Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 914 – 922
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Circulating interleukin-1β and interleukin-6 concentrations are closely associated with γ-glutamyltranspeptidase activity in middle-aged Japanese men without obvious cardiovascular diseases

Kazuki Mochizuki[a,1], Yasumi Misaki[a,2], Rie Miyauchi[a,2], Satsuki Takabe[a,2], Masaya Shimada[a,2], Noriyuki Miyoshi[b,3], Yoko Ichikawa[c,2], Toshinao Goda[a,*,4]

[a]*Laboratory of Nutritional Physiology and Global COE Program, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka, 422-8526, Japan*
[b]*Laboratory of Biochemistry, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*
[c]*Laboratory of Food Management, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka, 422-8526, Japan*

Received 16 March 2010; accepted 26 August 2010

Abstract

Interleukin (IL)-1β and IL-6 expressions are known to be induced by oxidant stress. In the present study, we examined the relationships between these interleukins and the activity of γ-glutamyltranspeptidase (γ-GTP), which was recently reported as a source of oxidant stress production, in the circulating blood of middle-aged Japanese men without obvious cardiovascular diseases. We conducted a cross-sectional study of 317 Japanese men without obvious cardiovascular diseases aged 40 to 69 years (mean ± SD, 58.6 ± 7.6 years) who participated in health checkups in Japan. We analyzed their clinical parameters in serum, lifestyle factors, and plasma IL-1β and IL-6 concentrations. We compared the relationships between these interleukin concentrations and the clinical parameters and lifestyle factors by Spearman correlation coefficients. Stepwise multiple linear regression analyses for interleukins based on the other parameters and γ-GTP, which were classified into 3 groups according to the concentrations, were performed. Interleukin-1β and IL-6 concentrations were closely associated with γ-GTP activity but less associated with alanine aminotransferase and aspartate aminotransferase activities by Spearman correlation coefficients. Stepwise multiple linear regression analyses showed that γ-GTP activity was the explanatory variable for elevated IL-1β and IL-6 concentrations. As natural logarithms, the IL-1β and IL-6 concentrations were estimated to be 1.734- and 1.157-fold higher, respectively, in subjects with high γ-GTP activity ranges than in subjects with a low γ-GTP activity range. The present results show that circulating IL-1β and IL-6 concentrations are strongly and independently associated with γ-GTP activity in middle-aged Japanese men without obvious cardiovascular diseases.

1. Introduction

Metabolic parameter abnormalities such as hyperglycemia and lipid abnormalities induce the development of diabetes and related complications such as cardiovascular diseases, hypertension, and inflammation of peripheral tissues by impairing insulin actions in various tissues [1-3]. In particular, it has been reported that the development of diabetes in people is associated with elevated plasma protein levels of circulating inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-12, IL-18, and tumor necrosis factor (TNF)–α [4-6]. In addition, these cytokines induce macrophage infiltration into the vascular endothelium and increase the risk of atherosclerosis [7,8]. Among these

* Corresponding author. Laboratory of Nutritional Physiology, School of Food and Nutritional Sciences, The University of Shizuoka, 52-1 Yada, Shizuoka-shi, Shizuoka 422–8526, Japan. Tel.: +81 54 264 5533; fax: +81 54 264 5565.
E-mail address: gouda@u-shizuoka-ken.ac.jp (T. Goda).
[1] Performed the analyses, contributed to the experiments and data collection, and wrote the manuscript.
[2] Contributed to the data collection.
[3] Contributed to raising the idea of a relationship between γ-glutamyltranspeptidase and reactive oxygen species production and to writing a discussion about it.
[4] Organized the study.

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.08.011

cytokines, IL-1β and IL-6 in particular are thought to be important for predicting the risk of inflammation and the onset of diabetes [4]. Indeed, several studies including ours have demonstrated that circulating IL-1β and IL-6 concentrations are positively associated with moderate abnormal glucose tolerance as well as obesity and type 2 diabetes mellitus in Western countries and Japan [4,9-13]. Thus, it is important to reduce circulating IL-1β and IL-6 concentrations to reduce the development and progression of diabetes and its complications.

Many studies using various cell lines such as T-cells, alveolar epithelial cells, and carcinoma cells have suggested that inflammatory cytokines including IL-1β and IL-6 are induced by oxidant stress such as the production of reactive oxygen species (ROS) [14-16]. The ROS production induced by hyperglycemia is known to be caused by activation of oxidative phosphorylation in mitochondria, glucose autoxidation, and some enzymes involved in ROS production such as nicotinamide adenine dinucleotide phosphate oxidase (Nox), cytochrome p450, lipoxygenases, xanthine oxidase, and nitric oxide synthase [17,18]. Recent studies have demonstrated that γ-glutamyltranspeptidase (γ-GTP), which is a marker for liver injury in blood as a hepatic disorder enzyme, is involved in oxidant stress production. γ-Glutamyltranspeptidase is expressed on the surface of many cells, and some of the enzyme is secreted into the blood. γ-Glutamyltranspeptidase is a key enzyme for glutathione (GSH) resynthesis because it catalyzes the first step of conversion of extracellular oxidized GSH (GSSG) and GSH to cysteine and glycine for resynthesis of GSH. During this reaction, γ-GTP produces ROS such as superoxide and hydrogen peroxide [19,20]. Indeed, higher γ-GTP activity is found in tumor cells; and the ROS production by γ-GTP induces cell proliferation [20]. Regarding diabetes, the function of γ-GTP activity for ROS production remains unknown, although several studies have demonstrated that circulating γ-GTP is associated with oxidant stress markers such as lipid peroxides and the inflammation marker C-reactive protein [21-23]. In addition, many studies have shown that circulating γ-GTP is elevated before the development of diabetes without hepatic disorder; and this elevation can predict the development of diabetes and metabolic syndrome in Western countries and Asian countries including Japan [24-27]. Because circulating IL-1β and IL-6 are also known as markers for predicting the development of diabetes [4,9-13] and are induced by ROS production, it is very likely that circulating IL-1β and IL-6 concentrations are strongly associated with circulating γ-GTP activity in subjects without obvious cardiovascular diseases. However, no studies have examined the associations between these interleukin concentrations and γ-GTP activity in blood.

