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Running title: GGT, CRP, and arterial stiffness

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**Abstract**

**Background and aims:** The relationships between γ-glutamyltransferase (GGT), C-reactive protein (CRP), and arterial stiffness have not been fully investigated. The aim of this study was to clarify whether serum GGT is related to CRP and arterial stiffness estimated using brachial-ankle pulse wave velocity (baPWV).

**Methods and results**: The subjects were 3412 males and 854 females. GGT, CRP, baPWV, and conventional risk factors were evaluated. On multiple regression analysis, after adjustment for the conventional risk factors, log GGT was significantly associated with log CRP in male and female subjects (male subjects: beta = 0.168, $p < 0.0001$; female subjects: beta = 0.098, $p < 0.05$). After adjustment for the conventional risk factors, log GGT was significantly associated with PWV in male subjects (beta = 0.060, $p < 0.0001$), but in female subjects, no significant relationships were found after adjustment (beta = 0.007, $p = 0.82$).

**Conclusion:** These results suggest that GGT is independently associated with an increased level of CRP in both males and females. In addition, in males, GGT is related to an increased level of arterial stiffness.

**Key Words**; gamma-glutamyltransferase; C-reactive protein; pulse wave velocity; arterial stiffness

## Introduction

Abnormal elevation of serum γ-glutamyltransferase (GGT) is used as a marker of alcohol consumption or liver disease [1]. Furthermore, several studies have indicated that slightly elevated serum GGT that is almost within the reference range is significantly associated with all-cause mortality [2, 3], as well as increased risks of myocardial infarction [4, 5] and stroke [6, 7]. These associations can be partly explained by the known correlation of GGT with cardiovascular risk factors, such as obesity [8, 9], dyslipidemia [10, 11], hypertension [12, 13], type 2 diabetes [13, 14], and metabolic syndrome [14].

Atherosclerosis is generally accepted to be an inflammatory disorder of the arterial wall [15]; the C-reactive protein (CRP) level is a strong predictor of cardiovascular events [16-18]. The CRP level is also correlated with many cardiovascular risk factors [19-22]. There have been several reports that there is a relationship between serum GGT and CRP levels [13, 23-25], but the relationship has not been fully investigated, particularly in the Asian population.

Pulse wave velocity (PWV) is a valid maker of arterial stiffness [25, 26], and there have been many reports dealing with PWV and the development of atherosclerotic diseases [27-30]. However, the specific role of serum GGT in relation to early atherosclerosis remains unclear, and the relationship between GGT and arterial stiffness has not been fully investigated.

In this study, we investigated the associations of serum GGT, serum CRP, which is a

cardiovascular risk factor, and arterial stiffness, which is as a marker of early-stage atherosclerosis.

## Methods

### Subjects

The subjects were local government employees (8229 men and 2194 women) aged 35 years or older who had their annual health checkup during the period from April 2003 through March 2004. We used a self-administered questionnaire to inquire about clinical history, family history, smoking, alcohol consumption, educational status, frequency of exercise, menopausal status, and hormone-replacement therapy. The questionnaires were distributed to the subjects in advance of their annual health checkup and were collected at the time of the checkup. The answers to the questionnaire and written consent to view health checkup data were obtained from 3907 men and 1044 women (response rate: men 47.5%, women 47.6%). A total of 708 subjects (516 men, 192 women) were excluded for the following reasons: past history of coronary disease or stroke (n=136; 124 men, 12 women), chronic hepatitis or cirrhosis (n= 26; 23 men, 3 women), low ankle/brachial pressure index (<0.9, n= 12; 11 men, 1 woman), PWV not measured (n= 600; 416 men, 184 women), or blood samples not analyzed (n=3; 3 women). The final study group thus consisted of 3391 male and 852 female subjects.

This study was approved by the institutional ethical board for epidemiological studies of Hokkaido University Graduate School of Medicine, and all subjects gave their written informed consent.

