Associations between Markers of Liver Injury and Cytokine Markers for Insulin Sensitivity and Inflammation in Middle-Aged Japanese Men Not Being Treated for Metabolic Diseases

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(Received May 11, 2011)

Summary Elevated circulating alanine aminotransferase (ALT) and γ-glutamyltranspeptidase (γ-GTP) activities in healthy and preclinical subjects are associated with increased risk for obesity, diabetes and related complications. In the present study, we examined the associations between these hepatic enzymes and circulating cytokines as markers for insulin sensitivity (adiponectin) and inflammation [interleukin-6 (IL-6)] in middle-aged Japanese men not being treated for metabolic diseases. We conducted a cross-sectional study of 310 Japanese men aged 40–69 y (mean±SD, 58.8±7.6 y) who were not being treated for metabolic diseases and who participated in health checkups in Japan. We analyzed their lifestyle factors, clinical factors, and plasma adiponectin and IL-6 concentrations. We determined associations between the concentrations of these cytokines and the clinical and lifestyle factors using Spearman’s correlation analysis, Jonckheere-Terpstra’s test and multiple linear regression. ALT activity was negatively associated with adiponectin (r=−0.302, p<0.001) but not with IL-6. γ-GTP activity was positively associated with IL-6 (r=0.335, p<0.001) and negatively associated with adiponectin (r=−0.129, p<0.05). Aspartate aminotransferase (AST) activity was positively associated with IL-6 (r=0.131, p<0.05) and negatively associated with adiponectin (r=−0.125, p<0.05). Multiple linear regression analyses showed that adiponectin was independently and negatively associated with ALT activity, while IL-6 was independently and positively associated with γ-GTP activity. Adiponectin and IL-6 were not independently associated with AST activity. The results of this study indicate that circulating ALT activity is negatively associated with adiponectin concentration, γ-GTP is positively associated with increased IL-6 concentration, and AST is not associated with these cytokines in middle-aged Japanese men not being treated for metabolic diseases.

Key Words adiponectin, IL-6, liver injury markers, Japanese men, subjects without treatment for metabolic diseases

Abnormalities in metabolic parameters, such as hyperglycemia and dyslipidemia, promote the development of diabetes, metabolic syndrome and related complications such as cardiovascular disease, hypertension, nephropathy and inflammation in peripheral tissues (1–3). Therefore, it is necessary to identify biomarkers that can accurately predict the development of these diseases. Recent studies have demonstrated that the circulating activity of alanine aminotransferase (ALT), a hepatic enzyme marker for liver injury, is associated with liver insulin resistance, which was determined by measuring hepatic glucose output during insulin infusion (4–6). Furthermore, other studies have shown that the circulating activity of γ-glutamyltranspeptidase (γ-GTP), another hepatic enzyme marker for liver injury, is also a marker for inflammation and oxidative stress in healthy and preclinical subjects (7–11). This is because the circulating γ-GTP activity is closely associated with markers for oxidative stress, such as lipid peroxides and the inflammatory marker C-reactive protein (CRP), in healthy subjects in Western countries and in Japan, and that γ-GTP itself contributes to the production of reactive oxygen species (ROS) (7–11). Indeed, γ-GTP is a key enzyme involved in glutathione (GSH) re-synthesis because it catalyzes the first stage in the conversion of extracellular oxidized GSH (GSSG) and GSH to cysteine and glycine for re-synthesis of GSH, and produces ROS...
such as superoxide and hydrogen peroxide (10, 11). Several studies in Western and Asian countries, including Japan, have demonstrated that the subsequent incidence of diabetes was greater in healthy and preclinical subjects with elevated circulating ALT and/or γ-GTP activities within the normal range or without fatty liver or hepatic dysfunction, as compared with subjects with lower levels of these enzymes. In contrast, the associations between the activity of aspartate aminotransferase (AST), another hepatic enzyme marker for liver injury, and homeostasis model assessment–insulin resistance (HOMA-IR) and insulin concentrations were weaker than those for ALT activity (4, 12–15). These findings suggest that the hepatic enzyme markers of ALT and γ-GTP, but not AST activities may be useful to assess the severity of insulin resistance and inflammation in people attending health checkups and in clinical settings. However, no studies have examined the potential associations between these hepatic enzyme markers and markers for inflammation and insulin resistance. In addition, the associations between these hepatic enzyme activities and insulin resistance and inflammation have not been confirmed, based on the changes in the circulating molecules that are implicated in the induction of insulin resistance, inflammation and related complications. Therefore, studies were needed to examine the associations between these hepatic enzyme activities and insulin resistance or inflammation, to confirm that ALT activity is a marker of insulin resistance, and to confirm that γ-GTP activity is a marker of inflammation whereas AST activity is not related to inflammation or insulin resistance.

