Interaction of PPAR γ 2 gene Pro12Ala and PPAR α gene Leu162Val SNPs with dietary intake of fat in determination of plasma lipid concentrations in subjects at risk of the metabolic syndrome¹⁻³

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Abstract

Background: The peroxisome proliferator activated receptors (PPARs) are transcriptional regulators of lipid metabolism, activated by unsaturated fatty acids.

Objective: We investigated independent and interactive effects of PPAR γ 2 gene *PPARG* Pro12Ala (rs1801282) and PPAR α gene *PPARA* Leu162Val (rs1800206) SNP genotypes with dietary intake of fatty acids on concentrations of plasma lipids in subjects at increased cardiometabolic risk.

Design: The RISCK study is a parallel design, randomized controlled trial. Plasma lipids were quantified at baseline after a 4-wk high-SFA (HS) diet and after a 24-wk intervention with reference (HS), high-MUFA (HM) and low-fat (LF) diets. SNPs were genotyped in 466 subjects.

Results: At baseline, carriage of the *PPARG* Ala12 allele was associated with increased plasma total cholesterol ($n = 415$; $P = 0.05$), LDL-cholesterol ($P = 0.04$) and apoB ($P = 0.03$) after adjustment for BMI, age, gender and ethnicity. *PPARA* Val162Leu x *PPARG* Pro12Ala genotype interaction influenced total cholesterol ($P = 0.04$) and triglyceride ($P = 0.03$) concentrations after adjustment for covariates. After HM and LF diets, total cholesterol, LDL-cholesterol and apoB concentrations were reduced (P s < 0.001) and gene x gene interaction determined LDL-cholesterol ($P = 0.02$) and triglyceride ($P = 0.002$) concentrations after adjustments. After the HM diet, carriage of both variant alleles was associated with a greater reduction in LDL-cholesterol and increase in triglycerides compared to other genotype combinations.

Conclusions: Interaction between *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes is a significant determinant of plasma LDL-cholesterol and triglyceride concentrations after a high-MUFA diet.

26 **Keywords:** Keywords: peroxisome proliferator activated receptor- γ (PPAR γ), peroxisome
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28 fatty acid, saturated fatty acid, gene-nutrient interaction.

INTRODUCTION

The peroxisome proliferator activated receptors are master transcriptional regulators of lipid and carbohydrate metabolism (1). The major natural ligands of PPAR γ are polyunsaturated fatty acids and prostanoids (2) and PPAR α responds to several fatty acids (3), suggesting roles in transducing nutritional into metabolic signals (4). Once activated, PPARs heterodimerise with the retinoid X-receptor and bind to specific peroxisome proliferator response elements (PPREs) in the promoter regions of target genes. Activation of PPAR γ 2, expressed mainly in adipose tissue, stimulates adipogenesis by regulating a subset of genes involved in fatty acid transport and synthesis of triglycerides (5). PPAR α plays an important role in liver, where it increases uptake of fatty acids and activates expression of genes involved in peroxisomal and mitochondrial β -oxidation (3). The PPARs are thus major regulators of lipid metabolism, capable of responding to nutritional status.

Variants of the PPAR γ 2 gene *PPARG* and PPAR α gene *PPARA* may alter the function of the receptors and their response to dietary agonists. Numerous studies have investigated associations of the single nucleotide polymorphisms (SNPs) Pro12Ala (rs1801282) in *PPARG* and Leu162Val (rs1800206) in *PPARA* with variables characterising the metabolic syndrome, although the results have been equivocal. In the original study by Deeb et al. (6), *PPARG* Ala12 carriers had a significantly lower BMI, but some later studies demonstrated a modest increase (7-10). Carriage of *PPARA* Val162 has been associated with components of the metabolic syndrome in some studies (11, 12) but not in others (13). Inconsistencies in the outcomes of SNP association studies suggest that environmental influences or gene-gene interaction may be influential. Compared to wild-type receptors, the PPAR γ -Ala variant exhibits reduced binding to DNA and modest impairment of transcriptional activation by pharmacological ligands *in vitro* (6, 14) and the PPAR α -Val form shows lower transcriptional activation in transfectants activated with ω -fatty acids (15).

