

Interaction of PPAR- γ 2 gene Pro12Ala single nucleotide polymorphism with dietary intake of fatty acids in determination of plasma lipids in subjects at cardiometabolic risk

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ABSTRACT

Objective: The *PPARG* SNP rs1801282 (Pro12Ala C>G) has shown variable association with metabolic syndrome traits in healthy subjects. We investigated genotype association with plasma lipids and the influence of dietary polyunsaturated:saturated fat ratio (P:S) in subjects at increased cardiometabolic risk.

Methods: Habitual dietary intake was recorded at recruitment to the RISCK Study. *PPARG* rs1801282 was genotyped in 466 subjects aged 30-70 y. Genotype associations with plasma lipids were assessed at recruitment, after a 4-wk high-SFA (HS) diet and a 24-wk intervention with reference (HS), high-MUFA (HM) and low-fat (LF) diets. The interaction of habitual P:S intake x genotype on plasma lipid concentrations was investigated.

Results: *PPARG* rs1801282 G-allele frequency was 0.09. At recruitment, G-allele carriers had higher plasma total cholesterol concentration ($n=415$; $P=0.05$) after adjustment for BMI, gender, age and ethnicity. Dietary P:S ratio x genotype interaction influenced plasma LDL-cholesterol ($P=0.02$) and triglyceride ($P=0.03$) concentrations. At P:S ratio ≤ 0.33 , mean LDL-cholesterol concentration in G-allele carriers was higher than in non-carriers, but fell between 0.34-0.65. Triglyceride concentration followed a similar pattern. After the 4-wk HS diet, G-allele carriers had higher concentrations of total cholesterol ($P=0.03$), LDL-cholesterol ($P=0.04$) and apo B ($P=0.04$) than non-carriers, after adjustments. After the 24-wk interventions, diet x genotype interaction did not significantly influence either LDL-cholesterol ($P=0.58$) or triglyceride ($P=0.57$) concentrations.

Conclusion: A high dietary P:S ratio would help to reduce plasma LDL-cholesterol and triglyceride concentrations in *PPARG* rs1801282 G-allele carriers at increased cardiometabolic risk.

Keywords: Peroxisome proliferator-activated receptor- γ (PPAR γ); Single nucleotide polymorphism; Polyunsaturated fatty acid; Saturated fatty acid; Gene-nutrient-interaction.

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1. Introduction

The transcription factor peroxisome proliferator-activated receptor- γ (PPAR γ) is one of three PPARs and a member of the nuclear hormone receptor superfamily [1]. The major natural ligands are polyunsaturated fatty acids and prostanoids [2], suggesting a role in transducing nutritional to metabolic signals [3]. An increase in PPAR γ mRNA in adipose tissue of mice exposed to a high fat diet [4] suggested that dietary modulation might influence adipogenesis induced by PPAR γ in response to raised plasma concentration of fatty acid ligands.

Variants of the PPAR γ 2 gene *PPARG* could alter transcriptional activity of the activator through DNA- and/or ligand-binding affinity. The Pro12Ala C>G polymorphism (rs1801282) is present at a frequency of approximately 7.6 % in Caucasians (NCBI SNP database <http://www.ncbi.nlm.nih.gov/snp/build/132> accessed 15/12/10). Previous studies have investigated *PPARG* rs1801282 genotype associations with risk of obesity and diabetes, with equivocal results [5, 6, 7] suggesting that environmental influences such as dietary intake may be involved. Luan et al. [8] found BMI in G-allele carriers but not CC homozygotes, was influenced by ratio of habitual dietary polyunsaturated:saturated fatty acid intake (P:S). Others showed a relationship between intake of total fat and BMI [9] or waist circumference [10] in common CC homozygotes, but not in G-allele carriers. However, when MUFA rather than total fat was examined [9], intake was found to be inversely associated with BMI in G-allele-carriers and not in CC homozygotes. *In vitro*, the PPAR γ 2 Ala-variant exhibits reduced binding to DNA and modest impairment of transcriptional activation following treatment with pharmacological ligand thiazolidinediones (TZDs) [11, 12]. This raised the possibility that differential responses by CC homozygotes and G-allele carriers to unsaturated fatty acid ligands of PPAR γ might influence adipogenesis.

