Circulating Interleukin-1 β Concentrations Are Independently-Positively Associated with γ -Glutamyltransferase Activity within the Normal Range in Middle-Aged Apparently Healthy Japanese Women

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Summary We previously demonstrated that the circulating concentrations of interleukin (IL)-1 β , which is known to induce the development and progression of type 2 diabetes and its complications, were positively associated with γ -glutamyltransferase (γ -GTP) activity in middle-aged apparently healthy Japanese men. It was still unknown if the association between IL-1 β concentrations and γ -GTP activity is within the normal range in apparently healthy Japanese women. In this study, we conducted a cross-sectional study of 824 apparently healthy women aged 40–64 y [mean $\pm$ standard deviation age, 53.1 $\pm$ 7.1 y; body mass index (BMI), 22.0 $\pm$ 3.1 kg/m²] who participated in health checkups in Japan, and whose γ -GTP activity was within the normal range (<38 U/L). Associations of γ -GTP with IL-1 β and other clinical or lifestyle factors were determined using analysis of variance (ANOVA) and one-way analysis of covariance (ANCOVA) followed by Tukey's multiple-range test. Multivariate logistic regression analyses (MLRA) were performed with γ -GTP activity as the dependent variable; independent variables included IL-1 β plus clinical and lifestyle factors. ANOVA and ANCOVA indicated that IL-1 β concentrations were positively associated with γ -GTP activity. MLRA showed that γ -GTP activity showed trends for higher IL-1 β concentrations after adjusting for age, BMI, energy intake, alcohol intake, and smoking status. Together, IL-1 β concentrations are positively associated with γ -GTP activity within the normal range in middle-aged apparently healthy Japanese women. Our results suggest that γ -GTP activity would be useful for assessing inflammation from the healthy state in Japanese women.

Key Words IL-1 β , γ -GTP, health checkups, Japanese women, cross-sectional study

Metabolic abnormalities such as hyperglycemia and lipid abnormalities induce inflammation of peripheral tissues by impairing insulin action, resulting in the development of type 2 diabetes, hypertension, and cardiovascular diseases (1–3). It was reported that the development of type 2 diabetes is positively associated with plasma concentrations of inflammatory cytokines, including tumor necrosis factor, interleukin (IL)-1 β , IL-6, IL-12, and IL-18 (4–6). Oxidative stress upregulates the expression of inflammatory cytokines, which

promote the development of type 2 diabetes. In cellular studies, inflammatory cytokines such as IL-1 β and IL-6 are induced by oxidative stressors, including reactive oxygen species (ROS) (7–9).

Accumulating evidence has revealed that serum γ -glutamyltransferase (GTP) activity is positively correlated with the subsequent incidence of type 2 diabetes (10–14). Serum γ -GTP activity is widely used as a marker of liver diseases and alcohol intake (11, 12, 15). Recent studies have demonstrated that γ -GTP is involved in the generation of oxidative stress. It is expressed on the surface of many cells, and part of the enzyme is secreted into the blood. γ -GTP is a key enzyme for glutathione (GSH) re-synthesis by catalyzing the first step in the conversion of extracellular oxidized GSH (GSSG) and GSH to glutamine and cysteine-glycine. γ -GTP also generates ROS, including superoxide and hydrogen peroxide, in this step (16, 17). Thus, γ -GTP activity is a possible marker for oxidative stress. In fact, associations

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Abbreviations: ALT, alanine aminotransferase; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AST, aspartate aminotransferase; BMI, body mass index; FBG, fasting blood glucose; GSH, glutathione; GSSG, oxidized glutathione; γ -GTP, γ -glutamyltransferase; HDL, high-density lipoprotein; IL, interleukin; MLRA, multivariate logistic regression analysis; ROS, reactive oxygen species; SD, standard deviation.

of γ -GTP activity with oxidative stress-related diseases, such as type 2 diabetes (10), cardiovascular diseases (18), cancer (19), and chronic kidney diseases (20), have been reported. IL-1 β is a marker for the development of type 2 diabetes and is associated with oxidative stress. Thus, it is very likely that markers of oxidative stress, including IL-1 β , are positively associated with γ -GTP activity. We have previously reported that circulating IL-1 β concentrations are positively associated with γ -GTP activity in middle-aged apparently healthy Japanese men (21). However, in that study of middle-aged men, some had γ -GTP activity beyond the normal range, even though they were not diagnosed with metabolic diseases until the health checkups. In addition, alcohol intake is usually higher in men than in women and it has been reported in many studies that γ -GTP activity is positively associated with alcohol intake (22). Therefore, it is important to examine whether the possible association between circulating IL-1 β concentrations and γ -GTP activity within the normal range (<38 U/L) was observed in apparently healthy Japanese subjects with less alcohol intake, particularly women (23).

