Adiponectin gene polymorphisms are associated with long-chain ω3 polyunsaturated fatty acids in serum phospholipids in non-diabetic Koreans

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A Dissertation

Submitted to the Department of Science for Aging

and the Graduate School of Yonsei University

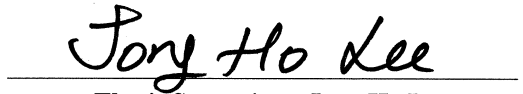
in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Bumsik Kim

June 2010

This certifies that the dissertation of Bumsik Kim is approved.



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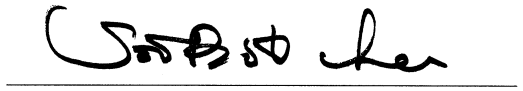

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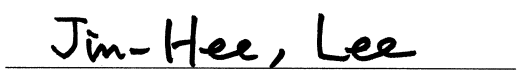

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감사의 글

새로운 환경에서, 그 동안 알지 못했던 새로운 사실을 깨닫는다는 것은 즐겁고도 힘든 일이었습니다. 이제는 작은 결실을 맺는 시간이 되어 지금까지 힘이 되어 주신 여러분들께 이 지면을 빌어 글로나마 감사의 마음을 전합니다.

Nutrigenomics라는 새로운 학문에서의 깨달음뿐만 아니라 인생에 있어서의 조언도 아끼지 않으셨던 이종호교수님께 깊은 감사를 드립니다. 바쁘신 와중에도 관심을 가져주신 장양수교수님, 이수복교수님 감사드립니다. 마지막까지 논문에 대한 도움을 주셨던 김오연교수님, 채지숙교수님, 김지영교수님께도 진심 어린 감사의 말씀을 올립니다. 아울러 이 모든 결과들이 제가 몸 담고 있는 CJ제일제당의 지원 없이는 이루어질 수 없었던 일인지라, 저에게 새로운 도전의 기회를 주신 손경식 회장님, 이재현 회장님, 김진수 사장님, 그리고 김태준 소장님께 지면을 빌어 깊은 감사의 말씀을 전하고 싶습니다. 아무것도 모르던 신입사원을 지금까지 있게 한 이강표소장님, 항상 옆에서 전폭적으로 지원해주시는 이진희박사님 정말 감사드립니다. 또한 항상 애정과 관심으로 격려해 주신 최준봉박사님, 정헌웅박사님, 권순희님께도 고마움을 전해드리고 싶습니다. 무엇보다도 청일점이라는 새로운 환경 속에서 잘 적응할 수 있도록 도와준 대학원 실험실 식구들, 떠날 때나 돌아올 때나 언제나 따뜻하게 반겨주었던 CJ제일제당 식품연구소 선배, 동료, 후배들 모두 감사합니다.

마지막으로 철없는 이 아들을 항상 믿어주시고 지원해주셨던 아버님, 어머님, 자주 찾아 뵙지는 못하지만 항상 사랑으로 감싸주시는 장인어른, 장모님 그리고 못난 남편 만나 희생만 강요당하는 사랑하는 아내 정주에게 무한한 감사의 마음을 전하며 나에게 가장 소중한 딸 서진이와 함께 이 작은 결실을 나누고 싶습니다.

2010년 7월

김범식

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Abstract

Adiponectin gene polymorphisms are associated with long-chain ω3 polyunsaturated fatty acids in serum phospholipids in non-diabetic Koreans

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Objective: Hypoadiponectinemia is caused by interactions between genetic and environmental factors, including the quality of dietary fats. We investigated the association of single-nucleotide polymorphisms (SNPs) in the adiponectin gene (*ADIPOQ*) with dietary fat intake or fatty acid (FA) composition in serum phospholipids, plasma adiponectin, and insulin resistance (IR).

Methods: Non-diabetic subjects (n=1194) were genotyped for three *ADIPOQ* SNPs (-11377C>G;45T>G;276G>T) after screening of eight sites. Dietary fat intake, FA composition in serum phospholipids, adiponectin, and homeostasis model assessment of IR (HOMA-IR) were also measured.

Results: 276G carriers (n=1082) showed lower HDL-cholesterol (*P*=0.024) and adiponectin (*P*<0.001) but higher glucose (*P*=0.015) and HOMA-IR (*P*=0.005) than 276T/T subjects (n=112). No associations were found in other SNPs. 276G carriers showed a higher proportion of myristic acid and lower proportions of total ω6FA, total ω3FA, 18:2ω6, 20:3ω6, 20:4ω6, 18:3ω3, 20:5ω3, 22:5ω3, and 22:6ω3 in serum phospholipids. After adjusted for age, sex, BMI, and the proportion of 18:2ω6 and 18:3ω3 (biomarkers of long-term essential FA intake), 276G>T was still associated with total ω3FA (*P*=0.026), 20:5ω3 (*P*=0.021), and 22:5ω3 (*P*=0.024). Among FAs in serum phospholipids, 18:2ω6 highly correlated with PUFA intake (r=0.260, *P*<0.001) and adiponectin (r=0.150, *P*<0.001). 276G carriers with higher proportion of 18:2ω6 (≥12.5%) exhibited more pronounced characteristics, i.e., lower adiponectin (*P*<0.001), HOMA-IR (*P*=0.013), and long-chain ω3PUFAs (20:5ω3, 22:5ω3, and 22:6ω3, *P*<0.05). Additionally, the effect of 276G>T on the relationship between adiponectin and HOMA-IR was modified by 18:2ω6 proportion.

Conclusion: *ADIPOQ* 276G is associated with reduced proportion of long-chain ω3PUFAs in serum phospholipids in non-diabetic Koreans.

Key words: *ADIPOQ*, adiponectin, insulin resistance, long-chain ω3PUFA

1. Introduction

Individuals with low serum concentrations of adiponectin, the protein product of the adipocyte C1q and collagen-domain-containing gene (*ADIPOQ*), are more likely to develop type 2 diabetes (T2DM) and cardiovascular disease (CVD) (1,2). Among the single-nucleotide polymorphisms (SNPs) studied in the *ADIPOQ* gene, 276G>T is associated with decreased levels of circulating adiponectin, greater insulin-resistance (IR) index, and an increased risk of T2DM in Japanese subjects (3). Menzaghi et al. (4) also showed that 276G>T, either independently or as a haplotype with 45T>G, is associated with several features of IR in non-diabetic Caucasians from Italy, including low serum adiponectin concentrations. In French subjects, a GG haplotype defined by the SNPs -11391G>A and -11377C>G in the promoter region of the *ADIPOQ* gene is significantly associated with hypoadiponectinemia, despite no association with IR and T2DM (5). The reasons for these partially discrepant results are unknown, but may result from the different genetic background of the study populations and potential gene-diet interactions in the different ethnicities.

Hypoadiponectinemia is caused by interactions between genetic and environmental factors, including the quality of dietary fats. Previous studies have reported significant association between circulating adiponectin and plasma fatty acids (FAs) (6,7). Furthermore, in rodents a diet rich in ω3 polyunsaturated

FAs (PUFAs) increases adiponectin concentration and up-regulates adipocytes (6,8,9), whereas saturated fat down-regulates *ADIPOQ* expression (10,11). However, no studies have been performed on the association between *ADIPOQ* polymorphisms and serum FA composition, even though FA composition was shown to be a reasonably accurate biochemical marker of long-term proportionate FA intake, especially for PUFAs and essential FAs (12).

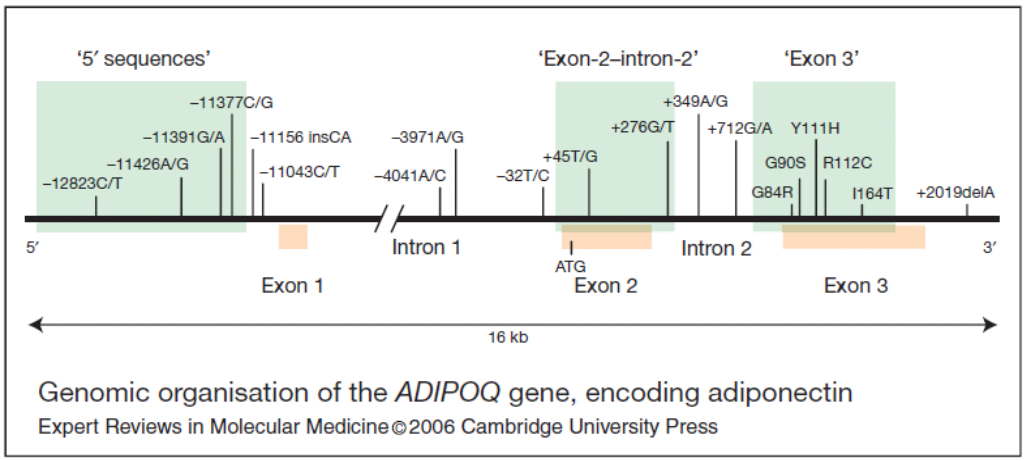
We hypothesized that genetic variation and dietary fat intake or FA composition of serum phospholipids may modulate plasma adiponectin and contribute to CVD risk, including IR index. Therefore, our objective was to examine the association between *ADIPOQ* SNPs, dietary fat intake or FA composition in serum phospholipids, plasma adiponectin, and IR index.

2. Background

2.1. Discovery of adiponectin

Adiponectin was independently identified by three different research groups about fifteen years ago. In 1995, Scherer described a 30 kDa protein exclusively produced by adipocytes, which was named ‘adipocyte complement related protein of 30 kDa’ (ACRP30) because of its structural similarity to the complement C1q fraction (13). The protein was also identified as the encoded product of two independently isolated cDNAs: an adipocyte rodent cDNA (adipoQ), described as encoding a 247 amino acid protein similar to complement C1q; and an abundant cDNA from adipose tissue named ‘adipose most abundant gene transcript 1’ (APM1), whose translated product was described as the plasma protein GBP28 (gelatin-binding protein 28) (14,15,16). Thus, several names were simultaneously given to this protein, and for a while the encoding gene was best known in the scientific community as the APM1 gene. Following the characterization of its genomic organization, comprising three exons (Fig. 1), and its 3q27 localization in 1999 (17), the protein was named adiponectin (18). In 2004, the gene was named ‘adipocyte C1q and collagen-domain-containing’ (ACDC), but the Human Genome Organization (HUGO) nomenclature is now ADIPOQ.

