Adiponectin Gene Variant Interacts with Fish Oil Supplementation to Influence Serum Adiponectin in Older Individuals

Aaseel AlSaleh, Daria Crepostnaia, Zoitsa Maniou, Fiona J. Lewis, Wendy L. Hall, Thomas A. B. Sanders, and Sandra D. O’dell,* on behalf of the MARINA study team

King’s College London, School of Medicine, Diabetes and Nutritional Sciences Division, London SE1 9NH, UK

Abstract

Marine n3 polyunsaturated fatty acids (PUFAs) activate the transcription factor peroxisome proliferator-activated receptor (PPARγ), which modulates the expression of adiponectin. We investigated the interaction of dietary n3 PUFAs with adiponectin gene (ADIPOQ) single nucleotide polymorphism (SNP) genotypes as a determinant of serum adiponectin concentration. The Modulation of Atherosclerosis Risk by Increasing Doses of n3 Fatty Acids study is a parallel design, double-blind, controlled trial. Serum adiponectin was measured in 142 healthy men and 225 women aged 45–70 y randomized to treatment with doses of 0.45, 0.9, and 1.8 g/d 20:5n3 and 22:6n3 (1.51:1), or placebo for 12 mo. The 310 participants who completed the study were genotyped for 5 SNPs at the ADIPOQ locus: −11391 G/A (rs17300539), −11377 C/G (rs266729), −10066 G/A (rs182052), +45 T/G (rs2241766), and +276 G/T (rs1501299). The −11391 A-allele was associated with a higher serum adiponectin concentration at baseline (n = 290; P < 0.001). The interaction between treatment and age as a determinant of adiponectin was significant in participants aged >58 y after the highest dose (n = 92; P = 0.020). The interaction between +45 T/G and treatment and age was a nominally significant determinant of serum adiponectin after adjustment for BMI, gender, and ethnicity (P = 0.029). Individuals homozygous for the +45 T-allele aged >58 y had a 22% increase in serum adiponectin concentration compared with baseline after the highest dose (P-treatment effect = 0.008). If substantiated in a larger sample, a diet high in n3 PUFAs may be recommended for older individuals, especially those of the +45 TT genotype who have reported increased risk of hypoadiponectinemia, type 2 diabetes, and obesity. J. Nutr. 143: 1021–1027, 2013.

Introduction

Adipose tissue stores TGs during energy surplus, but it is also a key endocrine organ secreting various biologically active adipokines involved in the regulation of energy homeostasis. Infiltration of macrophages and lymphocytes in obese adipose tissue (1) leads to increased production of proinflammatory adipokines and vasoconstrictors that induce endothelial dysfunction and vascular inflammation (2). By contrast, adiponectin secreted by adipocytes decreases in obesity (3) and its related pathologies (4–7) despite the greater adiposity, as local inflammation disrupts adiponectin transcription (8). Activities of adiponectin in glucose and lipid metabolism (9,10), vascular endothelial cells, smooth muscle cells, and macrophages (11) confer protection against obesity and metabolic syndrome traits. Thus, adiponectin or its signaling pathways are potential therapeutic targets.

The serum adiponectin concentration has a strong genetic component, with heritability estimated at 88% (12). Single nucleotide polymorphisms (SNPs) (5) associated with serum adiponectin have emerged from recent genome-wide association studies (13–19). However, associations between variants at the adiponectin gene (ADIPOQ) locus and adiponectin concentration in population studies have not always been confirmed (20). These inconsistencies may relate to the interaction between gene variants and nutrients with potential effects on expression.

5 Abbreviations used: ADIPOQ, adiponectin gene; MARINA, Modulation of Atherosclerosis Risk by Increasing Doses of n3 Fatty Acids; SNP, single nucleotide polymorphism.
In particular, heterogeneity of response to fish oil fatty acids within populations has been attributed to genetic variation (21).