Many recent studies have demonstrated that adiponectin, an adipose tissue-secreted cytokine, enhances insulin sensitivity by promoting glucose incorporation into skeletal muscle and adipose tissue, and activating fatty acid β-oxidation in the liver (16, 17). Higher plasma adiponectin concentrations in healthy and type 2 diabetic subjects in Western and Asian countries, including Japan, have been shown to be associated with lower fasting glucose, triacylglycerol, total cholesterol and low-density lipoprotein-cholesterol concentrations, and higher high-density lipoprotein (HDL)-cholesterol concentrations (18–23). In addition, it has been reported that insulin resistance and hyperglycemia in people with diabetes are 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 (24–26). These cytokines are induced by oxidative stress, such as the production of ROS (27–29). Of note, IL-6 is thought to be an important predictor for the risk of inflammation and the onset of diabetes (24), and directly induces insulin resistance as well as atherosclerosis in diabetes (30, 31). Taken together, these earlier findings indicate that circulating adiponectin induces insulin sensitivity and IL-6 induces inflammation and complications such as cardiovascular disease, hypertension and nephropathy during the development of metabolic diseases such as obesity and diabetes. Considering these findings, it seems likely that ALT activity is negatively associated with the circulating adiponectin concentration and that γ-GTP activity is positively associated with the circulating IL-6 concentration, whereas AST activity is not associated with adiponectin or IL-6 concentrations. However, the putative associations between these hepatic enzyme activities (ALT, γ-GTP and AST), adiponectin and IL-6 have not yet been reported.

Therefore, in this study, we determined the associations of circulating adiponectin and IL-6 concentrations with hepatic enzyme activities in 310 Japanese subjects who were not being treated for any metabolic diseases.

MATERIALS AND METHODS

Study population. This cross-sectional study was performed during health checkups offered by the city government of Izunokuni (Shizuoka Prefecture, Japan) between June 2005 and September 2005. A total of 2,484 subjects aged above 40 y participated in health checkups and 2,046 subjects who agreed to participate in this study were enrolled. We selected 310 men from among those aged 40–69 y and excluded subjects who were being treated for stroke, hypertension, cardiac disease, diabetes, hyperlipidemia, liver disease, kidney disease or gout. Anthropometric data and blood samples were collected from each participant by trained medical staff. The participants were also asked about their smoking status and physical activity. Smoking status was classified as never, past or current. Physical activity was classified as 0 times/wk, 1 time/wk, 2–3 times/wk, or 7 times/wk. 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 based on the Declaration of Helsinki (Shizuoka, Japan).

Measurements. After an overnight fast, height and weight were measured, and blood samples were collected from each subject. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Alcohol and energy intake during the preceding month was assessed with a brief self-administered diet history questionnaire (BDHQ) (32). Fasting plasma glucose, serum triacylglycerol, serum total cholesterol and serum HDL cholesterol were measured using a quick auto neo GLU-HK (Shino-Test Co., Ltd., Tokyo, Japan), detaminar-TGII (Kyowa Medex Co., Ltd., Tokyo, Japan), detaminar-TCH (Kyowa Medex Co., Ltd.) and metaboalead–HDL-C (Kyowa Medex Co., Ltd.), respectively. Plasma samples were kept at −80°C until used to measure insulin, adiponectin and IL-6 concentrations. Plasma insulin levels were measured using a solid-phase two-site enzyme immunoassay (Mercodia Ultrasensitive Insulin ELISA Kit; Mercodia, Uppsala, Sweden). Plasma total adiponectin levels (Adiponectin ELISA Kit; Otsuka Pharmaceutical Co., Tokyo, Japan) and plasma IL-6 levels (Quantikine IL-6 Kit; R&D Systems, Oxford, UK) were measured using enzyme-linked immunosorbent assays. All of the assays were performed according to the manufacturer’s instructions and the concentrations were determined by reference to
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standard curves calculated from several dilutions of each recombinant protein. The mean intra-assay and inter-assay coefficients of variation for the insulin, adiponectin and IL-6 assays were less than 5%.