**Data collection**

With respect to smoking habits, subjects were classified into a “nonsmoker” (including never- and ex-smokers) group and a “current smoker” group. The total average amount of alcohol consumed was calculated in grams per day, after taking into account the frequency, amount, and alcohol content of specific beverages. Alcohol consumption was categorized into the following groups for men: “rarely or never”, “≤19.9 g/day”, “20-39.9 g/day”, “40-59.9 g/day”, or “≥60 g/day”. For women, alcohol consumption was categorized into 3 groups: “rarely or never”, “≤19.9 g/day”, or “≥20 g/day”. Drinkers were defined as those with at least weekly alcohol consumption. Subjects were also grouped according to their answer to the question, ‘Other than at work, do you normally exercise so that you perspire?’ Those who exercised one or more times per week were placed in the “>1 per week” group, and those who exercised less than one time per week were placed in the “rarely or never” group. Educational attainment was categorized into “high school education or less (including junior high school and high school education)” or “more than high school education (including junior college, college, and graduate school education).

Anthropometric measures (height and body weight) were recorded using a standardized protocol. The body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

After a 12-hour fast, blood samples were drawn from the antecubital vein of seated subjects using a minimal tourniquet. Specimens were collected in siliconized glass vacuum tubes containing sodium fluoride for the glucose analysis and no additives for the serum.

The total cholesterol (TC) level was measured using an enzymatic method (Wako, Osaka, Japan.). The triglyceride (TG) level was measured using an enzymatic method (Daiichi Pure Chemicals, Tokyo, Japan). The high density lipoprotein cholesterol (HDL-C) level was measured using a direct method (Daiichi Pure Chemicals). Glucose was measured using an amperometric method (ARKRAY, Kyoto, Japan). Uric acid (UA) was measured using an enzymatic method (Daiichi Pure Chemicals). Enzyme activities for GGT, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using commercial reagent kits (Daiichi Pure Chemicals) based on the principles recommended by the Japan Society of Clinical Chemistry [29].

The CRP level was measured by nephelometry, using a latex particle-enhanced immunoassay (N Latex CRP II, Dade Behring, Tokyo, Japan). The assay could detect 0.004 mg/dL of CRP. Undetectable CRP values were recorded as 0.002 mg/dL.

All blood variables except CRP levels were measured at Daiichi Clinical Laboratories, Inc.

(Sapporo, Japan), a commercial hematology laboratory, where the measurements of TC and HDL cholesterol were all standardized by the Lipid Standardized Program, Centers for Disease Control and Prevention, Atlanta, Georgia. CRP was measured at Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan), a commercial hematology laboratory.

Brachial-ankle pulse wave velocity (baPWV) was measured using a volume-plethysmographic apparatus (Form PWV/AVI; model BP-203RPEII, Colin Co., Komaki, Japan). Details about this instrument and its use have been described elsewhere [32-34]. The subjects were examined in the supine position. This device records the phonocardiogram, electrocardiogram, volume pulse form, and the arterial blood pressure of both the left and right arms and ankles. The validation of this method has been previously reported; baPWV has been shown to be significantly correlated with arterial PWV measured directly by catheter pressure transducer ($n=41$, $r=0.87$, $P<0.01$), and the coefficients of variation were 8.4% for interobserver reproducibility and 10.0% for intraobserver reproducibility [32]. Since there was a significant positive correlation between the left and right baPWV ($r=0.95$, $P<0.0001$), we used a mean right and left baPWV value for analysis.

The blood pressure, heart rate (HR), and the ankle brachial index (ABI) were measured using the pulse-wave velocimeter at the same time as PWV was measured. The ABI is the ratio of the ankle systolic blood pressure to the brachial systolic blood pressure; the right and left ABIs were

measured simultaneously. In all of the studies, the baPWV was obtained after the patient had been at rest for at least 5 minutes.

**Statistical analysis**

Since various cardiovascular risk factors and GGT, CRP, and PWV were differently distributed (Mann-Whitney U Test: $P<0.01$ for GGT and CRP; Student's *t*-test for PWV : $p<0.01$), all analyses were performed separately for males and females. The data are presented as mean $\pm$ SD, median (and interquartile range) for variables with a skewed distribution, or percentages. Linear regression analysis was performed to evaluate the association between log CRP and other variables. Subsequently, multiple linear regression analysis was used to clarify the contributions of log GGT to log CRP after adjusting for age, BMI, systolic blood pressure, HR, TC, HDL, FBS, log TG, UA, ALT, smoking status, alcohol consumption, frequency of exercise, hypertension, hyperlipidemia, and diabetes for men; for women, all of the above variables were included, as well as menopausal status. Next, linear regression analysis was done to evaluate the association between PWV and other variables. Then, multiple linear regression analysis was done to clarify the contributions of log GGT to PWV adjusting for age, BMI, systolic blood pressure, HR, TC, HDL, FBS, log TG, UA, log CRP, ALT, smoking status, alcohol consumption, frequency of exercise, hypertension, hyperlipidemia, and diabetes for men; for women, all of the above

variables were included, as well as menopausal status.