Figure 1. Genomic organization of the ADIPOQ gene [adapted from ref.(18)]



The gene is highly polymorphic and associations with adiponectin level and/or phenotypes of the metabolic syndrome are restricted to three areas: the 50 sequences, the intron 2 and exon 2 region, and exon 3.

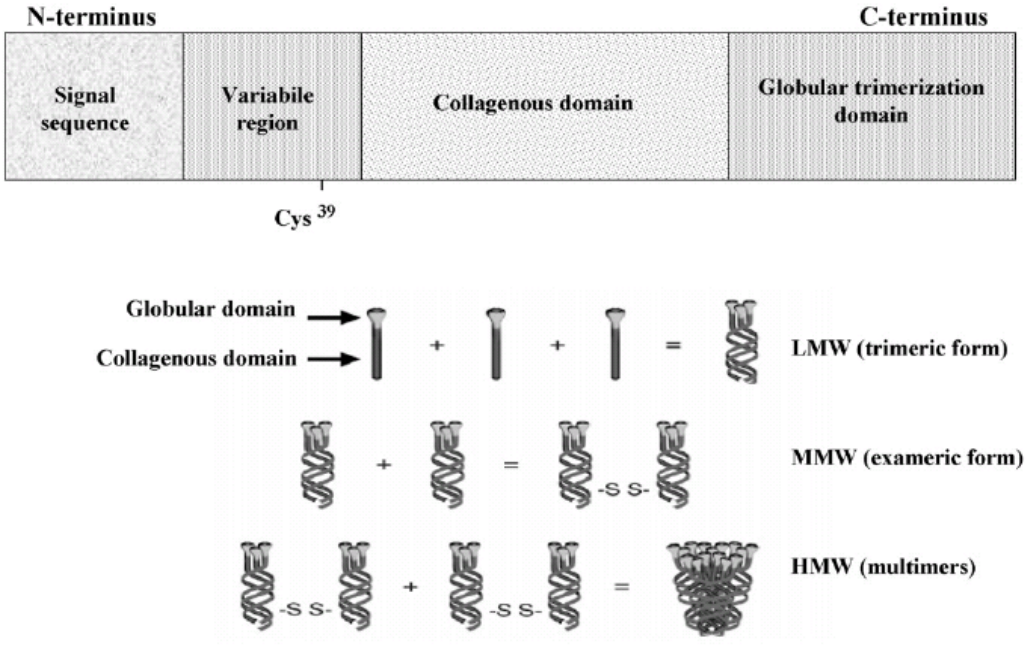
2.2. Molecular structure of adiponectin

Adiponectin is a protein of 247 aminoacids. It is constituted by four domains and presents a multimeric structure (19,20). As shown in Fig. 2, adiponectin is formed by a 20-residue amino-terminal signal sequence, a variable region, a collagenous domain and a carboxy-terminal globular domain. As demonstrated by X-ray crystallography of the globular domain, adiponectin presents a high structural homology with TNF-α, suggesting an evolutionary link between TNF- α family and this adipokine (21). By interactions at the collagenous domains, adiponectin is transformed within the adipocytes into multimeric forms (13,19,20), including the low molecular weight trimeric form (LMW, 75–90 kDa), made of three monomers strongly bounded at globular domain level, the middle molecular weight exameric form (MMW) and the high molecular weight form (HMW, about 500 kDa), constituted by eight or more monomers (Fig. 2). The trimer is formed by the association of the c-terminal globular domains with the triple helix formation at the collagenous domain level. The exameric form is obtained through disulphide bond formation at the cysteine (Cys)39 residue. HMW multimers are formed by noncovalent higher-order interactions. Both disulphide bond and the presence of cysteine at position 39 are necessary for multimerization; in fact, the replacement of cysteine with serine results in the lack of high-order structure formation. Similarly, heatdenaturated adiponectin is present only in the monomeric form, indicating that disulphide bridge is necessary for

multimerization.

The different multimers have been identified by different methods, namely gel filtration chromatography and electrophoresis on acrilamide gel in the presence of sodium dodecyl sulphate (SDS-PAGE) and Western blotting analysis. The monomeric form, 28–30 kD molecular weight, is not found in peripheral circulation, but only in the adipose tissue. Four multimers of adiponectin can be found in circulation: the three multimeric forms produced by adipocytes together with a further form consisting of the trimer bound to the serum albumin (Alb-LMW). Fragments from adiponectin proteolysis, including the globular domains, can also be found in the plasma (22). Both biological activity and transduction mode are different forthe different multimeric forms of adiponectin. In fact, it has been recently observed that the HMW form is an active form, and its ratio with total adiponectin appears to be closely correlated with insulin sensitivity (23,24). Moreover, it has been suggested that the percentage of each form with respect to the total adiponectin could vary as a function of the different physiopathological conditions (25). These observations indicate the importance, from the clinician point of view, of measuring the concentrations of each multimer besides the total adiponectin level.

Figure 2. Adiponectin structure (top); adiponectin multimeric forms (bottom) [adapted from ref.(26)].



2.3. Adiponectin Receptors

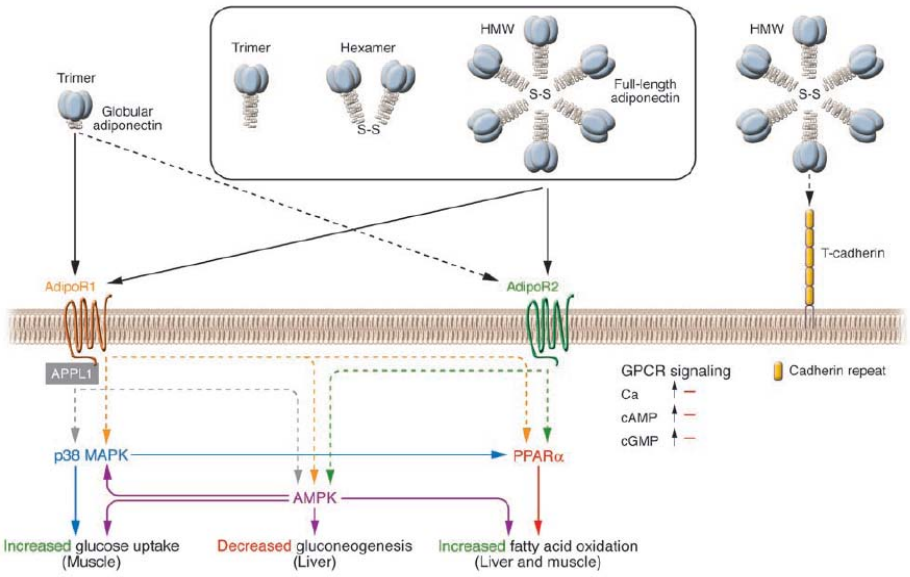
Two different receptor isoforms, AdipoR1 and AdipoR2, have been described to date (27). Both are 7-transmembrane proteins, and in contrast to the G protein–coupled receptor family, these receptors have internal N-terminal and external C-terminal regions (27). AdipoR1 has a high affinity for globular adiponectin and a low affinity for full-length adiponectin and is abundantly expressed in skeletal muscle and endothelial cells among other tissues. AdipoR2 has intermediate affinity for both forms of adiponectin and is predominantly expressed in the liver (28, 29). AdipoR1 and AdipoR2 serve as receptors for globular and full-length adiponectin and mediate increased AMPK, PPARα ligand activities, fattyacid oxidation, and glucose uptake by adiponectin (Fig. 3).

Although both receptors are present in almost every tissue, including pancreatic β cells and malignant cells, one or the other receptor usually predominates. Interestingly, HMW adiponectin has also been proposed to be a ligand for T-cadherin, but this remains to be fully elucidated.

AdipoR1 and AdipoR2 mediate the activation of adenosine monophosphate-activated protein kinase (AMPK), the oxidation of fatty acid, and glucose uptake. Specific receptors are expressed also in the cardiac tissue and particularly in cardiac myocytes, whose growth and intra-cellular interactions are modulated by adiponectin (22). In eukaryotes only, T-cadherin, an extra-cellular protein present in endothelial and smooth muscle cells, can function as a further receptor for

adiponectin MMW and HMW forms (30). (Fig. 3).

Figure 3. Signal transduction by adiponectin receptors [adapted from ref.(29)].



2.4. Association of hypoadiponectinemia with insulin resistance and type 2 diabetes

In contrast to the expression of adipokines such as TNF-α and resistin, which cause insulin resistance, adiponectin expression is reduced in obese, insulin-resistant rodent models (14). Plasma adiponectin levels are also decreased in an obese rhesus monkey model that frequently develops type 2 diabetes (31). Importantly, a decrease in plasma adiponectin levels preceded the onset of diabetes in these animals, in parallel with the observation of decreased insulin sensitivity (31). Plasma adiponectin levels have also been reported to be reduced in obese humans, particularly those with visceral obesity, and to correlate inversely with insulin resistance (32-35). Prospective and longitudinal studies (2,34,36-40) have shown that lower adiponectin levels are associated with a higher incidence of diabetes. Adiponectin, but not inflammatory markers such as C-reactive protein and IL-6, has been shown to be significantly related to the development of type 2 diabetes in Pima Indians (40). Hypoadiponectinemia has also been demonstrated to be independently associated with the metabolic syndrome — indeed, more strongly than are any other inflammatory markers (41). Reduced plasma adiponectin levels are also commonly observed in a variety of states frequently associated with insulin resistance, such as cardiovascular disease (42, 43) and hypertension (44, 45).

How is the level of plasma adiponectin physiologically regulated? There is a sexual dimorphism in the circulating levels of adiponectin. Indeed, female humans and rodents have higher plasma adiponectin levels than males, suggesting that sexual hormones regulate the production of adiponectin, although it is controversial how these hormones, such as estrogen and testosterone, are involved in the regulation of plasma adiponectin level (46-48). Nevertheless, this may partly account for the fact that females are more sensitive to insulin than males. Some dietary factors, such as soy protein (49), fish oils (6), and linoleic acid (50), are also suggested to increase plasma adiponectin levels, which is consistent with the fact that intake of these factors is thought to have a protective effect on the development of diabetes. On the other hand, a carbohydrate-rich diet appears to decrease plasma adiponectin level (51). Oxidative stress has also been suggested to inhibit the expression of adiponectin (52). Although the mechanism underlying this regulation is unclear, this may contribute to the decrease in plasma adiponectin in obesity, which is associated with increased oxidative stress in adipose tissue. Thus, the plasma adiponectin level is affected by multiple factors, including gender, aging, and lifestyle.

