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Associations between macronutrient intake and serum lipid profile depend on body fat in European adolescents: the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study

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Abstract

The present study aimed to investigate the relationships between macronutrient intake and serum lipid profile in adolescents from eight European cities participating in the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) cross-sectional study (2006–7), and to assess the role of body fat-related variables in these associations. Weight, height, waist circumference, skinfold thicknesses, total cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol, TAG, apoB and apoA1 were measured in 454 adolescents (44 % boys) aged 12·5–17·5 years. Macronutrient intake (g/4180 kJ per d (1000 kcal per d)) was assessed using two non-consecutive 24 h dietary recalls. Associations were evaluated by multi-level analysis and adjusted for sex, age, maternal education, centre, sum of four skinfolds, moderate-to-vigorous

Abbreviations: 24-HDR, 24 h dietary recall; HDL-C, HDL-cholesterol; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence; LDL-C, LDL-cholesterol; M:S, monosaturated:saturated fat ratio; P:S, polyunsaturated:saturated fat ratio; PA, physical activity; TC, total cholesterol; WHeR, waist:height ratio.

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physical activity, sedentary behaviours and diet quality index for adolescents. Carbohydrate intake was inversely associated with HDL-C ($\beta = -0{\cdot}189$, $P<0{\cdot}001$). An inverse association was found between fat intake and TAG ($\beta = -0{\cdot}319$, $P<0{\cdot}001$). Associations between macronutrient intake and serum lipids varied according to adiposity levels, i.e. an inverse association between carbohydrate intake and HDL-C was only observed in those adolescents with a higher waist:height ratio. As serum lipids and excess body fat are the major markers of CVD, these findings should be considered when developing strategies to prevent the risk of CVD among adolescents.

Key words: Macronutrient intake: Serum lipids: Body fat: Adolescents

High levels of LDL-cholesterol (LDL-C) in childhood and the onset of atherosclerosis in early life[1] could result in adult dyslipidaemias[2], which are important cardiovascular risk factors. Therefore, evidence for factors influencing lipid profiles is necessary for public health protection and promotion.

Dietary modifications that lower atherogenic lipids and lipoproteins are effective in the prevention and treatment of CVD risk[3]. Nonetheless, the optimal dietary pattern(s) to restrain atherosclerosis progression remains to be identified[4]. For instance, a high intake of total carbohydrates has been suggested to lower HDL-cholesterol (HDL-C) concentration and to increase TAG concentration in adults[5], and a protein-rich diet low in saturated fat has been shown to significantly decrease the concentrations of LDL-C, TAG and total cholesterol (TC) in comparison to a carbohydrate-rich diet and a diet rich in unsaturated fat[6]. However, findings about the role of dietary fat, mainly that of saturated fat, in CVD risk are still controversial[7] because of individual variability in serum lipid response to changes in dietary saturated fat and cholesterol[3]. Additionally, obesity has been reported to strongly affect serum lipid response to diet[3], making obese individuals less responsive to dietary interventions aimed to improve serum lipid profile.

Adolescence is a key period in life because of the growth spurt and sexual maturation that take place; therefore, having a healthy diet is essential to achieve optimal development and to prevent the appearance of chronic diseases later in life[8]. Furthermore, associations between macronutrient intake and serum lipids have mainly been investigated among adults, and there is a lack of the literature addressing this topic in adolescents. As these associations have not been examined yet among adolescents, we hypothesised that macronutrient intake was associated with serum lipids in a sample of healthy European adolescents and that body adiposity may exert a key role in this association. Therefore, the aims of the present study were (1) to investigate the relationships between macronutrient intake and serum lipid profile in European adolescents and (2) to assess the role of body fat-related variables in these associations.

Materials and methods

The present study sample was derived from the cross-sectional multi-centre HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study (n 3528) carried out in adolescents (12·5–17·5 years) between 2006 and 2007 in ten European cities (Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pécs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain). General HELENA procedures, characteristics and inclusion criteria can be found elsewhere[9,10]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the local ethical committee at each study centre[11]. Written informed consent was obtained from all adolescents and their parents.

Participants with complete data on TAG, TC, HDL-C, LDL-C, apoA1, apoB and two 24 h dietary recalls (24-HDR) were included (n 454; 44 % boys). Decreases from the original sample size are partially explained by the fact that blood samples were randomly drawn only in one-third of the HELENA participants and partially from the fact that Heraklion and Pécs were excluded from the 24-HDR analyses due to logistical reasons; therefore, eight out of the ten study centres were included in the 24-HDR analyses, resulting in a sample size decrease. Excluded participants (n 3074) were significantly ($P<0{\cdot}05$) older, heavier and had a higher mean BMI than those included in the present study (data not shown).

Macronutrient intake

Dietary intake was assessed using a self-administered computer-based tool called HELENA-DIAT (Dietary Assessment Tool), based on the previously developed software Young Adolescents' Nutrition Assessment on Computer (YANA-C) that was shown to be appropriate in assessing dietary information of European adolescents[12,13]. The software consists of a single, structured 24-HDR according to six meal occasions. Adolescents were asked to recall all food and drinks consumed the previous day. Within a time span of 2 weeks, two non-consecutive 24-HDR were obtained from each participant during school time and assisted by fieldworkers. Therefore, no information on Fridays and Saturdays was collected.

