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Postgraduate Symposium
Effect of interaction between *PPARG*, *PPARA* and *ADIPOQ* gene variants and dietary fatty acids on plasma lipid profile and adiponectin concentration in a large intervention study

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Unsaturated fatty acids are ligands of PPAR-γ, which up-regulates genes involved in fatty acid transport and TAG synthesis and the insulin-sensitising adipokine adiponectin, which activates fatty acid β-oxidation via PPAR-α action in liver. We investigated the effect of dietary fatty acid interaction with *PPARG, PPARA* and *ADIPOQ* gene variants on plasma lipid and adiponectin concentrations in the Reading Imperial Surrey Cambridge King's study, a five-centre, parallel design, randomised controlled trial of 466 subjects at increased cardiometabolic risk. After a 4-week run-in to baseline, SFA was replaced by MUFA or carbohydrate (low fat) in isoenergetic diets for 24 weeks. Habitual dietary PUFA:SFA ratio × *PPARG* Pro12Ala genotype interaction influenced plasma total cholesterol ($P = 0{\cdot}02$), LDL-cholesterol ($P = 0{\cdot}002$) and TAG ($P = 0{\cdot}02$) concentrations in White subjects. *PPARA* Val162Leu × *PPARG* Pro12Ala genotype interaction influenced total cholesterol ($P = 0{\cdot}04$) and TAG ($P = 0{\cdot}03$) concentrations at baseline. After high-MUFA and low-fat diets, total cholesterol and LDL-cholesterol were reduced ($P < 0{\cdot}001$) and gene × gene interaction determined LDL-cholesterol ($P = 0{\cdot}003$) and small dense LDL as a proportion of LDL ($P = 0{\cdot}012$). At baseline, *ADIPOQ* −10066 G/A A-allele was associated with lower serum adiponectin (*n* 360; $P = 0{\cdot}03$) in White subjects. After the high-MUFA diet, serum adiponectin increased in GG subjects and decreased in A-allele carriers ($P = 0{\cdot}006$ for difference). In GG, adiponectin increased with age after the high MUFA and decreased after the low-fat diet ($P = 0{\cdot}003$ for difference at 60 years). In conclusion, in Whites, high dietary PUFA:SFA would help to reduce plasma cholesterol and TAG in *PPARG* Ala12 carriers. In *ADIPOQ* −10066 GG homozygotes, a high-MUFA diet may help to increase adiponectin with advancing age.

Nutrient–gene interaction: PPAR: Adiponectin: Plasma lipids

The metabolic syndrome is defined by dyslipidaemia, glucose intolerance, hypertension and visceral obesity and is associated with an increase in the risk of type 2 diabetes and CVD[1]. Both environmental and genetic predisposition contribute to development. Among environmental factors, dietary habits (intake of fat, carbohydrate, alcohol and micronutrients) are of crucial importance. Low-fat (LF) diets reduce body weight[2] and LF and high complex carbohydrate diets produce a significant reduction in total cholesterol (TC), LDL-cholesterol (LDL-C) and TAG[3,4]. Recently, the type of fat consumed, SFA, MUFA or PUFA, has received more attention. Atherogenic dyslipidaemia is characterised by increased TAG-rich lipoproteins, small LDL-C particles and reduced HDL-cholesterol (HDL-C)[5].

Abbreviations: HM, high MUFA; HDL-C, HDL-cholesterol; HS, high saturated fat; LDL-C, LDL-cholesterol; LF, low fat; LPL, lipoprotein lipase; P:S, PUFA:SFA ratio; PPRE, peroxisome proliferator response element; RISCK, Reading Imperial Surrey Cambridge King's; sdLDL, small dense LDL; SREBP, sterol regulatory element-binding protein; TC, total cholesterol; TZD, thiazolidinedione.

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Diets rich in SFA have an adverse effect[6,7], whereas consumption of a MUFA-rich diet at the expense of SFA promotes healthy blood lipid profiles, improves insulin sensitivity and regulates glucose levels[8]. Substitution of carbohydrate by MUFA generally decreases TAG. Effects of high MUFA (HM) intake on LDL-C are less well defined, with reports of a reduction[9,10] or no effect[3].

The lack of consistent outcomes in dietary intervention studies could reflect variation in genetic background. Understanding the nature of multiple gene–gene interaction and gene–environment interactions is pivotal in understanding the causes and progression of the metabolic syndrome and its management[11]. In population-based studies, the habitual dietary intake of fat is an important consideration in determining an association of any SNP with risk of metabolic syndrome.

PPAR-γ

PPAR-γ is a member of the nuclear hormone receptor superfamily[12], a transcription factor with extensive influence over expression of genes related to inflammation, adipose cell differentiation, atherosclerosis and metabolism[13]. The major natural ligands of PPAR-γ are PUFA, as well as prostanoids[14], which suggests a role in transducing nutritional to metabolic signals[15]. Synthetic ligands include the thiazolidinediones (TZD)[12]. On ligand-dependent activation, PPAR-γ heterodimerises with retinoid-X receptor-α and binds to a peroxisome proliferator response element (PPRE) in the promoter region of the target genes (Fig. 1).

Role in lipid homoeostasis

Expression of the LDL receptor gene is activated by sterol regulatory element-binding protein (SREBP)-2[16]. Activated PPAR-γ up-regulates the insulin-induced gene *INSIG1*, the key regulator of SREBP activity[17]. Reported effects of PPAR-γ agonist TZD are mainly increased HDL-C, increased size/decreased density of LDL-C particles and increased lipoprotein (a)[18]. 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[19] and troglitazone and rosiglitazone have been shown to increase plasma LDL-C concentrations[20].

