AUTHOR Jari E Kaikkonen, Antti Jula, Jorma S A Viikari, Markus Juonala, Nina Hutri-Kähönen, Mika Kähönen, Terho Lehtimäki, Olli T Raitakari

TITLE Associations of Serum Fatty Acid Proportions with Obesity, Insulin Resistance, Blood Pressure, and Fatty Liver: The Cardiovascular Risk in Young Finns Study

YEAR 2021

DOI 10.1093/jn/nxaa409

VERSION Author's accepted manuscript

CITATION Jari E Kaikkonen, Antti Jula, Jorma S A Viikari, Markus Juonala, Nina Hutri-Kähönen, Mika Kähönen, Terho Lehtimäki, Olli T Raitakari, Associations of Serum Fatty Acid Proportions with Obesity, Insulin Resistance, Blood Pressure, and Fatty Liver: The Cardiovascular Risk in Young Finns Study, The Journal of Nutrition, 2021;, nxaa409, https://doi.org/10.1093/jn/nxaa409

19.11.2020 Revision

The Journal of Nutrition

Research Article

Nutritional Epidemiology

Associations of serum fatty acid proportions with obesity, insulin resistance, blood pressure and fatty liver: The Cardiovascular Risk in Young Finns Study

Jari E. Kaikkonen,[1,2,3] Antti Jula,[4] Jorma S. A. Viikari,[5] Markus Juonala,[5] Nina Hutri-Kähönen,[6] Mika Kähönen,[7] Terho Lehtimäki,[8] Olli T. Raitakari[1,3,9]

[1]*Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland*

[2]*Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland*

[3]*Centre for Population Health Research, University of Turku and Turku University Hospital, Turku, Finland.*

[4]*National Institute for Health and Welfare, Turku, Finland*

[5]*Department of Medicine, University of Turku and Division of Medicine, Turku University Hospital, Finland*

[6]*Department of Pediatrics, Tampere University Hospital and Tampere University, Tampere, Finland*

[7]*Department of Clinical Physiology, Tampere University Hospital and Tampere University, Tampere, Finland*

[8]*Department of Clinical Chemistry, Fimlab laboratories and Faculty of Medicine and Health Technology, Finnish Cardiovascular Research Center - Tampere, Tampere University, Tampere, Finland.*

[9]*Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland*

antti.jula@thl.fi; jorvii@utu.fi; mataju@utu.fi; nina.hutri-kahonen@uta.fi; mika.kahonen@uta.fi; terho.lehtimaki@tuni.fi; olli.raitakari@utu.fi;

Sources of Support: The Young Finns Study has been financially supported by the Academy of Finland: grants 322098, 287285, 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Competitive State Research Financing of the Expert Responsibility area of Kuopio, Tampere and Turku University Hospitals (grant X51001); Specified governments transfers to Kuopio University Hospital (5031364); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation for Cardiovascular Research ; Finnish Cultural Foundation; The Sigrid Juselius Foundation; Tampere

Tuberculosis Foundation; Emil Aaltonen Foundation; Yrjö Jahnsson Foundation; Signe and Ane Gyllenberg Foundation; Diabetes Research Foundation of Finnish Diabetes Association; EU Horizon 2020 (grant number 848146 for TO AITION and grant number 755320 for TAX-INOMISIS); European Research Council (grant 742927 for MULTIEPIGEN project); Tampere University Hospital Supporting Foundation; and The Paulo Foundation.

Author disclosures: J. E. Kaikkonen, A. Jula, J. S. A. Viikari, M. Juonala, N. Hutri-Kähönen, M. Kähönen, T. Lehtimäki, and O. T. Raitakari have no conflicts of interest

Correspondence: Jari Kaikkonen, Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Kiinamyllynkatu 10, 20520 Turku, Finland; Phone: +358400897018;

E-mail: jari.kaikkonen@utu.fi

Number of words: 4262

Number of figures: 3;

Number of tables: 1;

Supplemental data: 5 tables, 1 figure.

Running title: Fatty acids and metabolic outcomes

Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; E%, energy %; FA, fatty acid; HDL, high-density lipoprotein; HOMA, homeostatic model-based insulin resistance; MUFA, monounsaturated fatty acid; n-3, omega-3; n-6, omega-6; PUFA, polyunsaturated fatty acid; RCT, randomized clinical trial; SFA, saturated fatty acid; SBP, systolic blood pressure; T2D, type 2 diabetes

ABSTRACT

Background: The links between fatty acids (FAs) and cardiometabolic outcomes are topics of debate.

Objective: Our aim was to investigate the associations between serum standardized FA percentages and cardiometabolic outcomes.

Methods: We used cross-sectional (n=2187-2200, aged 24-39 years, women 54 %) and 10-year prospective data (n=975-1414) from the Young Finns Study. Outcomes included prevalent and incident obesity, insulin resistance (HOMA index in the upper quintile), elevated blood pressure (medication, or diastolic or systolic blood pressure in the upper quintile), and incident non-alcoholic fatty liver. Logistic regression models were used to calculate odds ratios per standard deviation increase in FAs. The models were adjusted for age and sex, and additionally for other potential confounders.

Results: Several cross-sectional findings were statistically significant also in prospective models (Bonferroni corrected $P<0.003$). In fully adjusted models for obesity, these consisted of saturated (SFAs) (*OR*=1.28) and monounsaturated (MUFAs) FAs (*OR*=1.38), including palmitoleic (*OR*=1.39) and oleic acids (*OR*=1.37). Furthermore, polyunsaturated FAs (PUFAs) (*OR*=0.70), including linoleic (*OR*=0.67) and docosahexaenoic acids (*OR*=0.75), were inversely related with obesity, whereas γ-linolenic acid (*OR*=1.32) was positively associated with obesity. In age and sex adjusted models for insulin resistance, MUFAs (*OR*=1.26) and oleic acid (*OR*=1.25) were positively, and PUFAs (*OR*=0.81), particularly linoleic acid (*OR*=0.78), were inversely associated with HOMA. Similarly with elevated blood pressure, palmitic acid (*OR*=1.22), MUFAs (OR=1.28) and oleic acid (*OR*=1.28) were positively associated with elevated blood pressure, whereas PUFAs (*OR*=0.77), n-6 (omega-6) PUFAs (*OR*=0.79) and linoleic acid (*OR*=0.77) were inversely associated. In fully adjusted models for incident fatty liver, the most consistent predictors were high palmitic (*OR*=1.61) and low linoleic acid (*OR*=0.63) percentages. The n-6/n-3 (omega-3) PUFA ratio was not linked with any adverse outcomes.

Conclusions: High serum percentages of total SFAs and MUFAs and low PUFAs, but also several specific FAs, predict future unfavorable cardiometabolic outcomes in Finnish adults.

300/300 words

Keywords: metabolic disease, prospective analysis, saturation degree, serum fatty acid

Introduction

Obesity, impaired insulin metabolism, elevated blood pressure (BP) and fatty liver are common cardiometabolic outcomes, and risk factors for atherosclerosis (1). These outcomes have been linked with an unhealthy diet, in particular with a high saturated fat intake. Therefore, the international dietary guidelines recommend that individuals should consume fat 20-35 energy % (E%) and saturated fatty acids (SFAs) less than 10 E% by replacing SFAs partly with both polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) to lower their cardiometabolic risk (2-5). These recommendations are based on results from randomized clinical trials (RCTs) (6-9), supported by epidemiological evidence, and confirmed in a series of meta-analyses and reviews (10-15). However, one recent meta-analysis has questioned the findings of the old RCTs and related meta-analyses because of inadequate randomization and controlling in some of these RCTs (16). In addition, some meta-analyses have suggested that an increase in the n-6 (omega-6) PUFA intake without a simultaneous increase in the n-3 (omega-3) PUFA intake could increase rather than decrease the risk of coronary heart disease (17-19). In line with these findings, one recent meta-analysis indicated that a higher intake of n-3 but not n-6 PUFAs, was associated with a lower risk of the metabolic syndrome (20). In addition, the role of MUFAs is unclear (21) since their serum percentages have been linked in large cohort studies with an increased risk of cardiometabolic outcomes, such as fatty liver, type 2 diabetes or cardiovascular disease (22-24). These are only a few examples in the recent literature reporting conflicting findings. For these reasons, the debate regarding the optimal dietary composition of fatty acids (FAs) is still far from finished, and remains an important topic for further investigations.

Dietary recommendations have tended to focus on total SFAs, MUFAs or PUFAs, without any particular emphasis on specific FAs. This may be a shortcoming since specific FAs may play important physiologic roles in outcome associations and body functions. For example, long-chain SFAs, such as 18:0 versus 12:0, have been suggested to increase the risk of obesity (25), and circulating SFAs with an even number of carbon atoms, such as 14:0, have been linked with the risk of incident type 2 diabetes (T2D) (26). With regard to serum n-6 PUFAs, cholesterol ester dihomo-γ-linolenic (20:3n-6)

acid, %, has shown a positive association, whereas the linoleic acid (18:2n-6), %, displayed an inverse association with the incidence of T2D (27). FA metabolism that leads to the production of longer and more desaturated FA chains may explain these varying associations (28). Furthermore, most of the published studies have investigated only individual cardiometabolic endpoints without any clear focus on the metabolic state as a whole.

For these reasons, in the present study, our objective was to investigate in depth not only the effects on health of circulating FAs, including total n-6 and n-3 PUFAs and their ratios, total MUFAs and SFAs but also to examine the importance of specific FAs with their varying chain lengths and saturation degrees. To clarify the links of serum FAs with the cardiometabolic state, both cross-sectional and prospective associations of FAs with obesity, insulin resistance (high homeostatic model-based insulin resistance, HOMA), fatty liver and elevated BP were investigated in young and middle-aged adults. We also formed summed variables from different FAs and outcomes to examine the association between the ratio of (SFAs+MUFA)/PUFAs and the metabolic state as a whole.

Methods

Study population (the Cardiovascular Risk in Young Finns Study)

In 1980, 4320 children and adolescents aged 3, 6, 9, 12, 15, and 18 years, living in 5 university cities and 12 adjacent rural communities, were randomly chosen from the Finnish national population register. A total of 3596 (83.1%) of those invited actually participated in the examination conducted in 1980 (29). Follow-up examinations which included basic laboratory analyses were carried out in 2283 subjects in 2001 and 2046 subjects in 2011. When examining the cross-sectional associations between serum FAs and cardiometabolic outcomes (with the year 2001 data), there were 2187-2200 participants, i.e., men (46.2%) and non-pregnant women (53.8%) for whom the anthropometric data was available. With regard to the prospective analyses (FAs analyzed in 2001 vs. incident outcomes in 2011), there were 975-1414 participants available, i.e., men (45.0%) and non-pregnant women (55.0%). One individual

having a HOMA index>1000 (2011 data) was removed as an outlier. Dietary intake data was available for 991 participants (the year 2001 data).

Clinical data assessment (2001 data)

Anthropometric data on waist circumference and body mass index (BMI) were measured during the study visits. All laboratory analyses were carried out on overnight fasting samples. Serum levels of glucose, lipids, activity of γ-glutamyl transferase, alanine aminotransferase and insulin were measured with standard clinical laboratory methods (see supplemental material for details). Data on daily cigarette smoking, pregnancy, medication for hypertension (no vs. yes), education-based socioeconomic status (comprehensive school vs. secondary education, not academic vs. academic, 1 to 3), the number of monthly portions for vegetables and fruits, alcohol consumption (g/day), use of additional salt, i.e., sodium or potassium (never added vs. added following tasting vs. always added, 1 to 3), and a leisure-time physical activity (an index score varying between 5 and 15) (30) were based on the participants' responses to the questionnaires. The intake of PUFAs, SFAs and MUFAs (either % serum total FAs, or E%) were calculated based on 48-hour dietary recall data (see supplemental material for details).