Unsaturated fatty acids are ligands for the transcription factor PPARγ (22), which upregulates ADIPOQ gene expression (23) and directly increases serum adiponectin concentration. Fish oil is a rich source of n3 PUFAs, especially EPA (20:5n3) and DHA (22:6n3). The serum adiponectin concentration could therefore be potentially influenced by the interaction between ADIPOQ SNPs and dietary intake of marine n3 PUFAs. The interaction with a MUFA-rich diet has been demonstrated by ourselves and others (24,25), but few studies have explored the effects of n3 PUFA dosage. One reported an increase in serum adiponectin in obese participants treated with 1.8 g/d purified 20:5n3 over a 3-mo period (26). In contrast, dietary n3 PUFAs comprising 3.5% of energy intake did not induce any significant changes in overweight-to-modestly obese men and women after 14 wk (27).

Here, we investigated associations of 5 SNPs at the ADIPOQ locus with serum adiponectin and variables characterizing metabolic syndrome in participants of the Modulation of Atherosclerosis Risk by Increasing Doses of n3 Fatty Acids (MARINA) study, a parallel, double-blind trial testing the effects of n3 PUFA supplementation in healthy individuals during 12 mo (28). Our aim was to discover whether SNP genotypes previously reported to associate with serum adiponectin significantly interacted with n3 PUFA dosage as a determinant of adiponectin concentration and associated plasma variables.

Materials and Methods

Subjects

The MARINA trial was a single-center, dietary intervention study conducted at King’s College London between April 2008 and October 2010. The study was approved by the St. Thomas’ Hospital NHS Research Ethics Committee (NREC 08/H0802/3) and written informed consent was given by participants, who were nonsmoking men and women aged between 45–70 y recruited through media advertisements and screened as previously described (28). Eligibility for the study was based on various medical exclusion criteria (28). The participants self-reported their ethnicity from a list that included white, South Asian, Southeast Asian, black, Middle Eastern, and Far Eastern. A total of 512 individuals were potentially eligible participants in the MARINA study, 475 were assessed for eligibility, 367 were randomly assigned to treatments, and 312 participants completed the 12-mo intervention. In the study reported here, the analysis was based on 310 participants completing the intervention for whom DNA and adiponectin data were available. Individuals without genotype and/or phenotype data were excluded from analysis.

Study design

The MARINA trial was a 12-mo dietary intervention study designed to assess the effects of increasing n3 PUFA intake on blood lipids and vascular variables (28). Prior to assignment to a treatment group, the participants attended a screening session in a fasted state and measurements of height, weight, waist circumference, seated blood pressure, liver function, glucose, lipids, and hematologic indicators were taken. Participants were randomly allocated to treatment by computer-generated sequence using the process of minimization to balance age, gender, and ethnicity between treatment groups. Each treatment comprised an initial run-in period of 4 wk, during which 3 olive oil (British Pharmacopoeia specification) placebo capsules per day were taken while also restricting oily fish intake. Baseline measurements of outcome variables were made at the end of the run-in period. The 12-mo treatment phase involved randomly assigned daily doses of 0.45, 0.9, and 1.8 g 20:5n3 and 22:6n3 (1.5:1:1), or placebo, in 3 capsules taken as dietary supplements. Oily fish intake was restricted to 1 portion/mo throughout this time. The present investigation was based on measurements made at baseline and after 12 mo of treatment. Compliance was assessed by measurement of the 20:5n3 and 22:6n3 content in erythrocyte phosphoglycerides at baseline and 6 and 12 mo (P-trend mean changes in proportions compared with placebo < 0.0001) (28). The participants were supplied with capsules at regular intervals and any that were unused were returned and their numbers recorded. The MARINA study was powered for flow-mediated dilatation as the primary outcome (28). Based on past studies, the mean flow-mediated dilatation was 6.7% with a common SD of 2%. To detect a 20% change, a sample size of 72 participants/treatment group was required for 90% power and significance level set at 0.01%. Allowing for a dropout rate of 20%, 90 participants/treatment group required the recruitment of 360 individuals, with 292 completing. In total, 312 participants completed the study. The oil blends used in the study were supplied by Croda Chemicals Europe and encapsulated in gelatin by Powerhealth as previously described (28). Quality control analysis was performed by Croda Chemicals Europe.

Blood sampling and analysis

Blood samples for the analysis of plasma lipids, glucose, and insulin and serum adiponectin were drawn after a minimum 8-h overnight fast preceded by a low-fat evening meal (<10 g fat, 3 MJ) and serum was stored at −45°C until analyzed.