Therefore, in the present study, we compared the circulating IL-1β and IL-6 concentrations and γ-GTP activity in 317 Japanese subjects who did not take medications for any metabolic diseases.

2. Methods

2.1. Study population

We conducted a cross-sectional study of 317 Japanese men without obvious cardiovascular diseases aged 40 to 69 years (mean ± SD, 58.6 ± 7.6 years) who participated in health checkups offered by the city government of Izunokuni (Shizuoka Prefecture, Japan) from June 2005 to September 2005. Anthropometric data and blood samples were collected from each participant by trained medical staff. The participants were also asked about their smoking status and self-reported physical activity. Smoking status was classified as never, past, or current; and self-reported physical activity was classified as none, once per week, 2 to 3 times per week, or every day. We excluded people who were being treated for stroke, hypertension, cardiac disease, diabetes, hyperlipidemia, liver disease, kidney disease, or gout. 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).

2.2. Measurements

Height, weight, fasting serum glucose, triacylglycerol, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured in the morning after an overnight fast. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Plasma samples were kept at −80°C for subsequent assays. Alcohol and energy intakes during the preceding month were assessed with a brief self-administered diet history questionnaire [28]. Plasma insulin levels were measured by a solid-phase 2-site enzyme immunoassay (Ultrasensitive Insulin ELISA Kit; Mercodia, Uppsala, Sweden). Plasma total adiponectin levels were measured using an enzyme-linked immunosorbent assay kit (Adiponectin ELISA Kit; Otsuka Pharmaceutical, Tokyo, Japan). Plasma IL-1β and IL-6 levels were measured by enzyme-linked immunosorbent assays (Quantikine IL-1β or IL-6 Kits; R&D Systems, Oxford, United Kingdom).

Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: fasting blood glucose (in milligrams per 100 milliliters) × fasting plasma insulin (in microunits per liter)/405.

2.3. Statistical analysis

The clinical and biochemical data of the subjects are presented as means ± SD. Spearman rank correlation coefficient analyses were used to calculate the correlations for all subjects. The Jonckheere-Terpstra test was used to calculate the correlations among 3 groups according to the IL-1β and IL-6 concentrations. In the multiple linear regression analyses, the natural logarithmic values of IL-1β and IL-6 were used because their distributions were

positively skewed. In addition, explanatory variables (excluding categorized explanatory variables) without linearity to each response value (IL-1β or IL-6) in scatter plots were converted to their natural logarithmic values because explanatory variables excluding categorized explanatory variables should have linearity in scatter plots with the response values. Stepwise multiple linear regression analyses were performed to extract the explanatory variables among the parameters for the IL-1β and IL-6 concentrations.

For all analyses, a value of $P < .05$ was considered significant. All statistical analyses were performed using SAS software version 9.1.3 (SAS Institute, Cary, NC).

3. Results

The study subjects were all Japanese men without obvious cardiovascular diseases who ranged in age from 40 to 69 years (mean ± SD, 58.6 ± 7.6 years). The mean BMI was 23.2 ± 2.9 kg/m^2, and the mean waist circumference was 84.4 ± 7.7 cm. The mean fasting blood glucose was 103 ± 23 mg/dL, and the mean fasting plasma insulin was 5.17 ± 6.2 mU/L. The mean aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-GTP concentrations were 23.3 ± 10, 24.1 ± 14, and 41.9 ± 75 U/L, respectively. The mean plasma adiponectin, IL-1β, and IL-6 concentrations were 5.50 ± 3.0 mg/L, 1.67 ± 2.6 pg/mL, and 3.38 ± 3.3 pg/mL, respectively (Table 1).

