# Genetic variants at the perilipin (PLIN) locus respond differently to mild weight loss in circulating free fatty acids levels and abdominal fat areas

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# 감사의 글

설레임으로 시작했던 지난 2년간의 대학원 생활도 어느덧 마무리를 해야 하는 시간이 되었습니다. 지나간 시간들은 저에게 많은 값진 것들을 배우고 느낄 수 있게 해주었던 소중한 시간이었습니다. 지금 이 순간까지 대학원 생활을 잘 마칠 수 있도록 많은 힘이 되어주셨던 분들께 글로나마 제 작은 감사의 마음을 전합니다.

여러모로 부족한 저에게 많은 관심과 배려를 아끼지 않으시고 임상영양학이라는 학문에 대해 많은 배움의 기회와 가르침을 주신 이종호 교수님께 진심으로 감사 드립니다. 언제나 인자하고 자상한 모습으로 몸소 진정한 학자의 모습을 보여주시고 일깨워 주신 장양수 교수님께도 깊은 감사를 드립니다.

학문적인 호기심을 일깨워 주시고 학문에 대한 깊이를 알게 해 주신 조홍근 교수님, 정지형 교수님, 이양자 교수님, 윤선 교수님, 곽동경 교수님, 양일선 교수님, 박태선 교수님, 이수복 교수님께도 감사 드립니다.

지난 2년동안 함께 했던 우리 연구실 가족들에게도 고마운 마음을 전합니다. 언제나 따뜻한 미소로 후배들에게 많은 귀감이 되어 주신 오연언니, 연구실 질서를 위해 후배들을 이끌어 가시느라 고생하신 지숙언니, 완벽하게 실험하는 모습으로 늘 모범을 보여주신 지영언니, 연구실의 패션리더 엘레강스 수정언니, 힘든 연구실 생활에 활력소가 되어 주었던 재치만점 예정언니, 언제나 따뜻한 조언을 아끼지 않았던 맘씨 고운 혜진언니에게도 감사의 마음을 전합니다.

힘들 때나 기쁠 때나 늘 많은 힘이 되어준 내겐 너무 소중한 인연 현지, 2년 동안 모든 동고동락을 같이 했던 친자매 같은 현양이, 문서작업의 귀재 컴퓨터 천재 승은이, 멋쟁이 패션리더 유쾌한 동기 유란이, 언제나 귀엽고 깜찍한 친구 계영이, 야무지고 똘똘한 고마운 후배 진경이, 매력 만점 인기녀 귀염둥이 현진이,

늘 맡은 일을 꼼꼼하게 해내는 부지런한 수현이, 모든일에 최선을 다하는 착하고 순수한 후배 수현이, 눈웃음이 참 예쁜 친절한 미진이, 성격 좋고 착한 친구 같은 후배 민지, 귀여운 동네주민 순수 소녀 유미, 노과연의 깜찍한 막내 정현이, 그리고 내게 많은 힘이 되어 주었던 노과연의 착한 동기 봉준이와 귀엽고 속 깊은 후배 강원이에게도 고맙다는 말을 전합니다. 늘 내 곁에서 한결 같이 나를 믿어주고 힘이 되어준 소중한 내 친구들 지은이와 성린이, 본주, 그리고 보연이에게도 감사의 마음을 전합니다. 마지막으로 부족한 딸을 항상 믿어주시고 사랑해 주시는 아버지, 언제나 기도로 든든한 후원군이 되어 주신 어머니, 자상하고 든든한 우리 오빠, 6년 동안 한결 같은 사랑으로 내 곁을 지켜준 사랑하는 종훈 오빠와 아버님, 어머님……세상 누구보다 소중한 나의 가족들과 대학원 생활을 잘 마칠 수 있도록 지켜주신 하나님께 감사드리며 이 작은 결실을 드립니다.

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## ABSTRACT

# Genetic variants at the perilipin(PLIN) locus respond differently to mild weight loss in circulating free fatty acids levels and abdominal fat area

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**Objective** : The purpose of this study was to investigate the allelic associations for each of the SNPs of the PLIN gene with circulating FFA concentrations and abdominal fat distribution in response to weight loss with caloric restriction.

**Methods and Results** : 177 nondiabetic/overweight-obese Koreans participated in a clinical intervention study lasting 12 weeks involving an intake reduction of 300kcal/d. Of 7 PLIN SNPs (6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T and 14995A>T) the strongest positive linkage disequilibrium was found between the PLIN 10171A>T and the PLIN 10076C>G (D'=0.923, R2=0.839, P<0.001). The less strong positive linkage disequilibrium was found between the 11482G>A and the 14995A>T (D'=0.824, R2=0.578, P<0.001). The low calorie diet induced a mild weight loss of 4.6% with reduced abdominal fat. For PLIN 10076C>G, GG subjects showed higher reduction of FFA

concentrations than those with CC (C/C:-16±23, C/G:-71±33, G/G:-159±73 μEq/L, P=0.010). However, subjects with homozygosity for the rare allele of either 11482G>A (G/G:-97±33, G/A:-56±27, A/A:86±44 μEq/L, P=0.045) or 14995A>T (A/A:-103±34, A/T:-59±28, T/T:75±37 μEq/L, P=0.029)showed an increase in FFA levels. There were no significant allelic differences of SNPs 6209T>C, 10171A>T, 13042A>G and 13048C>T in changes in FFA levels. For 11482G>A/14995A>T haplotypes, compared to GA/GA subjects who were G/G at 11482 and A/A at 14995, those with nGA/nGA haplotype (non-GA haplotype carriers) showed greater reduction in waist circumference (GA/GA:-2.0±0.4, GA/nGA:-3.5±0.4, nGA/nGA:-4.0±1.0cm, P=0.042) and visceral fat area (GA/GA:-4.8±1.7, GA/nGA:-12±2, nGA/nGA:-17±3cm2, P=0.004) at L4 level than those with GA/GA. Subjects with GA/GA haplotype showed the highest concentration of urinary excretion of PGF2αand circulating oxidized LDL at baseline and had greater reduction after weight loss.

**Conclusions** : The results have shown that genetic variants at the PLIN locus respond differently to mild weight loss in changes in circulating FFA levels, abdominal fat areas and lipid peroxides.

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Key words : PLIN variant, weight loss, free fatty acid, visceral fat and oxidative stress

# 1.INTRODUCTION

Obesity is the clear extremes of dysfunctional lipid mobilization or storage that is characterized by the excessive storage of triacylglyceride (TAG) in adipose tissue. Increased circulating non-esterified fatty acid derived from adipocyte lipolysis is frequently observed in overweight individuals (1). If elevated serum free fatty acid (FFA) concentrations are simply the reflection of increased adipose tissue, the values should be similar in all overweight subjects. However,it is apparent that fasting and daylong FFA concentrations are not similar in all overweight individuals (2). Similarly, variations in a change in FFA concentrations are found after moderate weight loss (2). The mechanisms behind this phenomenon cannot be easily explained by alterations in the action of the major lipolysis-regulating hormones, which are insulin (2) and catecholamines (3,4).

Lipolysis occurs at the surface of the intracellular lipid droplet where the perilipins, a family of phospohoproteins, are specially located (5,6). Perilipin is required to protect cellular triglycerides against hydrolysis in the basal state and is also necessary for a functional lipolytic activation in the stimulated state such as fasting and exercise (7). Genetic variation at the perilipin gene (PLIN) has been associated with modulation of the perilipin content and lipolytic rate in humans (1). Consistent with those functional

observations, we have found significant associations between genetic variants at this locus, body-weight and obesity risk in several ethnic groups (8-10).

Different single-nucleotide polymophisms (SNPs) at the PLIN locus could affect splicing during transcription and the expression of different perilipin isoforms. Recent data show that perilipin isoforms may function with different efficiency in protecting the stored fat from the protein kinase A (PKA) mediated lipolysis (11). Therefore, different SNPs at the PLIN locus might modulate the variation in circulating free fatty acids and fat areas through different perilipin isoforms in the basal state and the stimulated state such as weight loss. In this study, we determined the allelic associations for each of the SNPs of the PLIN gene with circulating FFA concentrations and abdominal fat distribution in response to weight loss with caloric restriction. In addition, lipid peroxides, such as oxidized LDL and urinary excretion of $PGF_{2\alpha}$, were analyzed due to the close relationship between circulating FFA levels and oxidative stress (12).