These findings raise the possibility that differential responses of PPAR gene variants to endogenous ligands might influence plasma variables controlled by target genes.

We hypothesised that carriage of *PPARG* Pro12Ala and *PPARA* Leu162Val allelic combinations might influence concentration of plasma lipids according to availability of dietary unsaturated fatty acid ligands. We studied 466 men and women aged 30-70 y at increased risk of the metabolic syndrome in the RISCK study, a highly-controlled intervention based on replacement of saturated fat with either monounsaturated fat or carbohydrate in isoenergetic diets (16). Independent and interactive effects of *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes in determination of plasma lipid concentrations were assessed at baseline after 4 wk run-in on the high saturated fat diet and after 24 wk of dietary intervention.

SUBJECTS AND METHODS

Subjects

Ethical approval for the RISCK study (ISRCTN29111298) was granted from the National Research Ethics Service and written informed consent from participants was obtained, including subsequent genetic analyses. Men and women (age range: 30-70 y) recruited from the general population, attended a clinic in a fasting state at the participating centers (University of Reading, Imperial College London, University of Surrey and the Medical Research Council Human Nutrition Research Centre (MRC-HNR), University of Cambridge and Kings College London). 47.5% of the subjects had metabolic syndrome according to the criteria of the International Diabetes Federation (17). 549 subjects completed the RISCK study. The current study involved 466 subjects for whom DNA samples were available. Self-reported ethnicity was recorded as White, South Asian, Black African, or other.

Study design

The RISCK study is a parallel 2×2 factorial design compared with a control intervention (16). The intervention diets were planned to provide similar intakes of dietary energy but to vary in the amount and type of fats and carbohydrates. All participants followed a 4-wk run-in period during which they consumed a high-saturated fat 'reference diet' before being randomised to the reference diet or one of four isoenergetic dietary interventions designed to lower saturated fat. In this study, the dietary intervention groups differing in carbohydrate quality were combined to focus the analyses on the manipulation of dietary fat. The resulting three dietary groups were: high saturated fat 'reference diet' (HS) designed to reflect saturated fat intake in a 'Western' diet (~18% of energy SFA, 12% MUFA, 38% total fat, 45% CHO), 'high-MUFA diet' (HM) in which SFA was reduced and replaced with MUFA (~10% of energy SFA, 20% MUFA, 38% total fat, 45% CHO) and 'low-fat diet' (LF), in which SFA was reduced through replacement of total fat with carbohydrate (~10 % of energy SFA, 11% MUFA, 28% total fat, 55% CHO). The dietary intervention is described in detail elsewhere (18). Measurements made after the run-in diet are referred to as 'baseline'. All participants followed their randomly prescribed diets for 24 wk, after which a further blood sample was collected and anthropometry measured. Unweighed 4-d food diaries (3 weekdays and 1 weekend day) were collected to record dietary intakes at baseline and in the third and the final month of the intervention. Nutrient intakes were estimated by using the food-composition database software DINO as described previously (16). Weight (in light clothing) and height (without shoes) were measured and an indwelling venous cannula was inserted into the forearm.

Blood analytic methods

Blood samples for analysis were drawn after a minimum 8-h overnight fast and serum was stored at -45 °C until analyzed. Fasting lipids including total cholesterol and triglycerides were measured as described previously (16). LDL-cholesterol was derived from the Friedwald equation. Fasted plasma phospholipid fatty acids were measured by GC at the University of Reading as described previously (18).

