Longitudinal interaction between *APOA5* -1131T>C and overweight in the acceleration of age-related increase in arterial stiffness through the regulation of circulating triglycerides

Hwa Jin Lee

The Graduate School

Yonsei University

Department of Science for Aging

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Hwa Jin Lee

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YONSEI UNIVERSITY

This certifies that the master's thesis of

Hwa Jin Lee is approved

Jong Ho Lee

Thesis Supervisor : Jong Ho Lee

Seungmin Lee

Thesis Committee Member : Seung-Min Lee

Oh Yoen Kim

Thesis Committee Member : Oh Yoen Kim

The Graduate School

Yonsei University

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또한 뒤에서 묵묵히 도와준 지민재, 김춘미, 장예은, 손명한, 이다형, 박지영, 김지현, 방영주, 김형윤, 김예리, 윤란희, 손요, 고주연, 서아림, 강민식 감사합니다. 후배님들의 도움이 있었기에 좋은 결실을 맺을 수 있었습니다.

마지막으로, 늘 같은 곳에서 한결같이 무한한 사랑과, 아낌없는 지지와 응원을 해주시는 사랑하는 부모님, 세상 그 누구보다 든든한 내동생 또야, 우리집 귀염둥이 부자, 그리고 항상 예뻐해주시고 사랑해주시는 이모, 이모부 사랑하고 감사합니다.

초심과 감사함을 잃지 않는 정직한 연구자로서의 역할과 책임을 다하겠다고 이 글을 빌어 다짐합니다. 다시 한 번 모든 분께 감사의 마음 드리며 이 글을 마칩니다.

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ABBREVIATION

ApoA5	Apolipoprotein A5
baPWV	Brachial-ankle pulse wave velocity
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
HDL	High density lipoprotein
PWV	Pulse wave velocity
LDL	Low density lipoprotein
TG	Triglycerides

ABSTRACT

Longitudinal interaction between *APOA5* -1131T>C and overweight in the acceleration of age-related increase in arterial stiffness through the regulation of circulating triglycerides

Hwa Jin Lee
Department of Science for Aging
The Graduate School
Yonsei University

The aim of this study was to evaluate whether the longitudinal interaction between *APOA5* -1131C variants and overweight could accelerate age-related increases in arterial stiffness and circulating triglycerides (TG) in healthy subjects. Brachial-ankle pulse wave velocity (baPWV) was measured in 503 healthy subjects at baseline and within a mean follow-up period of 3 years. triglycerides, *APOA5* -1131T>C SNPs, apolipoprotein (apo) A-V levels and low density lipoprotein (LDL) particle size were

measured. At the 3-year follow-up, the overweight group with the C allele showed increases in triglycerides and brachial-ankle pulse wave velocity relative to the baseline. Additionally, in the overweight group, there was a genotype effect on the changes in triglycerides: subjects with the C allele had greater increases in triglyceride concentrations than those with the TT genotype. Furthermore, overweight subjects with the C allele had greater increases in triglycerides concentrations than normal-weight subjects with the C allele (*P*-interaction=0.013). Overweight subjects with the C allele had greater increases in brachial-ankle pulse wave velocity than normal-weight subjects with the C allele (*P*-interaction=0.047). Changes in brachial-ankle pulse wave velocity were affected by age, baseline brachial-ankle pulse wave velocity, and changes in systolic blood pressure (BP) and triglycerides. Changes in triglycerides were affected by the *APOA5* -1131T>C genotype, age, baseline triglyceride levels, and changes in body mass index (BMI) and apolipoprotein A-V. In the overweight group, changes in brachial-ankle pulse wave velocity were affected by changes in systolic blood pressure, low density lipoprotein particle size and triglyceride. This prospective study shows that the interactive effect between *APOA5* -1131C variants and overweight can accelerate age-related increase in arterial stiffness through the regulation of circulating triglycerides in healthy subjects.

Keywords : *APOA5*; arterial stiffness; gene-environment interaction; overweight; triglycerides

1. INTRODUCTION

The *APOA5* gene, encoding apolipoprotein (apo) A-V, is one of the major genetic determinants of circulating triglycerides (TG) levels, as demonstrated in animal models [1]. In humans, both single-gene and genome-wide association studies in different populations have confirmed the importance of *APOA5* in affecting TG concentrations [2,3]. Circulating TG levels, which are also affected by overweight/obesity and insulin resistance [4], are a risk factor for atherosclerosis [5] and can initiate atherogenic dyslipidemia [6].

In clinical practice, pulse wave velocity (PWV) is widely used to reflect arterial stiffness, an index of atherosclerosis [7,8]. Brachial-ankle pulse wave velocity (baPWV) measurements, which are easier to perform than carotid-femoral PWV measurements, have been used as a marker to screen for vascular damage and cardiovascular risk in the general population [7,9]. A cardiovascular outcome resulting from hypertriglyceridemia is arterial stiffness, a pathological condition associated with vascular damage [7]. An inverse relationship between plasma TG and plasma apo A-V and between hepatic *APOA5* expression and plasma TG levels has been described in an animal-model study [1], whereas an unexpected and significant positive correlation between circulating apo A-V and TG levels in men and women has been demonstrated [10,11]. This difference might be because most human studies examining the relationship between apo A-V and TG are cross-sectional.