We investigated the correlations between the fasting plasma IL-1β and IL-6 concentrations and the serum clinical characteristics by Spearman correlation coefficient analyses. The IL-1β concentration was positively associated with IL-6, BMI, waist circumference, fasting blood glucose, triacylglycerol, AST, γ-GTP, insulin, and HOMA-IR, and negatively associated with HDL cholesterol and self-reported physical activity. The IL-6 concentration was positively associated with IL-1β, age, BMI, diastolic blood pressure, fasting blood glucose, triacylglycerol, AST, γ-GTP, insulin, and HOMA-IR, and negatively associated with HDL cholesterol and creatinine (Table 2). After dividing the subjects into 3 groups based on their IL-1β concentrations (0-0.16, 0.17-1.19, and 1.20-11.20 pg/mL), positive trends were observed for IL-6, BMI, fasting blood glucose, triacylglycerol, AST, γ-GTP, insulin, and HOMA-IR, whereas a negative trend was

Table 1
Physical characteristics, anthropometric and body composition measurements, and lifestyle habits in 317 middle-aged men

Characteristics	Category	Means ± SD or percentage	n
Age (y)		58.6 ± 7.6	317
Body height (cm)		166.4 ± 6.2	317
BMI (kg/m^2)		23.2 ± 2.9	317
Waist circumference (cm)		84.4 ± 7.7	317
Smoking			
No. of cigarettes (n/d)		12.2 ± 12.0	293
Duration of smoking (y)		15.8 ± 16.1	293
Self-reported physical activity	Every day	18.6%	59
	2-3 times per week	16.4%	52
	Once per week	10.7%	34
	Never	45.7%	145
	Unknown	8.5%	27
Alcohol intake (g/d)		30.2 ± 37.6	316
Energy intake (kcal/d)		2152.4 ± 520.4	303
Systolic blood pressure (mm Hg)		126.9 ± 16.9	317
Diastolic blood pressure (mm Hg)		76.9 ± 12.0	317
Fasting blood glucose (mg/dL)		103.2 ± 23.3	308
Total cholesterol (mg/dL)		196.9 ± 31.7	317
HDL cholesterol (mg/dL)		54.5 ± 15.1	317
Triacylglycerol (mg/dL)		134.1 ± 124.2	308
AST (U/L)		23.3 ± 10.4	317
ALT (U/L)		24.1 ± 13.9	317
γ-GTP (U/L)		41.9 ± 75.0	317
Creatinine (mg/dL)		0.80 ± 0.11	317
Insulin (mU/L)		5.17 ± 6.23	295
HOMA-IR[a]		1.40 ± 2.06	295
Adiponectin (mg/L)		5.50 ± 2.98	284
IL-1β (pg/mL)		1.67 ± 2.58	317
log(IL-1β)[b]		-0.667 ± 1.546	317
IL-6 (pg/mL)		3.38 ± 3.27	317
log(IL-6)[b]		0.647 ± 1.224	316

[a] HOMA-IR = fasting blood glucose × fasting insulin/405.
[b] Natural logarithmic transformation.

Table 2
Correlations between the cytokine concentrations and subjects characteristics