DNA extraction and SNP genotyping

Buffy coats removed from blood samples were stored in EDTA at -20°C. Genomic DNA was extracted from 200 µl buffy coat using an Illustra blood genomic prep mini spin kit (GE Healthcare, Amersham, UK) according to manufacturer's instructions. Genotyping was performed in the 466 participants for whom DNA was available. The *PPARG* Pro12Ala SNP (rs1801282) was genotyped by KBiosciences (Hoddesdon, UK). Genotype accuracy as assessed by inclusion of duplicates in the array was 98% and negative controls (water blanks) were included on each plate. Genotyping success rate was 89%. *PPARA* *Leu162Val* (rs1800206) was genotyped by Pyrosequencing (Qiagen, Crawley, UK). Template PCR amplifications were 1 cycle at 94 °C for 6 min; 50 cycles at 94 °C for 1 min, 60 °C for 45 s and 72 °C for 1 min; 1 cycle at 72°C for 10 min in a buffer containing 1X RedTaq polymerase buffer, 2.5 mM MgCl₂, 0.4 µM dNTP, 200 µM primers and 0.2 U RedTaq polymerase. Template primers were as follows: Forward: 5'-GCCAGTATTGTCGATTTTCAACAAGT-3'; reverse 5'-bio-TTACCTACCGTTGTGTGACATCC-3'. The forward sequencing primer was: 5'-TCGATTTCAACAAGTGC-3'. All reagents and primers were from Sigma-Aldrich, (Gillingham, Dorset, UK). Genotyping accuracy was 98% and success rate was 97%.

Statistical analyses

All genotype distributions were tested for deviation from the Hardy-Weinberg equilibrium by a χ^2 test with 1 df ($P > 0.05$). Statistical analyses were carried out using the SPSS version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). Data were analyzed by using analysis of covariance (ANCOVA), regressing follow-up measures against baseline measures with ethnicity, baseline age, gender and diet as covariates. Outliers were excluded from the ANCOVA and were defined as points > 2.5 times the interquartile range from the median on the transformed scale at baseline, follow-up, or change from baseline. All data presented in text and tables are expressed as means $\pm$ SD or 95% CI. The effect of each diet is expressed as the follow-up values above the baseline with 95% CI. Statistical significance was taken at $P < 0.05$.

RESULTS

Baseline characteristics of subjects

The characteristics of the 466 participants in this study after the baseline reference (HS) diet are presented in **Table 1**. There were significant differences between males and females ($P < 0.001$) in a number of variables as shown.

SNP allele and genotype frequencies

Online supplementary Table 1 shows the allele and genotype frequencies for *PPARG* Pro12Ala and *PPARA* Leu162Val in the 466 RISCK subjects. Genotype distributions did not deviate from Hardy-Weinberg expectations. In comparison to MAFs listed on the NCBI SNP database (build 132 accessed 01/01/11) (<http://www.ncbi.nlm.nih.gov/snp>), the *PPARG* Ala12 allele was more frequent in RISCK Whites than HapMap Europeans (0.076 in HapMap-CEU) and absent in Blacks, as in HapMap-YRI (Sub-Saharan African). The *PPARA* Val162 allele was also more frequent in RISCK Whites (0.042 in HapMap-CEU)

and absent in Blacks, as in HapMap-YRI trios. There is no comparative data available for the S. Asian subjects.

Independent associations of genotypes with concentrations of plasma lipids at baseline

Diet during the 4 wk run-in to baseline on the HS reference diet was monitored by weighed intake (18). **Table 2** shows plasma lipid measurements at baseline, stratified by *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes. Carriers of the *PPARG* Ala12 allele had 5.4% higher total cholesterol, 5.7% higher LDL-cholesterol and 11% higher apo B than Pro12 homozygotes, differences which were significant after adjustment for BMI, age, gender and ethnicity. There were no significant associations of *PPARA* Leu162Val genotype with plasma lipid concentrations.