Serum fatty acids

Serum concentrations and percentages of total FAs (free + esterified) were analyzed in a gas chromatography and flame ionization detector (31). Lipids were extracted from serum with chloroform-methanol (2:1). The FA methyl esters were synthesized using 14% boron trifluoride in methanol. The methyl esters were analyzed using a gas chromatograph (Varian CP-3800; Varian Inc, Walnut Creek, Calif) equipped with a 30-m × 0.25-mm glass capillary column (stationary phase 50% cyanopropylphenyl-methoxypolysiloxane; J & W Scientific, Folsom, Calif). The oven temperature increased by 5°C/min from 140°C to 220°C during the analysis run. The peaks were identified on the basis of retention times recorded for different standards. Heptadecanoic acid (C17:0) was used as an

internal standard. The FAs were quantified by peak areas relative to heptadecanoic acid using Star Chromatography Workstation software (Star Toolbar, version 5.50; Varian Inc).

Specific FAs were subdivided into (1) SFAs: myristic acid, 14:0; pentadecanoic acid, 15:0; palmitic acid, 16:0 and stearic acid, 18:0; (2) MUFAs: palmitoleic acid, 16:1n-7; octadecenoic acid, 18:1n-7; oleic acid, 18:1n-9; eicosenoic acid, 20:1n-9 and docosenoic acid, 22:1n-9; (3) PUFAs: linoleic acid, 18:2n-6; γ-linolenic acid, 18:3n-6; eicosadienoic acid, 20:2n-6; dihomo- γ-linolenic acid, 20:3n-6; arachidonic acid, 20:4n-6; docosatetraenoic acid, 22:4n-6, α-linolenic acid, 18:3n-3; eicosatetraenoic acid, 20:4n-3; eicosapentaenoic acid, 20:5n-3; docosapentaenoic acid, 22:5n-3 and docosahexaenoic acid, 22:6n-3.

Obesity and HOMA index

A subject was defined as being obese if her/his BMI was higher than 30 kg/m^2. The rest of the study population formed a non-obese group. At baseline, none of the individuals had type 2 diabetes. Glucose and insulin values were used to calculate the HOMA index (homeostatic model-based insulin resistance). Since there was a wide age distribution among the subjects (from 24 to 39 years in 2001), the HOMA index was categorized for logistic regression by forming age- and sex-specific percentiles, with 80% being applied as a cutoff point: ≥80%=1 vs. <80%=0.

Elevated blood pressure: definition and measurement

In our young cohort, the rates of elevated blood pressure (BP) increased as the subjects grew older and there were age-interactions in the associations between blood pressure and serum FAs, linoleic acid being one example. For these reasons, a participant was defined to have elevated BP if she/he was being prescribed medication for hypertension or her/his systolic (SBP) or diastolic (DBP) BP belonged to the highest age and sex specific 80% percentile. In women, the mean cutoff for the 80% percentile (the year 2001 data) was 122 mmHg for SBP and 77 mmHg for DBP. In men (the year 2001 data), the

corresponding values were 131 and 83 mmHg. Parallel analyses with common clinical criteria for hypertension (medication for hypertension or DBP≥90 or SBP≥140 mmHg) are presented in **Supplemental Table 4**. BP was measured in 2001 and 2011 by using a random-zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK) with the subject in the sitting position after 5 minutes of rest. Korotkoff's fifth phase was used as the sign of DBP, and the first phase was read as SBP. Readings were performed 3 times on each subject; the average of these values was calculated.

Imaging of the liver fat status (non-alcoholic fatty liver)

The liver fat was scanned using 4.0 MHz adult abdominal transducers with Acuson Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA). A trained sonographer graded the liver fat status from the ultrasonographic images using widely accepted criteria: 1) the liver-to-kidney contrast, 2) parenchymal brightness, 3) deep beam attenuation, 4) bright vessel walls, and 5) visibility of the neck of the gallbladder.

Statistical analyses

Variables with a skewed distribution were log-transformed prior to their statistical evaluation. T-test for independent samples was used to calculate baseline characteristics. Pearson correlation coefficients were calculated to examine the associations between dietary intake of FAs (% or E%) and their serum percentages. We also formed two summary variables: 1) the (SFAs+MUFAs)/PUFAs ratio and 2) the number of cardiometabolic outcomes (including obesity, high insulin resistance and elevated BP, values ranging from 0 to 3). Univariate general linear model was used to form a figure regarding age and sex-adjusted FA status versus the number of outcomes.

The two-step logistic regression models were conducted as follows: First, the associations of standardized FA variables with each cardiometabolic outcome were examined with models including age and sex as covariates. Then, additional covariates were specifically selected for each outcome to

construct fully-adjusted models. For prevalent and incident obesity, further adjustments were done for physical activity, educational socioeconomic status, smoking habits and the monthly portions for fruits and vegetables. For prevalent and incident HOMA, further covariates included BMI, leisure-time physical activity, alanine aminotransferase, the triglyceride/high-density lipoprotein (HDL) cholesterol ratio and smoking habits. For BP, further adjustments were made for BMI, leisure-time physical activity, HOMA levels, the triglyceride/HDL cholesterol ratio, smoking and salt use. With regard to incident outcomes, the follow-up time of 10 years was identical for all of the study participants. With regard to models for incident outcomes, individuals with the corresponding prevalent outcomes were removed prior to the analyses. For incident fatty liver, the same set of covariates was used as for BP, except that salt intake and the triglyceride/HDL cholesterol ratio were replaced with alcohol use. An ultrasound examination was not performed in 2001. Therefore, to exclude possible cases with prevalent fatty liver in 2001, we removed participants with the Bedogni's fatty liver index>30 (32) and those with a known risk level of alcohol use, i.e., over 20 g/day in women and 30 g per day in men in 2001, from the incident fatty liver models. Age and sex-interactions were characterized by the logistic regression models supplemented by age*FA or sex*FA-variable interaction terms. On the basis of principal component analysis, we calculated that 17 components would explain >99% of the variation among 33 serum FA variables (**Table 1).** Following Bonferroni-correction, a *P* value < 0.003 was defined as statistically significant and a *P*-value between 0.003 and 0.05 as borderline significant. IBM SPSS Statistics software (version 22) was used to perform the statistical analyses.

Ethics

The study was carried out in accordance with the recommendations of the Declaration of Helsinki. All participants provided written informed consent, and the study protocol was approved by the Ethics Committee, Hospital District of Southwest Finland.

Results

Characteristics

Baseline characteristics of the selected study variables are presented in **Table 1**. This includes clinical data, serum total FA percentages (FA classes and specific FAs) and serum total FA concentrations (summaries for desaturation degrees). In addition, outcome-specific characteristics have been presented for the participants without any investigated cardiometabolic outcome in 2001 and for the participants having prevalent obesity, high HOMA and/or elevated blood pressure in 2001 (**Supplemental Table 1**). With regard to incident outcomes, baseline characteristics have been presented separately for the participants without any investigated cardiometabolic outcome in 2001 or 2011, and for the participants having incident obesity, high HOMA, non-alcoholic fatty liver and/or elevated BP in 2011 (**Supplemental Table 2).**

Links between dietary intake and serum total FA percentages

Pearson correlation coefficients between dietary (either % total FA intake or E%) and serum FAs (% total FAs) are presented in **Supplemental Table 3**. The strongest associations were observed for n-3 PUFAs (r=0.3 to 0.4, P<0.003). With respect to the other FAs, the associations were weak, but statistically significant for at least one of these two dietary variables. With respect to dietary MUFAs, their E% exerted an inverse association with serum MUFAs. In addition, FA percentages have been compared between diet and serum in **Supplemental Figure 1**. In serum, n-6 and n-3 PUFA percentages were clearly higher than the corresponding percentages in dietary intake (visual assessment).

Cross-sectional logistic regression analyses for serum FA percentages versus outcomes

SFAs with an even number of carbon atoms, including myristic and palmitic acids, and MUFAs, including palmitoleic and oleic acids, were positively associated with prevalent obesity, insulin

resistance and elevated BP in the age and sex-adjusted models (P<0.003) **(Figure 1).** Stearic acid (18:0) did not show any age and sex-adjusted links, and the association of pentadecanoic acid with elevated BP was of an inverse nature. The percentages of PUFAs and n-6 PUFAs, linoleic acid in particular, were inversely associated with the outcomes (P<0.003). Furthermore, the n-3 PUFAs, showed borderline significant (P<0.05) inverse associations with obesity and elevated BP. The n-6/n-3 PUFA ratio exhibited inverse associations with obesity and high HOMA (P<0.003). However, specific PUFAs, such as γ-linolenic, dihomo-γ-linolenic and/or eicosatetraenoic acids, displayed positive rather than inverse associations with the outcomes (P<0.003). Docosatetraenoic acid, %, exhibited a statistically significant positive association with elevated BP. With respect to the long-chain n-3 PUFAs, docosapentaenoic acid and docosahexaenoic acid showed inverse links (some associations being only borderline significant) with the outcomes. In general, the associations between FA percentages and clinically determined hypertension (**Supplemental Table 4**) were similar, but somewhat stronger than the associations between FA percentages and elevated BP (Figure 1).

Most of the above-mentioned associations were also statistically significant in the fully-adjusted models reflecting their independence of common lifestyle cardiometabolic risk factors. The high PUFA/SFA ratio exhibited the strongest inverse fully-adjusted association with the outcomes.

We also tested associations between the actual serum FA concentrations, mg/L, and the outcomes. Regardless of FA class, specific FA concentrations were positively and nearly always significantly (P<0.003) associated with obesity (**Supplemental Table 5)**. The highest odds ratios were seen for palmitoleic and dihomo-γ-linolenic acids. Similar, consistently positive associations between FA concentrations and outcomes were also evident for blood pressure and HOMA (data not shown).

Finally, we tested whether the serum (SFAs+MUFAs) per PUFAs ratio would be linked with the number cardiometabolic outcomes. There was a strong overall positive association between these two variables, β=0.12, P<0.003 for the trend of increasing serum FA status across the number of outcomes (**Figure 2**).

Cross-sectional associations were confirmed with prospective data

The trend of associations between FA percentages at baseline and outcomes 10 years later (**Figure 3**) was very similar to the cross-sectional data presented above. Similarly as with the cross-sectional data, SFAs and MUFAs, particularly palmitoleic and oleic acids, were positively associated with obesity (P<0.003 in age and sex-adjusted and in fully-adjusted models). An increase in the carbon chain length in MUFAs was associated with a lower risk of obesity, since in contrast to palmitoleic and oleic acids (P<0.003), two longer chain fatty acids, eicosenoic and docosenoic acids, were not linked with obesity. PUFAs, including linoleic and docosahexaenoic acids, were inversely associated with obesity (P<0.003 in both models). In addition, γ-linolenic acid exhibited positive associations (P<0.003, both models) with obesity, whereas the associations of dihomo-γ-linolenic acid and eicosatetraenoic acid with obesity were only of borderline significant (both models).