Arterial stiffness increases with age, but the longitudinal interaction between genetic and environmental factors that might influence its progression are unknown. Thus, studies exploring the longitudinal effect of *APOA5* -1131T>C on the age-related progression of arterial stiffness and circulating concentrations of TG and apo A-V are necessary. In this study, the examined whether the longitudinal interaction between *APOA5* -1131T>C (TT vs C allele) and body weight (normal weight vs overweight) affects the age-related progression of arterial stiffness and the circulating levels of TG and apo A-V of healthy subjects.

2. BACKGROUND

2.1. Apolipoprotein A5 gene

Apolipoprotein A5 gene (*APOA5*) is located in the apolipoprotein *APOA1/C3/A4* gene on chromosome 11q23 [12,13]. *APOA5* is mainly expressed in liver cells and secreted into the plasma [13,14]. The *APOA5* is important function in the metabolism of TG [15]. Increased level of APOA5 is correlated with decreased TG concentration in the plasma [15]. Polymorphisms in the *APOA5* gene are strongly affected by TG levels [2]. Among the genetic variants associated with the expansion of hyperlipidemia there is a natural variant (-1131T>C) in the promoter region of APOA5 gene [2].

Mice expressing a human APOA5 transgene showed a decrease in plasma TG concentrations to one-third of those in control mice, reversely knockout mice lacking *APOA5* had four times as much plasma TG as controls [2]. In humans, single nucleotide polymorphisms (SNPs) across the *APOA5* locus were found to be significantly associated with plasma TG levels in two independent studies [2]. These findings indicate that *APOA5* is an important determinant of plasma TG levels, a major risk factor for coronary artery disease [2].

The -1131T>C polymorphism in the newly identified APOA5 gene has been associated with elevated plasma TG [16]. It has been shown for other genes that the effect of polymorphisms on plasma TG is modulated by body mass index (BMI) [17]. Study participants were divided into normal weight (BMI≤25 kg/m^2) and overweight

(BMI>25 kg/m^2) [16]. There was no significant difference TG level between carriers and noncarriers among normal-weight subjects, but in overweight subjects the level in carriers was very significantly higher than in noncarriers [16].

2.2. Brachial-ankle pulse wave velocity

In clinical practice, PWV is widely used to reflect arterial stiffness [7,8]. A noninvasive baPWV measurement, which is performed more easily than carotidfemoral PWV measurement, has been used as a marker for screening vascular damage and cardiovascular risk in the general population [18,19], in diabetes patients [20,21], in hypertension patients [22,23], in patients with end-stage renal disease [24,25], and in women with systemic lupus erythematosus [26].

The baPWV was assessed in a supine position after 5 minutes of bed rest [27]. Pneumonic cuffs were wrapped around both upper arm (brachial artery) and ankles (tibial artery) and connected to a plethysmographic sensor to determine the volume pulse waveform [27]. Waveforms were stored for 10-second sample times with automatic gain analysis and quality adjustment [27]. Requires simply placing blood pressure cuffs on the 4 extremities [27,28].

The baPWV has been shown to be a marker of atherosclerotic vascular damage or cardiovascular risk [7]. The present study conducted to evaluate the validity and reproducibility of noninvasive baPWV measurements and to examine the alteration of baPWV in patients with coronary artery disease (CAD) [29]. baPWV were significantly higher in CAD patients than in non-CAD patients with risk factors.

Furthermore, baPWV were higher in non-CAD patients with risk factors than in healthy subjects without risk factors [29]. Thus, the validity and reproducibility of baPWV measurements are considerably high, and this method seems to be an acceptable marker reflecting vascular damages. baPWV measured is suitable for screening vascular damages in a general population [29]. baPWV has been shown to increase with aging and hypertension [28].

3. MATERIALS AND METHODS

3.1. Subjects

This study was performed a 3-year prospective cohort study that included 800 heathy subjects (30-65 years old), who underwent triennial medical evaluations at the National Health Insurance Corporation Ilsan Hospital in Goyang, Korea during the period from January 2008 to December 2013. Of these, 503 nondiabetic and nonobese individuals were finally selected. Exclusion criteria included the following: 1) current and/or past history of cardiovascular disease, 2) diabetes mellitus (fasting glucose levels ≥126 mg/dL), 3) abnormal liver or renal function, 4) thyroid or pituitary disease, 5) pregnancy or lactation, and 6) regular use of any medication. Before participation, the purpose of the study was carefully explained to all participants, and their written informed consent was obtained. The study protocol was approved by the Institutional Review Board of the National Health Insurance Corporation Ilsan Hospital and Yonsei University and was carried out in accordance with the Helsinki Declaration.

3.2. Genotyping of rs662799 (*APOA5* -1131T>C)

Among the five common *APOA5* SNPs that have been studied, -1131T>C and 56C>G (S19W) are considered to be functional tag SNPs [30-32]. However, the S19W SNP was monomorphic in the population of this study. Thus, the *APOA5* -1131T>C SNP was selected as the functional SNP to investigate further [33].