Effect of interaction between genotypes on concentrations of plasma lipids at baseline

Subjects for whom *PPARG* Pro12Ala and *PPARA* Leu162Val genotype data was available ($n = 401$) were divided into four genotype groups defined by presence or absence of the variant alleles. Mean concentrations of plasma lipids with respect to genotype combinations are shown in **Table 3**. There was a significant interaction between genotypes as determinants of total cholesterol and triglyceride concentrations after adjustment of all data and *P*-values for BMI, gender, age and ethnicity. Carriage of *PPARA* Val162 was associated with higher plasma total cholesterol and triglyceride concentrations in *PPARG* Ala12 carriers than in Pro12 homozygotes at baseline.

Change in concentrations of plasma lipids after dietary intervention

Subjects were randomly assigned to continuation on the HS reference diet, or 24 wk on an isoenergetic diet, in which saturated fat was replaced with either MUFA (HM diet) or

carbohydrate (LF diet). There was no significant difference in intake of saturated fat during the run-in between groups randomised to the interventions. Body weight was relatively stable. Further information is provided elsewhere (16, 18). The changes in % SF and % MUFA differed between the diets over the 24 wk of intervention. The HM group had significantly lower plasma phospholipid % SF than the LF group and higher % MUFA, but other fatty acid classes [(n-3) PUFA, (n-6) PUFA and trans FA] were not affected (18). Total- and LDL-cholesterol concentrations were significantly lower after consumption of HM and LF than after the HS reference diet. ApoB concentrations differed between treatment groups and were lower after the HM and LF diets than the HS diet. There were no significant changes in plasma triglyceride concentrations (16).

Effect of interaction between genotypes on concentrations of plasma lipids after dietary intervention

Independent associations of *PPARG* Pro12Ala or *PPARA* Leu162Val genotypes with changes in concentrations of plasma lipids with respect to baseline were not significant after randomisation to continued HS reference, or switch to HM and LF diets (data not shown). Investigation of genotype interaction was based on subjects genotyped for both SNPs, with measurements of plasma lipids after HM ($n = 227$) and LF ($n = 236$) diets. There was a significant interaction between *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes as determinants of plasma LDL-cholesterol, ($P = 0.02$) and triglyceride ($P = 0.002$) concentrations after adjustment for change in BMI, age, gender and ethnicity. After the HM diet, carriage of the Val162 allele caused an 8-fold greater reduction in plasma LDL-cholesterol in Ala12 carriers than in Pro12 homozygotes. In the absence of the Val162 allele, reduction in plasma LDL-cholesterol in Ala12 carriers was less than in Pro12 homozygotes. After the LF diet, presence or absence of the Val12 allele had no effect on plasma LDL

cholesterol concentration. Carriage of the *PPARA* Val162 allele caused a 15-fold greater increase in plasma triglycerides in *PPARG* Ala12 carriers than in Pro12 homozygotes. In the absence of the Val162 allele, plasma triglycerides only varied by 10% between Ala12 carriers and Pro12 homozygotes. After the LF diet, plasma triglyceride concentration dependent on presence or absence of the Val162 allele varied by 20%.

Figure 1 shows the follow-up concentrations of plasma LDL-cholesterol and triglycerides after the HM and LF diets above the baseline, with respect to *PPARG* Pro12Ala and *PPARA* Leu162Val genotype combinations. Data on which Figure 1 is based are available in **Online Supplementary Material Table 2**. The differences in plasma lipid concentrations between co-carriers of *PPARG* Ala12 and *PPARA* Val162 and all other genotype combinations were significant ($P < 0.01$). We also included triglyceride as a covariate in the LDL-cholesterol model to see if the relationship between these variables might explain the similarity of the same small genotype group exhibiting differences. However, the covariate was not significant, suggesting that the effects are independent of one another.

DISCUSSION

We have shown independent and interactive effects of PPAR γ 2 gene *PPARG* Pro12Ala and PPAR α gene *PPARA* Leu162Val genotypes in determination of plasma lipid concentrations, after diets lowering intake of SFA by replacement with MUFA or carbohydrate.