# 2. BACKGROUND

## 2.1. The characteristics of perilipin

The Perilipins are a family of intracellular neutral lipid droplet storage proteinsthat are responsive to acute protein kinase A-mediated, hormonal stimulation. Perilipin was first identified as an ~60-kDa, lipid droplet associated phosphoprotien of rat primary (23). Perilipin expression appears to be limited to adipocytes and steroidoegnic cells, and its major demonstrated role is in the regulation of intracellular lipolysis in adipocytes (23).

The perilipins are encorded by a single-copy gene called PLIN. PLIN gives rise to mRNA by alternative and tissue-specific slicing (6,7); the murine perilipin gene generates a number of differently spliced mRNAs resulting in at least 4 isoforms. The A form is the most abundunt in fat cells, although small amounts of perilipin B are also expressed (6,7). The C and D isoforms are almost exclusivelyexpressed in steroidgenic cells (16). Peri A is the 57kDa and the major phosphorylation taget for 6 protein kinase A(PKA) sites only in adipocytes and steroidogenic cells (6).

## 2.2. Perilipin and lipolysis

The Perilipins are suggested to function as regulators of lipolysis. In pre-adipocytes or differentiating fibroblasts, lipid droplets are small (on the order of 1-5 microns) and are coated with ADRP (adipocyte differentiation related protein) (18). Once differentiated into mature adipocytes, these cells amass much larger fat droplets, that may comprise 95% of the volume of the primary adipocytes(18).While ADRP mRNA remains elevated during differentiation, perilipin gene expression ensues and perilipin protein, for reasons still unclear, displacesADRP from the droplet surface. Like ADRP, perilipin is found exclusively on the outer surface of lipid storage droplets and in no other subcellular compartment (24).

Activation of lipolysis is mediated by an increment of intracellular cAMP concentrations and activation of PKA. The two main targets for PKA-mediated phosphorylation in the adipocyte are HSL and the perilipins, and phosphorylation of these proteins dramatically increase lipolysis(1). A mechanism precluding HSL binding to the lipid droplets in nonstimulated intact cells seems to rely on the perilipins. Unphosphorulated perilipin may function as a barrier to lipolysis, preventing the interaction of HSL with TAG in the droplet (1). On stimulation of the adipocytes, perilipin phosphorylation would reduce the inhibition and allow phosphorylated HSL free access to the lipid droplet substrate, leading to initiation of TAG hydrolysis. Perilipin actually

inhibited lipolysis by blocking access of HSL to its lipid substrate, with phosphorylation of perilipin in response to lipolytic stimylation removing the perilipin 'barrier'(9). Thus, Perilipin functions to increase cellular TAG storage by decreasing the rate of TAG hydrolysis and serves an additional role in controlling the release of TAG at times of need. Furthermore, the regulation of this process may contribute to obesity and alterations in lipid metabolism.

## 2.3. The genetics of PLIN in obesity

Current evidence has supported the view that genetic factors play a critical role in the etiology of human obesity. However, less is known about the specific genes involved in human obesity and their specific contribution to the current epidemic of obesity. Several genes expressed in the adipocyte have been studied, and their variants have been associated with body fat and obesity risk, supporting the notion that these genes contribute to variability in measures of obesity in the general population.

Perilipins are a family of proteins that coat the intracellular lipid droplets. The expression of perilipin appears to be primarily in adipocytes and steroidogenic cells (9-12), and its major demonstrated role is in the regulation of intracellular lipolysis in adipocytes (13, 14). In experimental animal models, the absence of perilipin has resulted in lean phenotypes and has counterbalanced both genetic- and dietary- induced obesity in mice (15,

16). In contrast, elevated expression of perilipin has correlated with increased adiposity in human (17). Moreover, the perilipin 11482 G>A polymorphism in the human PLIN gene has been found to be associated with decreased perilipin content and increased lipolytic activity in women (18). The overall evidence has supported the notion that PLIN may be a candidate gene for human obesity.

Perilipin is encoded by a single-copy gene called PLIN. In humans, the PLIN has been assigned to chromosomal location 15q26.1 (23,25), in the neighborhood of previously reported susceptibility loci for obesity, diabetes and hypertriglyceridemia (23,25). PLIN gives rise to multiple mRNA by alternative and tissue-specific splicing, thus it can yield three protein isoforms . Perilipin A is the most abundant isoform expressed in adipocyte although small amount of perilipin B are also expressed. The C and D isoforms are almost exclusively expressed in steroidogenic cells (26). Perilipin A and B are identical through their amino terminal 406 amino acids, thereupon their sequences diverge.

Qi et al. (1, 9) found that common variance at the PLIN locus are associated with BMI and obesity risk in women from two different studies carried out in Spain and the United States, suggesting that PLIN variation may play a role in human obesity. In a Mediterranean Spanish population, the PLIN 1 and the PLIN 4 polymorphisms are significantly associated with the obesity risk in women but not in men.(1,9). The same gender specific

effect was noted in the study of population in the United States (9). In the present study, although the interaction term for heterogeneity of the PLIN effects by gender was not statistically significant, the effects were observed mainly in women because the prevalence of obesity in Malays and Indians was three times higher in women than men.An apparent paradox is the fact that in white women the PLIN 4 was found protective against obesity; however, it should be noted that whereas this polymorphism is in positive linkage disequilibrium (LD) with the PLIN 1 in whites (1,9), it is found in negative LD in Asians, and therefore the opposite association should be expected.

Mottagui Tabar et al. recently reported that the A allele at the PLIN 11482G>A SNP is associated with enhanced basal and noradrenaline induced lipolysis. Moreover, the same allele was associated with lower perilipin content in obese women. According to this finding , a negative association would be expected between PLIN 11482AA genotype and body fat.(1)

Jose M. Ordovas (8) demonstrated that ethnic differences in the association between obesity and polymorphism in the perilipin gene. Among 5 common single nucleotide polymorphisms (SNPs) at the PLIN locus PLIN 6209T>C, 10171A>T, 11482G>A, 13041A>G, and 14995A>T), the PLIN 14995A>T SNP is the most informative single genetic marker for the observed haplotype association, being significantly associated with increased obesity risk in both Malays OR2.28 (95%CI 1.45-3.57) and Indians OR 2.04 (95%CI

1.08-3.64) (8).Each individual SNP for its association with the risk of obesity in Asians: No significant association was found with PLIN 6209T>C or 11482G>A. Homozygosity for the T allele at 14995A>T was significantly associated with higher risk of obesity than other genotypes in both Malays and Indians (multivariate OR=2.28, 95%CI 1.45-3.47) in Malays; multivariate OR=2.04, 95% CI 1.08-3.48 in Indians) (8). Homozygosity for the rare allele of either the 11482G>A or 13041A>G was also found associated with increased risk of obesity in Indians and Malays, but only in the later group did it reach statistical significance (8). No significant association was found between any of the individuals SNPs analyzed and the risk of obesity in Chinese (8). Some researchers have proposed that a lower cutoff should be applied to define obesity in Asians (8).

## 2.4. The effect of perilipin in animal study

The regulation of basal lipolysis is not known but the adipocyte specific protein perilipin is an attractive candidate according to several animal studies. An animal study has shown that targeted disruption of the perilipin gene results in mice that are leaner than controls and are resistant to diet-induced obesity (6). Martinez-Botas et al. generated Plin-null mice by targeted disruption of Plin (27). White adipocytes from $Plin^{-/-}$ mice were 62% smaller than those of controls. Magnetic resonance imaging (MRI) showed

that the fat imaging represented approximately 7.6% of the total cross-sectional area in a slice through the heart and the liver region of $Plin^{/-}$, compared with approximately 15% in wild type(27). Thus the fat area reduction in individual sections was 50-65%. Similar analysis of $Lepr^{db/db}$ (genetically predisposed obesity)/$Plin^{+/+}$ and Lepr $^{db/db}$/$Plin^{-/-}$ mice, revealed that the subcutaneous and visceral fat in Lepr $^{db/db}$ was diminished; the total lipid signal fell from approximately 63% in $Lepr^{db/db}$/$Plin^{+/+}$ mice to approximately 27% in $Lepr^{db/db}$/$Plin^{-/-}$ mice, a value approximating that in wild type (~24%). These MRI analysis confirm that inactive of PLIN in mice reverses the obesity phenotype of $Lepr^{db/db}$ mice (27).Isolated adipocytes of perilipin null mice exhibit elevated basal lipolysis because of the loss of the protective function of perilipin. These have greater lean body mass, increase metabolic rate and increase tendency to develop glucose intolerance and peripheral insulin resistance more readily, probably due to the elevated levels of NEFA. When fed a high-fat diet, the perilipin null mice are resistant to diet-induced obesity. (5,27).