	IL-1β					IL-6				
Groups by interleukin conc (n)	Spearman correlation coefficient	Low (105)	Middle (112)	High (100)	*P* for Trend	Spearman correlation coefficient	Low (105)	Middle (110)	High (102)	*P* for trend
Ranges (pg/mL)		0-0.16	0.17-1.19	1.20-11.20			0-1.09	1.10-3.99	4.00-16.60	
IL-1β	–	0.11 ± 0.03	0.43 ± 0.26	4.69 ± 2.77	<.001	0.758‡	0.18 ± 0.18	0.77 ± 1.11	4.14 ± 3.13	<.001
IL-6	0.758‡	1.18 ± 1.34	2.67 ± 2.22	6.62 ± 3.30	<.001	–	0.56 ± 0.29	2.39 ± 0.88	7.34 ± 2.7	<.001
Age (y)	0.093	58.2 ± 8.4	58.1 ± 6.7	59.6 ± 7.7	.224	0.185‡	56.7 ± 8.0	59.4 ± 7.0	59.7 ± 7.7	.002
Body height (cm)	−0.046	167.2 ± 6.0	166.4 ± 6.2	165.8 ± 6.6	.133	−0.296	167.3 ± 5.6	166.2 ± 6.6	165.9 ± 6.5	.135
BMI (kg/m^2)	0.187‡	22.8 ± 2.8	23.0 ± 2.9	23.9 ± 2.8	.005	0.138*	23.0 ± 2.6	22.8 ± 3.0	23.9 ± 2.9	.029
Waist circumference (cm)	0.139*	83.8 ± 7.4	83.8 ± 7.9	85.6 ± 7.5	.107	0.083	84.0 ± 7.1	83.5 ± 7.7	85.6 ± 8.0	.167
Systolic blood pressure (mm Hg)	0.010	127.7 ± 18.9	126.9 ± 17.0	126.0 ± 14.6	.914	0.081	125.3 ± 19.1	127.8 ± 16.7	127.6 ± 14.7	.13
Diastolic blood pressure (mm Hg)	0.084	75.6 ± 12.3	77.1 ± 13.2	78.2 ± 10.0	.157	0.166†	74.7 ± 13.8	77.2 ± 11.1	79.0 ± 10.6	.006
Fasting blood glucose (mg/dL)	0.335‡	98.8 ± 13.1	100.7 ± 28.6	110.4 ± 23.6	<.001	0.297‡	100.6 ± 29.8	100.3 ± 13.0	108.7 ± 23.7	<.001
Total cholesterol (mg/dL)	−0.019	197.9 ± 32.7	199.2 ± 30.0	193.3 ± 32.6	.379	−0.073	199.6 ± 31.9	197.2 ± 31.4	193.9 ± 31.9	.214
Triacylglycerol (mg/dL)	0.158†	118.8 ± 94.3	147.2 ± 168.6	135.2 ± 86.7	.01	0.122*	137.1 ± 177.9	127.4 ± 85.3	138.1 ± 89.3	.038
HDL cholesterol (mg/dL)	−0.139*	55.7 ± 13.6	55.6 ± 17.0	52.1 ± 14.1	.029	−0.111*	54.5 ± 14.0	57.3 ± 16.1	51.6 ± 14.5	.058
AST (U/L)	0.175†	21.5 ± 7.1	23.5 ± 11.7	25.0 ± 11.6	.002	0.124*	21.6 ± 5.8	22.7 ± 7.8	25.6 ± 15.2	.06
ALT (U/L)	0.070	23.0 ± 12.2	23.1 ± 15.1	26.2 ± 14.1	.134	0.017	23.7 ± 12.0	21.9 ± 9.4	26.8 ± 18.7	.57
γ-GTP (U/L)	0.486‡	23.3 ± 19.0	35.8 ± 35.0	68.1 ± 122.9	.001	0.414‡	24.5 ± 17.9	32.3 ± 22.5	70.1 ± 124.6	<.001
Creatinine (mg/dL)	−0.090	0.81 ± 0.12	0.82 ± 0.11	0.78 ± 0.11	.059	−0.156†	0.82 ± 0.12	0.81 ± 0.12	0.77 ± 0.10	<.001
Insulin (mU/L)	0.215‡	4.59 ± 5.67	4.66 ± 4.93	6.34 ± 7.76	.003	0.163†	4.13 ± 4.79	5.38 ± 6.06	6.02 ± 7.47	.002
HOMA-IR	0.254‡	1.16 ± 1.49	1.31 ± 2.43	1.76 ± 2.13	<.001	0.202‡	1.19 ± 2.47	1.36 ± 1.56	1.66 ± 2.05	<.001
Adiponectin (mg/L)	0.031	5.33 ± 2.92	5.28 ± 2.41	5.88 ± 3.55	.385	−0.019	5.53 ± 2.89	5.55 ± 3.01	5.42 ± 3.07	.569
Smoking										
No. of cigarettes (n/d)	0.070	10.9 ± 12.4	13.0 ± 11.8	12.5 ± 12.0	.266	0.063	11.8 ± 12.1	11.6 ± 12.3	13.1 ± 11.8	.417
Duration of smoking (y)	0.048	14.4 ± 15.9	16.9 ± 16.3	16.0 ± 16.1	.57	0.066	15.2 ± 15.2	14.4 ± 16.0	17.8 ± 16.9	.259
Self-reported physical activity[a]	−0.151†	3.0 ± 1.2	2.9 ± 1.2	2.8 ± 1.3	.138	−0.110	3.1 ± 1.2	2.8 ± 1.2	2.8 ± 1.3	.17
Alcohol intake (g/d)	−0.063	29.7 ± 33.0	32.6 ± 42.6	28.0 ± 36.1	.211	−0.085	31.4 ± 36.9	30.6 ± 39.7	28.6 ± 36.2	.245
Energy intake (kcal/d)	−0.005	2111.9 ± 485.0	2194.1 ± 551.0	2147.5 ± 522.5	.73	0.927	2189.2 ± 508.8	2133.3 ± 526.9	2135.0 ± 528.7	.326

P values for trends were calculated using the Jonckheere-Terpstra test among the 3 groups based on the interleukin concentrations.
Spearman correlation coefficients between the interleukin concentrations and other parameters were calculated for all subjects:

* $P < .05$.

† $P < .01$.

‡ $P < .001$.

[a] Self-reported physical activity: 1 = every day; 2 = 2 to 3 times per week; 3 = once per week; 4 = never.

Table 3
Regression analyses of parameters as explanatory variables for the IL-1β and IL-6 concentrations

Response variables	Explanatory variables	Category	Initial model[c]			Final model[d]		
			β	SE	P	β	SE	P
log(IL-1β)[a] (n = 260)	Intercept		−13.142	56.380	.82	−8.522	2.234	<.001‡
	Age		0.025	0.011	.03*	0.024	0.010	.02*
	BMI		−0.002	0.034	.96			
	log(fasting blood glucose)		2.208	9.377	.81	1.240	0.491	.01*
	log(triacylglycerol)		−0.135	0.190	.48			
	log(HDL cholesterol)		−0.335	0.393	.4			
	log(AST)		0.094	0.289	.74			
	γ-GTP	Low (0-20 U/L)	Reference	–	–	Reference	–	–
		Middle (21-34 U/L)	1.100	0.207	<.001‡	1.095	0.188	<.001‡
		High (≥35 U/L)	1.624	0.234	<.001‡	1.734	0.198	<.001‡
	log(insulin)		1.352	9.358	.89			
	log(HOMA-IR)		−1.220	9.371	.9			
	Alcohol intake		−0.005	0.002	.040*	−0.006	0.002	0†
	Smoking (no. of cigarettes)		0.007	0.007	.29			
	Self-reported physical activity[b]		−0.053	0.07	.450			
log(IL-6)[a] (n = 259)	Intercept		−15.233	47.569	.75	−1.704	0.722	.02*
	Age		0.034	0.009	<.001‡	0.032	0.008	<.001‡
	BMI		−0.020	0.030	.49			
	Diastolic blood pressure		0.014	0.006	.03*	0.014	0.005	.01†
	log(fasting blood glucose)		2.503	7.911	.75			
	log(triacylglycerol)		−0.098	0.158	.54			
	HDL cholesterol		−0.002	0.006	.72			
	AST		0.005	0.007	.44			
	γ-GTP	Low (0-20 U/L)	Reference	–	–	Reference	–	–
		Middle (21-34 U/L)	0.727	0.175	<.001‡	0.709	0.151	<.001‡
		High (≥35 U/L)	1.106	0.196	<.001‡	1.157	0.156	<.001‡
	Creatinine		−1.499	0.635	.02*	−1.339	0.559	.02*
	log(insulin)		2.099	7.896	.79			
	log(HOMA-IR)		−2.062	7.907	.79			
	Alcohol intake		−0.006	0.002	0†	−0.006	0.002	0†
	Smoking (no. of cigarettes)		0.01	0.01	.06			
	Self-reported physical activity[b]		−0.02	0.060	.76			