Insulin resistance was estimated by the HOMA-IR method using the formula: fasting blood glucose (mg/dL) / fasting plasma insulin (mU/L) / 405.

Statistical analysis. Clinical and biochemical data are presented as means±SD, percentage, or as natural logarithm (ln)-transformed means (range), which were calculated using Excel 2007 (Microsoft, Tokyo, Japan). Spearman’s rank correlation coefficient analyses were used to calculate correlations among variables for all subjects. Jonckheere-Terpstra’s test was used to calculate the correlations among tertiles of ALT, γ-GTP and AST activities. The values of ALT, γ-GTP and AST activities transferred to natural logarithms were used in multiple linear regression analyses because their distributions were positively skewed. Explanatory variables (excluding categorized explanatory variables) that did not show linearity to each response value in scatter plots were also converted to values of natural logarithms, because the explanatory variables, excluding categorized explanatory variables, should show linearity with the response values in scatter plots. Multiple linear regression analyses were performed to identify variables independently associated with the ALT, γ-GTP and AST concentrations. For all analyses, a value of $p<0.05$ was considered significant. All statistical analyses were performed using Excel Statistics 2008 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

RESULTS

The study subjects were all Japanese men not being treated for metabolic diseases. The participants ranged in age from 40 to 69 y (mean±SD, 58.8±7.6 y). The mean BMI was 23.2±2.8 kg/m² and the mean waist circumference was 84.6±7.7 cm. The mean fasting blood glucose was 103.7±23.5 mg/dL and the mean fasting plasma insulin was 5.25±5.57 mU/L. The mean ALT, γ-GTP and AST activities were 24.7±14.9, 44.0±76.1 and 23.8±11.0 U/L, respectively. The plasma adiponectin and IL-6 concentrations were 5.47±2.96 mg/L and 3.49±3.34 pg/mL, respectively.

Table 1. Physical characteristics, anthropometric and body composition measures, and lifestyle habits of 310 middle-aged men.

| Characteristic            | Criterion value | Means±SD or percent | n   |
|--------------------------|-----------------|----------------------|-----|
| Age (y)                  | 58.8±7.6        | 310                  |
| Height (cm)              | 166.7±6.2       | 310                  |
| BMI (kg/m²)              | 18.5–24.9       | 310                  |
| Waist circumference (cm) | <85.0           | 302                  |
| Smoking                  |                 |                      |
| Number of cigarettes/d   | 11.8±11.7       | 280                  |
| Duration of smoking (y)  | 15.5±16.1       | 280                  |
| Physical activity        |                 |                      |
| 7 times/wk               | 19.0%           | 59                   |
| 2–3 times/wk             | 16.8%           | 52                   |
| 1 time/wk                | 11.0%           | 34                   |
| 0 times/wk               | 46.8%           | 145                  |
| Unknown                  | 6.5%            | 20                   |
| Alcohol intake (g/d)     | 29.0±35.7       | 309                  |
| Energy intake (kcal/d)   | 2,145.4±510.2   | 297                  |
| Systolic blood pressure (mmHg) | <130   | 126.5±16.3          | 310 |
| Diastolic blood pressure (mmHg) | <85    | 76.9±11.6          | 310 |
| Fasting blood glucose (mg/dL) | 70–109   | 103.7±23.5         | 302 |
| Total cholesterol (mg/dL) | 128–219      | 5.27 (32.79–310)   | 302 |
| HDL cholesterol (mg/dL)  | 40–96          | 4.72 (30–1,442)    | 310 |
| Triacylglycerol (mg/dL)  | 30–149         | 3.95 (15.5–310)    | 302 |
| ALT (U/L)                | 6–30           | 24.7±14.9           | 310 |
| γ-GTP (U/L)              | 10–47          | 44.0±76.1           | 310 |
| AST (U/L)                | 13–33          | 23.8±11.0           | 310 |
| Creatinine (mg/dL)       | 0.6–1.1        | 0.80±0.11           | 310 |
| Insulin (mU/L)           | 3.0–15.0       | 5.25±5.57           | 290 |
| HOMA-IR1                 | <1.6           | 1.44±2.02           | 290 |
| Adiponectin (mg/L)       |                 |                      |
| IL-6 (pg/mL)             | 3.49±3.34      | 287                  |