## 2.5. The important role of perilipin in the regulation of human adipocyte lipolysis

Perilipin seems important for the regulation of lipolysis in human fat cells. Obesity and a polymorphism in the perilipin gene associate with low adipocyte perilipin content. Low perilipin cotent also associate with high basal and catcholatmin-induced rate of lipolysis in the fat cells and also with a high in vivo lipolytic activity in Swedish obese women (28). Furthermore, obese women with low perilipin adipocyte content had higher in vivo lipolytic activity and higher adipocyte lipolytic activity in comparison with obese women with high perilipin content. So that results demonstrated the PLIN gene may be involed in human lipid metabolism (26). And other study, common variants at the PLIN locus are associated with body mass index (BMI) and obesity risk in white women from two different studies carried out in Spain and the United States (6,23), suggesting that PLIN variation may play a role in human obesity.

## 2.6. Free fatty acid metabolism in obesity

Plasma free fatty acids (FFA), originating from the release of adipose tissue triglyceride fatty acid, represent virtually the only route by which these fat stores can be transferred to non fat tissue for net loss via oxidation. Free

fatty acid metabolism in obesity has, therefore, been the subject of many studies over the last years. Unfortunately, conflicting conclusions regarding several aspects of FFA metabolism have appeared, including the antilipolytic effectiveness of insulin in obesity(29,30), the relationship of FFA release to the amount of body fat, and the lipolytic responsiveness of obese individuals to catecholamines. These potential differences in FFA metabolism between lean and obese humans may be of considerable importance in that they reflect either abnormalities of adipose tissue lipolysis. Plasma FFA concentrations may not accurately reflect FFA flux. Increased FFA flux is associated with hypertriglyceridemia and failure to suppress lipolysis could result in glucose intolerance via the glucose-fatty acid cycle.

## 2.7.Medical Consequences of Obesity

### 2.7.1. Type 2 Diabetes Mellitus

Obesity , particularly when centrally distributed, predisposes to diabetes via increased portal delivery of fattyacids to the liver from lipolytically active visceral adiposetissue. This induces both hepatic insulin resistance and reduced hepatic insulin clearance.(36) This, supplemented by increased pancreatic insulin secretion, maintains normal blood glucose concentrations until, with islet cell exhaustion, hyperglycemia and eventually type 2 diabetes mellitus supervene in genetically susceptible individuals.(36)

Obese women (BMI _30) have a 28-fold greater relative risk of diabetes; this increases to 93-fold in the morbidly obese (BMI _35)(37).Furthermore, a waist circumference of _40 inches (102 cm) increases the risk of diabetes 3.5-fold even after controlling for BMI. Fortunately, diabetes may be prevented or at least delayed by relatively modest degrees of weight loss. In two recent intervention studies, lifestyle interventions resulting in average weight loss of _7 kg over 6 months with some later regain resulted in a 58% reduction of cumulative diabetes incidence in the intervention groups. (38)

### 2.7.2. Hypertention

Although the precise mechanisms involved in the etiology of obesity-related hypertension are not fully understood, they are thought to involve activation of the sympathetic nervous system, physical pressure on the kidney, cytokine effects, and excessive sodium reabsorption.(39)

Although weight loss undoubtedly produces modest beneficial effects on blood pressure, these are rarely sufficient to obviate the need for adjuvant pharmacologic therapy. A recent systematic review by the Cochrane Collaboration(40) suggests that a 4-8% weight loss may be expected to reduce systolic/diastolic blood pressures by approximately 3/3 mm Hg. Although this is clearly a useful reduction at a population level, it may not be sufficient for the individual hypertensive patient even if achieved and maintained.

### 2.7.3. Dyslipidemia

The mechanism underlying the characteristic dyslipidemia of obesity is not fully understood but involves the combination of insulin resistance and hyperinsulinemia stimulating hepatic triglyceride synthesis from an increased free fatty acid pool. This in turn is thought to lead to enhanced postprandial lipidemia, smaller and denser low-density lipoprotein (LDL) particles, and reduced high-density lipoprotein (HDL) cholesterol concentration. (41)

The characteristic dyslipidemia of obesity includes elevated plasma concentrations of triglyceride, LDLcholesterol (particularly of highly atherogenic small, dense low-density lipoprotein [LDLIII] particles) and apolipoprotein B together with low circulating plasma concentrations of antiatherogenic HDL.(42)

Although weight loss, increased physical activity, and dietary change may all confer independent benefits on dyslipidemia, it isnot yet clear which exerts the greatest effect.(41,42)

# 3. SUBJECTS AND METHODS

## 3.1. SUBJECTS

One hundred and seventy seven overweight-obese subjects (BMI>25kg/$m^2$) were recruited concomitantly from participants in a prospective human genetic study, supported by a Genome Research Development Project on Health and Medicine (project #: HMG-00-GN-01-0001), Ministry of Health & Welfaren and participated in 12 week weight loss program conducted by the Clinical Nutrition Research Team at Yonsei University. Patients with orthopedic limitations, weight loss/gain over the previous 6 months or any diagnosis of diabetes mellitus, liver disease, renal disease, thyroid or pituitary disease were excluded. None of them were taking any medication or had diagnosis of coronary vascular disease and any disease mentioned above. Written informed consent was obtained from all subjects and the protocol was approved by the Institute of Review Board of Yonsei University.

## 3.2. GENETIC ANALYSIS

Genomic DNA was isolated from white blood cells. Polymorphisms were named according to the most recent recommendations. The reference sequence was GI21431190 (Genbank). Genotyping was carried out using Single Nucleotied Extension. First, the DNA fragments were amplified by multiplex polymerase chain reaction (PCR). The PCR products were 422bp, 350bp, 190bp, 469bp, 111bp and 96bp for PLIN1, PLIN4, PLIN5, PLIN6, A194P and V373V respectively. PCR amplification was carried out in a 10μl reaction volume containg 0.2mmol/l of each dNTP, 0.2μmol/l of each primer, 3.0mmol/l magnesium chloride, and 0.8U of Qiagen Hotstar Taq polymerase. PCR cycling conditions were 95℃ for 10min followed by seven cycles of 95℃ 30s, 70℃ for 30s, and 72℃for 1min, then followed by 41 cycles of 95℃ for 30s, 65℃ for 30s, and 72℃for 1min. A final extension phase of 2min at 72℃was included at the end of the protocol. The PCR products were incubated for 60min at 37℃ with 2.5U each of Exonuclease I (Nex England Biolabs, Inc. Beverly, MA, USA) and Calf Intestinal Phosphatase (New England Biolabs) to remove un-incorporated dNTPs and primers. This was followed by incubation for 15min at 75℃ to inactivate the enzymes. Subsequently, Single Nucreltide Extension was carried out using the ABI Prism SnaPshot multiplex system (Applied Biosystems, Foster City, CA, USA). The extension reaction was carried out using PCR thermocycler in a 5μl

reaction mixture containing 1.5μl of the Snapshot Ready Reaction Mastermix (Applied Biosystems), 1.0μl of water, 1.5μl of multiplex PCR products, and 1.0μl of the probe mixture (1.5μmol/l for PLIN1, PLIN4, AND 2.0μmol/l for PLIN5 and PLIN6). The reaction conditions were 35 cycles of 96℃ for 30s, 50℃ for 30s, and 60℃ for 30s. The reaction products were incubated for 60min at 37℃ with 3U Calf Intestinal Phosphatase to remove un-incorporated dNTPs, followed by incubation for 15min at 75℃ to inactivate the enzyme. Genotyping was carried with the final products on an ABI Prism 3100 genetic analyzer (Applied Biosystems) using Genotyper version 3.7 (Applied Biosystems). Standard good laboratory practices were undertaken to assure the accuracy of genotype data. Internal controls and repetitive experiments were used. Any sample that yielded a weak signal was repeated. In addition, 20% of samples were repeated at random in order to verify the reproducibility. Genomic DNA was extracted from 5ml of whole blood using a commercially available DNA isolation kit (WIZARD$^{R}$Genomic DNA purification kit, Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. We genotyped 7 site of PLIN SNPs already reported (6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T and 14995A>T) both in 438 CAD patients and 659 healthy subjects. Each genotyping was performed by SNP-IT$^{TM}$ assays using single primer extension technology (SNPstream 25K$^{TM}$ System, Orchid Biosystems, NJ, USA). The DNA fragments were visualized by UV illumination using Image Analyzer (AlphaImager$^{TM}$ 1220,

Alpha Innotech Corp., California, USA) respectively.

