Lipoprotein (a) concentration is associated with plasma arachidonic acid in subjects with familial hypercholesterolemia

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Abstract

Elevated lipoprotein (a) (Lp[a]) is associated with cardiovascular disease (CVD) and is mainly genetically determined. Studies suggest a role of dietary fatty acids (FAs) in the regulation of Lp(a), however, no studies have investigated the association between plasma Lp(a) concentration and omega-6 FAs. We aimed to investigate whether plasma Lp(a) concentration was associated with dietary omega-6 FA intake, and plasma levels of arachidonic acid in subjects with familial hypercholesterolemia (FH). We included FH subjects with (n=68) and without (n=77) elevated Lp(a) defined as ≥75 nmol/L, and healthy subjects (n=14). Total fatty acid profile was analyzed by Gas Chromatography-Flame Ionization Detector analysis, and the daily intake of macronutrients (including the sum of omega-6 FAs: 18:2n-6, 20:2n-6, 20:3n-6 and 20:4n-6) were computed from completed food frequency questionnaires. FH subjects with elevated Lp(a) had higher plasma levels of arachidonic acid (AA) compared to FH subjects without elevated Lp(a) (P=0.03). Furthermore, both FH subjects with and without elevated Lp(a) had higher plasma levels of AA compared to controls (P<0.001). The multivariable analyses showed associations between dietary omega-6 FA intake and plasma levels of AA (P=0.02), and between plasma levels of Lp(a) and AA (P=0.006). Our data suggest a novel link between plasma Lp(a) concentration, dietary omega-6 FAs and plasma AA concentration, which may contribute to explain the small diet-induced increase in Lp(a) levels associated with lifestyle changes. Although the increase may not be clinically relevant, this association may be mechanistically interesting in understanding more of the role and regulation of Lp(a).

Introduction

Lipoprotein (a) (Lp[a]) is a low density lipoprotein (LDL)-like particle bound to apolipoprotein (a).[1] Increasing evidence supports elevated Lp(a), which may be defined as >75 nmol/L or 30 mg/dL, for being an independent and important risk factor for cardiovascular disease (CVD).[2] The plasma level of Lp(a) is considered to be determined mostly by the *LPA* gene locus,[3] however, lifestyle-induced changes in the plasma level of Lp(a) have been reported.[4; 5; 6; 7; 8]

Dietary fatty acids (FAs) differently impact CVD risk through divergent effects on the lipid profile.[9] Despite a large body of evidence documenting the beneficial effect of replacing saturated FAs (SFA) with polyunsaturated FAs (PUFA) in the prevention of CVD,[10; 11] recent data from Chowdhury *et al* and Ramsden *et al*, have reported conflicting results and concluded that there is no clear support of replacing SFA with PUFA. However, in 2017, the American Heart Association presidential advisory on dietary fats and CVD, strongly concluded that the incidence of CVD will be reduced when the intake of SFA is replaced with PUFA.[12] Nevertheless, one of the cornerstones in diet recommendations when performing lifestyle changes and in the prevention of CVD, is replacing SFA with PUFA.[13] The essential omega-6 (n-6) PUFA linoleic acid is the predominant dietary PUFA, and lower tissue/blood concentrations of linoleic acid have been shown to be inversely associated with CVD risk,[14] and mortality.[15] Furthermore, Marklund *et al* recently concluded that higher circulating/tissue levels of linoleic acid and possibly arachidonic acid were inversely associated with risk of major cardiovascular events after analyzing 30 prospective studies from 13 countries.[16] However, some inconsistent data have also been reported. In the reanalysis of the Sydney Diet Heart study, it was shown that the intervention group had higher rates of death than controls,[17] and in a Mendelian randomization study, genetically predicted linoleic acid was shown not to be associated with ischemic heart disease, but was associated with lower diabetes risk.[18] Recently, Berk *et al* found increased Lp(a) levels elicited by diet-induced weight loss after a low-fat diet in overweight and obese subjects.[4] However, they found no change in Lp(a) levels after bariatric surgery[4], suggesting a role of dietary FAs in the regulation of Lp(a), rather than weight loss *per se*. Indeed, randomized controlled trials (RCTs) have found Lp(a)-increasing effects of low-fat versus low carbohydrate diets.[5; 6; 7; 8]

Subjects with familial hypercholesterolemia (FH) are characterized by increased plasma levels of total- and LDL-cholesterol, accelerated atherosclerosis and increased risk of premature CVD,[19; 20] and may have higher Lp(a) levels than unaffected relatives.[21] Thus, FH subjects may serve as a suitable human model to investigate underlying mechanisms of Lp(a) modification.

To our knowledge, no studies have investigated whether there is an association between Lp(a) levels and n-6 PUFAs. Although the magnitude of the effect may not be clinically relevant, this association may be mechanistically interesting in understanding more of the role and the regulation of Lp(a). We aimed to investigate whether plasma Lp(a) concentration was associated with dietary omega-6 FA intake and plasma levels of arachidonic acid in subjects with FH.