Erythrocyte fatty acid composition.

The plasma lipid profile and erythrocyte lipid composition were determined in blood collected at the end of the run-in period and after 6 and 12 mo of intervention. Erythrocyte lipids were extracted from washed RBCs within 3 d of blood collection as previously described (29) and extracts were stored at −20°C until derivatized for analysis by GC as previously described (28).

Plasma lipoproteins and TG.

The methods used for the measurement of plasma lipid concentrations were previously described (30). Inter-assay CVs for total cholesterol were 1.1, 1.5, and 1.0% at 3.9, 5.1, and 5.7 mmol/L, respectively; for HDL-cholesterol, they were 2.2, 2.1, and 2.5% at concentrations of 0.91, 1.39, and 1.95 mmol/L, respectively, and for TG they were 2.5 and 1.5% at concentrations of 1.32 and 2.36 mmol/L, respectively. LDL-cholesterol was derived from the Friedewald equation if fasting plasma TG concentrations were <4.49 mmol/L.

Serum adiponectin.

Serum adiponectin analysis was carried out using Quantikine Adiponectin ELISA kit (R&D Systems). The assay measures total (low, middle, and high molecular weight) human adiponectin. All samples were diluted 1/100 before the assay. The inter-assay CV was 6.8% and the intra-assay CV was 2.5%.

Plasma glucose and insulin.

Plasma glucose and insulin quantitation was carried out on the ADVIA 2400 analyzer (Siemens Healthcare Diagnostics) using reagents supplied by the manufacturer. The plasma glucose concentration was measured by the hexokinase-glucose-6-phosphate dehydrogenase method. The inter-assay CV was 1.6% and the intra-assay CV was 0.6%. The plasma insulin concentration was determined by Siemens Advia Centaur assay (Siemens Healthcare Diagnostics) according to the manufacturer’s instructions. The inter-assay CV was 5.9% and the intra-assay CV was 4.6%. HOMA-IR was ascertained by calculation: fasting glucose (mmol/L) fasting insulin (mU/L)/22.5.

DNA extraction and SNP genotyping

Buffo crops 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) according to the manufacturer’s instructions and stored at −20°C. Five SNPs at the ADIPOQ gene locus were genotyped [−11391 G/A (rs17300539), −11377 C/G (rs266729), −10066 G/A (rs182052), +43 T/G (rs2241766), and +276 G/T (rs1301299)]. Their relative positions with respect to the first coding base in exon 2 are indicated. Genotyping by K Biosciences, using the KASPar system (31), was performed for the 310 participants for whom DNA was available. Genotypes were assigned by simultaneously using all of the data from the study. We used internal
controls, and the accuracy, as assessed by inclusion of duplicates in the arrays, was 98%. Genotyping success rates were between 91.0 and 93.5%.

**Statistical analysis**

All genotype distributions were tested for deviation from the Hardy-Weinberg equilibrium by a \( \chi^2 \) test with 1 d.f. \((P > 0.05)\). Statistical analyses were carried out using the SPSS version 20.0 for Windows (SPSS). Normal distribution of outcome variables was evaluated by Q-Q plots. Where needed, variables were log-transformed to obtain better approximations of the normal distribution prior to analysis. Due to the limited sample size, SNP genotype association analyses were based on a dominant inheritance model. Potential population stratification was not addressed. Linear regression was used to assess SNP associations with phenotypes and the interaction with dietary treatment. All data presented in the text and tables are expressed as means or geometric means ± SDs or 95% CIs. Ethnicity, sex, age, and BMI were added to the models as covariates to adjust for possible confounding effects. The interaction between ADIPOQ SNPs genotype and dosage of 20:5n3 and 22:6n3 was explored by adding the interaction terms to the linear regression models. Correlations are presented as Spearman’s correlation coefficient \( (\rho) \) with significance set at \( P < 0.05 \) (2-tailed test). Multivariate ANOVA was used to allow for multiple testing of genotype associations with adiponectin, insulin, and glucose concentrations and HOMA-IR. Significance was accepted at \( P < 0.05 \).

