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The Journal of Nutrition
2021 AUG 07 ; 151(8): 2317-2329
2021 APR 13 (first published online: final publication)
doi: 10.1093/jn/nxab077

Publisher: Oxford University Press on behalf of the American Society for Nutrition

Dietary macronutrient composition in relation to circulating HDL and non-HDL cholesterol: a federated individual-level analysis of cross-sectional data from adolescents and adults in eight European studies

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Sources of support: The European Nutritional Phenotype Assessment and Data Sharing Initiative (ENPADASI) and its infrastructure are part of the Joint Programming Initiative "A Healthy Diet for a Healthy Life" (JPI-HDHL) and is funded by national funding agencies in 9 European countries. This work is financially supported by the German Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant number 2814ERA01F, and the German Ministry of Education and Research (BMBF), grant number 2814ERA03F. The GINIplus and LISA studies were mainly supported by Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research - UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, LMU Munich, TU Munich and also from IUF - Leibniz Research-Institute for Environmental Medicine at the University of Düsseldorf, and a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). The GINIplus and LISA studies were mainly supported by the Federal Ministry for Education, Science, Research and Technology, Helmholtz Zentrum Munich (former GSF), Research Institute at Marien-Hospital Wesel, IUF - Leibniz Research-Institute for Environmental Medicine at the University of Düsseldorf, the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296), and the Commission of the European Communities, the 7th Framework Program: MeDALL project. The GINIplus study was also supported by LMU Munich, TU Munich, and the companies Mead Johnson and Nestlé. The LISA study was also supported by the Helmholtz Centre for Environmental Research - UFZ, Leipzig, Pediatric Practice, Bad Honnef. C.L. and C.Y. were supported by FWO Research Foundation

Flanders, grant number G0D4815N. C.Y. is funded by a scholarship from the Chinese Scholarship Council.

Conflict of interest and Funding disclosure: "no conflicts of interest"

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Manuscript word count: 4,980

Number of figures: 2

Number of tables: 4

Supplementary data submitted: Supplemental Tables 1 to 5 and Supplemental Figures 1 to 4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

Running title (50 or fewer characters and spaces): Dietary fats and HDL and non-HDL cholesterol

List of abbreviations:

AC: Analysis Computer

BVS II: Bavarian Food Consumption Survey II

CVD: Cardiovascular disease

DASH-IN: Data Sharing Initiative for Nutrition

DC: Data Computer

DONALD Study: DOrtmund Nutritional and Anthropometric Longitudinally Designed Study

ENPADASI: European Nutritional Phenotype Assessment and Data Sharing Initiative

EPIC-Potsdam sub-study: European Prospective Investigation into Cancer and Nutrition-Potsdam sub-study

FAIR: Findable, Accessible, Interoperable, and Re-usable

GINIplus: German Infant Study on the Influence of Nutrition Intervention plus environmental and genetic influences on allergy development

GLM: Generalized linear models

HDL-C: High-Density Lipoprotein cholesterol

INGI-FVG: Italian Network of Genetic Isolates -Friuli Venezia Giulia

IPD: individual person data

LDL-C: Low-Density Lipoprotein cholesterol

LISA: Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany

MUFA: Monounsaturated fatty acids

NESCaV: Nutrition, Environment and Cardiovascular Health

PUFA: Polyunsaturated fatty acids

REML: Restricted maximum likelihood

SFA: Saturated fatty acids

SLMA: Study-level meta-analysis

TC: Total cholesterol

ABSTRACT

Background: Associations between increased dietary fat and decreased carbohydrate intake with circulating HDL and non-HDL cholesterol have not been conclusively determined.

Objective: We assessed these relationships in eight European observational human studies participating in the European Nutritional Phenotype Assessment and Data Sharing Initiative (ENPADASI) using harmonized data.

Methods: Dietary macronutrient intake was recorded using study-specific dietary assessment tools. Main outcome measures were lipoprotein-cholesterol levels: HDL-C (mg/dL) and non-HDL-C (mg/dL). A cross-sectional analysis on 5,919 participants (54% female) aged 13-80 years was undertaken using the statistical platform DataSHIELD that allows remote/federated non-disclosive analysis of individual-level data. Generalized linear models (GLM) were fitted to assess associations between the replacement of 5% of energy from carbohydrates with equivalent energy from total fats, saturated (SFAs), monounsaturated (MUFAs) or polyunsaturated (PUFAs) fatty acids with circulating HDL-C and non-HDL-C. GLM were adjusted for age, sex, smoking status, and body mass index (BMI).

Results: Replacement of 5% of energy from carbohydrates with total fats or MUFAs was statistically significantly associated with 0.67 mg/dL (95% CI 0.40, 0.94) or 0.99 mg/dL (95% CI 0.37, 1.60) higher HDL-C, respectively, but not with non-HDL-C concentrations. Replacement of 5% of energy from carbohydrates with SFAs or PUFAs was not associated with HDL-C, but SFAs were statistically significantly associated with 1.94 mg/dL (95% CI 0.08, 3.79) higher non-HDL-C, and PUFAs with -3.91 mg/dL (95% CI -6.98, -0.84) lower non-HDL-C concentrations. A statistically significant interaction by sex for the association of replacement of carbohydrates with MUFAs and non-HDL-C was observed, showing a statistically significant inverse association in

males and no statistically significant association in females. We observed no statistically significant interaction by age.

Conclusions: Replacement of dietary carbohydrates with fats had favorable effects on lipoprotein-cholesterol levels in European adolescents and adults when fats were consumed as MUFAs or PUFAs but not as SFAs.

Keywords (5-10): energy density models, substitution, blood lipids, dietary intake, fatty acids, carbohydrates, adults, adolescents, data sharing, data integration

Word count: 300

INTRODUCTION

Cardiovascular diseases (CVDs) are the most common cause of death worldwide, causing over 4 million deaths (45% of all deaths) each year across Europe (1). Key risk factors accounting for about 50% of CVDs are alterations in the lipoprotein metabolism such as high concentrations of total (TC) and low-density lipoprotein (LDL-C) cholesterol, and low concentrations of high-density lipoprotein cholesterol (HDL-C) (2). Non-high-density lipoprotein cholesterol (non-HDL-C) reflects the full burden of cholesterol carried by all potentially atherogenic particles, including LDL-C, intermediate density lipoproteins, very low-density lipoproteins, and remnant lipoproteins (3). European guidelines recommend a reduction of TC and LDL-C concentrations as primary targets in therapeutic interventions for both primary and secondary prevention of CVD (4). However, several meta-analyses found that non-HDL-C correlated more closely with cardiovascular risk than LDL-C, and non-HDL-C has therefore recently emerged as a new target for the prevention of cardiovascular events (5). Non-HDL-C is considered a better parameter because it includes remnant cholesterol and is independent of triglyceride variability (6). In addition, indirect measurement of LDL-C using the traditional Friedewald equation, as is common in clinical practice, tends to underestimate LDL-C concentrations (7), particularly in those with lower LDL-C (<70 mg/dL) and higher triglyceride concentrations (≥150 mg/dL) (8).

It has been estimated that diet-related risks accounted for 2.1 million deaths from CVDs (95% uncertainty interval (UI), 1.7–2.5 million) in the WHO European Region within one year in 2016, reflecting 22.4% of all deaths and 49.2% of CVD deaths (9). Modifying the macronutrient composition of habitual diet can have beneficial effects on lowering CVD risk via lipid risk factors (10). For example, diets low in saturated fatty acids (SFAs) are recommended for the prevention of CVD by lowering LDL-C levels (11), whereas diets rich in carbohydrates have shown detrimental effects on blood lipids by reducing HDL-C concentrations and raising fasting levels of

triglycerides (12, 13). There is a need to better understand the effects of replacement of carbohydrates by different types of fats in relation to lipoprotein profiles, especially in relation to non-HDL cholesterol. In randomized dietary intervention trials, substitution of carbohydrates with unsaturated fatty acids, predominantly monounsaturated fatty acids (MUFAs), increased HDL-C and reduced LDL-C concentrations (10, 14, 15). Moreover, replacement of carbohydrates with polyunsaturated fatty acids (PUFAs) increased HDL-C and decreased TC and LDL-C concentrations, whereas substitution of carbohydrates with SFAs increased TC, HDL-C and LDL-C (15, 16). However, there is little evidence on modified macronutrients composition and non-HDL-C. In addition, randomized trials often used strictly-controlled dietary interventions (14, 15), were conducted in special study collectives (prehypertension or Stage 1 hypertension (14), overweight or obese (17)), had narrow age-ranges and/or small sample size (14, 15) precluding sex- or age-specific analyses and inferences to the habitual diet in the general population.

Therefore, in the present study we investigated the association of the isocaloric replacement of carbohydrates with total fat or different types of fat with blood lipoproteins (HDL-C, non-HDL-C, and the ratio of HDL-C to TC (HDL-C/TC)) by sex and age in eight European observational studies participating in the European Nutritional Phenotype Assessment and Data Sharing Initiative (ENPADASI) project (18) covering a broad age range. Harmonized datasets were analyzed in a federated way in the ENPADASI Data Sharing Initiative for Nutrition (DASH-IN) (www.enpadasi.eu) implementation of DataSHIELD (19, 20), a statistical platform that allows remote/federated non-disclosive analysis of individual-level data from multiple studies without physically pooling or sharing them.

METHODS

Study population

The observational studies included in the present study were identified in the ENPADASI initiative where a total of 26 observational studies were identified (18). Briefly, a consortium was built to identify studies from Consortium partners with a wealth of data and metadata, particularly on dietary intake and traditional and omics biomarkers, as well as to develop the DASH-IN infrastructure to facilitate data exchange and data interpretation in order to increase the robustness of results from future joint (pooled or federated) data analysis in nutritional epidemiology (www.enpadasi.eu).

We planned to include studies with data on dietary macronutrient intake and blood lipids such as TC and HDL-C. From the 26 observational studies identified in ENPADASI, 10 studies fulfilled the inclusion criteria and were therefore pre-selected. An invitation to participate in the present study was sent to the principal investigators of the pre-selected studies. Those who accepted the invitation (8 out of 10) were included in our analyses. The studies included in this federated analysis are described in **Table 1**. Briefly, we included eight studies comprising a total number of 12,983 participants from the general population aged 13-80 years: one study from Belgium (NESCaV) (21), five from Germany (BVSII (22), ActivE (23), EPIC (Potsdam) sub-study (24), DONALD (25), GINIplus and LISA (26), one from Italy (INGI-FVG) (27) and one from Spain (Pizarra) (28). Three studies were cross-sectional and five were longitudinal by design, in which case data at baseline or at a single follow-up (GINIplus and LISA) were used for the cross-sectional analysis. All of the participants of the studies provided informed consent, and studies were approved by their local ethics committee (18).

Data assessments

Exposure variables

Dietary macronutrient intake (fat, carbohydrates and protein intake) was assessed using multiple 24-h dietary recalls (BVS-II (22), EPIC sub-study (24), and Pizarra (28)), self-completed, paper- or computer-based semi-quantitative food frequency questionnaires (NESCaV (21), GINIplus and LISA (29)), self-completed and paper-based food records (ActivE (23), and DONALD (25)), or other methods, e.g. dietary history interview (INGI-FVG) (27) (**Table 1**). Dietary assessment instruments were validated and validation results have been published elsewhere (30-35), with the exception of ActivE, in which the food record was validated against doubly-labeled water (for total energy expenditure/total energy intake, unpublished data). From the respective dietary assessment instrument, energy intake (kcal/day) and macronutrient intakes (total fat, SFA, MUFA, PUFA, protein and carbohydrate, all in g/d) were calculated using country-specific food composition tables.