Data are expressed as means±SD, or as natural logarithmic transformed means (range). BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; AST, aspartate aminotransferase; HOMA-IR, homeostasis model assessment–insulin resistance; IL-6, interleukin-6.

1 HOMA-IR = fasting blood glucose×fasting insulin/405.
Changes in AST (U/L) levels exceeding the normal ranges.

Eight subjects had activities of all three enzymes and negatively associated with BMI, waist circumference, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin, HOMA-IR and IL-6, and negatively associated with plasma adiponectin concentrations (Table 3). AST activity was positively associated with BMI, waist circumference, alcohol intake, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin, HOMA-IR and IL-6, and negatively associated with plasma adiponectin concentrations (Table 3). After dividing the subjects into three groups based on ALT activity (0–17, 18–25 and ≥26 U/L), positive trends for ALT activity were observed for BMI, waist circumference, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin and HOMA-IR, and negative trends were observed for HDL cholesterol and plasma adiponectin concentrations (Table 2). Regarding γ-GTP activity, positive trends were seen for all three groups (0–22, 23–36 and ≥37 U/L) in terms of BMI, waist circumference, alcohol intake, energy intake, diastolic blood pressure, fasting blood glucose, triacylglycerol, ALT, AST, insulin, HOMA-IR and IL-6, and negative trends were observed for HDL cholesterol and plasma adiponectin concentrations (Table 2). Regarding AST activity, positive trends were observed for all three groups (0–19, 20–24 and ≥25 U/L) in terms of BMI, waist circumference, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin, HOMA-IR and IL-6, and negative trends were observed (Table 1). Twenty-four, 105, 80 and 55 subjects had fasting glucose ≥125 mg/dL, triacylglycerol ≥149 mg/dL, systolic blood pressure >139 mmHg and diastolic blood pressure >89 mmHg, respectively. Twenty-two, 23 and 41 subjects had AST (>40 U/L), ALT (>45 U/L) and γ-GTP (≥79 U/L) levels exceeding the normal ranges. Eight subjects had activities of all three enzymes exceeding the normal ranges.

We investigated the correlations between ALT, γ-GTP and AST activities, serum parameters and plasma cytokine concentrations using Spearman’s correlation coefficient analyses. ALT activity was positively associated with BMI, waist circumference, fasting blood glucose, triacylglycerol, AST, γ-GTP, insulin and HOMA-IR, and negatively associated with age, HDL cholesterol and plasma adiponectin concentrations (Table 2). γ-GTP activity was positively associated with BMI, waist circumference, smoking status (number of cigarettes, duration of smoking), alcohol intake, energy intake, diastolic blood pressure, fasting blood glucose, triacylglycerol, ALT, AST, insulin, HOMA-IR and IL-6, and negatively associated with HDL cholesterol, creatinine and plasma adiponectin concentrations (Table 3). AST activity was positively associated with BMI, waist circumference, alcohol intake, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin, HOMA-IR and IL-6, and negatively associated with plasma adiponectin concentrations (Table 3). After dividing the subjects into three groups based on ALT activity (0–17, 18–25 and ≥26 U/L), positive trends for ALT activity were observed for BMI, waist circumference, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin and HOMA-IR, and negative trends were observed for HDL cholesterol and plasma adiponectin concentrations (Table 2). Regarding γ-GTP activity, positive trends were seen for all three groups (0–22, 23–36 and ≥37 U/L) in terms of BMI, waist circumference, alcohol intake, energy intake, diastolic blood pressure, fasting blood glucose, triacylglycerol, ALT, AST, insulin, HOMA-IR and IL-6, and negative trends were observed for HDL cholesterol and plasma adiponectin concentrations (Table 2). Regarding AST activity, positive trends were observed for all three groups (0–19, 20–24 and ≥25 U/L) in terms of BMI, waist circumference, fasting blood glucose, triacylglycerol, ALT, γ-GTP, insulin, HOMA-IR and IL-6, and negative trends were observed (Table 1). Twenty-four, 105, 80 and 55 subjects had fasting glucose ≥125 mg/dL, triacylglycerol ≥149 mg/dL, systolic blood pressure >139 mmHg and diastolic blood pressure >89 mmHg, respectively. Twenty-two, 23 and 41 subjects had AST (>40 U/L), ALT (>45 U/L) and γ-GTP (≥79 U/L) levels exceeding the normal ranges. Eight subjects had activities of all three enzymes exceeding the normal ranges.