DNA was extracted from 5 mL of whole blood using a commercially available DNA isolation kit (Promega Corp., Madison, USA) according to the manufacturer's protocol. Genotyping was performed via SNP-IT™ assays using single primer extension technology (SNPstream 25K™ System; Orchid BioSciences, NJ, USA).

3.3. Clinical and biochemical assessments

Body weight, height and waist circumference were measured, and BMI was calculated in units of kilograms per square meter (kg/m^2). Systolic and diastolic blood pressure (BP) was assessed in a supine position after a resting period. Blood samples were collected following an overnight fast of at least 12 hours.

Fasting triacylglycerol were measured via enzymatic assay using Pureauto S TG-N and Pureauto S CHO-N kits (Daiichi, Tokyo, Japan). Total- and high density lipoprotein (HDL) cholesterol was measured via selective inhibition using Cholestest N-HDL kits (Daiichi, Tokyo, Japan). Glucose levels were measured via the hexokinase method using glucose kits (Siemens, NY, USA), insulin levels were measured via an immunoradiometric assay using commercial kits (DIAsource ImmunoAssays S.A., Louvainla-Neuve, Belgium). Low density lipoprotein (LDL) particle size were isolated using sequential flotation ultracentrifugation and particle size distribution (1.019–1.063 g/mL) examined on non-denaturing polyacrylamide gels containing a linear gradient of 2-16 % acrylamide (CBS Scientific, CA, USA) using a pore gradient lipoprotein system (CBS Scientific Company Inc., CA, USA), and apo A-V were measured by turbidity at 340 nm using specificanti-serum (Roche, Basel, Switzerland).

baPWV were measured using an automatic waveform analyzer (model VP-1000; Nippon Colin Ltd., Komaki, Japan).

3.4. Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (IBM/SPSS, Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) was tested for using PLINK version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). Differences in clinical variables between two groups (Normal weight vs. Overweight; TT vs. C allele) were tested by independent *t*-test. Paired *t*-tests were performed to determine the differences at the 3-year follow-up from the baseline in each group. The interactions between genotype and bodyweight were tested using a two-way ANOVA. Multiple linear regression analyses were performed to identify major independent predictors of changes in baPWV and TG levels. Pearson's correlation coefficient was used to examine the relationships between variables. Heat maps were created to visualize and evaluate the relationships among metabolites and the biochemical measurements in the study population. Logarithmic transformations were performed on skewed variables. For descriptive purposes, the mean values are presented as untransformed values. The results are expressed as the means ± standard error (SE). A two-tailed *P*-value <0.05 was considered statistically significant.

4. RESULTS

4.1. Distribution of rs662799 (*APOA5* -1131T>C) in normal-weight and overweight subjects

Study participants were divided the cohort into 2 groups: the normal-weight group (18.5 kg/m^2 ≤ BMI < 25 kg/m^2, *n*=349) and the overweight group (25 kg/m^2 ≤ BMI < 30 kg/m^2, *n*=154). The genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium ($P > 0.05$). The frequencies of the minor alleles of *APOA5* -1131T>C were 0.297 and 0.315 in normal-weight and overweight individuals, respectively, which are similar to the values observed in the Korean population [34]. Among the 349 normal-weight individuals, 168 were homozygous (TT) for the T allele, 155 were heterozygous for the C allele (TC) and 26 were homozygous (CC) for the C allele of the *APOA5* -1131T>C polymorphism. Among the 154 overweight individuals, 68 were homozygous (TT) for the T allele, 75 were heterozygous for the C allele (TC) and 11 were homozygous (CC) for the C allele of the *APOA5* -1131T>C polymorphism. The pooled the heterozygotes (TC) and rare-allele homozygotes (CC) to increase statistical power.

4.2. Clinical characteristics and biochemical parameters at baseline and the 3-year follow-up

With regard to the *APOA5* -1131T>C polymorphism, there were no significant differences between the normal-weight and overweight groups at baseline in their age and sex distributions across the genotypes (Table 1). Additionally, there were no significant differences in smoking or drinking status across the genotypes in the normal-weight and overweight groups at baseline and at the 3-year follow-up (data not shown).

In the normal-weight group, there was no *APOA5* -1131T>C genotype effect at baseline, but at the 3-year follow-up, a genotype-effect was found in relation to HDL cholesterol: HDL cholesterol levels were lower in normal-weight individuals with the C allele than in those with the TT genotype (Table 1).

The overweight group showed higher BMI values, systolic and diastolic BP, and glucose and insulin levels and lower HDL cholesterol levels at both baseline and the 3-year follow-up than the normal-weight group, irrespective of genotype. Overweight individuals with the C allele showed higher total cholesterol levels at baseline and a smaller LDL particle size at both baseline and the 3-year follow-up. After 3 years, overweight C allele carriers showed significant increases in systolic BP and a significant decrease in HDL cholesterol relative to baseline. At the 3-year follow-up in the overweight group, C allele carriers showed lower HDL cholesterol levels and a smaller LDL particle size than TT carriers (Table 1).