It is well known that *n*-3 fatty acids, ligands of PPAR-γ, decrease the plasma concentration of TAG[21]. PPAR-γ may mediate this effect through enhancement of synthesis, clearance or hydrolysis. Troglitazone has been shown to decrease SREBP-1 target genes fatty acid synthase (*FASN*) and glycerol-3-phosphate acyltransferase (*GPAM*) resulting in reduction of TAG synthesised from *de novo*-derived fatty acids, intracellular and secreted TAG concentrations[22]. Other PPAR-γ targets are fatty acid transport protein and CD36[23], which facilitate the transport of fatty acids across cell membranes, and acyl-CoA synthetase, which facilities esterification to prevent their efflux[24], so PPAR-γ also enhances clearance of TAG from plasma by this route. Lipoprotein lipase (LPL) is a rate-limiting determinant of plasma TAG hydrolysis and as the *LPL* gene is a target of PPAR-γ[25] TAG could also be reduced by this mechanism. In summary, PPAR-γ activation by unsaturated fatty acids is expected to decrease TAG and possibly increase plasma LDL-C concentration.

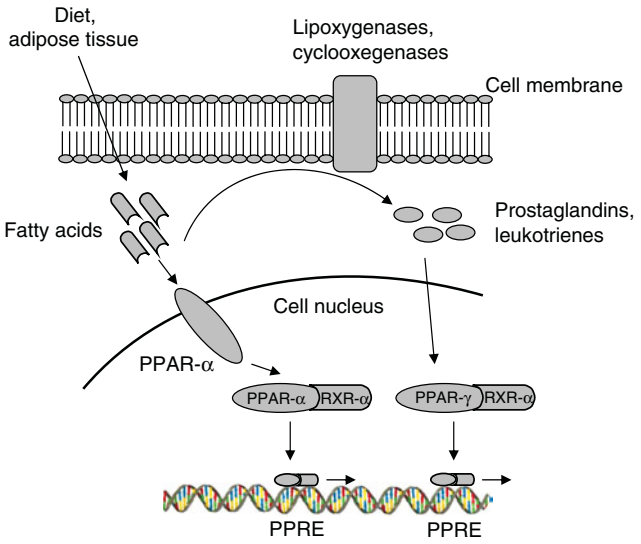

Fig. 1. (Colour online) PPAR mechanism of action. The major natural ligands of PPAR-γ and PPAR-α are PUFA, as well as prostanoids. Upon ligand-dependent activation, both PPAR heterodimerise with retinoid-X receptor-α (RXR-α) and bind to a peroxisome proliferator response element (PPRE) in the promoter region of the target genes to initiate transcription.

PPARG gene Pro12Ala polymorphism

Four subtypes of PPAR-γ mRNA transcribed from different promoters give rise to two different PPAR-γ proteins[26]. The PPAR-γ2 protein is exclusively expressed in adipose tissue[12]. Since PPAR-γ regulates several genes in different tissues, variation in the *PPARG* gene is likely to be associated with an alteration of the expression levels of targets[13]. The most widely studied SNP is Pro12Ala in the PPAR-γ2 isoform, located in codon 12 of exon 3[27]. The frequency of the minor allele is 0·076 in Europeans[28], lower in non-Caucasians[29].

Numerous studies have investigated association of Pro12Ala with the risk of obesity and diabetes. Results generally indicate a favourable effect of Ala12 carriage, but there are contrary findings. A meta-analysis of over 30 000 subjects, reported a significant association between Ala12 and the lowest risk of type 2 diabetes mellitus in overweight Caucasians[30]. Ala12 associates with reduced risk of obesity in some studies[31], but not others[32]. Contrary findings indicate association with increased risk of weight gain in obese patients[33], and higher BMI, waist circumference and fat mass[34,35]. In a recent meta-analysis, Ala12 carriers had significantly increased TC and HDL-C and lower plasma TAG compared with Pro homozygotes[36]. Other studies have reported no association between Pro12Ala and TAG concentrations[37] or plasma lipids[38,39].

Pro12Ala and diet

An increase in PPAR-γ mRNA in adipose tissue of mice exposed to a high-fat diet[40] suggested that dietary modulation might influence adipogenesis induced by

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PPAR-γ in response to raised plasma concentration of fatty acid ligands. PUFA affinities for PPAR-γ depend largely on their chain length and degree of saturation[14]. Thus, the metabolic impact of this polymorphism is potentially dependent on gene interaction with different types of dietary fat. A direct effect was reported in functional studies, in which the PPAR-γ Ala variant had decreased binding affinity for the PPRE and thus reduced transactivation ability, both in TZD-induced adipogenesis and a luciferase reporter gene assay[31,41].

The outcomes of previous studies on dietary interaction with Pro12Ala have been variable. Total fat intake was positively associated with increased BMI and waist circumference[34] and inversely correlated with plasma TC[42] in Pro12 homozygotes but not in Ala12 allele carriers. Memisoglu *et al.*[42] found that intake of MUFA was inversely associated with BMI in Ala12 carriers, but not in Pro12 homozygotes. Thus, the responsiveness of Ala12 carriers to dietary fat only emerged when MUFA rather than total fat intake was analysed. Luan *et al.*[43] had previously shown greater sensitivity of Ala12 carriers to dietary PUFA in determination of BMI. Interaction between the PUFA:SFA (P:S) ratio and genotype in determining BMI was highly significant. As P:S increased, BMI decreased in Ala12 carriers but not in Pro12 homozygotes. Both findings[42,43] are compatible with unsaturated fatty acids acting as specific ligands for PPAR-γ[14] and lower transcriptional activity of the Ala variant reducing PPAR-γ-mediated adipogenesis[31]. The Ala12 variant appears to be a diet-dependent metabolic sensor, whose protective effect appears to depend on the amount and type of dietary fat.

Pro12Ala interaction with habitual dietary PUFA:SFA ratio in the Reading Imperial Surrey Cambridge King's study

The Reading Imperial Surrey Cambridge King's (RISCK) study is a parallel 2 × 2 factorial design compared with a control intervention, to investigate effects of dietary fat intake on variables characterising the metabolic syndrome[44]. After a 4-week run-in on a high-SFA Western-type 'reference diet' (HS (high saturated fat)), subjects were randomised to continuation on the HS diet, a 'HM' diet in which SFA was reduced and replaced with MUFA and 'LF diet', in which SFA was reduced through replacement of total fat with carbohydrate. All participants followed prescribed diets for 24 weeks. A total of 549 subjects completed the RISCK study. Based on self-reported ethnicity, individuals of White, S. Asian, Black African and 'other' ancestry were distinguished. In view of the small sample size of the S. Asian and other ancestries and absence of the Pro12Ala SNP in Blacks, we chose to focus our genetic investigation on the White subjects only. Initially we were interested in the effect of P:S interaction with Pro12Ala genotype on plasma lipid concentrations. For this we utilised habitual intake at recruitment, as PUFA intake was constant in the interventions.