With regard to FA associations with high HOMA, MUFAs and oleic acid had positive, whereas PUFAs and linoleic acid exhibited inverse associations (P<0.003, age and sex-adjusted models). With respect to elevated BP (and hypertension in Supplemental Table 4), palmitic acid, %, percentages of MUFAs and oleic acid, %, displayed positive associations, with those of PUFAs, n-6 PUFAs and linoleic acid having inverse associations (P<0.003, age and sex-adjusted models). Fully-adjusted models for incident HOMA or elevated BP did not reveal any statistically significant FA associations (P≥0.05).

In the prospective models, high percentages of palmitic and low linoleic acids consistently predicted incident fatty liver (P<0.003, both models). In addition, palmitoleic acid, %, had a positive association with the fatty liver in age and sex-adjusted models.

Discussion

In this study, we have examined both cross-sectional and prospective associations of serum FAs with cardiometabolic outcomes, including obesity, insulin resistance, elevated BP or hypertension and fatty liver in young or middle-aged Finnish males and females.

Even though serum total FAs have been used commonly as a marker of dietary FA intake (33), we have demonstrated here that their correlations with dietary intake are weak, although n-3 PUFAs represent something of an exception (Supplemental Table 3).

Several of the cross-sectional outcome findings were statistically significant also in the prospective data. These consisted of circulating percentages of SFAs and MUFAs, including palmitoleic and oleic acids which displayed positive associations with obesity. Some PUFAs, e.g. linoleic and docosahexaenoic acids had inverse, whereas others, γ-linolenic acid had positive associations with obesity. With regard to insulin resistance, total MUFAs and oleic acid showed positive associations, whereas there were inverse associations for total PUFAs and linoleic acid (age and sex-adjusted model). With regard to elevated BP, palmitic acid, total MUFAs and oleic acid exhibited positive outcome associations, whereas the links of total PUFAs, n-6 PUFAs and linoleic acid were of an inverse character (age and sex-adjusted model). High palmitic acid and low linoleic acid percentages consistently predicted the incidence of fatty liver. In contrast to earlier findings (17-20), we did not find any evidence that a high n-6/n-3 PUFA ratio would associate with adverse cardiometabolic outcomes.

Regarding trends emerging from the cross-sectional data, an increase in the carbon chain length of MUFAs, i.e., not SFAs (25), was most consistently linked to a declining risk of obesity. In line with an earlier study for T2D (26), the percentages of myristic and palmitic acids, i.e. FAs with an even number of carbon atoms in their chains, were positively associated with several outcomes. Finally, the ratio of (SFA+MUFA)/PUFA, was positively and linearly associated with an increasing number of cardiometabolic outcomes. This suggests that there is a consistent link between the serum FA profile and the cardiometabolic state as a whole.

Obesity

All FAs have similar energy contents i.e. 37 kJ per gram of fat. However, in animal models, an increased consumption of long-chain n-3 PUFAs has been suggested to exert anti-obesity effects (34). Based on human experiments with labelled FAs, an elevated dietary intake of long-chain SFAs may lead to weight gain since long-chain SFAs are more poorly oxidized in the human body than other fats (25).

Recent meta-analyses and reviews have concluded that the intakes of long-chain SFAs and trans-FAs should be reduced and substituted with PUFAs to reduce body weight (35, 36). In addition, an increased intake of MUFAs from animal sources has been associated with a weight gain, whereas MUFAs from plant sources do not exert such an influence (35). The role of increased n-3 PUFAs intake is unclear, since human trials have found reductions in waist circumference but not in weight (37). With regard to erythrocyte phospholipid FA percentages, PUFAs have been lower and SFAs higher in obese children and adolescents, as compared to controls (38). When one considers the specific FAs, the plasma dihomo-γ-linolenic acid, %, was reported to be elevated in overweight or obese individuals in a review of 21 case-control studies (39). In a Swedish study, the percentages of serum CE palmitic, palmitoleic, stearic, γ-linolenic, dihomo-γ-linolenic, arachidonic and eicosapentaenoic acids displayed positive associations, whereas there were inverse associations between the markers of obesity with the linoleic acid percentage (40).

Our study confirms most of these findings (particularly SFAs vs. PUFAs). In Finland, dietary MUFAs are mainly of an animal origin. Thus, our serum total MUFA findings seem to support those earlier animal-source MUFA observations that these types of MUFAs are associated with weight gain. On the other hand, MUFAs and SFAs are metabolically linked together via Δ9-desaturase activity. With regard to n-3 PUFAs, only docosahexaenoic acid was inversely associated with weight in the prospective models.

Insulin resistance and type 2 diabetes

The strongest evidence of the beneficial health effects of unsaturated dietary fats has emerged not only from RCTs planned to study the association between dietary fats and insulin resistance (41) but also from life-time dietary interventions, such as the Finnish STRIP study (42-44).

The literature confirms the beneficial effects of circulating n-6 PUFAs and linoleic acid, in particular, in reducing the risk of T2D (45). An inverse association has been reported between T2D and the circulating levels of plant-origin phospholipid n-3 PUFA (α-linolenic acid) whereas no convincing associations have been detected between T2D and marine-derived n-3 PUFAs (46). With respect to SFAs, the odd-numbered chain 15:0 and 17:0 SFAs (26, 47), or very long-chain 20:0, 22:0 and 24.0 SFAs have been inversely associated with incident T2D (48). In several studies, levels of palmitoleic acid, γ-linolenic acid and/or dihomo-γ-linolenic acid have consistently exhibited positive links with T2D, impaired glucose and/or insulin metabolism (27, 46, 49, 50).

Our study confirms most of these findings, such as the beneficial role of linoleic acid. However, α-linolenic and eicosatetraenoic acids exerted or tended to exert positive baseline associations with insulin resistance, whereas for some longer chain n-3 PUFAs, particularly for docosapentaenoic acid, there were inverse associations. In addition, 15:0 was inert without any associations with insulin resistance. We did not investigate the very long-chain SFAs.

Blood pressure

The strongest evidence with respect to the BP lowering capabilities of FAs has emerged from RCT meta-analyses with long-chain n-3 PUFAs (51), instead for the other FAs, both the intake and biomarker data are inconsistent. For example, large studies from the US, i.e. the Nurses' Health Study (58 218 women) and the Health Professionals' Follow-up Study (30 681 men), have not found any associations between BP and the intakes of SFAs, MUFAs or PUFAs (52, 53).

In our study, the inverse associations of n-3 PUFAs with elevated BP were of borderline significance both in the age and sex adjusted, as well as in the fully-adjusted models (cross-sectional

data), supporting the literature. Overall, the FA associations of BP were very similar with those found for both obesity and HOMA. In contrast to other outcomes, the 15:0, %, was inversely associated with elevated BP in fully-adjusted models (cross-sectional data).

Fatty liver

According to a recent RCT meta-analysis (54), dietary n-3 PUFA supplementation may lower the liver fat content in individuals suffering from fatty liver. In our study, α-linolenic acid showed only borderline significant inverse associations with the incident fatty liver. In fact, our study highlighted the role of palmitic and linoleic acids which may exert obesity and insulin resistance-independent effects on future fatty liver. These findings support our earlier observations with the metabolomics data obtained from nuclear magnetic resonance-based serum FA analyses in which the serum total SFAs, %, increased, whereas those of total n-6 PUFAs lowered the risk of incident fatty liver (23).

Limitations

One shortcoming is that observational studies cannot establish causality. Due to the relatively young study population, “hard” outcomes, such as T2D or cardiovascular diseases, are not yet available in meaningful numbers. In addition, the generalizability of the observations is limited to white European subjects. There were age-interactions in the outcome models tested. For this reason, we used age and sex-specific categorized BP values (80% percentile as a cutoff point). Furthermore, although ultrasound is generally used method for fatty liver, it has a somewhat limited performance, compared to magnetic resonance imaging when the steatosis is <30% on liver biopsy (55). Smoking may increase the circulating levels of MUFAs (33), and statins may elevate the levels of serum long-chain PUFAs by increasing the enzymatic activities of elongase, Δ5-desaturase and Δ6-desaturase (31). Of these confounders, only smoking was taken as a covariate into consideration in our statistical models.

Conclusions

Serum FA percentages showed rather similar association profiles with obesity, insulin resistance, non-alcoholic fatty liver and BP. The ratio of (SFAs+MUFAs)/PUFAs was linked with the number of cardiometabolic outcomes. Our findings suggest that circulating FAs are associated with future cardiometabolic outcomes in young and middle-aged Finnish adults. The percentages of PUFAs, n-6 PUFAs and linoleic acid in particular were associated with a lowered risk, and SFAs (with an even number of carbon atoms) and MUFAs (with shorter carbon chains) with an increased risk of these disease outcomes. The γ-linolenic acid percentage displayed consistent positive outcome associations. Overall, these serum-based findings support the current dietary recommendations to replace saturated fat with PUFAs and with n-6 PUFAs in attempts to prevent cardiometabolic outcomes. However, the correlations between FA intakes and serum total FA percentages do seem to be rather weak.

Acknowledgments and statement of authors' contributions to manuscript

The authors thank Irina Lisinen and Johanna Ikonen for their expert technical assistance in data management. J.E.K. performed the statistical analyses, wrote the paper and is responsible for the final content; A.J., J.S.A.V, M.J., K.H.-K., M.K., T.L. and O.T.R designed and conducted the clinical research and provided scientific advice and/or wrote the paper; Additionally, A.J. was responsible for biochemical analyses, including serum fatty acids, and O.T.R. supervised the work. All authors read and approved the final manuscript.

References

1. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112:2735-52.

2. WHO, FAO. Fats and Fatty Acids in Human Nutrition. Report of an Expert Consultation, Food and Agriculture organization of the United Nations, Rome. 2010.

3. AHA online recommendations. https://healthyforgood.heart.org/Eat-smart/Articles/Dietary-Fats, visited 1.10. 2020.

4. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006;114:82-96.

5. Recommendations of USDA and US Department of Health and Human Services. Dietary Guidelines for Americans. Washington, DC: US Government Printing Office. 2010.

6. Morris JN and the committee. Controlled trial of soya-bean oil in myocardial infarction. Lancet. 1968;2:693-9.

7. Dayton S, Pearce ML, Hashimoto S, Dixon WJ, Tomiyasu U. A controlled clinical trial of a diet high in unsaturated fat in preventing complications of atherosclerosis. Circulation. 1969;40 (suppl II):II-63.

8. Leren P. The Oslo diet-heart study. Eleven-year report. Circulation. 1970;42:935-42.

9. Turpeinen O, Karvonen MJ, Pekkarinen M, Miettinen M, Elosuo R, Paavilainen E. Dietary prevention of coronary heart disease: the Finnish Mental Hospital Study. Int. J. Epidemiol. 1979;8:99-118.

10. Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Balter K, Fraser GE, Goldbourt U, Hallmans G, Knekt P, Liu S et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am. J. Clin. Nutr. 2009;89:1425-32.

11. Farvid MS, Ding M, Pan A, Sun Q, Chiuve SE, Steffen LM, Willett WC, Hu FB. Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. Circulation. 2014;130:1568-78.

12. Sacks FM, Lichtenstein AH, Wu JHY, Appel LJ, Creager MA, Kris-Etherton PM, Miller M, Rimm EB, Rudel LL, Robinson JG et al. Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. Circulation. 2017;136:e1-e23.

13. Hooper L, Martin N, Abdelhamid A, Davey Smith G. Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst. Rev. 2015;(6):CD011737. doi:CD011737.

14. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med. 2010;7:e1000252.

15. Skeaff CM, Miller J. Dietary fat and coronary heart disease: summary of evidence from prospective cohort and randomised controlled trials. Ann. Nutr. Metab. 2009;55:173-201.