PPAR γ target genes include several involved in cholesterol and triglyceride metabolism [13, 14, 15]. We hypothesised that dietary intake of unsaturated fats might

interact with *PPARG* rs1801282 genotype to influence concentration of plasma lipids. We have investigated 466 men and women aged 30-70 y at increased risk of metabolic syndrome in the RISCK Study [16]. We tested association of genotype with plasma lipids at recruitment screening (habitual diet), after 4 wk on a diet high in SFA (HS diet) and with changes in lipid concentrations after a 24-wk intervention with reference (HS), high-MUFA (HM) and low-fat (LF) diets. We also examined the influence of interaction between genotype and BMI, gender, ethnicity and habitual dietary P:S ratio on concentration of plasma lipids.

2. Methods

2.1 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 centres (University of Reading, Imperial College London, University of Surrey and the Medical Research Council Human Nutrition Research Unit [MRC-HNR] and Kings College London). Eligibility for entry to the study was assessed by a point system and implementation of exclusion criteria described previously [16]. 549 subjects completed the study. Self-reported ethnicity was recorded as White, South Asian, Black African, or 'other'.

2.2 Study design

The RISCK study is a parallel 2×2 factorial design compared with a control intervention [16]. At screening, unweighed 4-d food diaries (3 weekdays and 1 weekend day) were collected to record the habitual diet. Nutrient intakes were estimated by using the food-composition database software DINO as described previously [17]. 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 a higher saturated fat intake than habitual '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 [17]. 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. Weight (in light clothing) and height (without shoes) were measured. An indwelling venous cannula was inserted into the forearm.

2.2 Biochemical analysis

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, HDL-cholesterol and triglycerides were measured as described previously [16]. LDL-cholesterol was derived from the Friedwald equation.

2.3 DNA extraction and 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. The *PPARG*

rs1801282 SNP 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%.

2.4 Statistical analysis

PPARG rs1801282 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). Where needed, variables were log transformed to obtain better approximations of the normal distribution prior to analysis. Data were analyzed by using analysis of covariance (ANCOVA) with ethnicity, BMI, 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 recruitment or after HS diet. All data presented in text and tables are expressed as means or geometric means \pm SD or 95% CI. Statistical significance was taken at $P < 0.05$.

3. Results

3.1. Characteristics of subjects

A total of 549 subjects completed the study and data from 548 subjects were analyzed. Based on self-reported ethnicity, individuals of White, S. Asian, Black African and 'other' ancestry were distinguished. 47.5% of the subjects had metabolic syndrome according to the criteria of the International Diabetes Federation [18]. The characteristics at recruitment of the participants who completed the study are presented in Table 1.

3.2. PPARG rs1801282 allele and genotype frequencies

All available DNA samples were genotyped ($n = 466$) and data was obtained for 415 subjects. Table 2 shows the allele and genotype frequencies for *PPARG* rs1801282 in subjects stratified

by ethnic group. Genotype distributions did not deviate from Hardy-Weinberg expectations. The minor allele frequencies were similar to those listed on the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/snp>; build 132 accessed 15/12/10). The G-allele was more frequent than expected in White Europeans (0.10 in RISCK compared to 0.076 in HapMap-CEU (European)) and absent in Black Africans, as in Hap-Map trios (HapMap-YRI (Sub-Saharan African)). There are no comparative data available for S. Asians.

3.3. PPARG rs1801282 genotype associations with phenotypes at recruitment screening

Table 3 shows anthropometry and plasma lipid concentrations in subjects at recruitment screening with respect to *PPARG* rs1801282 genotype. Carriers of the minor G-allele had significantly higher plasma total cholesterol concentration compared to non-carriers, after adjustment for BMI, gender, age and ethnicity.