[a] Natural logarithmic transformation.
[b] Self-reported physical activity: 1 = every day; 2 = 2 to 3 times per week; 3 = once per week; 4 = never.
[c] Criteria for selecting parameters in the initial model.
[d] Explanatory variables were selected by a backward elimination procedure ($P < .05$) from the initial model.
* $P < .05$.
† $P < .01$.
‡ $P < .001$.

observed for HDL cholesterol. After dividing the subjects into 3 groups based on their IL-6 concentrations (0-1.09, 1.10-3.99, and 4.0-16.60 pg/mL), positive trends were observed for IL-1β, age, BMI, diastolic blood pressure, fasting blood glucose, triacylglycerol, γ-GTP, creatinine, insulin, and HOMA-IR.

Next, we performed multiple linear regression analyses based on the results of the Spearman correlation coefficient analyses. Twelve variables were selected for the initial multiple linear regression model for log(IL-1β). After backward elimination selection, 4 variables, namely, age, log(fasting blood glucose), γ-GTP, and alcohol intake, were selected for the final model. Multiple linear regression analyses for IL-1β using the initial model showed that age was a positive explanatory variable for the plasma IL-1β concentration, whereas alcohol intake was a negative explanatory variable for the plasma IL-1β concentration. Regarding the γ-GTP activity, we performed multiple linear regression analyses in 3 groups divided according to the γ-GTP activity (low, middle, and high), and found not only that γ-GTP activity was a strong positive explanatory variable for the plasma IL-1β concentration, but also that the middle– and high–γ-GTP activity groups had plasma IL-1β concentrations as natural logarithms that were 1.100-fold and 1.624-fold higher, respectively, than the natural logarithm in the low–γ-GTP activity group. Stepwise multiple linear regression analyses of the final model, comprising a model that is analyzed using all parameters with values of $P < .05$ and then modified by decreasing the parameters step by step from the greatest P value, revealed that age and fasting blood glucose were positive explanatory variables for the plasma IL-1β concentration, whereas alcohol intake was a negative explanatory variable for the plasma IL-1β concentration. The middle– and high–γ-GTP

activity groups had plasma IL-1β concentrations as natural logarithms that were 1.095-fold and 1.735-fold higher, respectively, than the natural logarithm in the low–γ-GTP activity group.

Based on the results of the Spearman correlation coefficients, 14 variables were included in the initial multiple linear regression model for log(IL-6). After backward elimination selection, 5 variables, namely, age, diastolic blood pressure, γ-GTP, creatinine, and alcohol intake, were selected for the final model. Multiple linear regression analyses for IL-6 using the initial model showed that age and diastolic blood pressure were positive explanatory variables for the plasma IL-6 concentration, whereas creatinine and alcohol intake were negative explanatory variables for the plasma IL-6 concentration. γ-GTP activity was a positive explanatory variable for the plasma IL-6 concentration; and the middle– and high–γ-GTP activity groups had plasma IL-6 concentrations as natural logarithms that were 0.727-fold and 1.106-fold higher, respectively, than the natural logarithm in the low–γ-GTP activity group. Stepwise multiple linear regression analyses of the final model revealed that age and diastolic blood pressure were positive explanatory variables for the IL-6 concentration, whereas creatinine and alcohol intake were negative explanatory variables for the IL-6 concentration. The middle– and high–γ-GTP activity groups had IL-6 concentrations as natural logarithms that were 0.709-fold and 1.157-fold higher, respectively, than the natural logarithm in the low–γ-GTP activity group (Table 3).

4. Discussion

In the present study, we focused on the relationships between the IL-1β and IL-6 concentrations and metabolic risk factors in Japanese middle-aged men. As shown in Tables 1 and 2, the plasma IL-1β concentration was positively associated with BMI, fasting blood glucose, triacylglycerol, AST, γ-GTP, insulin, and HOMA-IR, and negatively associated with HDL cholesterol. On the other hand, the plasma IL-6 concentration was positively associated with age, BMI, diastolic blood pressure, fasting blood glucose, triacylglycerol, γ-GTP, creatinine, insulin, and HOMA-IR. These correlations are consistent with previous reports in Western countries and Japan [4,9-13]. Interestingly, we found strong positive correlations between these interleukin concentrations and γ-GTP activity, but lower associations with other liver injury markers (AST and ALT). Recent studies have demonstrated that γ-GTP is involved in oxidant stress through ROS production [19,20]. In addition, many studies have demonstrated that circulating γ-GTP and circulating IL-1β and IL-6 are elevated before the development of diabetes without hepatic disorder and can predict the development of diabetes and metabolic syndrome in Western countries and Asian countries including Japan, and that the interleukin elevations are induced by ROS production [4,9-13,24-27]. Therefore, we hypothesized that the circulating γ-GTP activity is involved in the circulating IL-1β and IL-6 concentrations in subjects without obvious cardiovascular diseases.