2.5. Adiponectin and Fatty acid composition in serum phospholipids

The interaction among diet-induced insulin resistance, adiponectin concentrations, and lipid metabolism could be exerted at the level of the inflammatory cascade. The amount and quality of fat in the diet seem to be of importance for development of insulin resistance and related inflammatory activity (53). A low proportion of long-chain unsaturated fatty acids and a high proportion of saturated fatty acids in the diet have been associated with impaired insulin action (54). Adiponectin possesses antiinflammatory (55) and antiatherogenic properties (56,57). On the other hand, highly unsaturated fatty acids, ω-3 fatty acids in particular, are also receiving increasing attention as potential antiinflammatory agents (58) because these dietary fatty acids appear to modulate the release of different cytokines (59,60) (Fig. 4).

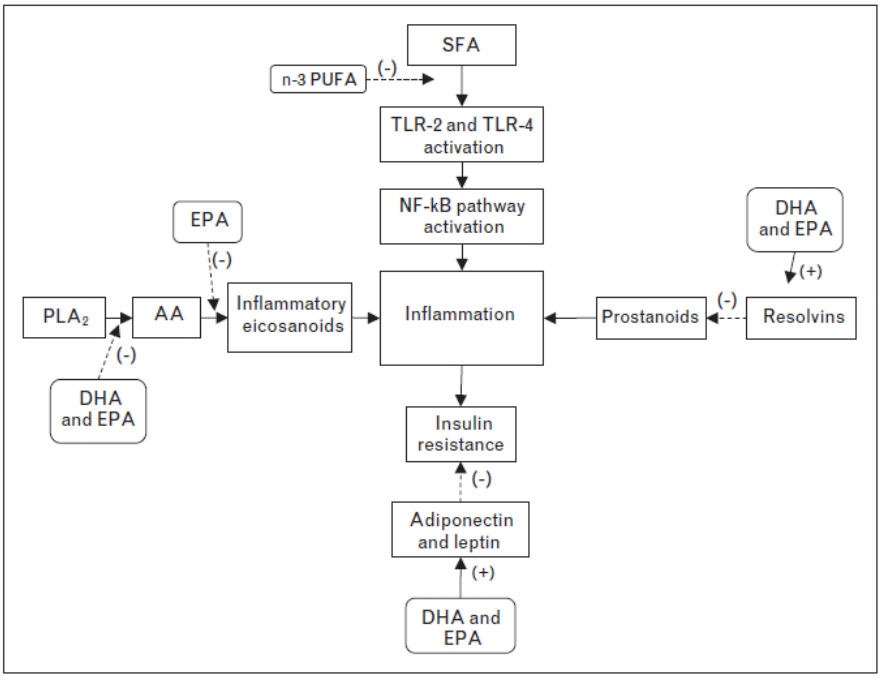
How dietary fat may impact on peripheral adiponectin concentration, or vice versa, is unknown. One potential pathway is activation of the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR- γ). In fact, fatty acids activate PPAR- γ (61), and pharmacologic activation with PPAR- γ agonists leads to increased plasma adiponectin concentrations (62,63) (Fig. 5).

Knowledge of the possible influence of dietary habits on circulating adiponectin concentrations could be helpful in delineating dietary measures aimed at preventing type 2 diabetes. However, studies of the associations between dietary habits in relation to the development of chronic diseases are hampered by

several methodological problems, including imprecision of dietary surveys (55). One way to monitor the type of fat in the diet is to record the fatty acid composition in serum phospholipids.

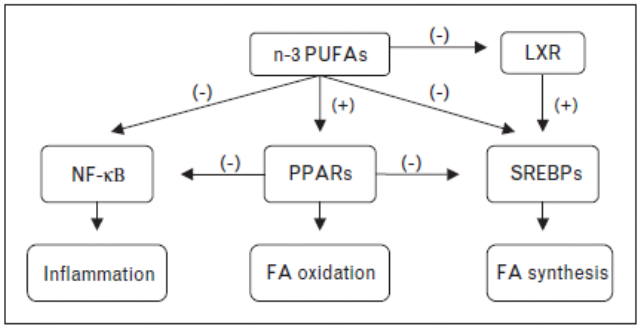
The fatty acid compositions of cholesterol esters, phospholipids, and triacylglycerols are objective biological markers of fat intake (64-66). They reflect actual rather than reported intakes and thus are unaffected by respondents' underreporting or overreporting of fat intakes that is typical of diet records and recalls (67). Moreover, serum fatty acids are particularly good markers of myristic and linoleic acid intakes; in controlled trials, the dietary intakes of these fatty acids are the major dietary predictors of serum cholesterol concentrations (68,69).

Figure 4. Anti-inflammatory effects of n-3 PUFA [adapted from ref.(70)]



AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PLA2, phospholipase A2; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TLR, toll-like receptor.

Figure 5. Transcription factors regulated by n-3 PUFAs [adapted from ref.(70)]



FA, fatty acid; PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acids; LXR, liver X receptor; SREBP, sterol receptor element binding protein.

2.6. Impact of Adiponectin gene polymorphisms on insulin resistance and type 2 diabetes

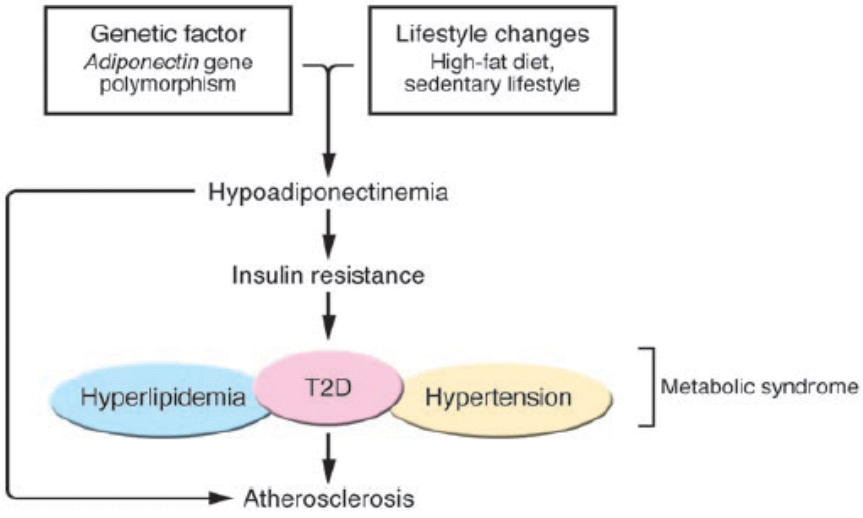
The *Adiponectin* gene is located on chromosome 3q27, which has been reported to be linked to type 2 diabetes and the metabolic syndrome (5,71,72). Therefore, the *Adiponectin* gene appears to be a promising candidate susceptibility gene for type 2 diabetes. Among the SNPs in the *Adiponectin* gene, 276G>T is associated with decreased levels of circulating adiponectin, greater insulin-resistance (IR) index, and an increased risk of T2DM in Japanese subjects (3). Menzaghi et al. (4) also showed that 276G>T, either independently or as a haplotype with 45T>G, is associated with several features of IR in non-diabetic Caucasians from Italy, including low serum adiponectin concentrations. In French subjects, a GG haplotype defined by the SNPs -11391G>A and -11377C>G in the promoter region of the *ADIPOQ* gene is significantly associated with hypoadiponectinemia, despite no association with IR and T2DM (5).

Taken together, these data strongly support the hypothesis that adiponectin plays a pivotal role in the pathogenesis of type 2 diabetes. A recent haplotype analysis based on a dense SNP map in a large sample clarified a 2-block linkage disequilibrium structure of the *Adiponectin* gene, the first block including the promoter SNPs and the second spanning the exons and introns (73). It is noteworthy that neither block has more than 1 SNP significantly associated with

the plasma adiponectin level. The haplotypes in the first block were associated with increased adiponectin level, whereas the haplotypes in the second block were associated with decreased adiponectin level. This result indicated the existence of at least 2 causal haplotypes or SNPs in the *Adiponectin* gene.

Based on the significant body of evidence discussed above, we have proposed the "adiponectin hypothesis," in which reduced plasma adiponectin levels caused by interactions between genetic factors, such as SNPs in the *Adiponectin* gene itself, and environmental factors causing obesity, such as a sedentary lifestyle, may play a crucial role in the development of insulin resistance, type 2 diabetes, and the metabolic syndrome (74) (Fig. 6).

Figure 6. Adiponectin hypothesis for insulin resistance, the metabolic syndrome, and atherosclerosis [adapted from ref. (29)].



3. Subjects and Methods

3.1. Subjects

This study involved 1,194 unrelated Korean adults (580 men and 614 women). All participants were recruited during routine check-ups at a health promotion center at Yonsei University Hospital and Ilsan Hospital. Exclusion criteria were: clinical or electrocardiographic evidence of CAD, stroke, myocardial infarction, or peripheral arterial occlusive disease; diabetes mellitus (or fasting glucose≥ 126 mg/dL); abnormal liver or renal function; thyroid or pituitary disease; acute or chronic inflammatory disease; and regular use of any medications. The purpose of the study was carefully explained to all participants, and their informed consent was obtained. The study protocol was approved by the Institutional Review Board of Yonsei University.

3.2. Methods

3.2.1. Anthropometric parameters, blood pressure, and blood collection

Body weight and height were measured unclothed and without shoes in the morning for the calculation of body mass index (BMI, kg/m^2). Waist and hip circumferences were measured for the calculation of waist hip ratio (WHR).

Blood pressure (BP) was measured in the left arm of the seated patient with an automatic BP monitor (TM-2654, A&D, Tokyo, Japan) after a 20-min rest. After a 12-hour fasting period, venous blood specimens were collected in EDTA-treated and plain tubes, centrifuged to give plasma or serum, and stored at -70°C until analysis.