3.4. Interaction between PPARG rs1801282 genotype and habitual dietary P:S ratio

There was a significant interaction between dietary P:S ratio and genotype as determinants of plasma LDL-cholesterol ($P = 0.02$) and triglyceride ($P = 0.03$) concentrations. Figure 1 shows plasma LDL-cholesterol and triglyceride concentrations adjusted for BMI, age, gender and ethnicity, with respect to *PPARG* rs1801282 genotype in quartiles of P:S intake. There were a number of significant differences in concentration of LDL-cholesterol and triglycerides in G-allele carriers between quartiles of P:S ratio as shown in Figure 1 and detailed in OSM Table 1. When the P:S ratio was low (≤ 0.33), mean plasma LDL-cholesterol concentration in G-allele carriers was higher than in non-carriers, but fell by up to 17% through the remaining quartiles. There was a difference of only 9% in LDL-cholesterol concentration in non-carriers across all quartiles. Plasma triglyceride concentration in G-allele carriers in the lowest quartile of P:S intake was 21.4% lower than in non-carriers. In the second quartile (0.34 to 0.47) G-allele carriers had higher mean concentration than non carriers, but showed a fall of 35.3%

from second to fourth (>0.65) quartile. There was 0% difference in triglyceride concentration in non-carriers between second and fourth quartiles.

3.5. Change in plasma lipid concentrations after dietary intervention

After the 4-wk run-in on HS diet, subjects were randomly assigned to continuation on the HS reference diet or the HM or LF diets. The HM group had lower plasma phospholipid %SF than the LF group ($P \leq 0.03$) and higher %MUFA ($P = 0.0001$). The dietary interventions did not affect other fatty acid classes [(n-3) PUFA, (n-6) PUFA and trans FA] [17]. Total cholesterol and LDL-cholesterol concentrations were significantly lower with the HM and LF than the HS diet ($P < 0.001$ and $P < 0.001$). Apo B concentrations differed between treatment groups ($P < 0.001$) and were lower with the HM and LF diets than with the HS diet. HDL cholesterol concentrations were lower with the LF than with the HS or HM diets ($P < 0.001$ and $P = 0.002$, respectively). There were no significant changes in concentration of plasma triglycerides following interventions [16].

3.6. PPARG rs1801282 genotype associations with plasma lipid concentrations at baseline

Table 4 shows anthropometry and plasma lipid measurements with respect to *PPARG* rs1801282 genotype after the 4-wk HS diet at baseline. Carriers of the minor G-allele had significantly higher plasma total cholesterol, LDL-cholesterol and apo B concentrations than non-carriers, after adjustment for gender, BMI, age and ethnicity.

3.7. PPARG rs1801282 genotype associations with change in plasma lipid concentrations after dietary intervention

In order to investigate the effect of decrease in SFA without alteration in MUFA intake, we compared change in plasma lipid concentrations after continuation on the HS and switching to LF diet (18% versus 10% SFA), with respect to *PPARG* rs1801282 genotype. Both diets

contained the same proportion of PUFA. There was no significant difference in the change in either plasma LDL-cholesterol or triglyceride concentration with respect to genotype ($n = 244$, respectively $P = 0.48$ and $P = 0.86$) after adjustment for change in BMI, age, gender and ethnicity. To examine the effect of increased intake of MUFA without alteration in SFA, we compared change in plasma lipid concentrations after the HM and LF diets (20% versus 11% MUFA), which also contained the same proportion of PUFA. There was no significant difference in the change in either plasma LDL-cholesterol or triglyceride concentrations with respect to genotype (respectively $n = 338$, $P = 0.34$; $n = 340$, $P = 0.24$) after adjustments. Interaction between the three diets and genotype did not significantly influence either LDL-cholesterol ($P = 0.58$) or triglyceride concentrations ($P = 0.57$).