16. Hamley S. The effect of replacing saturated fat with mostly n-6 polyunsaturated fat on coronary heart disease: a meta-analysis of randomised controlled trials. Nutr. J. 2017;16:30-5.

17. Ramsden CE, Hibbeln JR, Majchrzak SF, Davis JM. n-6 fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. Br. J. Nutr. 2010;104:1586-600.

18. Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, Ringel A, Davis JM, Hibbeln JR. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. BMJ. 2013;346:e8707.

19. Calder PC. Old study sheds new light on the fatty acids and cardiovascular health debate. BMJ. 2013;346:f493.

20. Jang HPK. Omega-3 and omega-6 polyunsaturated fatty acids and metabolic syndrome: A systematic review and meta-analysis. Clin Nutr. 2020;39:765-73.

21. Joris PJ, Mensink RP. Role of cis-Monounsaturated Fatty Acids in the Prevention of Coronary Heart Disease. Curr. Atheroscler. Rep. 2016;18:38-y.

22. Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation. 2015;131:774-85.

23. Kaikkonen JE, Würtz P, Suomela E, Lehtovirta M, Kangas AJ, Jula A, Mikkilä V, Viikari JS, Juonala M, Rönnemaa T et al. Metabolic profiling of fatty liver in young and middle-aged adults: Cross-sectional and prospective analyses of the Young Finns Study. Hepatology. 2017;65:491-500.

24. Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, Uusitupa M, Ala-Korpela M, Laakso M. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. Diabetes Care. 2013;36:3732-8.

25. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. Am. J. Clin. Nutr. 2000;72:905-11.

26. Huang L, Lin JS, Aris IM, Yang G, Chen WQ, Li LJ. Circulating Saturated Fatty Acids and Incident Type 2 Diabetes: A Systematic Review and Meta-Analysis. Nutrients. 2019;11:10.3390/nu11050998.

27. Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH, ARIC Study Investigators. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am. J. Clin. Nutr. 2003;78:91-8.

28. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu. Rev. Nutr. 2004;24:345-76.

29. Raitakari OT, Juonala M, Rönnemaa T, Keltikangas-Järvinen L, Räsänen L, Pietikäinen M, Hutri-Kähönen N, Taittonen L, Jokinen E, Marniemi J et al. Cohort profile: the cardiovascular risk in Young Finns Study. Int. J. Epidemiol. 2008;37:1220-6.

30. Telama R, Yang X, Viikari J, Välimäki I, Wanne O, Raitakari O. Physical activity from childhood to adulthood: a 21-year tracking study. Am. J. Prev. Med. 2005;28:267-73.

31. Jula A, Marniemi J, Ronnemaa T, Virtanen A, Huupponen R. Effects of diet and simvastatin on fatty acid composition in hypercholesterolemic men: a randomized controlled trial. Arterioscler. Thromb. Vasc. Biol. 2005;25:1952-9.

32. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:33.

33. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog. Lipid Res. 2008;47:348-80.

34. Buckley JD, Howe PR. Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids. Obes. Rev. 2009;10:648-59.

35. Liu X, Li Y, Tobias DK, Wang DD, Manson JE, Willett WC, Hu FB. Changes in Types of Dietary Fats Influence Long-term Weight Change in US Women and Men. J. Nutr. 2018;148:1821-9.

36. Hammad SS, Jones PJ. Dietary Fatty Acid Composition Modulates Obesity and Interacts with Obesity-Related Genes. Lipids. 2017;52:803-22.

37. Zhang YY, Liu W, Zhao TY, Tian HM. Efficacy of Omega-3 Polyunsaturated Fatty Acids Supplementation in Managing Overweight and Obesity: A Meta-Analysis of Randomized Clinical Trials. J. Nutr. Health Aging. 2017;21:187-92.

38. Tang J, Yan Y, Li J, Yang B, Zhao X, Wan Y, Zheng JS, Mi J, Li D. Relationship between erythrocyte phospholipid fatty acid composition and obesity in children and adolescents. J. Clin. Lipidol. 2019;13:70,79.e1.

39. Fekete K, Gyorei E, Lohner S, Verduci E, Agostoni C, Decsi T. Long-chain polyunsaturated fatty acid status in obesity: a systematic review and meta-analysis. Obes. Rev. 2015;16:488-97.

40. Warensjö E, Ohrvall M, Vessby B. Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. Nutr. Metab. Cardiovasc. Dis. 2006;16:128-36.

41. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia. 2001;44:312-9.

42. Pahkala K, Laitinen TT, Niinikoski H, Kartiosuo N, Rovio SP, Lagström H, Loo BM, Salo P, Jokinen E, Magnussen CG, et al. Effects of 20-year infancy-onset dietary counselling on cardiometabolic risk factors in the Special Turku Coronary Risk Factor Intervention Project (STRIP): 6-year post-intervention follow-up. Lancet Child. Adolesc. Health. 2020;4:359-69.

43. Simell O, Niinikoski H, Rönnemaa T, Raitakari OT, Lagström H, Laurinen M, Aromaa M, Hakala P, Jula A, Jokinen E et al. Cohort Profile: the STRIP Study (Special Turku Coronary Risk Factor Intervention Project), an Infancy-onset Dietary and Life-style Intervention Trial. Int. J. Epidemiol. 2009;38:650-5.

44. Jula A, Marniemi J, Huupponen R, Virtanen A, Rastas M, Rönnemaa T. Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men: a randomized controlled trial. JAMA. 2002;287:598-605.

45. Wu JHY, Marklund M, Imamura F, Tintle N, Ardisson Korat AV, de Goede J, Zhou X, Yang WS, de Oliveira Otto, MC, Kroger J et al. Omega-6 fatty acid biomarkers and incident type 2 diabetes: pooled analysis of individual-level data for 39 740 adults from 20 prospective cohort studies. Lancet Diabetes Endocrinol. 2017;5:965-74.

46. Forouhi NG, Imamura F, Sharp SJ, Koulman A, Schulze MB, Zheng J, Ye Z, Sluijs I, Guevara M, Huerta JM et al. Association of Plasma Phospholipid n-3 and n-6 Polyunsaturated Fatty Acids with Type 2 Diabetes: The EPIC-InterAct Case-Cohort Study. PLoS Med. 2016;13:e1002094.

47. Santaren ID, Watkins SM, Liese AD, Wagenknecht LE, Rewers MJ, Haffner SM, Lorenzo C, Hanley AJ. Serum pentadecanoic acid (15:0), a short-term marker of dairy food intake, is inversely associated with incident type 2 diabetes and its underlying disorders. Am. J. Clin. Nutr. 2014;100:1532-40.

48. Lemaitre RN, Fretts AM, Sitlani CM, Biggs ML, Mukamal K, King IB, Song X, Djousse L, Siscovick DS, McKnight B et al. Plasma phospholipid very-long-chain saturated fatty acids and incident diabetes in older adults: the Cardiovascular Health Study. Am. J. Clin. Nutr. 2015;101:1047-54.

49. Kurotani K, Sato M, Ejima Y, Nanri A, Yi S, Pham NM, Akter S, Poudel-Tandukar K, Kimura Y, Imaizumi K et al. High levels of stearic acid, palmitoleic acid, and dihomo-gamma-linolenic acid and low levels of linoleic acid in serum cholesterol ester are associated with high insulin resistance. Nutr. Res. 2012;32:669,675.e3.

50. Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes. 1994;43:1353-7.

51. Miller PE, Van Elswyk M, Alexander DD. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of randomized controlled trials. Am. J. Hypertens. 2014;27:885-96.

52. Witteman JC, Willett WC, Stampfer MJ, Colditz GA, Sacks FM, Speizer FE, Rosner B, Hennekens CH. A prospective study of nutritional factors and hypertension among US women. Circulation. 1989;80:1320-7.

53. Ascherio A, Rimm EB, Giovannucci EL, Colditz GA, Rosner B, Willett WC, Sacks F, Stampfer MJ. A prospective study of nutritional factors and hypertension among US men. Circulation. 1992;86:1475-84.

54. Yan JH, Guan BJ, Gao HY, Peng XE. Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. Medicine (Baltimore). 2018;97:e12271.

55. Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. Eur. Radiol. 2011;21:87-97.

TABLE 1 Baseline characteristics of the young and middle-aged Finnish adults in 2001 (n=2200)[1]

Characteristic	Values		Values
Sex, % females	54	PUFAs	39.9 ± 4.4
Age, y (range 24 to 39 years)	31.7 ± 5.0	n-6 PUFAs	35.0 ± 4.2
Body mass index, kg/m2	25.1 ± 4.4	Linoleic acid, 18:2n-6	27.5 ± 4.0
Waist circumference, cm	84.2 ± 12.3	γ-Linolenic acid, 18.3n-6	0.38 ± 0.15
Physical activity index, 1 to 15	9.9 ± 2.4	Eicosadienoic acid, 20:2n-6	0.17 ± 0.04
Smoking, %	25	Dihomo-γ-linolenic acid, 20:3n-6	1.4 ± 0.3
Systolic blood pressure, mm Hg	117 ± 13	Arachidonic acid, 20:4n-6	5.5 ± 1.1
Diastolic blood pressure, mm Hg	71 ± 11	Docosatetraenoic acid, 22:4n-6	0.13 ± 0.03
Serum glucose, mmol/L	5.07 ± 0.84	n-3 PUFAs	4.7 ± 1.2
Serum insulin, mU/L	7.76 ± 5.77	α-Linolenic acid, 18:3n-3	0.92 ± 0.26
Serum LDL cholesterol, mmol/L	3.27 ± 0.84	Eicosatetraenoic acid, 20:4n-3	0.14 ± 0.06
Serum HDL cholesterol, mmol/L	1.28 ± 0.31	Eicosapentaenoic acid, 20:5n-3	1.1 ± 0.6
Serum triglycerides, mmol/L	1.33 ± 0.85	Docosapentaenoic acid, 22:5n-3	0.50 ± 0.11
Serum alanine aminotransferase, U/L	11.4 ± 8.5	Docosahexaenoic acid, 22:6n-3	2.0 ± 0.7
Serum γ-glutamyltransferase, U/L	26.3 ± 27.0		
		PUFAs/SFAs	1.2 ± 0.2
Serum fatty acids, % total fatty acids:		n-6/n-3 PUFAs	7.8 ± 2.0
SFAs[2]	32.4 ± 2.3		
Myristic acid, 14:0	1.18 ± 0.44	Serum total fatty acid concentration, mg/L:	
Pentadecanoic acid, 15:0	0.24 ± 0.05	SFAs	840 ± 290
Palmitic acid, 16:0	24.0 ± 2.1	MUFAs	730 ± 290
Stearic acid, 18:0	6.9 ± 0.8	n-6 PUFAs	880 ± 170
		n-3 PUFAs	120 ± 50
MUFAs	27.7 ± 3.1		
Palmitoleic acid, 16:1n-7	2.3 ± 0.9		
Oleic acid, 18:1n-9	23.5 ± 2.6		
Octadecenoic acid, 18:1n-7	1.7 ± 0.6		
Eicosenoic acid, 20:1n-9	0.17 ± 0.05		
Docosenoic acid, 22:1n-9	0.06 ± 0.04		

[1]Values are mean ± SD.
[2]MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

FIGURE 1 Cross-sectional associations (odd ratios) of fatty acid percentages with different cardiometabolic outcomes among Finnish adults in 2001[1]

