Mann–Whitney test	Mann–Whitney on tertiles of cholesterol	–	TC	–	NR	NS
Lindquist *et al.*, 2000[51]	4	Linear regression	Log increase in lipid levels (mmol/l) per g increase in protein intake	Fat mass, ethnicity, SES, intake of carbohydrate and fat	TAG	–	−0·000	0·79
					TC		0·001	0·06
Mia *et al.*, 2000[54]	4	Pearson's correlation	Correlation between protein intake (g/d) and blood lipid levels	–	TAG	–	NR	NS
					LDL-C	Total protein	NR	NS
					LDL-C	Animal protein	0·20	0·0002
Morrison *et al.*, 1980[55]†	4	Correlation	Partial correlation between protein intake (g/d) and blood lipid levels	Sex, age, weight, height, ethnicity	TAG	–	−0·05	NS
					TC		0·02	NS
					LDL-C		0·03	NS
					HDL-C		0·00	NS
Nicklas *et al.*, 1993[56]	5	Correlation	Correlation between protein intake (g/d) and blood lipid levels	–	TC	–	−0·02	NS
					LDL-C		−0·01	NS
					HDL-C		−0·037	NS
Perry *et al.*, 1997[58]	4	Linear regression	Increase in TAG levels (mg/dl) per E% increase in protein intake	Energy intake, intake of carbohydrates, fat, cholesterol, fibre, Na, and vitamins A and C	TAG	–	NR	NS

Table 4. *Continued*

First author, year	Quality score	Statistical analysis	Measure of association	Covariate adjustments	Outcome	Subgroups	Effect estimate	*P*
Pistulkova *et al.*, 1992[59]	3	*t* test	Comparison of protein intake (E%) between children with high *v.* low cholesterol levels	–	TC TC	M F	NR Positive	NS <0·05
Potter *et al.*, 1989[60]	2	Correlation	Partial correlation between the ratio of animal: vegetable protein intake and blood lipid levels	Sex	TAG TC LDL-C HDL-C	–	−0·26 −0·22 −0·28 0·08	0·06 0·114 0·047 0·566
Räsänen *et al.*, 1978[61]	2	*t* test	Comparison of protein intake (g/4184 kJ (1000 kcal)) between children with high *v.* median *v.* low cholesterol levels	–	TC	–	NR	NS
Sanchez-Bayle *et al.*, 2008[64]	5	ANOVA	Comparison of TAG levels in tertiles of protein intake (E%)	–	TAG TC LDL-C	–	NR NR NR	NS NS NS
Sarría Chueca *et al.*, 1997[65]	2	Linear regression	Increase in cholesterol levels (mg/dl) per E% increase in protein intake	Body size and dietary variables (unspecified)	TC LDL-C HDL-C	–	NR NR NR	NS NS
Starc *et al.*, 1998[72]	4	Pearson's correlation	Correlation between protein intake (energy-adjusted, residual method) and HDL-C levels	–	HDL-C	–	0·28	<0·05
Suter *et al.*, 1993[74]	4	Spearman's correlation	Correlation between protein intake (E%) and blood lipid levels	–	TAG TC LDL-C HDL-C	–	NR NR NR NR	NS NS NS NS
Ventura *et al.*, 2008[76]	4	Pearson's correlation	Correlation between protein intake (E%) and TAG levels	–	TAG HDL-C	–	NR NR	NS <0·05
Vobecky *et al.*, 1979[77]	1	*t* test‡	Comparison of protein intake between infants with high *v.* normal cholesterol levels	–§	TC TC TC	M F Total	NR Positive Positive	NS <0·01 <0·01
Ward *et al.*, 1980[78]	2	Stepwise linear regression	Increase in cholesterol levels (mg/dl) per E% increase in protein intake	Dietary and biochemical variables (unspecified)	TC	–	NR	NS
Weidman *et al.*, 1978[79]	2	Linear regression	Increase in cholesterol levels (mg/dl) per E% increase in protein intake	Weight, height, energy intake, intake of fat, carbohydrate, fibre, and cholesterol	TC	–	NR	NS

NR, not reported; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; E%, percentage of energy; M, male; F, female; SES, socio-economic status.
* The intensive intervention group received most of their foods for free and had a higher adherence to the intervention diets.
† Partly overlapping populations.
‡ Statistical analysis used was not clearly reported.
§ Matched for sex and age.

Many studies received a low score for the items study design and adjustment for potential confounders. Most of the studies included in the present review (forty-seven of the fifty-six) were cross-sectional, only five were longitudinal and four were intervention studies. In observational studies, even after adjustment for multiple potential confounders, residual confounding may exist. Intakes of several other nutrients, for example, could be correlated not only with protein intake, but also with several other (unmeasured) determinants of cardiometabolic health such as exercise, BMI and dietary patterns. In many studies included in the present review, the results were not at all or not sufficiently adjusted for important confounding variables, which limits the validity of their results.