4. Discussion

Numerous studies have investigated associations between *PPARG* Pro12Ala (rs1801282 C>G) and risk of obesity and diabetes, with equivocal outcomes. One meta-analysis of studies of type 2 diabetes has found a significant increase in risk with the Pro12 allele [5] but another revealed no significant effect on related traits [6]. Contrary findings of associations with obesity have also been reported [7]. These inconsistencies suggest that environmental modifiers of the effects of genetic variation in *PPAR* γ 2 may be involved. We have investigated associations between *PPARG* rs1801282 genotype and plasma lipid concentrations in subjects at risk of the metabolic syndrome, following habitual intake and dietary interventions differing in proportions of saturated and unsaturated fatty acids. After the habitual diet, minor G-allele carriers had significantly higher plasma total cholesterol concentration than non-carriers and the dietary P:S ratio interacted with genotype to influence the concentrations of plasma LDL-cholesterol and triglyceride. After the HS diet, G-allele carriers had significantly higher plasma total cholesterol, LDL-cholesterol and apo B

concentrations. A decrease in SFA intake after the HM and LF diets had no significant effect on either plasma LDL-cholesterol or triglyceride concentrations.

Memisoglu et al. [9] found total fat intake was inversely correlated with total plasma cholesterol in *PPARG* rs1801282 CC subjects but no effect among G-allele-carriers. They were the first to report an interaction between genotype and intake of MUFA, which was inversely associated with BMI in G-allele-carriers, but not in CC homozygotes. Thus, the responsiveness of G-carriers to dietary manipulation only emerged when MUFA rather than total fat intake was analysed. Luan et al. [8] had previously shown greater sensitivity of G-allele carriers to dietary PUFA in determination of BMI. Without reference to diet, genotype was not significantly associated, but interaction between the P:S ratio and genotype in determining BMI was highly significant ($n = 592$, $P = 0.0038$). As the ratio of P:S increased, BMI decreased in G-allele carriers but not in CC homozygotes. Both findings [8, 9] are compatible with unsaturated fats acting as specific ligands for *PPAR* γ [2] and lower transcriptional activity of the *PPAR* γ -Ala variant reducing *PPAR* γ -mediated adipogenesis [11].

Our study is the first to report significant interaction between the P:S ratio and *PPARG* rs1801282 genotype influencing plasma LDL and triglyceride concentrations. Switching from habitual to HS diet was accompanied by an increase in plasma LDL in carriers of the less transcriptionally active *PPAR* γ -Ala variant, compared to those homozygous for the normal *PPAR* γ -Pro. As the P:S ratio increased beyond the first P:S quartile, the concentration of plasma LDL-cholesterol fell by 17% in G-carriers and by only 9% in CC homozygotes. When PUFA replaces SFA in the diet, the major portion of cholesterol lowering is seen in the LDL fraction, with reduction in circulating particles revealed by a fall in apo B concentration [19]. This occurs primarily through receptor-mediated clearance of LDL, by reversing suppression of receptor activity induced by SFA [20]. Expression of the LDL receptor gene is activated by sterol regulatory element-binding protein-2 (SREBP-2) [21]. Insulin-induced

gene *INSIG1*, the key regulator of SREBP activity, is up-regulated by activation of PPAR γ [13]. PPAR γ activation by troglitazone has been shown to reduce nuclear SREBP-2 and down-regulate LDL clearance from plasma by the liver LDL receptor [22]. The less active PPAR γ -Ala variant would be expected to bind to the *INSIG1* PPRE with less affinity than the Pro variant. As a result, G-carriers would express less Insig-1, SREBP2 activity would be higher, and increased expression of the *LDLR* gene would lead to increased LDL-cholesterol clearance from the plasma. As the PPAR γ PUFA ligand concentration increased, the difference in abilities of the Pro- and Ala- variants to increase transcriptional activity could become more pronounced. In the highest P:S quartile we found plasma LDL-cholesterol concentration was significantly higher in carriers of the PPAR γ -Ala variant. Hamada et al. [23] showed G-allele carriers have a significantly larger electrophoretic sub-fractional area of small dense lipoprotein (sdLDL4-7) particles than those with the CC genotype, suggesting impaired ability to clear this atherogenic LDL sub-fraction in particular.

