Genes Nutr (2015)10:43
DOI 10.1007/s12263-015-0493-z

RESEARCH PAPER

Dietary polyunsaturated fatty acids and the Pro12Ala polymorphisms of *PPARG* regulate serum lipids through divergent pathways: a randomized crossover clinical trial

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Received: 19 March 2015 / Accepted: 25 September 2015
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Abstract Human and animal studies suggest an interaction between the Pro12Ala polymorphism of *PPARG* and dietary fat. In this randomized crossover clinical trial, we investigated whether subjects with the Pro12Pro and Ala12Ala genotypes of *PPARG* respond differently to a diet supplemented with high saturated (SAFA) or polyunsaturated fatty acid (PUFA).We recruited non-diabetic men from a population-based METSIM study (including 10,197 men) to obtain men with the Ala12Ala and the Pro12Pro genotypes matched for age and body mass index. Seventeen men with the Pro12Pro genotype and 14 with the Ala12Ala genotype were randomized to both a PUFA diet and a SAFA diet for 8 weeks in a crossover setting. Serum lipids and adipose tissue mRNA expression were measured during the diet intervention. At baseline, subjects with the Ala12Ala genotype had higher levels of HDL cholesterol and lower levels of LDL cholesterol, total triglycerides, and apolipoprotein B compared to those subjects with the Pro12Pro genotype ($P < 0.05$, FDR < 0.1). The Ala12Ala genotype also associated with higher mRNA expression of *PPARG2, LPIN1,* and *SREBP-1c* compared to participants with the Pro12Pro genotype (FDR < 0.001). On the other hand, PUFA diet resulted in lower levels of fasting glucose, total cholesterol, total triglycerides, and apolipoprotein B ($P < 0.05$, FDR < 0.1) but did not affect *PPARG2* mRNA expression in adipose tissue. We conclude that individuals with the Pro12Pro genotype, with higher triglyceride levels at baseline, are more likely to benefit from the PUFA diet. However, the beneficial effects of dietary PUFA and the Ala12Ala genotype of *PPARG* on serum lipids are mediated through divergent mechanisms.

Keywords *PPARG* · Randomized clinical trial · Serum triglycerides · Dietary fat · Human

Abbreviations

LDL	Low-density lipoprotein
HDL	High-density lipoprotein
FDR	False discovery rate
BMI	Body mass index
PPARG	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acid
SAFA	Saturated fatty acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ALA	α-Linolenic acid

Introduction

Diet and physical activity are the major lifestyle determinants of the risk of type 2 diabetes, as demonstrated in diabetes prevention studies, e.g., in the Finnish Diabetes

Electronic supplementary material The online version of this article (doi:10.1007/s12263-015-0493-z) contains supplementary material, which is available to authorized users.

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Published online: 07 October 2015

Prevention study (Knowler et al. 2002; Tuomilehto et al. 2001). Recent genome-wide association analyses have identified >70 gene variants that contribute to type 2 diabetes (Huyghe et al. 2012; Morris et al. 2012) and >30 genes that contribute to obesity (Sandholt et al. 2012). However, none of these variants explain >5 % of the risk of these conditions, and altogether, they explain <10 % of the total disease risk (Manolio et al. 2009). Interactions between gene variants and the environment, i.e., our inherited responses to environmental changes, are likely to be crucial in the development of obesity and type 2 diabetes (Cornelis and Hu 2012). Despite a relatively small sample size, interactions between several genes, including *PPARG,* and lifestyle intervention have been demonstrated in randomized lifestyle intervention trials (Lindi et al. 2002; Uusitupa 2005).

We were the first to identify the Pro12Ala polymorphism of *PPARG* as a risk factor for type 2 diabetes. This polymorphism is expressed only in the adipose tissue-specific isoform *PPARG2* of *PPARG,* and the Ala12 allele associates with lower transcriptional activity (Deeb et al. 1998). Low prevalence of type 2 diabetes (Altshuler et al. 2000) and dyslipidemias (Huang et al. 2011) has been associated with the Ala12 allele of *PPARG*. However, other studies suggest that association between the genotype and BMI may depend on obesity (Ek et al. 1999), age (Pihlajamaki et al. 2004), dietary uptake of monounsaturated fatty acids (Garaulet et al. 2011), and dietary ratio of polyunsaturated to saturated fatty acid (P/S ratio) (Luan et al. 2001). Interestingly, dietary P/S ratio has also been shown to have an interaction with *PPARG* genotype on the effect on serum lipids in cross-sectional studies (Alsaleh et al. 2011; Bouchard-Mercier et al. 2011), and also in an intervention trial in which saturated fatty acid was replaced by monounsaturated fatty acid (AlSaleh et al. 2012). Accordingly, animal studies suggest a complex interaction between *PPARG2* and dietary fat. Mice lacking the *PPARG2* isoform are more insulin-resistant compared to wild-type mice on normal chow diet but not on high-fat diet (Medina-Gomez et al. 2005). Furthermore, in Pro12Ala knock-in mice, the beneficial effects of the Ala12 allele on adiposity, plasma lipids, and insulin sensitivity are lost with a high-fat diet (Heikkinen et al. 2009).