Important potential confounders or mediators in the association between protein intake and cardiometabolic health are energy intake and measures of obesity. Since protein is a macronutrient, protein intake is strongly associated with energy intake, but energy intake might also be associated with BP, insulin sensitivity and blood lipids. Therefore, not adjusting for energy intake could confound the results. In only twelve of the fifty-two observational studies included in the present review, energy intake was considered in some way (e.g. by expressing protein intake in E% or by adjusting for energy intake)[21,26,31,32,46,62,63,67,69,70,73,75]. Nevertheless, even for the studies that did control for energy intake, we cannot exclude that the observed associations of increased protein intake are, in fact, caused by a decreased intake in energy from carbohydrate or fat. In the four trials included in the present review, a higher protein level of diets was accomplished by lowering the percentage of energy from carbohydrate. The effects of these diets could thus also be ascribed to a decrease in carbohydrate intake rather than to an increase in protein intake.

An important potential mediator in the relationship between protein intake and cardiometabolic health is body weight or body fat. Protein intake has been positively linked to childhood obesity[3–5], while in adults, it has been inversely associated with obesity[9]. Since obesity is strongly associated with cardiometabolic health, it is interesting to investigate the association of protein intake with cardiometabolic outcomes both with and without adjustment for measures of body composition. However, in only nine of the fifty-two observational studies, the results were adjusted for a measure of body weight or composition[20,27,31,51,52,68,69,71,72,96]. We did not observe any trends after comparing the results from the studies included in the present review that did with those that did not adjust for measures of obesity. Of the included studies, ten were performed in overweight children only[31–33,37,38,47,63,71,76] and three studies included both overweight and normal-weight children[21,41,57]. The latter three studies reported no clear differences in the associations between protein intake and insulin sensitivity or blood lipid levels among the overweight *v.* the normal-weight group.

Only four intervention studies met the selection criteria for the present review, of which two were short term (29 and 31 d)[33,38], limiting the ability to observe an effect. The other two trials had a duration of 6 months, but consisted of dietary advice only[30,37]. In one of these trials, the actual protein intake did not even differ significantly between the two groups among participants who received dietary instructions only (18·6 (SEM 1·3) *v.* 17·6 (SEM 1·3) E%, $P=0·31$)[30]. However, the latter trial also included a subgroup that received free foods and had a higher adherence to the intervention diet (23·7 (SEM 1·4) *v.* 16·9 (SEM 1·3) E% protein; $P = 0·001$) . In this more intensive treatment group, beneficial effects of protein intake on BP and insulin sensitivity were observed, whereas in the total group, no differences were observed[30].

Strengths and limitations of the present review

The main strength of the present review is that it gives a comprehensive overview of the currently available evidence on the effects of dietary protein on BP, insulin sensitivity and blood lipids in children. A very extensive literature search in multiple databases was used to identify articles. We aimed to reduce the problem of publication bias by also searching for publications that did not explicitly mention protein intake in their title or abstract, and by contacting authors to identify unpublished studies. Studies were independently screened and data extracted by two reviewers using a predefined and meticulous procedure. We assessed the quality of the included studies with a scoring system in order to more objectively distinguish between the higher- and lower-quality studies. Unfortunately, many of the included studies were of relatively low quality. This made it difficult to draw conclusions regarding the absence or presence of the association evaluated. A meta-analysis was not possible due to the large levels of heterogeneity in study design, outcomes and age range of the children. Moreover, we were limited by a lack of reported effect estimates. Therefore, we conducted only a qualitative synthesis of evidence, in which we took into account the quality score of the included studies and the consistency of the reported results.

Conclusions

The fifty-six studies included in the present systematic review provide insufficient evidence for effects of protein intake on BP, insulin sensitivity or blood lipids in children. Although a substantial number of studies addressed these associations, data from high-quality studies investigating the independent effects of protein intake are scarce. Results from the few high-quality studies were not consistent. Further research of high methodological quality is needed to understand the effects of protein intake on cardiometabolic health in children. Specifically, in order to investigate the independent effects of protein intake, future studies should take into account important potential confounding factors such as total energy intake and other dietary factors, and measures of body weight. A better evaluation of the effect of protein intake on cardiometabolic outcomes in children is important since cardiometabolic risk factors in childhood have been shown to predict CVD and type 2 diabetes in adulthood. Therefore, insight into early-life determinants of cardiometabolic risk

may contribute to the prevention of CVD and type 2 diabetes in adulthood.

Supplementary material

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