Outcome variables

Blood lipids (HDL-C and TC) were measured in plasma samples in the EPIC sub-study and in serum in all other studies (**Table 1)**. Non-HDL-C and the ratio of HDL-C to TC (HDL-C/TC) were calculated (see data harmonization process).

Covariables

Covariables such as age, sex, smoking status, and alcohol consumption, were obtained from study-specific questionnaires. Height (cm) and weight (kg) were measured in each study (**Supplemental Table 1**).

Data harmonization process

For the purpose of data harmonization, a catalogue with the exact name of the variables, a description of each variable, the units, an example of their value as well as a column for comments was prepared following the FAIR (Findable, Accessible, Interoperable, and Re-usable) principles (36). The harmonized datasets were uploaded by the study partners on local servers together with their data dictionary. The following harmonized variables were requested: age (years), sex, height (cm), weight (kg), smoking status (never, former, and current), HDL-C (mg/dL), LDL-C (mg/dL), TC (mg/dL), alcohol consumption (g/d), total energy intake (kcal/d), dietary intakes of carbohydrates (g/d), protein (g/d), total fat (g/d), SFA (g/d), MUFA (g/d), and PUFA (g/d) (**Supplemental Table 1**).

The following variables were computed after the harmonization process: the percentage of energy available from carbohydrates, proteins, and fats were obtained by multiplying the number of daily grams of carbohydrate, protein, and fat (including SFA, MUFA and PUFA) by their energy content per gram (4.0, 4.0, and 9.0 kcal, respectively), and divided by the total energy intake (kcal/day). Non-HDL-C was calculated as TC minus HDL-C. The HDL-C/TC ratio was calculated as the percentage of HDL-C with respect to TC (2). Smoking status was recoded into two categories (never/former, and current), a categorical variable "non-drinkers and drinkers" (non-drinkers if alcohol consumption <0.3g/d) created and body mass index (BMI, kg/m^2) calculated from weight (kg) and height (in meters).

Statistical analysis

Across the eight studies (total *n*=12,983 participants), we only included participants with complete data on HDL-C and total energy intake in the analyses (total *n*=5,960, 45.9%). We further excluded participants with missing values in the outcome variable non-HDL-C (*n*=1 in GINI/LISA, *n*=1 in

NESCaV) or in the macronutrient intake variables (*n*=38 in NESCaV, *n*=1 for type of fats in Pizarra), resulting in a total of 5,919 individuals. Statistical analyses were performed using the DataSHIELD tool, which allowed remote federated analysis of harmonized datasets across the studies without physically sharing their individual-level data (19). Briefly, individual participant data from contributing studies were held securely on servers at each study location (data computers, DC) (19). A computer within the network (analysis computer, AC) sent analytical commands that requested each local server (DC) to undertake an analysis locally and to return non-identifiable summary statistics (e.g. estimates and confidence intervals) for each individual study. Participants' characteristics were described by means (standard deviation) for (approximately) normally-distributed and medians (25th–75th percentile) for skewed distributed continuous variables, or counts (%) for categorical variables. As statistical normality tests were not available in DataSHIELD, normality was assessed by visual inspection of histograms. Generalized linear regression models (GLM) were carried out to determine the cross-sectional associations between macronutrient composition (independent variables) and HDL-C and non-HDL-C concentrations, as well as the HDL-C/TC ratio (dependent variables). DataSHIELD offers two complementary approaches: (a) A full-likelihood-based individual person data (IPD) methodology (also known as the "virtual IPD analysis") where data are effectively analyzed on an individual person basis, but without physically moving them from their usual trusted repository. This approach generates the same results as if the data from all sources were physically transferred to a central warehouse and analyzed jointly (19). (b) A study level meta-analysis (SLMA), sometimes called federated meta-analysis, where the analysis is undertaken in each study separately and then all the resultant estimates and standard errors are combined using conventional SLMA methods.

We conducted virtual IPD GLM as primary analysis approach. In order to compare the results, we conducted SLMA GLM as secondary analysis. For the virtual IPD analysis, each GLM model was

fitted in a federated manner using the iterative reweighted least squares process. At each iteration, DataSHIELD transmitted the score vectors and information matrices – which are fully efficient non-disclosive summary statistics – from each study to the AC (37). For the SLMA, GLM models were fitted to completion in each study and DataSHIELD then transmitted the study-specific effect estimates and standard errors – again, non-disclosive – to the AC (19), where they were combined across studies using random effects meta-analysis under restricted maximum likelihood (REML) using R "metafor" packages v 3.3.2. Heterogeneity was tested using Chi^2 and I^2 statistics (38). Significance was set as $P < 0.05$ for the Chi^2 test. Careful interpretation of the value of I^2 depends on the magnitude and direction of effects and strength of evidence for heterogeneity I^2 values of 0–40%, 30–60%, 50–90%, and 75–100% were considered to indicate low, moderate, substantial, and considerable heterogeneity, respectively.

Multivariable nutrient density models were used to estimate the association of isocaloric replacement (as 5% of energy) of carbohydrate with total fats or with different types of fats namely SFA, MUFA and PUFA. The 5% increment was chosen to be comparable to previous investigations on macronutrient composition (12). Percentages of energy from total fat or different types of fats (SFA, MUFA and PUFA) were included as exposure variables along with percentage of energy from proteins and total energy intake as covariates (39). The coefficients of these multivariable nutrient density models indicate differences in blood lipid concentrations associated with the replacement of 5% of energy intake from carbohydrates with equivalent energetic amounts of dietary fats.

The adjustment variables were chosen *a priori* and were comparable to the set of covariates used in similar analyses on macronutrient intake and lipoprotein profiles described elsewhere (40, 41). Missing values for smoking status were found in BVSII (*n*=1), INGI-FVG (*n*=14), DONALD (*n*=50), GINIplus and LISA (*n*=69) and NESCaV (*n*=2) studies. In addition, missing values for

BMI were found in INGI-FVG (*n*= 62), GINIplus and LISA (*n*=11) and NESCaV (*n*=1) studies. Missing data were handled separately for each study by simple imputations. Briefly, missing data for smoking status were handled by imputing the missing values with the value for the most frequent category among the total study population since no sex-specific differences were observed, and missing data for BMI was handled by imputing missing values with sex-specific median values. Regression models were computed separately for each blood lipid and macronutrient association. We constructed two models with different adjustments for covariates. Model 1 included percentage of energy from proteins (continuously), total energy intake (kcal/day), alcohol consumption (nondrinker (yes/no) and continuous intake in g/day), and study source. Model 2 was further adjusted for age (years), sex, smoking status (never/former, current) and BMI (kg/m^2). Analyses were conducted in males and females combined as well as stratified by sex.

We assessed linear regression assumptions through histograms and scatter plots of regression residuals and fitted values using privacy-preserving variants of standard regression diagnostics recently implemented in DataSHIELD (42). We also investigated the potential for collinearity between model terms. In addition, in order to investigate whether potentially non-linear associations exist we added quadratic terms of percentage of energy from total fat, SFA, MUFA and PUFA separately to the models along with the linear terms and checking their significance using the Wald test.

In order to examine whether observations were consistent across different age groups, we also conducted stratified analyses by age (≤30 (*n*=6 studies), 31-40 (*n*=4), 41-50 (*n*=4), 51-60 (*n*=5) and >60 years (*n*=5)). All participants from GINIplus and LISA fell into the age category ≤30 years. Participants from the DONALD study fell into the first two categories (≤30, and 31-40 years). However, aggregated results for the age category of 31-40 years were not returned by DataSHIELD because they were disclosive. A contingency table is considered as providing a potential disclosure

risk, if any of its cells have less counts than a pre-specified threshold (43). To address this problem under DataSHIELD, each DC tested any contingency table that was created and only returned a full table to the AC if all cells were empty or contained at least 5 observations. The EPIC sub-study had participants in the last three age categories (41-50, 51-60 and >60 years); however, aggregated results for the age category of 41-50 years could not be used since they were potentially disclosive. ActivE was removed from the age-stratified analyses due to disclosive results in all age categories. Statistical interactions were investigated in GLM virtual IPD analyses by including a cross-product term for macronutrient intake e.g. total fats or type of fats (continuous), and the stratification variable (age (continuous) or sex), along with the main effect terms of each in the model with each blood lipid as the dependent variable. All studies were included in the interaction analysis. The *P*-value for interaction was determined by a Wald test.

Results were considered statistically significant at a level of $P<0.05$ throughout. All statistical analyses were performed in DataSHIELD version 5.0.0 (19).

RESULTS

The number of included participants with complete data from the eight studies ranged between 50 and 2,126, totaling 5,919 participants' data available for a combined analysis, of which 3,197 (54%) were female (**Table 2**). Percentage of female sex ranged between 49% (EPIC sub-study) to 65% (Pizarra). Mean HDL-C concentrations ranged between 46.6 mg/dL (BVSII) and 67.1 mg/dL (Pizarra). Mean non-HDL-C concentrations ranged between 108 mg/dL (DONALD study) and 184 mg/dL (Pizarra). Median intakes of total fats ranged from 30.6% (GINIplus and LISA) to 42.6% (Pizarra), SFA ranged from 9.13% (INGI-FVG) to 16.7% (EPIC sub-study), MUFA ranged from 10.9% (GINIplus and LISA) to 18.9% (Pizarra) and PUFA ranged from 2.98% (INGI-FVG) to 6.63% (EPIC sub-study).

We assessed linear regression assumptions and no violations were observed. Furthermore, little or no multicollinearity was observed in the data (data not shown). The associations between (5% of energy) replacement of carbohydrates with total and different types of fats and HDL-C and non-HDL-C are depicted in **Table 3** and stratified by sex in **Table 4**. In the fully adjusted model including sex, age, smoking status and BMI (model 2) replacing 5% of energy from carbohydrates with the same amount of energy from total fat was statistically significantly associated with 0.67 mg/dL (95% CI 0.40, 0.94; $P<0.0001$) higher HDL-C. No statistically significant associations between replacement of carbohydrates with total fats and non-HDL-C concentrations were observed (-0.37 mg/dL, 95% CI -1.10, 0.36; $P=0.32$) (**Table 3**). While isocaloric replacement of carbohydrates with SFAs was not associated with higher HDL-C, it was statistically significantly associated with 1.94 mg/dL (95% CI 0.08, 3.79; $P=0.04$) higher non-HDL-C in model 2. Higher intake of MUFAs in place of carbohydrates was statistically significantly associated with 0.99 mg/dL (95% CI 0.37, 1.60; $P=0.002$) higher HDL-C, but no associations were found with non-HDL-C concentrations. Higher PUFAs intake in place of carbohydrates yielded no statistically

252 significant associations with HDL-C, but a statistically significant association with lower (-3.91
253 mg/dL, 95%CI -6.98, -0.84; *P*=0.01) non-HDL-C concentrations (**Table 3**). Overall the models
254 followed a linear trend, with no indication of non-linear associations (data not shown).