P-values <0.05 were considered to be statistically significant. All analyses were conducted using the SPSS software package Version 14 for Windows (SPSS Inc., Chicago, U.S.A.).

**Results**

The characteristics of the male and female subjects are presented in Table 1. The mean ages were 48.3 (SD 6.8) years for males and 46.8 (SD 7.2) years for females. The median CRP values were 0.044 (interquartile range: 0.023-0.089) mg/dL for males and 0.025 (interquartile range: 0.012-0.052) mg/dL for females. The median GGT values were 44 (interquartile range: 29-73) U/L for males and 21 (interquartile range: 16-31) U/L for females. The mean PWV values were 1638 (SD 199) cm/sec for males and 1250 (SD 180) cm/sec for females.

Table 2 shows the correlation coefficients obtained in the linear regression analysis of log CRP and other variables in male and female subjects. In male subjects, age, BMI, hemodynamic variables, parameters that reflect either atherosclerotic risk factors or metabolic disorders (except for total cholesterol), smoking status, frequency of exercise, education, hypertension, hyperlipidemia, AST, ALT, and log GGT were significantly associated with log CRP. In female subjects, age, BMI, hemodynamic variables, parameters that reflect either atherosclerotic risk factors or metabolic disorders, education, hypertension, hyperlipidemia, menopausal status, AST,

ALT, and log GGT were significantly associated with log CRP.

Table 3 shows the results of the multiple regression analysis of log CRP and other variables in male and female subjects. In male subjects, age, BMI, heart rate, log triglycerides, HDL cholesterol, UA, smoking, education, hypertension, and log GGT were significantly associated with log CRP. In female subjects, age, BMI, SBP, heart rate, TC, log triglycerides, HDL cholesterol, UA, and log GGT were significantly associated with log CRP.

Table 4 shows the correlation coefficients obtained in the linear regression analysis of PWV and other variables in male and female subjects. In male subjects, age, BMI, hemodynamic variables, parameters that reflect either atherosclerotic risk factors or metabolic disorders, exercise, education, hypertension, hyperlipidemia, diabetes, AST, ALT, and log GGT were significantly associated with PWV. In female subjects, age, BMI, hemodynamic variables, parameters that reflect either atherosclerotic risk factors or metabolic disorders, education, hypertension, hyperlipidemia, diabetes, menopausal status, AST, ALT, and log GGT were significantly associated with PWV.

Tables 5 shows the results of the multiple regression analysis of PWV and other variables in male and female subjects. In male subjects, log GGT was significantly associated with PWV, but in female subjects, no significant relationships were found.

## Discussion

One previous prospective study found a significant association between serum GGT and CRP levels [13] in the United States. Though the prospective study was valuable, the analysis was adjusted only for race, gender, and age. Two cross-sectional studies (one in the United States [23] and the other Italy [24]) have reported a significant association between serum GGT and CRP levels after adjustment for age, smoking, alcohol consumption, and BMI. Yamada et al. reported that there was a significant association between serum GGT and CRP levels [13] in Japanese males, but the analysis was not adjusted for alcohol consumption and BMI [25]. As previously noted, serum GGT and CRP levels are related to many conventional cardiovascular risk factors; to the best of our knowledge, this is the first study to identify the significant association between serum GGT and CRP levels in Japanese males and females after adjustment for conventional cardiovascular risk factors.

In male subjects, the serum GGT level was significantly associated with PWV after adjustment for conventional cardiovascular risk factors, alcohol consumption, ALT, and CRP. Recent review papers have reported that the serum GGT level is a possible marker of oxidative stress [35, 36]. Some experimental studies have reported that cellular GGT plays an important role in the antioxidant defense system [37-39], but other experimental studies have indicated that ectoplasmic GGT is involved in the generation of reactive oxygen species [40-42].

Epidemiological data have shown that lower dietary intake and lower plasma levels of some antioxidants can predict a future increase in serum GGT levels [43, 44]. However, another report indicated that the serum GGT level could predict future elevation of $F_2$-isoprostanes, which are sensitive markers of oxidative stress [13]. Thus, though the actual role that GGT plays in oxidative stress is controversial, GGT is surely related to oxidative stress.