## 3.3. MATERIALS AND METHODS

For laboratory assay, all measurements were done in a single batch at the end of the study, and the laboratory staff was blinded to the clinical data.

### 3.3.1. General characteristics

At the time of subject enrollment, the baseline characteristics were reported in all study subjects; these included age, smoking status (non- or ex-smoker and current smoker), history of disease, and frequency of alcohol intake. Alcohol consumption was calculated as the grams of ethanol ingested per day and cigarettes smoking data were reported as the number of cigarettes smoked per day.

### 3.3.2. Anthropometric parameters and blood pressure

Body weight and height were measured in the morning, light clothed without shoes. The body mass index (BMI) was calculatedas body weight in kilograms divided by height in meters squared. Waist and hip circumference were measured and waist to hip ratio (WHR) was computed as an indication

of the index of body fat distribution. Waist circumference was measured with a flexible tape midway between the lower rib margin and the iliac crest, and the hip girth was measured at the widest part of the hip. Both circumferences were measured in the standing position after normal expiration.

Blood pressure was measured from the left arm while the subjects remained seated. An average of three measurements was recorded for each subject. When the systolic blood pressure was greater than or equal to 140mmHg, or the systolic blood pressure was greater than or equal to 90mmHg, they were classified as having hypertension according to the $6^{th}$report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure.

### 3.3.3. Abdominal fat distribution at levels of L1 and L4 using CT scanner

Abdominal fat areas were measured by computerized tomography (CT) scanning using a General Electric (GE) High Speed Advantage 9800 scanner (Milwaukee, WI). Two cross-sectional images were made for each subjects; abdomen at the levels of 1st lumbar (L1) vertebra and 4th lumbar (L4) vertebra. Each CT slice was analyzed for the cross-sectional area of fat using a density control program available in the standard GE computer software. Parameters for total abdominal fat density at the levels of L1 and

L4 were selected between the range of -150 and -50 Hounsfield Units (HU). Total abdominal fat area was divided into visceral and subcutaneous fat areas to calculate specific fat areas.

### 3.3.4. Fasting blood collection

To reduce the influence of circadian variation, all blood specimens were collected between 08:00 and 10:00 after the subjects had fasted overnight. Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-hour fast. The tubes for antioxidant analysis were immediately covered with aluminum foil and placed on ice in the dark until they arrived at the laboratory room (within 1-3 hours) and stored at -70℃ until analysis after plasma and serum were separated.

### 3.3.5. Serum lipid profile

Fasting serum concentrations of total cholesterol and TAG were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd. Tokyo, Japan). After precipitation of serum chylomicron, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) with dextran sulfate-magnesium, high density lipoprotein (HDL) cholesterol left in the supernatant was measured by an enzymatic method. LDLcholesterol was estimated indirectly using the Friedewald formula for subjects with serum TAG

concentrations <4.52 mol/l (400mg/ml) and directly measured for subjects with serum TAG concentration 4.52 mol/l. Serum apolipoprotein A-I and B were determinedby turbidometry at 340nm using a specific anti serum (Roche, Switzerland).

### 3.3.6. Serum glucose, insulin and free fatty acid

Glucose was measured by a glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Insulin was measured by radioimmunoassays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA). Free fatty acid (FFA) was analyzed with a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). IR was calculated with the homeostasis model assessment (HOMA) using the following equation: IR = {fasting insulin (IU/ml) fasting glucose (mmol/l)}/ 22.5. The glucose criteria, newly developed and modified by the National Diabetes Data Group (NDDG) and the World Health Organization (WHO) Expert Committee on Diabetes Mellitus, were used to categorize subjects as diabetic : fasting glucose ≥7.0mmol/L (126mg/dL)

### 3.3.7. Urinary excretion of prostaglandin F2

Urine was collected after a 12-h fast in polyethylene bottles containing 1% butylated hydroxytoluene before blood collection. The tubes were immediately covered with aluminum foil and stored at -70℃ until analysis. 8-epi-prostaglandin F2 (8-epi-PGF2) was measured using an enzyme immunoassay (BIOXYTECH urinary 8-epi-PGF2TM Assay kit, OXIS International Inc., OR, U.S.A.). The resultant color reaction was read using a Victor2 (Perkin Elmer life sciences, Turka, Finland) at 650nm. Urinary creatinine was determined by the alkaline picrated (Jeffe) reaction and urinary 8-epi-PGF2 concentrations were expressed as picogram per milligram (pg/mg) of creatinine.

## 3.4. Statistical analysis

We used SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, USA) for all our statistical analyses. Linkage disequilibrium (LD), was examined using Executive SNP Analyzer Executive SNPAnalyzer1.0(http://www.istech.info/SilicoSNP/index.html). We selected SNPs showing relatively strongly positive LD from LD test and made haplotype. The associations between haplotype and continuous variables before and after 12 weeks were examined using paired t-test and Pearson correlation coefficient. Each variable was examined for normal distribution and significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented on untransformed and unadjusted variables. Results are expressed as meanSE. A two tailed value of $P<0.05$ was considered statistically significant.

# 4. RESULTS

## 4.1. Clinical characteristics of study subjects

### 4.1.1. General characteristics

Table1 shows anthropometries and clinical parameters for the overweight-obese participants (n=177) before and at the end of a 12-wk low calorie diet. The low calorie diet induced a mild mean weight loss of 4.6% and significant reductions in abdominal fat areas and blood pressure. Cardiovascular risk factors such as serum lipids and HOMA-IR showed a significant change toward clinical improvement at the end of the diet (Table 1). No significant differences were, however, found in serum insulin levels when comparing values before and after weight loss (10.6±0.4 vs 10.3±0.9μIU/ml).

| | before | | | After | | | % change |
|---|---|---|---|---|---|---|---|
| Age (years) | | 40.0 ± 1.04 | | | | | |
| Weight (kg) | 71.1 | ± | 0.69 | 67.8 | ± | 0.68*** | -4.6 |
| Body mass index ($kg/m^2$) | 27.1 | ± | 0.22 | 25.9 | ± | 0.21*** | -4.5 |
| Waist (cm) | 90.3 | ± | 0.54 | 87.2 | ± | 0.53*** | -3.4 |
| 1st lumbar vertebra | | | | | | | |
| Total fat ($cm^2$) | 255.9 | ± | 5.69 | 235.4 | ± | 6.22*** | -8.0 |
| Visceral fat ($cm^2$) | 104.5 | ± | 3.28 | 96.2 | ± | 3.51*** | -7.9 |
| 4th lumbar vertebra | | | | | | | |
| Total fat ($cm^2$) | 299.6 | ± | 5.25 | 278.8 | ± | 5.92*** | -6.9 |
| Visceral fat ($cm^2$) | 88.3 | ± | 2.81 | 77.8 | ± | 2.58*** | -11.8 |
| Blood pressure | | | | | | | |
| Systolic (mmHg) | 123.3 | ± | 1.37 | 120.0 | ± | 1.19*** | -2.6 |
| Diastolic (mmHg) | 77.1 | ± | 0.85 | 75.2 | ± | 0.81* | -2.4 |
| Triglyceride (mg/dl) | 143.3 | ± | 5.65 | 126.1 | ± | 4.99*** | -12.0 |
| Total cholesterol (mg/dl) | 194.9 | ± | 2.44 | 188.4 | ± | 2.76** | -3.3 |
| LDL cholesterol (mg/dl) | 122.4 | ± | 2.23 | 116.1 | ± | 2.40** | -5.1 |
| HDL cholesterol (mg/dl) | 43.9 | ± | 0.77 | 47.1 | ± | 0.75*** | 7.2 |
| Glucose (mg/dl) | 86.2 | ± | 0.93 | 84.0 | ± | 1.03** | -2.5 |
| HOMA-IR[1] | 2.25 | ± | 0.09 | 2.15 | ± | 0.19*** | -4.4 |
| Free fatty acid (μEq/l) | 596.0 | ± | 21.1 | 532.0 | ± | 21.2** | -10.7 |