Table 1. Biochemical parameters according to rs662799 (*APOA5* -1131 T>C) genotypes at baseline and 3-year follow-up in healthy individuals with normal weight or overweight

	Normal weight				Overweight			
	TT (n=168)		C allele (n=181)		TT (=68)		C allele (n=86)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Baseline age (year)	47.7±0.67		47.3±0.70		47.6±1.14		47.4±1.05	
Male/Female n, (%)	76 (45.2) / 92 (54.8)		79 (43.6) / 102 (56.4)		28 (41.2) / 40 (58.8)		41 (47.7) / 45 (52.3)	
BMI (kg/m^2)	22.1±0.14	22.2±0.16	22.0±0.14	22.1±0.15	26.8±0.18^{e}	27.0±0.22^{f}	26.8±0.19^{g}	26.7±0.23^{h}
Change	0.10±0.08		0.10±0.07		0.08±0.15		-0.08±0.11	
Systolic BP (mmHg)	115.5±1.08	116.7±1.03	115.2±0.90	115.1±1.01	121.6±1.65^{e}	123.4±1.73^{f}	122.7±1.38^{g}	126.1±1.35^{h,*}
Change	1.15±0.92		-0.10±0.89		1.76±1.71		3.35±1.34^{l}	
Diastolic BP (mmHg)	70.9±0.83	71.8±0.81	70.7±0.74	71.5±0.76	75.2±1.22^{e}	76.8±1.26^{f}	75.4±1.01^{g}	77.4±1.04^{h}
HDL-cholesterol (mg/dL)$^{\phi}$	56.3±1.08	55.3±0.93	53.9±1.11	52.0±1.05^{b,*}	52.4±1.68^{e}	49.0±1.53^{f,*}	47.2±1.27^{c,g}	44.6±1.16^{d,h,*}
Total-cholesterol (mg/dL)$^{\phi}$	187.1±2.34	195.0±2.45***	184.8±2.43	194.0±3.07***	193.2±3.80	195.6±4.43	197.7±3.42^{g}	200.7±3.38
Glucose (mg/dL)$^{\phi}$	91.2±0.65	92.0±0.59	90.0±0.64	90.5±0.75	95.2±1.18^{e}	97.4±1.53^{f}	97.0±0.93^{g}	96.7±1.22^{h}
Insulin (μIU/dL)$^{\phi}$	8.12±0.27	7.31±0.20*	7.93±0.22	7.13±0.23***	10.2±0.46^{e}	9.72±0.51^{f}	9.77±0.42^{g}	9.02±0.44^{h,*}
LDL particle size (nm)$^{\phi}$	23.8±0.07	23.9±0.10	23.8±0.07	23.8±0.08	23.5±0.18	23.6±0.16	23.3±0.14^{g}	23.2±0.17^{d,h}

Mean ± SE. $^{\phi}$ tested by logarithmic transformation. ^{a}P<0.05 comparison between TT and C allele in Normal weight group at baseline. ^{b}P<0.05 comparison between TT and C allele in Normal weight group at 3 year follow-up. ^{c}P<0.05 comparison between TT and C allele in Overweight group at baseline. ^{d}P<0.05 comparison between TT and C allele in Overweight group at 3 year follow-up. ^{e}P<0.05 comparison between TT in Normal weight and Overweight groups at baseline. ^{f}P<0.05 comparison between TT in Normal weight and Overweight groups at 3 year follow-up. ^{g}P<0.05 comparison between C allele in Normal weight and Overweight groups at baseline. ^{h}P<0.05 comparison between C allele in Normal weight and Overweight groups at 3 year follow-up. ^{i}P<0.05 comparison between TT and C allele in Normal weight group at change values. ^{j}P<0.05 comparison between TT and C allele in Overweight group at change values. ^{k}P<0.05 comparison between TT in Normal weight and Overweight groups at change values. ^{l}P<0.05 comparison between C allele in Normal weight and Overweight groups at change values. $^{*}P$<0.05,$^{**}P$<0.01,$^{***}P$<0.001 compared with the levels at baseline in each group by paired t-test.

4.3. Interaction between the *APOA5* -1131T>C genotype and body weight in relation to 3-year changes in TG, apo A-V, systolic BP and baPWV

The effects of the *APOA5* -1131T>C genotype on mean changes in TG, apo A-V, and baPWV in the normal-weight and overweight groups at 3 years are shown in Figure 1. In the normal-weight group, individuals with the C allele showed higher serum TG levels than those with the TT genotype. The overweight group showed higher serum TG levels at both baseline and the 3-year follow-up than the normal-weight group, irrespective of genotype. After 3-years, overweight C allele carriers showed significant increases in TG and baPWV. At the 3-year follow-up, C allele carriers showed higher TG than TT carriers in the overweight group. Furthermore, at the 3-year follow-up, overweight TT and C allele carriers showed higher baPWVs than normal-weight subjects with the TT and C alleles, respectively (Figure 1).