To examine this hypothesis, we carried out multiple linear regression analyses for the subjects after dividing them into 3 groups based on the γ-GTP activity (low, middle, and high), using other parameters with significant differences by Spearman rank correlation coefficient analyses. As shown in Table 3, γ-GTP activity was an independent and positive explanatory variable for the plasma IL-1β and IL-6 concentrations in the initial multiple linear regression analysis models. These associations were strong because γ-GTP remained as a positive explanatory variable after performing the stepwise multiple linear regression analyses and the β-values for γ-GTP for the IL-1β and IL-6 concentrations were higher. Because these associations were observed even though HOMA-IR was added as an explanatory variable for each regression analysis, the positive associations between IL-1β or IL-6 and γ-GTP could be independent of insulin resistance. It should be noted that we found these associations in subjects without obvious cardiovascular diseases. Therefore, it should be examined in detail whether these circulating cytokine concentrations are associated with γ-GTP activity independently of insulin resistance, fatty liver, and hepatic disorder, which are observed in many subjects with metabolic diseases including diabetes, obesity, and metabolic syndrome.

It has been reported that antagonism of IL-1β by interleukin 1 receptor antagonists (eg, IL-1Ra) inhibits apoptosis of β-cells in the pancreas and maintains insulin secretion from β-cells [29]. Treatment of diabetic patients with anakinra, a recombinant human IL-1Ra developed as a drug for rheumatoid arthritis, improved the glycemic status, maintained high C-peptide secretion, and reduced plasma IL-6 levels [30,31]. However, it remains unknown whether an antagonist for IL-6, tocilizumab, which is also a drug for rheumatoid arthritis, improves the metabolic parameters in diabetic animals and patients. Indeed, we observed positive associations of IL-1β with BMI, fasting blood glucose, triacylglycerol, AST, γ-GTP, insulin, and HOMA-IR, and positive associations of IL-6 with age, BMI, diastolic blood pressure, fasting blood glucose, triacylglycerol, γ-GTP, creatinine, insulin, and HOMA-IR. However, stepwise multiple regression analyses showed that the only positive explanatory variables were age and fasting blood glucose for the plasma IL-1β concentration, and age and diastolic blood pressure for the plasma IL-6 concentration, except for γ-GTP. These results indicate that elevation of these circulating interleukins accompanied by increased γ-GTP activity could be directly and/or indirectly involved in the development of metabolic diseases.

The mechanisms involved in the positive associations between circulating IL-1β and IL-6 concentrations and γ-GTP activity in middle-aged Japanese men without obvious cardiovascular diseases are still unknown. In this

study, associations of the IL-1β and IL-6 concentrations with ALT activity were observed. Thus, the positive associations between the IL-1β and IL-6 concentrations and the γ-GTP activity may be partially caused by liver injury or insulin resistance in the liver because recent studies have indicated that circulating ALT activity is a marker for insulin resistance in the liver [32]. Furthermore, many previous studies have suggested that hyperglycemia and obesity are closely associated with circulating IL-1β and IL-6 concentrations in healthy and diabetic patients [4,9-13]. In the present study, we also found positive associations between these interleukins and parameters indicating hyperglycemia and obesity such as BMI, fasting blood glucose, triacylglycerol, insulin, and HOMA-IR. Therefore, these factors may be involved in the positive associations between the IL-1β and IL-6 concentrations and the γ-GTP activity. Many recent studies have demonstrated that hyperglycemia and insulin resistance induce ROS production [17,18]. In particular, ROS production decreases the GSH levels in cells and increases extracellular GSSG [19,20]. Because γ-GTP is a rate-controlling enzyme for resynthesizing GSH from GSSG, it is speculated that increased oxidant stress induces γ-GTP activity to resynthesize GSH from GSSG [17,18] and that the induced γ-GTP may further enhance oxidant stress by producing ROS. Indeed, the messenger RNA (mRNA) levels of inflammatory cytokines including IL-1β and IL-6 are induced by oxidant stress such as ROS production [14-16]. Consequently, ROS production by the increased γ-GTP activity may lead to increases in the circulating IL-1β and IL-6 concentrations. Recently, it was reported that peripheral blood mononuclear cells isolated from patients with chronic granulomatous disease lacking expression of $p22^{phox}$, a protein required for the functions of Nox 1-4, showed normal induction of IL-1β protein in response to treatment with uric acid, silica, imiquimod, and lipopolysaccharide, although inhibition of ROS production by the inhibitor diphenylene iodonium repressed the IL-1β production [33,34]. These results indicate that IL-1β production is independent of ROS produced by Nox, but may be dependent on ROS from other sources such as activation of oxidative phosphorylation in mitochondria, glucose autoxidation, and some enzymes involved in ROS production other than Nox such as cytochrome p450, lipoxygenases, xanthine oxidase, and nitric oxide synthase. It should be examined whether IL-1β production associated with increased γ-GTP activity is caused by ROS production and which sources of ROS production are important for IL-1β production. In addition, future studies should investigate the associations among IL-1β and IL-6; markers for ROS production in blood such as 8-hydroxydeoxyguanosine (a marker for DNA), carbonylated proteins (markers for proteins), and lipid peroxides (markers for lipids); and GSSG in blood.