### 4.1.2. Genotype distribution at the PLIN locus in subjects

We genotyped seven (6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T and 14995A>T) at the PLIN locus in 177 non-diabetic and overweight-obese subjects. PLIN genotypes, allele frequencies (1 for the common variant and 2 for the rare allele), linkage disequilibrium coefficients and correlation coefficients for the 7 SNPs are given in Table 2. Genotype distributions did not deviate from Hardy-Weinberg expectations. Allele 2 (T) at the PLIN 14995A>T was the most prevalent gene variant (0.364); whereas allele 2 (T) at the PLIN 13048C>T was the least prevalent (0.280). The strongest positive linkage disequilibrium was found between the PLIN 10076C>G and the PLIN 10171A>T (D'=0.923, $R^2$=0.839, P<0.001). The less strong positive linkage disequilibrium was observed between the PLIN 11482G>A and the PLIN 14995A>T (D'=0.824, $R^2$=0.578, P<0.001). Therefore, we analyzed the haplotypic association based on either 10076C>G/10171A>T or 11482G>A/14995A>T haplotypes. For subsequent statistical analyses with 10076C>G/10171A>T haplotype, subjects were subdivided into 3 haplotype groups; homozygous carriers of the CA haplotype (CA/CA, i.e., individuals who were C/C at 10076 and A/A at 10171), heterozygous carriers of the CA haplotype (CA/nCA, i.e., individuals with CA/GA, CA/CT and CA/GT) and non-CA haplotype carriers (nCA/nCA, i.e., subjects with all haplotypes other than CA/CA and CA/nCA). Similarly, for statistical analyses with

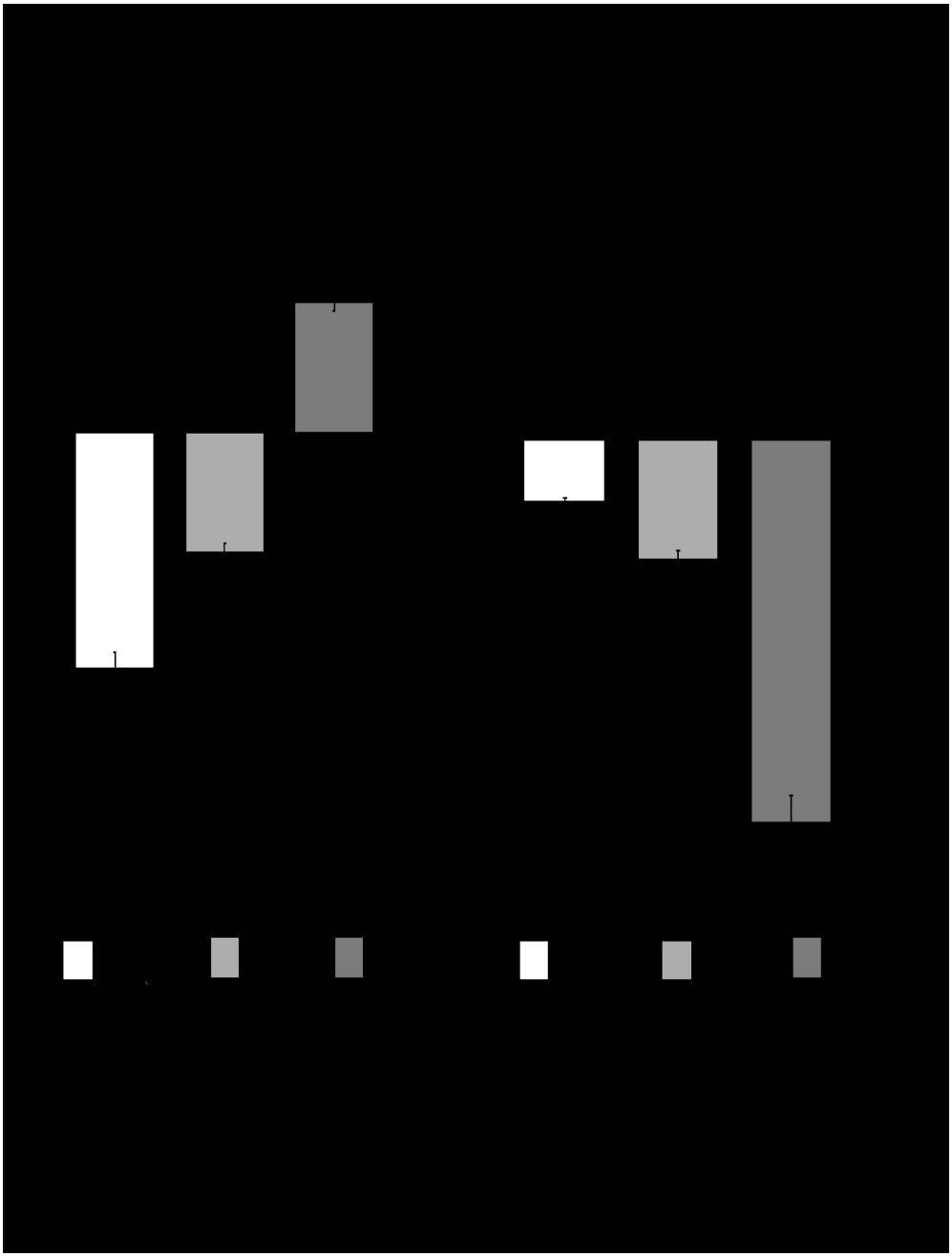
11482G>A/14995A>T haplotype, subjects were subdivided into 3 haplotype groups; homozygous carriers of the GA haplotype (GA/GA, i.e., individuals who were G/G at 11482 and A/A at 14995), heterozygous carriers of the GA haplotype (GA/nGA, i.e., individuals with GA/AA, GA/GT and GA/AT) and non-GA haplotype carriers (nGA/nGA, i.e., subjects with all haplotypes other than GA/GA and GA/nGA).

| | PLIN 6209T>C | | 10076C>G | | 10171A>T | | 11482 G>A | | 13042 A>G | | 13048 C>T | | 14995 A>T | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | men (%) | women(%) | men (%) | women(%) | men (%) | women(%) | men (%) | women(%) | men (%) | women(%) | men (%) | women(%) | men (%) | women(%) |
| 11 | 16(40) | 56(40.9) | 22(55) | 64(48.7) | 20(50) | 65(47.4) | 18(45) | 58(42.3) | 18(40) | 71(51.8) | 17(42.5) | 79(57.8) | 13(32.5) | 54(39.4) |
| 12 | 19(47.5) | 64(46.7) | 13(32.5) | 57(41.6) | 15(37.5) | 59(43.1) | 18(45) | 68(49.6) | 19(47.5) | 51(37.2) | 16(40) | 47(34.3) | 22(55) | 69(50.4) |
| 22 | 5(12.5) | 17(12.4) | 5(12.5) | 16(11.7) | 5(12.5) | 13(9.5) | 4(10) | 11(8.1) | 5(12.5) | 15(10.9) | 7(17.5) | 11(8.1) | 5(12.5) | 14(10.2) |
| Rare allele frequency and 95% CI | | | | | | | | | | | | | | |
| Allele 2 | 0.359 (0.342–0.376) | | 0.316 (0.300–0.332) | | 0.311 (0.296–0.327) | | 0.328 (0.309–0.348) | | 0.311 (0.297–0.325) | | 0.280 (0.266–0.294) | | 0.364 (0.346–0.381) | |
| Linkage disequilibrium between variants: D' and $R^2$(p-value) | | | | | | | | | | | | | | |
| 6209T>C | - | | 0.917, 0.202 (<0.001) | | -1.000, 0.237 (<0.001) | | -1.000, 0.302 (<0.001) | | -0.139, 0.005 (0.328) | | -0.302, 0.021 (0.060) | | -0.693, 0.170 (<0.001) | |
| 10076C>G | | | - | | 0.923, 0.839 (<0.001) | | -0.777, 0.127 (<0.001) | | -0.381, 0.023 (0.016) | | -0.497, 0.039 (<0.001) | | -0.777, 0.149 (<0.001) | |
| 10171A>T | | | | | - | | -1.000, 0.207 (<0.001) | | -0.334, 0.018 (0.023) | | -0.399, 0.025 (0.020) | | -0.655, 0.104 (<0.001) | |
| 11482G>A | | | | | | | - | | 0.264, 0.067 (<0.001) | | 0.335, 0.084 (<0.001) | | 0.824, 0.578 (<0.001) | |
| 13042A>G | | | | | | | | | - | | 0.694, 0.494 (<0.001) | | 0.231, 0.038 (0.001) | |
| 13048C>T | | | | | | | | | | | - | | 0.388, 0.057 (<0.001) | |
| 14995 A>T | | | | | | | | | | | | | - | |