Lipoprotein lipase activity is a rate-limiting determinant of triglyceride hydrolysis in plasma. Plasma triglyceride concentration in G-allele carriers fell consistently beyond the second P:S quartile. It is well known that *n*-3 fatty acids decrease the concentration of serum triglycerides [24]. PPAR γ may mediate this effect, since PUFAs are PPAR γ ligands [2] and *LPL* is a PPAR γ target gene [15]. Lindi et al. [25] found a significantly greater decrease in serum triglyceride concentration in healthy G-allele carriers than in CC homozygotes in response to *n*-3 fatty acid supplementation, when the total dietary fat intake was below 37% energy intake or the intake of SFA was below 10%. This is consistent with our finding of a fall in plasma triglyceride concentration in G-allele carriers as P:S intake increased. However, both results suggest that the G-allele is associated with increased LPL activity in the presence of high PUFA and by implication, higher transactivation of the *LPL* gene by the PPAR γ Ala-variant, contrary to findings *in vitro* [11, 12]. Lindi et al. [25] found no difference in post-heparin plasma LPL activity following placebo or fatty acid supplementation, suggesting that

any change in LPL expression elicited by PPAR γ was not functionally significant. An explanation of the genotype x diet interaction which significantly influenced plasma triglyceride concentration is not evident at present.

We found that G-allele carriers had significantly higher plasma total cholesterol, LDL-cholesterol and apo B concentrations, compared to CC homozygotes after the 4-wk HS diet. An increase in plasma cholesterol by dietary SFA is well established [20]. Hence, a shift from a mean ~13% of energy SFA in the habitual intake [17] to ~18% of energy SFA on the HS diet would be expected to increase plasma cholesterol. As SFAs bind to PPAR γ with less affinity than PUFAs [2], increasing SFA would reduce activation of the receptor. The PPAR γ Pro- and Ala-variants do not differ in their affinities for pharmacological ligands, but the Ala-variant has lower affinity for target gene PPREs [11]. Both variants could show lower activation as SFA intake increases but gene activation by the Ala-variant would remain lower than by the Pro form. On the HS diet, G-carriers had 5.4% higher total cholesterol and 5.7% higher LDL-cholesterol, but 11% higher apo B than CC homozygotes. This suggests a higher number of plasma LDL-particles were circulating in carriers of the less transcriptionally active PPAR γ -Ala variant.

In order to establish whether effects of interaction between with the P:S ratio of habitual intake and genotype that we had observed were related to increased PUFA, as distinct from decreased SFA, we first compared change in plasma lipid concentrations after HS and LF diets, in which SFA was reduced and MUFA remained constant. Carriage of the G-allele was not significantly associated with change in either plasma LDL-cholesterol or triglyceride concentrations, so the decrease in SFA had no significant effect. We obtained the same results when we compared changes in lipids after HM and LF diets, in which MUFA was raised and SFA remained constant. An increase in MUFA might have been expected to have had an effect, but they are weaker PPAR γ activators than PUFAs [2]. Therefore we cannot confirm that the interaction between the P:S ratio of habitual intake and *PPARG*

rs1801282 genotype in determining plasma LDL-cholesterol and triglyceride concentrations depends specifically on an increase in consumption of PUFA, but it does appear that it does not depend on a decrease in SFA.

Limitations of our study include a relatively small sample size and the small observed changes in plasma lipid concentrations. Wide inter-individual variation in concentrations could have limited the significance of associations with phenotype. The significance of the effect of dietary P:S x gene interactions on plasma LDL-cholesterol and triglyceride concentrations should be treated with caution, as they were of modest significance in mainly overweight subjects. If substantiated in a larger cohort, a recommendation to *PPARG* rs1801282 G-allele carriers to maintain a high dietary intake of PUFA:SFA, to reduce plasma concentrations of atherogenic LDL-cholesterol and triglycerides, would be justified. Identification of individuals who are genetically more likely to respond to particular dietary changes may be important for successful intervention in the prevention of cardiovascular disease.