The German Food Code and Nutrition Data Base (Bundeslebensmittelschlüssel, BLS version II.3.1)[14] was used to calculate energy and nutrient intakes. Usual food and nutrient intakes were estimated by the multiple source method in order to account for within-person variability[15]. Energy intake was estimated in kJ/d and macronutrient intake (fat, protein and carbohydrate) in g/d. Subsequently, intakes of each macronutrient were divided by energy intake and are expressed as g/4180 kJ per d (1000 kcal per d) to account for total energy intake[16]. Additionally, the mono-unsaturated:saturated fat ratio (M:S), the polyunsaturated:

saturated fat ratio (P:S) and the cholesterol–saturated fat index (CSI) were computed as follows[17]:

$$CSI = (1{\cdot}01 \times g\,saturated\,fat) + (0{\cdot}05 \times mg\,cholesterol).$$

Diet Quality Index for Adolescents

A previously validated Diet Quality Index for Adolescents (DQI-A)[18] was used to adjust for all dietary factors simultaneously. The technical aspects regarding the development of the DQI-A have been published elsewhere[18].

Physical examinations

All anthropometric measurements were taken following a standardised protocol described elsewhere[19]. Weight and height were measured in underwear and barefoot using an electronic scale (Type SECA 861) and a stadiometer (Type SECA 225). BMI was calculated as body weight (in kg) divided by the square of height (in m) and was categorised as described by Cole *et al.*[20,21]. Skinfold thicknesses were measured with a calliper (Holtain Ltd) in triplicate on the left side at the biceps, triceps, subscapular and suprailiac sites. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest using an anthropometric tape (SECA 200). The waist:height ratio (WHeR) was calculated.

Blood sampling

Blood sampling procedures have been described elsewhere[22]. Briefly, blood samples were drawn after a 10 h overnight fast and analysed in centralised laboratories. Serum TAG, TC, HDL-C, TAG and LDL-C concentrations were measured on a Dimension RxL clinical chemistry system (Dade Behring) with enzymatic methods using the manufacturer's reagents and instructions. ApoB and apoA1 were measured in an immunochemical reaction with a BN II analyser (Dade Behring), according to the manufacturer's instructions. The technique consists of the following: the proteins contained in the serum sample form immune complexes with specific antibodies. These complexes scatter a beam of light when it passes through the sample. As the intensity of the scattered light is proportional to the concentration of the relevant protein in the sample, the result is evaluated by comparison with a standard of known concentration. Quantitative evaluation was made by comparison with standard concentrations. Intra-assay CV for all blood variables were $<3{\cdot}9\,\%$, and inter-assay CV were $<4{\cdot}3\,\%$. The TC:HDL-C and the apoB:apoA1 ratios were computed.

Education

Maternal education was used as a proxy of socio-economic status and was assessed via a questionnaire according to the following four categories: (1) lower education; (2) lower secondary education; (3) higher secondary education; (4) higher education/university degree.

Sedentary behaviours

Average time spent in two sedentary behaviours (television viewing and playing with videogames) was estimated by means of a self-administered questionnaire that has been previously found to demonstrate good reliability[23].

Physical activity

Physical activity (PA) was objectively measured by uniaxial accelerometers during seven consecutive days (Actigraph MTI, model GT1M; Manufacturing Technology, Inc.)[24]. At least 3 d of recording, with a minimum of 8 h registration per d, was set as an inclusion criterion. The time sampling interval was set at 15 s. The time spent in moderate-to-vigorous PA (>3 metabolic equivalents) was calculated on the basis of the following cut-off point: ≥2000 counts/min for moderate-to-vigorous PA[24,25].

Statistical analysis

The normality of all variables was checked and non-normally distributed variables (TAG, TC, HDL-C, LDL-C, TC:HDL-C ratio, apoB:apoA1 ratio, fat intake and P:S ratio) were log transformed before the analysis. Normality for the CSI was reached by using the power of 2. The M:S ratio was converted to 1/(M:S). Differences across the groups were tested by means of the independent-samples *t* test for normally distributed variables and the Mann–Whitney *U* test for non-normally distributed variables. The χ^2 test was applied for categorical variables.

Multi-level linear regression analyses were performed to investigate the associations between the intakes of macronutrients (independent variables) and plasma lipid concentrations (dependent variables). As no interaction by sex was found, the analyses were conducted with boys and girls combined. Study centre was included as the random intercept. Sex, age, maternal education, sum of four skinfolds, moderate-to-vigorous PA, sedentary behaviours and DQI-A were entered as covariates. Collinearity tests showed no collinearity among the covariates.

Since serum lipid profile has previously been associated with body fat in the HELENA adolescents[26] and excess adiposity has been shown to have an influence on serum lipid response to diet[3], participants were categorised into low and high body fat content according to three body fat indicators, i.e. *z*-score of BMI and sum of skinfolds as the measures of general body fatness, and the WHeR as an indicator of central adiposity. These cut-offs were calculated specifically by sex and by 1-year groups (12·5–13·49, 13·5–14·49, 14·5–15·49, 15·5–16·49 and 16·5–17·5) based on the median of each subgroup. Multi-level linear regression analyses on the associations between macronutrient intake and serum lipid concentrations were performed separately for each group of low/high body fat indicator and adjusted for potential confounders, i.e. sex, age, maternal education, sum of four skinfolds, moderate-to-vigorous PA, sedentary behaviours and DQI-A, where study centre was entered as the random intercept. No collinearity was observed among

the covariates. The level of statistical significance was controlled for multiple testing (0·05/number of tests = 0·05/8 = 0·006); therefore, statistical significance was considered at $P \leq 0{\cdot}006$. Multi-level linear regression analyses were re-run by using tertiles of protein, carbohydrate and fat intake. Bonferroni correction was used for the *post hoc* multiple comparison test, and statistical significance was set at $P < 0{\cdot}05$. Statistical analysis was performed using the statistical software package STATA version 12.0 (Stata Corporation).