The deleterious effects of dietary SFA on circulating lipids and lipoproteins are well known (19). When MUFA isocalorically replace SFA in the diet, plasma LDL-cholesterol decreases and HDL-cholesterol concentration is maintained. High MUFA diets cause significant reductions in triglycerides compared to high carbohydrate (low fat) diets (20, 21). In RISCK participants, as expected, plasma total- and LDL-cholesterol and ApoB

concentrations were significantly lower after consumption of HM and LF than after the HS reference diet. However, unexpectedly, there were no significant reductions in plasma triglyceride concentrations (16).

After the 4-wk run-in on HS diet, carriers of the *PPARG* Ala12 allele had significantly higher plasma total cholesterol, LDL-cholesterol and apoB concentrations than Pro12 homozygotes. Although SFAs are relatively poor stimulators of PPAR γ activity (22) the difference between genotypes is likely to reflect lower activation of target genes by the PPAR γ -Ala form (6). The *PPARA* Leu162Val genotype was not associated with plasma lipids at baseline. Others have reported similarly (13, 23, 24, 25), but associations of Val162 with higher (11, 26) and lower (27) concentrations of plasma triglycerides have previously been found. Co-carriage of *PPARA* Val162 was associated with higher concentrations of plasma total cholesterol and triglycerides in *PPARG* Ala12 carriers than in Pro12 homozygotes at baseline. One other investigation found no significant interaction between these SNPs in determination of plasma lipid concentrations (28).

Increased expression of PPAR γ mRNA in adipose tissue (29) and PPAR α mRNA and target genes in liver (30) after a high-fat diet in mice suggests that fat intake might influence target gene expression in humans. Neither *PPARG* Pro12Ala nor *PPARA* Leu162Val genotypes were independently associated with changes in concentrations of plasma lipids after intervention, although after randomisation, the power to detect significant changes was reduced. Various dietary interactions with Pro12Ala and Leu162Val have been reported. An initial study found total fat intake to be inversely correlated with total plasma cholesterol in *PPARG* Pro12 homozygotes, but MUFA intake was inversely associated with BMI in Ala12 allele carriers (31), i.e. the responsiveness of Ala12 carriers depended on the type of fat. Luan et al. (32) previously found greater sensitivity of Ala12 allele carriers to dietary PUFA in determination of BMI. Both findings are compatible with unsaturated fatty acids acting as

specific ligands for *PPAR* γ (22) and lower transcriptional activity of the *PPAR* γ -Ala variant (6) reducing adipogenesis. Reports of effects of *PPARA* Leu162Val interaction with fatty acid intake on plasma lipids are inconsistent, including no interaction with PUFA (33), association of Val162 with higher total- and LDL-cholesterol after a high-PUFA diet (34) and higher triglyceride after low PUFA intake (33).

Our study is the first to report significant interaction between *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes as a determinant of plasma lipid concentrations. After the HM diet there was a significantly larger reduction in plasma LDL-cholesterol and a significantly larger increase in triglyceride concentration in co-carriers of *PPARG* Ala12 and *PPARA* Val162 alleles than in any other genotype group. There were no such effects after the LF diet. PUFA is a stronger activator of PPARs than MUFA (22), but was constant in both interventions. As PPAR variant carriage affected plasma lipids only after the HM diet, the effects may depend on high MUFA concentration.

