

ONLINE SUPPLEMENTAL MATERIAL

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

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Supplemental Table 1 Literature search

Embase (via Embase.com)

Population	((infan* OR newborn* OR (new NEXT/1 born*) OR baby OR babies OR neonat* OR perinat* OR postnat* OR child OR 'child s' OR childhood* OR children* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors* OR underag* OR (under NEXT/2 ag*) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepuberty* OR pediatric* OR peadiatric* OR school* OR preschool* OR highschool* OR suckling):de,ab,ti OR (adoles*:de,ab,ti NOT adult/exp) OR child/exp OR newborn/exp)
Exposure	AND ('protein intake'/de OR (protein NEXT/1 supplement*) OR ((diet* OR intake* OR consumption* OR consumed* OR feeding OR food* OR nutrition* OR 'energy percentage' OR 'percentage of energy' OR 'caloric percentage' OR 'percentage of calories') NEAR/6 (protein* OR macronutrient* OR nutrient*)):ab,ti OR (diet* NEXT/1 intake*):ab,ti OR (diet* NEAR/3 composition*):ab,ti)
Outcomes	AND (('cardiovascular disease'/exp NOT ('cardiovascular disease'/exp/dm_cn OR 'congenital disorder'/exp)) OR 'non insulin dependent diabetes mellitus'/de OR hypertension/exp OR obesity/exp OR 'body mass'/de OR 'Metabolic Syndrome X'/de OR (((cardiovascular OR cardiac OR heart OR vascular OR cardiometabolic) NOT congenital) OR (diabetes NEAR/6 ('type 2' OR 'type ii' OR 'non insulin' OR noninsulin)) OR ((glucose OR insulin) NEAR/3 (level* OR concentration OR plasma OR blood OR serum OR metabolism OR tolerance OR intolerance OR sensitivit* OR insensitivity* OR resistance OR homeosta*)) OR 'blood pressure' OR hypertensi* OR ((cholesterol OR LDL* OR HDL* OR triglyceride* OR lipoprotein* OR lipid*) NEAR/3 (plasma OR blood OR serum OR level* OR profile*)) OR hyperlip* OR dyslip* OR obesity OR obese OR 'over weight' OR overweight OR adiposity OR 'metabolic syndrome' OR 'body mass index' OR BMI OR Quetelet OR (body NEXT/1 (composition* OR fat* OR weight*)) OR (weight NEXT/1 gain*) OR 'abdominal fat' OR (fat NEAR/3 (mass OR percentage*)) OR skinfold* OR (waist NEAR/3 (hip OR circumference*)))ab,ti)
Limits	NOT ([animals]/lim NOT [humans]/lim) NOT ([Review]/lim OR [Conference Abstract]/lim OR [Conference Paper]/lim OR [Short Survey]/lim OR [Editorial]/lim OR [Letter]/lim OR [Note]/lim OR [Conference Review]/lim OR [Erratum]/lim)

Medline (via OvidSP)

Population	((infan* OR newborn* OR (new born*) OR baby OR babies OR neonat* OR perinat* OR postnat* OR child OR child s OR childhood* OR children* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors* OR underag* OR (under ADJ2 ag*) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepuberty* OR pediatric* OR peadiatric* OR school* OR preschool* OR highschool* OR suckling).de,ab,ti. OR (adoles*.de,ab,ti. NOT exp adult/) OR exp child/ OR exp newborn/)
Exposure	AND (exp dietary proteins/ OR protein supplement*.ab,ti. OR ((diet* OR intake* OR consumption* OR consumed* OR feeding OR food* OR nutrition* OR energy percentage OR percentage of energy OR caloric percentage OR percentage of calories) ADJ6 (protein* OR macronutrient* OR nutrient*)):ab,ti. OR (diet* ADJ intake*):ab,ti. OR (diet* ADJ3 composition*):ab,ti.)