Results

The main characteristics of the study sample are shown in Table 1. Table 2 presents the means and medians of dietary intake and blood lipid levels according to high/low body WHeR, i.e. above or below the sex- and age-specific median-based cut-offs of the WHeR. Adolescents in the high-WHeR group showed significantly higher protein intake (g/4180 kJ) and percentage of protein intake, M:S ratio, TAG, TC:HDL-C ratio and apoB:apoA1 ratio and lower total energy intake than adolescents in the low-WHeR group.

The associations between macronutrient intake (g/4180 kJ) and serum lipid profile are shown in Table 3. Carbohydrate intake was inversely associated with HDL-C ($P = 0{\cdot}001$), and a trend towards significance ($P = 0{\cdot}010$) was observed for apoA1. Inverse associations were found between fat intake and TAG ($P < 0{\cdot}001$) and TC:HDL-C ratio ($P = 0{\cdot}005$). LDL-C, apoB and apoB:apoA1 ratio were not significantly associated,

Table 1. Descriptive characteristics of the study sample stratified by sex
(Mean values and standard deviations; percentages; medians and 25th–75th percentiles)

	Boys (*n* 200)		Girls (*n* 254)		
	Mean	SD	Mean	SD	*P*
Age (years)	14	1·2	14	1·2	0·085
Weight (kg)	59	13	56	11	<0·001*
Height (cm)	170	9·8	162	7·5	<0·001*
BMI (kg/m^2)	20	3·4	21	3·5	0·097
Underweight (%)†	8·2		6·6		–
Normal weight (%)	75		73		–
Overweight (%)	10		16		–
Obese (%)	6·7		4·5		–
Maternal education (%)					0·035‡
Lower education	6·2		10		–
Lower secondary education	18		23		–
Higher secondary education	30		34		–
Higher education/University degree	46		33		–
ApoA1 (g/l)	1·48	0·01	1·55	0·01	<0·001*
ApoB (g/l)	0·61	0·01	0·67	0·01	<0·001*
	Median	25th–75th percentile	Median	25th–75th percentile	
Sum of four skinfolds (mm)	34	26–52	54	40–75	<0·001§
Waist:height ratio	0·42	0·40–0·44	0·43	0·40–0·47	0·005§
MVPA (min/d)	64	50–82	48	34–60	<0·001§
Sedentary behaviours (min/d)	133	77–209	107	65–167	<0·001§
DQI-A	51	37–62	55	44–65	<0·001§
Total energy intake					
kJ/d	10 263	8506–12 560	7665	6330–9243	<0·001§
kcal/d	2453	2033–3002	1832	1513–2209	<0·001§
Protein intake (g/4180 kJ)	40	35–45	40	35–46	0·881
Carbohydrate intake (g/4180 kJ)	120	112–132	123	112–135	0·193
Fat intake (g/4180 kJ)	41	36–47	42	34–48	0·594
Saturated fat intake (g/4180 kJ)	16	13–17	15	14–17	0·417
Monounsaturated fat intake (g/4180 kJ)	14	12–15	13	12–15	0·114
Polyunsaturated fat intake (g/4180 kJ)	5	4–6	5	4–6	0·594
Monounsaturated:saturated ratio	0·86	0·78–0·99	0·86	0·77–0·99	0·966
Polyunsaturated:saturated ratio	0·32	0·26–0·41	0·33	0·27–0·42	0·468
Cholesterol–saturated fat index	23	21–26	24	21–27	0·267
TAG (mmol/l)	0·64	0·49–0·87	0·71	0·54–0·95	<0·001§
TC (mmol/l)	3·99	3·57–4·35	4·33	3·83–4·77	<0·001§
HDL-C (mmol/l)	1·40	1·22–1·53	1·45	1·30–1·66	<0·001§
LDL-C (mmol/l)	2·33	1·99–2·59	2·51	2·07–2·95	<0·001§
TC:HDL-C	2·8	2·5–3·2	2·9	2·5–3·4	0·340
ApoB:apoA1	0·43	0·33–0·48	0·42	0·35–0·50	0·169

MVPA, moderate-to-vigorous physical activity; DQI-A, Diet Quality Index for Adolescents; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.
* $P < 0{\cdot}05$ (independent-samples *t* test).
† BMI categories as described by Cole *et al.*[20,21].
‡ $P < 0{\cdot}05$ (χ^2 test).
§ $P < 0{\cdot}05$ (Mann–Whitney *U* test).