There was a significant interaction between dietary P:S ratio and genotype as a determinant of plasma concentrations of TC ($P = 0{\cdot}02$), LDL-C ($P = 0{\cdot}002$) and TAG ($P = 0{\cdot}02$) after adjustment for BMI, age and gender. When the P:S ratio was low (≤0·33), mean plasma TC concentration in Ala12 carriers was significantly higher than in non-carriers ($P = 0{\cdot}003$). As P:S increased, the concentration of TC fell by 10%. The trend in reduction as the ratio increased from ≤0·33 to >0·65 was significant ($P = 0{\cdot}02$). An even more significant difference was seen in LDL-C concentration between carriers and non-carriers in the lowest P:S quartile ($P = 0{\cdot}0001$). As P:S increased, the concentration fell by 19·5% in Ala12 carriers, but here the trend was NS ($P > 0{\cdot}05$). There were no significant differences in plasma TAG concentrations between Ala12 carriers and non-carriers in any P:S quartile. However, there was a significant trend in the reduction of plasma TAG in Ala12 carriers as the P:S ratio increased from 0·34 to >0·65, in which concentration fell by 50·0% ($P = 0{\cdot}002$). Plasma TAG concentrations stratified by genotype and P:S quartile are shown in Fig. 2.

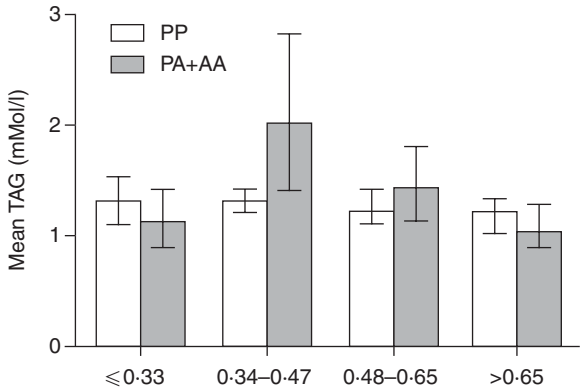

Fig. 2. Mean TAG concentration with respect to quartiles of habitual dietary PUFA:SFA (P:S) ratio and *PPARG* Pro12Ala genotype in White subjects. The numbers of genotyped subjects with measurements in each quartile of P:S ratio ≤ 0·33, 0·34–0·47, 0·48–0·65 and >0·65, were as follows: PP: 64, 76, 59, 49; PA + AA:16, 8, 19 and 17. The geometric mean concentrations of TAG are shown. Bars represent 95% CI. Dietary P:S ratio × genotype interaction determined by univariate analysis of covariance (ANCOVA) significantly influenced plasma TAG ($P = 0{\cdot}02$), after adjustment for BMI, gender and age. There was a significant trend in reduction of plasma TAG concentration between P:S ratio 0·34 to >0·65 ($P = 0{\cdot}002$) in Ala12 allele carriers.

As mentioned earlier, PPAR-γ activation by troglitazone has been shown to raise circulating LDL-C[19] and increased plasma concentration has been observed following TZD treatment[20]. As the PPAR-γ-Ala12 form has lower transactivational ability than the wild-type[31], Ala12 allele carriers would be expected to show a fall in LDL-C concentration, as we observed in the higher P:S quartiles. However, at P:S <0·33, the concentration of PUFA ligand may not have been sufficient to activate LDL clearance in carriers of the low-activity isoform.

Plasma TAG concentration in Ala12 carriers fell consistently in the higher P:S quartiles. As mentioned earlier, PPAR-γ activity is expected to reduce plasma TAG[21]. Lindi *et al.*[45] found a significantly greater decrease in serum TAG concentration in Ala12 carriers than in Pro12 homozygotes in response to *n*-3 fatty acid supplementation, when the intake of SFA was below 10%, i.e. at high P:S intake. This is consistent with our finding of a fall in

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plasma TAG concentration in Ala12 carriers as P:S intake increased, but is inconsistent with reduced lipase activity associated with a less-active PPAR-γ-Ala isoform.

In order to determine whether gene interaction was related to decreased SFA, rather than increased PUFA, we utilised data from dietary interventions. As these did not differ in PUFA content, we were only able to investigate change in SFA. The HS and LF diets allowed comparison of high and low SFA, with constant MUFA and PUFA intake. As carriage of Ala12 was not significantly associated with change in either plasma LDL-C or TAG concentrations, the interaction does not appear to depend on a decrease in SFA.

PPAR-α

PPAR-α is a nuclear receptor mostly expressed in tissues with high levels of fatty acid oxidation, such as liver and muscle[46] and regulates target genes involved in the transportation and oxidation of fatty acids[47]. PPAR-α ligands can be both exogenous lipid-lowering drugs such as fibrate and fenofibrate and endogenous SFA and unsaturated fatty acid[48,49].

Like PPAR-γ, ligand-activated PPAR-α heterodimerises with retinoid-X receptor-α before binding to target gene promoters[26], which usually contain one or more PPRE[50]. In addition, PPAR-α transactivation is modulated by co-factors or co-repressors[48], which in the absence of a ligand inhibit its activity[51]. AMP-activated protein kinase activation increases expression of PPAR-α target genes in muscle[52]. PPAR-α also appears to alter its own expression[51,53] and transcriptional activity is also regulated by phosphorylation, which stabilises its binding to the PPRE[48].