In this study, we hypothesized that subjects with Pro12Pro and Ala12Ala genotypes respond differently to a diet supplemented with high saturated (SAFA) or polyunsaturated fatty acid (PUFA). This hypothesis was based on the knowledge that polyunsaturated fatty acids are ligands for PPARG (Kliewer et al. 1997; Xu et al. 1999). As opposed to previous studies investigating the putative modifying effect of the *PPARG* genotype in randomized dietary interventions independent of the genotype, we randomized 17 men with the Pro12Pro genotype and 14 matched controls with the Ala12Ala genotype, from a population-based METSIM study (Stancakova et al. 2012), to receive PUFA and SAFA diets for 8 weeks in a cross-over setting. The specific objective of the study was to investigate whether SAFA and PUFA supplementation interferes with genetic regulation of lipid metabolism by Pro12Ala genotype.

Materials and methods

Subjects

We recruited lean and overweight subjects (BMI > 20 kg/m^2 < 29 kg/m^2) with the Pro12Pro and Ala12Ala genotypes of *PPARG* from the METSIM study (METabolic Syndrome in Men), including 10,197 men originally identified from the population register of the Kuopio town (Stancakova et al. 2012). Obese individuals were excluded to avoid the confounding effect of *PPARG* polymorphism on obesity and thus the secondary effects of obesity on serum lipids. One hundred and forty-seven non-diabetic subjects with the Ala12Ala genotype and an equal number of age- and BMI-matched subjects with the Pro12Pro genotype were selected from the METSIM study. A total of 31 subjects (17 with the Pro12Pro genotype and 14 with the Ala12Ala genotype) were willing to participate and were recruited. These participants did not differ from non-participants with respect to anthropometric and laboratory measurements (age 59.2 ± 6.0 vs. 59.0 ± 6.0 years, BMI 26.5 ± 2.9 vs. 27.2 ± 3.1 kg/m^2, fasting glucose 6.0 ± 0.6 vs. 5.8 ± 0.7 mmol/l, and fasting insulin 49.1 ± 22.9 vs. 53.2 ± 25.6 pmol/l in participants and non-participants, respectively). There were no dropouts during the study.

Dietary interventions

Both the SAFA diet and the PUFA diet were isocaloric and contained 30 % of energy as fat, 18 % as protein, and 52 % as carbohydrates. The SAFA diet had a polyunsaturated/saturated (P/S) ratio of 0.3 and the PUFA diet 1.0. This was pursued in the SAFA diet by the consumption of medium-fat liquid dairy products, i.e., 1.5 % milk and sour milk, 2–2.5 % yoghurt, and fatty cheese (fat 24–30 %) and by using a butter and vegetable oil mixture as a spread and in cooking. Vegetable oil-based salad dressings were not allowed. Meals made of low-fat fish, e.g., perch and pike, were allowed once per week. In the PUFA diet, the subjects were instructed to use fat-free liquid dairy products (fat less than 1 %) and low-fat cheese (fat max. 17 %). Soft vegetable oil-based margarine was used as a spread and vegetable oil (low erucic acid rapeseed oil) and liquid

margarine in cooking. Vegetable oil-based salad dressings were used in salads. Butter and butter-based products were not allowed during this period. Three fish meals per week (150 g of fish) with fatty fish (e.g., rainbow trout, salmon) were to be consumed. Low-fat fish was not recommended. In order to ensure compliance, the participants were given spreads, vegetable oils, liquid margarine, and cheeses free of charge during both periods. The extra costs for fish consumption during the PUFA period were reimbursed.