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Table 2. Dietary characteristics and serum lipid parameters stratified by high *v.* low waist:height ratio‡
(Mean values and standard deviations; medians and 25th–75th percentiles)

	Waist:height ratio			
	High (*n* 217)		Low (*n* 218)	
	Median	25th–75th percentile	Median	25th–75th percentile
ApoA1 (g/l)				
Mean		1·49*		1·55
SD		0·21		0·22
ApoB (g/l)				
Mean		0·65		0·64
SD		0·16		0·15
Protein intake (g)	79†	63–103	87	70–105
Protein intake (g/4180 kJ)	41†	36–46	38	34–43
Protein intake (% energy)	16†	14–19	15	14–17
Carbohydrate intake (g)	235†	190–292	267	220–347
Carbohydrate intake (g/4180 kJ)	121	112–135	124	112–134
Carbohydrate intake (% energy)	48	45–54	50	45–53
Fat intake (g)	83	60–104	92	67–118
Fat intake (g/4180 kJ)	42	36–49	41	34–47
Fat intake (% energy)	38	32–44	37	31–42
Saturated fat intake (g/4180 kJ)	15	13–17	16	14–17
Monounsaturated fat intake (g/4180 kJ)	14†	12–16	13	11–15
Polyunsaturated fat intake (g/4180 kJ)	5	4–6	5	4–6
Monounsaturated:saturated ratio	0·89†	0·80–1·01	0·84	0·74–0·97
Polyunsaturated:saturated ratio	0·33	0·28–0·41	0·33	0·26–0·42
Cholesterol–saturated fat index	24	21–26	23	21–26
TAG (mmol/l)	0·71†	0·52–1·03	0·66	0·52–0·86
TC (mmol/l)	4·20	3·65–4·58	4·17	3·70–4·61
HDL-C (mmol/l)	1·40	1·24–1·55	1·45	1·30–1·68
LDL-C (mmol/l)	2·43	2·07–2·82	2·41	2·05–2·77
TC:HDL-C	3·0†	2·6–3·4	2·8	2·4–3·2
ApoB:apoA1	0·44†	0·36–0·50	0·41	0·33–0·50

TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.
* Mean value was significantly different from that of adolescents in the low-adiposity waist:height ratio group ($P<0·05$; independent-samples *t* test).
† Median value was significantly different from that of adolescents in the low-adiposity waist:height ratio group ($P<0·05$; Mann–Whitney *U* test).
‡ High- and low-waist:height ratio groups were defined by means of sex- and age-specific medians.

but an inverse trend towards significance was observed. In addition, a positive trend towards significance was found between fat intake and HDL-C ($P=0·047$). Protein intake was not significantly associated with TAG, but an inverse trend towards significance was observed ($P=0·027$).

Analyses were conducted for the three body fat indicators; however, the results have been focused on only one measure of adiposity, specifically central adiposity, to ease its interpretation. Table 4 presents the associations between macronutrient intake and blood lipid profile for the low and high WHeR. Carbohydrate intake was inversely associated ($P=0·001$) with HDL-C in adolescents with the high WHeR and a negative trend was found for apoA1 ($P=0·043$), but not in those with the low WHeR. A positive trend towards significance ($P=0·011$) was observed between the intake of carbohydrate and TAG concentration only in those adolescents within the high WHeR group. An inverse trend between fat intake and TAG ($P=0·008$) and TC:HDL-C ratio ($P=0·044$) was found in adolescents with the high WHeR. Although not significantly associated, inverse trends between protein intake and TAG ($P=0·021$) and LDL-C ($P=0·049$) concentrations were observed only in adolescents with the high WHeR. The results were consistent with the other measures of adiposity, namely sum of four skinfolds and *z*-score of BMI (data not shown).

Fig. 1 shows the means of TAG, TC, HDL-C and LDL-C concentrations by tertiles of fat intake in adolescents with the high and low WHeR. Overall, adolescents with the high WHeR in the lower tertile of fat intake (tertile 1) showed clinically adverse values of serum lipid concentrations, i.e. higher TAG, TC and LDL-C concentrations, and lower HDL-C concentration, compared with those in the higher tertile of fat intake (tertile 3). Within the upper tertile of fat intake, adolescents with the high WHeR had better values of blood parameters, whereas slight differences were observed across the tertiles of fat intake in adolescents with lower central adiposity levels. Indeed, adolescents with the high WHeR (Fig. 1(a)) in the lowest tertile of fat intake showed significantly higher TAG concentrations than those in the upper tertile of fat intake ($P<0·05$). Furthermore, a significant interaction ($P<0·05$) was observed between fat intake and WHeR for TAG (Fig. 1(a)). In contrast, adolescents with high adiposity levels, i.e. high WHeR, in the upper tertile of carbohydrate intake showed significantly higher TAG levels than those in the lower tertile of carbohydrate intake ($P<0·05$, data not shown). No significant differences were observed across tertiles of protein intake (data not shown).

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Table 3. Multi-level regression analysis addressing the associations between intakes of protein, carbohydrates and fat, monounsaturated:saturated fat ratio, polyunsaturated:saturated fat ratio and cholesterol–saturated fat index and serum lipid profile
(*β*-Coefficients and 95 % confidence intervals)