3.2.2. Glucose, Insulin and HOMA-IR

Fasting glucose was measured by a glucose oxidase method using the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Insulin was measured by radioimmunoassays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA). Insulin resistance (IR) was calculated with the homeostasis model assessment (HOMA) using the following equation: IR=[fasting insulin (μIU/ml) × fasting glucose (mmol/l)]/22.5.

3.2.3. Serum lipid profile

Fasting total cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of serum chylomicron using dextran sulfate magnesium, the concentrations of LDL-cholesterol and HDL-cholesterol in the supernatant were measured by an enzymatic method. LDL-cholesterol was indirectly estimated in subjects with serum triglyceride concentrations <400 mg/mL using the Friedewald

formula [LDL-cholesterol=total-cholesterol–{HDL-cholesterol+(triglycerides/5)}].

3.2.4. Plasma adiponectin

Plasma adiponectin concentration was measured using an enzyme immunoassay (Human Adiponectin ELISA kit, B-Bridge International Inc., CA, USA). Assays were read using a Victor^2 (Perkin Elmer Life Sciences, Turku, Finland) at 450 nm and wavelength correction was set to 540 nm.

3.2.5. Genotyping

Genomic DNA was extracted from 5 mL whole blood using a commercially available DNA isolation kit (WIZARD Genomic DNA purification kit, Promega, Madison, WI, USA) according to the manufacturer's protocol. We first prescreened eight sites of previously reported *ADIPOQ* SNPs (-11391G>A; -11377C>G; H241P; Y111H; G90S; R221S; 45T>G; 276G>T)) to identify the allele frequency of each SNP (75). Each genotyping reaction was performed with SNP-IT assays using single primer extension technology (SNPstream 25K System, Orchid Biosystems, Princeton, NJ, USA).

3.2.6. Fatty acid composition in serum phospholipids

Serum phospholipid fatty acid (FA) composition was analyzed using the modified method of Folch et al. (76) and Lepage et al. (77) with gas chromatography (Hewlett Packard 5890A, CA, USA). Individual FAs were calculated as a relative percentage with the elevated FAs set at 100% using Chemstation software.

3.2.7. Dietary intervention program and assessment of dietary intake/physical activity level

Subjects' diets were assessed using a 24-h recall method to perform analyses and a semi-quantitative food frequency questionnaire to check whether the data collected by 24-h recall methods was representative of the usual dietary pattern. A registered dietitian gave all subjects written and verbal instructions on completion of a 3-day dietary record (2 weekdays and 1 weekend day). Dietary energy values and nutrient content were calculated using CAN-pro 2.0 (Korean Nutrition Society). Total energy expenditures (kcal/day) were calculated from activity patterns including basal metabolic rate, physical activity for 24 h, and the specific dynamic action of food.

3.3. Statistical Analysis

Statistical analyses were performed with SPSS ver12.0 (Statistical Package for the Social Sciences, SPSS Ins., Chicago, IL, USA). Executive SNP Analyzer (http://www.istech.info/SilicoSNP/index.html?) was used for Hardy-Weinberg equilibrium (HWE) testing of each SNP and linkage disequilibrium (LD) test of SNPs. The Kolmogorov-Smirnov test was used to test the normality of distribution, and skewed variables were logarithmically transformed for statistical analysis. For descriptive purposes, mean values are presented using untransformed values. Results are expressed as mean ± S.E. or frequency. A two-tailed value of $P < 0.05$ was considered statistically significant. Differences in continuous variables among genotype groups were tested by one-way analysis of variance followed by the Bonferroni method, and non-continuous variables were tested by chi-square test. Pearson's correlation coefficients were used to examine the relationships between variables. Differences in clinical variables between the two groups were tested by independent t-test. General linear model was also performed with adjustment for confounders.

4. Results

4.1. **Detection** of SNPs in the ADIPOQ gene

The allele frequencies of eight SNPs in *ADIPOQ* in the prescreening test were as follows: -11391G>A (G:A=1:0); -11377C>G (C:G=0.73:0.27); 45T>G (T:G=0.69:0.31), 276G>T (G:T=0.70:0.30); H241P (A:C=1:0); Y111H (T:C=1:0); G90S (G:A=1:0); and R221S (C:A=0.98:0.02). We included only SNPs -11377C>G, 45T>G, and 276G>T in further analysis because the others were rare mutations with minor allele frequency (<2%) in these study subjects.

4.2. Distribution of -11377C>G, 45T>G, and 276G>T genotype in the entire population

This study included 1,194 non-diabetic subjects with the following genotypes: 647 C/C, 453 C/G, and 94 G/G for -11377C>G; 577 T/T, 498 T/G, and 119 G/G for 45T>G; and 580 G/G, 502 G/T, and 112 T/T for 276G>T. Genotype distributions did not deviate from HWE for any of the SNPs. The minor allele frequency of each SNP was consistent with previous reports in Korean populations (75,78).

There was low LD between 45T>G and -11377C>G (D´=0.710; P<0.001) and between 276G>T and -11377C>G (D´=0.041; P=0.459). 45T>G and

276G>T were found to be highly linked by LD test (D´=-1; P<0.001). Based on these results, haplotype analysis was performed for 45T>G with 276G>T. Estimated 45-276 haplotype frequencies were 13.8% for TG/TG, 25.1% for TG/TT, 24.8% for TG/GG, 9.4% for TT/TT, 16.9% for TT/GG, and 10.0% for GG/GG. For subsequent statistical analyses, subjects were divided into three haplotype groups: homozygous TG haplotype (TG/TG; n=165), heterozygous carriers of the TG haplotype (TG/X; n=596), and non-TG haplotype carriers (X/X; n=433). However, haplotype analysis did not provide information beyond that revealed by each SNP (Table 1), therefore we presented the results of individual *ADIPOQ* polymorphisms.

4.3. Clinical characteristics according to ADIPOQ SNP genotypes

Table 1 shows general characteristics of the subjects according to each SNP genotype. No significant genotype-associated differences were observed in age, sex distribution, BMI, WHR, cigarette smoking, alcohol consumption, blood pressure, and serum levels of triglyceride, total cholesterol, and LDL-cholesterol. Compared with 276T/T subjects, 276G allele carriers showed lower concentrations of HDL-cholesterol (P=0.024) and adiponectin (P<0.001), and higher levels of glucose (P=0.015), insulin (P=0.006), and HOMA-IR (P=0.005) (Table 1). None of the other SNPs were significantly associated with these variables.

4.4. Dietary intake according to ADIPOQ SNP genotypes

No significant differences were observed in total energy intake and proportions of energy intake derived from macronutrients according to *ADIPOQ* SNP genotypes (Table 2). However, G allele carriers of SNP276G>T showed lower intake of polyunsaturated fat with lower PUFA/saturated FA (SFA) than 276T/T subjects despite incomplete tabulation of fatty acid (FA) contents in the Korean Food Composition Table.

4.5. Fatty acid composition in serum phospholipids according to ADIPOQ SNP genotypes

Table 3 presents FA composition in serum phospholipids according to SNP genotypes. SNP-11377 was not associated with FA composition in serum phospholipids. Compared with 45G/G subjects, T allele carriers of SNP45T>G showed a lower proportion of total SFA (*P*=0.014) and higher proportions of total monounsaturated FA (MUFA) (*P*=0.039), oleic acid (18:1ω9, *P*=0.042), total ω3FA (*P*=0.033), docosapentaenoic acid (DPA, 22:5ω3, *P*=0.020), and docosahexaenoic acid (DHA, 22:6ω3, P=0.022) in serum phospholipids.

SNP276G>T was significantly associated with FA proportion in serum phospholipids (Table 3). Compared with 276T/T subjects, G allele carriers of SNP276G>T showed higher levels of myristic acid (14:0, *P*=0.007) and lower

total ω6 FAs (*P*=0.003), total ω3 FAs (*P*<0.001), linoleic acid (18:2ω6, *P*=0.001), dihomo-γ-linolenic acid (20:3ω6, *P*=0.005), arachidonic acid (20:4ω6, *P*=0.015), α-linolenic acid (18:3ω3, *P*=0.026), eicosapentaenoic acid (EPA, 20:5ω3, *P*=0.003), 22:5ω3 (*P*<0.001), and 22:6ω3 (*P*=0.001) in serum phospholipids. After adjusting for the proportion of 18:2ω6 and 18:3ω3 (biomarkers of long-term essential FA intake), 276G>T was still associated with the proportion of total ω3 (*P*=0.026), 20:5ω3 (*P*=0.021), and 22:5ω3 (*P*=0.024) but the significance between SNP276 and the proportion of total ω6 (*P*=0.639), 20:3ω6 (*P*=0.232), 20:4ω6 (*P*=0.639), and 22:6ω3 (*P*=0.079) disappeared.