Outcomes	AND ((exp cardiovascular diseases/ NOT (exp cardiovascular diseases/cn OR exp congenital abnormalities/)) OR "Diabetes Mellitus, Type 2"/ OR exp hypertension/ OR exp obesity/ OR Body Mass Index/ OR Metabolic Syndrome X/ OR (((cardiovascular OR cardiac OR heart OR vascular OR cardiometabolic) NOT congenital) OR (diabetes ADJ6 ("type 2" OR "type ii" OR "non insulin" OR noninsulin)) OR ((glucose OR insulin) ADJ3 (level* OR concentration OR plasma OR blood OR serum OR metabolism OR tolerance OR intolerance OR sensitivit* OR insensitivity* OR resistance OR homeosta*)) OR "blood pressure" OR hypertensi* OR ((cholesterol OR LDL* OR HDL* OR triglyceride* OR lipoprotein* OR lipid*) ADJ3 (plasma OR blood OR serum OR level* OR profile*)) OR hyperlip* OR dyslip* OR obesity OR obese OR "over weight" OR overweight OR adiposity OR "metabolic syndrome" OR "body mass index" OR BMI OR Quetelet OR (body ADJ (composition* OR fat* OR weight*)) OR (weight ADJ gain*) OR "abdominal fat" OR (fat ADJ3 (mass OR percentage*)) OR skinfold* OR (waist ADJ3 (hip OR circumference*)))ab,ti.)
Limits	NOT (exp animals/ NOT humans/) NOT (review OR Congresses OR Clinical Conference OR Editorial OR comment OR letter OR Collected Correspondence).pt.

Cochrane library

Population	((infan* OR newborn* OR (new born*) OR baby OR babies OR neonat* OR perinat* OR postnat* OR child OR child s OR childhood* OR children* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors* OR underag* OR (under NEAR/2 ag*) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepuberty* OR pediatric* OR peadiatric* OR school* OR preschool* OR highschool* OR suckling):ab,ti OR adoles*:ab,ti)
Exposure	AND (protein supplement*:ab,ti OR ((diet* OR intake* OR consumption* OR consumed* OR feeding OR food* OR nutrition* OR energy percentage OR percentage of energy OR caloric percentage OR percentage of calories) NEAR/6 (protein* OR macronutrient* OR nutrient*)):ab,ti OR (diet* NEAR/1 intake*):ab,ti OR (diet* NEAR/3 composition*):ab,ti)
Outcomes	AND (((cardiovascular OR cardiac OR heart OR vascular OR cardiometabolic) NOT congenital) OR (diabetes NEAR/6 ('type 2' OR 'type ii' OR 'non insulin' OR noninsulin)) OR ((glucose OR insulin) NEAR/3 (level* OR concentration OR plasma OR blood OR serum OR metabolism OR tolerance OR intolerance OR sensitivit* OR insensitivity* OR resistance OR homeosta*)) OR 'blood pressure' OR hypertensi* OR ((cholesterol OR LDL* OR HDL* OR triglyceride* OR lipoprotein* OR lipid*) NEAR/3 (plasma OR blood OR serum OR level* OR profile*)) OR hyperlip* OR dyslip* OR obesity OR obese OR 'over weight' OR overweight OR adiposity OR 'metabolic syndrome' OR 'body mass index' OR BMI OR Quetelet OR (body NEXT/1 (composition* OR fat* OR weight*)) OR (weight NEXT/1 gain*) OR 'abdominal fat' OR (fat NEAR/3 (mass OR percentage*)) OR skinfold* OR (waist NEAR/3 (hip OR circumference*)))ab,ti

Pubmed (not Medline)