255 Replacing 5% of energy from carbohydrates with the same amount of energy from total fats was
256 more strongly associated with higher HDL-C concentrations in females (0.84 mg/dL, 95% CI 0.46,
257 1.21) than in males (0.44 mg/dL, 95% CI 0.07, 0.82; *P*-interaction=0.05) (**Table 4**). No statistically
258 significant associations between replacement of carbohydrates with total fats and non-HDL-C
259 concentrations were observed either in males or in females, although there was an indication for a
260 statistically significant interaction by sex (*P*-interaction= 0.01). A statistically significant
261 interaction by sex was observed for the association of replacement of carbohydrates with MUFAs
262 and non-HDL-C, such that a statistically significant inverse association was found in males and no
263 significant association in females (*P*-interaction= 0.002). No other statistically significant
264 interactions by sex were observed. Findings for HDL-C were comparable with HDL/TC ratio
265 where HDL-C was expressed as percentage of TC (**Supplemental Table 2 and Supplemental Fig.**
266 **1**).

267 SLMAs yielded similar results as in the virtual IPD DataSHIELD analyses (**Figure 1 and**
268 **Supplemental Figs. 1-4** and **Supplemental Table 3**). For example, replacement of 5% of energy
269 from carbohydrates with total fats in model 2 was statistically significantly associated with 0.63
270 mg/dL (95% CI 0.35, 0.90; *P*-value for heterogeneity=0.26) higher HDL-C in the SLMA, and 0.67
271 mg/dL (95% CI 0.40, 0.94) higher HDL-C in the virtual IPD analysis. Substantial heterogeneity
272 was observed in the fully adjusted model for the replacement of 5% of energy from carbohydrates
273 with SFAs and HDL-C in males (I^2=66.7%, *P*<0.01) and non-HDL-C in females (I^2=54.2%,
274 *P*=0.04) (**Supplemental Table 3**).

Figure 2 shows associations between (5% of energy) replacement of carbohydrates with total fats and different types of fats and HDL-C and non-HDL-C stratified by age groups. Positive associations between replacement of carbohydrates with total fats and HDL-C concentrations were most pronounced in the middle age groups, e.g. between 41 and 50 years (1.23 mg/dL per 5% energy, 95% CI 0.50, 1.97), as well as between 51 and 60 years (0.94 mg/dL per 5% energy, 95% CI 0.13, 1.75). Positive associations between replacement of carbohydrates with SFA and non-HDL-C concentrations were most pronounced between 41 and 50 years (10.01 mg/dL per 5% energy, 95% CI 3.91, 16.11). No statistically significant interactions of the different types of fat with age on either HDL-C or non-HDL-C concentrations were observed (all *P*-values for interaction >0.05). Age-stratified findings for the HDL-C/TC ratio were comparable to those for HDL-C (**Supplemental Table 4**). The corresponding SLMAs for HDL-C, non-HDL-C and HDL-C/TC ratio showed similar findings in the age-stratified analysis (**Supplemental Table 5**). Substantial heterogeneity was observed for the associations between replacement of carbohydrates with MUFA (I^2=62.8%, P=0.03) and PUFA (I^2=63.3%, P=0.04), and HDL-C concentrations in the age groups ≥ 60 years and 41- 50 years, respectively. Substantial heterogeneity was also observed for the associations between replacement of carbohydrates with total fats (I^2=69.9%, P=0.02), MUFA (I^2=74.8%, P<0.01) and PUFA (I^2=68.8%, P=0.02) and non-HDL-C concentrations in the age group between 31 and 40 years (**Supplemental Table 5**).

DISCUSSION

In this large federated cross-sectional analysis of eight observational studies, we found that isocaloric replacement of carbohydrates with total fats or MUFAs was positively associated with HDL-C, while replacement of carbohydrates with SFAs was positively associated with non-HDL-C concentrations. Replacement of carbohydrates with PUFAs was inversely associated with non-HDL-C concentrations. Although most associations were similar and in the same direction in males and females, replacement of carbohydrates with MUFAs was inversely associated with non-HDL-C in males but not in females. We observed no statistically significant interaction by age, although estimates varied across age groups.

In agreement with our findings, there is convincing evidence from randomized trials that the replacement of carbohydrates with total fat or MUFAs increases HDL-C in adults (15, 16, 44, 45). For example, a meta-analysis of 395 published dietary intervention studies conducted under controlled conditions with diets persisting at least two weeks (so called metabolic ward studies) found that isocaloric increases in MUFA (replacing carbohydrates) increased HDL-C concentrations (45).

It is well-known that higher intake of SFAs increases LDL-C concentrations, which is considered a major risk factor for cardiovascular diseases (46, 47). The replacement of carbohydrates with SFAs has been consistently associated with higher LDL-C in randomized trials (15, 16, 48) but not in observational studies (26). However, to our knowledge, no intervention or observational studies relating the isocaloric replacement of carbohydrates with SFAs to non-HDL-C concentrations are currently available.

Literature addressing non-HDL-C in the context of replacing dietary carbohydrates with PUFAs or MUFAs is scarce, while results on LDL-C from existing intervention (14-16, 45) and observational

studies (26) are conflicting. For example, a randomized, 3-period, crossover feeding study observed that partial substitution of carbohydrates with unsaturated fats (mainly MUFAs) showed no effects on LDL-C concentrations (14), whereas a meta-analysis of 27 trials (16) and a large systematic review of 84 trials (15) showed that isocaloric substitution of total carbohydrates with MUFA or PUFA significantly decreased LDL-C concentrations. In addition, a meta-analysis of 395 published metabolic ward studies found that isocaloric increases in PUFA intake (replacing carbohydrates) decreased LDL-C, whereas MUFA had no significant effect on LDL-C (45). Differences in results may be partly explained by study-specific differences in the *n*-6/*n*-3 PUFA ratio, since *n*-6 versus *n*-3 PUFAs may exert differential effects on lipid profiles (26, 46).

Isocaloric macronutrient exchange models should be interpreted cautiously, as any observed association may be attributed to either the macronutrient of interest (in our case, types of fat), or to the substituted macronutrient. Similar to other studies (12), we chose carbohydrates as reference macronutrient for our isocaloric exchange models. We conducted additional substitution models in which fats were replaced at the expense of protein intake (instead of carbohydrates) and similar results were found, further supporting that our observations can largely be attributed to fat intake. Most studies in the present analysis had median carbohydrate intakes <50%, which is lower than the recommended intake by many European nutrition societies (49-52). To avoid unhealthy weight gain, the German guidelines (49) recommend to limit total fat intake to less than 30% of total energy intake (from age 15 years, 30-35% between 4 and 15 years), whilst the Belgian (51), Spanish (52) and Italian (53) guidelines recommend to limit total fat intake up to 35% of total energy intake. However, there is an ongoing debate on limiting the intake of total fats to less than 30% of the total energy intake as recent studies suggest that diets with a higher fat intake are not associated with higher cardiovascular disease or mortality (40). In addition, in terms of unhealthy weight gain, total

calories intake rather than macronutrient composition is the determinant, which underlines the special importance of the isocaloric replacement of macronutrients.

A number of experimental studies in animal models aimed at elucidating the mechanisms by which different types of fatty acids modulate circulating cholesterol concentrations (54-59). Resultant plausible mechanisms that could explain how dietary fats affect circulating LDL-C concentrations include alterations in LDL-C receptor activity, LDL-C receptor protein levels and mRNA abundance (55-58); whilst SFAs markedly decrease the LDL-C receptor activity and protein and mRNA levels (59), PUFAs upregulated them (55). Furthermore, (*n*-6) PUFA reduces circulating cholesterol by upregulating LDL-C receptor and increasing the activity of cholesterol 7α-hydroxylase (CYP7) - the initial and rate-limiting enzyme in the conversion of cholesterol to bile acids (60). In human studies, key components of cholesterol metabolism are the cholesterol efflux (a measure of HDL-C functionality), and proprotein convertase subtilisin-kexin type 9 (PCSK9) concentrations, a protein involved in the degradation of LDL-C receptors (60). A randomized trial has shown that higher intake of PUFAs reduces PCSK9 concentrations (61), which could be another mechanism that might explain why PUFAs exert lipoprotein benefits. However, to our knowledge there are no experimental studies examining specifically biological mechanisms for the effects of replacement of carbohydrates with types of fat on HDL or non-HDL concentrations.

A major strength of this study is that it used federated data from large studies conducted in several European countries covering the South and Central Europe, as well as with broad age ranges, showing consistent results across the diverse studies. Another strength is that *a priori* FAIRyfied harmonization of data before individual-level and study-level meta-analyses were carried out. In addition, the remote federated analysis approach through DataSHIELD allowed us to perform both virtual IPD and study-level meta-analyses without the need to physically pool or share individual-level data, and hence substantially reduced the governance burdens, and ethico-legal challenges.

Similar effect estimates were observed between virtually pooled analysis of individual-level data and study-level meta-analyses; the latter though provided, as expected, larger confidence intervals, sometimes losing the statistical significance observed in the virtual IPD analyses. However, by using both virtual IPD GLM analyses and study-level meta-analyses, we demonstrated that there are no serious flaws in the analytic assumption that could disturb either approach: in particular no serious heterogeneity in the underlying etiological models. The implementation of DataSHIELD in DASH-IN made it possible to perform individual-level analysis. Collection of data via this type of solution may simulate research on existing data.

Our study has several limitations. First, given the cross-sectional nature of our analyses, we can neither confirm the temporal relationship between the substitution of carbohydrates for fats and the lipoprotein profiles in our study population nor infer causality. Second, we included eight studies from four European countries among the observational studies identified within the ENPADASI Consortium. Therefore –although participants were recruited from the general population– our studies may not be representative of the European population. Third, residual confounding cannot be ruled out, since not all the studies had potentially important confounding variables such as education, physical activity and waist circumference available. Fourth, small studies had to be left out in stratified analysis by age and sex due to the risk of potentially disclosive results, which lowered statistical power and hence reduced the chance of detecting a true effect. Fifth, an increased risk of making a Type I error could not be ruled out, especially since we tested multiple outcomes in our exploratory analysis. However, we did not find substantial differences with the level of significance after applying a conservative Bonferroni correction for 12 independent tests (3 outcomes, 4 exposures) although the associations between isocaloric replacement of carbohydrates with SFA or PUFA with higher and lower non-HDL-C concentrations, respectively, were no longer significant after Bonferroni correction for 12 tests (*P*-value >0.004). Sixth, it is known that the

effects of replacement of carbohydrates may depend in part on the quality of the carbohydrates, however, the quality of carbohydrates was not taken into account, e.g. by considering glycemic index (41, 47). In addition, we did not assess differences in the *n*-6/*n*-3 PUFA ratio or differences in the food sources of the considered nutrients, e.g. animal-derived MUFA versus plant-derived MUFA, which may have exerted differential effects on lipid profiles. Seventh, random measurement error cannot be ruled out from having diluted real associations between nutrients and lipoprotein profiles. One potential source of random measurement error may reside in the methods used for the assessment of dietary macronutrients intake, which was obtained from self-reported food-frequency questionnaires, food records or 24-hour recall as well as the methods and medium used to measure lipoproteins (plasma, which was used in EPIC sub-study versus serum, which was used in all other studies). However, from the forest plots we did not observe substantial heterogeneity among studies for most associations (substantial heterogeneity was only observed in a few associations after stratification by sex or age), meaning that we largely did not find differences between studies with different dietary collection methods, lipoprotein measurement methods, nor studies using plasma versus serum as analysis medium nor differences between studies located in the South or Central Europe. Whilst it is true that center-specific effects could not be fully elucidated, we adjusted for study source, which partly accounted for center-specific effects. Eighth, data on smoking and BMI were missing for only a few participants. However, we used simple imputations for missing values in two covariables, namely BMI and smoking status, to minimize the loss of statistical power, since multiple imputation was not yet available in DataSHIELD version 5.0.0 and the relative simplicity of the underlying data structure was such that the approach to simple imputation was intuitive.