Table 1. Clinical characteristics according to the genotypes of *ADIPOQ* SNPs of healthy Koreans

	SNP 45			SNP 276			SNP -11377			SNP45-276 haplotype		
	T/T+T/G	G/G	P	G/G+G/T	T/T	P	C/C+C/G	G/G	P	TG/TG+TG/X	X/X	P
n	1075	119		1082	112		1100	94		761	433	
Male / Female, (%)	48.7 / 51.3	47.1 / 52.9	0.727	49 / 51.0	44.6 / 55.4	0.382	47.8 / 52.2	57.4 / 42.6	0.073	49.4 / 50.6	47.1 / 52.9	0.445
Age (yr)	50.3 ± 0.34	51.1 ± 1.02	0.466	50.4 ± 0.34	51 ± 1.14	0.538	50.3 ± 0.34	51.3 ± 1.14	0.409	50.5 ± 0.40	50.3 ± 0.54	0.732
BMI (kg/m^2)	23.8 ± 0.09	23.8 ± 0.25	0.807	23.8 ± 0.09	23.8 ± 0.26	0.914	23.8 ± 0.08	23.8 ± 0.30	0.919	23.9 ± 0.10	23.7 ± 0.13	0.226
Waist hip ratio	0.9 ± 0.00	0.9 ± 0.01	0.320	0.9 ± 0.00	0.91 ± 0.01	0.552	0.9 ± 0.00	0.91 ± 0.01	0.108	0.9 ± 0.00	0.9 ± 0.00	0.516
Smoker, n (%)	205 (19.1)	19 (16.0)	0.411	209 (19.3)	15 (13.4)	0.126	208 (18.9)	16 (17.0)	0.653	151 (19.8)	73 (16.9)	0.204
Alcohol drinker, n (%)	630 (58.6)	71 (59.7)	0.824	642 (59.3)	59 (52.7)	0.173	650 (59.1)	51 (54.3)	0.361	444 (58.3)	257 (59.4)	0.734
Systolic BP (mm Hg)	121.8 ± 0.46	120.2 ± 1.47	0.269	121.5 ± 0.46	122.7 ± 1.43	0.411	121.6 ± 0.45	121.6 ± 1.54	0.964	121.8 ± 0.54	121.3 ± 0.73	0.581
Diastolic BP (mm Hg)	75.5 ± 0.34	73.8 ± 1.11	0.113	75.3 ± 0.34	75.4 ± 1.11	0.968	75.3 ± 0.34	75.5 ± 1.15	0.880	75.6 ± 0.40	74.9 ± 0.55	0.271
Triglyceride (mg/dL) $\sharp$	115.4 ± 2.02	119.3 ± 6.28	0.697	116.1 ± 2.02	112.6 ± 6.43	0.569	115.4 ± 1.99	120.2 ± 7.36	0.640	115.1 ± 2.42	117.1 ± 3.18	0.581
TC (mg/dL)	193.1 ± 1.06	189.2 ± 3.04	0.239	192.5 ± 1.05	195.5 ± 3.36	0.383	193.2 ± 1.05	187.2 ± 3.39	0.103	191.4 ± 1.21	195.1 ± 1.75	0.081
LDLc (mg/dL)	114.5 ± 1.01	110.4 ± 2.78	0.200	114 ± 1.00	114.8 ± 3.24	0.805	114.5 ± 0.99	108.4 ± 3.42	0.083	113.4 ± 1.17	115.3 ± 1.63	0.335
HDLc (mg/dL) $\sharp$	55.8 ± 0.46	56 ± 1.52	0.836	55.5 ± 0.47	58.8 ± 1.46	0.024	55.9 ± 0.47	55 ± 1.51	0.636	55.2 ± 0.55	56.9 ± 0.76	0.106
Glucose (mg/dL) $\sharp$	90.4 ± 0.33	91.1 ± 1.02	0.516	90.7 ± 0.33	87.9 ± 1.17	0.015	90.4 ± 0.33	91.6 ± 1.04	0.248	91.1 ± 0.39	89.5 ± 0.53	0.012
Insulin (mU/mL) $\sharp$	9.07 ± 0.15	9.18 ± 0.44	0.898	9.18 ± 0.15	8.04 ± 0.33	0.006	9.1 ± 0.15	8.82 ± 0.36	0.944	9.27 ± 0.19	8.75 ± 0.21	0.064
HOMA-IR $\sharp$	2.04 ± 0.04	2.08 ± 0.11	0.779	2.07 ± 0.04	1.79 ± 0.09	0.005	2.05 ± 0.04	2.01 ± 0.09	0.793	2.1 ± 0.04	1.96 ± 0.05	0.022
Adiponectin (mg/mL) $\sharp$	6.33 ± 0.11	7.05 ± 0.39	0.056	6.26 ± 0.11	7.54 ± 0.34	<0.001	6.41 ± 0.11	6.25 ± 0.33	0.841	6.09 ± 0.13	6.94 ± 0.19	<0.001
Smoker, n (%)	205 (19.1)	19 (16.0)	0.411	209 (19.3)	15 (13.4)	0.126	208 (18.9)	16 (17.0)	0.653	151 (19.8)	73 (16.9)	0.204

Values are mean ± S.E. $\sharp$ tested by logarithmic transformation, *P*-values tested by independent *t*-test or chi-square test.
BP: blood pressure, TC: total cholesterol, LDLc : LDL cholesterol, HDLc : HDL cholesterol, HOMA-IR: homeostasis model assessment of IR

Table 2. Energy and macronutrient intake according to the genotypes of *ADIPOQ* SNPs in healthy Koreans

	SNP -11377			SNP 45			SNP 276		
	C/C+C/G	G/G	P	T/T+T/G	G/G	P	G/G+G/T	T/T	P
n	1100	94		1075	119		1082	112	
Total energy expenditure (kcal)	2057±10.5	2050±35.9	0.856	2062±10.5	2012±33.6	0.132	2057±10.6	2052±31.3	0.879
Estimates of daily nutrient intakes†									
Total energy intake (kcal)	2150±10.7	2124±37.6	0.509	2151±10.9	2121±31.7	0.375	2148±10.9	2151±32.3	0.923
Carbohydrate (% of energy)	62.0±0.06	61.9±0.18	0.764	62.0±0.06	62.0±0.16	0.874	62.0±0.06	62.1±0.16	0.307
Protein (% of energy)	16.9±0.04	17.0±0.14	0.325	16.9±0.04	17.1±0.12	0.094	16.9±0.04	16.8±0.12	0.421
Fat (% of energy)	21.3±0.06	21.3±0.14	0.945	21.3±0.06	21.3±0.16	0.848	21.3±0.06	21.3±0.20	0.825
SFA (g)	10.9±0.14	10.5±0.47	0.367	11.0±0.14	10.8±0.37	0.742	11.0±0.14	10.4±0.42	0.211
MUFA (g)	13.1±0.18	12.5±0.53	0.387	13.1±0.18	12.9±0.44	0.792	13.1±0.18	12.9±0.46	0.765
PUFA (g)	13.2±0.13	12.9±0.45	0.536	13.2±0.13	12.9±0.38	0.389	13.0±0.13	14.9±0.42	<0.001
PUFA/SFA	1.32±0.02	1.32±0.04	0.987	1.32±0.02	1.28±0.04	0.364	1.29±0.01	1.58±0.05	<0.001

Values are mean±S.E.$^{\sharp}$ tested by logarithmic transformation, *P*-values tested by independent *t*-test,

†Nutrient intakes, obtained from weighed food records and calculated using the database of the computerized Korean food code.

Table 3. Mean proportion (%) of fatty acid composition in serum phospholipids according to the genotypes of *ADIPOQ* SNPs in healthy Koreans

	SNP -11377			SNP 45			SNP 276		
	C/C+C/G	G/G	P	T/T+T/G	G/G	P	G/G+G/T	T/T	P
n	1100	94		1075	119		1082	112	
SFA	54.5±0.21	54.3±0.77	0.821	54.3±0.21	56.0±0.75	0.014	54.6±0.21	53.5±0.66	0.122
12:0$^{\phi}$	0.37±0.01	0.34±0.03	0.394	0.37±0.01	0.37±0.02	0.809	0.37±0.01	0.34±0.02	0.537
14:0$^{\phi}$	0.63±0.02	0.60±0.03	0.767	0.61±0.01	0.80±0.16	0.301	0.64±0.02	0.55±0.02	0.007
16:0	32.2±0.18	32.1±0.61	0.918	32.1±0.18	32.7±0.62	0.307	32.2±0.18	31.6±0.51	0.258
18:0$^{\phi}$	18.9±0.11	18.9±0.37	0.978	18.9±0.11	19.7±0.44	0.107	19.0±0.12	18.6±0.36	0.266
MUFA$^{\phi}$	11.3±0.08	11.3±0.22	0.858	11.3±0.08	10.9±0.26	0.039	11.3±0.08	11.4±0.22	0.686
16:1$^{\phi}$	0.71±0.03	0.64±0.02	0.611	0.71±0.03	0.72±0.08	0.721	0.71±0.03	0.67±0.02	0.258
18:1 (ω-9)	6.85±0.06	6.93±0.15	0.695	6.89±0.06	6.53±0.18	0.042	6.84±0.06	6.99±0.16	0.390
ω-6 PUFA	20.3±0.16	20.1±0.57	0.832	20.3±0.16	19.7±0.58	0.306	20.1±0.16	21.6±0.52	0.003
18:2 (ω-6)	12.6±0.11	12.4±0.37	0.578	12.6±0.11	12.3±0.39	0.417	12.5±0.11	13.7±0.34	0.001
18:3 (ω-6)$^{\phi}$	0.25±0.01	0.26±0.04	0.890	0.25±0.01	0.26±0.03	0.173	0.25±0.01	0.24±0.02	0.898
20:3 (ω-6)	1.45±0.02	1.43±0.07	0.765	1.46±0.02	1.33±0.05	0.055	1.43±0.02	1.62±0.06	0.005

Table 3. continued

	SNP -11377			SNP 45			SNP 276		
	C/C+C/G	**G/G**	**P**	**T/T+T/G**	**G/G**	**P**	**G/G+G/T**	**T/T**	**P**
20:4 (ω-6)	4.53±0.06	4.61±0.23	0.717	4.56±0.06	4.30±0.20	0.177	4.49±0.06	4.96±0.19	0.015
ω-3 PUFA$^{\phi}$	4.80±0.08	4.80±0.20	0.375	4.84±0.07	4.37±0.22	0.033	4.70±0.07	5.71±0.26	<0.001
18:3 (ω-3)$^{\phi}$	0.15±0.01	0.17±0.03	0.915	0.15±0.00	0.22±0.07	0.886	0.15±0.01	0.16±0.01	0.026
20:5 (ω-3)$^{\phi}$	1.17±0.02	1.26±0.07	0.102	1.19±0.02	1.08±0.07	0.056	1.16±0.02	1.39±0.09	0.003
22:5 (ω-3)$^{\phi}$	0.54±0.01	0.54±0.04	0.819	0.54±0.01	0.46±0.02	0.020	0.52±0.01	0.66±0.04	<0.001
22:6 (ω-3)$^{\phi}$	2.85±0.05	2.74±0.14	0.996	2.88±0.05	2.52±0.14	0.022	2.78±0.05	3.42±0.17	0.001

Values are mean ± S.E. $^{\phi}$ tested by logarithmic transformation, *P*-values tested by independent *t*-test.