Methods

Subjects and study design

In this cross-sectional study, we invited FH subjects (>18 years of age) with or without elevated Lp(a) as defined by Lp(a) ≥ or <75 nmol/L, respectively, who were regularly followed-up at the outpatient Lipid Clinic, Oslo University Hospital, Norway. Other inclusion criteria were a definite FH diagnosis as defined by a positive DNA test (genetic FH) or a Dutch Lipid Clinic Network score >8[19] (clinical FH) and willingness to give a blood sample. All FH subjects continued with their current lipid-lowering therapy during the study. Exclusion criteria were: diabetes mellitus type 1, or pregnant or lactating women. The study visit was coordinated simultaneously with their next prescheduled consultation at the Lipid Clinic. Age- and sex-matched (by percentage) healthy controls were recruited among employees and friends of employees at the Department of Nutrition, University of Oslo, Norway and Oslo University Hospital, Norway. Exclusion criteria for the controls were: Lp(a) levels ≥75 nmol/L, cardiovascular or metabolic disease, use of lipid-lowering therapy, severe illness such as cancer the last 5 years, or pregnant or lactating women. All participants were recruited in the period September 2016-September 2017. For all participants, a non-fasting blood sample was obtained, and weight, height, and blood pressure were measured. Informed consent was obtained from all the participants. The study protocol was approved by the Regional Committee of Medical and Health Research Ethics, south-east region of Norway (no. 2015/1577), and by the Privacy Ombudsman at Oslo University Hospital. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Total plasma fatty acid profile, FA ratios and other plasma analyses

Plasma was obtained by EDTA tubes and kept dark, on ice until centrifugation at 4ºC for 15 minutes, before aliquoted and stored at -80ºC until further analysis. We analysed total plasma fatty acid profile by Gas Chromatography-Flame Ionization Detector analysis at the commercial laboratory Vitas Analytical Services as previously described,[22] and show the results as percentage of total fatty acids. We estimated certain ratios between the product and the precursor of the individual fatty acids in plasma (γ-linolenic acid [18:3n-6]/linoleic acid [18:2n-6]; arachidonic acid

[20:4n-6]/dihomo γ-linolenic acid [20:3n-6], and eicosadienoic acid [20:2n-6]/linoleic acid 18:2n-6]), as described elsewhere.[23] The plasma n-6/n-3 ratio was calculated as the total percentage of n-6 (linoleic acid, γ-linolenic acid, eicosadienoic acid, dihomo γ-linolenic acid and arachidonic acid) divided by the total percentage of n-3 fatty acids (α-linolenic acid, eicosapentaenic acid, n-3 docosapentaenic acid and docosahexaenic acid). Lp(a) was analysed by an immunoturbidimetric method by Roche Diagnostics at an accredited medical laboratory, Oslo University Hospital, Rikshospitalet, Oslo, Norway (NS-EN ISO 15189:2007). Other biochemical analyses were measured in plasma or serum by standard methods at the same medical laboratory.

Dietary intake

Nutrient intake was recorded by a self-administered 256-item food frequency questionnaire (FFQ) completed according to a description, both received by mail. In case of incorrect completion, the participants were interviewed at the study visit or by phone. The FFQ is developed at the Department of Nutrition, University of Oslo, Norway and has been validated and described in detail elsewhere.[24] Briefly, intake frequencies (monthly, weekly or daily) and portion sizes in predefined household units were registered. Open spaces were available for description of unlisted food items, which were included in the nutrient calculations. The food database AE-14 and the software "Kostberegningssystem" (version 7.3, 2017) were used to compute the daily intake of energy and nutrients. The food database used (AE-14) is based on the official Norwegian Food Composition Table (http://www.norwegianfoodcomp.no/), supplemented with data from calculated recipes and other databases, and has a large number of fatty acids including n-6 PUFAs. Total dietary n-6 PUFA is the sum of 18:2n-6, 20:2n-6, 20:3n-6 and 20:4n-6.

Statistics

Kruskal-Wallis test was used for all comparisons between the three groups as regards continuous variables. Pair-wise comparisons (posthoc tests) were performed with Mann-Whitney test, and presented both unadjusted and Bonferroni-adjusted. The results are presented as medians (25th - 75th percentile) in the tables and the text, and median (min-max) in figures. Chi-square test or Fisher's exact test were used for categorical data and presented as frequency (percentage) in tables. Linear regression analysis was performed to study the association between the dietary intake of total n-6 PUFA, linoleic acid and arachidonic acid (all in energy percentage [E%]), and plasma arachidonic acid after adjustment for potential confounding variables among FH patients that completed FFQ. Furthermore, logistic regression analysis was used to adjust for potential confounding variables when studying the association between plasma arachidonic acid and plasma Lp(a) concentration. Results are presented as regression coefficients and odds ratios (ORs),

respectively, with 95% confidence intervals (CIs). The regression analyses were guided by directed acyclic graphs. Statistical analyses were performed by IBM SPSS Statistics 24.ink. P-values (two-tailed) <0.05 were considered significant.

Results

Characteristics

In total, FH subjects with (n=68) and without (n=77) elevated Lp(a) levels defined as ≥ and < 75 nmol/L plasma Lp(a), and 14 healthy controls were included in the study (Supplementary Figure 1). All the FH subjects had a genetically verified diagnosis except for four who had clinical FH[19] (Table 1). All FH patients used lipid-lowering therapy, and had generally received dietary counselling as part of their standard follow-up at the Lipid Clinic. Both groups of FH subjects had significantly higher body mass index (BMI) (P>0.001) and systolic blood pressure (P=0.003) than the healthy controls. More FH subjects with elevated Lp(a) levels had experienced CVD (P=0.02) and were more often treated with PCSK9-inhibitors (P=0.01) than the FH subjects without elevated Lp(a). Although blood samples were taken non-fasting, median triglyceride levels were not elevated according to European guidelines, where triglyceride levels ≥1.7 mmol/L is considered elevated.[25] However, 19.1%, 19.5% and 14.3% of the FH subjects with elevated Lp(a), FH subjects without elevated Lp(a) and controls had levels ≥1.7 mmol/L, respectively.