On the other hand, CRP has been found to be deposited in coronary artery plaque, and it has a pro-oxidative effect on cultured coronary artery smooth muscle cells [45]. The significant relationship that has been found between GGT and CRP is possibly due to their association with oxidative stress, which is a cardiovascular risk factor, since the analyses were not adjusted for markers of oxidative stress.

Waist circumference as a marker of central obesity was better correlated with the GGT level than the BMI [46]. The serum CRP concentration had a significantly positive correlation with parameters of obesity; the correlations with CRP were stronger with parameters of visceral obesity (waist circumference, waist-to-hip ratio, and visceral adipose tissue accumulation) than with the BMI [19]. Therefore, GGT and CRP are possibly related via their relationship to visceral obesity.

Seropositivity for hepatitis B and C viruses may have a role to play in the pathogenesis of carotid atherosclerosis [47, 48]. Seropositivity for hepatitis C virus is an independent predictor for

coronary artery disease [49]. We excluded subjects with chronic hepatitis or cirrhosis, but hepatitis virus carriers were included. Thus, the relationship of GGT to CRP and atherosclerosis that was found may be partially explained by hepatitis virus infection.

The serum GGT level is significantly associated with all-cause mortality [2, 3], myocardial infarction risk [4, 5] and stroke risk [6, 7]. However, previous analyses were not adjusted for the serum CRP level, and the associations that were found might be diluted after such adjustment. Therefore, further studies are needed to clarify whether a high GGT value is an independent predictor of cardiovascular events after adjustment for the serum CRP level.

In female subjects, the adjusted analyses showed that either the serum GGT level was not associated with baPWV value. However, the smaller sample size of females (a quarter of that of male subjects) could have reduced the statistical power of the analyses. In the multiple regression analysis, when adjustment was restricted to demographic variables (age and education), health behavior variables (smoking, alcohol, and exercise), and medical history (hypertension, hyperlipidemia, and diabetes), log GGT was significantly associated with baPWV (beta=0.134, $p<0.0001$). So, overadjustment may reduce the significant association between log GGT and baPWV in female subjects. Furthermore, it is known that females have lower GGT [46] and CRP [20] levels than males, and the baPWV of females is significantly lower than in males among subjects $\leq$55 years old [33]. Therefore, the GGT might have a limited effect on

arterial stiffness in females.

The present study has several limitations. First, this study could not identify a causal role for the relationship that was found between GGT, CRP, and arterial stiffness. Second, we could not obtain the subjects' income data, though we know that the all worked for one local government. Therefore, it is likely that the subjects had similar socioeconomic backgrounds. Furthermore, since our data were adjusted for educational attainment, the influence of socioeconomic status on the adjusted analysis would be practically nil. Finally, the number of female subjects was rather small. Thus, a further study with a larger number of female subjects is required.

In summary, these results suggest that GGT is associated with increased CRP levels in males and females, and that GGT is related to arterial stiffness in males. Given that GGT is easily measured and is extensively used, further studies are needed to elucidate the mechanism responsible for the relationship that exists between GGT and CRP, and to clarify whether GGT is related to atherosclerotic disease after adjustment for cardiovascular risk factors.

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Table 1 Characteristics of the male and female subjects

| | Males (n=3391) | Females (n=852) |
|---|---|---|
| Age (y) | 48.3 ± 6.8 | 46.8 ± 7.2 |
| BMI (kg/m$^2$) | 23.8 ± 2.9 | 21.8 ± 3.4 |
| SBP (mmHg) | 122.8 ± 15.3 | 114.5 ± 15.7 |
| DBP (mmHg) | 77.9 ± 10.9 | 69.3 ± 10.2 |
| Heart rate (bpm) | 60.6 ± 9.5 | 59.6 ± 8.1 |
| Total cholesterol (mg/dl) | 207.5 ± 33.4 | 208.5 ± 32.0 |
| Triglycerides (mg/dl) | 106 (75-153) | 50 (67-92) |
| HDL cholesterol (mg/dl) | 56.7 ± 14.4 | 69.8 ± 15.0 |
| Fasting glucose (mg/dl) | 95.8 ± 20.8 | 88.7 ± 14.4 |
| Uric acid (mg/dl) | 5.9 ± 1.2 | 4.5 ± 1.0 |
| AST (U/L) | 25.5 12.1 | 21.2 8.3 |
| ALT (U/L) | 29.7 20.4 | 19.4 14.2 |
| GGT (U/L) | 44 (29-73) | 21 (16-31) |
| CRP (mg/dl) | 0.044 (0.023-0.089) | 0.025 (0.012-0.052) |
| Current smoker (%) | 49.7 | 24.3 |
| Frequency of exercise (%) | | |
| Rarely or never | 54.6 | 75.7 |
| >1week | 45.4 | 24.3 |
| Educational attainment (%) | | |
| High school education or less | 57.3 | 43.8 |
| More than high school education | 42.7 | 56.2 |
| Alcohol consumption (%) | | |
| Rarely or never | 26.9 | 47.2 |
| ≤19.9g/day | 25.0 | 36.7 |