The two 8-week diet periods were started after a habitual diet and separated by a 2-week washout period with a habitual diet. To monitor the dietary intake, the subjects kept 4-day food records during four predefined consecutive days (Sunday–Wednesday or Wednesday–Saturday) in week 0 and twice during the diet periods (third and seventh week). The food records were checked at return by a clinical nutritionist. The physical activity was instructed to remain unchanged during the study.

The diets were planned, and the nutrient intake during the diet periods was calculated by Diet32 dietary analysis software (AivoFinland Ltd, Turku, Finland).

Clinical measurements and laboratory measurements

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Body composition was determined by bioelectrical impedance (RJL Systems) in subjects in the supine position after a 12-h fast. A 2-h OGTT (75 g of glucose) was performed, and samples for plasma glucose and insulin were drawn at 0, 30, and 120 min. Plasma glucose was measured by enzymatic hexokinase photometric assay (Konelab Systems Reagents, Thermo Fischer Scientific, Vantaa, Finland). Serum insulin was determined by immunoassay (ADVIA Centaur Insulin IRI, no 02230141, Siemens Medical Solutions Diagnostics, Tarrytown, NY). The $InsAUC_{30}/GluAUC_{30}$ ratio and Matsuda Insulin Sensitivity Index (ISI) (Matsuda and DeFronzo 1999) were used as surrogate markers of insulin secretion and insulin sensitivity, as previously reported (Stancakova et al. 2009).

Serum fatty acid composition

Serum lipids were extracted with chloroform–methanol (2:1), and the lipid classes were separated by solid-phase extraction with an aminopropyl column as a marker of dietary compliance (Agren et al. 1992; Vessby et al. 1980; Vidgren et al. 1997). Fatty acids were analyzed with Agilent GC 7890A gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, Wilmington, DE) equipped with a 25-m FFAP column ($I = 25$ m, ID = 0.2 mm, film thickness 0.33 μm; Agilent Technologies Inc.).

Adipose tissue biopsies

After an overnight fast and 30-min resting, adipose tissue samples were taken as needle biopsies from subcutaneous abdominal adipose tissue at baseline, after the first dietary period (8 weeks), and at the end of the study (18 weeks) under local anesthesia (lidocaine 10 mg/ml without adrenaline). Samples were immediately frozen in liquid nitrogen.

Genotyping

PPARG2 polymorphism (rs1801282) was determined with the TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA, USA). The TaqMan genotyping call rate was 100 %, with an error rate of 0 % in 4.5 % of DNA samples genotyped in duplicate.

mRNA expression

Total RNA was extracted using Tri-Reagent [Applied Biosystems (ABI) Foster City, CA, USA] and reverse transcribed using High Capacity cDNA Reverse Transcription Kit (ABI) according to the manufacturer's protocol. Quantitative real-time PCR was carried out in the Applied Biosystems 7500 Real-Time PCR System. *PPARG2, ADIPOR1,* and *ADIPOR2* expressions were determined by the TaqMan Gene Expression Assays (Applied Biosystems) (Hs01115510_m1, Hs00360422_m1, and Hs00226105_m1, respectively) according to the protocol. *PPARG2, ADIPOR1,* and *ADIPOR2* expressions were normalized to *RPLP0* expression (Hs99999902_m1). Expression of *LPIN1, FASN, SREBP-1c* was determined using SYBR Green (KAPA SYBR FAST qPCR Kit, Kapa Biosystems, Woburn, MA), and gene expression was normalized to *RPLP0*. Primer information is presented in Supplementary Table 1.

Statistical analysis

Variables with non-normal distribution were logarithmically transformed before statistical analysis. Differences between the genotype groups at baseline and after the dietary periods were examined with general linear model using the SPSS version 19 programs (SPSS, Chicago, IL, USA).

To adjust for possible confounding factors, mixed-model analyses were performed using linear mixed effect models (nlme package version 3.1-105) (Pinheiro et al. 2015). Models were fit using a restricted maximum likelihood method ignoring missing observations. For investigating the association of a particular genotype with selected phenotypes, we included type of diet, phase of the

intervention period (start or end of the period), and the week of the study (0, 8, 10, or 18) as fixed effects. For investigating the association of diet with selected phenotypes, we examined diet–phase interaction, which includes type of diet, phase of the intervention period, and the week of the study as fixed effects. In all models, subject identifier was used as a random effect. We used Benjamini–Hochberg false discovery rate (FDR) to adjust for multiple testing (Benjamini and Hochberg 1995). All analyses were performed using R version 2.15.2.