[1]Values are odd ratios and their 95% confidence intervals per 1-SD increment in the fatty acid measures (logistic regression). Outcome variables included prevalent obesity (BMI>30 kg/m^2 vs. ≤30 kg/m^2, n=271 obese out of 2200 participants), high HOMA-IR (age and sex specific percentiles ≥80% vs. <80%, n=444 high HOMA out of 2199 participants) and elevated blood pressure (age and sex specific diastolic or systolic blood pressure percentiles≥80% or medication for hypertension vs. <80% without medication, n=647 hypertensive out of 2187 participants). Each fatty acid measure was tested separately in the logistic regression models adjusted for sex and age[2] (odds ratios above the bars) and additionally for the outcome-specific cardiometabolic risk or preventive factors[3] (fully adjusted models, odds ratios below the bars). BP, blood pressure; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

FIGURE 2 Serum FAs (SFAs, %+MUFAs, %)/PUFAs, % versus the number of cardiometabolic outcomes (the year 2001 cross-sectional data in Finnish adults, n=2200)[1]

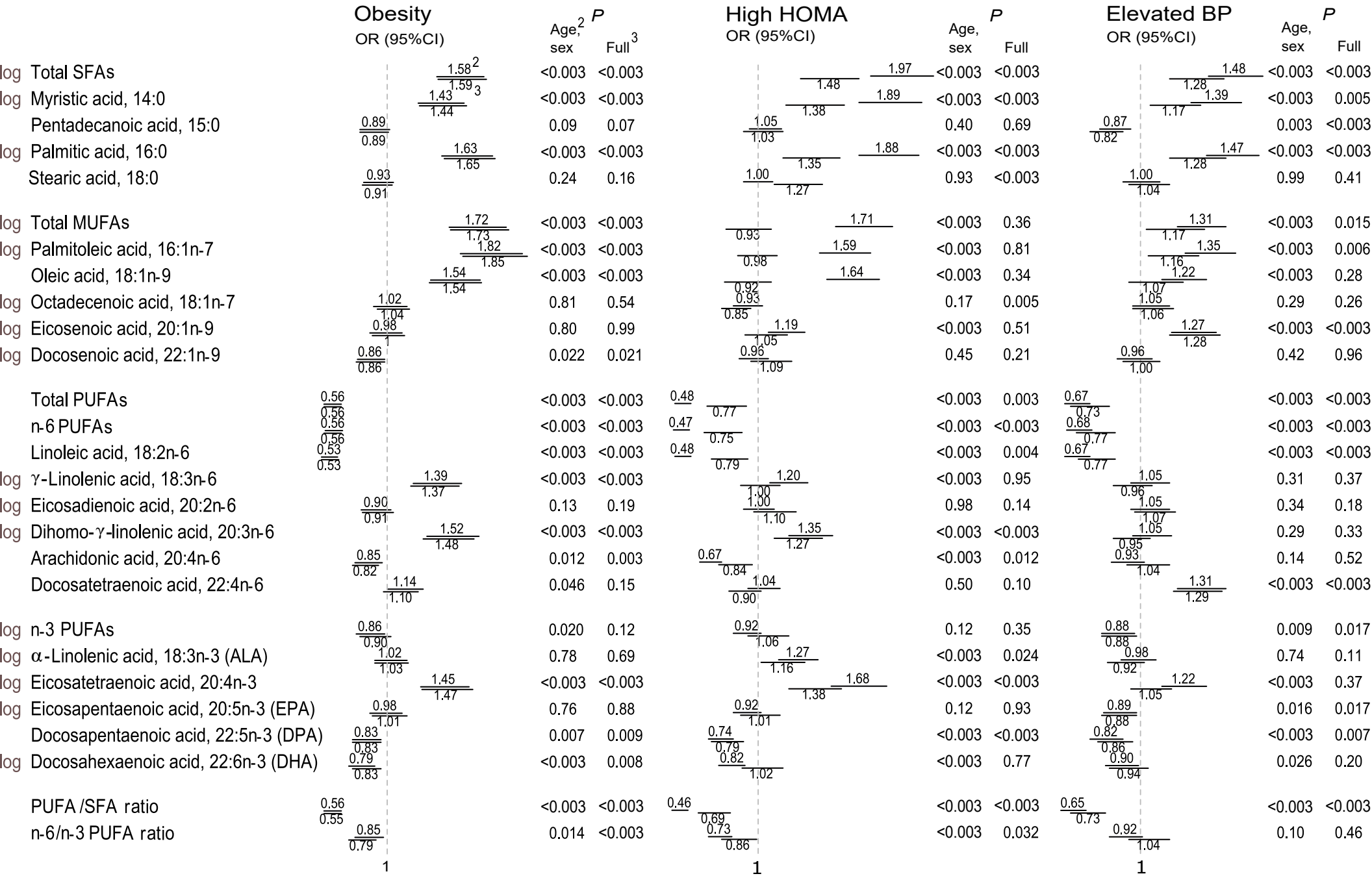
[1]Bars denote estimated marginal means and their standard errors, adjusted for age and sex (β=0.12, P<0.003 for the trend in FAs across the number of outcomes, Unianova). Liver fat was not included in the outcome score since it was not estimated by ultrasound in 2001. Number of subjects per group is given next to the error bars. MUFA, monounsaturated FA; PUFA, polyunsaturated FA; SFA, saturated FA.

FIGURE 3 Prospective associations (odd ratios) of fatty acid percentages in 2001 with incident cardiometabolic outcomes among Finnish adults in 2011[1]

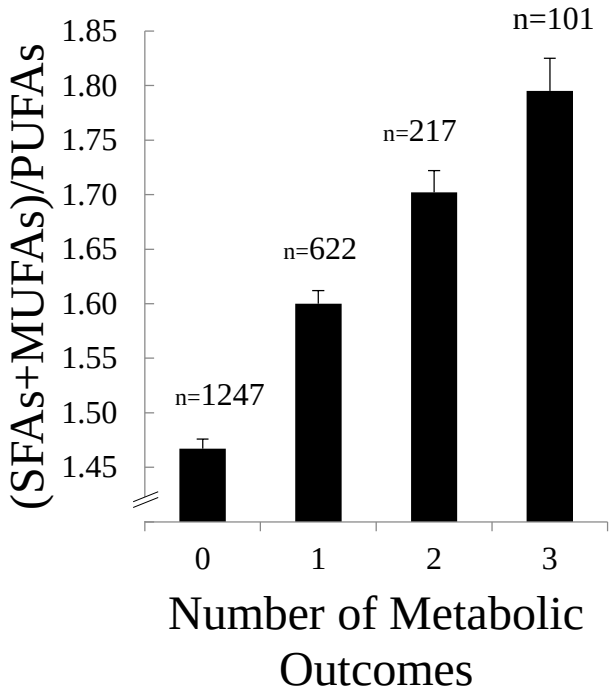
[1]Values are odd ratios and their 95% confidence intervals per 1-SD increment in the fatty acid measures (logistic regression). Outcomes include incident obesity (BMI>30 kg/m^2 vs. ≤30 kg/m^2, 163 obese out of 1414 participants), high HOMA (age and sex specific percentiles≥80% vs. <80%, n=255 high HOMA out of 1289 participants), fatty liver (n=70 fatty liver out of 975 individuals) and elevated blood pressure (age and sex specific diastolic and systolic blood pressure percentiles≥80% or medication for hypertension vs. <80% without medication, n=342 hypertensive out of 1088 participants). Each fatty acid measure was tested separately in the logistic regression models adjusted for sex and age[2] (odds ratios above the bars). Further adjustments were carried out for the outcome-specific cardiometabolic risk and preventive factors[3] (fully-adjusted models, odds ratios below the bars). BP, blood pressure; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Figure

Click here to access/download;Figure;kuva1r.eps

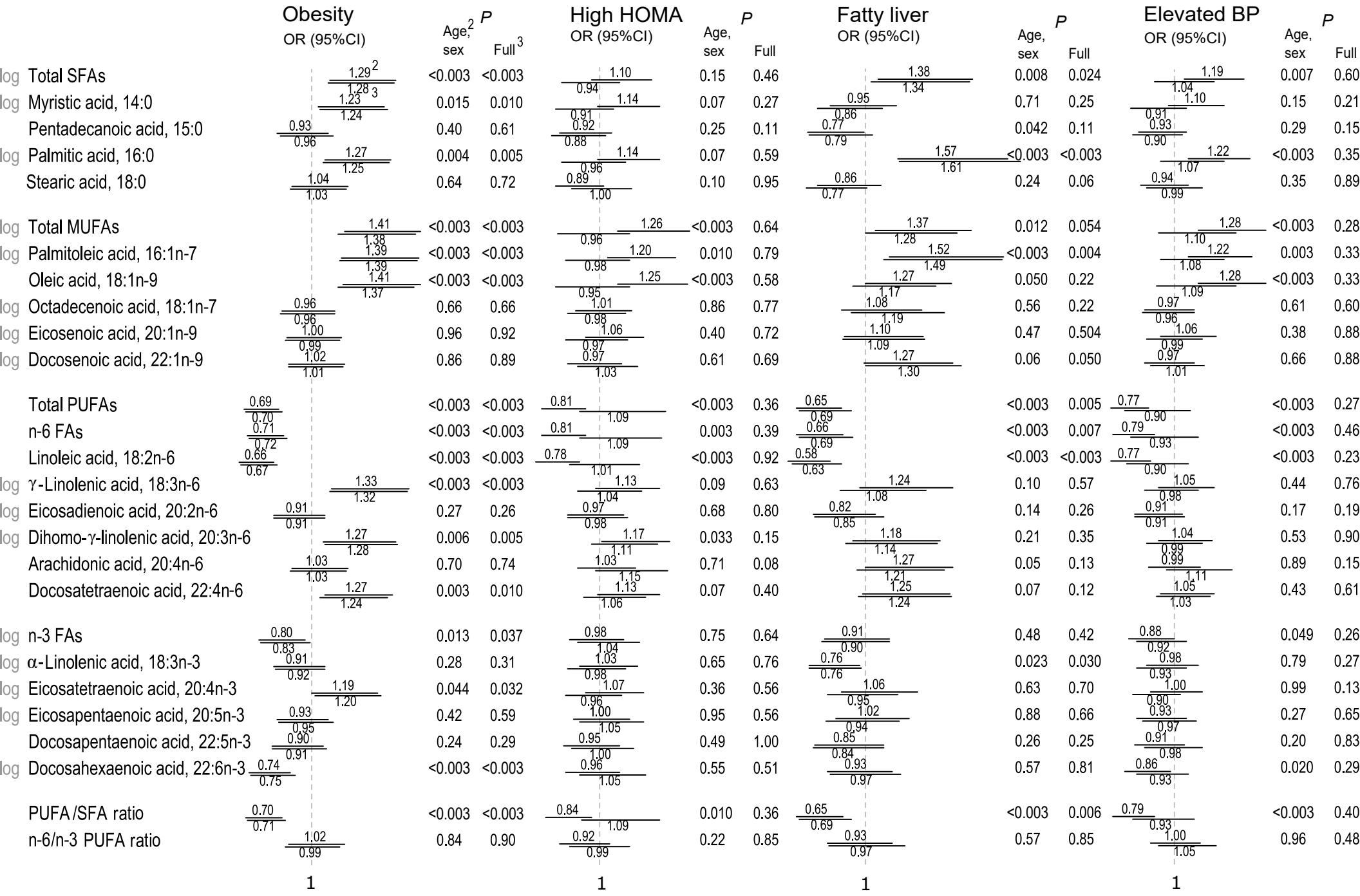


Figure



Figure

Click here to access/download;Figure;kuva 3r.eps



Online Supplemental Material

Serum fatty acid proportions: associations with obesity, insulin resistance, blood pressure and fatty liver: The Cardiovascular Risk in Young Finns Study

Jari E. Kaikkonen

Supplemental Methods

Collection of dietary data

Dietary interviewers, all trained dietitians, collected information on foods and beverages consumed by participants during the 2 days prior to the interview. The latest version of the National Food Composition Database (FND2) was used to calculate the intakes of energy and nutrients (including different oils) for each participant (www.fineli.fi).