| | | |
|---|---|---|
| 20-39.9g/day (≥20g/day for female) | 19.0 | 16.1 |
| 40-59.9g/day | 12.1 | |
| ≥60g/day | 16.9 | |
| Medication | | |
| Hypertension (%) | 9.3 | 4.3 |
| Hyperlipidemia (%) | 5.0 | 4.7 |
| Diabetes (%) | 2.2 | 0.8 |
| Menopausal Status (%) | | |
| Postmenopausal | | 39.3 |
| PWV (cm/s) | 1368 ± 199 | 1250 ± 180 |

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage

Table 2 Correlation coefficients on linear regression analysis between log CRP and the relevant variables for male and female subjects

| Variables | Males (n=3391) | | Females (n=852) | |
|---|---|---|---|---|
| Age (y) | 0.08 | ** | 0.20 | ** |
| BMI ($kg/m^2$) | 0.27 | ** | 0.39 | ** |
| SBP (mmHg) | 0.13 | ** | 0.21 | ** |
| DBP (mmHg) | 0.13 | ** | 0.16 | ** |
| Heart rate (bpm) | 0.14 | ** | 0.15 | ** |
| Total cholesterol (mg/dl) | 0.03 | | 0.07 | ** |
| Log Triglycerides (mg/dl) | 0.21 | ** | 0.29 | ** |
| HDL cholesterol (mg/dl) | -0.26 | ** | -0.28 | ** |
| Fasting glucose (mg/dl) | 0.13 | ** | 0.18 | ** |
| Uric acid (mg/dl) | 0.11 | ** | 0.25 | ** |
| AST (U/L) | 0.15 | ** | 0.25 | ** |
| ALT (U/L) | 0.20 | ** | 0.30 | ** |
| Current smoker (vs. ex- or non-smoker) | 0.13 | ** | -0.02 | |
| Exercise ($\geq$1/week vs. rarely or never) | -0.06 | ** | -0.03 | |
| Alcohol consumption [a] | 0.01 | | -0.05 | |
| High school education or less (vs. more than high school education) | 0.08 | ** | 0.11 | ** |
| Hypertension | 0.10 | ** | 0.10 | ** |
| Hyperlipidemia | 0.06 | ** | 0.09 | ** |
| Diabetes | 0.03 | | 0.05 | |
| Postmenopausal (vs. premenopausal) | | | 0.14 | ** |
| Log GGT (U/L) | 0.23 | ** | 0.28 | ** |

[a] Ordinal variables were used ( 0: rarely or never, 1: <19.9 g/day, 2: 20-39.9 g/day, 3: 40-59.9 g/day, 4: >60 g/day for males;  0: rarely or never, 1: <19.9 g/day, 2: >20 g/day for females

** P<0.01

Table 3 Multiple linear regression analysis with log CRP as the dependent variable in male and female subjects