In conclusion, the findings from this large cross-sectional federated analysis of eight European observational studies suggest that in adolescents and adults replacing dietary carbohydrates with

411 total fats and MUFAs is related to higher HDL-C concentrations. Our findings also suggest that
412 replacing dietary carbohydrates with either MUFAs or PUFAs is related to lower non-HDL-C,
413 whereas replacing dietary carbohydrates with SFAs is associated with higher non-HDL-C
414 concentrations. The findings on non-HDL-C warrant confirmation by future studies. Consumption
415 of fats in place of carbohydrates showed beneficial effects when fats were consumed in the form
416 of MUFAs or PUFAs but not SFAs. Thus, our findings support global dietary guidelines (62) about
417 detrimental effects of saturated fats intake, although –as has also been indicated in recent studies
418 (40, 63, 64) – intake of total fats showed no detrimental effects in the blood lipoprotein profiles.
419 Federated analysis on data is possible and can answer research questions without sharing individual
420 data.

421

Statement of authors' contributions to manuscript

K.N., M.P. and T.P. designed research; K.N., and M.P. conducted research; S.J, H.B., M.S-M., M. S., C.H., J.L, C.K., U.N., J.B, S.B., C.L, C.Y., P.G., A.R., G.R-M., A.F.D., D.A., P.B., provided essential reagents, or provided essential materials; M. P. analyzed data and wrote the paper; and K.N. and T.P had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1. List of the observational studies from ENPADASI consortium included in the analysis[1,2]

Study Name (Ref)	Country	Study design	Dietary assessment				Lipoproteins	
			24-h recall	FFQ	Food records	Other	HDL-C	TC
NESCaV (21)	Belgium	CS		a 134-food item FFQ: past 3 months based on 6 levels of frequency			enzymatic colorimetric method with PEG-modified enzymes (Modular P, Roche)	enzymatic method cholesterol oxidase (Modular P, Roche)
ActivE (23)	Germany	CS			7 to 13-day		enzymatic colorimetric method (Beckmann Coulter AU5800)	enzymatic colorimetric method (Beckmann Coulter AU5800)
BVS II (22)	Germany	CS	three telephone-based computer-assisted 24 h diet recall method (EPIC-SOFT) tool				enzymatic colorimetric method with PEG-modified enzymes (Roche)	enzymatic method cholesterol oxidase (Roche)
DONALD (25)	Germany	Cohort			3-day weighted		enzymatic colorimetric methods using the Advia 1650-Chemistry System analyser (Siemens Healthcare Diagnostics, Eschborn, Germany)	
EPIC sub-study (24)	Germany	Cohort	three telephone-based computer-assisted 24 h diet recall method (EPIC-SOFT) tool				enzymatic colorimetric methods using the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany)	
GINIplus/ LISA (26)	Germany	Cohort[†]		80-food item FFQ: past 12 months based on 9 levels of frequency			homogenous enzymatic colorimetric methods on a Modular Analytics System from Roche Diagnostics GmbH Mannheim according to the manufactures instructions	
INGI-FVG (27)	Italy	Cohort				dietary history interview	enzymatic colorimetric methods using the BIOTECNICA BT-3000 TARGA chemistry analyser	
Pizarra (28)	Spain	Cohort	three face-to-face or telephone-based computer-assisted 24-hour recall method				enzymatic colorimetric methods using a Dimension autoanalyzer (Dade Behring Inc., Deerfield, IL, USA)	

[1]The general population includes both random and convenience sampling designs.

[2] Abbreviations: CS: Cross-sectional; FFQ: food frequency questionnaires; BVS II: Bavarian Food Consumption Survey II; DONALD: DOrtmund Nutritional and Anthropometric Longitudinally Designed Study; EPIC: European Prospective Investigation in Cancer and Nutrition; GINIplus; German Infant Study on the Influence of Nutrition Intervention; INGI-FVG: Italian Network of Genetic Isolates–Friuli Venezia Giulia; LISAplus: Influences of Lifestyle-Related Factors on the Human Immune; [†]GINIplus and LISA are two German birth

cohorts whose harmonized data were pooled to increase statistical power; NESCaV: Nutrition, Environment, and Cardiovascular Health; HDL-C: High Density Lipoprotein Cholesterol; TC: Total Cholesterol.

Table 2. Baseline characteristics of the participants from eight European studies included in the federated analysis (*n*= 5,919)[1,2]

	ActivE	EPIC sub-study	BVSII	DONALD	GINI-LISA	NESCaV	INGI-FVG	Pizarra
n	50	786	514	277	2126	919	519	728
Female, *n* (%)	25 (50)	388 (49)	295 (57)	147 (53)	1118 (53)	457 (50)	294 (57)	473 (65)
Age, *y*	45.0 ± 14.9	65.4 ± 8.38	48.6 ± 15.3	21.5 ± 4.63	15.2 ± 0.30	44.6 ± 13.6	51.8 ± 15.71	47.6 ± 13.8
BMI, *kg/m²*	26.4 ± 4.10	27.5 ± 4.30	26.4 ± 4.84	23.1 ± 3.83	20.7 ± 3.04	26.2 ± 4.84	25.5 ± 4.75	28.5 ± 5.12
Current smoking, *n (%)*	12 (24)	84 (11)	123 (24)	48 (21)	124 (6)	222 (24)	110 (22)	217 (30)
Non-Drinkers, *n (%)*	7 (14)	25 (3)	123 (24)	134 (48)	2067 (97)	145 (16)	179 (35)	556 (76)
Alcohol, *g/d*	12.6 [3.17, 23.0]	9.43 [4.09, 18.2]	7.29 [0.33, 20.9]	0.36 [0.02, 5.42]	0.04 [0.02, 0.07]	5.87 [1.39, 15.9]	4.08 [0.00, 24.6]	0.00 [0.00, 0.00]
HDL-C, *mg/dL*	57.0 ± 12.4	56.5 ± 14.7	46.6 ± 8.0	59.2 ± 16.2	57.5 ± 14.0	60.7 ± 16.5	59.6 ± 16.3	67.1 ± 16.6
LDL-C, *mg/dL*	144 ± 33.1	134 ± 38.8	*N.A.*	93.8 ± 30.6	91.6 ± 26.2	119 ± 34.0	139 ± 39.8	162 ± 47.2
TC, *mg/dL*	216 ± 42.7	217 ± 42.7	208 ± 38.0	167 ± 37.1	169 ± 32.5	198 ± 38.6	220 ± 43.7	251 ± 53.6
Non-HDL-C, mg/dL	159 ± 41.7	161 ± 40.8	161 ± 36.8	108 ± 32.5	111 ± 29.8	137 ± 38.8	161 ± 45.0	184 ± 50.1
HDL-C/TC ration, (%)	27.2 ± 6.74	26.6 ± 7.15	23.0 ± 4.93	36.0 ± 9.21	34.7 ± 8.22	31.6 ± 9.29	28.0 ± 9.01	27.4 ± 7.29
Total Energy, *kcal/day*	2180 [1810, 2580]	2025 [1724, 2359]	1980 [1589, 2388]	2079 [1693, 2543]	2001 [1558, 2539]	2292 [1833, 2815]	2587 [2116, 3087]	1870 [1473, 2370]
Carbohydrate intake, *% energy*	45.2 [41.8, 49.0]	39.9 [36.9, 42.9]	43.2 [38.7, 48.8]	49.3 [44.2, 53.9]	53.2 [48.4, 57.6]	42.6 [38.6, 47.2]	48.3 [43.5, 52.6]	42.0 [34.9, 49.1]
Protein intake, *% energy*	15.7 [14.5, 17.5]	14.5 [13.5, 15.6]	14.3 [12.6, 16.2]	14.0 [12.6, 16.1]	14.8 [13.0, 16.7]	15.7 [14.1, 17.6]	15.4 [14.1, 16.7]	15.0[12.3, 18.0]
Total fat intake, *% energy*	33.3 [30.2, 36.5]	40.7 [37.8, 43.4]	36.7 [32.9, 40.6]	34.5 [30.5, 38.7]	30.6 [27.1, 34.9]	37.7 [34.1, 41.3]	35.0 [31.9, 38.8]	42.6 [35.9, 49.6]
SFA intake, *% energy*	14.3 [13.3, 15.7]	16.7 [15.2, 18.3]	14.7 [12.6, 17.2]	14.6 [12.6, 16.9]	12.7 [10.8, 14.8]	13.8 [12.1, 15.3]	9.15 [7.66, 11.4]	9.36 [6.77, 12.5]
MUFA intake, *% energy*	11.3 [10.3, 13.0]	14.3 [13.2, 15.5]	12.8 [11.1, 14.7]	14.5 [12.5, 16.4]	10.9 [9.31, 12.6]	15.7 [13.8, 17.7]	14.9 [12.9, 16.9]	18.9 [13.6, 24.2]
PUFA intake, *% energy*	5.04 [4.30, 5.81]	6.63 [5.66, 7.77]	5.92 [4.63, 7.37]	5.21 [4.26, 6.54]	4.51 [3.85, 5.38]	5.32 [4.64, 6.39]	2.98 [2.57, 350]	4.19 [3.17, 5.74]

[1]Values are mean ± SD or median [25th, 75th percentiles] or counts (%).

[2] Abbreviations: TC: Total cholesterol; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Table 3. Associations between replacement of 5% of energy from carbohydrates with total fats or types of fats and HDL-C and non-HDL-C among adolescents and adults from eight European studies (*n*=5,919)[1,2]

Type of fat	HDL-C	*P* value	Non-HDL-C	*P* value
	β (95%CI)		**β (95%CI)**	
Total fats				
Model 1	0.81 (0.52, 1.09)*	<0.0001	-0.12 (-0.86, 0.63)	0.76
Model 2	0.67 (0.40, 0.94)*	<0.0001	-0.37 (-1.10, 0.36)	0.32
SFA				
Model 1	1.37 (0.64, 2.10)*	0.0002	2.20 (0.31, 4.09)*	0.02
Model 2	0.55 (-0.13, 1.23)	0.11	1.94 (0.08, 3.79)*	0.04
MUFA				
Model 1	0.46 (-0.20, 1.12)	0.17	-0.77 (-2.47, 0.93)	0.37
Model 2	0.99 (0.37, 1.60)*	0.002	-0.85 (-2.51, 0.81)	0.32
PUFA				
Model 1	0.12 (-1.09 1.33)	0.85	-2.80 (-5.94, 0.34)	0.08
Model 2	-0.30 (-1.43, 0.83)	0.61	-3.91 (-6.98, -0.84)*	0.01

[1] Data are beta coefficients, upper and lower 95% confidence intervals for all participants. Values of HDL-C and non-HDL-C are expressed in mg/dL and total fat, SFA, MUFA and PUFA intakes as 5% energy. General linear regression models were used. Model 1was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day) and study source (*EPIC sub-study, ActivE, BVSII, DONALD, Pizarra, NESCaV, GINIplus and LISA, INGI-FVG*); and model 2 was additionally adjusted for age (years), sex, smoking status (never/former, current), and BMI (kg/m^2). *P< 0.05.