Role in lipid homoeostasis

In the liver, fibrate agonists of PPAR-α enhance fatty acid transport protein and acyl-CoA synthetase, which generate fatty acyl-CoA, carnitine palmitoyl transferase-1, essential for facilitating the entry of fatty acyl carnitine into mitochondria, and genes involved in mitochondrial β-oxidation[54]. Other genes involved in peroxisomal[55,56] and microsomal β-oxidation[57] are tightly regulated by PPAR-α. *INSIG1*, the key regulator of SREBP activity, is up-regulated by activation of PPAR-α in liver by clofibrate[58], leading to reduction in expression of the SREBP-2 target LDL receptor gene *LDLR*[16] and an increase in plasma LDL-C concentration. The hypotriglyceridaemic action of fibrates involves effects on LPL and apoC-III expression[59,60] and on enzymes involved in TAG synthesis. PPAR-α induces *LPL* gene transcription[60] and represses expression of apoC-III, a natural inhibitor of LPL activity[59], which further enhances LPL-mediated catabolism of very low density lipoprotein (VLDL) production[60]. Treatment with PPAR-α agonist WY14 643 reduces *FASN* and *GPAM*, resulting in reduced synthesis of TAG[22].

PPARA *gene Leu162Val polymorphism*

The human PPAR-α gene *PPARA* gene contains fifteen coding SNP. The active isoform *PPARA1* encodes the entire region, whereas *PPARA2* is truncated[61]. The most widely studied SNP Leu162Val is located in codon 162 of exon 5[62]. The frequency of the minor allele (Val162) is 0·042 in Europeans[28]. Many studies have examined association of *PPARA* Leu162Val with plasma lipid profiles, with conflicting results. Associations of Val162 with higher[63–66] and lower[67] concentrations of plasma TAG have been found. Val162 has been associated with higher levels of LDL-C[62,64] and with higher[68] and lower[65,69] concentrations of HDL-C. Higher concentrations of apoA-1[68], apoC-III[62,66] and apoB[70] have been found in Val162 carriers. However, several studies have found no associations with lipid profile, BMI, body fat composition or insulin sensitivity[71,72]. Only one other investigation has examined *PPARA* Leu162Val and *PPARG* Pro12Ala interaction in determination of plasma lipid concentrations, which found no effect in obese subjects[73].

Reports of the relative activities of the Leu162 and Val162 PPAR-α isoforms *in vitro* have been contradictory, possibly owing to dependence on ligand concentration. Sapone *et al.*[74] found Val162 allele had greater activity than Leu162 at high, but lower activity at low ligand concentration. Flavell *et al.*[68] originally found Val162 showed greater transactivation in a reporter construct. However, recently Rudkowska *et al.* found transcription to be higher in Leu162 than Val162 constructs containing the *LPL* PPRE, after *n*-3 fatty acid transactivation[75]. They also found an inverse correlation between LPL activities and plasma TAG levels in Leu162 homozygotes but not in Val162 carriers[76], suggesting that Val162 has lower transactivational ability than Leu162 under physiological conditions.

Leu162Val and diet

Reports of *PPARA* Leu162Val interaction with fatty acid intake in determination of plasma lipids are inconsistent, including no interaction with PUFA[77], Val162 allele association with higher TC, LDL-C and apoA1 after a high-PUFA diet[78] and higher TAG and apoCII after low PUFA intake[77]. In the latter, when PUFA intake was less than 4%, Val162 carriers had higher plasma TAG compared with Leu162 homozygotes, but when PUFA intake was more than 8%, Val162 allele carriers had lower plasma TAG. In Leu162 homozygotes, waist circumference increased with a higher intake of dietary fat, but no significant interaction was found in determining TC, LDL-C, HDL-C or apoB concentrations[63]. Only one other study has examined *PPARG* Pro12Ala and *PPARA* Leu162Val after dietary intervention. After a 2·5-year low-energy diet, in non-diabetic obese women there were significant favourable changes in lipid profile, but no significant interactive effects on anthropometric or biochemical characteristics at baseline or at the follow-up[79].

PPARG *Pro12Ala and* PPARA *Leu162Val interaction in the Reading Imperial Surrey Cambridge King's study: effect of MUFA*

We hypothesised that carriage of *PPARG* Pro12Ala and *PPARA* Leu162Val allelic combinations might influence

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concentration of plasma lipids according to the availability of dietary unsaturated fatty acid ligands. At baseline, after a 4-week run-in on the HS diet, carriage of the *PPARG* Ala12 allele was associated with a modest increase in plasma TC (*n* 415; $P = 0{\cdot}05$), LDL-C ($P = 0{\cdot}04$) and apoB ($P = 0{\cdot}03$) after adjustment for BMI, age, gender and ethnicity. Although SFA are relatively poor stimulators of PPAR-γ activity[14] these outcomes are likely to reflect lower transactivation of target genes by the PPAR-γ-Ala form[31]. The *PPARA* Leu162Val genotype was not associated with concentrations of plasma lipids at baseline, but *PPARA* Val162Leu × *PPARG* Pro12Ala genotype interaction influenced TC ($P = 0{\cdot}04$) concentration after adjustment for covariates.

After HM and LF diets, plasma TC, LDL-C and apoB concentrations were reduced ($P<0{\cdot}001$), but surprisingly there was no change in TAG concentration[44]. Independent associations of *PPARG* Pro12Ala or *PPARA* Leu162Val genotypes with changes in concentrations of plasma lipids with respect to baseline were NS after randomisation to diets. However, there was significant interaction between the two genotypes as determinants of plasma LDL-C concentration, ($P = 0{\cdot}003$) and small dense LDL (sdLDL) as a proportion of LDL ($P = 0{\cdot}012$) after adjustment for change in BMI, age, gender and ethnicity. Carriage of both variant alleles was associated with a greater reduction in LDL-C and proportion as sdLDL after HM diet than after LF diet. PUFA is a stronger activator of PPAR than MUFA[14], but was constant in both interventions. As PPAR variant carriage affected plasma lipids only after the HM diet, the effects may depend on HM concentration.

Fig. 3 shows the follow-up concentrations of plasma LDL-C and sdLDL as a proportion of LDL after the HM and LF diets above the baseline, with respect to *PPARG* Pro12Ala and *PPARA* Leu162Val genotype combinations. The results of gene × gene interaction were highly significant for these data. Our ANOVA model used the variability of the whole dataset to measure the background variation, and produced evidence of a significant effect of gene–gene interaction on LDL-C and proportion as sdLDL. The significance should nevertheless be treated with caution and confirmation awaits replication in a larger sample.