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for plasma adiponectin concentrations (Table 4).

Next, we performed multiple linear regression based on the results of the Spearman’s correlation coefficient analyses. Ten variables were included in the multiple linear regression model for ln(ALT), ln(γ-GTP) and ln(AST). Ln(ALT) was positively and independently associated with BMI and triacylglycerol, and negatively associated with adiponectin. Ln(γ-GTP) was positively associated with fasting blood glucose, triacylglycerol, IL-6 and alcohol intake. Finally, ln(AST) was only positively associated with alcohol intake (Table 5).

**DISCUSSION**

In the present study, we focused on the associations between circulating hepatic enzyme activities, metabolic risk factors, and the concentrations of cytokines as markers for insulin sensitivity (adiponectin) and inflammation (IL-6) in Japanese middle-aged men not being treated for metabolic diseases. In this study, the associations between ALT activity and HOMA-IR and insulin concentrations were stronger than those for AST and γ-GTP activities. These correlations are consistent with those of previous reports in Western countries and Japan (4–6, 12–15). Interestingly, we found a strong inverse association between adiponectin and ALT activity ($r = -0.302, p<0.001$) by Spearman’s correlation coefficient analysis, while weak associations between adiponectin and γ-GTP ($r = -0.129, p<0.05$) or AST ($r = -0.125, p<0.05$) were found. In addition, we found a strong positive association between IL-6 and γ-GTP activity ($r = 0.440, p<0.001$), but a weak negative association between IL-6 and AST ($r = -0.129, p<0.05$), and no association between IL-6 and ALT ($r = 0.050, p=0.248$). Previous studies have demonstrated that the circulating ALT activity is associated with liver insulin resistance, which was assessed by measuring hepatic glucose output during insulin infusion, in studies performed in Western countries and in Japan (4–6). However, it is not reported whether ALT activity was associated with insulin resistance in other tissues or with other parameters which indicate insulin resistance. Recent studies have demonstrated that the circulating adiponectin concentration is associated with insulin sensitivity. Adiponectin targets insulin-sensitive tissues, including skeletal muscle and liver, and enhances insulin sensitivity in these tissues (16, 17).