**Results**

**Baseline data**

**Characteristics of participants.** In total, 312 participants completed the 12-mo intervention and DNA samples were available for 310. The number of participants allocated and drop-out rates did not significantly differ between the treatment groups (28). Women, mostly postmenopausal, outnumbered men by ~1.6:1. The mean BMI was higher than the desirable range (20–25 kg/m²). Based on self-reported ethnicity data, the majority of the participants were of a white background (82%) and the rest of the participants were of other ethnicities, including South and Southeast Asian, black, Middle Eastern, and Far Eastern (28). Table 1 shows the characteristics of participants after the 4-wk run-in consuming the placebo for whom DNA samples were available. These measures did not differ between the 4 treatment groups at baseline \((P > 0.05)\).

**Serum adiponectin with respect to BMI, age, gender, and ethnicity.** The association of mean serum adiponectin concentration with both age and BMI was significant \((\beta = 0.16; \text{BMI} \beta = -0.18; P < 0.001)\). Table 2 shows the serum adiponectin concentration at baseline stratified by age, BMI, and ethnicity. The adiponectin concentration increased with age after adjustment for BMI, gender, and ethnicity \((P < 0.01)\) and decreased with increasing BMI after adjustment for age, gender, and ethnicity \((P < 0.001)\). Participants of a white background had a higher concentration compared than other ethnic groups after adjustment for age, BMI, and gender \((P < 0.001)\) and the mean concentration in men \((6.8 \text{ (95\% CI: 6.2, 7.4) ng/mL})\) was less than in women \((14.0 \text{ (95\% CI: 13.0, 15.0) ng/mL})\) \((P < 0.001)\). There was no significant interaction between gender and age tertiles, between gender and BMI groups, or between gender and ethnicity as determinants of the serum adiponectin concentration.

**Correlation between concentrations of serum adiponectin and plasma variables.** There was a negative correlation between serum adiponectin and TG \((\rho = -0.18; P < 0.01)\), insulin \((\rho = -0.36; P < 0.0001)\), glucose \((\rho = -0.18; P < 0.01)\), and

**HOMA-IR \((\rho = -0.35; P < 0.0001)\). A positive correlation was found between serum adiponectin and HDL-cholesterol \((\rho = 0.53; P < 0.0001)\).**

<table>
<thead>
<tr>
<th>n</th>
<th>Adiponectin (\text{ng/mL})</th>
<th>(P) value (^{1})</th>
<th>(P) value (^{5})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;52</td>
<td>101</td>
<td>8.7 (7.7,9.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>52–58</td>
<td>114</td>
<td>11.0 (9.8,12.4)</td>
<td></td>
</tr>
<tr>
<td>≥59</td>
<td>92</td>
<td>12.5 (11.2,13.9)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>152</td>
<td>12.5 (11,13.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25–29</td>
<td>98</td>
<td>9.1 (8,10.3)</td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>57</td>
<td>8.7 (7.3,10.4)</td>
<td></td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>251</td>
<td>11.3 (10.5,12.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-white</td>
<td>56</td>
<td>8.0 (7.8,8.3)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\) Values are geometric means (95% CIs) for participants with available DNA samples and serum adiponectin data at baseline after the 4-wk run-in of the background diet with placebo supplement.

\(^{2}\) Self-reported ethnicity: non-white are black, Far Eastern, South Asian, Southeast Asian, Middle Eastern, and other ethnic groups.

\(^{5}\) Significance of differences among age, BMI, and ethnic groups determined by univariate ANCOVA \((P\) values with adjustments).
SNP allele and genotype frequencies. Five SNPs at the ADIPOQ locus (see “Materials and Methods”) were genotyped in participants who completed the study (n = 310). The minor allele and genotype frequencies in the total and ethnic groups are shown in Supplemental Table 1. Genotype distributions for all SNPs except −10066 G/A did not deviate from Hardy-Weinberg expectations (P > 0.05). SNP −10066 G/A was not further analyzed. Minor allele frequencies were in close agreement with those listed for Europeans on the National Center for Biotechnology Information SNP database (32).