As explained earlier, PPAR-γ activation by troglitazone reduces nuclear SREBP-2 and down-regulates LDL clearance from plasma by SREBP-2 target, the liver LDL receptor[19]. Expression of the LDL receptor is also reduced by clofibrate[58]. Activation of PPAR-α and PPAR-γ would thus impair LDL receptor expression, down-regulate LDL clearance from plasma and increase circulating LDL-C concentration, as found in response to TZD[20], but LDL apoB-100 levels generally decrease in response to fibrates[22]. PPAR-γ-Ala12 and PPAR-α-Val162 forms have lower transactivational ability than the wild types[31,76]. Hence, carriers of *PPARG* Ala12 and *PPARA* Val162 would express higher LDL receptor activity, leading to maximum clearance and the largest fall in LDL-C concentration, as we observed. All the other genotype combinations showed smaller reductions in LDL-C after the HM diet. As Ala12 was associated with higher TC concentration and interaction with Val162 yielded higher

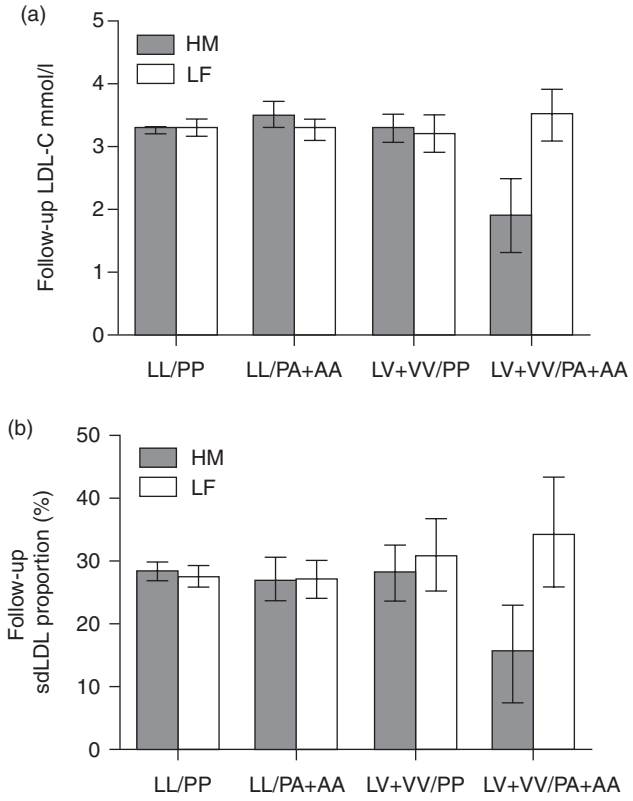

Fig. 3. Interaction between *PPARG* Pro12Ala and *PPARA* Leu162Val genotype groups after dietary treatments influences plasma LDL-cholesterol (LDL-C) concentration and small dense LDL (sdLDL) as proportion of LDL. Interaction between *PPARG* Pro12Ala and PPARA Leu162Val genotypes was a significant determinant of change in plasma concentrations of (a) LDL-C ($P = 0{\cdot}003$) and (b) sdLDL as proportion of LDL ($P = 0{\cdot}012$) after high MUFA (HM) and low fat (LF) diets, after adjustment for baseline values, change in BMI, age, gender and ethnicity using three-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-C (mmol/l) and sdLDL as proportion of LDL (%) adjusted for baseline values after 24 weeks on HM or LF diets are shown. Bars indicate 95% CI. The figure is based on subjects with genotypes for both SNP and measurements of plasma lipids after HM and LF diets. The numbers of subjects in each genotype group LL/PP, LL/PP + PA, LV + VV/PP and LV + VV/PP + PA were as follows: HM diet: 121, 24, 17, 4; LF diet: 126, 34, 9 and 4.

LDL-C after the HS diet, the lower LDL-C in carriers of both variants after the HM diet appears to be a response to increased availability of MUFA.

One of the most consistent effects of TZD is to increase the mean LDL particle size and/or reduce LDL density[80]. Were PPAR-γ to be implicated directly, carriage of the lower activity PPAR-γ-Ala form would be expected to associate with a higher proportion of small LDL particles. This was found to be the case by Hamada *et al.*[81], where *PPARG* Ala12 carriers had a significantly higher proportion of sdLDL fractions four to seven independent of lipid concentration. As mentioned previously, high-fat intake is associated with an increase in large LDL and decrease in

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sdLDL[82]. Bouchard-Mercier *et al.*[83] found no significant change in LDL peak particle diameter in *PPARG* Pro12 homozygotes or Ala12 carriers after high SFA intake, but a significant increase in LDL peak particle diameter in Ala12 carriers after high intake of PUFA, which unlike SFA are PPAR-γ activators[14]. They found that high SFA intake associated with larger LDL particle size in *PPARA* Leu162 homozygotes, but with a higher proportion of sdLDL in Val162 carriers. Fibrate ligands of PPAR-α can reduce production of VLDL[22] and lower sdLDL[84,85], and so in carriers of the less-active PPAR-α-Val form, activation by dietary ligands could result in a shift to a higher proportion of sdLDL. We found no significant change in the proportion of sdLDL in carriers of *PPARG* Ala12 or *PPARA* Val162 on switching from the HS diet at baseline to the HM or LF diets, but a significant reduction in the proportion of sdLDL in carriers of both *PPARA* Val162 and *PPARG* Ala12 alleles after the HM diet. This cannot be explained by reduced activity of both variants, because as indicated above, this would be expected to lead to a higher proportion of sdLDL.

Adiponectin

Adiponectin is a 244-amino-acid plasma protein secreted exclusively by adipocytes. It is an insulin sensitising adipokine with anti-atherogenic, anti-diabetic and anti-inflammatory functions[86]. Plasma adiponectin concentration is negatively correlated with human obesity, hypertension, insulin resistance and increased plasma TAG concentrations[87,88]. In the circulation, adiponectin is present in three oligomeric complexes, with different biological functions, acting though distinct signalling pathways. The basic trimer is the low-molecular-weight isoform[89]. The hexametric isoform is formed through the association of two homotrimers[90]. High-molecular-weight adiponectin is the biologically active form.