Results

Thirty-one non-diabetic men (17 with the Pro12Pro genotype and 14 with the Ala12Ala genotype) were randomized to receive either the PUFA or SAFA diets for 8 weeks, separated by a 2-week washout period. No significant difference in clinical parameters indicating a carryover effect after the first diet was observed. Therefore, we combined the data for SAFA and PUFA diets from the first and second periods according to the original study design. The calculated intake of energy nutrients, dietary fiber, cholesterol, and sucrose from food records during the SAFA and PUFA diets is presented in Supplementary Table 2. Estimated P/S ratio was 0.25 during the SAFA diet and 0.63 during the PUFA diet. The intake of dietary cholesterol was higher in the SAFA diet due to the use of higher fat dairy products as compared with the PUFA diet, as instructed. The intake of dietary fiber and sucrose did not differ between the diets.

The results of serum fatty acid composition analysis indicated a good compliance of the study subjects with the diets (Supplementary Table 3). Levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) increased, as expected in agreement with previous studies (Karvonen et al. 2002; Vessby et al. 1980; Vidgren et al. 1997), both in cholesterol esters (by 32 and 108 %, respectively, $P < 1 \times 10^{-4}$) and in phospholipids (by 32 and 101 %, $P < 0.001$) during the PUFA diet. In contrast, levels of DHA and EPA decreased in cholesterol esters (by 14 and 16 %, $P < 0.001$) and in phospholipids (by 12 and 15 %, $P < 0.001$) during the SAFA diet. In addition, levels of α-linolenic acid (ALA) increased by 11 % in cholesterol esters during the PUFA diet ($P = 2 \times 10^{-5}$). Overall, FDRs for a dietary effect on DHA and EPA were $<10^{-10}$, verifying that participants followed the instructed diets.

Effect of the Ala12Ala genotype on serum lipids and PPARG2 mRNA expression

As expected (Huang et al. 2011), individuals with the Ala12Ala genotype had higher levels of HDL cholesterol and lower levels of LDL cholesterol, total triglycerides, and apolipoprotein B at baseline (Table 1; Fig. 1a, $P < 0.05$, FDR < 0.01). Although we aimed to match individuals with the Pro12Pro and Ala12Ala genotypes for age and BMI, carriers with the Ala12Ala genotype were slightly older than carriers of the Pro12Pro genotype at baseline ($P = 0.048$, Table 1). No difference in glucose and insulin levels was observed between the genotypes. In Table 1, we also present the effects of the genotypes on serum lipids taking into account all time points during the study. In that analysis, total and LDL cholesterol, total triglycerides, and apolipoprotein were associated with the genotype. More specifically, total and LDL cholesterol, total triglycerides, and apolipoprotein were associated with the genotype at the end of the diets ($P < 0.05$).

The Ala12Ala genotype was associated with higher adipose tissue expression of *PPARG2* (Fig. 2a). Levels of adipose tissue *PPARG2* mRNA expression were consistently higher in carriers of the Ala12Ala genotype than in carriers of the Pro12Pro genotype regardless of the diet (FDR = 0.001 for genotype effect in mixed-model analysis including all time points). In addition, expression of *LPIN1* and *SREBP-1c* was higher in individuals with the Ala12Ala genotype ($P = 0.009$ and $P = 0.032$, respectively) (Fig. 2c, e). Expression of *FASN* did not associate with the genotype ($P = 0.058$) (Fig. 2g).

PPARG2 mRNA expression correlated negatively with serum total triglycerides at all three time points (all $P < 0.05$, Table 2, all time points combined in Supplementary Figure 1). We could not observe a significant difference in the correlation between *PPARG2* mRNA expression and serum triglycerides between the genotypes ($z = 0.71$, $P = 0.477$, Fisher *r*-to-*z* transformation test, Supplementary Figure 1). There was an inverse correlation of *PPARG2* mRNA expression with total and LDL cholesterol at 8 weeks but not at baseline or at the end of the study. There was no correlation between adipose tissue *PPARG2* mRNA expression and the levels of glucose and insulin (data not shown).