It should be noted that the secretion of IL-1β protein into the blood requires many steps. Most IL-1β mRNA is degraded without significant elongation of the protein when IL-1β mRNA assembles into a large polyribosome. IL-1β protein in monocytes is translated from the mRNA by stimulation with Toll-like receptor ligands or IL-1 itself. The translated protein is the IL-1β precursor, which is an inactive form located in the cytosol that is cleaved by caspase-1 to form active IL-1β for secretion [35]. In addition, it should be noted that the circulating IL-1β concentration reported in the present study is likely to be underestimated because the concentration of IL-1β was found to be much higher when it was stabilized by injecting healthy subjects and patients with cryopyrin-associated periodic syndromes with an antibody against IL-1β [36]. Thus, further studies are needed to investigate the association between γ-GTP and stabilized active IL-1β in plasma, as well as the mRNA and immature protein levels of IL-1β in peripheral leukocytes such as monocytes, neutrophils, and T-lymphocytes.

It should also be mentioned that creatinine was a strong negative explanatory variable for the IL-6 concentration. Because circulating creatinine was reported to be positively associated with muscle mass and physical activity [37], the negative association between IL-6 and creatinine may be caused by muscle mass and physical activity. Indeed, inverse associations between the IL-1β and IL-6 concentrations and creatinine were observed by Spearman correlation coefficient analyses. In addition, alcohol intake had weak associations with the IL-1β and IL-6 concentrations in the stepwise multiple regression analyses. The circulating γ-GTP activity was reported to be positively associated with alcohol intake [38]. In the present study, we also observed an association between γ-GTP activity and alcohol intake ($r = 0.277$, $P < .001$, by Spearman correlation coefficient analysis). It is known that alcohol intake also induces ROS production and reduces intracellular GSH [39,40]. Thus, the elevation of γ-GTP activity by alcohol intake may be caused by ROS production and reduced intracellular GSH. However, alcohol intake was not associated with elevation of circulating interleukins in the present study. Therefore, the elevated levels of interleukins may be prerequisites for the elevation of other metabolic parameters such as blood glucose, blood pressure, triacylglycerol, and insulin, which are all associated with elevation of γ-GTP activity.

In addition, many previous studies have demonstrated that TNF-α is closely associated with the development of metabolic diseases and that circulating TNF-α protein is elevated before the development of diabetes [41]. It is therefore necessary to examine the associations between circulating TNF-α and γ-GTP activity in many subjects with or without obesity, diabetes, and cardiovascular diseases.

In summary, in our middle-aged Japanese men without obvious cardiovascular diseases, the circulating levels of IL-1β and IL-6 were positively associated with γ-GTP activity. The results of the present study suggest that circulating γ-GTP is involved in the increased circulating levels of IL-1β and IL-6 in middle-aged Japanese men without obvious cardiovascular diseases.

Funding

This study was financially supported by the Global COE Program for the Center of Excellence for Innovation in Human Health Sciences from the Ministry of Education, Science, Sports, and Culture of Japan, and a grant from the Ministry of Health, Labour, and Welfare of Japan.

Acknowledgment

We thank Drs S Sasaki and K Murakami for providing the brief self-administered diet history questionnaire and for helpful discussions.