There was no significant gene and body weight interaction on the changes in fasting apo A-V (Figure 1), BP, HDL and total cholesterol, glucose, insulin, and LDL particle size values (data not shown). At the 3-year follow-up, after adjusting for age, sex, smoking and drinking, the results show a significant interaction between the *APOA5* -1131T>C genotype and body weight (overweight compared with normal-weight) in relation to changes in TG (*P* for interaction=0.013) and baPWV (*P* for interaction=0.047). In the overweight group, there was an *APOA5* -1131T>C genotype effect on changes in TG: subjects with the C allele had greater increases in serum TG concentrations than did those with the TT genotype; however, this genotypic effect on

changes in TG was not observed in normal-weight subjects. Additionally, overweight subjects with the C allele had greater increases in their serum TG concentration than normal-weight subjects with the C allele. Overweight subjects with the C allele had greater increases in baPWV than normal-weight subjects with C allele. Similarly, overweight subjects with C allele had greater increase in systolic BP than normal-weight subjects with C allele (Table 1); however, there was no significant effect of a gene × body weight interaction on systolic BP.

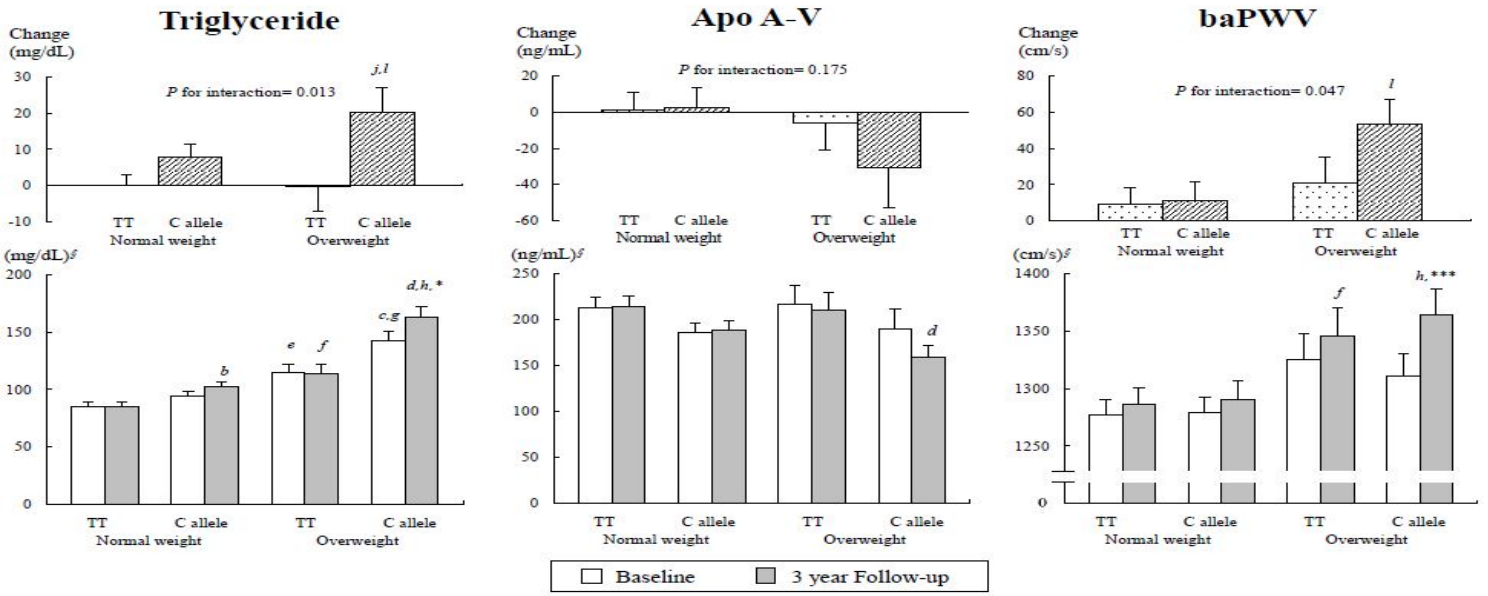

Figure 1. Genotype effect of *APOA5* -1131T>C on changes in TG, LDL particle size, apo A-V, and baPWV by nomal weight and overweight groups at 3-year follow-up from baseline.

Mean ± SE. [§]tested by logarithmic transformation.[a]$P<0.05$ comparison between TT and C allele in Normal weight group at baseline. [b]$P<0.05$ comparison between TT and C allele in Normal weight group at 3 year follow-up. [c]$P<0.05$ comparison between TT and C allele in Overweight group at baseline. [d]$P<0.05$ comparison between TT and C allele in Overweight group at 3 year follow-up. [e]$P<0.05$ comparison between TT in Normal weight and Overweight groups at baseline. [f]$P<0.05$ comparison between TT in Normal weight and Overweight groups at 3 year follow-up. [g]$P<0.05$ comparison between C allele in Normal weight and Overweight groups at baseline. [h]$P<0.05$ comparison between C allele in Normal weight and Overweight groups at 3 year follow-up. [i]$P<0.05$ comparison between TT and C allele in Normal weight group at change values. [j]$P<0.05$ comparison between TT and C allele in Overweight group at change values. [k]$P<0.05$ comparison between TT in Normal weight and Overweight groups at change values. [l]$P<0.05$ comparison between C allele in Normal weight and Overweight groups at change values. [*]$P<0.05$, [**]$P<0.01$, [***]$P<0.001$ compared with the levels at baseline in each group by paired t-test.