Measurement of clinical data

Serum total cholesterol levels were measured by the enzymatic cholesterol esterase –cholesterol oxidase method (Cholesterol reagent, Olympus, Ireland). The same reagent was used for estimating HDL-cholesterol levels after precipitation of LDL and VLDL with dextran sulfate-Mg^{2+}. LDL-cholesterol was estimated by the Friedewald formula in subjects with triglyceride levels <4.0 mmol/L. The serum concentration of triglycerides was assayed using the enzymatic glycerol kinase – glycerol phosphate oxidase method (Triglyceride reagent, Olympus). Serum glucose concentration was determined by the enzymatic hexokinase method (Glucose reagent, Olympus). Serum ALT and GGT activities were measured enzymatically (ALT and GGT System Reagent, Olympus). A clinical chemistry analyzer (AU400; Olympus Optical Ltd, Mishima, Japan) was used for all of the above-mentioned measurements.

Serum insulin concentration was determined by a microparticle enzyme immunoassay (IMx insulin reagent, Abbott Diagnostics, USA) with an IMx instrument (Abbott).

Analysis of serum total FA concentrations

Following blood drawing, the serum samples were immediately frozen and stored at −70°C until assayed within a couple of years.

For the determination of serum total fatty acid composition, lipids were extracted from serum with chloroform-methanol (2:1). The fatty acid methyl esters were synthesized using 14% boron trifluoride in methanol. The methyl esters were analyzed using a gas chromatograph (Varian CP-3800; Varian Inc, Walnut Creek, Calif) equipped with a 30-m × 0.25-mm glass capillary column (stationary phase 50% cyanopropylphenyl-methoxypolysiloxane; J & W Scientific, Folsom, Calif). The oven temperature increased by 5°C/min from 140°C to 220°C during the analysis run. The peaks were identified on the basis of retention times recorded for different standards. Heptadecanoic acid (C17:0) was used as an internal standard. The fatty acids were quantified by peak areas relative to heptadecanoic acid using Star Chromatography Workstation software (Star Toolbar, version 5.50; Varian Inc).

Supplemental References

Jula A, Marniemi J, Rönnemaa T, Virtanen A, Huupponen R. Effects of diet and simvastatin on fatty acid composition in hypercholesterolemic men: a randomized controlled trial. Arterioscler. Thromb. Vasc. Biol. 2005;25:1952-9.

van Wijngaarden D. Modified rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem**.** 1967;39:848–9.

Supplemental data

SUPPLEMENTAL TABLE 1 Baseline characteristics of the participants without any metabolic outcome in 2001 and in participants having prevalent obesity, high HOMA and/or elevated blood pressure in 2001[1]

	No outcome n=1247	Obesity n=271	High HOMA n=444	Elevated BP n=647
	Values	Values	Values	Values
Sex, % females	53.8	49.1	53.8	53.8
Age, y (range 24 to 39 years)	31.6 ± 5.0	32.9 ± 4.7	31.7 ± 5.0	31.7 ± 5.0
Body mass index, kg/m2	23.3 ± 2.8	33.6 ± 3.7	29.0 ± 5.3	26.8 ± 5.2
Waist circumference, cm	79.8 ± 9.0	104.8 ± 10.0	94.6 ± 13.9	88.4 ± 13.9
Physical activity index, 1 to 15	10.0 ± 2.4	9.2 ± 2.3	9.2 ± 2.3	10.0 ± 2.3
Smokers, %	27.2	22.7	22.8	19.1
Systolic blood pressure, mm Hg	111 ± 9	125 ± 14	122 ± 14	130 ± 11
Diastolic blood pressure, mm Hg	66 ± 7	79 ± 12	76 ± 12	82 ± 10
Serum glucose, mmol/L	4.91 ± 0.40	5.43 ± 1.32	5.55 ± 1.54	5.24 ± 1.15
Serum insulin, mU/L	5.56 ± 2.07	13.83 ± 9.34	15.63 ± 8.36	9.53 ± 6.79
Serum LDL cholesterol, mmol/L	3.20 ± 0.80	3.63 ± 0.90	3.43 ± 0.89	3.33 ± 0.88
Serum HDL cholesterol, mmol/L	1.31 ± 0.30	1.10 ± 0.27	1.16 ± 0.30	1.27 ± 0.32
Serum triglycerides, mmol/L	1.12 ± 0.60	1.82 ± 0.99	1.87 ± 1.22	1.52 ± 0.90
Serum alanine aminotransferase, U/L	9.8 ± 6.5	17.6 ± 13.2	15.1 ± 11.9	13.7 ± 10.5
Serum γ-glutamyltransferase, U/L	21.4 ± 17.2	44.3 ± 38.4	36.0 ± 30.6	32.4 ± 36.5
SFAs[2]	31.9 ± 2.0	33.4 ± 2.6	33.7 ± 2.7	33.0 ± 2.5
Myristic acid, 14:0	1.10 ± 0.39	1.31 ± 0.44	1.40 ± 0.51	1.27 ± 0.45
Pentadecanoic acid, 15:0	0.24 ± 0.05	0.23 ± 0.05	0.24 ± 0.05	0.23 ± 0.06
Palmitic acid, 16:0	23.6 ± 1.8	25.0 ± 2.2	25.1 ± 2.3	24.6 ± 2.2
Stearic acid, 18:0	6.9 ± 0.7	6.9 ± 0.9	6.9 ± 0.8	6.9 ± 0.8
MUFAs	27.2 ± 2.9	29.2 ± 2.8	29.0 ± 3.2	28.3 ± 3.2

Palmitoleic acid, 16:1n-7	2.2 ± 0.8	2.7 ± 0.8	2.6 ± 0.9	2.5 ± 1.0
Oleic acid, 18:1n-9	23.1 ± 2.4	24.4 ± 2.7	24.4 ± 2.9	23.8 ± 2.7
Octadecenoic acid, 18:1n-7	1.7 ± 0.3	1.8 ± 1.4	1.8 ± 1.1	1.8 ± 1.0
Eicosenoic acid, 20:1n-9	0.17 ± 0.04	0.17 ± 0.04	0.18 ± 0.07	0.18 ± 0.05
Docosenoic acid, 22:1n-9	0.06 ± 0.04	0.06 ± 0.04	0.06 ± 0.04	0.06 ± 0.04
PUFAs	40.9 ± 3.9	37.5 ± 4.4	37.3 ± 4.8	38.7 ± 4.6
n-6 PUFAs	36.0 ± 3.7	32.7 ± 4.3	32.5 ± 4.5	33.9 ± 4.4
Linoleic acid, 18:2n-6	28.4 ± 3.5	25.2 ± 3.8	25.2 ± 4.1	26.4 ± 4.1
γ-Linolenic acid, 18.3n-6	0.37 ± 0.14	0.42 ± 0.14	0.40 ± 0.15	0.38 ± 0.14
Eicosadienoic acid, 20:2n-6	0.17 ± 0.04	0.16 ± 0.03	0.17 ± 0.04	0.17 ± 0.04
Dihomo-γ-linolenic acid, 20:3n-6	1.4 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.4 ± 0.3
Arachidonic acid, 20:4n-6	5.6 ± 1.1	5.3 ± 1.1	5.2 ± 1.1	5.4 ± 1.1
Docosatetraenoic acid, 22:4n-6	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03
n-3 PUFAs	4.8 ± 1.2	4.6 ± 1.2	4.6 ± 1.2	4.6 ± 1.2
α-Linolenic acid, 18:3n-3	0.91 ± 0.25	0.93 ± 0.26	0.98 ± 0.30	0.92 ± 0.26
Eicosatetraenoic acid, 20:4n-3	0.13 ± 0.06	0.16 ± 0.07	0.17 ± 0.07	0.15 ± 0.06
Eicosapentaenoic acid, 20:5n-3	1.1 ± 0.6	1.1 ± 0.5	1.1 ± 0.6	1.1 ± 0.5
Docosapentaenoic acid, 22:5n-3	0.51 ± 0.11	0.49 ± 0.11	0.47 ± 0.12	0.48 ± 0.11
Docosahexaenoic acid, 22:6n-3	2.1 ± 0.7	1.9 ± 0.6	1.9 ± 0.6	2.0 ± 0.7
PUFA/SFA ratio	1.3 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2
n-6/n-3 PUFA ratio	8.0 ± 2.0	7.5 ± 1.9	7.4 ± 1.9	7.7 ± 1.9
Serum total fatty acid concentration, mg/L				
SFAs	780 ± 210	990 ± 350	1000 ± 410	910 ± 310
MUFAs	670 ± 210	870 ± 340	870 ± 400	790 ± 310
n-6 PUFAs	860 ± 160	940 ± 210	930 ± 210	900 ± 180
n-3 PUFAs	120 ± 40	140 ± 60	140 ± 60	130 ± 50

[1]Values are mean ± SD.
[2]MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

SUPPLEMENTAL TABLE 2 Baseline characteristics of the participants without any metabolic outcome in 2001 or 2011, and in participants having incident obesity, high HOMA, non-alcoholic fatty liver and/or elevated blood pressure in 2011[1]