Evidence for influence of *PPAR* γ and *PPAR* α on plasma lipoprotein concentrations has mainly come from studies of pharmacological ligands *in vitro* and *in vivo*. Reported effects of *PPAR* γ agonist thiazolidinediones are mainly increased HDL-cholesterol, increased size/decreased density of LDL-cholesterol particles and increased lipoprotein (a) (35). Reported effects of *PPAR* α agonist fibrates generally relate to reduced plasma triglycerides and an increase in HDL-cholesterol levels (36). However, there is evidence for potential influence of *PPAR* γ and *PPAR* α on LDL-cholesterol concentration. The insulin-induced gene *INSIG1*, a key regulator of SREBP activity, is up-regulated by activation of *PPAR* γ in rat adipose tissue by rosiglitazone (37) and of *PPAR* α in rat liver by clofibrate (38). *PPAR* γ activation by troglitazone reduces nuclear SREBP-2 and down-regulates LDL clearance from plasma by SREBP-2 target, the liver LDL receptor (39). Expression of the LDL receptor is also reduced by clofibrate (33). Activation of *PPAR* α and *PPAR* γ would thus impair LDLR

278 expression, down-regulate LDL clearance from plasma and increase circulating LDL-
279 cholesterol concentration. Ovalle and Bell (40) found that troglitazone and rosiglitazone
280 increased plasma LDL-cholesterol concentrations in humans, but LDL apoB-100 levels
281 generally decrease in response to fibrates (36). PPAR γ -Ala12 and PPAR α -Val162 forms
282 have lower transactivational ability than the wild-types (6,15). Carriers of *PPARG* Ala12 and
283 *PPARA* Val162 would express higher LDLR activity leading to maximum clearance and the
284 largest fall in LDL-cholesterol concentration, as observed. All the other genotype
285 combinations showed smaller reductions in LDL-cholesterol after the HM diet. As Ala12
286 was associated with higher total cholesterol concentration and interaction with Val162
287 yielded higher LDL-cholesterol after the HS diet, the lower LDL-cholesterol in carriers of
288 both variants after the HM diet appears to be a response to increased availability of MUFA.

289 Goldberg et al. (35) found troglitazone and pioglitazone lowered triglyceride levels,
290 by a mechanism that may involve enhancement of triglyceride clearance, reduction of hepatic
291 lipase and increase in ATP binding cassette A1 (ABCA1) activity. Another mechanism may
292 involve inhibition of hepatic very low-density lipoprotein (VLDL) secretion. In general,
293 fibrates act to reduce VLDL apoB-100 through enhanced clearance and reduced production
294 (36). Reduced production of VLDL is also associated with *n*-3 PUFA consumption (41).

295 Lipoprotein lipase (LPL) is a rate-limiting determinant of triglyceride hydrolysis and as the
296 *LPL* gene is a target of both PPAR γ (42) and PPAR α (15) the triglyceride-lowering effect of
297 both receptors could be mediated by this route. Rudkowska et al. (15) showed that after *n*-3
298 PUFA dietary supplementation, LPL activity increased more in *PPARA* Leu162
299 homozygotes than in Val162 carriers and enzyme activities were inversely related to plasma
300 triglyceride concentration. Transcription after *n*-3 fatty acid transactivation was also found to
301 be higher in Leu162- than Val162-constructs containing the *LPL* PPRE (43). These findings
302 are compatible with the increased triglyceride concentration that we found in carriers of the

less transcriptionally active Val162 allele after the HM diet. Co-carriage of the less active *PPARG* Ala12 allele could maximise the effect. One other study has examined *PPARG* Pro12Ala and *PPARA* Leu162Val interaction after dietary intervention: a 2.5 y low energy diet in obese women, in whom there were significant favourable changes in lipid profile, but no significant interactive effects at baseline or at the follow-up (44).

The strength of our study lies in its design as a randomized, tightly controlled feeding trial with high adherence and retention rates and diets with practical relevance to the general population. Limitations include reduced power after randomisation to dietary treatments, compared to that at baseline. However, the results of gene-gene interaction were highly significant for this data. Our ANOVA model used the variability of the whole dataset to measure the background variation, which was able to produce significant results even though the number of subjects was small. The results for LDL-cholesterol and triglycerides seem to be independent, which further strengthens both results and conclusion. The significance should nevertheless be treated with caution and confirmation awaits replication in a larger sample.