4.4. Correlation among changes in BMI, systolic and diastolic BP, TG, HDL and total cholesterol, glucose, insulin, LDL particle size, apo A-V, and baPWV.

Figure 2 shows the correlation among the changes (differences from baseline) in BMI, systolic and diastolic BP, TG, HDL and total cholesterol, glucose, insulin, LDL particle size, apo A-V, and baPWV values in the normal-weight and overweight groups in relation to the *APOA5* -1131T>C genotype. In the normal-weight group with the TT genotype, changes in TG correlated positively with changes in BMI, glucose, and insulin values and negatively with changes in LDL particle size and apo A-V level (P<0.001, Figure 3).

Changes in baPWV were positively correlated with changes in glucose level and systolic and diastolic BP in the normal-weight group with the TT genotype. In the normal-weight group with the C allele, changes in TG correlated negatively with changes in HDL cholesterol level and LDL particle size and positively with changes in BMI, insulin level and baPWV (P=0.011, Figure 3), which was positively correlated with changes in systolic and diastolic BP in the normal-weight group with the C allele. In the overweight group with the TT genotype, changes in TG were negatively correlated with changes in LDL particle size and apo A-V level (P=0.002, Figure 3). Changes in baPWV were positively correlated with changes in glucose level and systolic and diastolic BP in the normal-weight group with the C allele. In the overweight group with the C allele, changes in TG correlated negatively with changes in HDL cholesterol and apo A-V levels (P=0.028, Figure 3) and LDL particle size and

positively with changes in BMI, insulin level and baPWV (P=0.004, Figure 3), which was positively correlated with changes in systolic and diastolic BP in the overweight group with the C allele. Other correlations among the changes in clinical and biochemical parameters are shown in Figure 2.

Because the regulation of changes in TG levels is complex, a multiple linear regression analysis was performed in all subjects (n=503) to determine the independent effects of the following variables on changes in TG: age, *APOA5* -1131T>C genotype, and baseline TG levels, as well as changes in BMI, systolic and diastolic BP and the levels of glucose, insulin, total and HDL cholesterol, and apo A-V. Changes in TG in all subjects (n=503) were affected by the *APOA5* -1131T>C genotype, age, baseline TG level and by changes in BMI and apo A-V level (R^2=0.258, P<0.001). In the normal-weight group (n=349), changes in TG were affected by baseline TG levels and changes in BMI, glucose, apo A-V and systolic BP values (R^2=0.286, P=0.034). In the overweight group (n=154), changes in TG were affected by baseline TG levels and changes in apo A-V levels (R^2=0.209, P=0.013).

Also performed a multiple linear regression analysis including all subjects (n=503) to determine the independent effects of the following variables on changes in baPWV: age, *APOA5* -1131T>C genotype, baseline baPWV, as well as changes in BMI and systolic and diastolic BP and in the levels of glucose, insulin, total and HDL cholesterol, and apo A-V and LDL particle size. Changes in baPWV in all subjects (n=503) were affected by age, baseline baPWV, and changes in systolic BP and TG level (R^2=0.193, P<0.001). In the normal-weight group (n=349), changes in baPWV

were affected by age, baseline baPWV and changes in TG and apo A-V levels (R^2=0.204, P=0.032). In the overweight group (n=154), changes in baPWV were affected by changes in systolic BP, LDL particle size and TG level (R^2=0.413, P=0.002).