Effect of gender, age and ethnicity

The sexual dimorphism of adiponectin is well known; males have significantly lower plasma concentrations than females[91]. The gender differences have been attributed primarily to the inhibitory effect of testosterone on adiponectin production established *in vitro*[92]. Adiponectin concentrations generally increase with age[93], mainly explained by changes in sex hormones[87]. As insulin sensitivity declines with age, this may reflect development of resistance, or survival in those with higher concentrations. Cohen *et al.*[94] reported significantly lower concentrations in Black than in White individuals.

Effect on insulin sensitivity

Adiponectin acts on two receptors AdipoR1 in skeletal muscle and AdipoR2, more abundant in the liver[95] (Fig. 4). In the liver, adiponectin activates PPAR-α and AMP-activated protein kinase, a key energy sensor that maintains cellular energy homoeostasis, via AdipoR2. Activation of AMP-activated protein kinase down-regulates enzymes involved in gluconeogenesis, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. It also increases the inhibitory phosphorylation of acetyl coenzyme A carboxylase, promoting fatty acid oxidation, and inhibits the action of genes such as *SREBP*, required for fatty acid synthesis. The activation of PPAR-α by AMP-activated protein kinase decreases TAG in the liver by stimulating fatty acid oxidation[95]. In muscle, adiponectin acts via AdipoR1 to stimulate fatty acid oxidation and glucose utilisation. AdipoR1 targets genes such as CD36, involved in fatty acid transport, acyl-CoA oxidase, involved in fatty acid oxidation and uncoupling protein-2, involved in energy dissipation as heat[95]. Therefore, adiponectin increases fatty acid oxidation in liver and muscle, leading to reduced adipose tissue mass, a fall in pro-inflammatory cytokines and promotion of insulin signalling.

Effect on plasma lipid profile

Adiponectin is correlated negatively with plasma TAG[96] and positively with HDL-C concentration[97]. The mechanism may relate to insulin resistance. Insulin is a well-known stimulator of adipose tissue LPL activity[98], which catalyses the rate-limiting step in the hydrolysis of the TAG component in circulating VLDL and chylomicrons[99]. Adiponectin promotes mitochondrial fatty acid oxidation and reduction in circulating fatty acids, which in turn promotes LPL activity[100]. Adiponectin also activates PPAR-α, which up-regulates expression of apo-proteins A-I and A-II, promoting hepatic HDL-C secretion[101].

ADIPOQ *gene polymorphisms*

Fifty-three SNP have been identified at the adiponectin gene *ADIPOQ* locus[102]. There are many, often conflicting, reports of SNP associations with circulating adiponectin concentrations[103] and various metabolic syndrome traits. In an earlier study of SNP at the *ADIPOQ* locus −11391 G/A, −10066 G/A, −7734 A/C and +276 G/T in this laboratory, we found −10066G, −11391A, −7734A and +276T were significantly associated with higher serum adiponectin concentration in two large cohorts[104]. Association of elevated adiponectin with the −11391 A-allele has been reported widely[102,105–108], although one group found lower adiponectin in G-allele carriers[109]. Associations between +276G and lower adiponectin concentrations have also been reported in Spanish[110], European[111], Korean[112] and Japanese[113] subjects. The −10066G allele has also been associated with higher adiponectin concentration elsewhere[107].

Association between *ADIPOQ* gene variants and metabolic syndrome risk factors has been established in many studies. +276G carriage predisposed to higher CVD risk in Koreans[112]. In Italians +276T was a risk allele in one study[114] and protective in another[115]. In Spanish subjects +276G was associated with impaired glucose tolerance[110] and higher homoeostatic model assessment of insulin resistance in Korean[112], Italian[116] and Japanese[113] subjects. +276T has been associated with lower[116,117] and higher[118] homoeostatic model assessment of insulin resistance. Higher LDL-C and lower HDL-C levels have been

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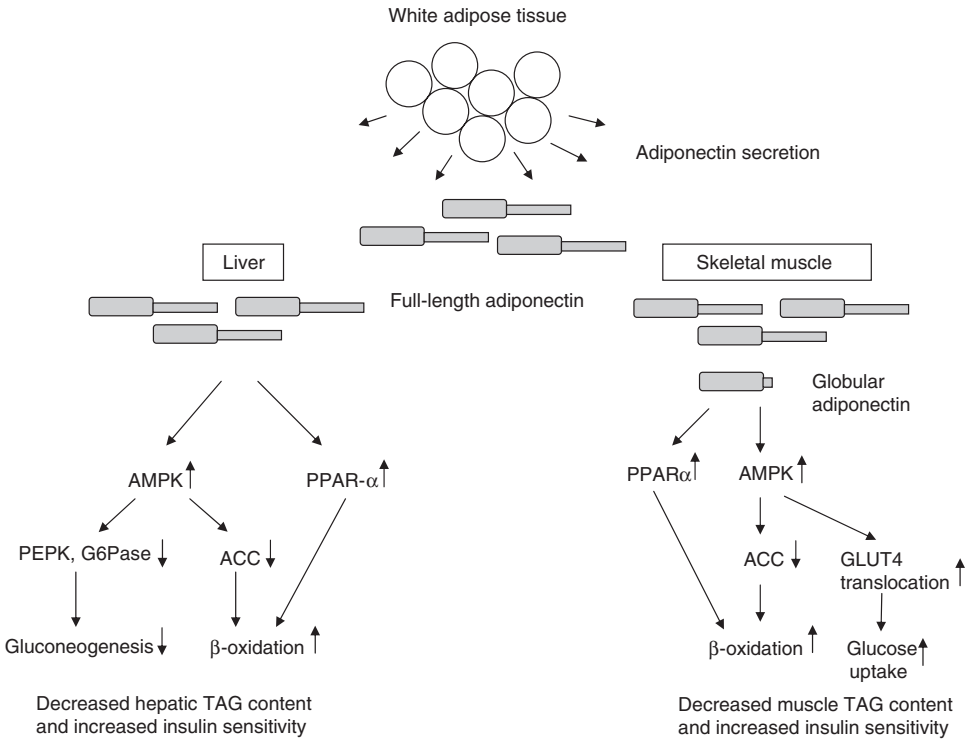