Plasma FA profile and ratios of FAs

For plasma levels of n-6 PUFAs, we found several significant differences between the groups (Figure 1). First, we observed higher level of arachidonic acid (20:4n-6) in FH subjects with elevated Lp(a) compared to FH subjects without elevated Lp(a) (P=0.03, Figure 1a). Furthermore, the levels of arachidonic acid (20:4n-6), dihomo-γ-linolenic acid (20:3n-6) and γ-linolenic acid (18:3n-6) were all higher in both FH groups compared to the healthy controls (P<0.001 for all, Figure 1a, b, c, respectively). Contrary, the level of linoleic acid (18:2n-6) was lower in both FH groups compared to healthy subjects (P<0.001 for both, Figure 1d), and the level of eicosadienoic acid (20:2n-6) was lower in FH with elevated Lp(a) compared to healthy subjects (P<0.01, Figure 1e). For the plasma levels of n-3 PUFA (Table 2), we found lower levels of α-linolenic acid (18:3n-3) in both FH groups compared to the healthy controls (P<0.005 for both). The level of docosahexaenoic acid (22:6n-3) was lower in the FH subjects without elevated Lp(a) than in the healthy controls (P=0.02). A few significant differences between the groups were also observed among the plasma levels of monounsaturated fatty acids (MUFA) and SFA (Table 2).

In order to investigate the n-6 PUFA pathway more in detail, we estimated the ratios of certain n-6 PUFAs (as described in methods). The ratios 18:3n-6/18:2n-6 and 20:4n-6/20:3n-6 were higher in both FH groups than in the healthy controls (P<0.05 for all, Figure 2a).

Dietary intake and dietary pattern

FH subjects with elevated Lp(a) had higher intake of dietary fibre (E %) and lower intake of cholesterol (mg) compared to FH subjects without elevated Lp(a), and lower intake of trans-fatty acids (E %) than healthy controls (P=0.03 for all, Table 3). There was an overall significant difference in SFA intake between the three groups (P=0.03), however, no significant differences were observed in the pair-wise comparisons with Bonferroni-adjustment. We found no significant differences between the three groups regarding MUFA, total PUFA, n-3 or n-6 intake (E % for all, Table 3). In order to explain the differences in dietary intake between the FH groups, we explored dietary pattern between FH subjects with and without elevated Lp(a). Compared to FH subjects without elevated Lp(a), FH subjects with elevated Lp(a) had lower intake of cakes 7.9 (2.1-18.8) vs. 13.6 (7.5-21.4) g/day P=0.006; sweets 12.0 (4.6-29.1) vs 18.8 (12.0-38.2) g/day P=0.02, and non-significantly lower intake of cheese 22.2 (12.7-35.1) vs 28.2 (16.0-49.2) g/day P=0.08. There was no difference in total intake of food (g/day) between the FH groups (data not shown).

Multivariable analyses

The results of multiple linear regression analyses of intake of n-6 PUFA in relation to plasma arachidonic acid among FH subjects (n=139) were unchanged after adjustment for age, sex and BMI (crude regression coefficients: 0.32 [95% CI 0.05, 0.60], P=0.02 and adjusted regression coefficients: 0.32 [95% CI 0.05, 0.59], P=0.02, for every E % change in n-6 PUFA). Furthermore, the results of multiple linear regression analyses of intake of linoleic acid in relation to plasma arachidonic acid among FH subjects (n=139) were unchanged after adjustment for age, sex and BMI (crude regression coefficients: 0.32 [95% CI 0.05, 0.59], P=0.02 and adjusted regression coefficients: 0.32 [95% CI 0.05, 0.60], P=0.02, for every E % change in linoleic acid). The results of multiple linear regression analyses of intake of arachidonic acid in relation to plasma arachidonic acid among FH subjects (n=139) were unchanged after adjustment for age, sex and BMI (crude regression coefficients: 4.9 [95% CI -11.3, 21.1], P=0.55 and adjusted regression coefficients: 0.79 [95% CI -15.8, 17.3], P=0.93, for every E % change in arachidonic acid).

The results of the logistic regression analyses comparing plasma arachidonic acid levels between the two FH groups were unchanged after adjustment for age, sex, BMI, use of PCSK9-inhibitors and cholesterol intake (crude and adjusted ORs were 1.29 [95 % CI 1.08, 1.54], P=0.005 and 1.31

[95 % CI 1.08, 1.58], *P*=0.006 for every unit change in plasma arachidonic acid for FH subjects with elevated Lp[a] versus FH subjects without elevated Lp[a]).

Discussion

In the present study, we found that FH subjects with elevated Lp(a) levels had higher plasma levels of arachidonic acid compared to FH subjects without elevated Lp(a). Furthermore, both FH subjects with and without elevated Lp(a) had higher plasma levels of arachidonic acid compared to controls. Additionally, the associations between n-6 PUFA intake and plasma arachidonic acid, and between plasma arachidonic acid and Lp(a) in FH subjects, further support our hypothesis of a relationship between n-6 PUFA and Lp(a). We propose a novel link between n-6 PUFA intake, plasma arachidonic acid and elevated Lp(a) levels.

Arachidonic acid and other higher n-6 PUFA derivatives are derived during desaturation and elongation processes from the precursor and essential FA linoleic acid,[26] however, the plasma levels of arachidonic acid may also depend on other processes such as turnover, oxidation etc.[27] Our results from both univariable and multivariable analyses suggest an association between plasma arachidonic acid level and plasma Lp(a) level in FH subjects. Furthermore, the multivariable analyses show associations between plasma arachidonic acid and n-6 PUFA intake in FH subjects, whereof dietary linoleic acid, but not arachidonic acid, as expected, is the n-6 PUFA associated with plasma arachidonic acid level. These results support that linoleic acid, which is the most abundant n-6 PUFA in the diet, is the driver of the association between dietary n-6 PUFA and plasma level of arachidonic acid. Collectively, this may support an association between plasma Lp(a) concentration, dietary n-6 PUFAs and plasma arachidonic acid level. Consistent with our results, Hikita *et al* have shown higher plasma Lp(a) concentration and lower ratio of eicosapentaenoic acid/arachidonic acid in patients with compared to without increased risk of CVD.[28] Recently, lipid apheresis was found to reduce linoleic acid and arachidonic acid in plasma of hyperlipidemic patients, concomitant with a reduction in Lp(a) levels, further indicating an indirect link between n-6 PUFAs and lipoproteins such as LDL and Lp(a).[29; 30] Previously, Li *et al* showed that omnivores had both higher Lp(a) levels and higher concentration of arachidonic acid in serum phospholipids,[31] possibly due to increased direct availability of arachidonic acid from their meat intake. However, the present study is, to our knowledge, the first to show a link between n-6 PUFA intake, plasma arachidonic acid and plasma Lp(a) concentration in FH subjects.