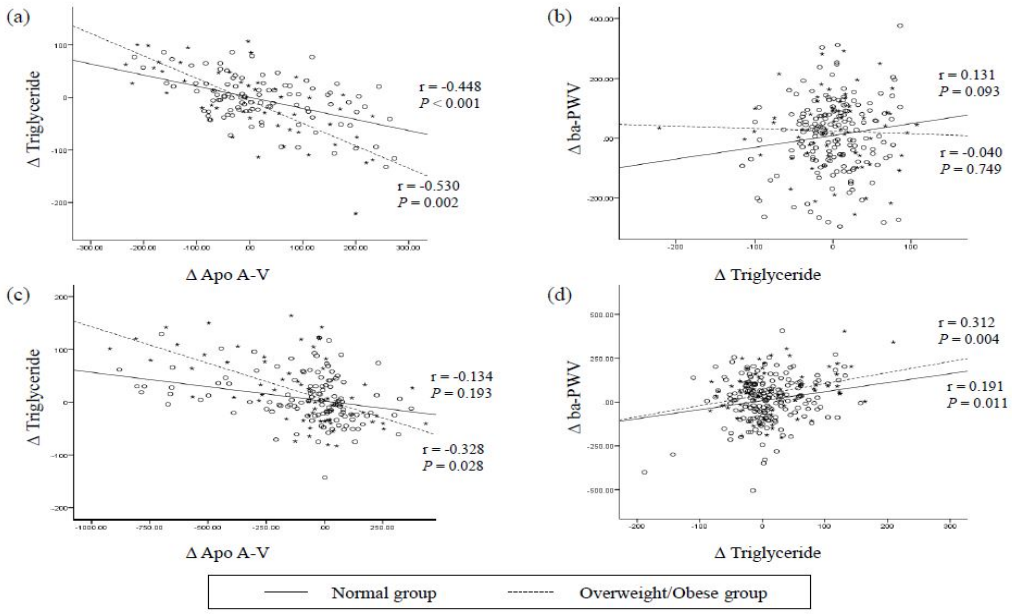

Figure 2. Correlation between changes (Δ) in apo A-V and TG and between changes in triglyceride and baPWV in normal weight (○) and overweight/obese (*) groups according to *APOA5* -1131 T>C genotype

(a) Correlation between changes in apo A-V and TG in TT genotype. (b) Correlation between changes in TG and baPWV in TT genotype. (c) Correlation between changes in apo A-V and triglyceride in TC+CC genotype. (d) Correlation between changes in TG and baPWV in TC+CC genotype.

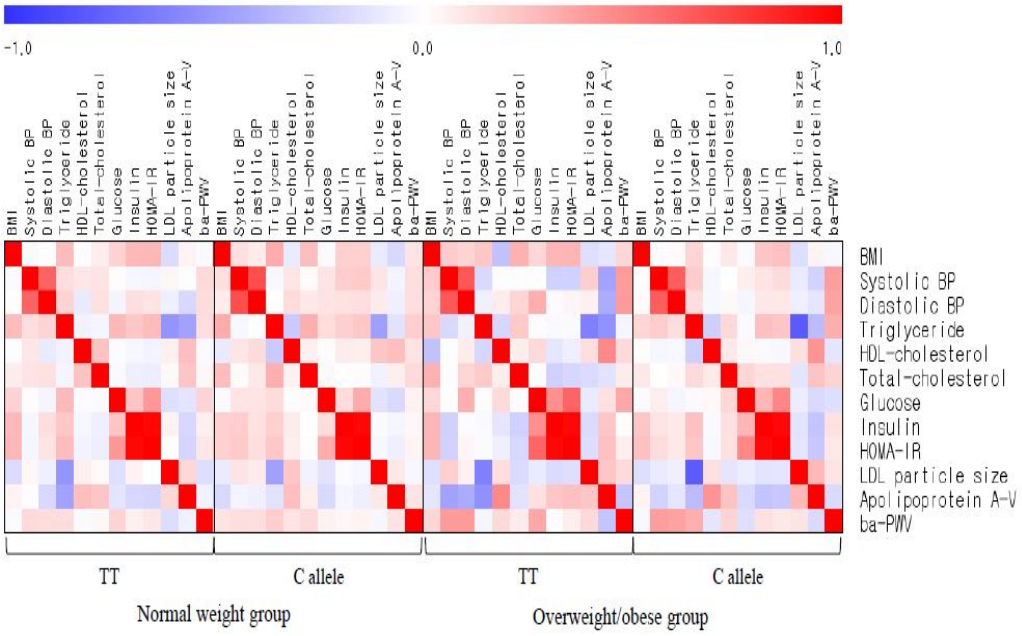

Figure 3. Correlation matrix among changes in clinical parameters in normal weight and overweight/obese groups according to *APOA5* -1131 T>C genotype

Correlations were obtained by deriving a Pearson correlation coefficient. *Red* is a positive correlation and *Blue* is a negative correlation.

5. DISCUSSION

Arterial stiffness increases with age, but the interaction between genetic and environmental factors that might influence its progression is unknown. This prospective study examined the longitudinal interactive effects of *APOA5* -1131T>C and body weight on circulating TG and the progression rate of arterial stiffness. baPWV, which reflects arterial stiffness [9,35] was measured at baseline and re-examined within a mean follow-up period of 3 years. The main finding of this study is that the interactive effect between *APOA5* -1131C variants and overweight can accelerate the age-related elevation of arterial stiffness through the regulation of circulating TG in healthy subjects.

At 3-years, the overweight group showed higher baPWVs than the normal-weight group, without considering genotype, but only the overweight subjects with the C allele showed an increase in baPWV over the 3-year period. Therefore, the overweight subjects with the C allele had greater increases in baPWV than the normal-weight subjects with the C allele. This result suggests that an acceleration in the age-related enhancement of arterial stiffness can partly result from a longitudinal interaction between *APOA5* -1131C variants and overweight. The existence of a strong association between the presence of metabolic syndrome, which includes elevated levels of circulating TG, and arterial stiffness has been shown in many cross-sectional studies [9,36,37] and follow-up studies [7,8,38]. However, the longitudinal effect of triglyceride-related genes on arterial stiffness in the general population has not been

clarified.