**Table 3. Correlations between characteristics and γ-GTP.**

| Groups by γ-GTP range (U/L) | Spearman’s correlation coefficient | Low 0–22 | Middle 23–36 | High ≥37 | p for trend |
|-----------------------------|-----------------------------------|---------|-------------|---------|------------|
| γ-GTP (U/L)                 |                                   | 14.4±4.9| 29.2±3.9    | 89.4±119.6 |            |
| n                           | 317                               |         |             |         |            |
| Age (y)                     | -0.036                            | 59.2±7.4| 59.0±7.8    | 58.1±7.6 | 0.455      |
| Height (cm)                 | 0.027                             | 166.6±6.1| 166.6±6.5  | 167.6±6.1 | 0.624      |
| BMI (kg/m²)                 | 0.222***                          | 22.4±2.8| 23.5±2.6    | 23.9±2.8 | <0.001     |
| Waist circumference (cm)    | 0.277***                          | 81.5±7.7| 85.4±7.0    | 86.8±7.2 | <0.001     |
| Smoking                     |                                   |         |             |         |            |
| Number of cigarettes/d      | 0.141*                            | 10.5±11.0| 11.0±11.9  | 14.0±11.7| 1.000      |
| Duration of smoking (y)     | 0.167**                           | 12.6±14.7| 15.2±17.0  | 18.9±15.7| 0.904      |
| Physical activity1**        | -0.051                            | 2.9±1.18 | 2.96±1.18   | 2.73±1.28| 1.000      |
| Alcohol intake (g/d)        | 0.245***                          | 21.6±31.0| 26.9±31.0  | 38.6±41.9| 0.026      |
| Energy intake (kcal/d)      | 0.129*                            | 2.09±58.0| 2.099±468.2| 2.234±529.7| 0.044     |
| Systolic blood pressure (mmHg) | 0.081                            | 125.9±17.7| 127.5±16.5| 126.2±14.3| 0.495      |
| Diastolic blood pressure (mmHg) | 0.221***                        | 74.1±12.4| 77.6±11.3  | 78.9±10.5| 0.005      |
| Fasting blood glucose (mg/dL) | 0.377***                          | 96.2±11.8| 103.5±15.5| 111.8±34.2| <0.001     |
| Total cholesterol (mg/dL)   | 0.057                             | 5.26±299 | 5.29±289   | 6.74±307 | 0.358      |
| HDL cholesterol (mg/dL)     | -0.137*                           | 4.00±102 | 3.93±97    | 5.40±127 | 0.008      |
| Triacylglycerol (mg/dL)     | 0.362***                          | 4.49±554 | 4.73±1022  | 6.48±1442 | <0.001     |
| ALT (U/L)                   | 0.394***                          | 19.5±7.3 | 21.5±8.1   | 33.2±20.9| <0.001     |
| AST (U/L)                   | 0.376***                          | 20.4±5.3 | 21.8±5.2   | 28.9±16.3| <0.001     |
| Creatinine (mg/dL)          | -0.122*                           | 0.82±0.11| 0.81±0.12  | 0.78±0.11| 0.191      |
| Insulin (mU/L)              | 0.291***                          | 3.63±4.1 | 5.60±6.18  | 6.60±5.83| <0.001     |
| HOMA-IR                     | 0.355***                          | 0.88±0.95| 1.44±1.60  | 2.04±2.82| <0.001     |
| Adiponectin (mg/L)          | -0.129*                           | 5.89±3.34| 5.61±2.70  | 4.90±2.70| 0.012      |
| IL-6 (pg/mL)                | 0.440***                          | 1.98±3.21| 3.59±1.32  | 4.98±3.72| <0.001     |

Data are expressed as means±SD, or as natural logarithmic transformed means (range in natural number). Spearman’s correlation coefficients (*$p<0.05$, **$p<0.01$, ***$p<0.001$) were determined between each interleukin and each parameter for all subjects.

1 Physical activity: 1=7 times/wk, 2=2–3 times/wk, 3=1 time/wk, 4=0 times/wk.

$p$ for trend was determined using Jonckheere-Terpstra’s test among three groups divided by concentrations of γ-GTP.
Table 4. Correlations between characteristics and AST.