	Protein (g/4180 kJ)		Carbohydrates (g/4180 kJ)		Fat (g/4180 kJ)		Monounsaturated: saturated fat ratio		Polyunsaturated: saturated fat ratio		Cholesterol–saturated fat index	
	β†	95 % CI	*β*†	95 % CI	*β*†	95 % CI	*β*†	95 % CI	*β*†	95 % CI	*β*†	95 % CI
TAG	−0·242	−0·456, −0·028	0·230	−0·033, 0·493	−0·319*	−0·494, −0·143	0·042	−0·145, 0·230	0·025	−0·097, 0·146	−0·053	−0·143, 0·038
TC	−0·056	−0·142, 0·030	−0·048	0·153, 0·056	−0·073	−0·147, −0·000	−0·071	−0·147, 0·005	0·027	−0·018, 0·760	0·003	−0·032, 0·038
HDL-C	0·014	−0·080, 0·109	−0·189*	−0·302, −0·076	0·086	0·001, 0·172	−0·034	−0·118, 0·049	0·003	−0·048, 0·055	0·041	0·003, 0·080
TC:HDL-C	−0·080	−0·179, 0·018	0·153	0·032, 0·274	−0·118*	−0·200, −0·035	−0·005	−0·091, 0·080	0·023	−0·033, 0·080	−0·039	−0·081, 0·003
LDL-C	−0·120	−0·251, 0·010	0·077	−0·084, 0·238	−0·131	−0·240, −0·022	−0·061	−0·174, 0·051	0·049	−0·025, 0·124	−0·035	−0·091, 0·020
ApoA1	−0·011	−0·123, 0·102	−0·177	−0·311, −0·042	0·031	−0·638, 0·130	−0·075	−0·172, 0·023	0·012	−0·050, 0·075	0·011	−0·036, 0·057
ApoB	−0·065	−0·143, 0·011	0·022	−0·073, 0·118	−0·068	−0·133, −0·003	−0·062	−0·129, 0·004	0·038	−0·006, 0·082	−0·022	−0·055, 0·011
ApoB:apoA1	−0·084	−0·223, 0·055	0·136	−0·035, 0·307	−0·117	−0·234, −0·001	−0·047	−0·167, 0·073	0·035	−0·045, 0·114	−0·034	−0·093, 0·025

TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.
* $P < 0.006$ (Bonferroni correction).
† Adjusted for sex, age, study centre, maternal education, sum of four skinfolds, moderate-to-vigorous physical activity, sedentary behaviours and dietary quality index.

Identical findings across the tertiles of fat intake were observed in adolescents with the high/low sum of skinfolds.

Discussion

The present study examined the associations between energy-adjusted macronutrient intake and serum lipid profile, as well as the potential role that body adiposity may exert on these associations among healthy European adolescents. Overall, the results suggested that dietary fat has a beneficial role in serum lipid levels by lowering serum TAG levels and TC:HDL-C ratio, whereas carbohydrate intake was adversely associated with lipid profile by decreasing serum HDL-C concentrations. These above-mentioned associations varied according to the body fat status of adolescents, i.e. significant associations and trends towards significance between intakes of fat and carbohydrate and blood lipids were observed mainly among adolescents in the high-body fat group. To the best of our knowledge, the present study is a novel study as it is the first to address such relationships among adolescents.

The associations between carbohydrate intake and HDL-C add further evidence to the complex and adverse role that dietary carbohydrates appear to play in serum lipid profile[5]. Indeed, a high intake of total carbohydrate is also associated with lower HDL-C and higher TAG concentrations in adults[5] and children[27]. Nevertheless, it is important to take into account that we did not discriminate among the types of carbohydrates, i.e. simple and complex carbohydrates, meaning that the observed associations may have differed among the subtypes of carbohydrates. However, it is known that serum lipid levels are controlled not only by dietary carbohydrates but also by dietary proteins[28]. Although available data addressing the associations between dietary protein intake and serum lipids are still limited, vegetal sources of protein *per se* have been shown to lower plasma cholesterol concentrations[29]. Furthermore, Appel *et al.*[6] observed that a healthy diet, rich in protein and low in saturated fat, significantly decreased the concentrations of LDL-C, TAG and TC among adults compared with a carbohydrate-rich diet and a diet rich in unsaturated fat. The inverse association that we found between protein intake and TAG concentration is partially in concordance with these findings.

The role of fat intake in serum lipid levels differs according to the type of fat consumed. Indeed, the fatty acid profile of the diet seems to be the major determinant of serum cholesterol concentrations[30]. The findings from a follow-up study[31] have suggested that replacing dietary saturated fat with polyunsaturated fat rather than with monounsaturated fat or carbohydrates protects middle-aged and older men and women from the risk of CHD. Other studies have observed that replacement of dietary saturated fat with polyunsaturated fat, monounsaturated fat and/or carbohydrates reduces LDL-C concentration[32,33]. PUFA intake decreases LDL-C concentration[30] and MUFA intake decreases the TC:HDL-C ratio when isoenergetically compared with SFA intake[34]. Monounsaturated fat-rich diets have been shown to have comparable effects on serum lipid concentrations

Table 4. Multi-level regression analysis addressing the associations between macronutrient intake and serum lipid profile categorised by high *v.* low waist:height ratio (WHeR)‡

(β-Coefficients and 95 % confidence intervals)