## 4.2.Association between the PLIN polymorphisms and change in FFA levels

The subjects who completed the 12-wk trial (n=177) had a mean FFA reduction of 64.1± 19.9 μEq/L. Table 3 showed the allelic associations for each of the SNPs with change in FFA levels. Genotype distributions at 10076C>G (P=0.010), 11482G>A (P=0.045) and 14995A>T (P=0.029) affected the change in fasting FFA levels. For PLIN 10076C>G, GG subjects showed significantly higher reduction of FFA concentrations than those with CC (Table 3). After mild weight loss, G carriers at the 11482G>A and A carriers at the 14995A>T showed a reduction of FFA concentration. However, subjects with homozygosity for therare allele of either 11482G>A or 14995A>T showed an increase in FFA levels. We also found haplotypic differences of 10076C>G/10171A>T (P=0.044) or 11482G>A/14995A>T (P=0.018) haplotypes in change in circulating FFA levels (Fig. 1). There were no significant allelic differences of SNPs 6209T>C, 10171A>T, 13042A>G and 13048C>T in changes in FFA levels (Table 3).

| | Mean changes in FFA (μEq/L) | | | | | |
|---|---|---|---|---|---|---|
| | 11 | | 12 | | 22 | |
| PLIN SNPs | Baseline | Change | Baseline | Change | Baseline | Change |
| 6209T>C | 604.1 ± 34.7 | -51.7 ± 32.8 | 568.7 ± 30.3 | -59.6 ± 29.0 | 678.0 ± 50.3 | -102.4 ± 48.4 |
| 10076C>G | 572.8 ± 29.1 | -15.6 ± 23.6[a] | 595.8 ± 33.4 | -71.2 ± 33.4[ab] | 688.6 ± 68.9 | -205.0 ± 68.7[b] |
| 10171A>T | 578.7 ± 29.4 | -40.8 ± 24.5 | 588.7 ± 32.5 | -60.8 ± 33.5 | 708.7 ± 74.1 | -158.5 ± 73.4 |
| 11482G>A | 634.4 ± 32.0 | -96.6 ± 32.5[b] | 572.1 ± 30.8 | -55.7 ± 27.1[ab] | 534.1 ± 65.3 | 86.0 ± 43.9[a] |
| 13042A>G | 600.5 ± 32.8 | -73.1 ± 29.9 | 613.5 ± 29.1 | -67.1 ± 31.1 | 521.8 ± 60.0 | 5.21 ± 48.8 |
| 13048C>T | 612.3 ± 31.0 | -87.3 ± 28.5 | 592.8 ± 31.3 | -50.9 ± 32.0 | 524.3 ± 62.5 | 30.7 ± 49.9 |
| 14995 A>T | 614.5 ± 32.3 | -103.2 ± 33.6[b] | 597.5 ± 31.6 | -58.6 ± 27.8[ab] | 525.2 ± 56.5 | 74.9 ± 37.4[a] |

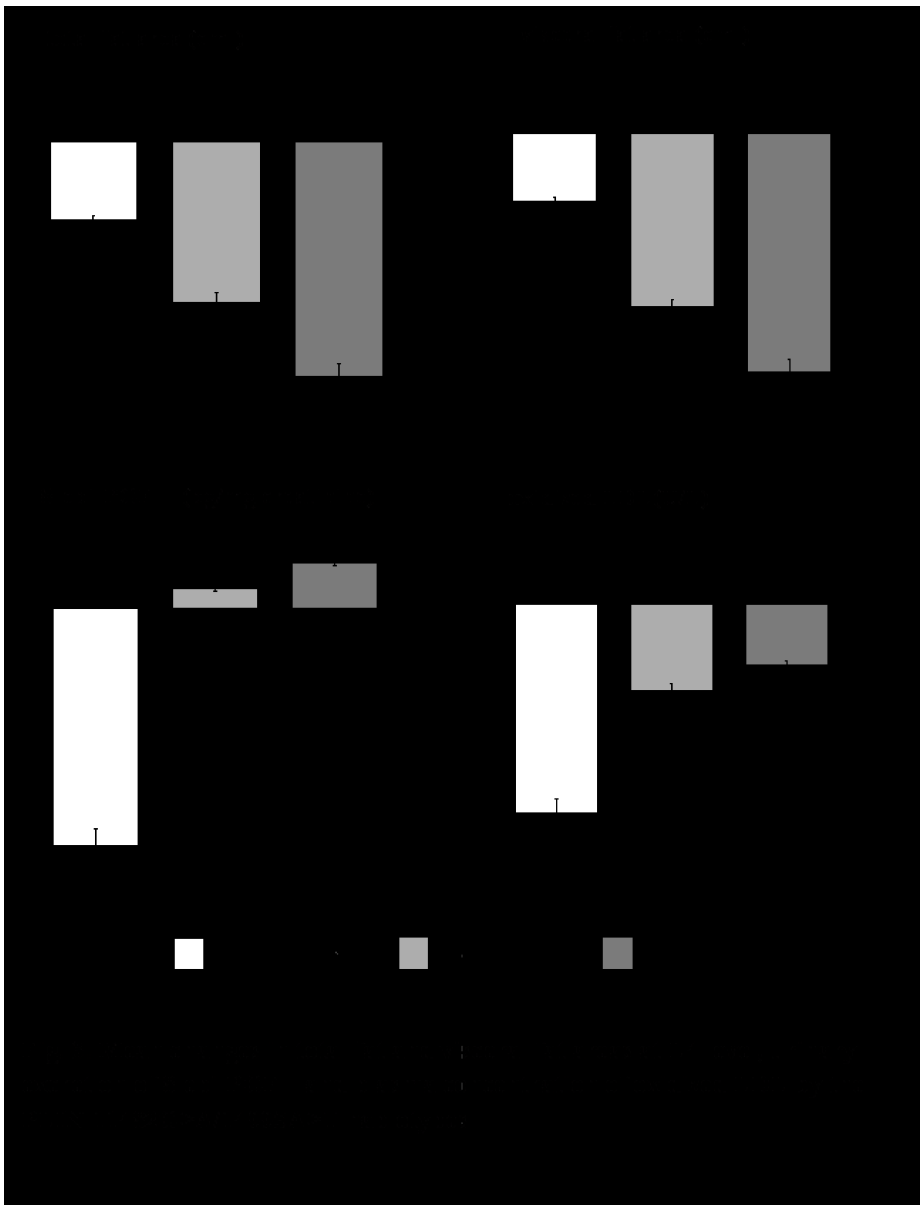


## 4.3. Relationship between change in FFA levels and baseline FFA concentrations according to 11482G>A /14995A>T or 10076C>G/10171A>T haplotypes in PLIN gene

There was a negative correlation between the change in serum FFA levels and baseline FFA concentration ($r=-0.531$, $P<0.001$) in GA carriers of 11482G>A/14995A>T haplotypes; the higher baseline FFA levels, the greater reduction in FFA levels after weight loss (Fig. 2). However, subjects with nGA/nGA haplotype at 11482-14995 SNPs showed a positive correlation ($r=0.444$, $P<0.05$); the higher baseline FFA levels, the greater increase in FFA levels after weight loss. For 10076C>G/10171A>T haplotypes, change in fasting FFA in CA carriers were negatively proportional to baseline FFA levels ($r=-0.423$, $P<0.001$). Similarly, a correlation between change in FFA and baseline FFA was observed in the subjects with nCA/nCA haplotype in PLIN 10076C>G/10171A>T haplotypes, but with 1.25-fold change in slope (Fig.2).