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338 provided access to databases; AA and TABS analysed data and performed statistical
339 analyses; SDO wrote the manuscript; SDO and TABS had primary responsibility for the final
340 content.

341 The authors and their research groups have a number of links with the food industry. In a
342 personal capacity, GSF is a consultant to Coca-Cola, Premier Foods, and Unilever and TABS
343 has acted as a consultant to Seven Seas and is a member of the Scientific Advisory
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TABLE 1

Characteristics of RISCK study subjects at baseline

	All	M	F	<i>P</i>
Characteristic	(<i>n</i> = 466)	(<i>n</i> = 191)	(<i>n</i> = 275)	M vs F
Ethnicity ¹				
South Asian [n (%)]	44 (9.4)	19 (9.9)	25 (9.1)	
Black African [n (%)]	38 (8.2)	10 (5.2)	28 (10.2)	
White European [n (%)]	367 (78.8)	155 (81.2)	212 (77.1)	
Others	17 (3.6)	7 (3.7)	10 (3.6)	
Age (y)	52.4±9.9	53.5±10.3	51.6±9.5	0.04
BMI (kg/m ²)	28.7±4.7	28.6±3.9	28.8±5.2	0.56
Triglycerides (mmol/L)	1.5±0.7	1.7±0.8	1.4±0.6	<0.001
Total cholesterol (mmol/L)	5.6±1.0	5.6±1.0	5.6±1.0	0.57
HDL cholesterol (mmol/L)	1.4±0.3	1.3±0.3	1.5±0.3	<0.001
ApoA1 (g/L)	1.2±0.3	1.2±0.2	1.3±0.2	0.002
LDL cholesterol (mmol/L)	3.5±0.8	3.6±0.8	3.5± 0.8	0.14
ApoB (g/L)	0.97±0.3	1.00±0.3	0.96±0.3	0.09

Data is presented for all subjects who completed the study and for whom DNA was available

(*n* = 466). Values presented as mean ± SD. All variables were measured at baseline after 4-

wk run-in on reference HS diet. Significance of differences between males and females was

determined by students t-test. ¹Self-reported ethnicity.

TABLE 2Independent associations of *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes with phenotypes at baseline

Phenotype	<i>PPARG</i> Pro12Ala genotype		<i>P</i>	<i>PPARA</i> Leu162Val genotype		<i>P</i>
	PP	PA + AA		LL	LV + VV	
Males <i>n</i> (%)	132 (77%)	40 (23%)		165 (41)	22 (43)	
Females <i>n</i> (%)	210 (86%)	33 (14%)		237 (59)	29 (57)	
BMI (kg/m ²) ¹	28.8 (28.3,29.3)	27.9 (26.9,29.0)	0.14 ¹	28.7 (28.2,29.1)	29.5 (28.2,30.8)	0.17
Total cholesterol (mmol/L)	5.5 (5.4,5.6)	5.8 (5.6,6.0)	0.03	5.6 (5.5,5.7)	5.5 (5.3,5.8)	0.15
Triglycerides (mmol/L)	1.5 (1.4,1.5)	1.6 (1.5,1.8)	0.14	1.5 (1.4,1.6)	1.4 (1.2,1.6)	0.26
HDL cholesterol (mmol/L)	1.4 (1.3,1.4)	1.4 (1.3,1.4)	0.88	1.4 (1.3,1.4)	1.4 (1.3,1.5)	0.97
ApoA1 (g/L)	1.2 (1.2,1.2)	1.2 (1.2,1.3)	0.55	1.2 (1.2,1.2)	1.2 (1.2,1.3)	0.58
LDL Cholesterol (mmol/L)	3.5 (3.4,3.6)	3.7 (3.5,3.9)	0.04	3.6 (3.5,3.6)	3.5 (3.3,3.7)	0.22
ApoB (g/L)	0.9 (0.9,1.0)	1.0 (1.0,1.1)	0.03	1.0 (0.9,1.0)	1.0 (0.9,1.0)	0.53

Data is presented for subjects for whom DNA samples were available (*n* = 466). All variables were measured at baseline after 4-wk run-in on reference HS diet. Values are mean (95% CI). *P*-values for analysis of variance based on a dominant model are shown, adjusted for age, gender,

BMI and ethnicity nominally significant ($P < 0.05$) **and remained significant after** adjustment for multiple comparisons by 2-way ANOVA are shown in bold. ¹ P -value BMI adjusted for gender, age and ethnicity.