References

[1] Vettor R, Milan G, Rossato M, Federspil G. Review article: adipocytokines and insulin resistance. Aliment Pharmacol Ther 2005;22(Suppl 2):3-10.
[2] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.
[3] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24:816-23.
[4] Spranger J, Kroke A, Mohlig M, Hoffmann K, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: Results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study. Diabetes 2003;52: 812-7.
[5] Kim ES, Im JA, Kim KC, Park JH, et al. Improved insulin sensitivity and adiponectin level after exercise training in obese Korean youth. Obesity (Silver Spring) 2007;15:3023-30.
[6] Bruun JM, Stallknecht B, Helge JW, Richelsen B. Interleukin-18 in plasma and adipose tissue: effects of obesity, insulin resistance, and weight loss. Eur J Endocrinol 2007;157:465-71.
[7] Hoge M, Amar S. Role of interleukin-1 in bacterial atherogenesis. Drugs Today (Barc) 2006;42:683-8.
[8] Mahmoudi M, Curzen N, Gallagher PJ. Atherogenesis: the role of inflammation and infection. Histopathology 2007;50:535-46.
[9] Bastard JP, Jardel C, Bruckert E, Blondy P, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000;85: 3338-42.
[10] Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000;148:209-14.
[11] Deepa R, Velmurugan K, Arvind K, Sivaram P, et al. Serum levels of interleukin 6, C-reactive protein, vascular cell adhesion molecule 1, and monocyte chemotactic protein 1 in relation to insulin resistance and glucose intolerance—the Chennai Urban Rural Epidemiology Study (CURES). Metabolism 2006;55:1232-8.
[12] Matsushita K, Yatsuya H, Tamakoshi K, Wada K, et al. Comparison of circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in Japanese men. Arterioscler Thromb Vasc Biol 2006;26:871-6.
[13] Misaki Y, Miyauchi R, Mochizuki K, Takabe S, et al. Plasma IL-1β concentrations are closely associated with fasting blood glucose levels in healthy and preclinical middle-aged, non-overweight and overweight Japanese men. Metabolism 2010;59(10):1465-71.
[14] Ginn-Pease ME, Whisler RL. Redox signals and NF-kappaB activation in T cells. Free Radic Biol Med 1998;25:346-61.
[15] Haddad JJ. Redox regulation of pro-inflammatory cytokines and IkappaB-α/NF-kappaB nuclear translocation and activation. Biochem Biophys Res Commun 2002;296:847-56.
[16] Zhang J, Johnston G, Stebler B, Keller ET. Hydrogen peroxide activates NFkappaB and the interleukin-6 promoter through NFkappaB-inducing kinase. Antioxid Redox Signal 2001;3:493-504.
[17] Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 2003;26: 1589-96.
[18] Niedowicz DM, Daleke DL. The role of oxidative stress in diabetic complications. Cell Biochem Biophys 2005;43:289-330.
[19] Lieberman MW, Barrios R, Carter BZ, Habib GM, et al. γ-Glutamyl transpeptidase. What does the organization and expression of a multipromoter gene tell us about its functions? Am J Pathol 1995;147:1175-85.
[20] Pompella A, Corti A, Paolicchi A, Giommarelli C, et al. γ-Glutamyltransferase, redox regulation and cancer drug resistance. Curr Opin Pharmacol 2007;7:360-6.
[21] Yamada J, Tomiyama H, Yambe M, Koji Y, et al. Elevated serum levels of alanine aminotransferase and γ-glutamyltransferase are markers of inflammation and oxidative stress independent of the metabolic syndrome. Atherosclerosis 2006;189:198-205.
[22] Bo S, Gambino R, Durazzo M, Guidi S, et al. Associations between γ-glutamyl transferase, metabolic abnormalities and inflammation in healthy subjects from a population-based cohort: a possible implication for oxidative stress. World J Gastroenterol 2005;11:7109-17.
[23] Lee DH, Steffen LM, Jacobs Jr DR. Association between serum γ-glutamyltransferase and dietary factors: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Clin Nutr 2004;79:600-5.
[24] Nakanishi N, Suzuki K, Tatara K. Serum γ-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. Diabetes Care 2004;27:1427-32.
[25] Kim CH, Park JY, Lee KU, Kim JH, et al. Association of serum γ-glutamyltransferase and alanine aminotransferase activities with risk of type 2 diabetes mellitus independent of fatty liver. Diabetes Metab Res Rev 2009;25:64-9.
[26] Andre P, Balkau B, Born C, Charles MA, et al. Three-year increase of γ-glutamyltransferase level and development of type 2 diabetes in middle-aged men and women: the D.E.S.I.R. cohort. Diabetologia 2006;49:2599-603.
[27] Meisinger C, Lowel H, Heier M, Schneider A, et al. Serum γ-glutamyltransferase and risk of type 2 diabetes mellitus in men and women from the general population. J Intern Med 2005;258:527-35.
[28] Murakami K, Mizoue T, Sasaki S, Ohta M, et al. Dietary intake of folate, other B vitamins, and ω-3 polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults. Nutrition 2008;24:140-7.
[29] Maedler K, Donath MY. β-Cells in type 2 diabetes: a loss of function and mass. Horm Res 2004;62(Suppl 3):67-73.
[30] Larsen CM, Faulenbach M, Vaag A, Ehses JA, et al. Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. Diabetes Care 2009;32:1663-8.
[31] Larsen CM, Faulenbach M, Vaag A, Volund A, et al. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007; 356:1517-26.
[32] Vozarova B, Stefan N, Lindsay RS, Saremi A, et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002;51: 1889-95.
[33] van Bruggen R, Koker MY, Jansen M, van Houdt M, et al. Human NLRP3 inflammasome activation is Nox1-4 independent. Blood 2010; 115:5398-400.
[34] van de Veerdonk FL, Smeekens SP, Joosten LA, Kullberg BJ, et al. Reactive oxygen species–independent activation of the IL-1b inflammasome in cells from patients with chronic granulomatous disease. Proc Natl Acad Sci U S A 2010;107:3030-3.
[35] Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 2009;27:519-50.

[36] Lachmann HJ, Lowe P, Felix SD, Rordorf C, et al. In vivo regulation of interleukin 1β in patients with cryopyrin-associated periodic syndromes. J Exp Med 2009;206:1029-36.
[37] Baxmann AC, Ahmed MS, Marques NC, Menon VB, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin J Am Soc Nephrol 2008;3:348-54.
[38] Niemela O. Biomarkers in alcoholism. Clin Chim Acta 2007;377:39-49.
[39] Das SK, Vasudevan DM. Alcohol-induced oxidative stress. Life Sci 2007;81:177-87.
[40] Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. Nat Rev Cancer 2007;7:599-612.
[41] Gnacinska M, Malgorzewicz S, Stojek M, Lysiak-Szydlowska W, et al. Role of adipokines in complications related to obesity: a review. Adv Med Sci 2009;54:150-7.