The enzymes Δ-6 desaturase (D6D, rate-limiting) and D5D convert linoleic acid to γ-linolenic acid, and dihomo-γ-linolenic acid to arachidonic acid, respectively, and the ratios 18:3n-6/18:2n-6 and 20:4n-6/20:3n-6 may be used as surrogate markers of D6D and D5D activities, respectively.[23]

Thus, our results may suggest higher D6D and D5D activity leading to lower plasma levels of linoleic acid and higher levels of downstream n-6 PUFAs such as γ-linolenic acid, dihomo- γ-linolenic acid and arachidonic acid in FH subjects compared to control subjects. Contrary, plasma levels of eicosadienoic acid were lower in FH subjects compared with controls. The conversion of linoleic acid to eicosadienoic acid is a "side track" from the direct conversion towards arachidonic acid[32], possibly indicating a preferred metabolism towards arachidonic acid in FH subjects. Desaturases are regulated through the transcription factor, sterol regulatory element binding protein-1c, by dietary fat and cholesterol.[33] Lower dietary intake of SFA and cholesterol may potentially be surrogate markers of higher PUFA intake, and may thus differentially impact the regulation of D6D in FH subjects with elevated Lp(a). Thus, FA regulation of D6D may also partially explain the previously observed increased plasma levels of Lp(a) after lifestyle-induced, but not bariatric, weight reduction in the study by Berk *et al*,[4] further supporting the possible biological link between Lp(a) and arachidonic acid. However, the link between n-6 PUFA and Lp(a) needs further investigation.

Common for the previously mentioned dietary RCTs, the intervention groups lowered the intake of SFA, but also increased the intake of carbohydrates [5; 6; 7; 8], in particular dietary fibre intake[6; 8]. In line with this, we also find a higher intake of dietary fibre between the FH subjects with versus without elevated Lp(a). Hence, it cannot be ruled out that the diet-induced Lp(a) response may be a synergy effect of lowering SFA/increasing PUFA and increasing dietary fibre. In the present study it is likely that all FH individuals received the same dietary advices regarding their diagnosis. However, the adherence to a beneficial diet is potentially better in the FH subjects with elevated Lp(a) concentration since they had experienced more events of CVD and were more often treated with PCSK9-inhibitors, potentially increasing the awareness of their increased CVD risk. Data on dietary pattern showed a lower intake of cakes, sweets and cheese in FH subjects with elevated Lp(a) compared to FH subjects without elevated Lp(a), indicating healthier food choices among FH subjects with elevated Lp(a). Although being mechanistically interesting, the diet-induced increase in plasma Lp(a) concentration is, however, probably less clinically significant since a reduction of approximately 100 mg/dL (~240 nmol/L when converted according to Gencer et al[34]) in Lp(a) in a short-time perspective may be required to reduce CVD risk with the same magnitude achieved by lowering LDL-cholesterol by 1 mmol/L.[35]

The FA content in phospholipids is dominated by n-6 PUFAs, in particular linoleic acid and arachidonic acid, rather than n-3 PUFAs.[36] Since, Lp(a) transport more than 90% of oxidized phospholipids (OXPL) in plasma, representing an atherogenic feature,[37] and there is an association between a dietary marker of increased PUFA intake and the proportion of different phospholipids,

[38] it is tempting to speculate that increased levels of arachidonic acid change the proportion of phospholipids, possibly increasing OXPL. Furthermore, linoleic and arachidonic acid have been shown to be antagonists of farnesoid X receptor (FXR),[39] and FXR activation has been shown to decrease Lp(a).[40] Thus, n-6 PUFA may inhibit FXR, mediating an increase in Lp(a) required for transporting increased OXPL, and this small increase in Lp(a) could therefore represent a counteracting and even atheroprotective mechanism mediated by n-6 PUFA.

Major strengths of the study are that we show associations between plasma arachidonic acid and Lp(a) concentrations in both univariable and multivariable analyses. Further, we included mainly genetically verified FH subjects with and without elevated Lp(a) with dietary data. A major limitation is the low number of control subjects. The main aim of the study was to compare FH subjects with and without elevated Lp(a) levels, however, we chose to include a small number of healthy controls to have a reference "point" since a number of the analyses we measured (including the plasma fatty acid profile) does not have established prespecified cut-off points. Other limitations are that the study is explorative and hypothesis generating; the use of FA ratios as markers of enzyme activities since the plasma level of the product and the precursor FAs may be biased by synthesis, turnover, oxidation, medication etc; and measuring plasma FAs as relative rather than absolute concentrations. However, a major scope of the study was to explore the relation between Lp(a) concentration and plasma levels of specific FAs. Thus, we believe that relative values of FA are more meaningful in the present manuscript. Furthermore, FAs in red blood cells would have been a better marker for long-term diet intake reflecting FFQ data, compared to plasma FAs from non-fasting blood samples that mainly reflect short-term dietary intake.[41] Nevertheless, the FH patients had generally been followed-up at the Lipid Clinic for years and received dietary counselling as part of their treatment. Furthermore, we have previously shown a more beneficial diet in children and young adults with FH compared to the general population.[42] Also, we recently showed that 87% of all FH adults, that had been treated at 3 Norwegian lipid clinics, received dietary counselling.[43] Dietary pattern was measured using a score divided into three categories. LDL-C levels were lower among those with a diet score in the healthiest category at last visit, than in subjects with a score in the most unhealthy category (3.2 [1.2] vs. 4.4 [2.1] mmol/L, $P<0.001$). After follow-up at the lipid clinics, the number of subjects with a diet score in the healthiest diet category doubled. These data are also supported by data from the SAFEHEART-study which showed that adults with FH have healthier dietary habits with lower consumption of SFA compared to non-FH.[44] Taken together, this may support the notion that FH subjects have long-lasting improved dietary habits, which may be reflected in plasma FAs even if plasma FAs mainly reflect short-term dietary intake.