Therefore, in this study, we measured plasma IL-1 β concentrations in Japanese women who were not taking medications for any metabolic disease and whose γ -GTP activity was within the normal range.

MATERIALS AND METHODS

Subjects. We conducted a cross-sectional study of 840 apparently healthy women aged 40–64 y who participated in health checkups offered by the city government of Izunokuni (Shizuoka Prefecture, Japan) from June to September 2005. None of the subjects had been diagnosed with metabolic diseases, such as stroke, hypertension, cardiac disease, diabetes, hyperlipidemia, liver disease, kidney disease, gout, psychiatric disease, cancer, or ulcer, before the health checkups. Anthropometric data and blood samples were collected from each subject by trained medical staff. The subjects were also asked about their smoking status and physical activity. Smoking status was classified as never, past, or current. Self-reported physical activity was classified as none, once/wk, 2–3 times/wk, or every day. We excluded women with γ -GTP activity >38 U/L. Analyses were ultimately performed in 824 women.

Alcohol and energy intake during the preceding month was assessed by the Brief Self-administered Diet History Questionnaire (24, 25). All subjects gave informed consent for the use of their personal information in this study. The study protocol was approved by the Ethics Committee of the University of Shizuoka (Shizuoka, Japan) in accordance with the Declaration of Helsinki.

Measurements. Subjects underwent an overnight fast, starting at the latest at 9:00 PM on the day before the health check-ups, and blood samples were collected between 8:30 AM and 9:00 AM on the day of the health check-ups. Thus, all subjects fasted for at least 11.5 h before blood collection. Other parameters including

Table 1. Physical, clinical, and lifestyle characteristics of the study subjects.

Characteristic (n=824)	
Age (y)	53.1 $\pm$ 7.1
BMI (kg/m ²)	22.0 $\pm$ 3.1
Systolic blood pressure (mmHg)	119.9 $\pm$ 18.4
Diastolic blood pressure (mmHg)	70.1 $\pm$ 11.3
Fasting blood glucose (mg/dL)	93.3 $\pm$ 11.6
Total cholesterol (mg/dL)	205.0 $\pm$ 33.2
HDL cholesterol (mg/dL)	66.8 $\pm$ 14.7
Triacylglycerol (mg/dL)	86.0 $\pm$ 44.8
AST (U/L)	18.7 $\pm$ 5.3
ALT (U/L)	16.0 $\pm$ 8.1
γ -GTP (U/L)	14.8 $\pm$ 7.9
IL-1 β (pg/mL)	0.32 $\pm$ 0.52
Alcohol intake (g/d)	5.4 $\pm$ 15.0
Energy intake (kcal/d)	1,706 $\pm$ 405
Smoking	
Number of cigarettes (number/d)	2.5 $\pm$ 6.1
Duration of smoking (y)	3.2 $\pm$ 1.1
Physical activity (times/wk)	
1. Everyday	12.1%
2. 2–3 times/wk	15.0%
3. Once/wk	14.3%
4. Never	58.5%

BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine transferase; γ -GTP, γ -glutamyltransferase; IL-1 β , interleukin-1 β .