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Conflicts of interest

The authors and their research groups have a number of links with the food industry. In a personal capacity, GSF is a consultant to Coca-Cola, Premier Foods, and Unilever and TABS has acted as a consultant to Seven Seas and is a member of the Scientific Advisory Committee for the Global Dairy Platform and external scientific review committee of the Malaysian Palm Oil Board, and chairs Cadbury's Global Nutrition Advisory Panel. TABS, BAG, JAL, SAJ and GSF have received ad hoc honoraria for lectures or writing articles. In a non-personal capacity, BAG was formerly a member of an expert group known as the Fat Panel, which was supported by Dairy Crest, Kerry Gold, and Unilever; SAJ is a member of Scientific Advisory Boards for Coca-Cola, Heinz, PepsiCo, Nestlé and Kellogg's. SAJ sits on UK government advisory boards that also include food industry members. All research groups received products from a range of food companies gratis for research purposes, including Archer

Daniel Mills, Croda, Matthews Foods, Nestle, PepsiCo, Jordan, GSK, and Unilever. AA and SDO reported no conflicts of interest.

Appendix A Supplementary data associated with this article: OSM Table 1.

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Table 1

Characteristics of RISCK study subjects at recruitment screening

	Male	Female
Phenotype	(<i>n</i> = 230)	(<i>n</i> = 318)
Ethnicity ¹ [<i>n</i> (%)]		
White European	192 (83.5)	249 (78.3)
South Asian	21 (9.1)	31 (9.7)
Black African	12 (5.2)	28 (8.8)
Other	5 (2.2)	10 (3.2)
Age (y)	52 ± 10	51 ± 9
Waist circumference (cm)	102 ± 10	94 ± 12
BMI (kg/m ²)	28.3±3.8	28.6±5.3
Triglycerides (mmol/L)	1.4±0.8	1.2±0.7
Total cholesterol (mmol/L)	5.5±0.9	5.5±1.0
LDL-cholesterol (mmol/L)	3.6±0.8	3.5±0.9
HDL-cholesterol (mmol/L)	1.2±0.3	1.5±0.4

Data measured at recruitment is presented for all subjects who completed the study (*n* = 548). Values are mean ± SD. ¹Self-reported ethnicity.

Table 2

PPARG rs1801282 allele and genotype frequencies

	Ethnic group ¹				
	S. Asian [<i>n</i> (%)]	Black African [<i>n</i> (%)]	White European [<i>n</i> (%)]	Other [<i>n</i> (%)]	All [<i>n</i> (%)]
	44 (9)	38 (8)	366 (79)	18 (4)	466 (100)
MAF	0.07	0.0	0.10	0.10	0.09
CC	36 (86)	36 (100)	258 (80)	12 (80)	342 (82)
CG	6 (14)	0 (0)	61 (19)	3 (20)	70 (17)
GG	0 (0)	0 (0)	3 (1)	0 (0)	3 (1)
Total	42 (100)	36 (100)	322 (100)	15 (100)	415 (100)

All subjects for whom DNA samples were available were genotyped ($n = 466$); n (%) is number of each ethnic group genotyped, as % of total. Total with genotype data ($n = 415$); n (%) refers to number of each genotype obtained, with % genotype frequency. MAF Minor allele frequency; C = major allele; G = minor allele. ¹Self-reported ethnicity.