| | Males (n=3391) | | | Females (n=852) | | |
|---|---|---|---|---|---|---|
| Variables | Beta | 95% CI | P value | Beta | 95%CI | P value |
| Age (y) | 0.052 | 0.019 , 0.085 | <0.01 | 0.099 | 0.008 , 0.190 | <0.05 |
| BMI ($kg/m^2$) | 0.148 | 0.111 , 0.185 | <0.0001 | 0.242 | 0.168 , 0.316 | <0.0001 |
| SBP (mmHg) | 0.003 | -0.035 , 0.040 | 0.89 | -0.011 | -0.084 , 0.062 | 0.77 |
| Heart rate (bpm) | 0.081 | 0.048 , 0.114 | <0.0001 | 0.088 | 0.024 , 0.151 | <0.01 |
| Total cholesterol (mg/dl) | 0.000 | -0.035 , 0.035 | 0.99 | -0.092 | -0.169 , -0.016 | <0.05 |
| Log Triglycerides (mg/dl) | -0.053 | -0.096 , -0.010 | <0.05 | 0.085 | 0.004 , 0.166 | <0.05 |
| HDL cholesterol (mg/dl) | -0.206 | -0.247 , -0.165 | <0.0001 | -0.094 | -0.170 , -0.018 | <0.05 |
| Fasting glucose (mg/dl) | 0.036 | -0.001 , 0.073 | 0.06 | 0.018 | -0.052 , 0.088 | 0.62 |
| Uric acid (mg/dl) | 0.043 | 0.010 , 0.075 | <0.05 | 0.085 | 0.018 , 0.153 | <0.05 |
| ALT (U/L) | 0.032 | -0.006 , 0.069 | 0.10 | 0.074 | -0.002 , 0.150 | 0.06 |
| Current smoker (vs. ex- or non-smoker) | 0.102 | 0.070 , 0.135 | <0.0001 | -0.020 | -0.083 , 0.043 | 0.54 |
| Exercise ($\geq$1/week vs. rarely or never) | -0.010 | -0.042 , 0.021 | 0.52 | -0.018 | -0.079 , 0.043 | 0.56 |
| Alcohol consumption [a] | -0.028 | -0.065 , 0.008 | 0.12 | -0.038 | -0.103 , 0.028 | 0.26 |
| High school education or less (vs. more than high school education) | 0.038 | 0.006 , 0.069 | <0.05 | 0.042 | -0.023 , 0.107 | 0.20 |
| Hypertension | 0.049 | 0.015 , 0.082 | <0.01 | -0.037 | -0.102 , 0.028 | 0.26 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Hyperlipidemia | 0.002 | -0.029 , | 0.034 | 0.88 | 0.005 | -0.058 , | 0.068 | 0.87 |
| Diabetes | -0.010 | -0.045 , | 0.024 | 0.56 | 0.024 | -0.040 , | 0.088 | 0.46 |
| Postmenopausal (vs. premenopausal) | | | | | -0.003 | -0.087 , | 0.080 | 0.94 |
| Log GGT (U/L) | 0.168 | 0.126 , | 0.210 | <0.0001 | 0.098 | 0.020 , | 0.176 | <0.05 |

[a] Ordinal variables were used ( 0: rarely or never, 1: <19.9 g/day, 2: 20-39.9 g/day, 3: 40-59.9 g/day, 4: >60 g/day for males; 0: rarely or never, 1: <19.9 g/day, 2: >20g /day for females

Table 4 Correlation coefficients on linear regression analysis between PWV and relevant variables in male and female subjects

| Variables | Males (n=3391) | | Females (n=852) | |
|---|---|---|---|---|
| Age (y) | 0.39 | ** | 0.46 | ** |
| BMI ($kg/m^2$) | 0.10 | ** | 0.20 | ** |
| SBP (mmHg) | 0.69 | ** | 0.74 | ** |
| DBP (mmHg) | 0.64 | ** | 0.65 | ** |
| Heart rate (bpm) | 0.33 | ** | 0.25 | ** |
| Total cholesterol (mg/dl) | 0.06 | ** | 0.31 | ** |
| Log Triglycerides (mg/dl) | 0.18 | ** | 0.34 | ** |
| HDL cholesterol (mg/dl) | 0.01 | ** | -0.12 | ** |
| Fasting glucose (mg/dl) | 0.30 | ** | 0.36 | ** |
| Uric acid (mg/dl) | 0.10 | ** | 0.26 | ** |
| Log CRP (mg/dl) | 0.15 | ** | 0.22 | ** |
| AST (U/L) | 0.15 | ** | 0.17 | ** |
| ALT (U/L) | 0.11 | ** | 0.19 | ** |
| Log GGT (U/L) | 0.28 | ** | 0.26 | ** |
| Current smoker (vs. ex- or non-smoker) | -0.01 | | 0.01 | |
| Exercise ($\geq$1/week vs. rarely or never) | -0.05 | ** | 0.00 | |
| Alcohol consumption [a] | 0.17 | ** | 0.01 | |
| High school education or less (vs. more than high school education) | 0.07 | ** | 0.18 | ** |
| Hypertension | 0.30 | ** | 0.27 | ** |
| Hyperlipidemia | 0.10 | ** | 0.19 | ** |
| Diabetes | 0.12 | ** | 0.11 | ** |