In conclusion, FH subjects with elevated plasma Lp(a) levels had higher plasma levels of the n-6 PUFA arachidonic acid. Furthermore, dietary n-6 PUFA intake was associated with plasma arachidonic acid, where dietary linoleic acid seemed to be the main driver of the dietary n-6 PUFAs in this association. Our data suggest a novel link between plasma Lp(a) concentration, dietary omega-6 FAs and plasma arachidonic acid concentration, which may contribute to explain the small diet-induced increase in Lp(a) levels associated with lifestyle changes. Although the increase may not be clinically relevant, this association may be mechanistically interesting in understanding more of the role and the regulation of Lp(a).

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Conflict of Interest

Dr. Bogsrud has received research grants and/or personal fees from Amgen, Sanofi, MSD, Boehringer Ingelheim, Mills DA and Kaneka, none of which are related to the content of this manuscript. Dr. Retterstøl has received research grants and/or personal fees from Amgen, Mills DA, The Directorate for Health in Norway, The Norwegian Medical Association, Sanofi, Chiesi, Takeda, Bayer, MSD, none of which are related to the content of this manuscript. Dr. Ulven has received research grants and/or personal fees from Mills DA, Tine BA, and Rimfrost, none of which are related to the content of this manuscript. Dr Roeters van Lennep reports honoraria from Akcea and grants from Aegerion/Amryt none of which are related to the content of this manuscript. Dr. Holven reports grants and/or personal fees from Tine SA, Mills DA, Olympic Seafood, Amgen, Sanofi, Kaneka and Pronova, none of which are related to the content of this manuscript. The other authors have no financial relationships relevant to disclose.

Authorship

Authors' contribution: I.N., M.P.B., and K.B.H. conceived and designed research; I.N., M.P.B., L.K.L.Ø., T.U., and K.B.H. conducted research; I.N., L.K.L.Ø., M.B.V. and K.B.H. performed

statistical analyses; I.N., M.P.B., L.K.L.Ø., S.M.U., K.R., M.M., J.R.v.L., B.H., P.A., M.B.V. and K.B.H., interpreted results; I.N., M.P.B., M.B.V., and K.B.H were responsible for drafting the manuscript; I.N., M.P.B., M.B.V. and K.B.H. were responsible for final content; all authors read, critically revised, and approved the final manuscript.