SNP genotype associations with plasma variables. Adiponectin, lipid, and insulin sensitivity measures were stratified by the −11391 G/A, −11377 C/G, +45 T/G, and +276 G/T genotypes after a 4-wk run-in while consuming the normal diet with placebo supplement. Based on a dominant model, serum adiponectin was associated with the −11391 G/A genotype only, with carriers of the minor A-allele showing a higher concentration than the common allele genotype GG (n = 290; P < 0.001). This association remained significant after correction for multiple comparisons (P = 0.012). No significant associations were found between SNPs −11377 C/G, +45 T/G, and +276 G/T and the serum adiponectin concentration at baseline, and no significant associations between SNP genotypes and plasma glucose, insulin, or lipid concentrations were detected. Data are shown in Supplemental Table 2.

Effect of n3 PUFA treatment Changes in adiponectin and plasma variables. In the second part of our study, we investigated the effects of dietary supplementation with 20:5n3 and 22:6n3 (1:1:1) for 12 mo. Measurements of proportions of fatty acids in erythrocyte phosphoglycerides confirmed that similar concentrations of n3 PUFAs were established in all treatment groups during the run-in while receiving the placebo and compliance during the 12-mo intervention (28). There was no significant change in the adiponectin concentration in groups receiving placebo and 3 doses of n3 PUFAs for 12 mo after adjustment for BMI, age, ethnicity, and gender. We then tested the significance of the interaction between the n3 PUFA dose and covariates of adiponectin concentration: age, BMI, gender, and ethnicity as possible influences on concentration after intervention. P values for the interaction between n3 PUFA dose and BMI, gender, and ethnicity were not significant and were not investigated further. Only the interaction between treatment and age was significant (P = 0.047). Further analysis revealed that the effect of dose on change in adiponectin compared with baseline was not significant in the first age tertile (<52 y) (n = 101) or the second (52–58 y) (n = 114), but change from baseline was significant in the third tertile (>58 y) (P = 0.02; n = 92). In these oldest participants, the serum adiponectin concentration increased by 1.2% compared with baseline after the highest intake of n3 PUFA (1.8 g/d) for 12 mo. There were no significant changes in fasting glucose, insulin, or HOMA-IR after treatment.

Interaction with SNP genotype as a determinant of serum adiponectin concentration. No significant interactions were found between treatment and genotype for any SNP (P > 0.05). However, because interaction between treatment and age as a determinant of serum adiponectin was significant in participants aged >58 y after the highest dose and we previously detected a significant association between −11391 G/A genotype and serum adiponectin at baseline in the oldest tertile, we were interested to discover whether treatment interacted with any genotype to modulate adiponectin in an age-specific manner.

Interactions among SNP −11391 G/A, −11377 C/G, and +276 T/G genotypes, treatment, and age were not significant. Only the interaction among SNP +45 T/G genotype × treatment × age was found to be a nominally significant determinant of serum adiponectin after adjustment for BMI, gender, and ethnicity (P = 0.029). Further analysis revealed that the interaction with treatment and age tended to be significant in participants homozygous for the common T-allele (P = 0.05) but not in minor allele G-carriers. Among the TT homozygotes, only those in the oldest age tertile (aged >58 y) had a significant change in serum adiponectin compared with baseline after adjustment for BMI, gender, and ethnicity (P = 0.008). Table 3 shows the effect of the n3 PUFA dose on percent change in the serum adiponectin concentration in +45 TT homozygotes and G-allele carriers. Although there were inconsistent effects in the lower age tertiles, in common TT homozygotes aged >58 y, the adiponectin concentration increased by 22% compared with baseline after the highest dose. In G-allele carriers, there was no significant change in serum adiponectin concentration compared with baseline, with increasing age, at any dose of n3 PUFA. We tested a 3-way interaction between treatment, +45 T/G genotype, and each of BMI, gender, ethnicity, and age, but only interaction with age was nominally significant.

Discussion

We have investigated interaction between dietary intake of n3 PUFA, covariates of serum adiponectin, and ADIPOQ SNP genotypes as a determinant of serum adiponectin concentration. Carriers of the minor A-allele of −11391 G/A had a significantly higher mean serum adiponectin concentration than noncarriers at baseline. This association was not modulated by intake of 20:5n3 and 22:6n3, but the interaction among +45 T/G genotype, treatment, and age was a significant determinant of serum adiponectin concentration. Participants aged >58 y homozygous for the common +45 T-allele had a 22% increase in serum adiponectin concentration compared with baseline after consuming the highest n3 PUFA dose.