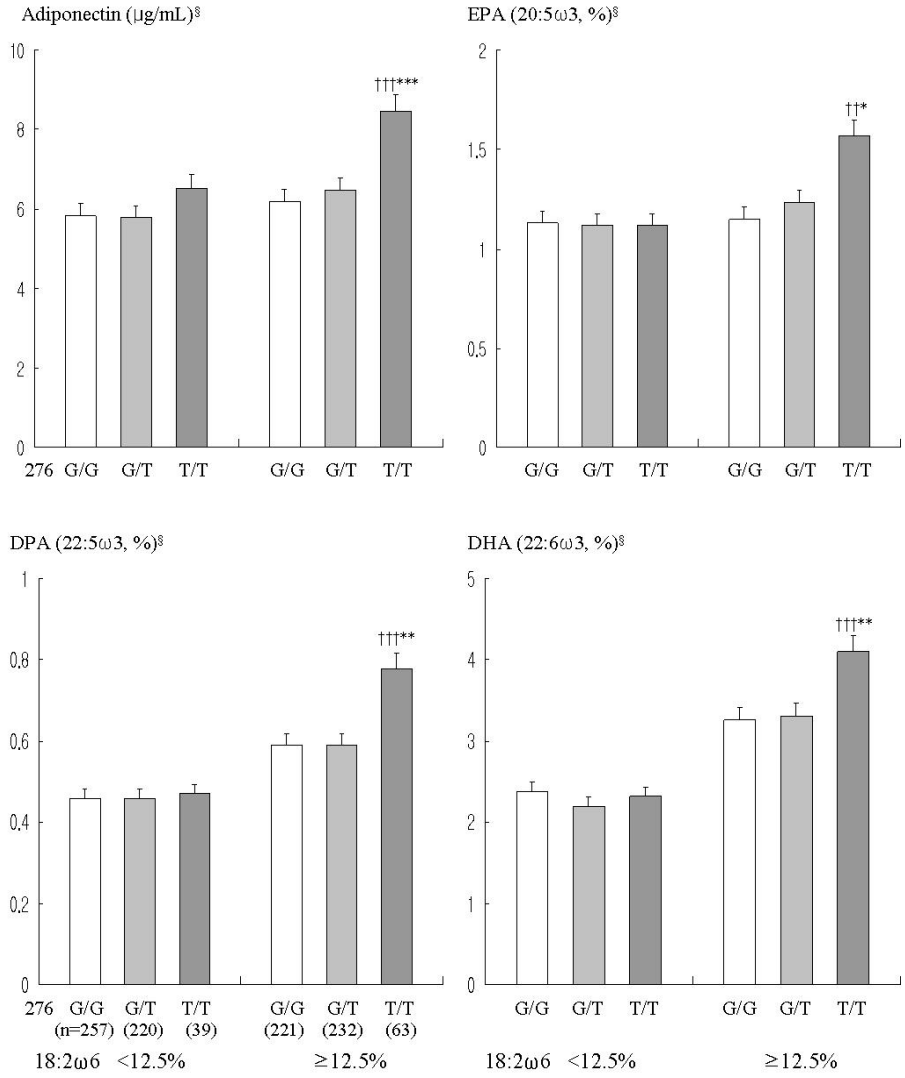
4.6. Effect of ADIPOQ 276G>T on plasma adiponectin and long-chain ω3 PUFAs in serum phospholipids according to the proportion of linoleic acid

We found significantly positive correlations between the proportion of 18:2ω6 in serum phospholipids and PUFA intake (r=0.260, *P*<0.001) and plasma adiponectin (r=0.150, *P*<0.001). Therefore, subjects were divided into two subgroups according to the median value of 18:2ω6: below the median (<12.5% of total fatty acids in serum phospholipids) and above the median (≥12.5%). These two subgroups differed significantly in plasma adiponectin (5.87±0.15 vs. 6.64±0.17 μg/mL; *P*<0.001), total SFAs (57.4±0.31 vs. 51.5±0.19 %; *P*<0.001), total ω6 (16.9±0.17 vs. 23.6±0.15 %; *P*<0.001), and total ω3 FAs (4.14±0.09 vs. 5.46±0.10 %; *P*<0.001) in serum phospholipids (Table 4,5). In the subgroup with a higher proportion of 18:2ω6, 276G allele carriers showed lower adiponectin (*P*<0.001) (Fig. 7), higher glucose (G/G: 92.1±0.64, G/T: 90.2±0.62, T/T: 85.4±1.39 mg/dL; *P*<0.001), and higher HOMA-IR (G/G: 2.03±0.07, G/T: 2.10±0.07, T/T: 1.72±0.11; *P*=0.013) than 276T/T subjects (Table 6). Furthermore, 276G allele carriers showed lower 20:5ω3 (*P*=0.005), 22:5ω3 (*P*=0.003), and 22:6ω3 (*P*=0.012) after adjusting for the proportions of 18:2ω6 and 18:3ω3 in serum phospholipids (Fig. 7). They still remained significant after adjusting for age, sex and BMI (P=0.006 for 20:5ω3, P=0.001 for 22:5ω3, P=0.003 for 22:6ω3). In contrast, in the subgroup with lower 18:2ω6, 276G>T

was not associated with adiponectin (Fig. 7), glucose, HOMA-IR, or FA composition in serum phospholipids (Table 6,7).

Fig.8 shows the relationship between adipnecntin levels and HOMA-IR according to 276G>T and the levels of 18:2ω6 proportion. G allele carriers had the negative relationship between the two variables regardless of 18:2ω6 proportion. On the other hand, TT subjects showed the significant negative correlation when 18:2ω6 proportion is above the median (≥12.5%). These patterns still maintained after adjusting for age, sex, BMI and the proportions of 18:2ω6 and 18:3ω3 in serum phospholipid

Figure 7. Effect of *ADIPOQ* 276 G>T on plasma adiponectin and proportions of long-chain ω3 PUFAs in serum phospholipids by 18:2 ω6 median value



Data are mean ± S.E.

§tested by logarithmic transformation; ††P<0.01, †††P<0.001 compared with wild type and *P<0.05, **P<0.01, ***P<0.001 compared with heterozygote for each group, tested by general linear model with adjustment for 18:2ω6 and 18:3ω3.

Table 4. Anthropometric and biochemical variables by 18:2 ω6 median value

	Total	**18:2 ω6 < 12.5%**	**18:2 ω6 ≥12.5%**	***P***
n	1194	516	516	
Age (yr)	50.4 ± 0.32	49.4 ± 0.44	48.4 ± 0.51	0.121
Body Mass Index (kg/m^2)	23.8 ± 0.08	23.8 ± 0.12	23.6 ± 0.13	0.276
Waist hip ratio	0.90 ± 0.00	0.90 ± 0.00	0.90 ± 0.00	0.437
Systolic BP	121.6 ± 0.44	120.8 ± 0.64	119.9 ± 0.66	0.308
Diastolic BP	75.3 ± 0.32	75.3 ± 0.47	74.2 ± 0.51	0.110
Triglyceride (mg/dL)[ʃ]	115.8 ± 1.93	119.8 ± 2.95	112.0 ± 2.78	0.032
Total cholesterol (mg/dL)	192.7 ± 1.00	192.3 ± 1.47	193.3 ± 1.56	0.648
LDL cholesterol (mg/dL)	114.0 ± 0.95	113.8 ± 1.40	113.8 ± 1.50	0.998
HDL cholesterol (mg/dL)[ʃ]	55.8 ± 0.44	55.1 ± 0.70	57.0 ± 0.67	0.028
Fasting glucose (mg/dL)[ʃ]	90.5 ± 0.32	91.3 ± 0.51	90.4 ± 0.43	0.287
Fasting insulin (μU/mL)[ʃ]	9.08 ± 0.14	8.47 ± 0.22	8.99 ± 0.19	0.020
[1]HOMA-IR[ʃ]	2.05 ± 0.03	1.93 ± 0.05	2.02 ± 0.05	0.074
Adiponectin (μg/mL)	6.39 ± 0.11	5.87 ± 0.15	6.64 ± 0.17	<0.001

Values are mean ± S.E. [ʃ] Tested by logarithmic transformation. *P*-values are tested by paired *t*-test. [1]HOMA-IR = {fasting insulin(μU/mL) x fasting glucose(mmol/L)}/22.5

Table 5. Mean proportion (%) of fatty acid composition in serum phospholipids by 18:2 ω6 median value

	Total (n=1194)	**18:2 ω6 < 12.5%**	**18:2 ω6 ≥ 12.5%**	***P***
n	1194	516	516	
SFA	54.5 ± 0.20	57.4 ± 0.31	51.5 ± 0.19	<0.001
12:0 $^{\phi}$	0.37 ± 0.01	0.35 ± 0.01	0.38 ± 0.01	0.096
14:0 $^{\phi}$	0.63 ± 0.02	0.70 ± 0.03	0.56 ± 0.01	<0.001
16:0	32.2 ± 0.17	33.8 ± 0.27	30.5 ± 0.18	<0.001
18:0 $^{\phi}$	18.9 ± 0.11	20.1 ± 0.17	17.8 ± 0.12	<0.001
MUFA $^{\phi}$	0.51 ± 0.01	0.52 ± 0.01	0.49 ± 0.01	0.006
16:1 $^{\phi}$	11.3 ± 0.07	11.2 ± 0.12	11.4 ± 0.09	0.057
18:1 (ω-9)	0.71 ± 0.02	0.77 ± 0.04	0.65 ± 0.02	0.001
ω-6 PUFA	6.86 ± 0.05	6.72 ± 0.08	6.99 ± 0.07	0.010
18:2 (ω-6)	20.2 ± 0.16	16.9 ± 0.17	23.6 ± 0.15	<0.001
18:3 (ω-6) $^{\phi}$	12.6 ± 0.10	10.0 ± 0.08	15.2 ± 0.09	<0.001
20:3 (ω-6)	0.25 ± 0.01	0.21 ± 0.01	0.29 ± 0.01	<0.001
20:4 (ω-6)	1.45 ± 0.02	1.19 ± 0.03	1.70 ± 0.02	<0.001
ω-3 PUFA $^{\phi}$	4.53 ± 0.06	3.61 ± 0.07	5.46 ± 0.07	<0.001
18:3 (ω-3) $^{\phi}$	4.80 ± 0.07	4.14 ± 0.09	5.46 ± 0.10	<0.001
20:5 (ω-3) $^{\phi}$	0.15 ± 0.01	0.16 ± 0.02	0.15 ± 0.00	0.052
22:5 (ω-3) $^{\phi}$	1.18 ± 0.02	1.12 ± 0.03	1.24 ± 0.03	0.001
22:6 (ω-3) $^{\phi}$	0.54 ± 0.01	0.46 ± 0.01	0.61 ± 0.02	<0.001
22:6 (ω-3) $^{\phi}$	2.84 ± 0.05	2.30 ± 0.06	3.39 ± 0.06	<0.001

Values are mean ± S.E. $^{\phi}$ Tested by logarithmic transformation.