Fig. 4. Adiponectin mechanism of action. Adiponectin activates AMP kinase (AMPK) and PPAR-α in liver and skeletal muscle. In muscle, globular and full-length adiponectin activate AMPK, stimulating inhibitory phosphorylation of acetyl-CoA carboxylase (ACC), promoting fatty-acid oxidation, and GLUT4 translocation promoting glucose uptake. Activation of PPAR-α also leads to stimulation of fatty-acid oxidation and decreased TAG. In the liver, full-length adiponectin activates AMPK, thereby reducing enzymes involved in gluconeogenesis, also increasing phosphorylation of ACC and stimulating fatty-acid oxidation. Activation of PPAR-α decreases TAG as in muscle. All actions increase insulin sensitivity. PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase. (Adapted from Kadowaki and Yamauchi[95]).

found in +276T allele carriers[119] and +276G was associated with higher concentration of TAG in Koreans[112]. The +276G allele was associated with higher BMI in Italians[116] but with lower BMI in Swedish and African Americans[120,121]. Higher waist:hip ratio was found in carriers of the −11391A allele[122] and there are reports of increased risk of insulin resistance and type 2 diabetes associated with the −11391 G/G genotype[105,109].

ADIPOQ *polymorphisms and diet*

Inconsistent associations between the *ADIPOQ* variants and serum adiponectin, BMI and insulin resistance suggest that environmental influences may be influential. A few studies have explored the relationship between dietary factors and adiponectin concentrations or gene–nutrient interactions involving SNP at the *ADIPOQ* locus. In the largest study to date, in American Whites, −11391 A-allele carriers in the highest fiftieth percentile of MUFA intake had lower BMI and risk of obesity compared with G/G homozygotes[123]. In another study, after switching from an SFA- to MUFA-rich diet, −11377 C/C homozygotes were significantly less insulin resistant compared with G-allele carriers[124]. In a recent study, an interaction between *ADIPOQ* −11377 C/G genotype with SFA, but not MUFA or PUFA, significantly affected homoeostatic model assessment of insulin resistance, but there were no significant effects on serum adiponectin concentration[125].

ADIPOQ *and* PPAR-γ

One potential pathway for dietary interaction with *ADIPOQ* is via activation of PPAR-γ[126]. PPAR-γ agonists such as TZD have been clearly shown to increase serum adiponectin concentrations in both human subjects and rodents[127]. PUFA have been reported to increase plasma adiponectin concentrations and may up-regulate *ADIPOQ* by acting as ligands of PPAR-γ[127]. Both natural and artificial ligands of PPAR-γ enhance the expression of adiponectin mRNA in adipose tissue and dramatically increase plasma concentration of adiponectin[128]. The mechanism involves a functional PPRE and a responsive element of liver receptor homolog-1 in the *ADIPOQ* promoter[127].

ADIPOQ *−10066 G/A in the Reading Imperial Surrey Cambridge King's study: effect of MUFA*

Diets low in carbohydrate[129] and high in unsaturated fat increase adiponectin[130]. We hypothesised that variants in

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ADIPOQ could interact with dietary intake of unsaturated fat and age to influence serum adiponectin in the absence of significant change in BMI, in RISCK study participants.

After 4-week run-in on the HS diet, there were significant differences between males and females in fasting glucose and TAG (higher in males), HDL-C, adiponectin, insulin sensitivity and percentage body fat (lower in males). Adiponectin positively correlated with age ($\beta = 0{\cdot}217$, $P<0{\cdot}001$) and negatively with BMI ($\beta = -0{\cdot}161$, $P<0{\cdot}001$) in agreement with previous reports[87,93]. Adiponectin was significantly higher in White Europeans than in S. Asians ($P = 0{\cdot}001$) and Black Africans ($P = 0{\cdot}001$) as reported previously[94] and higher in females (mean 11·1 (SD 6·2) μg/ml) than males (mean 8·5 (SD 4·1) μg/ml) ($P<0{\cdot}001$), as is well known[91]. However, there were no significant interactions between gender × age ($P = 0{\cdot}697$), gender × BMI ($P = 0{\cdot}139$) or gender × ethnicity ($P = 0{\cdot}15$) in determination of serum adiponectin concentration.

Surprisingly, replacement of SFA by isoenergetic MUFA or carbohydrate diets for 24 weeks did not significantly improve adiponectin concentration. Previously, we reported no significant effect on insulin sensitivity following this dietary regimen[44]. Small changes in adiponectin concentration after dietary intervention may not have been sufficient to affect insulin sensitivity, or the intervention period may not have been long enough to produce an effect. This is consistent with other reports[131–133]. Long-term effects were seen only after a 10-year Mediterranean diet in diabetic women[134]. These data suggest that adiponectin concentrations are unlikely to be affected by relatively short-term dietary changes, but reflect intakes over longer time periods[129].

We hypothesised that stratification by genotype might uncover influential interaction between diet and *ADIPOQ* variants in determination of serum adiponectin concentration following dietary intervention. Our genetic investigations were based on the White subjects. We investigated four SNP which we previously showed to have the strongest replicated associations with serum adiponectin[104]: −11391 G/A is located in the promoter region −10066 G/A and −7734 A/C are both located in intron 1 and +276 G/T is in intron 2.

After the 4-week run-in on HS diet, +276T was associated with higher (n 340; $P = 0{\cdot}006$) and −10066A with lower serum adiponectin concentration (n 360; $P = 0{\cdot}03$) after adjustment for covariates, in agreement with previous reports[104,107].There were no significant differences in the change in serum adiponectin concentration after HM or LF diets, with the exception of −10066 G/A. After the HM diet GG subjects showed a 3·8% increase (95% CI −0·1, 7·7) and GA+AA subjects a 2·6% decrease (95% CI −5·6, 0·4) in serum adiponectin ($P = 0{\cdot}006$ for difference, after adjustment for change in BMI, age and gender). However, gene × diet interaction in determination of serum adiponectin was NS ($P = 0{\cdot}12$) after adjustments[135].