	High WHeR (*n* 217)		Low WHeR (*n* 218)		High WHeR (*n* 217)		Low WHeR (*n* 218)	
	β†	95 % CI	β†	95 % CI	β†	95 % CI	β†	95 % CI
	Protein (g/4180 kJ)				Monounsaturated:saturated fat ratio			
TAG	−0·400	−0·738, −0·061	−0·040	−0·301, 0·221	0·349	0·052, 0·647	−0·239	−0·461, −0·017
TC	−0·118	−0·248, 0·012	−0·008	−0·116, 0·099	−0·025	−0·143, 0·093	−0·092	−0·183, −0·000
HDL-C	0·028	−0·106, 0·161	0·012	−0·116, 0·141	−0·084	−0·202, 0·035	−0·009	−0·120, 0·101
TC:HDL-C	−0·145	−0·292, 0·002	−0·021	−0·155, 0·114	0·081	−0·048, 0·211	−0·083	−0·198, 0·032
LDL-C	−0·198	−0·395, −0·001	−0·042	−0·217, 0·134	0·007	−0·168, 0·182	−0·135	−0·284, 0·015
ApoA1	−0·030	−0·185, 0·124	0·036	−0·120, 0·193	−0·100	−0·236, 0·036	−0·077	−0·211, 0·057
ApoB	−0·108	−0·221, 0·005	−0·019	−0·125, 0·088	−0·002	−0·103, 0·098	−0·116	−0·206, −0·026
ApoB:apoA1	−0·128	−0·333, 0·076	−0·059	−0·251, 0·133	0·060	−0·120, 0·240	−0·122	−0·286, 0·042
	Carbohydrates (g/4180 kJ)				Polyunsaturated:saturated fat ratio			
TAG	0·573	0·130, 1·016	−0·065	−0·368, 0·238	−0·018	−0·227, 0·190	0·036	−0·105, 0·178
TC	−0·114	−0·284, 0·056	−0·009	−0·134, 0·115	0·043	−0·034, 0·120	0·015	−0·043, 0·074
HDL-C	−0·286*	−0·455, −0·116	−0·128	−0·277, 0·020	0·047	−0·031, 0·126	−0·038	−0·107, 0·032
TC:HDL-C	0·181	−0·011, 0·374	0·119	−0·036, 0·274	−0·007	−0·097, 0·083	0·053	−0·019, 0·126
LDL-C	−0·029	−0·289, 0·232	0·141	−0·061, 0·344	0·046	−0·075, 0·167	0·062	−0·032, 0·157
ApoA1	−0·205	−0·403, −0·006	−0·186	−0·366, −0·006	0·066	−0·026, 0·157	−0·029	−0·113, 0·057
ApoB	0·012	−0·137, 0·162	0·025	−0·099, 0·148	0·030	−0·040, 0·099	0·046	−0·012, 0·103
ApoB:apoA1	0·083	−0·185, 0·352	0·175	−0·047, 0·397	−0·021	−0·146, 0·104	0·084	−0·019, 0·188
	Fat (g/4180 kJ)				Cholesterol−saturated fat index			
TAG	−0·379	−0·659, −0·099	−0·172	−0·389, 0·044	−0·039	−0·185, 0·108	−0·051	−0·162, 0·060
TC	−0·068	−0·181, 0·044	−0·088	−0·177, 0·001	0·001	−0·053, 0·056	−0·002	−0·048, 0·044
HDL-C	0·110	−0·009, 0·229	−0·007	−0·115, 0·100	0·004	−0·051, 0·060	0·066	0·012, 0·121
TC:HDL-C	−0·125	−0·247, −0·003	−0·081	−0·193, 0·031	−0·006	−0·069, 0·057	−0·069	−0·125, −0·012
LDL-C	−0·124	−0·288, 0·040	−0·131	−0·276, 0·015	−0·013	−0·098, 0·071	−0·053	−0·128, 0·021
ApoA1	0·026	−0·107, 0·161	0·016	−0·114, 0·147	−0·034	−0·099, 0·030	0·051	−0·015, 0·117
ApoB	−0·081	−0·174, 0·013	−0·050	−0·138, 0·039	−0·012	−0·061, 0·137	−0·027	−0·072, 0·018
ApoB:apoA1	−0·115	−0·284, 0·054	−0·102	−0·262, 0·058	0·015	−0·073, 0·102	−0·074	−0·156, 0·007

TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

* $P<0·006$ (Bonferroni correction).

† Adjusted by sex, age, study centre, maternal education, moderate-to-vigorous physical activity, sedentary behaviours and diet quality index for adolescents.

‡ High and low waist:height ratio was defined by means of sex- and age-specific medians.

with diets rich in PUFA, although they tend to reduce TAG concentration less, but elevate HDL-C concentration more than PUFA-rich diets[34]. Accordingly, further analysis carried out in the present study sample showed a positive association between monounsaturated fat intake and HDL-C and apoA1. For this reason, the inverse association observed between total fat intake and TAG and TC:HDL-C ratio could be partially explained by the presence of polyunsaturated and monounsaturated fat, although no significant associations were observed between the M:S and P:S ratios and serum lipids. However, adolescents with higher levels of adiposity showed significantly higher intakes of monounsaturated fat and a higher M:S ratio. The results from a recent meta-regression[35] have revealed that increases in HDL-C concentrations are associated with higher amounts of total fat mainly derived from monounsaturated fat in high-fat diets, whereas higher intakes of carbohydrates are associated with increases in TAG levels. Lyu *et al.*[36] also found a positive association between total fat intake and apoA1 in both men and women. Such findings could denote a positive role of dietary fat in different fractions of serum lipids and are consistent with several experimental studies that observed unfavourable effects of low-fat diets on HDL-C, TC:HDL-C ratio and postprandial TAG concentrations in women when compared with men[37–40].