There was a significant and weakly positive correlation between the changes in TG and in baPWV in the normal-weight group with the -1131C allele; however, a strongly positive correlation was present between the changes in TG and baPWV in the overweight group with -1131C allele. This is partly due to the greater increases in TG at the 3-year follow-up in the overweight C allele carriers than in those in the normal-weight group. These interactive effects between the *APOA5* gene and overweight on circulating TG levels and arterial stiffness supports the role of circulating TG as one of the major inducers of arterial stiffness in overweight/obese subjects [4,39,40]. Recently, Guardiola et al. [2] have shown that *APOA5* variants predispose hyperlipidemic patients to atherogenic dyslipidemia and subclinical atherosclerosis, and this observation is significantly stronger in subjects with a BMI $\geq$25 kg/m^2 than in those with a normal weight. In this study, a multiple linear regression analysis revealed that changes in TG, systolic BP, and LDL particle size were independent determinants of the longitudinal progression of arterial stiffness in the subjects in the overweight groups.

The rare *APOA5* -1131C alleles were associated with an atherogenic profile characterized by high levels of small, dense LDL [2]. Atherogenic changes associated with LDL cholesterol induced by TG have been found to occur at concentrations as low as 1.7 mmol/L, which is the level at which small, dense LDLs become predominant [41]. Recently, Vishnu et al. [8] found that baPWV was significantly associated with atherogenic small-sized LDL particles, independent of other

cardiovascular risk factors. Takahashi et al. [42] also reported a significant association between very small LDL cholesterol and baPWV. Similarly, in the overweight group in this study, a change in LDL particle size was one of the independent determinants of a change in baPWV. At the 3-year follow-up, overweight subjects with the C allele showed smaller LDL particle sizes and lower plasma apo A-V concentrations than those with the TT genotype.

In all subjects, changes in TG were independently affected by *APOA5* -1131T>C genotype, age, baseline TG level, and changes in BMI and apo A-V levels. There were significant negative correlations between changes in TG and plasma apo A-V concentrations in overweight individuals with the C allele and in TT carriers. This is in contrast to previous observations of significant positive correlations between circulating apo A-V and TG levels in men and women [10,11]. These different results could be partly explained by difference in study design. In general, previous human studies on the relationship between apo A-V and TG levels have been cross-sectional; however, the current study was longitudinal.

6. CONCLUSIONS

In terms of limitations, this study only included normal-weight and overweight subjects without diabetes or cardiovascular diseases; therefore, the present results may not extrapolate to subjects who are obese, diabetic or have cardiovascular diseases. Despite this limitation, this study found that the longitudinal interaction between *APOA5* -1131C variants and overweight could accelerate the age-related increase in arterial stiffness through the regulation of circulating TG in healthy subjects. Therefore, the management of hypertriglyceridemia is important for preventing the progression to advanced arterial stiffness, especially in overweight subjects with the *APOA5* -1131C allele.

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국문요약

노화에 따른 혈관 경직도와 혈류 내 중성지방에 *APOA5* -1131T>C와 과체중의 상호작용이 미치는 영향

연구 목적: 본 연구에서는 건강한 사람을 대상으로 노화에 따른 혈관경직도의 가속화와 혈류 내 중성지방에 *APOA5* -1131T>C와 과체중이 미치는 상관관계를 알아보기 위해 연구를 진행하였다.

연구 방법: 3년 추적 코호트 연구로 설계되었으며, 건강한 성인 503명을 정상체중군 (349명)과 과체중군 (154명) 두 그룹으로 나누어 비교 관찰하였다. 혈관 경직도는 brachial-ankle PWV (baPWV)을 통하여 측정하였으며, 중성지방, *APOA5* -1131T>C SNP, apo A-V, LDL 입자 크기를 측정하였다.

연구 결과: C allele (86명)을 지닌 과체중군에서 3년 후, 혈중 중성지방과 baPWV의 증가를 나타냈다. 또한 과체중군 (154명)에서 혈중 중성지방의 변화는 risk C allele을 가진 대상자에서 TT 유전자형 (68명)을 가진 대상자보

다 변화가 더 크게 나타났다. 뿐만 아니라 risk C allele을 가진 과체중군에서 C allele (181명)을 가진 정상체중군보다 혈중 중성지방 농도가 더 많이 증가했다. baPWV 변화에서도 risk C allele을 지닌 과체중군에서 C allele을 가진 정상체중군보다 변화의 폭이 더 컸다. 분석 결과 모든 대상자에서 baPWV의 변화는 나이, 기준 baPWV, 수축기 혈압의 변화에 영향을 받았으며, 혈중 중성지방의 변화는 *APOA5* -1131T>C 유전자형, 나이, 수축기 혈압의 변화, 기준 중성지방의 변화 및 BMI와 apo A-V의 변화에 영향을 받는 것으로 나타났다. 과체중군에서 baPWV의 변화는 수축기 혈압, LDL 입자 크기 및 중성지방의 변화에 영향을 받았다.

연구 결론: 본 연구에서는 *APOA5* -1131T>C 유전자형과 과체중 사이의 상호 작용이 건강한 대상자에서 혈중 중성지방 농도에 영향을 미치며, 연령 증가에 따른 혈관 경직도를 가속화 할 수 있음을 보여준다.

핵심되는 말 : *APOA5*; 혈관경직도; 유전자-환경 상호작용; 과체중; 중성지방