	No outcome n=437	Obesity n=163	High HOMA n=255	Fatty liver n=70	Elevated BP n=342
	Values	Value	Values	Values	Value
Sex, % females	63.2	55.8	54.1	50.0	52.0
Age, y (range 24 to 39 years)	31.9 ± 5.0	32.1 ± 4.8	32.1 ± 4.9	33.3 ± 4.9	32.1 ± 5.0
Body mass index, kg/m2	22.3 ± 2.1	27.7 ± 1.6	25.8 ± 3.9	23.9 ± 2.2	25.3 ± 4.5
Waist circumference, cm	76.4 ± 7.0	90.3 ± 8.0	85.8 ± 10.9	82.5 ± 6.2	84.9 ± 12.3
Physical activity index, 1 to 15	10.2 ± 2.3	9.9 ± 2.3	9.8 ± 2.4	9.3 ± 2.3	9.7 ± 2.4
Smokers, %	20.5	31.4	24.4	30.4	28.3
Systolic blood pressure, mm Hg	108 ± 9	119 ± 13	117 ± 13	119 ± 12	115 ± 9
Diastolic blood pressure, mm Hg	64 ± 7	73 ± 11	72 ± 11	71 ± 9	69 ± 7
Serum glucose, mmol/L	4.85 ± 0.39	5.09 ± 0.70	5.00 ± 0.69	5.12 ± 0.40	5.05 ± 0.73
Serum insulin, mU/L	5.06 ± 1.90	9.06 ± 5.26	6.82 ± 2.08	6.96 ± 3.17	7.85 ± 4.31
Serum LDL cholesterol, mmol/L	3.12 ± 0.74	3.32 ± 0.83	3.31 ± 0.82	3.10 ± 0.87	3.34 ± 0.86
Serum HDL cholesterol, mmol/L	1.36 ± 0.28	1.23 ± 0.29	1.25 ± 0.32	1.34 ± 0.31	1.27 ± 0.31
Serum triglycerides, mmol/L	0.97 ± 0.36	1.59 ± 1.40	1.37 ± 0.87	1.03 ± 0.41	1.41 ± 1.06
Serum alanine aminotransferase, U/L	8.3 ± 4.2	12.9 ± 10.0	12.4 ± 9.2	9.6 ± 5.0	10.9 ± 7.4
Serum γ-glutamyltransferase, U/L	17.0 ± 9.5	27.4 ± 18.2	26.7 ± 21.2	21.2 ± 9.7	26.0 ± 23.1
SFAs[2]	31.6 ± 1.9	32.8 ± 2.4	32.2 ± 2.2	32.2 ± 2.2	32.4 ± 2.4
Myristic acid, 14:0	1.06 ± 0.39	1.23 ± 0.45	1.16 ± 0.42	1.06 ± 0.43	1.18 ± 0.47
Pentadecanoic acid, 15:0	0.24 ± 0.06	0.23 ± 0.05	0.23 ± 0.05	0.23 ± 0.07	0.24 ± 0.05
Palmitic acid, 16:0	23.4 ± 1.8	24.3 ± 2.2	24.0 ± 2.1	24.0 ± 1.8	24.1 ± 2.2
Stearic acid, 18:0	7.0 ± 0.7	7.0 ± 0.9	6.9 ± 0.8	6.9 ± 0.7	6.9 ± 0.8
MUFAs	26.5 ± 2.6	28.4 ± 3.7	27.9 ± 3.0	27.3 ± 2.8	28.0 ± 3.1
Palmitoleic acid, 16:1n-7	2.1 ± 0.7	2.5 ± 0.9	2.3 ± 0.9	2.2 ± 0.8	2.3 ± 0.8
Oleic acid, 18:1n-9	22.5 ± 2.1	24.0 ± 3.2	23.6 ± 2.5	23.1 ± 2.4	23.7 ± 2.6
Octadecenoic acid, 18:1n-7	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.2	1.7 ± 0.2

Eicosenoic acid, 20:1n-9	0.16 ± 0.04	0.17 ± 0.09	0.17 ± 0.04	0.17 ± 0.05	0.17 ± 0.07
Docosenoic acid, 22:1n-9	0.06 ± 0.04	0.07 ± 0.05	0.06 ± 0.04	0.07 ± 0.04	0.06 ± 0.04
PUFAs	41.8 ± 3.5	38.8 ± 5.0	39.9 ± 4.3	40.5 ± 3.8	39.6 ± 4.6
n-6 PUFAs	36.8 ± 3.4	34.1 ± 4.7	35.1 ± 4.2	35.6 ± 3.5	34.8 ± 4.4
Linoleic acid, 18:2n-6	29.3 ± 3.3	26.4 ± 4.2	27.4 ± 3.9	27.6 ± 3.2	27.3 ± 4.0
γ-Linolenic acid, 18.3n-6	0.35 ± 0.13	0.41 ± 0.16	0.38 ± 0.15	0.39 ± 0.14	0.38 ± 0.15
Eicosadienoic acid, 20:2n-6	0.17 ± 0.04	0.17 ± 0.03	0.17 ± 0.03	0.16 ± 0.04	0.17 ± 0.03
Dihomo-γ-linolenic acid, 20:3n-6	1.4 ± 0.3	1.5 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
Arachidonic acid, 20:4n-6	5.6 ± 1.0	5.5 ± 1.3	5.6 ± 1.2	5.9 ± 1.3	5.5 ± 1.3
Docosatetraenoic acid, 22:4n-6	0.12 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03
n-3 PUFAs	4.9 ± 1.4	4.6 ± 1.2	4.7 ± 1.1	4.8 ± 1.2	4.7 ± 1.2
α-Linolenic acid, 18:3n-3	0.91 ± 0.24	0.90 ± 0.28	0.92 ± 0.27	0.84 ± 0.23	0.92 ± 0.28
Eicosatetraenoic acid, 20:4n-3	0.13 ± 0.06	0.15 ± 0.07	0.14 ± 0.06	0.13 ± 0.06	0.14 ± 0.07
Eicosapentaenoic acid, 20:5n-3	1.2 ± 0.6	1.1 ± 0.5	1.1 ± 0.5	1.2 ± 0.6	1.1 ± 0.6
Docosapentaenoic acid, 22:5n-3	0.51 ± 0.11	0.49 ± 0.11	0.50 ± 0.11	0.50 ± 0.10	0.50 ± 0.11
Docosahexaenoic acid, 22:6n-3	2.2 ± 0.7	1.9 ± 0.7	2.1 ± 0.6	2.1 ± 0.7	2.0 ± 0.7
PUFA/SFA ratio	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
n-6/n-3 PUFA ratio	8.0 ± 2.1	7.9 ± 2.1	7.8 ± 2.0	7.9 ± 2.0	7.9 ± 2.0
Serum total fatty acid concentration, mg/L					
SFAs	740 ± 160	910 ± 440	850 ± 280	750 ± 180	860 ± 330
MUFAs	620 ± 150	810 ± 470	740 ± 290	630 ± 160	760 ± 350
n-6 PUFAs	850 ± 140	900 ± 200	900 ± 180	810 ± 150	890 ± 190
n-3 PUFAs	110 ± 40	130 ± 70	120 ± 40	110 ± 40	120 ± 60

[1]Values are mean ± SD.
[2]MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

SUPPLEMENTAL TABLE 3 Pearson correlations between dietary intake of fatty acids vs. serum total fatty acids in Finnish adults (n=991)[1]

	log Serum SFAs, %	log Serum MUFAs, %	Serum n-6 PUFAs, %	log Serum n-3 PUFAs, %
SFAs/total FA intake	*r*= 0.14 *P*< 0.003	*r*= -0.01 NS	*r*= -0.02 NS	*r*= -0.16 *P*< 0.003
MUFAs/total FA intake	*r*= -0.07 *P*= 0.03	*r*= 0.05 NS	*r*= -0.01 NS	*r*= 0.02 NS
log n-6 PUFAs/total FA intake	*r*= -0.14 *P*< 0.003	*r*= 0.00 NS	*r*= 0.04 NS	*r*= 0.14 *P*= 0.004
log n-3 PUFAs/total FA intake	*r*= -0.14 *P*< 0.003	*r*= -0.11 *P*= <0.003	*r*= 0.03 NS	*r*= 0.40 *P*< 0.003
SFAs, E%	*r*= -0.04 NS	*r*= -0.12 *P*< 0.003	*r*= 0.15 *P*< 0.003	*r*= -0.13 *P*< 0.003
MUFAs, E%	*r*= -0.15 *P*< 0.003	*r*= -0.10 *P*= <0.003	*r*= 0.17 *P*< 0.003	*r*= -0.04 NS
log n-6 PUFAs, E%	*r*= -0.21 *P*< 0.003	*r*= -0.09 *P*= 0.005	*r*= 0.16 *P*< 0.003	*r*= 0.09 *P*= 0.006
log n-3 PUFAs, E%	*r*= -0.20 *P*< 0.003	*r*= -0.17 *P*< 0.003	*r*= 0.14 *P*< 0.003	*r*= 0.31 *P*< 0.003

[1]Shades of blue = levels of inverse association; shades of red = levels of direct association. MUFA, monounsaturated fatty acid; NS, $P \geq 0.05$; PUFA, polyunsaturated fatty acid; SFA=saturated fatty acid.

SUPPLEMENTAL TABLE 4 Odds ratios between serum total fatty acid percentages and hypertension in 199 hypertensive out of 2187 Finnish participants at baseline (cross-sectional data) and for 113 hypertensive out of 1088 participants in prospective analyses (incidence data)[1]

	Cross-sectional data (2001)				Prospective incidence data (2001-2011)			
	Age and sex-adjusted		Fully-adjusted		Age and sex-adjusted		Fully-adjusted	
	OR (95%CI)	*P*	*OR* (95%CI)	*P*	*OR* (95%CI)	*P*	*OR* (95%CI)	*P*
Total SFAs. %	1.53 (1.34, 1.76)	<0.003	1.09 (0.92, 1.29)	0.31	1.27 (1.06, 1.54)	0.011	1.09 (0.88, 1.35)	0.44
Myristic acid, 14:0	1.38 (1.19, 1.61)	<0.003	0.97 (0.81, 1.17)	0.75	1.08 (0.88, 1.32)	0.45	0.86 (0.67, 1.10)	0.22
Pentadecanoic acid, 15:0	0.83 (0.71, 0.96)	0.015	0.78 (0.66, 0.92)	0.003	0.85 (0.69, 1.04)	0.11	0.83 (0.67, 1.03)	0.09
Palmitic acid, 16:0	1.59 (1.38, 1.83)	<0.003	1.14 (0.96, 1.37)	0.14	1.40 (1.15, 1.69)	<0.003	1.23 (0.98, 1.55)	0.07
Stearic acid, 18:0	0.91 (0.79, 1.06)	0.25	0.97 (0.83, 1.14)	0.75	0.84 (0.68, 1.03)	0.09	0.88 (0.71, 1.08)	0.22
Total MUFAs, %	1.58 (1.36, 1.83)	<0.003	1.23 (1.00, 1.50)	0.048	1.45 (1.19, 1.77)	<0.003	1.34 (1.02, 1.77)	0.036
Palmitoleic acid, 16:1n-7	1.57 (1.35, 1.83)	<0.003	1.19 (1.00, 1.42)	0.052	1.41 (1.15, 1.72)	<0.003	1.25 (0.99, 1.56)	0.06
Oleic acid, 18:1n-9	1.41 (1.22, 1.64)	<0.003	1.05 (0.87, 1.28)	0.59	1.40 (1.15, 1.70)	<0.003	1.29 (0.97, 1.70)	0.08
Octadecenoic acid, 18:1n-7	1.19 (1.04, 1.37)	0.013	1.21 (1.02, 1.43)	0.025	1.00 (0.81, 1.23)	0.99	1.02 (0.83, 1.27)	0.83
Eicosenoic acid, 20:1n-9	1.25 (1.09, 1.45)	<0.003	1.17 (0.99, 1.38)	0.07	1.18 (0.97, 1.44)	0.09	1.15 (0.91, 1.44)	0.23
Docosenoic acid, 22:1n-9	0.85 (0.73, 0.98)	0.030	0.89 (0.76, 1.05)	0.17	0.84 (0.69, 1.03)	0.10	0.88 (0.71, 1.09)	0.24
Total PUFAs, %	0.60 (0.52, 0.68)	<0.003	0.80 (0.66, 0.98)	0.033	0.70 (0.58, 0.84)	<0.003	0.76 (0.58, 1.00)	0.05
n-6 PUFAs, %	0.61 (0.53, 0.70)	<0.003	0.85 (0.69, 1.04)	0.11	0.71 (0.59, 0.85)	<0.003	0.77 (0.58, 1.01)	0.06
Linoleic acid, 18:2n-6	0.60 (0.52, 0.69)	<0.003	0.86 (0.70, 1.04)	0.12	0.67 (0.55, 0.82)	<0.003	0.74 (0.57, 0.96)	0.023
γ-Linolenic acid, 18.3n-6	1.01 (0.87, 1.18)	0.87	0.87 (0.74, 1.03)	0.11	1.16 (0.94, 1.43)	0.17	1.05 (0.84, 1.31)	0.65
Eicosadienoic acid, 20:2n-6	0.92 (0.79, 1.08)	0.33	0.96 (0.81, 1.14)	0.63	0.87 (0.70, 1.08)	0.21	0.89 (0.71, 1.11)	0.30
Dihomo-γ-linolenic acid, 20:3n-6	1.00 (0.86, 1.17)	0.97	0.88 (0.75, 1.04)	0.13	1.04 (0.85, 1.27)	0.72	0.97 (0.78, 1.21)	0.81
Arachidonic acid, 20:4n-6	0.84 (0.72, 0.98)	0.023	1.05 (0.88, 1.25)	0.61	0.99 (0.81, 1.20)	0.89	1.13 (0.90, 1.41)	0.30
Docosatetraenoic acid, 22:4n-6	1.26 (1.10, 1.46)	<0.003	1.21 (1.04, 1.41)	0.013	1.18 (0.97, 1.43)	0.09	1.17 (0.96, 1.43)	0.12
n-3 PUFAs, %	0.82 (0.70, 0.95)	0.009	0.86 (0.73, 1.02)	0.08	0.84 (0.68, 1.03)	0.10	0.90 (0.73, 1.11)	0.32
α-Linolenic acid, 18:3n-3	1.00 (0.87, 1.16)	0.95	0.92 (0.78, 1.07)	0.29	0.93 (0.77, 1.13)	0.49	0.87 (0.71, 1.07)	0.20
Eicosatetraenoic acid, 20:4n-3	1.20 (1.03, 1.39)	0.021	0.94 (0.80, 1.11)	0.49	1.08 (0.88, 1.33)	0.44	0.95 (0.77, 1.17)	0.63
Eicosapentaenoic acid, 20:5n-3	0.82 (0.70, 0.95)	0.011	0.84 (0.71, 1.00)	0.046	0.90 (0.73, 1.11)	0.32	0.94 (0.76, 1.17)	0.59
Docosapentaenoic acid, 22:5n-3	0.83 (0.70, 0.97)	0.022	0.93 (0.79, 1.11)	0.43	0.84 (0.68, 1.05)	0.13	0.94 (0.75, 1.18)	0.62