Data are expressed as means $\pm$ standard deviation for continuous variables or as the percentage.

height, weight, and blood pressure were measured at the health check-up between 9:00 AM and 11:00 AM. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood biochemical parameters were measured on a JCA-BM2250 analyzer using specific kits for fasting plasma glucose (quick auto neo GLU-HK; Shino-Test Co., Ltd., Tokyo, Japan), serum triacylglycerol (detaminar-TGII; Kyowa Medex Co., Ltd., Tokyo, Japan), total cholesterol (detaminar-TCII; Kyowa Medex Co., Ltd.), high-density lipoprotein (HDL) cholesterol (metabolead HDL-C; Kyowa Medex Co., Ltd.), aspartate aminotransferase (AST) (quick auto neo AST; Shino-Test Co., Ltd.), alanine transaminase (ALT) (quick auto neo ALT; Shino-Test Co., Ltd.), and γ -GTP (quick auto neo γ -GT; Shino-Test Co., Ltd.). Plasma IL-1 β concentrations were measured using an enzyme-linked immunosorbent assay (Quantikine IL-1 β ; R&D Systems, Oxford, UK).

Statistical analysis. The clinical and biochemical data are presented as means $\pm$ standard deviation (SD). Analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to calculate differences among tertiles of subjects divided by γ -GTP activity. Tukey's multiple-range tests based on one-way analysis of covariance (ANCOVA) were performed for systolic blood pressure, diastolic blood pressure, fasting blood glucose (FBG),

Table 2. Characteristics of the subjects according to tertile of γ -GTP activity.

	Low (<11) <i>n</i> =274	Middle (11–16) <i>n</i> =261	High (17–38) <i>n</i> =289	ANOVA	
				<i>F</i> value	<i>p</i> value
Age (y)	52.8±7.3 ^A	52.1±7.5 ^A	54.3±6.3 ^B	7.18	0.0008
BMI (kg/m ²)	21.4±3.1 ^A	22.1±3.0 ^B	22.6±3.1 ^B	10.73	0.0001
Systolic blood pressure (mmHg)	118.8±17.9	119.3±18.2	121.4±18.9	1.64	0.1938
Diastolic blood pressure (mmHg)	67.7±11.3 ^A	69.8±10.9 ^A	72.6±11.3 ^B	13.76	0.0001
Fasting blood glucose (mg/dL)	89.5±8.2 ^A	93.8±12.7 ^B	96.5±12.2 ^C	27.83	0.0001
Total cholesterol (mg/dL)	201.6±31.8 ^A	200.0±32.6 ^A	212.9±33.7 ^B	12.83	0.0001
HDL cholesterol (mg/dL)	67.1±14.0	66.1±14.7	67.2±15.4	0.43	0.6519
Triacylglycerol (mg/dL)	76.6±34.1 ^A	85.9±47.3 ^B	94.8±49.5 ^C	11.94	0.0001
AST (U/L)	17.9±4.0 ^A	18.1±4.7 ^A	20.0±6.6 ^B	13.48	0.0001
ALT (U/L)	15.0±5.5 ^A	14.9±6.3 ^A	18.0±10.8 ^B	13.91	0.0001
IL-1 β (pg/mL)	0.13±0.17 ^A	0.37±0.54 ^B	0.46±0.65 ^B	32.85	0.0001
Alcohol intake (g/d)	6.4±16.7	3.4±8.6	6.2±17.4	3.33	0.0362
Energy intake (kcal/d)	1,745±409	1,664±411	1,706±394	2.66	0.0707
Smoking					
Number of cigarettes (number/d)	2.7±6.0	2.4±6.2	2.3±6.2	0.41	0.6624
Duration of smoking (y)	3.6±8.2	3.3±8.7	2.8±7.9	0.59	0.5547
Physical activity (times/wk) ¹	3.2±1.1	3.2±1.1	3.1±1.1	1.13	0.3226

Post-hoc analyses of the ANOVA were performed using Tukey's multiple-range test.

^{A, B, C} Different letters denote significant differences between groups at $p<0.05$.

BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ -GTP, γ -glutamyltransferase; IL-1 β , interleukin-1 β .

¹ Physical activity: 1, every day; 2, 2 to 3 times per week; 3, once per week; 4, never.

total cholesterol, HDL cholesterol, triacylglycerol, ALT, AST, and IL-1 β in tertiles of γ -GTP activity, with adjustment for age, BMI, smoking, physical activity, alcohol intake, and energy intake. Multivariate logistic regression analysis (MLRA) was performed to identify the factors that were independently associated with γ -GTP activity. The p for trend was assessed using MLRA with ordinal numbers 1–3 assigned to each tertile of γ -GTP activity. For all analyses, a value of $p<0.05$ was considered significant. All statistical analyses were performed using Excel Statistics 2008 software version 1.10 (SSRI Co., Ltd., Tokyo, Japan).