P-values are tested by paired *t*-test

Table 6. Effects of a *ADIPOQ* SNP 276G>T on circulating levels of biochemical variables by 18:2 ω6 median value

	18:2 ω6 < 12.5%				18:2 ω6 ≥ 12.5%			
	GG	**GT**	**TT**	***P***	**GG**	**GT**	**TT**	***P***
n	257	220	39		221	232	63	
Age (yr)	49.6±0.62	49.2±0.69	49.6±1.41	0.905	47.9±0.74	48.2±0.74	50.8±1.80	0.208
Male / Female, (%)	58.0/42.0	56.4/43.6	46.2/53.8	0.382	50.2/49.8	54.3/45.7	34.9/65.1	0.024
BMI (kg/m^2)	23.9±0.17	23.6±0.17	23.9±0.39	0.282	23.5±0.18	23.6±0.20	23.7±0.37	0.815
Waist hip ratio	0.90±0.00	0.90±0.00	0.91±0.01	0.589	0.90±0.00	0.90±0.00	0.91±0.01	0.310
Systolic BP (mm Hg)	119.8±0.92	122.1±0.99	120.3±2.17	0.211	119.4±1.01	119.7±0.95	122.5±2.04	0.327
Diastolic BP(mm Hg)	74.6±0.66	76.2±0.73	74.6±1.68	0.216	74.0±0.78	74.3±0.77	74.0±1.50	0.958
TG (mg/dL)$^{\phi}$	119.2±4.32	119.6±4.10	124.2±13.6	0.789	112.9±4.24	113.6±4.29	102.8±7.02	0.553
TC (mg/dL)	189.5±2.06	193.9±2.26	201.3±5.40	0.080	191.2±2.22	195.7±2.47	191.7±4.55	0.378
LDLc (mg/dL)	112.5±1.93	113.3±2.19	125.0±5.24	0.073	112.6±2.19	116.4±2.34	108.3±4.25	0.192
HDLc (mg/dL)$^{\phi}$	54.0±0.94	56.7±1.14	53.6±2.36	0.179	56.0±1.00	56.3±0.99	62.8±1.94	0.004
Glucose (mg/dL)$^{\phi}$	91.7±0.73	90.8±0.74	91.4±2.07	0.700	92.1±0.64	90.2±0.62	85.4±1.39	<0.001
Insulin (μU/mL)$^{\phi}$	8.88±0.38	8.04±0.22	8.03±0.62	0.159	8.87±0.30	9.37±0.29	8.03±0.44	0.053
[1]HOMA-IR$^{\phi}$	2.03±0.09	1.82±0.06	1.88±0.17	0.184	2.03±0.07	2.10±0.07	1.72±0.11	0.013
Adiponectin (μg/mL)$^{\phi}$	5.84±0.21	5.80±0.23	6.53±0.48	0.185	6.19±0.23	6.46±0.25	8.45±0.48	<0.001

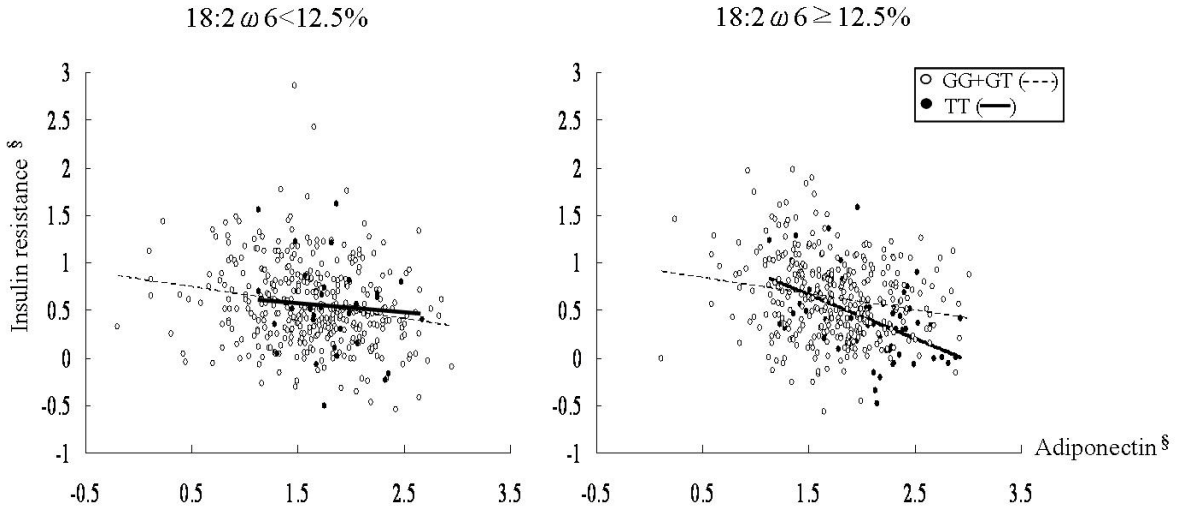
Mean ± S.E. $^{\phi}$ tested by logarithmic transformation, *P*-value tested by one-way analysis of variance with Bonferroni method, BP: blood pressure, [1]HOMA-IR $^{\phi}$

Table 7. Effects of a *ADIPOQ* SNP 276G>T on circulating levels of plasma fatty acid by 18:2 ω6 median value

	18:2 ω6 < 12.5%				18:2 ω6 ≥ 12.5%			
	GG	**GT**	**TT**	**P**	**GG**	**GT**	**TT**	**P**
n	257	220	39		221	232	63	
SFA	57.5±0.48	57.1±0.42	58.4±1.14	0.517	51.7±0.31	51.7±0.27	50.5±0.51	0.122
12:0$^{\phi}$	0.37±0.02	0.33±0.02	0.25±0.02†	0.006	0.37±0.02	0.39±0.02	0.40±0.03	0.740
14:0$^{\phi}$	0.76±0.07	0.65±0.02	0.55±0.04††	0.005	0.56±0.01	0.57±0.01	0.54±0.02	0.832
16:0	33.8±0.40	33.7±0.39	34.7±0.87	0.663	30.5±0.30	30.8±0.25	29.7±0.50	0.200
18:0$^{\phi}$	20.1±0.25	20.0±0.24	20.3±0.60	0.907	17.9±0.19	17.7±0.17	17.6±0.40	0.527
MUFA$^{\phi}$	11.0±0.17	11.5±0.17	11.4±0.46	0.094	11.4±0.15	11.3±0.13	11.3±0.21	0.768
16:1$^{\phi}$	0.81±0.07	0.73±0.05	0.68±0.04	0.972	0.70±0.06	0.60±0.01	0.67±0.02	0.069
18:1 (ω-9)	6.51±0.11	6.95±0.13†	6.83±0.34	0.041	7.00±0.10	6.95±0.10	7.09±0.17	0.797
ω-6 PUFA	16.7±0.26	17.1±0.25	16.7±0.65	0.586	23.6±0.24	23.3±0.20	24.7±0.39*	0.017
18:2 (ω-6)	9.80±0.13	10.2±0.12	10.1±0.32	0.063	15.2±0.15	15.1±0.12	15.9±0.26*	0.025
18:3 (ω-6)$^{\phi}$	0.23±0.02	0.19±0.01	0.17±0.03	0.220	0.29±0.02	0.28±0.02	0.29±0.03	0.308
20:3 (ω-6)	1.18±0.04	1.20±0.03	1.22±0.08	0.906	1.67±0.04	1.68±0.04	1.86±0.06†	0.043
20:4 (ω-6)	3.52±0.11	3.71±0.11	3.69±0.27	0.453	5.46±0.11	5.38±0.10	5.75±0.19	0.238
ω-3 PUFA$^{\phi}$	4.25±0.14	4.00±0.13	4.14±0.35	0.641	5.22±0.14	5.35±0.14	6.69±0.31	<0.001
18:3 (ω-3)$^{\phi}$	0.19±0.03	0.14±0.01	0.12±0.01	0.450	0.14±0.01	0.14±0.01	0.18±0.01	<0.001
20:5 (ω-3)$^{\phi}$	1.13±0.05	1.12±0.05	1.12±0.11	0.870	1.15±0.04	1.23±0.05	1.57±0.13	0.002
22:5 (ω-3)$^{\phi}$	0.46±0.02	0.46±0.02	0.47±0.04	0.935	0.59±0.03	0.59±0.02	0.78±0.06	<0.001
22:6 (ω-3)$^{\phi}$	2.38±0.10	2.20±0.09	2.32±0.24	0.597	3.26±0.10	3.31±0.09	4.10±0.19	<0.001

Values are mean ± S.E. $^{\phi}$ tested by logarithmic transformation, *P*-value tested by one-way analysis of variance (ANOVA) with Bonferroni method

Figure 8. Relationship between adiponectin and insulin resistance according to ADIPOQ 276G>T polymorphism and the proportion of 18:2ω6 in serum phospholipids (below or above the median level, 12.5%)



	r_0	p_0	r_1	p_1
GG+GT	-0.201,	<0.001	-0.130,	0.012
TT	-0.231,	0.211	-0.025,	0.904

	r_0	p_0	r_1	p_1
GG+GT	-0.189,	<0.001	-0.112,	0.038
TT	-0.486,	<0.001	-0.279,	0.039

§tested by logarithmic transformation, tested by Pearson correlation (r_0) or partial correlation analysis ($r1$)

r_0, correlation coefficient, unadjusted; r_1, correlation coefficient after adjusted for age, sex, BMI, proportions of 18:2ω6 and 18:3ω3 in serum phospholipids

5. Discussion

The current study shows a significant association of ADIPOQ 276G>T genotypes with plasma adiponectin and long chain ω3PUFAs in serum phospholipids after adjustment for the proportions of 18:2ω6 and 18:3ω3, biomarkers of long-term essential FA intake (79). The level of circulating adiponectins is modulated by ingestion of certain FAs or by changes in serum FA composition (80). Dietary factors such as fish oil and linoleic acid are associated with higher plasma concentration and gene expression of adiponectin (6,50), whereas saturated fat down-regulates *ADIPOQ* expression (10,11). In carriers of the 276G allele, higher proportions of 14:0 FAs and lower proportions of total ω6 and total ω3FAs in serum phospholipids might reflect a dietary intake with lower PUFA and lower PUFA/SFA. Since there were no differences in the proportion of energy intake derived from macronutrients according to 276G>T genotypes, G allele carriers appeared to have dietary habits involving consumption of less fish, soy, or vegetable oil, and more animal fat as a fat source. Moreover, the significantly higher levels of 20:5ω3 and 22:5ω3 in 276T/T subjects after adjustment for proportions of 18:2ω6 and 18:3ω3 could indicate a close link between ω3 FAs and adiponectin. Fernandez-Real et al. (81) have also observed that the proportion of ω3 FAs in plasma FA profiles is highest in individuals with increased circulating adiponectin, and that this association persists after controlling for age, BMI, and remaining individual FAs.