[2] Abbreviations: HDL-C: High Density Lipoprotein Cholesterol; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 4. Sex-stratified associations between replacement of 5% of energy from carbohydrates with total fats or types of fats and HDL-C and non-HDL-C among adolescents and adults from eight European studies[1,2,3]

Type of fat	HDL-C	*P* value	Non-HDL-C	*P* value
Total fats	**β (95%CI)**		**β (95%CI)**	
Males[2] (*n*=2,697)	0.44 (0.07, 0.82)*	0.02	-0.27 (-1.40, 0.85)	0.63
Females (*n*=3,197)	0.84 (0.46, 1.21)*	<0.0001	-0.12 (-1.08, 0.83)	0.80
P-interaction by sex	0.05		0.01*	
SFA				
Males[2] (*n*=2,697)	0.68 (-0.30, 1.66)	0.18	3.73 (0.83, 6.63)*	0.01
Females (*n*=3,197)	0.43 (-0.51, 1.37)	0.37	0.63 (-1.77, 3.02)	0.61
P-interaction by sex	0.59		0.06	
MUFA				
Males[2] (*n*=2,697)	0.67 (-0.25, 1.59)	0.16	-3.03 (-5.76, -0.30)*	0.03
Females (*n*=3,197)	1.16 (0.34, 1.98)*	0.006	0.84 (-1.24, 2.92)	0.43
P-interaction by sex	0.16		0.002*	
PUFA				
Males[2] (*n*=2,697)	-0.49 (-2.17, 1.18)	0.56	-2.11 (-7.09, 2.87)	0.41
Females (*n*=3,197)	-0.02 (-1.54, 1.52)	0.43	-4.08 (-7.95, -0.21)*	0.04
P-interaction by sex	0.21		0.38	

[1]Data are beta coefficients, upper and lower 95% confidence intervals. Values of HDL-C and non-HDL-C are expressed in mg/dL and total fat, SFA, MUFA and PUFA intakes as 5% energy. General linear regression models were used. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI (kg/m^2), and study source (*EPIC sub-study, ActivE, BVSII, DONALD, Pizarra, NESCaV, GINIplus and LISA, INGI-FVG*). **P*< 0.05.

[2]ActivE study excluded in all datasets for males.

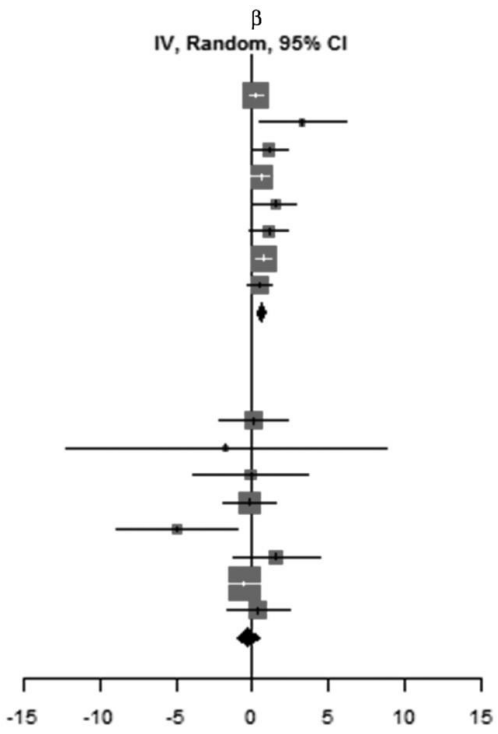
[3]Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

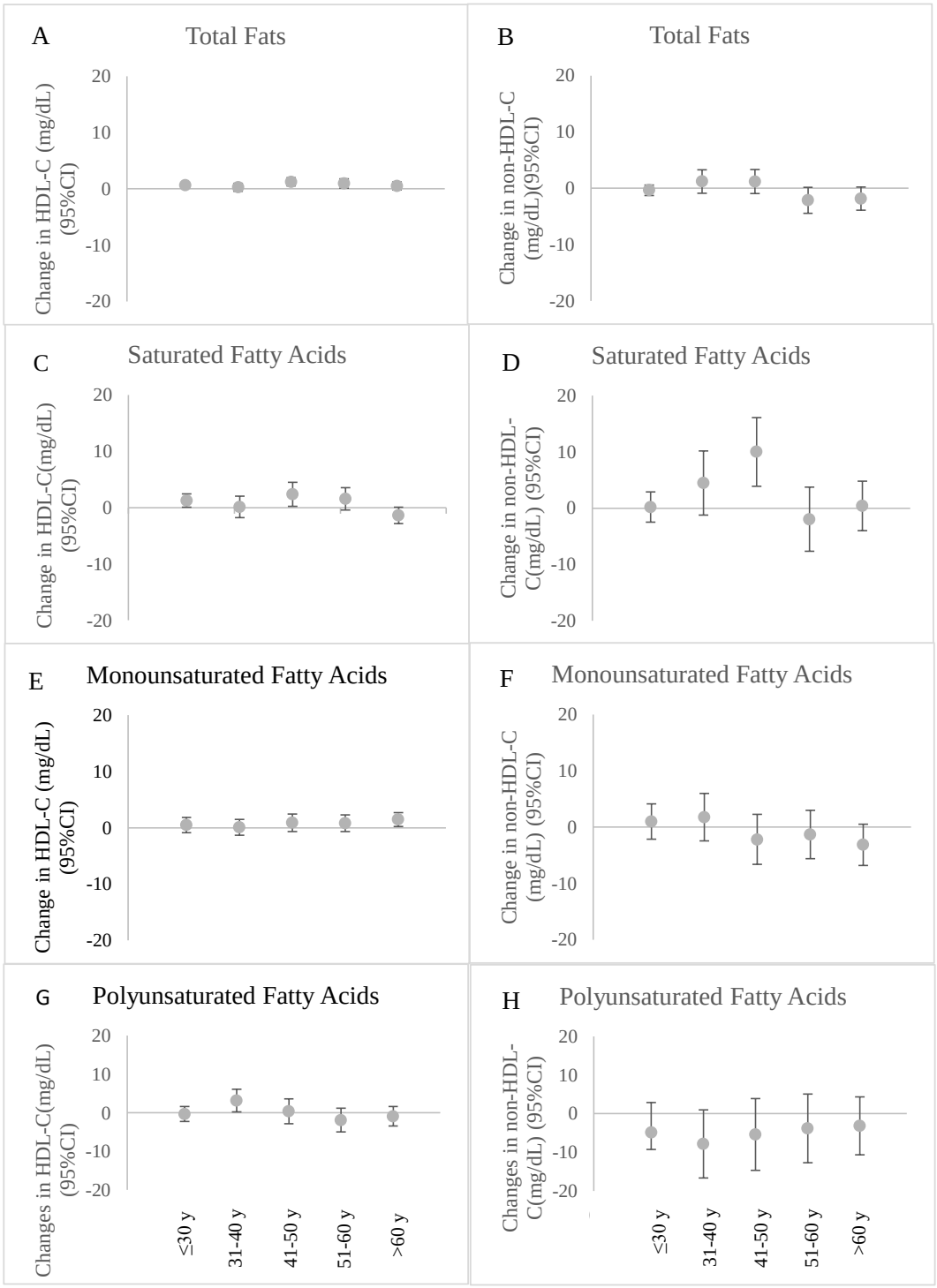
FIGURE LEGEND

Figure 1. Forest plot of random-effects study-level meta-analysis for the association between lipoprotein profiles and percentage of 5% of energy intake from total fats in replacement of carbohydrates among adolescents and adults from eight European studies. Values are beta coefficients, upper and lower 95% confidence intervals for all the participants (*n*=5,919). HDL-C and non-HDL-C were expressed in mg/dL and total fat intake was expressed as 5% energy. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI (kg/m2), and study source. The shaded circles represent the point estimate for each individual study, and the horizontal line extending from each square represents the upper and lower limits of the 95% CI. The size of the shaded square indicates the relative weight of the study in the meta-analysis. The diamonds represent the overall beta coefficient of the studies.

Figure 2. Age-stratified analyses on the association between replacement of 5% of energy from carbohydrates with total fats (A and B), SFA (C and D), MUFA (E and F) or PUFA (G and H) and HDL-C and non-HDL-C among adolescents and adults from eight European studies. Values are beta coefficients, upper and lower 95% confidence intervals for all the participants (n=5,919). HDL-C and non-HDL-C were expressed in mg/dL and total fat, SFA, MUFA, PUFA intakes were expressed as 5% energy. The circles represent the point estimate for each age group, and the horizontal line extending from each circle represents the upper and lower limits of the 95% CI. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI (kg/m2), and study source. Included studies for age category ≤30 years (BVSII, Pizarra, NESCaV, INGI-FVG, GINI-LISA and DONALD); 31-40 and 41-50 years (BVSII, Pizarra, INGI-FVG and NESCaV); 51-60, and >60 years (BVSII, EPIC sub-study, Pizarra, INGI-FVG and NESCaV); Age categories: ≤30 years (*n*=2,758); 31-40 years (*n*=561); 41-50 years (*n*=603); 51-60 years (*n*=761), and >60 years (*n*=1,141). No significant interactions between fats and age were found.

Study or subgroup	TE	SE	β IV, Random, 95% CI
Outcome = HDL-C			
BVS II	0.24	0.25	0.24 (–0.24; 0.73)
ActivE	3.30	1.47	3.30 (0.42; 6.17)
EPIC	1.09	0.61	1.09 (–0.10; 2.28)
Pizarra	0.58	0.29	0.58 (0.00; 1.15)
INGI_FVG	1.53	0.71	1.53 (0.14; 2.91)
DONALD	1.08	0.65	1.08 (–0.20; 2.35)
GINI_LISA	0.75	0.26	0.75 (0.25; 1.26)
NESCaV	0.44	0.40	0.44 (–0.35; 1.23)
Total (95% CI)			0.63 (0.35; 0.90)
Heterogeneity: $tau^2 = 0.0109$; $chi^2 = 8.92$, df = 7 ($P = 0.26$); $I^2 = 21\%$			
Outcome = non-HDL-C			
BVS II	0.09	1.14	0.09 (–2.14; 2.32)
ActivE	-1.74	5.38	-1.74 (–12.29; 8.81)
EPIC	-0.10	1.92	-0.10 (–3.86; 3.66)
Pizarra	-0.15	0.87	-0.15 (–1.86; 1.55)
INGI_FVG	-4.96	2.03	-4.96 (–8.93; -0.98)
DONALD	1.58	1.47	1.58 (–1.30; 4.46)
GINI_LISA	-0.55	0.56	-0.55 (–1.65; 0.55)
NESCaV	0.38	1.07	0.38 (–1.71; 2.47)
Total (95% CI)			-0.29 (-1.03; 0.44)
Heterogeneity: $tau^2 = 0$; $chi^2 = 7.76$, df = 7 ($P = 0.35$); $I^2 = 10\%$			