PUFA diet improves lipid profile without affecting adipose tissue mRNA expression

Table 3 demonstrates a decrease in fasting glucose, total cholesterol, and total triglycerides in response to the PUFA diet ($P < 0.05$). In contrast, the SAFA diet led to a significant increase in total cholesterol and LDL cholesterol ($P < 0.05$). Importantly, at the end of the dietary periods, the levels of total cholesterol, LDL cholesterol, total triglycerides, and apolipoprotein B were higher after the SAFA diet compared to the PUFA diet ($P < 0.05$). To control for multiple testing and for the crossover setting, we also analyzed the data in a mixed model including diet, phase of the study, and the genotype. Diet had an effect on

Table 1 Clinical characteristics at baseline according to the *PPARG* genotype

	Pro12Pro ($n = 17$)		Ala12Ala ($n = 14$)		Baseline	Whole study	
	Mean	SD	Mean	SD	$P^{\dagger}$	$P^{\ddagger}$	FDR[§]
Age (years)	56.7	7.2	62.1	7.3	0.048*		
Weight (kg)	82.1	8.4	83.5	12.7	0.715	0.660	0.777
Body mass index (kg/m^2)	26.0	2.4	26.9	3.4	0.406	0.271	0.492
Fasting plasma glucose (mmol/l)	5.8	0.4	5.7	0.5	0.857	0.479	0.713
Fasting plasma insulin (mU/l)	49.2	24.6	49.0	20.1	0.967	0.758	0.803
Fasting plasma FFA (mmol/l)	0.44	0.13	0.42	0.11	0.601	0.751	0.803
Total cholesterol (mmol/l)	5.38	0.81	4.81	0.81	0.062	0.003*	0.009*
HDL cholesterol (mmol/l)	1.24	0.25	1.52	0.45	0.039*	0.077	0.365
LDL cholesterol (mmol/l)	3.38	0.66	2.76	0.62	0.013*	2×10^{-4} *	9×10^{-4} *
Total triglycerides (mmol/l)	1.38	0.49	0.85	0.28	0.001*	4×10^{-4} *	0.001*
Apolipoprotein A1 (g/l)	1.39	0.17	1.43	0.24	0.596	0.661	0.777
Apolipoprotein B (g/l)	1.05	0.18	0.83	0.14	0.001*	2×10^{-5} *	3×10^{-4} *

Whole study results are from mixed-model analysis for the genotype effect utilizing data from the whole duration of the study, adjusted for diet, phase, and week of the intervention

[†] Independent samples *t* test; [‡] Mixed-model analysis for the genotype effect, adjusted for diet, phase, and week of the intervention; [§] Mixed-model analysis *P* values FDR adjusted for multiple testing. Baseline measures were taken after habitual diet. * $P < 0.05$

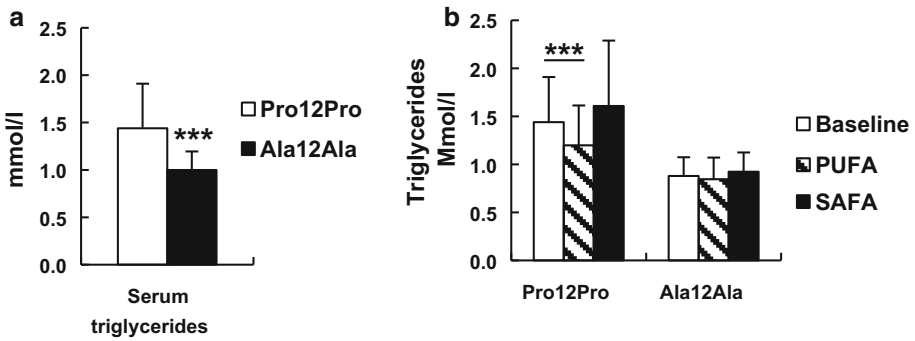


Fig. 1 Levels of serum triglycerides (mmol/l) are lower in individuals with the Ala12Ala genotype compared to individuals with the Pro12Pro genotype of *PPARG* at baseline (**a**) and lower in response to PUFA diet. The difference between genotypes was observed also after PUFA and SAFA diets ($P < 0.01$, not shown) (**b**). Mean ± SD. ***$P < 0.001$, *t* test at baseline

total cholesterol, LDL cholesterol, total triglycerides, and apolipoprotein B (FDR < 0.1). The PUFA diet did not affect mRNA expression of *PPARG2* (Fig. 2b), *LPIN1* (Fig. 2d), *SREBP-1c* (Fig. 2f), *FASN* (Fig. 2h), *ADIPOR1,* and *ADIPOR2* (data not shown). There was no interaction between the genotype and diet.