Interestingly, the association of 276G>T genotype with plasma adiponectin and long chain ω3PUFAs was more pronounced in subjects with 18:2ω6 proportions above the median (≥12.5% of total FAs in serum phospholipids). In the subgroup with a higher proportion of 18:2ω6, 276G carriers showed lower circulating adiponectin and lower proportions of 20:5ω3, 22:5ω3, and 22:6ω3. Similarly, a recent study in mice fed a high-fat diet showed that 20:5ω3 and 22:6ω3 stimulated *ADIPOQ* expression and increased circulating adiponectin independent of food intake or adiposity (6). In our study, subjects with 18:2ω6 above median had higher PUFA intake and exhibited higher total ω6 and total ω3FAs in serum phospholipids and higher plasma adiponectin than those with 18:2ω6 below median.

The major biological functions of adiponectin depend on the activation of adenosine monophosphate-activated protein kinase primarily in skeletal muscles, which increases FA oxidation and glucose uptake, thereby improving insulin sensitivity (82). In the current study, the association between *ADIPOQ* SNPs, adiponectin, and IR was found only for SNP276, consistent with previous findings that the 276G allele is associated with lower circulating adiponectin and is a significant contributor to increased CVD risk in Koreans independent of common environmental factors (75). In this study population, SNP11391 in the *ADIPOQ* promoter region was monomorphic (G:A=1:0) and SNP11377 was not significantly associated with adiponectin and HOMA-IR, similar to previous

findings in Korean women (78).

The observation of lower adiponectin, higher glucose and IR, and lower proportions of long-chain ω3PUFAs in carriers of the 276G allele supports the idea that adiponectin plays an important role in modulating insulin sensitivity, circulating glucose and FAs (82,83). The mechanism whereby serum phospholipid ω3PUFAs might impact peripheral adiponectin concentration, or vice versa, was not elucidated in this study. One potential pathway involves activation of the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ), a transcriptional regulator that interacts directly with the *ADIPOQ* promoter. In fact, EPA and DHA may up-regulate *ADIPOQ* by acting as ligands of PPAR-γ (84), and pharmacologic activation with PPAR-γ agonists leads to increased plasma adiponectin (61,62). Furthermore, genetic variations in *ADIPOQ* SNPs 276 and 45 can affect the PPAR-γ agonist response of circulating adiponectin and blood glucose control in T2DM (85). In this study, SNP45 was also weakly associated with total ω3FAs, 22:5ω3, and 22:6ω3 in serum phospholipids. However, all 45G/G subjects had a 276G/G genotype and 45T>G did not have significant association with adiponectin and HOMA-IR, therefore the weak association between SNP45 and ω3PUFAs could be due to the strong LD between SNP45 and SNP276. In contrast, 45T>G was significantly associated with insulin sensitivity in a German population without a family history of diabetes. This discrepancy might be explained by differences in family history

and ethnicity (86).

Circulating adiponectin levels are known to be highly heritable (~50%). Additionally, dietary FAs and gene-diet interactions have been found to modulate plasma adiponectin and contribute to IR, a risk factor for T2DM and CVD (87). Our results indicate associations between dietary PUFAs or serum phospholipid PUFAs, 276G>T, circulating adiponectin, and HOMA-IR, despite a much lower percentage of fat energy intake in the subjects of this study and in the general Korean population than that reported in white populations (88). In recent decades, Koreans have increased the percentage of calories from fat in their diets from 6% in 1969 to 20% in 2005 (88), and the mortality rate of ischemic heart disease has rapidly increased from 6.8% in 1988 to 27.5% in 2005 (89). The increase in rates and risk of CVD in Koreans is partially related to the rapid increase in fat intake. The fact that this increase in disease rate is occurring in the context of dietary habits considered healthy in white populations suggests that the Korean population is genetically predisposed to be negatively affected by adverse dietary changes at a lower threshold than whites.

Our results suggest that when diets with low intake of PUFA or PUFA/SFA are superimposed on a high-risk 276G carrier background, the lower proportion of PUFAs, especially long-chain ω3PUFAs, in serum phospholipids could result in more pronounced hypoadiponectinemia and IR than for other genotypes, thus accounting for the increased CVD risk. Therefore, substitution of soy, vegetable,

or fish oil for saturated fat, even in a low-fat diet, might be beneficial for 276G allele carriers. In fact, it has been suggested that diet plays a major role in triggering IR by interaction with genetic variants of candidate genes for dyslipidemia and IR (80). It should be noted that our results have the limitations of all cross-sectional and observational studies. We evaluated associations, rather than prospective predictions; thus cause-effect relationships and the mechanistic interactions between *ADIPOQ* genetic variability, adiponectin concentration, dietary fat, and other biochemical factors cannot be easily established. Despite these limitations, our data confirmed the relationship between *ADIPOQ* 276G>T and circulating adiponectin and IR shown in multiple independent populations (3,75,78,86,90) and identified an interesting association of the 276G allele with reduced proportion of long chain ω3PUFAs in serum phospholipids after adjustment for long-term essential fatty acid intake.

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국문요약

비당뇨 한국인에서 아디포넥틴 유전자 다형성과 혈청 인지질 중의 오메가-3 장쇄 다중불포화지방산과의 관련성 연구

아디포넥틴은 지방세포에서 분비되는 아디포카인으로써, 인슐린 감수성을 증가시키고 혈관염증을 억제하여 항염과 항동맥경화 작용과 관련되어 있는 것으로 알려져 있다. 혈중 아디포넥틴 농도는 유전적인 요인과 식이지방의 종류와 같은 환경적인 요인의 상호작용에 따라 변화한다. 본 연구에서는 아디포넥틴 유전자 다형성이 식이지방섭취 또는 혈청인지질 중의 지방산 조성, 그리고 혈중 아디포넥틴 농도 및 인슐린 저항성에 어떠한 영향을 미치는지 살펴보고자 하였다.

당뇨병에 걸리지 않은 한국인 1194명을 대상으로 8개의 SNPs (-11391G>A, -11377C>G, H241P, Y111H, G90S, R221S, 45T>G, 276G>T), 식이지방, 혈청인지질 중의 지방산 조성, 아디포넥틴 농도, 인슐린 저항성 지수 (HOMA-IR) 등을 측정 비교 하였다.

총 1,082명의 276G carrier군은 276T/T군에 비해 HDL-cholesterol (P=0.024)과 아디포넥틴 농도 (P<0.001)가 낮았으나,

혈당 (P=0.015)과 HOMA-IR (P=0.005) 은 높았다. 반면에, 다른 SNP에서는 어떤 관련성도 보이지 않았다. 276G carrier군은 혈청인지질중의 미리스티산(14:0)의 비율은 높았고, 총 오메가-6, 18:2 오메가-6, 20:3 오메가-6, 20:4 오메가-6, 18:3 오메가-3, 20:5 오메가-3, 22:5 오메가-3, 22:6 오메가-3 지방산의 비율은 낮았다. 나이, 성별, 체질량지수 그리고 장기간의 필수지방산 섭취량의 지표인 18:2 오메가-6와 18:3 오메가-3 지방산의 비율을 보정한 후에도 276G>T와 총 오메가-3 지방산 (P=0.026), 20:5 오메가-3 (P=0.021), 22:5 오메가-3 (P=0.024) 지방산과의 관련성은 여전히 유의하였다. 혈청인지질의 지방산 중에서 18:2 오메가-6 지방산은 다중불포화지방산 섭취량 (r=0.260, P<0.001) 및 아디포넥틴 농도 (r=0.260, P<0.001)와 유의한 상관성을 보였다. 혈중 인지질 총지방산 중 12.5% 이상의 높은 18:2 오메가-6 지방산 비율을 가지는 276G carrier 군은 더 낮은 아디포넥틴 농도 (P<0.001), HOMA-IR (P=0.013), 그리고 오메가-3 장쇄 다중불포화지방산 비율을 보여 더욱 확연한 특징을 보였다. 또한, 276G>T가 아디포넥틴 농도와 HOMA-IR에 미치는 영향은 18:2 오메가-6 지방산 비율에 따라 변화하였다.

결론적으로, 아디포넥틴 유전자 다형성은 비당뇨 한국인에 있어서 혈청인지질 중의 오메가-3 장쇄 다중불포화지방산의 조성에 영향을 주었다. 특히, *ADIPOQ* 276G은 다른 유전자형에 비해 적은 양의 다중불포화지방산을 섭취할 경우, 혈청인지질 중의 오메가-3 장쇄 다중불포화지방산의 낮은 비율로 인해 저아디포넥틴혈증으로 이를 수 있고, 이는 심혈관질환의 위험성을 증가시킬 수 있다고 제안할 수 있다.

핵심되는 말: 아디포넥틴, 아디포넥틴 유전자 다형성, 인슐린저항성, 오메가-3 장쇄다중불포화 지방산