A
Total Fats
Change in HDL-C (mg/dL) (95%CI)
20
10
0
-10
-20
B
Total Fats
Change in non-HDL-C (mg/dL)(95%CI)
C
Saturated Fatty Acids
Change in HDL-C(mg/dL) (95%CI)
D
Saturated Fatty Acids
Change in non-HDL-C(mg/dL) (95%CI)
E
Monounsaturated Fatty Acids
Change in HDL-C (mg/dL) (95%CI)
F
Monounsaturated Fatty Acids
Change in non-HDL-C (mg/dL) (95%CI)
G
Polyunsaturated Fatty Acids
Changes in HDL-C(mg/dL) (95%CI)
H
Polyunsaturated Fatty Acids
Changes in non-HDL-C(mg/dL) (95%CI)
≤30 y
31-40 y
41-50 y
51-60 y
>60 y

Supplementary data

Dietary macronutrient composition in relation to high-density lipoprotein (HDL) cholesterol and non-HDL cholesterol: a federated individual-level analysis of cross-sectional data from eight ENPADASI studies- Pinart et al., Online Supplementary Material

Supplemental Table 1. Harmonized variables used in the federated meta-analysis of eight European studies

Harmonized variable name	Description	Units or categories
Covariates		
AGE	Age at blood collection	years
SEX	male or female sex	1=male; 2=female
WEIGHT	Body weight	Kg
HEIGHT	Height	cm
SMOKE_ST	Smoking status	1=never smoker; 2=former smoker; 3=current smoker
ENERGY	energy intake (total energy from fat, carbohydrates, protein and alcohol)	kcal/day
ALC	alcohol (ethanol) intake	g/day
Exposure variables		
CARB	carbohydrate intake	g/day
FAT	total fat intake	g/day
SFA	saturated fatty acid intake	g/day
MUFA	monounsaturated fatty acid intake	g/day
PUFA	polyunsaturated fatty acid intake	g/day
PROT	protein intake	g/day
Outcome variables		
HDL-C	High-Density Lipoproteins cholesterol	mg/dL
LDL-C	Low-Density Lipoproteins cholesterol	mg/dL
TC	Total cholesterol	mg/dL

Supplemental Table 2. Associations between replacement of 5% of energy from carbohydrates with total fats or types of fat and HDL-C/TC ratio among adolescents and adults from eight European studies (*n*=5,919)[1,2,3]

	HDL-C/TC ratio (%)	*P* value
Men and women combined	**β (95%CI)**	
Total fat		
Model 1	0.30 (0.15, 0.46)*	0.0002
Model 2	0.30 (0.15, 0.45)*	0.0001
Types of fat		
SFA		
Model 1	0.12 (-0.28, 0.52)	0.57
Model 2	-0.13 (-0.51, 0.25)	0.51
MUFA		
Model 1	0.35 (-0.01, 0.71)	0.06
Model 2	0.54 (0.20, 0.88)*	0.002
PUFA		
Model 1	0.34 (-0.33, 1.01)	0.31
Model 2	0.37 (-0.26, 1.00)	0.25
Stratified by sex (Model 2)		
Total fats		
Males[2] (*n*= 2,697)	0.17 (-0.06, 0.41)	0.14
Females (*n*= 3,197)	0.35 (0.15. 0.55)*	0.0005
P-value for interaction by sex	0.003*	
Types of fat		
SFA		
Males[2] (*n*=2,697)	-0.31 (-0.91, 0.30)	0.32
Females (*n*=3,197)	-0.04 (-0.53, 0.46)	0.89
P-value for interaction by sex	0.69	
MUFA		
Males[2] (*n*=2,697)	0.66 (0.09, 1.23)*	0.02
Females (*n*=3,197)	0.44 (0.01, 0.87)*	0.04

P-value for interaction by sex	0.01*	
PUFA		
Males[2] (*n*=2,697)	-0.08 (-1.12, 0.96)	0.88
Females (*n*=3,197)	0.61 (-0.19, 1.40)	0.14
P-value for interaction by sex	0.33	

[1]Data are beta coefficients, upper and lower 95% confidence intervals for all participants. HDL-C/TC ratio was expressed as percentage of HDL-C with respect to TC. Values of total fat, SFA, MUFA and PUFA intakes as 5% energy. General linear regression models were used. Model 1 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day) and study source (*EPIC sub-study, ActivE, BVSII, DONALD, Pizarra, NESCaV, GINIplus and LISA, INGI-FVG*); Model 2 was additionally adjusted for age (years), sex, smoking status (never/former, current), and BMI (kg/m^2). [¥]ActivE study excluded in all datasets for males; *$P < 0.05$.

[2]ActivE study excluded in all datasets for males.

[3]Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Supplemental Table 3. Study-level meta-analysis of association between replacement of 5% of energy from carbohydrates with total fats or types of fats and HDL-C, non-HDL-C, and HDL-C/TC ratio among adolescents and adults from eight European studies (*n*=5,919) [1,2,3]

	HDL-C	Non-HDL-C	HDL-C/TC ratio (%)
	β (95%CI)	β (95%CI)	β (95%CI)
Total fat			
Model 1	0.80 (0.37, 1.24); I^2=47.5%, *P*=0.06	-0.15 (-1.02, 0.72); I^2=30.3%, *P*=0.19	0.32 (-0.03, 0.67); I^2=69.7%, *P*<0.01*
Model 2	0.63 (0.35, 0.90); I^2=21.5%, *P*=0.26	-0.29 (-1.03, 0.44); I^2=9.7%, *P*=0.35	0.34 (0.02, 0.67); I^2=64.5%, *P*<0.01*
Males[2]	0.56 (0.20, 0.92); I^2=0.0%, *P*=0.92	0.19 (-0.95, 1.32); I^2=0.0%, *P*=0.57	0.20 (-0.02, 0.42); I^2=0.0%, *P*=0.65
Females	0.80 (0.26, 1.35); I^2=44.2%, *P*=0.08	-0.65 (-2.29, 0.99); I^2=51.0%, *P*=0.05	0.56 (-0.04, 1.16); I^2=78.0%, *P*<0.01*
Types of fats			
SFA			
Model 1	1.43 (0.15, 2.71); I^2=61.1%, *P*=0.01*	2.91 (-0.24, 6.06); I^2=49.7%, *P*=0.05	-0.06 (-0.57, 0.45); I^2=34.6%, *P*=0.15
Model 2	0.41 (-0.33, 1.16); I^2=0.0%, *P*=0.56	1.71 (-0.41, 3.03); I^2=25.9%, *P*=0.22	-0.22 (-0.60, 0.16); I^2=0.0%, *P*=0.60
MUFA			
Model 1	0.36 (-0.52, 1.24); I^2=27.4%, *P*=0.21	-1.47 (-3.90, 0.96); I^2=24.9%, *P*=0.23	0.32 (-0.02, 0.67); I^2=32.5%, *P*=0.17
Model 2	0.82 (0.17, 1.47); I^2=0.0%, *P*=0.52	-0.75 (-2.71, 1.21); I^2=0.0%, *P*=0.87	0.53 (0.06, 0.99); I^2=26.8%, *P*=0.21
PUFA			
Model 1	0.40 (-1.34, 2.14); I^2=56.0 %, *P*=0.03*	-1.89 (-5.18, 1.40); I^2=0.0%, *P*=0.73	0.22 (-0.40, 0.84); I^2=0.8%, *P*=0.42
Model 2	-0.05 (-1.31, 1.21); I^2=51.0%, *P*=0.05	-3.26 (-6.46, -0.06); I^2=0.0%, *P*=0.95	0.29 (-0.29, 0.88); I^2=26.0%, *P*=0.22
SFA			
Males[2]	1.02 (-0.78, 2.82); I^2=66.7%, *P*<0.01*	3.04 (-0.15, 6.22); I^2=0.0%, *P*=0.63	-0.12 (-0.85, 0.62); I^2=31.1%, *P*=0.19
Females	0.07 (-0.90, 1.04); I^2=0.0%, *P*=0.78	0.97 (-3.02, 4.97); I^2=54.2%, *P*=0.04*	-0.33 (-0.83, 0.18); I^2=0.0%, *P*=0.79
MUFA			
Males[2]	0.61 (-0.81, 2.02); I^2=45.1%, *P*=0.09	-1.59 (-4.95, 1.76); I^2=0.0%, *P*=0.74	0.48 (-0.05, 1.01); I^2=0.0%, *P*=0.76

Supplementary data

Females	1.13 (0.22, 2.03); I^2=0.0%, P=0.76	0.00 (-2.37, 2.38); I^2=15.7%, P=0.31	0.82 (-0.05, 1.68); I^2=42.9%, P=0.09
PUFA			
Males[2]	-0.42 (-1.92, 1.08); I^2=14.4%, P=0.32	-2.41 (-7.67, 2.85); I^2=0.0%, P=0.52	0.03 (-0.89, 0.94); I^2=22.1%, P=0.26
Females	-0.23 (-1.68, 1.21); I^2=17.1%, P=0.30	-3.36 (-7.37, 0.64); I^2=0.0%, P=0.98	0.44 (-0.32, 1.20); I^2=0.0%, P=0.87

[1]Data are beta coefficients, upper and lower 95% confidence intervals for all participants. HDL-C/TC ratio was expressed as percentage of HDL-C with respect to TC. Values of HDL-C and non-HDL-C are expressed in mg/dL and total fat, SFA, MUFA and PUFA intakes as 5% energy. Study-level meta-analysis using random effect models were used. Model 1 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day) and study source (*EPIC sub-study, ActivE, BVSII, DONALD, Pizarra, NESCaV, GINIplus and LISA, INGI-FVG*); Model 2 was additionally adjusted for age (years), sex, smoking status (never/former, current), and BMI (kg/m^2). For stratified analyses only the regression outcomes of model 2 are shown. *$P < 0.05$.

[2]ActivE study excluded in all datasets for males.

[3]Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Supplemental Table 4. Age-stratified association between replacement of 5% of energy from carbohydrates with total fat and HDL-C/TC ratio, stratified by age among adolescents and adults from eight European studies[1]

	HDL-C/TC ratio (%)
Total fats	**β (95%CI)**
≤30 years (*n*= 2,758)	0.34 (0.10, 0.59)*
31 to 40 years (*n*= 561)	0.05 (-0.33, 0.43)
41 to 50 years (*n*= 603)	0.29 (-0.10, 0.67)
51 to 60 years (*n*= 761)	0.46 (0.06, 0.86)*
> 60 years (*n*= 1,141)	0.41 (0.04, 0.77)*
P-value for interaction by age	0.58
Types of fats	
SFA	
≤30 years (*n*= 2,758)	0.39 (-0.31, 1.09)
31 to 40 years (*n*= 561)	-0.74 (-1.76, 0.28)
41 to 50 years (*n*= 603)	-0.57 (-1.69, 0.54)
51 to 60 years (*n*= 761)	0.81 (-0.18, 1.79)
> 60 years (*n*= 1,141)	-0.56 (-1.32, 0.21)
P-value for interaction by age	0.50
MUFA	
≤30 years (*n*= 2,758)	0.16 (-0.66, 0.98)
31 to 40 years (*n*= 561)	0.01 (-0.75, 0.76)
41 to 50 years (*n*= 603)	0.71 (0.11, 1.52)
51 to 60 years (*n*= 762)	0.19 (-0.55, 0.93)
> 60 years (*n*= 1,141)	0.99 (0.35, 1.62)*
P-value for interaction by age	0.72
PUFA	
≤30 years (*n*= 2,758)	0.78 (-0.38, 1.94)
31 to 40 years (*n*= 561)	2.55 (0.97, 4.13)*
41 to 50 years (*n*= 603)	0.52 (-1.18, 2.22)
51 to 60 years (*n*= 762)	-0.08 (-1.61, 1.46)
> 60 years (*n*= 1,141)	-0.18 (-1.48, 1.12)
P-value for interaction by age	0.80

[1]Data are beta coefficients, upper and lower 95% confidence intervals. HDL-C/TC ratio was expressed as percentage of HDL-C with respect to TC. Values of total fat, SFA, MUFA and PUFA intakes as 5% energy. General linear regression models were used. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy

(continuously), total energy intake (kcal/day), age (years), sex, smoking status (never/former, current), BMI (kg/m^2), and study source. Included studies for age category ≤30 years (BVSII, Pizarra, NESCaV, INGI-FVG, GINIplus and LISA, and DONALD); 31-40 and 41-50 years (BVSII, Pizarra, INGI-FVG and NESCaV); 51-60, and >60 years (BVSII, EPIC sub-study, Pizarra, INGI-FVG and NESCaV); *P< 0.05.