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Legends

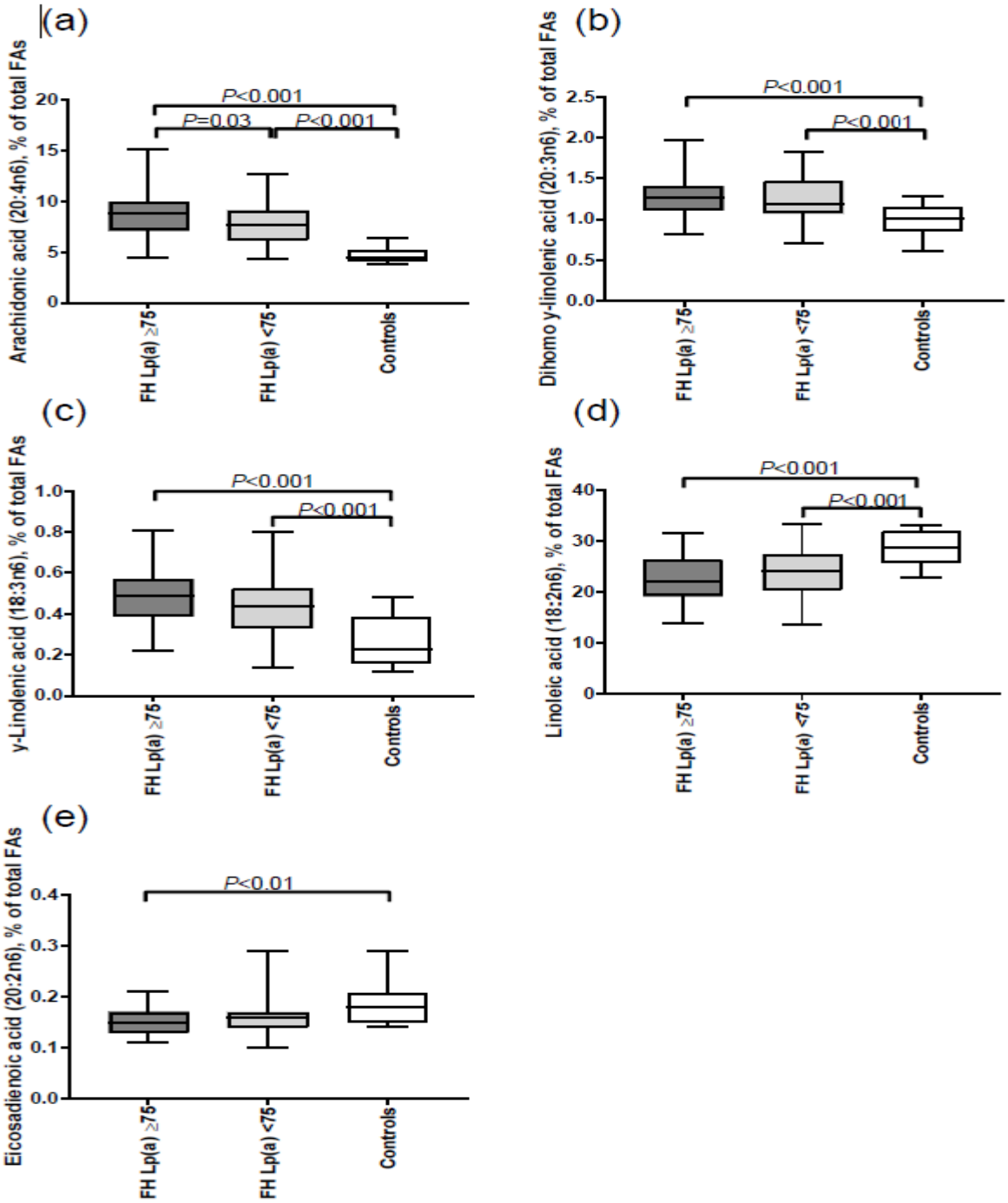

Figure 1. Plasma levels of arachidonic acid (a), dihomo-γ-linolenic acid (b), γ-linolenic acid (c), linoleic acid (d) and eicosadienoic acid (e) in FH subjects with (n=68) or without (n=77) elevated Lp(a) and healthy controls (n=14). Data are analysed by Kruskal-Wallis test with Bonferroni corrected posthoc comparisons between the groups when significant and given as median (min-max) percent of total fatty acids. FAs, fatty acids; FH familial hypercholesterolemia; Lp(a), lipoprotein (a)

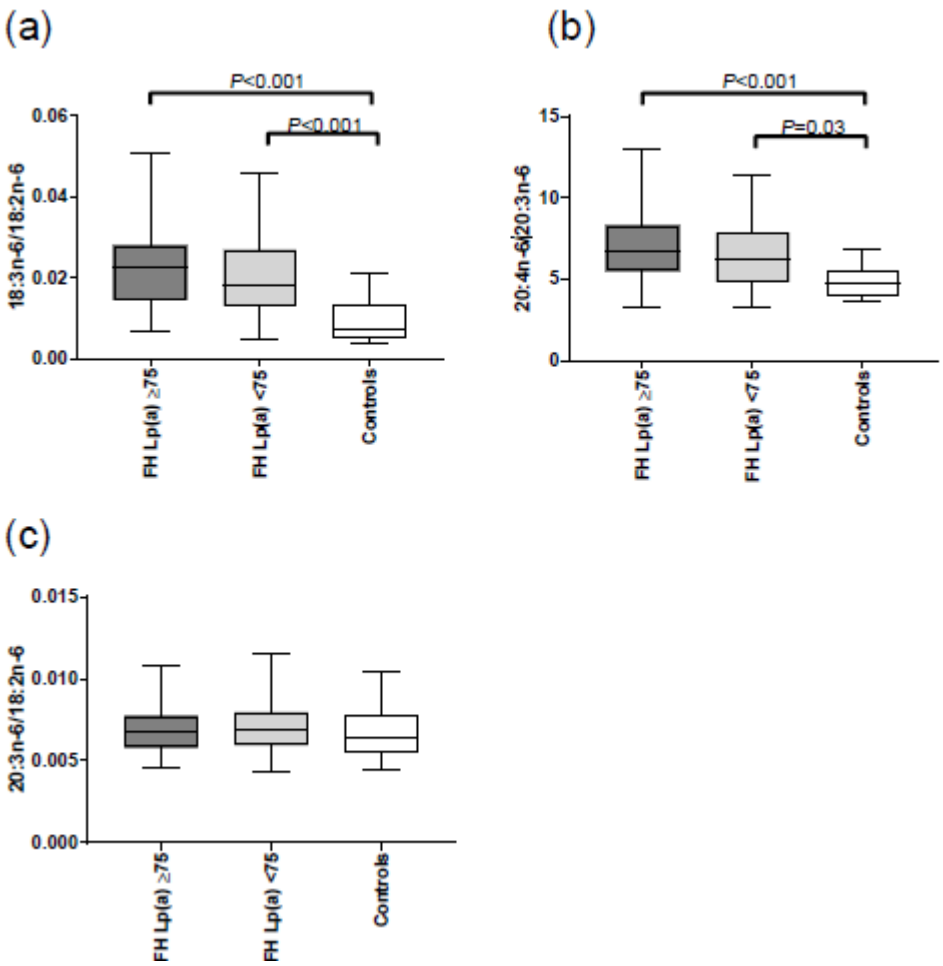

Figure 2. Estimated ratios as surrogate markers of delta 6 desaturase (a), delta 5 desaturase and elongase 5 in FH subjects with (n=68) or without (n=77) elevated Lp(a) and healthy controls (n=14). The ratios as calculated as product divided by precursor as indicated on the y-axes. Data are analysed by Kruskal-Wallis test with Bonferroni corrected posthoc comparisons between the groups when significant and given as median (min-max). FH familial hypercholesterolemia; Lp(a), lipoprotein (a).

Supplementary Figure 1. Flow chart showing the recruitment process of the cross-sectional study. FH familial hypercholesterolemia; Lp(a), lipoprotein (a).