Population	((infan*[tiab] OR newborn*[tiab] OR (new born*[tiab]) OR baby[tiab] OR babies[tiab] OR neonat*[tiab] OR perinat*[tiab] OR postnat*[tiab] OR child[tiab] OR "child s"[tiab] OR childhood*[tiab] OR children*[tiab] OR kid[tiab] OR kids[tiab] OR toddler*[tiab] OR teen*[tiab] OR boy*[tiab] OR girl*[tiab] OR minors*[tiab] OR underag*[tiab] OR (under ag*[tiab]) OR juvenil*[tiab] OR youth*[tiab] OR kindergar*[tiab] OR puber*[tiab] OR pubescen*[tiab] OR prepubescen*[tiab] OR prepuberty*[tiab] OR pediatric*[tiab] OR peadiatric*[tiab] OR school*[tiab] OR preschool*[tiab] OR highschool*[tiab] OR suckling[tiab]) OR (adoles*[tiab] NOT adult*[tiab]))
Exposure	AND (((diet*[tiab] OR intake*[tiab] OR consumption*[tiab] OR consumed*[tiab] OR feeding[tiab] OR food*[tiab] OR nutrition*[tiab] OR "energy percentage"[tiab] OR "percentage of energy"[tiab] OR "caloric percentage"[tiab] OR "percentage of calories"[tiab]) AND (protein*[tiab] OR macronutrient*[tiab] OR nutrient*[tiab])) OR (diet*[tiab] AND intake*[tiab]) OR (diet*[tiab] AND composition*[tiab]))
Outcomes	AND (((((cardiovascular[tiab] OR cardiac[tiab] OR heart[tiab] OR vascular[tiab] OR cardiometabolic[tiab]) NOT congenital[tiab])) OR (((diabetes[tiab] AND ("type 2"[tiab] OR "type ii"[tiab] OR "non insulin"[tiab] OR noninsulin[tiab])) OR ((glucose[tiab] OR insulin[tiab]) AND (level*[tiab] OR concentration[tiab] OR plasma[tiab] OR blood[tiab] OR serum[tiab] OR metabolism[tiab] OR tolerance[tiab] OR intolerance[tiab] OR sensitivit*[tiab] OR insensitivity*[tiab] OR resistance[tiab] OR homeosta*[tiab]))) OR (((("blood pressure"[tiab] OR hypertensi*[tiab] OR ((cholesterol[tiab] OR LDL*[tiab] OR HDL*[tiab] OR triglyceride*[tiab] OR lipoprotein*[tiab] OR lipid*[tiab]) AND (plasma[tiab] OR blood[tiab] OR serum[tiab] OR level*[tiab] OR profile*[tiab])) OR hyperlip*[tiab] OR dyslip*[tiab])) OR ((obesity[tiab] OR obese[tiab] OR "over weight"[tiab] OR overweight[tiab] OR adiposity[tiab] OR "metabolic syndrome"[tiab] OR "body mass index"[tiab] OR BMI[tiab] OR Quetelet[tiab] OR (body[tiab] AND (composition*[tiab] OR fat*[tiab] OR weight*[tiab])) OR weight gain*[tiab] OR abdominal fat[tiab] OR (fat[tiab] AND (mass[tiab] OR percentage*[tiab])) OR skinfold*[tiab] OR (waist[tiab] AND (hip [tiab] OR circumference*[tiab]))))))
Limits	NOT (animal*[tiab] NOT human*[tiab]) NOT (review*[tiab] OR Congres*[tiab] OR Conference*[tiab] OR Editorial*[tiab] OR comment*[tiab] OR letter*[tiab] OR Corresponden*[tiab])) AND publisher[sb]

Supplemental Table 2 Quality score

The score was composed of 5 items, and each item was allocated 0, 1 or 2 points. This allowed a total score between 0 and 10 points, 10 representing the highest quality. The following items were included in the score:

1. Study design

0 for studies with cross-sectional data analysis

1 for studies with longitudinal data analysis (retrospective or prospective) or non-randomized intervention studies

2 for randomized intervention studies

2. Population

Observational studies

0 if $n < 500$

1 if $n \geq 500$ and < 1000

2 if $n \geq 1000$

Intervention studies

0 if $n < 50$

1 if $n \geq 50$ and < 100

2 if $n \geq 100$

3. Exposure

Observational studies

0 if the study used no appropriate standard dietary assessment method (see below) or if not reported

1 if the study used a one-day food record, one 24h recall, or a short food frequency questionnaire that did not cover the full diet

2 if the study used multiple-day food records, multiple 24h recalls, or a full-diet food frequency questionnaire

Intervention studies

0 if the intervention diet was not described or not adequately blinded

1 if the intervention diet was adequately single-blinded

2 if the intervention was adequately double-blinded

4. Outcome

0 if the study used no appropriate outcome measurement method (see below) or if not reported

1 if the study used moderate quality outcome measurement methods:

- Blood pressure: only one measurement, in resting position, by a trained observer
- Insulin sensitivity: only either glucose or insulin measures, blood sampling after 12h or overnight fast
- Blood lipids: non-fasting blood sampling

2 if the study used adequate outcome measurement methods*:

- Blood pressure: at least two measurements, in resting position, by a trained observer
- Insulin sensitivity: both glucose and insulin or a composite measure, with blood sampling after 12h or overnight fast, or an appropriate glucose or insulin tolerance test
- Blood lipids: blood sampling after 12h or overnight fast

5. Adjustments

0 if a study was not randomized (for intervention studies) or if findings were not controlled** for at least the four key covariates mentioned below

1 if findings were controlled for:

- age or Tanner stage,
- sex,
- energy intake (including E%), and
- at least one measure of body weight (e.g., BMI, body weight, or body fat).

2 if a study was adequately randomized (for intervention studies) or if findings were additionally controlled for at least two of the following covariates:

- intake of other macronutrients,
- intake of micronutrients,
- physical activity,
- growth,
- birth characteristics (e.g. birth weight, gestational age),
- maternal characteristics (e.g. maternal BMI),
- socioeconomic status, or
- ethnicity.