Docosahexaenoic acid, 22:6n-3	0.82 (0.71, 0.95)	0.009	0.91 (0.78, 1.08)	0.29	0.80 (0.66, 0.98)	0.032	0.88 (0.71, 1.09)	0.24
PUFA/SFA ratio	0.59 (0.51, 0.68)	<0.003	0.84 (0.69, 1.02)	0.08	0.71 (0.59, 0.86)	<0.003	0.81 (0.63, 1.05)	0.11
n-6/n-3 PUFA ratio	0.90 (0.77, 1.05)	0.17	1.08 (0.91, 1.28)	0.37	0.99 (0.81, 1.22)	0.95	1.05 (0.85, 1.31)	0.64

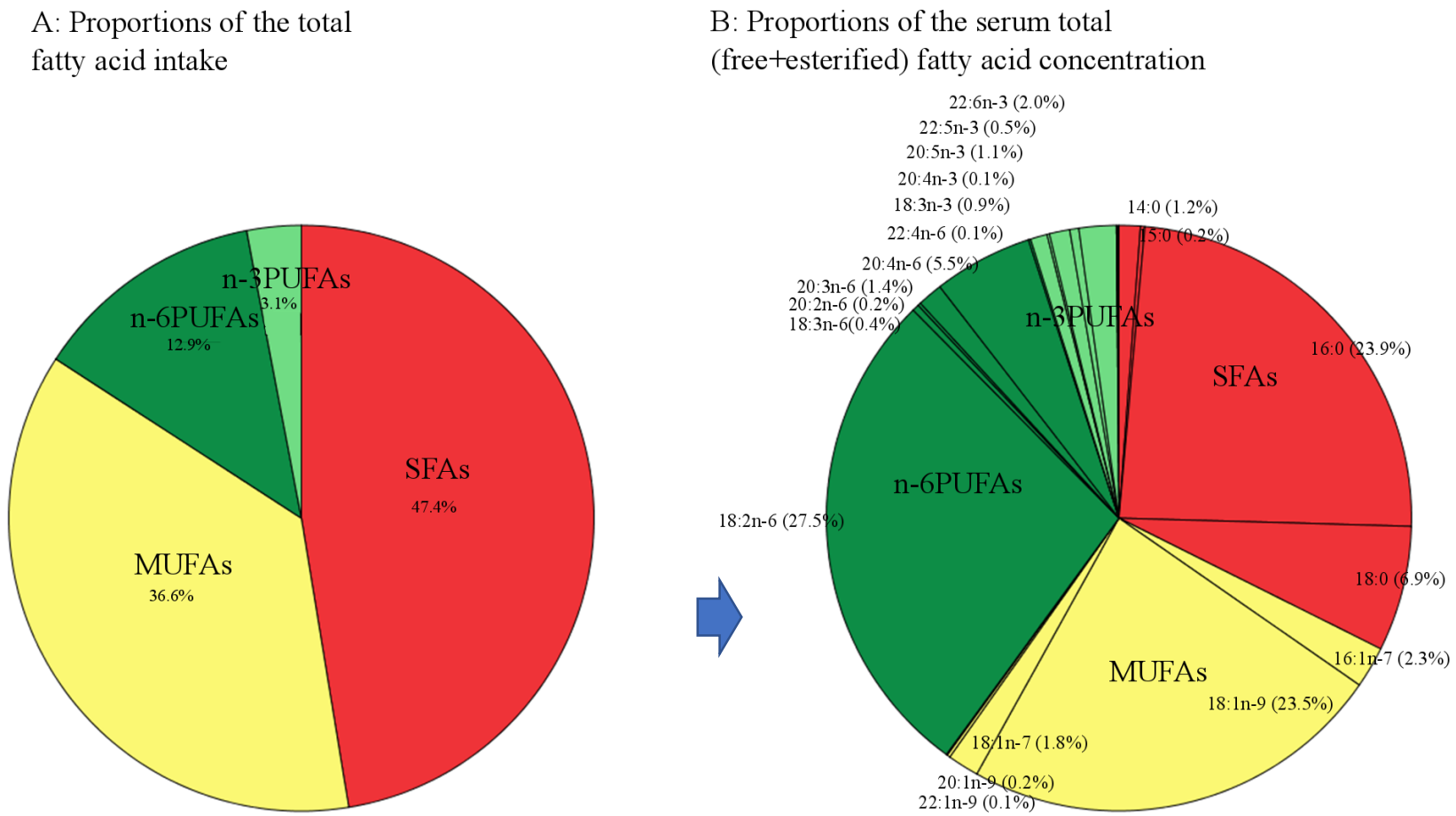
[1]In these models, participants were hypertensive if their systolic blood pressure was ≥140 mm Hg or diastolic blood pressure ≥ 90 mmHg or they had medication for hypertension. Fully-adjusted models were similar with the models used for blood pressure percentiles (Figures 1 and 3). MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA=saturated fatty acid.

SUPPLEMENTAL TABLE 5 Cross-sectional associations (odds ratios) of serum fatty acid concentrations (mg/L) with obesity among Finnish adults in 2001[1]

	Age and sex-adjusted		Fully-adjusted	
	OR (95%CI)	*P*	*OR* (95%CI)	*P*
Total SFAs	1.71 (1.51, 1.94)	<0.003	1.16 (0.96, 1.40)	0.11
Myristic acid, 14:0	1.62 (1.42, 1.84)	<0.003	1.62 (1.42, 1.84)	<0.003
Pentadecanoic acid, 15:0	1.36 (1.20, 1.55)	<0.003	1.35 (1.19, 1.54)	<0.003
Palmitic acid, 16:0	1.71 (1.51, 1.94)	<0.003	1.72 (1.51, 1.95)	<0.003
Stearic acid, 18:0	1.62 (1.42, 1.84)	<0.003	1.60 (1.41, 1.83)	<0.003
Total MUFAs	1.74 (1.53, 1.97)	<0.003	1.74 (1.53, 1.98)	<0.003
Palmitoleic acid, 16:1n-7	1.87 (1.64, 2.13)	<0.003	1.89 (1.66, 2.16)	<0.003
Oleic acid, 18:1n-9	1.67 (1.47, 1.90)	<0.003	1.67 (1.46, 1.90)	<0.003
Octadecenoic acid, 18:1n-7	1.49 (1.32, 1.69)	<0.003	1.51 (1.33, 1.72)	<0.003
Eicosenoic acid, 20:1n-9	1.34 (1.18, 1.52)	<0.003	1.35 (1.19, 1.53)	<0.003
Docosenoic acid, 22:1n-9	1.09 (0.96, 1.24)	0.20	1.08 (0.95, 1.23)	0.23
Total PUFAs	1.34 (1.19, 1.52)	<0.003	1.34 (1.18, 1.51)	<0.003
n-6 PUFAs	1.33 (1.17, 1.50)	<0.003	1.31 (1.16, 1.49)	<0.003
Linoleic acid, 18:2n-6	1.19 (1.06, 1.35)	0.004	1.19 (1.05, 1.34)	0.007
γ-Linolenic acid, 18.3n-6	1.76 (1.53, 2.03)	<0.003	1.74 (1.50, 2.01)	<0.003
Eicosadienoic acid, 20:2n-6	1.38 (1.22, 1.57)	<0.003	1.39 (1.22, 1.58)	<0.003
Dihomo-γ-linolenic acid, 20:3n-6	1.99 (1.73, 2.29)	<0.003	1.96 (1.70, 2.26)	<0.003
Arachidonic acid, 20:4n-6	1.43 (1.27, 1.62)	<0.003	1.40 (1.24, 1.58)	<0.003
Docosatetraenoic acid, 22:4n-6	1.60 (1.40, 1.83)	<0.003	1.57(1.37, 1.80)	<0.003
n-3 PUFAs	1.31 (1.16, 1.50)	<0003	1.37 (1.20, 1.56)	<0.003
α-Linolenic acid, 18:3n-3	1.38 (1.21, 1.56)	<0.003	1.38 (1.21, 1.57)	<0.003
Eicosatetraenoic acid, 20:4n-3	1.72 (1.50, 1.96)	<0.003	1.72 (1.50, 1.98)	<0.003
Eicosapentaenoic acid, 20:5n-3	1.29 (1.13, 1.47)	<0.003	1.33 (1.16, 1.52)	<0.003
Docosapentaenoic acid, 22:5n-3	1.39 (1.20, 1.59)	<0.003	1.38 (1.20, 1.60)	<0.003
Docosahexaenoic acid, 22:6n-3	1.15 (1.01, 1.31)	0.039	1.21 (1.06, 1.39)	0.005
PUFA/SFA ratio	0.56 (0.49, 0.63)	<0.003	0.55 (0.49, 0.63)	<0.003
n-6/n-3 PUFA ratio	0.85 (0.74, 0.97)	0.014	0.79 (0.69, 0.91)	<0.003

[1]Values are odd ratios and their 95% confidence intervals per 1-SD increment in the fatty acid measures (logistic regression). Outcome variables included prevalent obesity (BMI>30 kg/m^2 vs. ≤30 kg/m^2, n=271 obese out of 2200 participants). Each fatty acid measure was tested separately in the logistic regression models adjusted for sex and age, and additionally for the outcome-specific cardiometabolic risk or preventive factors (fully adjusted models). MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

SUPPLEMENTAL FIGURE 1 Fatty acid percentages in daily intake and in serum total concentration among Finnish adults, the year 2001 data (n=991)[1]



[1]MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; SFA=saturated fatty acid.