## 4.4. Effects of 12-wk LCD on abdominal fat distribution and lipid peroxides according to 11482G>A/14995A>Tor10076C>G/10171A>T haplotypes in PLIN gene

There were no significant haplotypic differences in age, sex distribution and reduced weight according to 11482G>A/14995A>T or 10076C>G /10171A>T haplotypes (Table4, Table5). For 11482G>A/14995A>T haplotypes, subjects with nGA/nGA showed greater reduction in waist circumference and total fat area at L4 level than those with GA/GA (Table 4). Reduction in visceral fat area at L4 was greater in nGA carriers than GA/GA subjects (Fig. 3). Urinary excretion of $PGF_{2\alpha}$ at baseline and change after weight loss was higher in GA/GA subjects than nGA carriers. GA/GA subjects showed the highest concentration of oxidized LDL at baseline and had greater reduction than nGA carriers. For 10076C>G/10171A>T haplotypes, there were no significant haplotypic differences in changes in abdominal fat distribution and lipid peroxides (Table 5).



| | GA/GA (n=60) | | GA/X (n=93) | | X/X (n=24) | |
|---|---|---|---|---|---|---|
| | Baseline | Change | Baseline | Change | Baseline | Change |
| Age (years) | 41.2 ± 1.87 | | 40.2 ± 1.42 | | 36.5 ± 2.55 | |
| Body mass index (kg/m²) | 26.9 ± 0.37 | −1.01 ± 0.11 | 27.3 ± 0.32 | −1.33 ± 0.10 | 26.4 ± 0.52 | −1.23 ± 0.23 |
| Male/Female | 13(22%) / 47(78%) | | 21(23%) / 72(77%) | | 6(25%) / 18(75%) | |
| Weight (kg) | 70.7 ± 1.1 | −2.6 ± 0.3 | 71.6 ± 1.0 | −3.5 ± 0.3 | 70.5 ± 2.0 | −3.3 ± 0.6 |
| Waist (cm) | 90.3 ± 0.8 | −2.0 ± 0.4[a] | 90.9 ± 0.8 | −3.5 ± 0.4[a] | 88.1 ± 1.6 | −4.0 ± 1.0[a] |
| 1[st] lumber vertebra | | | | | | |
| Total fat (cm²) | 255.6 ± 9.02 | −15.3 ± 7.55 | 259.5 ± 8.43 | −22.3 ± 3.31 | 238.9 ± 12.5 | −25.8 ± 6.64 |
| Visceral fat (cm²) | 103.3 ± 5.02 | −5.18 ± 4.94 | 107.3 ± 4.88 | −9.61 ± 2.43 | 97.8 ± 9.15 | −10.5 ± 3.87 |
| 4[th] lumber vertebra | | | | | | |
| Total fat (cm²) | 294.1 ± 8.54 | −11.2 ± 5.66[a] | 305.9 ± 7.65 | −23.2 ± 5.06[a,b] | 290.3 ± 13.3 | −34.0 ± 5.76[a] |
| Visceral fat (cm²) | 86.6 ± 4.67 | −4.79 ± 1.68[a] | 93.0 ± 4.11 | −12.3 ± 1.93[a] | 76.3 ± 6.10 | −16.8 ± 3.19[a] |
| Free fatty acid (μEq/l) | 637.9 ± 34.3 | −114.5 ± 37.2[a] | 582.0 ± 30.1 | −57.9 ± 26.9[a,b] | 542.3 ± 44.5 | −63.7 ± 31.3[a] |
| $PGF_{1\alpha}$ (pg/mg creatinine) | 1340.1 ± 197.0[a] | −358.3 ± 169.4[a] | 862.5 ± 87.4[a] | −28.9 ± 66.1[a] | 815.9 ± 199.0[a] | −69.0 ± 132.7[a] |
| Oxidized LDL (U/l) | 44.0 ± 2.74[a] | −6.32 ± 1.55[a] | 38.5 ± 2.00[a,b] | −2.59 ± 1.06[a] | 34.9 ± 2.84[a] | −1.84 ± 1.51[a] |

| | CA/CA (n=82) | | CA/X (n=73) | | X/X (n=22) | |
|---|---|---|---|---|---|---|
| | Baseline | Change | Baseline | Change | Baseline | Change |
| Age (years) | 39,5 ± 1,52 | | 41,0 ± 1,63 | | 38,6 ± 2,98 | |
| Body mass index (kg/m²) | 27,1 ± 0,33 | −1,20 ± 0,09 | 27,2 ± 0,35 | −1,32 ± 0,14 | 26,3 ± 0,54 | −0,88 ± 0,12 |
| Male/Female | 20(24%) / 62(76%) | | 14(19%) / 59(81%) | | 6(27%) / 16(73%) | |
| Weight (kg) | 72,1 ± 1,1 | −3,2 ± 0,2 | 70,6 ± 1,1 | −3,4 ± 0,3 | 69,6 ± 1,7 | −2,3 ± 0,3 |
| Waist (cm) | 90,6 ± 0,8 | −2,8 ± 0,4 | 90,5 ± 0,9 | −3,5 ± 0,6 | 88,5 ± 1,2 | −3,1 ± 0,3 |
| 1st lumbar vertebra | | | | | | |
| Total fat (cm²) | 249,4 ± 8,54 | −23,1 ± 2,94 | 260,5 ± 8,95 | −18,0 ± 6,98 | 258,5 ± 13,0 | −18,5 ± 3,19 |
| Visceral fat (cm²) | 104,1 ± 4,93 | −10,6 ± 1,94 | 107,0 ± 5,29 | −6,42 ± 4,67 | 98,7 ± 7,20 | −5,69 ± 3,42 |
| 4th lumbar vertebra | | | | | | |
| Total fat (cm²) | 290,8 ± 7,56 | −17,0 ± 4,35 | 312,3 ± 8,03 | −25,4 ± 6,37 | 290,5 ± 16,1 | −19,4 ± 5,65 |
| Visceral fat (cm²) | 86,5 ± 4,07 | −10,7 ± 1,84 | 90,5 ± 4,63 | −10,6 ± 2,33 | 88,3 ± 7,15 | −8,92 ± 2,14 |
| Free fatty acid (µEq/l) | 577,4 ± 28,9 | −29,6 ± 23,0* | 588,6 ± 32,2 | −57,8 ± 33,8** | 682,5 ± 63,3 | −185,7 ± 68,1** |
| $PGF_{2\alpha}$ (pg/mg creatinine) | 944,3 ± 112,9 | −111,1 ± 75,8 | 1107,3 ± 141,4 | −88,4 ± 133,0 | 1030,1 ± 323,0 | −126,5 ± 262,5 |
| Oxidized LDL (U/l) | 41,1 ± 2,27 | −5,34 ± 1,08 | 39,1 ± 2,31 | −2,34 ± 1,43 | 37,5 ± 3,22 | −3,57 ± 2,25 |

# 5. DISCUSSION

The important finding of this study is that change in fasting FFA concentrations in response to modest weight loss (4.6% of initial body weight) is significantly different in overweight-obese subjects as a function of whether they have genetic variations at the PLIN locus. For PLIN 10076C>G/10171A>T haplotypes, located in exon 5 and intron 5, respectively, reductions in FFA levels after mild weight loss were about 4 times greater in nCA/nCA subjects as compared with CA carriers. On the other hand, for 11482G>A/14995A>T haplotypes, located in intron 6 and exon 9, respectively, subjects with nGA/nGA showed an increase in FFA levels after weight loss. Circulating concentrations of FFA, which indirectly mirror in vivo lipolytic activity, are known to be inversely related to the adipocyte perilipin content (1). Thus, this discrepant association between genetic variability at the PLIN locus and changes in FFA levels could relate to the expression of different perilipin isoforms which might function with different efficiency in the PKA mediated lipolysis (11).

In the human perilipin gene PLIN 11482G>A polymorphism, but not PLIN 14995A>T, was found to be associated with decreased perilipin content and increased basal and noradrenaline induced lipolytic acitivity in Swedish obese women (BMI≥30kg/$m^2$) (1). In this study subjects with homozygosity for the rare allele of either the PLIN 11482G>A or PLIN 14995A>T showed a lower

tendency at baseline FFA and an increase in FFA levels after weight loss in comparison to major allele homozygotes. This discrepancy might relate to differences in gender distribution (23% males in our study), BMI (27.1kg/$m^2$in this study) and ethnicity between two studies. In fact, variations in expression levels of perilipin have been reported between men and women and between obese and nonobese individuals (13). Furthermore, another study has reported different associations between genetic variants at the PLIN locus across ethnic groups (8).

Obesity may decrease perilipin concentrations in the adipocyte resulting in an increased levels of circulating FFA, while non-obese or weight loss may increase the perilipin protein expression. Generally, modest weight loss of 5-10% was shown to decrease circulating FFA levels (2,14) and decreased FFA related to improved insulin sensitivity (14). In this study, no differences were found between haplotype groups at PLIN 11482G>A/14995A>T in change in HOMA-IR after weight loss. Therefore, increased FFA concentrations in subjects with nGA/nGA haplotype at 11482G>A/14995A>Tcould be a consequence of excessive lipolysis resulting in rapid fat loss, secondary to reduced perilipin expression in adipose tissue. Furthermore, subjects with nGA/nGA showed a greater reduction in visceral and total fat areas at L4 than those with GA/GA. Mottagui-Tabar et al.(1) have also suggested a negative association between homozygostiy for the rare allele (AA) of PLIN 11482G>A genotype and body fat.