Table 1. Characteristics of the participants

	FH subjects				Healthy subjects		P*	Unadjusted			Adjusted		
	Lp(a) ≥ 75 nmol/L		Lp(a) < 75nmol/L					P†	P‡	P§	P†	P‡	P§
	n=68		n=77		n=14								
Descriptives													
Age, years	48	(32-61)	44	(31-59)	44	(34-50)	0.74						
Sex, female	35	(51.5)	41	(53.2)	8	(57.1)	0.92						
BMI, kg/m^2	25.9	(22.2-28.6)	25.7	(22.5-29.2)	21.5	(19.1-22.4)	**<0.001**	0.81	**<0.001**	**<0.001**	1.00	**<0.001**	**<0.001**
SBP, mmHg	126	(120-136)‖	128	(115-137)	114	(107-121)	**0.002**	0.94	**0.001**	**0.001**	1.00	**0.003**	**0.003**
DBP, mmHg	74	(71-82)¶	77	(69-82)	72	(66-74)	0.06						
Current smoking	7	(10.3)	11	(14.3)	0		**0.04**						
Genetic diagnosis	66	(97.1)	75	(97.4)				1.00			1.00		
CVD	15	(22.1)	6	(7.8)				**0.02**			**0.02**		
Blood biochemistry													
Tchol, mmol/L	4.5	(3.9-5.3)	4.7	(4.0-5.6)	5.0	(4.5-5.3)	0.36						
HDL-C, mmol/L	1.3	(1.1-1.6)	1.5	(1.2-1.7)	1.6	(1.3-2.1)	0.08						
LDL-C, mmol/L	2.7	(2.1-3.3)	2.8	(2.3-3.6)	3.0	(2.4-3.4)	0.6						
Triglycerides, mmol/L	1.0	(0.7-1.4)	1.0	(0.7-1.6)	1.0	(0.8-1.5)	0.9						
ApoA1, g/L	1.4	(1.3-1.6)	1.4	(1.2-1.6)	1.5	(1.4-1.7)	0.39						
ApoB, g/L	1.0	(0.8-1.1)	0.9	(0.8-1.1)	0.8	(0.7-1.0)	0.15						
Lp(a), nmol/L	224	(170-326)	7	(7-20)	10	(7-28)							
Glucose, mmol/L	5.3	(5.0-5.8)	5.2	(4.9-5.6)	5.1	(4.6-5.5)	0.1						

Medical treatment						
Statins	63	(92.6)	70	(90.9)	0.94	0.94
PCSK9-inhibitor	18	(26.5)	8	(10.4)	**0.01**	**0.01**
Colesevelam	10	(14.7)	13	(16.9)	0.90	0.90
Ezetimibe	49	(72.1)	47	(61)	0.22	0.22
Acetylsalicylic acid	25	(36.8)	19	(24.7)	0.16	0.16

Data are presented as median (25th-75th percentile) for continuous variables, and frequency (%) for categorical variables.

Apo, apolipoprotein; BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; FH, familial hypercholesterolemia; g/L, grams per Litre; HDL-C, high density lipoprotein cholesterol; kg, kilograms; LDL-C, low density lipoprotein cholesterol; Lp(a), lipoprotein (a); m, meters; mmHg, millimeter mercury; mmol/L, millimoles per Litre; nmol/L, nanomoles per Litre; PCSK9, proprotein convertase subtilisin/kexin type 9; SBP, systolic blood pressure; Tchol, total cholesterol.

[*]Kruskal-Wallis test or chi-square test between the three groups

[†]Mann Whitney test or chi-square test between FH subjects Lp(a) ≥ 75 nmol/L and FH subjects Lp(a) < 75 nmol/L,

Mann Whitney test between: [‡]FH subjects Lp(a) ≥ 75 nmol/L and controls, [§]FH subjects Lp(a) < 75 nmol/L and controls

P-values from the Mann Whitney U test are shown as unadjusted and Bonferroni-adjusted.

[‖]n=62, ¶n=61

Table 2. Fatty acids in plasma

	FH subjects				Healthy subjects			Unadjusted				Adjusted		
	Lp(a) ≥ 75 nmol/L		Lp(a) < 75nmol/L				P*	P†	P‡	P§		P†	P‡	P§
	n=68		n=77		n=14									
n-3 PUFA, % of total FAs														
ALA, 18:3n-3	0.62	(0.46-0.77)	0.62	(0.49-0.83)	0.94	(0.73-1.12)	**<0.001**	0.66	**<0.001**	**0.001**		1.00	**<0.001**	**0.003**
EPA, 20:5n-3	1.72	(1.04-2.59)	1.42	(1.07-2.08)	1.66	(1.03-2.04)	0.60							
DPA, 22:5n-3	0.60	(0.50-0.67)	0.54	(0.49-0.61)	0.59	(0.55-0.61)	0.10							
DHA, 22:6n-3	2.48	(1.97-3.19)	2.38	(2.01-2.99)	3.03	(2.76-3.43)	**0.02**	0.59	**0.02**	**0.006**		1.00	0.06	**0.02**
MUFA, % of total FAs														
Palmitoleic acid, 16:1n-7	1.65	(1.34-2.02)	1.55	(1.26-1.90)	1.33	(1.17-1.81)	0.27							
Oleic acid, 18:1n-9	22.1	(19.4-24.5)	21.3	(19.5-24.3)	19.6	(18.7-20.3)	**0.02**	0.69	**0.01**	**0.04**		1.00	**0.03**	0.12
cis-Vaccenic acid, 18:1n-7	1.69	(1.53-1.88)	1.60	(1.46-1.77)	1.40	(1.35-1.53)	**0.001**	0.07	**<0.001**	**0.005**		0.21	**<0.001**	**0.02**