Discussion

In this study, we used a unique recruit-by-genotype approach to study the effect the Pro12Pro and Ala12Ala genotypes of *PPARG* on serum lipids in a dietary intervention trial. Similar approach has earlier been used to demonstrate an effect of the *PPARG* genotype on adipose metabolism (Tan et al. 2006). We demonstrated that the Ala12Ala genotype associated with lower levels of serum lipids at the baseline and at the end of the PUFA and SAFA diets and higher expression of *PPARG2* mRNA in adipose tissue compared to the Pro12Pro genotype, independent of the diet (Figs. 1, 2; Table 1). In contrast, the PUFA diet improved serum lipids without affecting *PPARG2* expression in adipose tissue. These results suggest that dietary PUFAs and the *PPARG* genotype regulate serum triglycerides through divergent pathways. More specifically, our results suggest that individuals with Pro12Pro genotype, with higher triglyceride levels, are more likely to benefit from the PUFA diet (Fig. 1; Table 2).

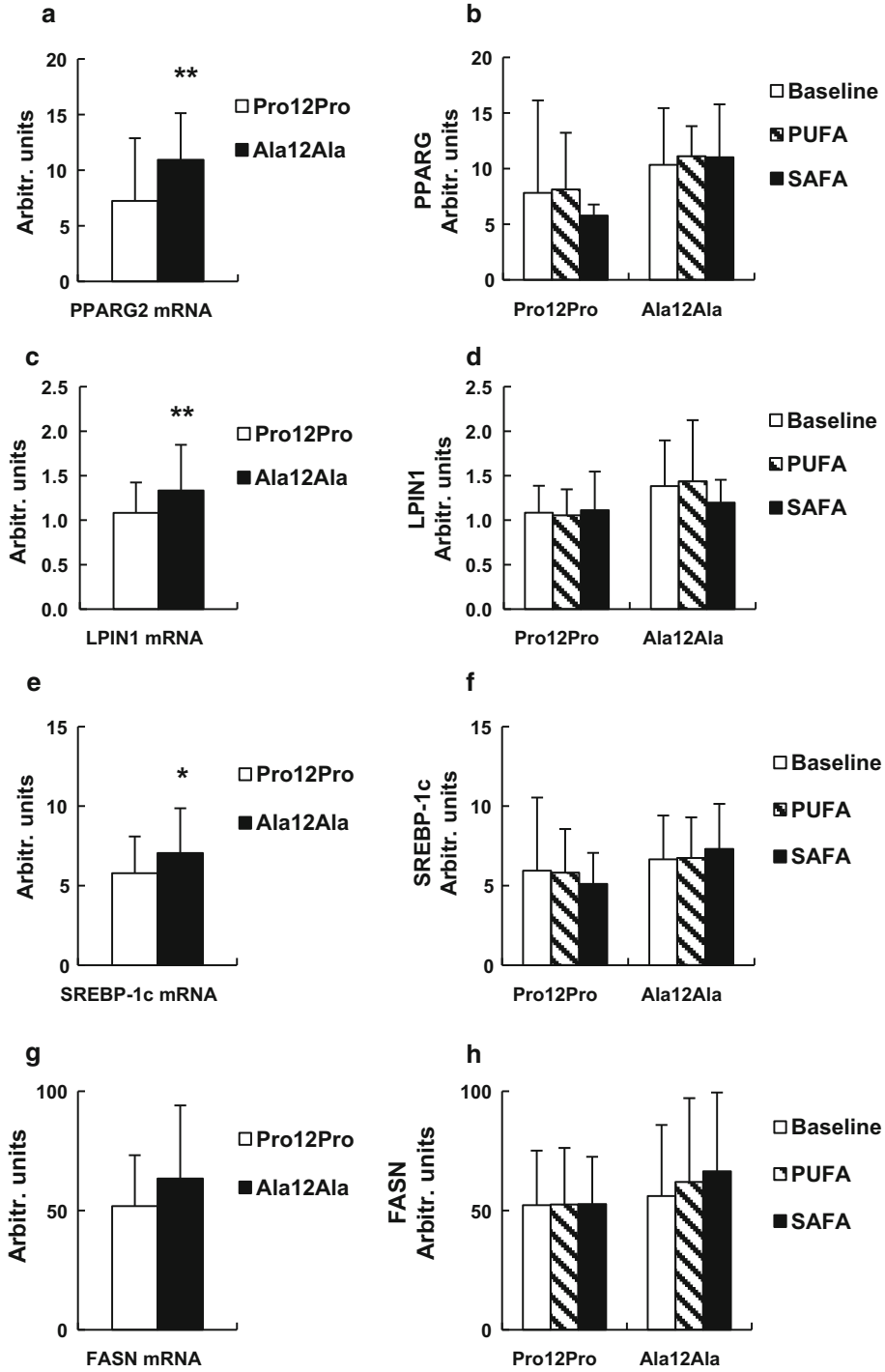


Fig. 2 Association of adipose tissue mRNA expression of *PPARG2* (**a**, **b**), *LPIN1* (**c**, **d**), *SREBP-1c* (**e**, **f**), *FASN* (**g**, **h**) with the *PPARG* genotype and PUFA diet. Mean ± SD. $*P < 0.05$, $**P < 0.01$ *t* test