[2]Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Supplemental Table 5. Study-level meta-analysis of age-stratified association between replacement of 5% of energy from carbohydrates with total fats or types of fats and HDL-C, non-HDL-C, and HDL-C/TC ratio among adolescents and adults from eight European studies[1]

	HDL-C	Non-HDL-C	HDL-C/TC ratio (%)
Total Fats	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
≤30 years (n=6) (*n*= 2,758)	0.52 (-0.02, 1.06); I^2=18.6%, *P*=0.29	0.03 (-1.33, 1.39); I^2=45.1%, *P*=0.10	0.19 (-0.38, 0.76); I^2=64.9%, *P*=0.01*
31 to 40 years (n=4) (*n*= 561)	0.08 (-0.59, 0.74); I^2=0.0%, *P*=0.50	-0.25 (-4.44, 3.94); I^2=69.9%, *P*=0.02*	0.13 (-0.56, 0.82); I^2=62.3%, *P*=0.05
41 to 50 years (n=4) (*n*= 603)	0.71 (-0.10, 1.52); I^2=0.0%, *P*=0.40	1.19 (-1.10, 3.48); I^2=10.0%, *P*=0.69	0.06 (-0.39, 0.51); I^2=0.0%, *P*=0.34
51 to 60 years (n=5) (*n*= 761)	0.81 (0.05, 1.57); I^2=0.0%, *P*=0.43	-1.74 (-4.54, 1.06); I^2=7.4%, *P*=0.36	0.41 (0.06, 0.77); I^2=0.0%, *P*=0.70
> 60 years (n=5) (*n*= 1,141)	0.58 (-0.09, 1.24); I^2=0.0%, *P*=0.47	-2.52 (-5.96, 0.93); I^2=57.4%, *P*=0.05	0.63 (-0.36, 1.62); I^2=74.1%, *P*<0.01*
Types of fat			
SFA			
≤30 years (n=6) (*n*= 2,758)	0.77 (-0.38, 1.91); I^2=0.0%, *P*=0.56	2.56 (-2.20 7.31); I^2=38.9%, *P*=0.15	-0.13 (-0.95, 0.70); I^2=17.4%, *P*=0.30
31 to 40 years (n=4) (*n*= 561)	-0.21 (-1.95, 1.52); I^2=0.0%, *P*=0.82	1.96 (-7.10, 11.01); I^2=57.3%, *P*=0.07	-0.46 (-1.43, 0.52); I^2=0.0%, *P*=0.43
41 to 50 years (n=4) (*n*= 603)	1.90 (-0.13, 3.93); I^2=0.0%, *P*=0.52	8.72 (2.11, 15.32); I^2=0.0%, *P*=0.43	-0.86 (-2.24, 0.53); I^2=30.2%, *P*=0.23
51 to 60 years (n=5) (*n*= 761)	1.56 (-0.27, 3.38); I^2=0.0%, *P*=0.84	-4.36 (-10.55, 1.83); I^2=0.0%, *P*=0.70	0.74 (-0.19, 1.67); I^2=0.0%, *P*=0.85
> 60 years (n=5) (*n*= 1,141)	-1.07 (-3.57, 1.42); I^2=57.9%, *P*=0.05	-0.56 (-5.27, 4.14); I^2=11.8%, *P*=0.34	-0.33 (-1.05, 0.39); I^2=0.0%, *P*=0.71
MUFA			
≤30 years (n=6) (*n*= 2,758)	0.49 (-0.92, 1.89); I^2=0.0%, *P*=0.73	0.43 (-4.83, 5.69); I^2=37.8%, *P*=0.15	0.22 (-1.02, 1.46); I^2=41.1%, *P*=0.13
31 to 40 years (n=4) (*n*= 561)	-0.17 (-1.66, 1.32); I^2=0.0%, *P*=0.91	7.68 (-12.55, 27.90); I^2=74.8%, *P*<0.01*	-0.29 (-1.04, 0.46); I^2=54.8%, *P*=0.08
41 to 50 years (n=4) (*n*= 603)	-0.80 (-3.75, 2.16); I^2=54.1%, *P*=0.09	-2.01 (-7.43, 3.41); I^2=0.0%, *P*=0.85	0.57 (-0.32, 1.46); I^2=0.5%, *P*=0.39
51 to 60 years (n=5) (*n*= 761)	1.00 (-0.58, 2.58); I^2=0.0%, *P*=0.74	1.76 (-5.23, 8.75); I^2=37.5%, *P*=0.17	0.14 (-0.80, 1.09); I^2=2.2%, *P*=0.39
> 60 years (n=5) (*n*= 1,141)	1.41 (-1.45, 4.28); I^2=62.8%, *P*=0.03*	-4.73 (-10.80, 1.34); I^2=39.3%, *P*=0.16	1.43 (-0.96, 3.82); I^2=81.3%, *P*<0.01*

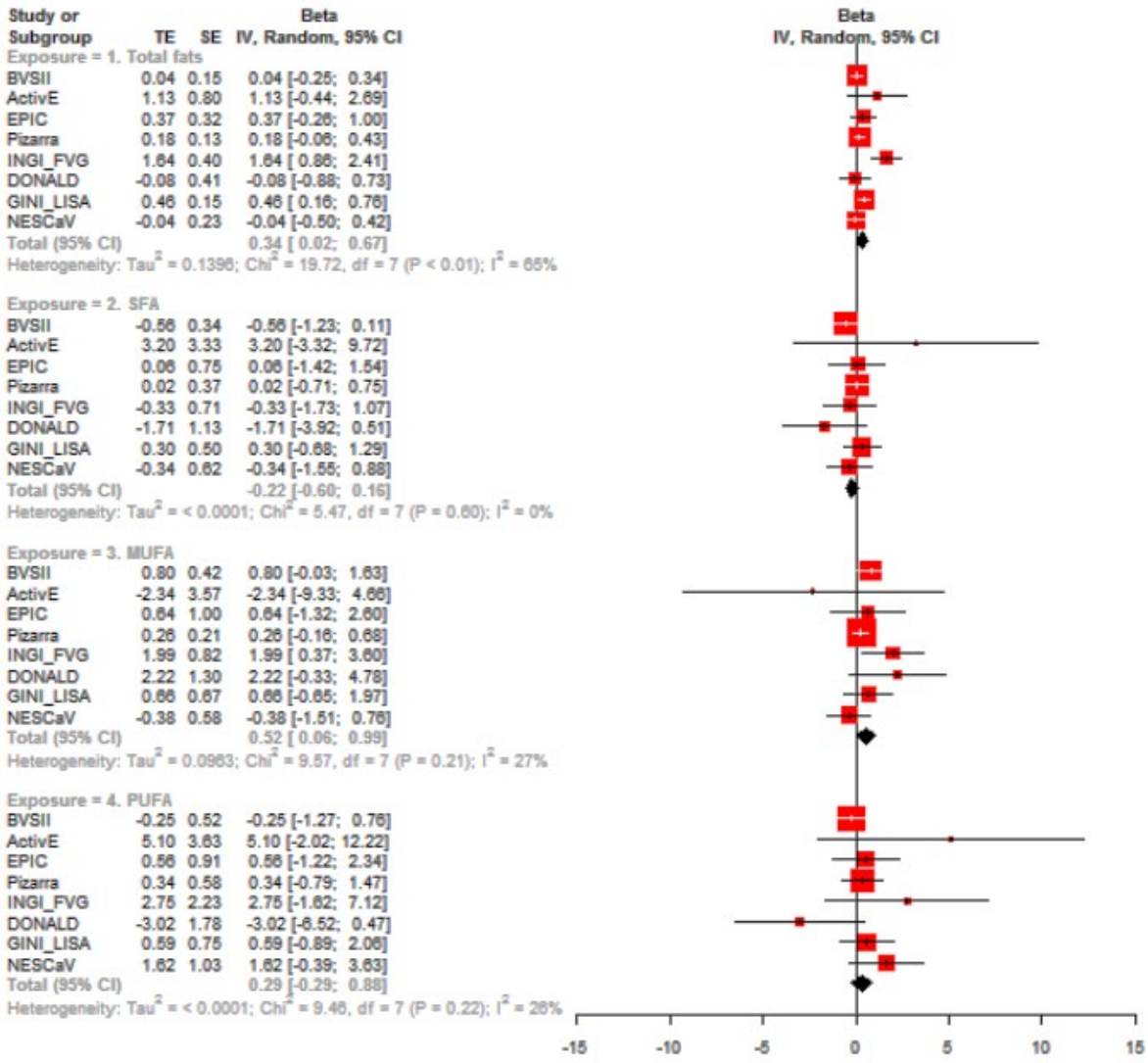
Supplementary data

PUFA			
≤30 years (n=6) (*n*= 2,758)	-0.65 (-2.44, 1.14); I^2=0.0%, *P*=0.63	-3.23 (-7.83, 1.37); I^2=0.0%, *P*=0.54	0.40 (-0.72, 1.53); I^2=19.8%, *P*=0.28
31 to 40 years (n=4) (*n*= 561)	2.31 (-0.39, 5.00); I^2=0.0%, *P*=0.54	-11.95 (-25.65, 1.74); I^2=68.8%, *P*=0.02*	3.47 (-0.11, 7.05); I^2=68.7%, *P*=0.02*
41 to 50 years (n=4) (*n*= 603)	2.27 (-3.41, 7.95); I^2=63.3%, *P*=0.04*	-4.68 (-14.71, 5.35); I^2=0.0%, *P*=0.63	0.52 (-1.82, 2.87); I^2=34.9%, *P*=0.20
51 to 60 years (n=5) (*n*= 761)	-1.57 (-4.32, 1.18); I^2=0.0%, *P*=0.59	-4.56 (-14.01, 4.89); I^2=2.5%, *P*=0.39	-0.16 (-1.54, 1.23); I^2=0.0%, *P*=0.57
> 60 years (n=5) (*n*= 1,141)	-0.37 (-2.77, 2.03); I^2=0.0%, *P*=0.76	-3.39 (-15.10, 8.33); I^2=44.9%, *P*=0.12	0.09 (-1.11, 1.29); I^2=0.0%, *P*=0.73

[1]Data are beta coefficients, upper and lower 95% confidence intervals. HDL-C/TC ratio was expressed as percentage of HDL-C with respect to TC. Values of HDL-C and non-HDL-C are expressed in mg/dL and total fat, SFA, MUFA and PUFA intakes as 5% energy. Study-level meta-analysis using random effect models were used. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), age (years), sex, smoking status (never/former, current), BMI (kg/m^2), and study source. Included studies for age category ≤30 years (BVSII, Pizarra, NESCaV, INGI-FVG, GINIplus and LISA and DONALD); 31-40 and 41-50 years (BVSII, Pizarra, INGI-FVG and NESCaV); 51-60, and >60 years (BVSII, EPIC sub-study, Pizarra, INGI-FVG and NESCaV); *P< 0.05.