Focusing on saturated fat, a meta-analysis of prospective cohort studies[7] did not find significant evidence to conclude that dietary saturated fat intake was associated with the increased risk of CVD. However, these findings should be interpreted with caution as, according to Kromhout *et al.*[41], existing sources of error in both dietary exposure and effect measure might have attenuated the correlation between dietary saturated fat intake and serum cholesterol concentration, leading to a correlation close to zero. Nevertheless, Mozaffarian *et al.*[4] observed that a greater intake of saturated fat was associated with higher HDL-C and apoA1 concentrations, and lower TAG concentration and TC:HDL-C ratio in postmenopausal women. It seems that not all SFA have identical effects on serum cholesterol levels[30], suggesting that the effects of saturated fat intake on serum lipids vary according to the specific SFA consumed[42]. The findings from a study conducted among Swedish adolescents revealed significant inverse associations between the dietary content of SFA with a chain length of four to fifteen carbon atoms and the serum concentrations of TC and apoB[43]. A meta-analysis of controlled feeding experiments has shown that SFA with twelve, fourteen, sixteen and eighteen carbon atoms increased the concentration of HDL-C when they isoenergetically replaced carbohydrate[44]. It should be noted that the increases in HDL-C concentration were greater as the chain

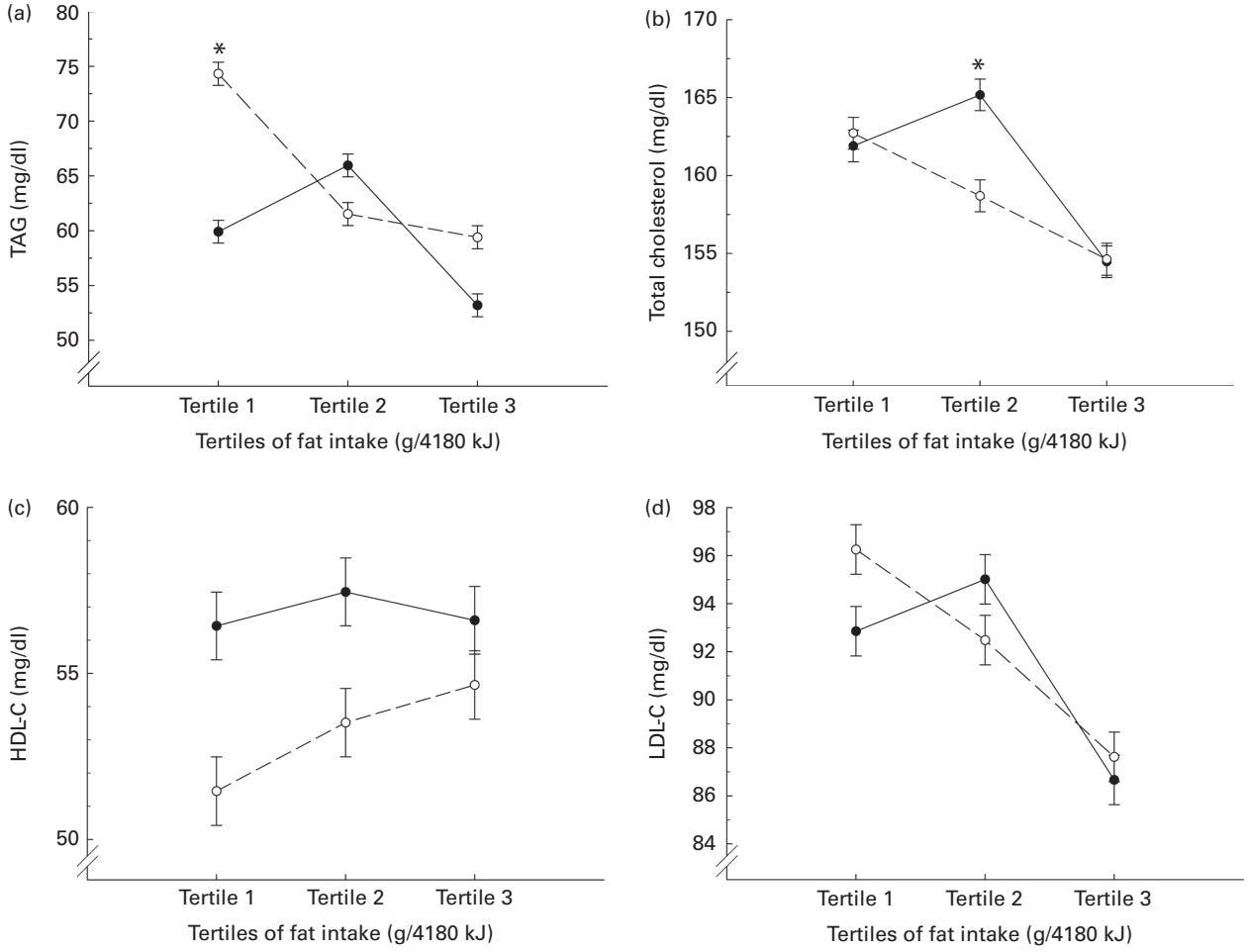

Fig. 1. TAG (a), total cholesterol (b), HDL-cholesterol (HDL-C) (c) and LDL-cholesterol (LDL-C) (d) by tertiles of fat intake for high (–○–) and low (–●–) waist: height ratio (WHeR) after adjustment for covariates: age; sex; study centre; socio-economic status; moderate-to-vigorous physical activity; sedentary behaviours; diet quality index for adolescents. Values are means, with their standard errors represented by vertical bars. To convert TAG in mg/dl to mmol/l, multiply by 0·0113. To convert cholesterol in mg/dl to mmol/l, multiply by 0·0259. * Mean value was significantly different from that of adolescents in the higher tertile of fat intake ($P<0·05$; Bonferroni's correction for *post hoc* multiple comparisons). Unlogged values are shown for easier interpretability. Median fat intake: tertile 1 – 32·1 g/4180 kJ (1000 kcal) (low WHeR), 33·6 g/4180 kJ (1000 kcal) (high WHeR); tertile 2 – 40·8 g/4180 kJ (1000 kcal) (low WHeR), 41·9 g/4180 kJ (1000 kcal) (high WHeR); tertile 3 – 50·0 g/4180 kJ (1000 kcal) (low WHeR), 51·4 g/4180 kJ (1000 kcal) (high WHeR).

length decreased. Overall, the TC:HDL-C ratio was not significantly affected by SFA with fourteen, sixteen and eighteen carbon atoms; however, it significantly decreased when SFA with twelve carbon atoms replaced carbohydrate[44]. In addition to the effect of the chain length, there may also be an effect of the source of saturated fat on TC and HDL-C concentrations. For example, despite its animal origin, milk fat elevates serum HDL-C concentration[45]. In addition, the unique position of SFA in milk fat, which is typically the position of unsaturated fatty acids in plant oils, may also affect postprandial metabolism, leading to the prevention of elevated serum TC and TAG concentrations[45].

Variability in lipid response to diet is affected by numerous factors, but excess adiposity seems to be one of the strongest factors[3]. Our findings showed that associations between macronutrient intake and serum lipids varied by body fat status in the present study sample of healthy adolescents regardless of the definition employed, i.e. general body fatness (*z*-score of BMI and sum of skinfolds) or central body fat (WHeR). Previous reports have pointed out that adiposity plays a key role in these associations among adults[36,46]. Lyu *et al.*[36] observed that general body fatness, but also body fat distribution, might exert different effects on HDL-C subclasses. Obese individuals have shown lower responses to dietary interventions focused on improving their serum lipid profile[3]. Indeed, Denke *et al.*[47] found that the response to a LDL-C-lowering diet was lower in obese participants than in those with a lower BMI ($<21\,kg/m^2$). However, our findings showed significant associations between macronutrient intake and blood lipids mainly in the high-adiposity groups, which initially would be in contrast to previous literature.