** Based on guidelines from the American Heart Association (blood pressure and blood lipids) and the American Diabetes Association (insulin sensitivity)*

*** 'Controlled for' here refers to: adjusted for in the statistical analyses (e.g. with multiple regression); stratified for in the analyses (e.g. boys and girls separately); or narrow selection criteria of study participants on this covariate (e.g. including only 7-year-old children would count as sufficiently controlling for age and including girls only does not require controlling for sex).*

Supplemental Table 3 Exposures and outcomes measured in the included studies

First author, year	Dietary assessment	Mean protein intake (g/d)	Mean protein intake (E%)	Outcomes
Aeberli et al, 2007	2x 24hR + 1d weighed FR	62.9	13.8%	LDL particle size
Aeberli et al, 2009	2x 24hR + 1d weighed FR	62.6	13.9%	SBP, fasting insulin levels, QUICKI
Akerblom et al, 1984	48hR	82.0	14.0%	Total and HDL cholesterol, TG
Andersen et al, 1979	1wk FR + 1 24hR	43.0 (0.7 y) 65.0 (4 y)	18.7% (0.7 y) 17.3% (4 y)	Total cholesterol
Berenson et al, 1979	4d weighed FR	30.0	12.6%	Total, HDL and LDL cholesterol
Boulton et al, 1995	1x 24hR	NR	NR	Total cholesterol, TG, BP
Casazza et al, 2009a	2x 24hR	71.9	15.3%	SBP, glucose levels, TG, HDL cholesterol
Casazza et al, 2009b	2x 24hR	NR	~15% (from figure)	Fasting insulin, insulin sensitivity index, acute insulin response
Colin-Ramirez et al, 2009	1x 24hR	67.3	13.7%	Hypertension, diastolic hypertension, and systolic hypertension [†]
Cowin et al, 2001	3x 1d FR	42.7 (M)/ 40.1 (F)*	15.0% (M)/ 15.1% (F)*	Total, HDL and LDL cholesterol
Damsgaard et al, 2013	Trial diet (target 10-15 vs. 23-28 E% protein), adherence measured with 3d weighed FR	NR	18.6 vs. 17.6% (whole group) 23.7 vs. 16.9% (intensive group)	MAP, fasting glucose and insulin levels, HOMA-IR, total, LDL and HDL cholesterol, TG
Davis et al, 2005	3d FR	78.3	16.5%	Insulin sensitivity, acute insulin response, disposition index
Davis et al, 2009	2x 24hR	67.7	15.3%	Insulin sensitivity, acute insulin response, disposition index
Duckworth et al, 2009	Trial diet (15 vs. 25 E% protein)	NR	15.0% (control) 25.0% (intervention)	SBP and DBP
Frank et al, 1977	1x 24hR	15 to 210	13%	BP, total cholesterol, TG
Frank et al, 1978	1x 24hR	69.0	12.9%	SBP, total cholesterol, TG
Garemo et al, 2006	7d FR (split in 2)	52.2	15.0%	Fasting glucose, insulin, HOMA-IR, HOMA-β, total cholesterol, TG
Garnett et al, 2013	Trial diets (15 vs. 25-30 E% protein)	NR	15% (control) 25-30% (intervention)	Insulin sensitivity index, TG, LDL and HDL cholesterol, SBP and DBP
Gately et al, 2007	Trial diets (15 vs. 22.5 E% protein)	NR	15.0% (control) 22.5% (intervention)	Total, LDL and HDL cholesterol, TG, SBP and DBP
Glueck et al, 1982	1x 24hR	75.7	14.2%	Total, HDL and LDL cholesterol, TG
Gonzalez-Requejo et al, 1995	1d FR	NR	14.0%	Total, HDL and LDL cholesterol