Correlations with adiponectin in MARINA participants at baseline confirmed those previously reported for age (5), gender (33), BMI (34), and ethnicity (35), but not with plasma lipids (7). The inverse relationship with glucose and insulin concentrations and HOMA-IR was in line with the well-known beneficial effect of adiponectin on insulin sensitivity (9).

There are many reports of adiponectin association with genotypes of SNPs at the ADIPOQ locus in population and genome-wide association studies. The only significant association in MARINA participants at baseline was with −11391 G/A. This SNP is one of the strongest genetic markers of adiponectin, with consistent evidence of a higher concentration associated with the A-allele in population studies (20). Its situation 5’ to the response element for the transcriptional activator PPARγ (peroxisome proliferator response element) in the ADIPOQ promoter region suggests a possible effect on transcription rate. The A-allele has been reported to increase promoter activity in transfected COS-7 cells (36), although we were not able to confirm this in 3T3-L1 adipocytes (37). Meta-analyses have shown significant associations with −11391 G/A and +276 G/T but not with +45 T/G genotypes (20). The associations with −11391 G/A and +276 G/T are likely to be independent, because the SNPs are sited in different linkage disequilibrium blocks.
We found that n3 PUFA dose interacted with age as a determinant of serum adiponectin concentration. In the oldest tertile (>58 y), concentration significantly increased with respect to baseline only after the highest dose (1.8 g/d). If aging is associated with development of adiponectin resistance, the increase observed in only the oldest participants may reflect an adaptation to the metabolic stress of aging (28). Limitations include a relatively small sample size, which reduced the power to detect some significant genotype associations and interactions, and changes in serum adiponectin concentration associated with genotype, which are small comparison with those with BMI, age, or gender. The observed differences in the increase in adiponectin with respect to baseline were very small, with an effect size in the 1-way ANOVA of only 0.005, which would be impossible to detect practically with 80% power. Although we measured total adiponectin, this is highly correlated with the most bioactive high-molecular weight form (47). Multiple testing of SNP associations was accounted for by multivariate ANOVA, but replication in other cohorts would offer the most reliable confirmation of true associations.

**TABLE 3** Serum adiponectin in participants aged >58 y stratified by ADIPOQ +45 T/G genotype and treatment group

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>0.45 g/d</th>
<th>0.9 g/d</th>
<th>1.8 g/d</th>
<th>P value ( ^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>+45 TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.7 (10.6, 17.9)</td>
<td>14.6 (11.8, 18.1)</td>
<td>12.9 (10.1, 16.4)</td>
<td>8.8 (5.8, 13.4)</td>
<td></td>
</tr>
<tr>
<td>12 mo</td>
<td>14.5 (10.5, 19.8)</td>
<td>13.4 (10.8, 16.5)</td>
<td>11.1 (8.5, 14.6)</td>
<td>11.0 (7.9, 15.4)</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>0.05 (−0.05, 0.15)</td>
<td>−0.09 (−0.19, 0.00)</td>
<td>−0.15 (−0.27, −0.02)</td>
<td>0.22 (−0.01, 0.46)</td>
<td>0.008</td>
</tr>
<tr>
<td>Treatment effect</td>
<td>0.000 (reference)</td>
<td>−0.14 (−0.33, 0.04)</td>
<td>−0.20 (−0.39, −0.02)</td>
<td>0.09 (−0.12, 0.29)</td>
<td></td>
</tr>
<tr>
<td>+45 TG+GG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.4 (8.5, 23.7)</td>
<td>8.3 (5.0, 13.6)</td>
<td>18.0 (16.0, 20.2)</td>
<td>10.2 (5.5, 19.2)</td>
<td></td>
</tr>
<tr>
<td>12 mo</td>
<td>13.6 (8.5, 21.7)</td>
<td>7.3 (4.4, 12.2)</td>
<td>15.7 (9.0, 27.3)</td>
<td>9.6 (6.6, 14.1)</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>0.09 (−0.18, 0.36)</td>
<td>−0.12 (−0.35, 0.10)</td>
<td>−0.14 (−0.74, 0.46)</td>
<td>−0.08 (−0.45, 0.30)</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment effect</td>
<td>0.000 (reference)</td>
<td>0.71 (0.45, 1.11)</td>
<td>0.90 (0.59, 1.37)</td>
<td>0.80 (0.52, 1.22)</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) Values are geometric means (95% CI) for participants with available DNA samples and serum adiponectin data at baseline after 4-wk run-in of the placebo diet and at follow-up after 12 mo on placebo, or 20:5n3 and 22:6n3 at doses shown. Changes were adjusted for BMI, gender, and ethnicity. NS, nonsignificant.