There were several limitations to our findings. Due to the cross-sectional nature of the study, we cannot determine causality. Hormonal changes during the menstrual cycle may influence serum lipid concentrations; however, blood samples were not taken at the consistent time of the menstrual cycle to account for that factor. Although the self-administered

24-HDR used to assess dietary intake is subject to measurement errors as occurs with other self-reporting methods, it has been shown to be appropriate to collect detailed dietary data in adolescents[12,13]. Collection of dietary data for more than 2 d would have been desirable to compensate for day-to-day variability[48]; nevertheless, dietary information was corrected for within-person variability to partially mitigate such limitation[49]. Additionally, our sample included under-reporters; however, results did not change when they were withdrawn from the analyses. The BLS food composition table was used to ensure that all countries used the same food composition data (obtained via the same definitions and analytical methods), though it might also represent a limitation as not all country-specific recipes and foods could be found in this German food composition table. However, recipes were generated to calculate nutrient intakes for those particular recipes. The sex- and age-specific median-based cut-offs used are sample specific and, therefore, limits their comparability with other studies. Also, adolescents included in the present study cannot be considered a true representative sample due to differences in age, weight and height compared with the original HELENA sample.

The present study has several strengths. Blood samples were collected following a standardised methodology and transported to a centralised laboratory in order to ensure the viability and stability of the samples[22]. Fieldworkers were trained and a manual of operation was developed to guarantee good clinical practice[22]. The present results were adjusted for multiple testing by the Bonferroni method, which is considered as a very conservative method.

In conclusion, the present study showed the associations between energy-adjusted macronutrient intake and serum lipid profile in adolescents. Fat intake was related to a better serum lipid profile while carbohydrate intake was observed to be associated with an adverse lipid profile. It is noteworthy that these associations differed according to body fat status and were consistent across the obesity definitions used. These findings emphasise the importance of considering body fat status when developing strategies to prevent the risk of CVD among adolescents since serum lipids and obesity are the major markers of CVD risk. More research is needed, preferably with a longitudinal design, to confirm these findings.

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The authors' contributions were as follows: L. A. M., F. G., A. K., Y. M., D. M., K. W. and M. S. participated in the conception and design of the study; S. B.-S., I. H., M. C.-G., G. P., C. B., R. R., K. V., M. S., L. L. and S. G.-M. conducted the research and collected the data; S. B.-S. wrote the manuscript, analysed the data and generated the figures; T. M., I. H., I. L. and L. A. M. participated in the interpretation of the data; S. B.-S., T. M., I. H., I. L., M. C.-G., G. P., C. B., D. M., R. R., K. W., F. G., A. K., Y. M., K. V., M. S., L. L., S. G.-M. and L. A. M. critically reviewed the manuscript. All authors read and approved the final manuscript.

There are no conflicts of interest.

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APPENDIX: HELENA Study Group

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