Activation of PPAR-γ by unsaturated fatty acids increases with chain length and degree of unsaturation[136]. The switch from SFA to MUFA could lead to increased expression of the *ADIPOQ* gene and serum adiponectin concentration through increased availability of PPAR-γ-activating ligands. The PPRE lies in a 1·3 kb linkage disequilibrium block[102]. If the −10066A-allele was in linkage disequilibrium with a variant in the PPRE reducing affinity for the receptor, this could account for higher serum adiponectin in response to MUFA in GG homozygotes and the lower concentration in A-allele carriers.

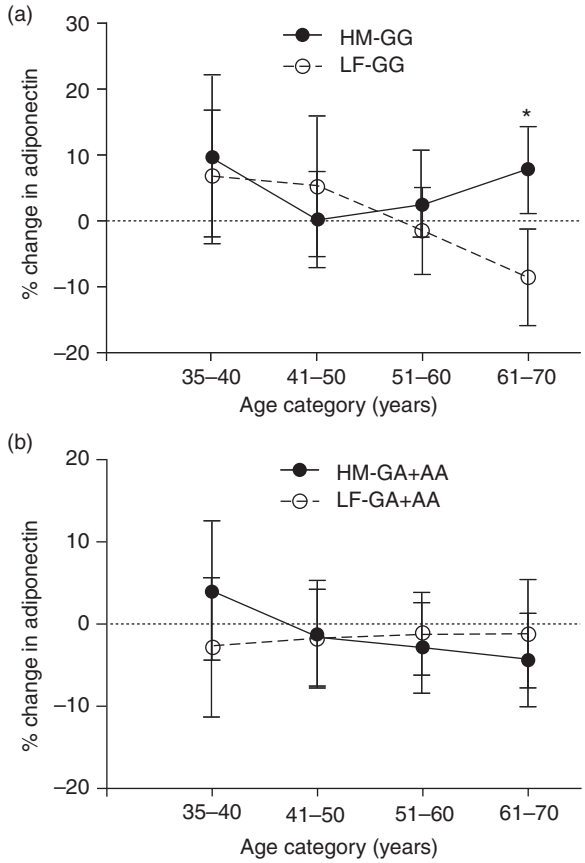

Fig. 5. Effect of high-MUFA (HM) and low-fat (LF) diets on adiponectin concentration with respect to −10066 G/A genotype and age in White subjects. % changes (95% CI) in geometric mean adiponectin concentration adjusted for change in BMI and gender are shown in each age group, after subjects consumed HM (n 151) or LF (n 152) diets. (a) −10066 GG subjects (n 111) and (b) −10066 GA+AA subjects (n 192). The number of genotyped subjects in each age group 35–40, 41–50, 51–60 and 61–70 years were as follows: HM diet: GG 6, 18, 13, 20; GA+AA 13, 22, 30 and 29; LF diet: GG 9, 8, 21, 16; GA+AA 13, 27, 36 and 22. Interaction between gene × age × diet in determination of change in serum adiponectin concentration found by analysis of covariance (ANCOVA) was NS after adjustment for change in BMI (n 303; $P = 0{\cdot}07$). *Denotes significant difference in % change in serum adiponectin between GG subjects on HM and LF diets ($P = 0{\cdot}003$). (From AlSaleh A *et al.*[135].)

We were interested to discover whether the strong relationship between adiponectin concentration and age seen at baseline was modified by diet. There was no significant interaction between either genotype or diet in determining adiponectin concentration. We then looked at whether age × genotype interaction was influential after dietary intervention. Fig. 5 compares the effect of HM and LF diets on % change in serum adiponectin concentration in White −10066 GG homozygotes and A-allele carriers. In GG homozygotes over 40 years of age, adiponectin

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concentration increased progressively after the HM diet and decreased after the LF diet. The difference in % change in serum adiponectin between GG subjects on HM and LF diets in the oldest 61–70-year age group was significant ($P = 0{\cdot}003$). In A-allele carriers there was little change in serum adiponectin concentration compared with baseline with increasing age, after HM or LF diet. Interaction between gene × age × diet in determination of change in serum adiponectin concentration approached significance after adjustment for gender and change in BMI (n 303; $P = 0{\cdot}07$). However, interaction between gene × age × diet × gender was NS after adjustment for change in BMI[135].

Serum adiponectin might be expected to be lower in GG subjects after the LF diet, in which carbohydrates replace PPAR-γ-activating fatty acids, than after the HM diet. In A-allele carriers, substitution of carbohydrate for MUFA would have little effect if reduced affinity of the PPRE, rather than ligand activation were to be the rate-limiting step. This would be compatible with other reports of lower serum adiponectin after high-carbohydrate[129] and higher serum adiponectin with a diet rich in MUFA[137]. If aging is associated with the development of adiponectin resistance, the change in adiponectin concentrations may reflect a capability of responding by increasing production after HM, but not LF, diets.

Conclusion

The strength of the RISCK study lies in its design as a randomised, tightly controlled feeding trial with high adherence and retention rates and diets with practical relevance to the general population. Analysis of White subjects showed that at the lowest PUFA:SFA intake, carriage of the less active PPAR-γ Ala12 isoform associated with higher plasma TC and LDL-C. The significant trends in the reduction of plasma TC and TAG in Ala12 carriers as the P:S ratio increased suggests that these subjects might be advised to maintain a high PUFA:SFA intake ratio to reduce plasma concentrations of atherogenic lipids. sdLDL particles are recognised as an important risk factor for CVD and numerous dietary elements have a significant impact on several characteristics of the LDL size phenotype. Significant predictive value of individual disease risk or responses to diet could potentially be gained by combining genotype information from the *PPARA* Leu162Val and *PPARG* Pro12Ala loci. The switch from SFA to MUFA could lead to increased expression of the *ADIPOQ* gene and serum adiponectin concentration through increased availability of PPAR-γ-activating ligands. In White *ADIPOQ* −10066 GG homozygotes, increase in adiponectin with age suggests that a HM diet may help to increase adiponectin concentrations with advancing years.

Limitations to these SNP association studies include relatively small sample sizes, and multiple testing remains a controversial issue in interpretation. Replication in other cohorts is the most reliable method to distinguish true from false-positive associations. Substantiated effects of common SNP in modifying the outcome of dietary intervention studies in larger samples should help in the identification of individuals at risk of complex disease who would benefit from personalized dietary recommendations.

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