RESULTS

The study subjects were all apparently healthy Japanese women. The characteristics of the subjects are shown in Table 1.

ANOVA followed by Tukey's test was used to assess associations between tertiles of γ -GTP (low, <11 U/L; middle, 11–16 U/L; high, 17–38 U/L) and clinical characteristics. Age, BMI, diastolic blood pressure, FBG, total cholesterol, triacylglycerol, AST, ALT, and IL-1 β were higher in the high tertile than in the low and/or normal tertiles. FBG and triacylglycerol were higher in the middle tertile than in the low tertile. There were no differences in HDL cholesterol, alcohol intake, energy intake, smoking (number of cigarettes or duration of smoking), or physical activity among the three tertiles (Table 2).

We then performed ANCOVA for tertiles of γ -GTP with adjustment for age, BMI, alcohol intake, energy intake,

smoking, and physical activity, and applied Tukey's post hoc test, with a significance level of $p<0.05$. Diastolic blood pressure, FBG, total cholesterol, triacylglycerol, AST, ALT, and IL-1 β were higher in the high tertile than in the low and/or middle tertiles. IL-1 β was also higher in the middle tertile than in the low tertile (Table 3).

We next performed MLRA for tertiles of γ -GTP activity in which age, BMI, diastolic blood pressure, FBG, triacylglycerol, ALT, IL-1 β , energy intake, smoking (number of cigarettes), and physical activity were included as independent variables. For FBG, the odds ratios (ORs) for tertiles 2 and 3 of γ -GTP activity relative to tertile 1 were 1.04 and 1.05, respectively. For IL-1 β , the ORs for tertiles 2 and 3 of γ -GTP activity relative to tertile 1 were 1.56 and 1.90, respectively. MLRA showed that the p for trend between FBG or IL-1 β and tertiles of γ -GTP activity (ordinals 1–3) was statistically significant (Table 4).

DISCUSSION

In the present study, we focused on the associations between γ -GTP activity and metabolic risk factors in apparently healthy Japanese women. Recent studies have demonstrated that γ -GTP is involved in oxidative stress via ROS production (16, 17). In addition, associations between γ -GTP activity and many oxidative stress-related diseases, including type 2 diabetes (10), cardiovascular diseases (18), cancer (19), and chronic kidney diseases (20), have been reported. However, it was also reported that inflammatory cytokines, particu-

Table 3. ANCOVA analysis for clinical parameters associated with tertiles of γ -GTP activity, with adjustment for age, BMI, alcohol intake, energy intake, smoking and physical activity.

	Low (<11 U/L) <i>n</i> =274	Middle (11–16 U/L) <i>n</i> =261	High (17–38 U/L) <i>n</i> =289	ANCOVA	
				<i>F</i> value	<i>p</i> value
Systolic blood pressure (mmHg)	120.2 $\pm$ 1.02	119.8 $\pm$ 1.04	119.6 $\pm$ 1.00	0.09	0.9144
Diastolic blood pressure (mmHg)	68.5 $\pm$ 0.64 ^A	70.1 $\pm$ 0.65 ^{AB}	71.7 $\pm$ 0.62 ^B	6.32	0.0019
Fasting blood glucose (mg/dL)	90.0 $\pm$ 0.66 ^A	94.1 $\pm$ 0.68 ^B	95.8 $\pm$ 0.65 ^B	20.67	0.0001
Total cholesterol (mg/dL)	202.8 $\pm$ 1.84 ^A	201.5 $\pm$ 1.88 ^A	210.4 $\pm$ 1.79 ^B	6.83	0.0011
HDL cholesterol (mg/dL)	66.2 $\pm$ 0.85	66.4 $\pm$ 0.87	67.8 $\pm$ 0.83	1.11	0.3315
Triacylglycerol (mg/dL)	79.6 $\pm$ 2.48 ^A	86.7 $\pm$ 2.54 ^{AB}	91.3 $\pm$ 2.42 ^B	5.73	0.0034
AST (U/L)	18.1 $\pm$ 0.31 ^A	18.4 $\pm$ 0.31 ^A	19.7 $\pm$ 0.30 ^B	7.54	0.0006
ALT (U/L)	15.4 $\pm$ 0.47 ^A	15.1 $\pm$ 0.48 ^A	17.4 $\pm$ 0.45 ^B	7.56	0.0006
IL-1 β (pg/mL)	0.13 $\pm$ 0.03 ^A	0.36 $\pm$ 0.03 ^B	0.47 $\pm$ 0.03 ^C	32.43	0.0001

^{A, B, C} Values not sharing common superscript letters are significantly different from one another by Tukey's test at $p < 0.05$ based on one-way ANCOVA.