In contrast to the haplotypic effects of PLIN 11482G>A/14995A>T on change in FFA levels, certain haplotypes inferred using the PLIN 10076C>G and PLIN 10171A>T SNPs were associated with greater reduction inFFA levels for the nCA haplotype in response to weight loss compared with CA/CA haplotype. This discrepant association between variability at the PLIN locus and changes in FFA levels is possible that the genetic effect underlying the associations with PLIN 11482G>A/14995A>T haplotypes or PLIN 10076C>G/10171A>T haplotypes is through affecting splicing and the expression of different perilipin isoforms. In fact, the perilipins are encoded by a single copy gene that gives rise to multiple mRNAs by alternativesplicing mechanisms (15), these mRNAs are translated to yield different protein isoforms (16,17) with different efficiency in the lipolysis.

The perilipins inhibit the actions of lipases on intracellular lipid, perhaps acting as a barrier to lipase access in the unstimulated, basal state (18,19). However, perilipins are also required to facilitate HSL traslocation to lipid droplet and hydrolysis of triglycierides in the stimulated state such as fasting (7). Thus, the greater reduction of FFA levels in nCA/nCA subjects in 10076C>G/10171A>T haplotypes may be partly responsible for blocking perilipin's inhibitory actions in PKA mediated lipolysis in response to calorie restriction. Recently, Souza et al.(20) found the mutant perilipin blocked PKA-stimulated lipolysis. In addition, perilipin was found to be necessary to demonstrate an effect of PKA on lipolysis in both cell model and adipocytes

from perilipin-null mice.

Excessive circulating FFA levels are known to increase the level of oxidative stress and cause lipid peroxidation in healthy subjects (12). Evidence in vitro also indicates that elevated FFA have numerous adverse effects on oxidative status including the generation of reactive oxygen species (21). For assessing oxidative stress the measurement of urinary 8-epi- $PGF_{2\alpha}$and circulating oxidized LDL is known to be a precise method (22). Subjects with GA/GA haplotype at PLIN 11482G>A/14995A>T showed greater reduction in $PGF_{2\alpha}$ and oxidized LDL in comparison to those with nGA/nGA even though they had lower reduction in total and visceral fat at L4. Thus, this haplotype effect on lipid peroxides might be related to the greater reduction in FFA levels after weight loss or higher values of oxidative stress at baseline rather than change in visceral fat areas.

In conclusion, the results of this study have shown that genetic variants at the PLIN locus respond differently to mild weight loss in changes in circulating FFA levels and visceral fat areas. Subjects with nGA/nGA haplotype at 11482G>A/14995A>T could expect to have a rapid loss in abdominal fat with increased FFA levels in response to calorie reduction. On the other hand, subjects with nCA/nCA at 10076C>G/10171A>T showed greater reduction in FFA levels in response to weight loss than those with CA/CA haplotype. Further studies are needed to elucidate the underlying mechanism of differential effects of genetic variants at the PLIN locus on the

expression of perilipin isoforms and their functional consequences in lipolysis.

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# 국 문 요 약

## Perilipin 유전자의 다형성이 체중감량시 FFA농도와 복부지방 면적에 미치는 영향

비만은 환경적 요인과 유전적 요인이 상호 연관되어 생기는 복합적인 질병으로, 최근 여러 연구에서 환경적인 요인 뿐만 아니라 유전자의 변이형이 비만에 중요한 영향을 미친다는 설이 제기되고 있다. 이러한 비만 관련 후보 유전자 중의 하나로 지목되고 있는 perilipin은 지방세포에서 중성지방의 분해 과정 (lipolysis)을 조절하는 중요한 역할을 하는 것으로 보고되고 있다. 즉, perilipin은 지방세포표면에 존재하는 단백질로, 평상시에는 hormone sensitive lipase (HSL)에 의해 중성지방이 가수분해되는 과정을 방지하는 역할을 하지만, 노르에피네프린이나 카테콜라민과 같은 자극이 주어지게 되면 활성화된 protein kinase A(PKA)에 의해 HSL과 perilipin이 인산화되어 중성지방을 글리세롤과 유리지방산으로 분해하게 된다.

비만은 흔히 지질대사 이상과 adipose tissue 내에 과도한 중성지방 축적을 동반한다. 비만인에게서 흔히 adipocyte lipolysis 에 따른 혈중 비에스테르화지방산의 증가 현상을 볼 수 있다. 이러한 증가된 혈중 유리지방산의 농도는 단순히 adipose tissue mass의 반영이라고 생각하기 쉽지만, 실제로 유사한 조건하의 비만인의 혈중 유리지방산의 농도는 매우 다양한 양상을 띄는 것으로 보인다. 또한 한 연구에서도 moderate weight loss 실시 후에 비만인에게서 유리지방산 농도 변화는 서로 차이가 있는 것으로 나타났다. 이것으로 보아 adipose tissue mass 이외에도 혈중 유리지방산의 농도에 영향을 미치는 다른 요인이 있을 것으로 생각할 수 있으며,

이와 관련하여 최근 perilipin이라는 유전자의 다형성이 인체에서 중성지방의 분해와 혈중 FFA농도, 복부지방 면적 등에 영향을 끼쳐 비만의 위험성을 조절한다는 연구들이 보고되고 있다.

따라서, 본 연구는 BMI 25이상의 177명의 다른 질병이 없는 과체중의 한국인 성인 남녀를 대상으로 (남자 40명, 여자 137명) 7개의 perilipin 유전자 ( 6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T, 14995A>T) 단일염기다형성과 이 중 가장 강한 LD를 보이는 두개의 haplotype (PLIN 10171A>T와 PLIN 10076C>G (D'=0.923, $R^2$=0.839, P<0.001), PLIN 11482G>A와 PLIN 14995A>T (D'=0.824, $R^2$=0.578, P<0.001))이 칼로리제한을 통한 12주의 체중 감량에 따라 FFA농도와 복부지방 면적, 지질과산화물 등과 같은 비만관련지표에 미치는 영향에 대해 알아보고자 하였다.

하루에 300Kcal를 제한하는 12주의 저 칼로리 다이어트 후에 대상자들의 몸무게는 평균 4.6Kg정도 감량되었고, 복부지방 면적도 이에 따라 감소한 것으로 나타났다. PLIN 10076C>G의 경우, GG군은 CC군에 비하여 혈중 유리지방산 농도의 감소 정도가 큰 것으로 나타났다. (C/C:-16±23, C/G:-71±33, G/G:-159±73μEq/L, P=0.010). 이에 반해 PLIN 11482G>A , 14995A>T의 경우에는 rare allele의 homozygosity가 높은 군에서 혈중 유리지방산 농도 증가가 큰 것으로 나타났다 (11482G>A; G/G:-97±33, G/A:-56±27, A/A:86±44μEq/L, P=0.045 , 14995A>T; A/A:-103±34, A/T:-59±28, T/T:75±37μEq/L, P=0.029). PLIN 6209T>C, 10171A>T, 13042A>G, 13048C>T의 경우에는 allele의 차이에 따른 혈중 유리지방산 농도의 유의적인 차이는 나타나지 않았다. 11482G>A/14995A>T haplotypes에서는 GA/GA haplotype군보다 nGA/nGA haplotype군에서 허리둘레(GA/GA:

-2.0±0.4, GA/nGA:-3.5±0.4, nGA/nGA:-4.0±1.0cm, P=0.042)와, $4^{th}$ lumbar vertebra 에서의 Visceral fat (GA/GA:-4.8±1.7, GA/nGA:-12±2, nGA/nGA:-17±3 $cm^2$, P=0.004)이 유의적으로 감소하는 경향이 큰 것으로 나타났다. 지질 과산화물인 $PGF_{2\alpha}$ 와 oxidized LDL의 경우는 GA/GA haplotype군에서 baseline 농도가 높은 경향을 띄는 것으로 나타났고, 베이스라인에서 농도가 높은 그룹이 체중감량 후 농도 감소가 큰 것으로 나타났다.

이상의 결과로 본 연구에서 perilipin 유전자의 다형성이 체중감량과 같은 자극시에 fasting FFA농도, 복부 지방 면적, 지질 과산화물 등의 비만과 대사증후군 관련 지표들에 영향을 주는 것을 확인하였고, 이러한 perilipin 유전자의 다형성에 따라 한국인의 비만관련 위험도는 서로 다른 영향을 받을 것으로 사료된다.

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핵심이 되는 말: perilipin 유전자 다형성, 체중감량, 유리지방산, 내장지방, 산화 스트레스