Table 2 Spearman correlation of the *PPARG2* mRNA with serum lipids at different time points ($n = 31$ at each time point)

	Baseline	8 weeks	End of the study	All time points combined
Total cholesterol (mmol/l)	−0.022	−0.466*	−0.305	−0.022
HDL cholesterol (mmol/l)	0.358	0.277	0.104	0.358
LDL cholesterol (mmol/l)	−0.186	−0.607*	−0.292	−0.186
Total triglycerides (mmol/l)	−0.434*	−0.544*	−0.436*	−0.434*

* $P < 0.05$

Table 3 Clinical characteristics before and after the diet with high PUFA or the diet with high SAFA content

	PUFA ($n = 31$)				PUFA effect	SAFA ($n = 31$)				SAFA effect	End of the study	Diet effect Mixed model	
	Start		End			Start		End					
	Mean	SD	Mean	SD	$P^{\dagger}$	Mean	SD	Mean	SD	$P^{\dagger}$	$P^{\dagger}$	$P^{\ddagger}$	FDR[§]
Weight (kg)	83.4	10.6	83.8	10.9	0.236	83.2	10.8	83.7	10.6	0.058	0.749	0.904	0.956
Fasting plasma glucose (mmol/l)	5.9	0.6	5.7	0.5	0.011*	5.8	0.5	5.7	0.6	0.146	0.861	0.998	0.998
Fasting plasma insulin (mU/l)	54.6	24.9	60.6	30.1	0.136	57.6	31.0	60.6	33.2	0.403	0.960	0.805	0.956
Fasting plasma FFA (mmol/l)	0.42	0.14	0.35	0.12	0.052	0.40	0.13	0.41	0.16	0.532	0.093	0.076	0.289
Total cholesterol (mmol/l)	5.17	0.93	4.99	0.82	0.015*	5.06	0.82	5.33	0.90	0.017*	0.002*	0.001*	0.011*
HDL cholesterol (mmol/l)	1.33	0.38	1.39	0.38	0.248	1.35	0.31	1.35	0.30	0.971	0.311	0.261	0.626
LDL cholesterol (mmol/l)	3.15	0.83	2.94	0.69	0.007*	3.07	0.67	3.18	0.75	0.023*	0.010*	0.008*	0.022*
Total triglycerides (mmol/l)	1.19	0.47	1.04	0.38	0.007*	1.18	0.56	1.30	0.61	0.071	0.001*	0.002*	0.011*
Apolipoprotein A1 (g/l)	1.37	0.21	1.38	0.21	0.818	1.39	0.17	1.40	0.16	0.687	0.355	0.897	0.956
Apolipoprotein B (g/l)	0.97	0.21	0.94	0.20	0.074	0.95	0.20	1.01	0.22	0.058	0.012*	0.003*	0.011*

[†] Paired samples *t*-test for the change during the diet (PUFA or SAFA) and for the difference at the end of the diets; [‡] Mixed-model analysis for the effect of diet during the intervention, adjusted for the diet, phase, and week of the intervention; [§] Mixed-model analysis *P* values FDR adjusted for multiple testing; * $P < 0.05$