Data are presented as the least-square mean $\pm$ standard error.

ANCOVA, analysis of covariance; BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ -GTP, γ -glutamyltransferase; IL-1 β , interleukin-1 β .

Table 4. Multivariable logistic regression analysis for tertiles of γ -GTP activity.

Variables	T2		T3		<i>p</i> for trend
	OR	CI	OR	CI	
Age (y)	0.97	(0.94, 0.99)	0.99	(0.96, 1.03)	0.7141
BMI (kg/m ²)	1.02	(0.95, 1.09)	1.00	(0.94, 1.08)	0.3428
Diastolic blood pressure (mmHg)	1.01	(0.99, 1.03)	1.03	(1.01, 1.04)	0.0064
Fasting blood glucose (mg/dL)	1.04	(1.01, 1.06)	1.05	(1.03, 1.08)	0.0001
Triacylglycerol (mg/dL)	1.01	(1.00, 1.01)	1.01	(1.00, 1.01)	0.0031
ALT (U/L)	1.01	(0.98, 1.05)	1.06	(1.02, 1.10)	0.0005
IL-1 β (pg/mL)	1.56	(1.30, 1.86)	1.90	(1.51, 2.39)	0.0001
Alcohol intake (g/d)	0.99	(0.97, 1.00)	1.01	(0.99, 1.02)	0.2237
Energy intake (kcal/d)	1.00	(0.99, 1.00)	1.00	(0.99, 1.00)	0.1599
Number of cigarettes (number/d)	0.99	(0.96, 1.02)	0.98	(0.95, 1.02)	0.5702
Physical activity (times/wk) ¹	0.95	(0.80, 1.13)	0.93	(0.78, 1.12)	0.3504

OR, odds ratio; CI, confidence interval; γ -GTP, γ -glutamyltransferase; ALT, alanine aminotransferase; IL-1 β , interleukin-1 β . Tertiles of γ -GTP: T1, 2–10 U/L (n =274); T2, 11–16 U/L (n =261); T3, 17–38 U/L (n =289).

T1 was coded as 0, and T2 or T3 was coded as 1 in multivariate logistic regression analyses.

The p for trend was determined by multivariate linear regression analysis with ordinal number 1–3 assigned to the tertiles of γ -GTP activity.

¹ Physical activity: 1, every day; 2, 2 to 3 times per week; 3, once per week; 4, never.

larly IL-1 β , are associated with the production of ROS (7–9). Therefore, we hypothesized that IL-1 β concentrations are positively associated with circulating γ -GTP activity. In fact, we previously reported that circulating IL-1 β concentrations are positively associated with γ -GTP activity in middle-aged apparently healthy Japanese men (21). However, it remained unclear whether IL-1 β concentrations were positively associated with γ -GTP activity within the normal range, and whether a positive association between these parameters would be found in apparently healthy Japanese women. Therefore, we examined whether IL-1 β concentrations were

positively associated with serum γ -GTP activity within the normal range (<38 U/L) in apparently healthy Japanese women.