The adiponectin data in the +45 G/T meta-analysis showed high heterogeneity, which may account for the lack of overall significance. Heterogeneous data may reflect different recruitment criteria and/or adiponectin assays, or variation in environmental factors, including diet.

As ligands of PPARγ, dietary n3 PUFAs, particularly at the highest dose of 1.8 g/d (equivalent to 4 portions of oily fish/wk), were expected to increase the adiponectin concentration in MARINA participants after 12 mo. Others have found modest effects of n3 PUFA on serum adiponectin at comparable dosage but over shorter timescales. In obese participants, 6 wk of supplementation with 1.1 g/d marine n3 PUFA increased serum adiponectin by 0.55 µg/mL compared with baseline \((P = 0.04; n = 50)\) (39) and in healthy individuals, 10 wk of dietary modification including 1.25 g/d of 20:5n3 and 22:6n3 led to a serum adiponectin concentration 1.1 µg/mL greater than in a control group \((P = 0.02; n = 17)\) (40). Dietary n3 PUFA comprising 3.5% of energy intake (0.24 g/d) did not significantly increase total or high-molecular weight adiponectin concentrations in overweight-to-moderately obese healthy men and women \((n = 26)\) after 14 wk (27). We also found no significant effects of treatment on fasting insulin or glucose concentrations. A recent meta-analysis found no evidence that n3 PUFA intervention had any effect on insulin sensitivity compared with placebo (41). In our case, the lack of an effect of n3 PUFAs on insulin and glucose concentrations may be explained by a failure to alter adiponectin.

We found that n3 PUFA dose interacted with age as a determinant of serum adiponectin concentration. In the oldest tertile (>58 y), concentration significantly increased with respect to baseline only after the highest dose (1.8 g/d). If aging is associated with development of adiponectin resistance, the increase observed in only the oldest participants may reflect an adaptation to the metabolic stress of aging (28). Limitations include a relatively small sample size, which reduced the power to detect some significant genotype associations and interactions, and changes in serum adiponectin concentration associated with genotype, which are small compared with those with BMI, age, or gender. The observed differences in the increase in adiponectin with respect to baseline were very small, with an effect size in the 1-way ANOVA of only 0.005, which would be impossible to detect practically with 80% power. Although we measured total adiponectin, this is highly correlated with the most bioactive high-molecular weight form (47). Multiple testing of SNP associations was accounted for by multivariate ANOVA, but replication in other cohorts would offer the most reliable confirmation of true associations.

**ADIPOQ** polymorphism, adiponectin, and fish oil
In conclusion, individuals of the +45 TT genotype are predisposed to hypoadiponectinemia and possibly increased risk of type 2 diabetes and obesity (42–45). If substantiated in a larger sample, a recommendation to +45 TT homozygotes to increase consumption of oily fish to maintain adiponectin concentrations with advancing years would be justified. The potential for the development of individualized strategies to reduce the risk of diseases linked to hypoadiponectinemia requires evidence from well-powered replicated studies with accurate dietary data.

Acknowledgments

The assistance of Roy Sherwood and Tracy Dew in the Clinical Biochemistry Department at King’s College Hospital and Robert Gray at King’s College London is gratefully acknowledged. T.A.B.S. and W.L.H. developed the overall research plan and S.D.O. conceived the current research project; Z.M. and F.J.L. organized and conducted the MARINA study; A.A. and D.C. analyzed data; and S.D.O. wrote the manuscript with contributions from other authors and had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited

13. Sanders TA, Lewis F, Slaughter S, Griffin BA, Griffin M, Davies I, Millward DJ, Cooper JA, Miller GJ. Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45–70 y: the OPTILIP study. Am J Clin Nutr. 2006;84:513–22.