Our hypothesis was that the effects of the PUFA diet on glucose and lipid metabolism could depend on the *PPARG* genotype. This hypothesis was based on the knowledge that polyunsaturated fatty acids, including derivatives of DHA and EPA, are ligands for PPARG (Kliewer et al. 1997; Xu et al. 1999). Therefore, we assumed that the modification of the fatty acid composition in the diet changes the ligands, i.e., PUFAs, for PPARG and thus modifies PPARG2 function depending on the genotype. Given the low frequency of the Ala12Ala genotype in the population (<1 %), we recruited the participants with either the Pro12Pro or the Ala12Ala genotypes of *PPARG* from a large population-based METSIM cohort including 10,197 participants (Stancakova et al. 2009, 2012). However, there was no effect of the PUFA diet on the expression of *PPARG2,* and we could also not observe any diet–genotype interaction effect on *PPARG2* expression. In addition, we did not observe any significant interaction in the effects of the *PPARG* genotype and the PUFA diet on serum lipids, or on glucose metabolism, which could be due to our small sample size. The correlation analysis between *PPARG2* mRNA expression and serum triglycerides suggested a difference between genotypes (Supplementary Figure 1). More importantly, the beneficial effect of PUFA diet on serum triglycerides was observed in individuals with Pro12Pro genotype, with higher triglyceride levels at baseline. Together with the earlier findings that dietary fat intake correlates with the components with the metabolic syndrome in individuals with the Pro12Pro genotype but not in those with Ala12Ala genotype (Robitaille et al. 2003), our results suggest that although dietary effect on serum lipids is not mediated through *PPARG2* mRNA expression in adipose tissue, *PPARG* genotype may still modify the effects of dietary fat on serum lipids.

The Ala12Ala genotype did associate with higher mRNA expression of *PPARG2* which correlated with serum triglyceride levels. These results suggest that the effects of the *PPARG* genotype on serum lipid levels are mediated through altered *PPARG2* expression in adipose tissue (Deeb et al. 1998), while the effect of the PUFA diet on serum triglyceride levels is independent of the *PPARG* genotype and *PPARG2* expression in adipose tissue. The mechanisms how the Ala12 allele, which leads to lower transcriptional activity (Deeb et al. 1998) and associates with higher mRNA expression (Kolehmainen et al. 2003), contributes to lower levels of serum triglycerides remain unknown. However, these mechanisms may include secondary responses in adipose tissue, such as higher expression of *LPIN1 and SREBP-1c* and potential other targets. Secondary effects also outside the subcutaneous adipose tissue, e.g., in the visceral depot and in the liver, are possible, but cannot be investigated in a dietary intervention. We acknowledge that PCR-based gene expression

analysis is always affected by the selection of endogenous control genes, and that the use of other control genes than *RPLP0* could have slightly modified the results.

In summary, our study suggests that the beneficial effects of dietary PUFA and Ala12Ala genotype of *PPARG* on serum lipids are mediated through divergent pathways. Ultimately, larger randomized intervention trials based on genetic background will be needed to fully evaluate the significance of interactions between Pro12Ala polymorphism and environmental factors.

Acknowledgments We thank Kaija Kettunen, Tuomas Onnukka, Päivi Turunen, and Sirkku Karhunen for their careful work in patient recruitment and laboratory analyses; Taisa Venäläinen for instructing the subjects and analyzing dietary intake; and Marketta Puttonen for practical assistance during the study.

Funding This study was supported by a grant from the Kuopio University Hospital (JPI, EVO project); the Academy of Finland (Contract No. 138006 JPI, 124243 ML and 141069 ML/MU); the Finnish Diabetes Research Foundation (JPI, MU, and ML); the Finnish Heart Foundation (ML); TEKES (ML, Contract No. 1510/31/06); and Commission of the European Community (ML, Contract No. LSHM-CT-2004-512013 EUGENE2). We thank Skånemejerier Ltd, Maitokolmio cooperative, Riitan Herkku Ltd, and Bunge Finland Ltd. for providing food items.

Authorship JPI designed the research, analyzed data, wrote paper, and had primary responsibility for final content. US was responsible for planning the diets, conduction of the dietary intervention, supervision of nutrient intake calculations and data entering, and analyzed data. DK conducted RNA analyses, analyzed data, and participated in writing the paper. JÅ was responsible for the fatty acid composition analysis. MK contributed to the study design and discussion. JPA was responsible for the statistical analysis. ML and JK are responsible for the METSIM study and contributed to statistical analyses and discussion. MU was responsible for the study design with JPI and contributed to the analysis and discussion.

Compliance with ethical standards

Conflict of interest Jussi Pihlajamäki, Ursula Schwab, Dorota Kaminska, Jyrki Ågren, Johanna Kuusisto, Marjukka Kolehmainen, Jussi Paananen, Markku Laakso, and Matti Uusitupa declare that they have no conflict of interest.

Ethical standard In this study protocol, all procedures followed were in accordance with the ethical standards of the Ethics Committee of the Northern Savo Hospital District (28/2010; ClinicalTrials.gov Identifier: NCT01274091) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all participants included in the study.

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