First author, year	Dietary assessment	Mean protein intake (g/d)	Mean protein intake (E%)	Outcomes
Hermelo et al, 1995	3d FR	NR	NR	Total, HDL, LDL, and VLDL cholesterol, TG
Heyman et al, 2012	4d FR	72.4	15.4%	Total, HDL and LDL cholesterol, TG, fasting glucose and insulin
Hitchcock et al, 1977	1x 24hR	NR	12.4%	SBP, DBP, total cholesterol
Hong et al, 2009	3d FR	76.0	15.9%	Total and HDL cholesterol, TG, fasting glucose
Il'chenko et al, 1989	1d FR	61.5	11.7%	Total and HDL cholesterol, TG, arterial pressure
Jenner et al, 1988	FFQ	62.7	13.93%	SBP and DBP
Keser et al, 2010	1x 24hR	NR	15-24%	Total, HDL and LDL cholesterol, TG, glucose, insulin
Knuiman et al, 1993	48hR	76.6 (Finland) 70.9 (Netherlands) 74.4 (Italy) 56.0 (Philippines) 39.2 (Ghana)	13.8% (Finland) 15.5% (Netherlands) 13.4% (Italy) 11.7% (Philippines) 9.2% (Ghana)	Total and HDL cholesterol
Kouvalainen et al, 1982	7d FR	55.0 (3 y) 82.7 (12 y)	14.7% (3 y) 14.2% (12 y)	Total and HDL cholesterol
Larsen et al, 1989	1x 24hR	70.5	13.8%	Total cholesterol
Lindquist et al, 2000	3x 24hR	63.7	14.0%	Total cholesterol, TG, insulin sensitivity, acute insulin response
Lucas et al, 1994	Lab analysis of breast milk and formula samples	2.3	9.4%	SBP and DBP
Menghetti et al, 2004	1x 24hR	NR	15-16%	Hypertension ^s
Mia et al, 2000	FFQ	91.7	13.0%	Total and LDL cholesterol, TG
Morrison et al, 1980	1x 24hR	78.2	14.0%	Total, HDL and LDL cholesterol, TG
Nicklas et al, 1993	1x 24hR	72.5	13.4%	Total, HDL and LDL cholesterol
Obuchowicz et al, 1997	3d diet history	65.4 (normal weight) 104.7 (overweight)	12.7% (normal weight) 13.6% (overweight)	Fasting insulin levels
Perry et al, 1997	3x 1d FR	63.0	15.0%	TG
Pistulkova et al, 1992	3d FR	86.8	12.9%	Total cholesterol
Potter et al, 1989	3x 1d FR	NR	NR	Total, HDL and LDL cholesterol, TG
Räsänen et al, 1978	1x 24hR	NR	NR	Total cholesterol
Regan et al, 2006	Hospital records	2.5	7.7%	Insulin sensitivity
Rinaldi et al, 2012	3x 24hR	NR	16.0%	Total, LDL and HDL cholesterol, TG

First author, year	Dietary assessment	Mean protein intake (g/d)	Mean protein intake (E%)	Outcomes
Sanchez-Bayle et al, 2008	1x 24hR & FFQ	NR	14.8%	Total, LDL and HDL cholesterol, TG
Sarria Chueca et al, 1997	1x 24hR	102.0	19.2%	Total, LDL and HDL cholesterol
Schachter et al, 1979	2d FR	NR	NR	BP
Schutte et al, 2003a	1x 24hR	60.5	NR	SBP, DBP, and MAP
Schutte et al, 2003b	1x 24hR	60.5	NR	Pulse pressure
Sharma et al, 2009	3d FR	65.5	14.8%	Total, HDL and LDL cholesterol, TG, HOMA-IR
Simons-Morton et al, 1997	3x 24hR	63.0	14.8%	SBP and DBP
Smith et al, 2003	1x 24hR	NR	NR	SBP and DBP
Starc et al, 1998	3d FR	57.5	16.5%	HDL cholesterol
Sugiyama et al, 2007	1x 24hR	79.2	13.5%	SBP and DBP
Suter et al, 1993	3d FR	75.3	15.8%	Total, HDL and LDL cholesterol, TG
Ulbak et al, 2004	7d FR	42.0	11.9%	SBP and DBP
Ventura et al, 2008	2x 24hR	71.8	16.1%	HDL cholesterol, TG, glucose tolerance, SBP and DBP
Vobecky et al, 1979	1x 24hR	4.3	NR	Total cholesterol
Ward et al, 1980	3d FR	47.8	14.1%	Total cholesterol
Weidman et al, 1978	7d FR	77.6	14.0%	Total cholesterol

Abbreviations: 24hR, 24-hour recall; BP, blood pressure; DBP, diastolic blood pressure; E%, energy percentage; FFQ, food frequency questionnaire; FR, food record; HOMA- β , homeostatic model assessment- β -cell function; HOMA-IR, homeostatic model assessment-insulin resistance; MAP, mean arterial pressure; NR, not reported; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TG, triglycerides.

[†]Defined using population-based percentiles for sex, age, and height: Hypertension SBP and DBP $\geq 90^{\text{th}}$ percentile; diastolic hypertension DBP $\geq 95^{\text{th}}$ percentile and SBP $< 90^{\text{th}}$ percentile; systolic hypertension SBP $\geq 95^{\text{th}}$ percentile and DBP $< 90^{\text{th}}$ percentile.

^{*}Retrieved from another paper from the same study (Cowin et al, 2001)

[§]Based on reference values for the Italian population