| Groups by AST range (U/L) | Spearman’s correlation coefficient | Low (n=108) | Middle (n=102) | High (n=100) | p for trend |
|---------------------------|------------------------------------|------------|---------------|-------------|------------|
| AST (U/L)                 |                                     | 16.7±2.2  | 21.8±1.3      | 33.1±14.8   |            |
| n                         | 317                                 |            |               |             |            |
| Age (y)                   | 0.052                               | 57.9±8.1  | 59.5±7.5      | 59.1±7.0    | 0.480      |
| Height (cm)               | -0.056                              | 167.6±6.3 | 165.6±6.2     | 166.9±6.0   | 0.522      |
| BMI (kg/m²)               | 0.139*                              | 22.0±2.6  | 23.5±2.7      | 23.6±2.9    | 0.011      |
| Waist circumference (cm)  | 0.167**                             | 83.0±7.1  | 84.5±7.6      | 86.4±7.9    | 0.004      |
| Smoking                   |                                     |            |               |             |            |
| Number of cigarettes/d    | -0.004                              | 12.1±11.6 | 11.3±11.6     | 12.0±11.8   | 1.000      |
| Duration of smoking (y)   | 0.021                               | 16.1±16.4 | 13.5±14.9     | 16.9±16.5   | 1.000      |
| Physical activity¹        | -0.099                              | 3.03±1.17 | 2.79±1.19     | 2.77±1.28   | 1.000      |
| Alcohol intake (g/d)      | 0.155**                             | 23.4±33.0 | 28.4±33.4     | 35.7±39.4   | 0.236      |
| Energy intake (kcal/d)    | 0.105                               | 2,122.5±547.6 | 2,117.0±453.7 | 2,199.9±513.8 | 0.082 |
| Systolic blood pressure (mmHg) | 0.051                          | 125.4±16.5 | 126.5±16.0    | 127.7±16.2  | 0.259      |
| Diastolic blood pressure (mmHg) | 0.055                            | 76.8±10.9  | 75.9±11.5     | 77.9±12.4   | 0.512      |
| Fasting blood glucose (mg/dL) | 0.221***                        | 101.5±29.2 | 103.4±20.6    | 106.5±18.6  | <0.001     |
| Total cholesterol (mg/dL) | -0.034                              | 5.28 (128-289) | 5.28 (122-273) | 5.26 (113-307) | 0.214     |
| HDL cholesterol (mg/dL)   | -0.060                              | 3.96 (25-104) | 3.99 (22-127) | 3.90 (27-102) | 0.098     |
| Triacylglycerol (mg/dL)   | 0.143*                              | 4.66 (39-1,442) | 4.67 (30-1,022) | 4.85 (37-678) | 0.008     |
| ALT (U/L)                 | 0.702***                            | 16.5±5.1  | 22.2±6.6      | 36.1±20.0   | <0.001     |
| γ-GTP (U/L)               | 0.376***                            | 26.2±17.4 | 36.6±36.0     | 70.9±122.9  | <0.001     |
| Creatinine (mg/dL)        | -0.066                              | 0.81±0.11 | 0.81±0.11     | 0.79±0.12   | 0.639      |
| Insulin (muU/L)           | 0.165*                              | 4.35±5.57 | 5.34±5.53     | 6.13±5.44   | <0.001     |
| HOMA-IR                   | 0.191**                             | 1.23±2.49 | 1.40±1.51     | 1.71±1.84   | <0.001     |
| Adiponectin (mg/L)        | -0.125*                             | 5.67±2.74 | 6.04±3.66     | 4.67±2.10   | 0.009      |
| IL-6 (pg/mL)              | 0.131*                              | 3.10±3.28 | 3.37±3.23     | 4.01±3.42   | 0.014      |

Data are expressed as means±SD, or as natural logarithmic transformed means (range in natural number).

Spearman’s correlation coefficients (*p<0.05, **p<0.01, ***p<0.001) were determined between each interleukin and each parameter for all subjects.

¹ Physical activity: 1=7 times/wk, 2=2–3 times/wk, 3=1 time/wk, 4=0 times/wk.

p for trend was determined using Jonckheere-Terpstra’s test among three groups divided by concentrations of AST.

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Table 5. Multivariate linear regression analysis of parameters associated with markers of liver injury.

| n=257 | ln(ALT)¹ | ln(γ-GTP)¹ | ln(AST)¹ |
|-------|----------|------------|----------|
|       | β        | SE        | p        | β        | SE        | p        | β        | SE        | p        |
| Intercept | 1.579 | 1.027 | 0.125 | -2.869 | 1.650 | 0.083 | 2.296 | 0.789 | 0.004** |
| BMI | -0.001 | 0.002 | 0.591 | 0.005 | 0.018 | 0.794 | -0.001 | 0.009 | 0.903 |
| Diastolic blood pressure | -0.091 | 0.126 | 0.061 | 0.038* | 0.148 | 0.170 | 0.384 | 0.067 | 0.089 | 0.481 | -0.003 | 0.097 | 0.900 | -0.012 | 0.017 | 0.066 |
| ln(fasting blood glucose)¹ | -0.011 | 0.055 | 0.037 | 0.145 | 0.000 | 0.000 | 0.902 | 0.000 | 0.000 | 0.469 | 0.000 | 0.000 | 0.451 |
| ln(Adiponectin)¹ | -0.157 | 0.005 | 0.005 | 0.005 | 0.000 | 0.902 | 0.000 | 0.000 | 0.469 | 0.000 | 0.000 | 0.451 |
| ln(IL-6)¹ | -0.011 | 0.022 | 0.631 | 0.005 | 0.000 | 0.001 | 0.902 | 0.000 | 0.000 | 0.469 | 0.000 | 0.000 | 0.451 |
| ln(Insulin)¹ | 0.055 | 0.037 | 0.145 | 0.000 | 0.000 | 0.902 | 0.000 | 0.000 | 0.469 | 0.000 | 0.000 | 0.451 |

¹ Natural logarithm (ln) transformation.