TABLE 3Effect of interaction of *PPARA* Leu162Val and *PPARG* Pro12Ala on plasma lipids at baseline

Phenotype	<i>PPARA</i> Leu162Val / <i>PPARG</i> Pro12Ala genotype				<i>P</i>		
	LL/PP	LL/PA+AA	LV+VV/PP	LV+VV/PA+AA	<i>PPARG</i>	<i>PPARA</i>	Interaction
	(<i>n</i> = 293)	(<i>n</i> = 64)	(<i>n</i> = 36)	(<i>n</i> = 8)	P12A	L162V	
Total cholesterol							
(mmol/L)	5.5 (5.4,5.6)	5.7 (5.5,6.0)	5.2 (4.9,5.5)	5.8 (5.1,6.5)	0.03	0.08	0.04
Triglycerides							
(mmol/L)	1.5 (1.4,1.6)	1.6 (1.4,1.7)	1.2 (1.0,1.4)	1.9 (1.4,2.4)	0.13	0.08	0.03
LDL cholesterol							
(mmol/L)	3.5 (3.4,3.4)	3.7 (3.5,3.5)	3.3 (3.0,3.0)	3.7 (3.1,3.1)	0.05	0.17	0.12
ApoB							
(g/L)	0.96 (0.92,0.99)	1.02 (0.95,1.09)	0.87 (0.78,0.97)	1.07 (0.85, 1.29)	0.05	0.18	0.08
HDL-cholesterol							
(mmol/L)	1.4 (1.3,1.4)	1.4 (1.3,1.4)	1.4 (1.3,1.5)	1.3 (1.1,1.5)	0.91	0.99	0.91
ApoA1							

(g/L)	1.2 (1.2,1.2)	1.2 (1.2,1.3)	1.2 (1.1,1.3)	1.4 (1.2,1.5)	0.59	0.62	0.46
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Data is presented for subjects for whom *PPARG* Pro12Ala and *PPARA* Leu162Val genotype data was available ($n = 401$). All variables were measured at baseline after 4-wk run-in on reference HS diet. Values are mean (95% CI). All data and *P*-values for **analysis of variance** are shown, adjusted for age, gender, BMI and ethnicity based on subjects. *P*-values nominally significant (< 0.05) **and remained significant after adjustment for multiple comparisons by 2-way ANOVA are shown in bold.**

Figure legend

Interaction between *PPARG* Pro12Ala and *PPARA* Leu162Val genotype groups after dietary treatments influences plasma lipid concentrations. Interaction between *PPARG* Pro12Ala and *PPARA* Leu162Val genotypes was a significant determinant of change in plasma concentrations of (A) LDL- cholesterol ($P = 0.02$) and (B) triglycerides ($P = 0.002$) after HM and LF diets, after adjustment for BMI, age, gender and ethnicity and corrected for multiple comparisons using 3-way ANOVA. PP represents subjects homozygous for the *PPARG* Pro12 allele and PA + AA carriers of the Ala12 allele. LL represents subjects homozygous for the *PPARA* Leu162 allele and LV + VV carriers of the Val162 allele. **Mean follow-up concentrations of LDL-cholesterol and triglycerides (mmol/L) above the baseline**, after 24 wk on HM or LF diets are shown. Bars indicate **95% CI**. The figure is based on subjects with genotypes for both SNPs and measurements of plasma lipids after HM and LF diets.