Table 3

Associations between *PPARG* rs1801282 genotypes and phenotypes at recruitment

Phenotype	<i>PPARG</i> rs1801282 genotype		<i>P</i>
	CC	CG + GG	
Males <i>n</i> (%)	132 (77)	40 (23)	
Females <i>n</i> (%)	210 (86)	33 (14)	
BMI (kg/m ²)	29.0 (28.5,29.4)	28.0 (26.9,29.0)	0.10
Waist circumference (cm)	98.5 (97.2,99.8)	97.9 (95.1,100.7)	0.16
Body fat (%)	34.6 (33.6,35.6)	31.6 (29.5,33.7)	0.26
Total cholesterol (mmol/L)	5.5 (5.4,5.6)	5.7 (5.5,6.0)	0.05
Triglycerides (mmol/L)	1.3 (1.2,1.3)	1.3 (1.2,1.5)	0.57
HDL-cholesterol (mmol/L)	1.4 (1.3,1.4)	1.4 (1.3,1.4)	0.92
LDL-cholesterol (mmol/L)	3.4 (3.3,3.5)	3.6 (3.4,3.8)	0.16

Data is presented for subjects for whom genotypic and phenotypic data was available (*n* = 415). Mean (95% CI), or GM mean (95% CI) values for triglycerides and HDL-cholesterol, stratified by genotype are shown at recruitment. Association was tested by univariate analysis of variance based on a dominant model. *P*-values adjusted for BMI, age, gender and ethnicity, shown in bold when nominally significant (< 0.05).

Table 4

Association between *PPARG* rs1801282 genotype and phenotypes after HS diet

Phenotype	<i>PPARG</i> rs1801282 genotype		<i>P</i>
	CC	CG + GG	
Males <i>n</i> (%)	132 (77)	40 (23)	
Females <i>n</i> (%)	210 (86)	33 (14)	
BMI (kg/m ²)	28.8 (28.3,29.3)	27.9 (26.9,29.0)	0.14
Waist circumference (cm)	97.9 (96.6,99.3)	98.4 (95.5,101.3)	0.48
Body fat (%)	34.3 (33.4,35.2)	31.6 (29.7,33.6)	0.25
Total cholesterol (mmol/L)	5.5 (5.4,5.6)	5.8 (5.6,6.0)	0.03
Triglycerides (mmol/L)	1.3 (1.3,1.4)	1.4 (1.3,1.6)	0.26
HDL-cholesterol (mmol/L)	1.4 (1.3,1.4)	1.4 (1.3,1.4)	0.85
Apolipoprotein A1 (g/L)	1.2 (1.2,1.2)	1.2 (1.2,1.3)	0.57
LDL-cholesterol (mmol/L)	3.5 (3.4,3.6)	3.7 (3.5,3.9)	0.04
Apolipoprotein B (g/L)	0.95 (0.92,0.98)	1.03 (0.96,1.10)	0.03

Data is presented for subjects for whom genotypic and phenotypic data was available (*n* = 415). Mean (95% CI), or GM mean (95% CI) values for triglycerides and HDL-cholesterol, stratified by genotype are shown after 4 wk on HS diet. Association was tested by univariate analysis of variance based on a dominant model. *P*-values adjusted for BMI, age, gender and ethnicity, shown in bold when nominally significant (< 0.05).

Figure legend

Figure 1. (A) Mean plasma LDL-cholesterol and (B) triglyceride concentrations with respect to quartiles of habitual dietary P:S ratio and *PPARG* rs1801282 genotype. C = major allele; G = minor allele. Number of genotyped subjects ($n = 415$) with plasma lipid measurements in each quartile of P:S ratio ≤ 0.33 , 0.34-0.47, 0.48-0.65, > 0.65 was as follows: (A) LDL-cholesterol: 88, 105, 102, 101 ($n = 396$); (B) Triglycerides: 88, 103, 101, 101 ($n = 393$). Mean concentration of LDL-cholesterol and geometric mean concentration of triglycerides are shown. Bars represent 95% CI. In CG + GG subjects, significant differences in LDL-cholesterol concentration with respect to P:S ≤ 0.33 and in triglycerides with respect to P:S > 0.65 are shown * $P < 0.05$; ** $P < 0.01$.