In this study, we used ANCOVA with adjustment for age, BMI, and lifestyle factors, such as alcohol intake, energy intake, smoking, and physical activity, and found that diastolic blood pressure, FBG, total cholesterol, LDL cholesterol, triacylglycerol, AST, ALT, and IL-1 β were higher in the higher tertiles of γ -GTP activity than in the lower tertiles. These trends are consistent with previous studies performed in other Western and Asian countries (12, 21, 26). Interestingly,

there was a very strong positive association between IL-1 β concentrations and circulating γ -GTP activity. Furthermore, MLRA showed that this association was independent of other clinical parameters, including blood pressure, FBG, triacylglycerol, and ALT, as well as lifestyle factors, particularly alcohol intake. Therefore, the present results indicate that IL-1 β concentrations are positively and independently associated with γ -GTP activity in apparently healthy Japanese women. In particular, γ -GTP activity of the subjects in this population was within the normal range, and alcohol intake in the women (5.4 ± 15.0 g/d) was lower than in the men in same population reported by a previous study (30.2 ± 37.6 g/d) (27). In addition, the association between alcohol intake and γ -GTP activity was not observed in middle-aged apparently healthy Japanese women in this population. These results indicate that γ -GTP activity in apparently healthy Japanese women was positively associated with IL-1 β concentrations and the association was less affected by the alcohol intake and liver injury. These insights suggest that higher levels of γ -GTP activity within normal range may be related to oxidative stress and inflammation in the body and γ -GTP activity would be useful for assessing inflammation from the healthy state in women. Future studies should examine whether the association observed in this cohort of women is also evident in apparently healthy Japanese men who consume no or very small amounts of alcohol. It should be examined whether higher levels of γ -GTP activity without liver injury and less alcohol intake in women and men have higher incidences of oxidative stress- and inflammation-associated diseases including type 2 diabetes, hypertension and CVD. In addition, there needs to be examined whether intakes of antioxidant such as vitamin C, vitamin E, carotenoids and flavonoids are associated with reduction of circulating γ -GTP activity and IL-1 β concentrations and subsequent incidences of oxidative stress- and inflammation-associated diseases.

γ -GTP is expressed on the surface of many cells and some of the enzyme is secreted into the blood. It plays a key role in GSH re-synthesis by catalyzing the first step in the re-synthesis of GSH. In this process, γ -GTP generates ROS, including superoxide and hydrogen peroxide (16, 17). It was reported that circulating γ -GTP activity is positively associated with oxidative stress markers, such as nitrotyrosine and lipid peroxide (28–30). Furthermore, it was shown that C-reactive protein, an inflammatory marker, is positively associated with circulating γ -GTP activity in healthy subjects and in patients with acute coronary syndrome (31, 32). Inflammatory cytokines, including IL-1 β , are also associated with the production of ROS (7–9). Therefore, future studies should examine the possible associations of γ -GTP activity with DNA oxidative stress markers such as 8-hydroxydeoxyguanosine in apparently healthy Japanese women and men.

It should be noted that FBG concentrations were associated with γ -GTP activity in this study. It was reported that hyperglycemia induces oxidative stress by induc-

ing the production of ROS (33, 34). We previously reported that constant hyperglycemia and intermittent postprandial hyperglycemia in rats upregulates IL-1 β mRNA expression in peripheral leukocytes (35–37). Furthermore, we demonstrated that FBG concentrations are strongly and positively associated with plasma IL-1 β concentrations in apparently healthy Japanese men (27). In addition, the self-reported rate of eating, a lifestyle that could increase postprandial hyperglycemia with faster eating in particular, was positively associated with plasma IL-1 β concentrations in apparently healthy Japanese men (38). In addition, our previous reports demonstrated that circulating IL-1 β , IL-6 and C-reactive protein (CRP) concentrations are also positively associated with FBG concentrations in apparently healthy Japanese men (27, 39). The associations of FBG concentrations with IL-1 β , IL-6 and CRP concentrations by Spearman's correlation coefficients were 0.344, 0.287 and 0.103, respectively. These results indicate that γ -GTP activity, and IL-1 β and IL-6 concentrations may be useful predictors for development of type 2 diabetes. In particular, the association of IL-1 β , but not IL-6, with FBG concentrations was observed after adjusting for age and BMI. The association between circulating IL-1 β and FBG concentrations in the apparently healthy Japanese women in this population should be examined.

Our study has a limitation, as our cross-sectional study design could not identify the causal relationship between circulating γ -GTP activity and IL-1 β concentrations. Therefore, it will be necessary to examine the association between IL-1 β concentrations and γ -GTP activity in prospective studies.

In conclusion, our results suggest that IL-1 β concentrations are positively and independently associated with serum γ -GTP activity within the normal range in apparently healthy Japanese women.

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