11-Eicosenoic acid, 20:1n-9	0.17	(0.14-0.21)	0.17	(0.11-0.22)	0.11	(0.11-0.14)	0.06						
SFA, % of total FAs													
Lauric acid, 12:0	0.08	(0.05-0.12)	0.08	(0.06-0.12)	0.12	(0.08-0.15)	0.29						
Myristic acid, 14:0	0.78	(0.64-0.94)	0.80	(0.64-1.02)	0.88	(0.80-0.97)	0.26						
Pentadecylic acid, 15:0	0.17	(0.14-0.19)	0.16	(0.14-0.20)	0.21	(0.19-0.22)	**0.001**	0.43	**<0.001**	**0.001**	1.00	**<0.001**	**0.003**
Palmitic acid, 16:0	19.3	(18.4-20.5)	19.6	(18.9-20.8)	19.7	(19.2-20.3)	0.40						
Stearic acid, 18:0	7.06	(6.73-7.56)	7.00	(6.51-7.52)	6.79	(6.47-7.04)	0.20						
Arachidic acid, 20:0	0.33	(0.29-0.38)	0.32	(0.28-0.36)	0.25	(0.22-0.26)	**<0.001**	0.4	**<0.001**	**<0.001**	1.00	**<0.001**	**<0.001** **1**
Behenic acid, 22:0	0.77	(0.68-0.89)	0.76	(0.66-0.83)	0.62	(0.60-0.70)	**0.02**	0.61	**0.02**	0.06	1.00	0.06	0.18
Lignoceric acid, 24:0	0.55	(0.46-0.69)	0.56	(0.47-0.67)	0.39	(0.36-0.62)	0.07						

Data are presented as median (25 th-75th percentile).

ALA, alpha-linolenic acid; C, carbon; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAs, fatty acids;

FH, familial hypercholesterolemia; Lp(a), lipoprotein (a); MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids

*Kruskal-Wallis test between the three groups

Mann Whitney test between: †FH subjects Lp(a) ≥ 75 nmol/L and FH subjects Lp(a) < 75 nmol/L, ‡FH subjects Lp(a) ≥ 75 nmol/L and controls, §FH subjects Lp(a) < 75 nmol/L and controls P-values from the Mann Whitney U test are shown as unadjusted and Bonferroni-adjusted.

Table 3. Intake of energy and macronutrients

	FH subjects		**Healthy controls**		**Unadjusted**			**Adjusted**		
	Lp(a) ≥ 75 nmol/L n=66	**Lp(a) < 75nmol/L n=73**	**n=14**	**P***	**P†**	**P‡**	**P§**	**P†**	**P‡**	**P§**
Energy, kJ	9187 (7588-10796)	9752 (8466-11468)	10400 (9334-12710)	0.08						
Protein, E%	17.9 (16.5-19.5)	17.8 (16.2-19.3)	18.7 (15.3-19.7)	0.91						
Fat, E%	31.2 (28.1-35.2)	33.2 (30.0-35.8)	33.3 (31.5-35.4)	0.21						
SFA, E%	9.1 (7.6-10.3)	9.5 (8.3-11.6)	10.0 (9.1-11.8)	**0.03**	**0.03**	**0.03**	0.41	0.09	0.09	1.00
TFA, E%	0.2 (0.1-0.2)	0.2 (0.1-0.3)	0.3 (0.2-0.3)	**0.03**	0.28	**0.01**	0.06	0.84	**0.03**	0.18
Cis-MUFA, E%	12.1 (10.6-14.1)	12.9 (11.6-14.0)	12.8 (11.5-13.6)	0.43						
Cis-PUFA, E%	6.9 (6.0-8.0)	6.8 (5.8-7.8)	6.7 (5.6-7.6)	0.64						
n-3 PUFA, E%	1.8 (1.4-2.4)	1.9 (1.2-2.4)	1.8 (1.2-2.2)	0.72						
n-6 PUFA E%	4.8 (4.1-5.6)	4.8 (4.1-5.4)	4.7 (4.1-6.0)	0.95						
Linoleic acid, E%	4.7 (3.9-5.5)	4.7 (4.0-5.3)	4.6 (4.0-5.9)	0.93						
Arachidonic acid, E%	0.05 (0.04-0.07)	0.06 (0.05-0.07)	0.06 (0.04-0.07)	0.16						
Carbohydrates, E%	44.0 (39.5-49.3)	43.4 (39.4-46.8)	43.3 (41.5-46.8)	0.64						

Starch, E%	21.8 (18.5-26.4)	21.6 (17.9-24.6)	23.8 (20.3-30.7)	0.19						
Dietary fibre, E%	2.9 (2.5-3.5)	2.6 (2.2-3.0)	2.7 (2.5-2.8)	**0.03**	**0.01**	0.19	0.6	**0.03**	0.57	1.00
Monodisaccharides, E%	18.0 (14.7-22.0)	18.1 (15.3-22.5)	16.7 (15.0-19.7)	0.52						
Sugar, E%	3.8 (2.4-6.4)	4.7 (3.3-6.8)	4.5 (2.3-5.5)	0.08						
Alcohol, E%	2.6 (1.1-4.1)	2.4 (1.0-4.6)	1.3 (0.9-5.1)	0.82						
Cholesterol, mg	232 (180-321)	289 (230-370)	268 (194-331)	**0.03**	**0.01**	0.4	0.37	**0.03**	1.00	1.00

Data are presented as median (25 th-75th percentile).

E%, energy percent; FH, familial hypercholesterolemia; kJ, kilo Joule; Lp(a), lipoprotein (a); mg, milligram; nmol/L; MUFA, monounsaturated fatty acids;

n-3/6, omega-3/6; nanomoles per litre; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans-unsaturated fatty acids.

*Kruskal-Wallis test between the three groups

Mann Whitney U test between: †FH subjects Lp(a) ≥ 75 nmol/L and FH subjects Lp(a) < 75 nmol/L,

‡FH subjects Lp(a) ≥ 75 nmol/L and controls, §FH subjects Lp(a) < 75 nmol/L and controls

P-values from the Mann Whitney U test are shown as unadjusted and Bonferroni-adjusted.