[2]Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

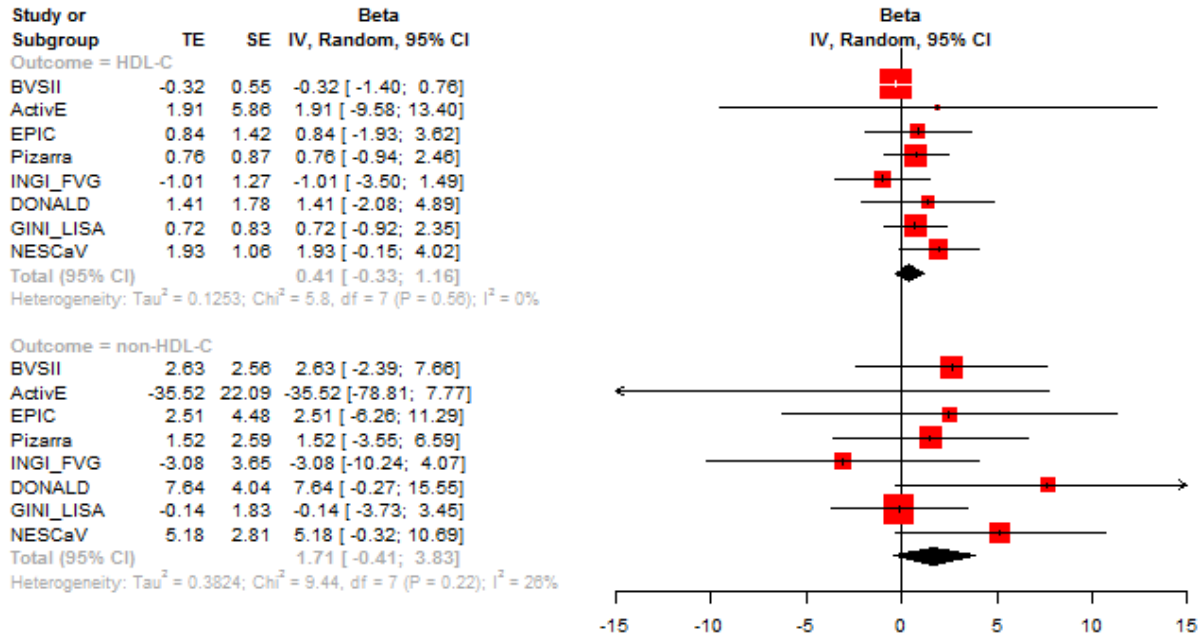
Supplementary data



Supplemental Figure 1. Forest plot of random-effects study-level meta-analysis for the association between ratio of HDL to total cholesterol (HDL-C/TC ratio) of 5% of Energy intake from total and different types of fats among adolescents and adults from eight European studies. Values are beta coefficients, upper and lower 95% confidence intervals for all the participants (n=5,919). HDL-C/TC ratio was expressed as percentage of HDL-C with respect to TC and total fat SFA, MUFA and PUFA intakes were expressed as 5% energy. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI

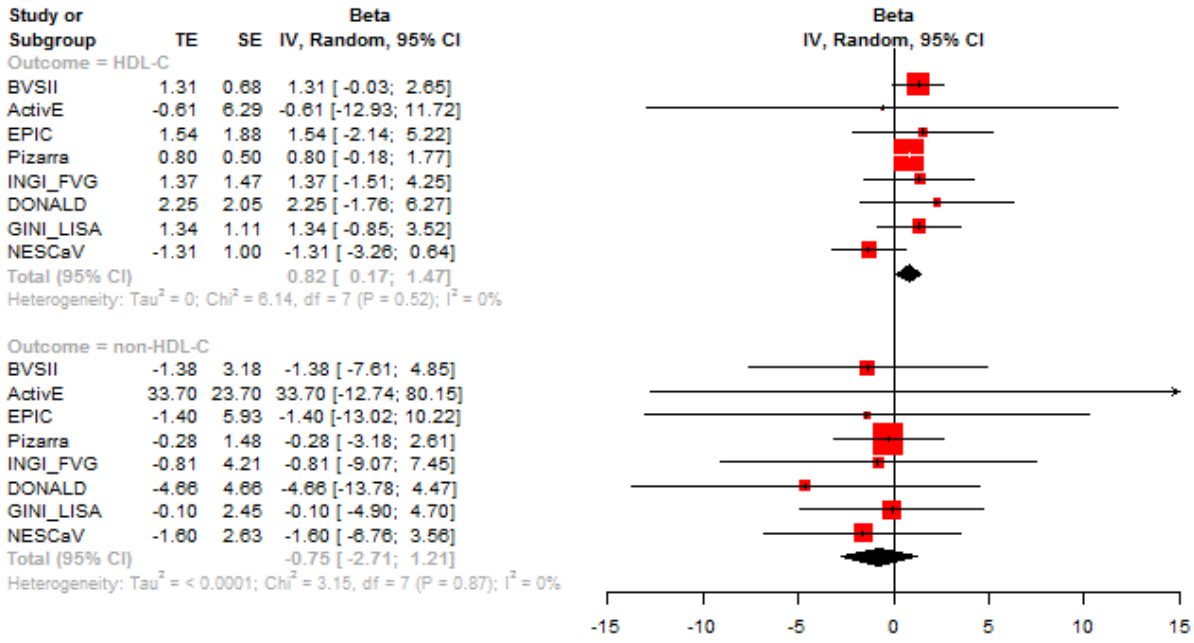
(kg/m^2), and study source. The shaded squares represent the point estimate for each individual study, and the horizontal line extending from each square represents the upper and lower limits of the 95% CI. The size of the shaded square indicates the relative weight of the trial in the meta-analysis. The diamonds represent the overall beta coefficient of the studies.

Supplementary data



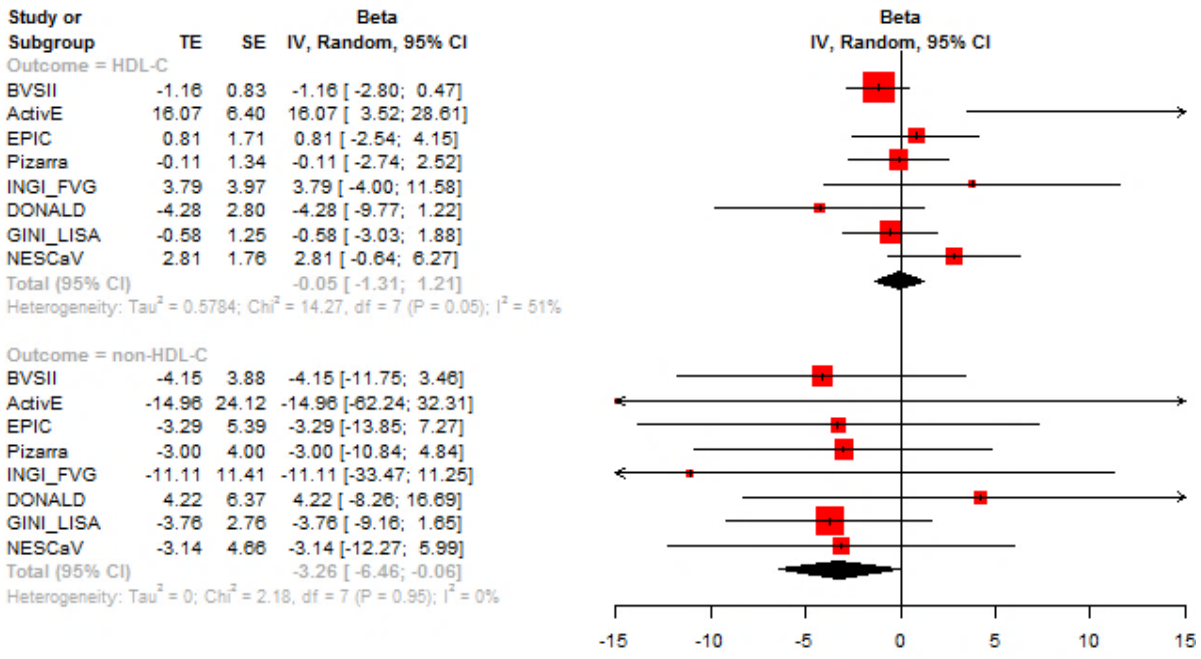
Supplemental Figure 2. Forest plot of random-effects study-level meta-analysis for the association between lipoprotein profiles and percentage of 5% of energy intake from SFA among adolescents and adults from eight European studies. Values are beta coefficients, upper and lower 95% confidence intervals for all the participants (*n*=5,919). HDL-C and non-HDL-C were expressed in mg/dL and SFA intake was expressed as 5% energy. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI (kg/m^2), and study source. The shaded squares represent the point estimate for each individual study, and the horizontal line extending from each square represents the upper and lower limits of the 95% CI. The size of the shaded square indicates the relative weight of the trial in the meta-analysis. The diamonds represent the overall beta coefficient of the studies.

Supplementary data



Supplemental Figure 3. Forest plot of random-effects study-level meta-analysis for the association between lipoprotein profiles and percentage of 5% of energy intake from MUFA among adolescents and adults from eight European studies. Values are beta coefficients, upper and lower 95% confidence intervals for all the participants (*n*=5,919). HDL-C and non-HDL-C were expressed in mg/dL and MUFA intake was expressed as 5% energy. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI (kg/m^2), and study source. The shaded squares represent the point estimate for each individual study, and the horizontal line extending from each square represents the upper and lower limits of the 95% CI. The size of the shaded square indicates the relative weight of the trial in the meta-analysis. The diamonds represent the overall beta coefficient of the studies.

Supplementary data



Supplemental Figure 4. Forest plot of random-effects study-level meta-analysis for the association between lipoprotein profiles and percentage of 5% of energy intake from PUFA among adolescents and adults from eight European studies. Values are beta coefficients, upper and lower 95% confidence intervals for all the participants (*n*=5,919). HDL-C and non-HDL-C were expressed in mg/dL and PUFA intake was expressed as 5% energy. Model 2 was adjusted for alcohol (yes, no and continuously in g/day), protein intake, 5% energy (continuously), total energy intake (kcal/day), for age (years), sex, smoking status (never/former, current), BMI (kg/m^2), and study source. The shaded squares represent the point estimate for each individual study, and the horizontal line extending from each square represents the upper and lower limits of the 95% CI. The size of the shaded square indicates the relative weight of the trial in the meta-analysis. The diamonds represent the overall beta coefficient of the studies.