| | |
|---|---|
| Postmenopausal (vs. premenopausal) | 0.36 ** |

[a] Ordinal variables were used ( 0: rarely or never, 1: <19.9 g/day, 2: 20-39.9 g/day, 3: 40-59.9 g/day, 4: >60g/day for males; 0: rarely or never, 1: <19.9 g/day, 2: >20 g/day for females

** P<0.01

Table 5 Multiple linear regression analysis with PWV as the dependent variable in male and female subjects

| | Male (n=3391) | | | Female (n=852) | | |
|---|---|---|---|---|---|---|
| Variables | Beta | 95% CI | P value | Beta | 95%CI | P value |
| Age (y) | 0.228 | 0.204 , 0.251 | <0.0001 | 0.148 | 0.084 , 0.212 | <0.0001 |
| BMI ($kg/m^2$) | -0.138 | -0.164 , -0.112 | <0.0001 | -0.147 | -0.200 , -0.094 | <0.0001 |
| SBP (mmHg) | 0.564 | 0.538 , 0.590 | <0.0001 | 0.618 | 0.567 , 0.670 | <0.0001 |
| Heart rate (bpm) | 0.157 | 0.133 , 0.180 | <0.0001 | 0.100 | 0.055 , 0.144 | <0.0001 |
| Total cholesterol (mg/dl) | -0.004 | -0.028 , 0.021 | 0.77 | 0.069 | 0.015 , 0.124 | <0.05 |
| Log Triglycerides (mg/dl) | 0.034 | 0.003 , 0.064 | <0.05 | 0.010 | -0.047 , 0.068 | 0.72 |
| HDL cholesterol (mg/dl) | 0.003 | -0.026 , 0.033 | 0.82 | -0.021 | -0.075 , 0.033 | 0.45 |
| Fasting glucose (mg/dl) | 0.089 | 0.063 , 0.116 | <0.0001 | 0.097 | 0.047 , 0.146 | <0.001 |
| Uric acid (mg/dl) | 0.043 | 0.020 , 0.067 | <0.001 | 0.055 | 0.007 , 0.102 | <0.05 |
| Log CRP (mg/dl) | 0.031 | 0.007 0.055 | <0.05 | 0.036 | -0.012 0.084 | 0.14 |
| ALT (U/L) | -0.001 | -0.028 , 0.025 | 0.92 | 0.002 | -0.051 , 0.056 | 0.93 |
| Current smoker (vs. ex- or non-smoker) | 0.016 | -0.007 , 0.040 | 0.16 | 0.035 | -0.010 , 0.080 | 0.12 |
| Exercise ($\geq$1/week vs. rarely or never) | -0.039 | -0.062 , -0.017 | <0.001 | -0.001 | -0.044 , 0.042 | 0.95 |
| Alcohol consumption [a] | -0.024 | -0.050 , 0.001 | 0.06 | -0.034 | -0.080 , 0.012 | 0.15 |
| High school education or less (vs. more than high school education) | 0.009 | -0.013 , 0.032 | 0.41 | 0.017 | -0.029 , 0.063 | 0.47 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Hypertension | 0.049 | 0.025 , | 0.072 | <0.0001 | 0.035 | -0.010 , | 0.081 | 0.13 |
| Hyperlipidemia | 0.010 | -0.013 , | 0.032 | 0.39 | 0.032 | -0.013 , | 0.076 | 0.16 |
| Diabetes | 0.018 | -0.006 , | 0.043 | 0.15 | -0.033 | -0.079 , | 0.012 | 0.15 |
| | | | | | 0.026 | -0.032 , | 0.085 | 0.38 |
| Log GGT (U/L) | 0.060 | 0.030 , | 0.090 | <0.0001 | 0.007 | -0.049 , | 0.062 | 0.82 |

[a] Ordinal variables were used ( 0: rarely or never, 1: <19.9 g/day, 2: 20-39.9 g/day, 3: 40-59.9 g/day, 4: >60 g/day for males; 0: rarely or never, 1: <19.9 g/day, 2: >20 g/day for females