*p<0.05, **p<0.01, ***p<0.001.

An example of the regression equation for ln(ALT)=1.579+0.001×BMI+0.001×diastolic blood pressure+0.148×ln(fasting blood glucose)+0.126×ln(triacylglycerol)+0.091×ln(HDL cholesterol)+0.157×ln(adiponectin)+0.011×ln(IL-6)+0.005×ln(Insulin)+0.000×alcohol intake+0.000×energy intake.
This suggests that the circulating adiponectin concentration is a suitable marker for whole-body insulin sensitivity. In contrast, γ-GTP activity is closely associated with markers for oxidative stress, such as lipid peroxides and the inflammatory marker CRP in healthy subjects in Western countries (7–11). Based on the results of our study, we hypothesized that the circulating ALT activity is independently and negatively associated with circulating adiponectin concentrations, while γ-GTP activity is independently and positively associated with circulating IL-6 concentrations, and AST is not associated with these circulating cytokine concentrations.

To confirm this hypothesis, we performed multiple linear regression analyses with ALT, γ-GTP and AST activities as the dependent variable, using the parameters showing statistically significant differences on Jonckheere-Terpstra’s test. As shown in Table 5, adiponectin was independently and negatively associated with ALT activity, but not with γ-GTP or AST activities. In the present study, multiple linear regression analyses revealed that diastolic blood pressure, fasting blood glucose, triacylglycerol, alcohol intake and IL-6 were positively associated with γ-GTP activity. In addition, we found that IL-6 was not associated with ALT or AST activities. We first reported in this study that ALT activity, but not γ-GTP or AST activities, is negatively associated with the circulating adiponectin concentration, whereas γ-GTP activity, but not ALT or AST activities, is positively associated with the circulating IL-6 concentration. These results indicate that ALT may be a useful marker for predicting decreases in the circulating adiponectin concentration, while γ-GTP activity may predict increases in the circulating IL-6 concentration. In addition, these results provide further evidence that the circulating ALT activity is a marker for insulin resistance, while the circulating γ-GTP activity is a marker for inflammation. In addition, our results indicate that AST activity is not affected by the presence of insulin resistance or inflammation. In this study, only eight subjects had all three enzyme activities exceeding the normal ranges, although a large proportion of subjects had ALT or γ-GTP activities exceeding the normal ranges (23 and 41, respectively), suggesting that the elevated activities of these hepatic enzymes, including AST, may be related to liver damage. At health check-ups, it is difficult to assess insulin resistance, inflammation, and the extent of liver damage because the circulatory concentrations of adiponectin and IL-6, and liver ultrasonography are not always determined. Our results suggest that it would be useful to assess insulin resistance, inflammation and liver damage at checkups because determining the activities of these hepatic enzyme markers could predict the circulating adiponectin and IL-6 concentrations, as well as liver damage. Thus, assessing these states based on these markers of liver injury would be useful to prevent the development of metabolic diseases. Clearly, more detailed assessment of the associations between liver injury markers and cytokines for insulin sensitivity (adiponectin) and inflammation (IL-6), as well as liver damage assessed by ultrasonography, is necessary. In addition, it is essential to investigate whether the elevated serum ALT or γ-GTP activities are correlated with the severity of insulin resistance or inflammation in subjects without liver injury in cross-sectional and cohort studies.

It should be noted that alcohol intake was positively associated with γ-GTP and AST activities, supporting the previous findings that alcohol intake is independently and positively associated with circulating γ-GTP and AST activities (33). This may be because alcohol intake stimulates ROS production and decreases intracellular GSH levels (34, 35).

The mechanisms involved in the negative association between circulating ALT activity and adiponectin concentrations and the positive association between γ-GTP activity and IL-6 concentrations in middle-aged Japanese men not being treated for metabolic diseases are still unknown. ALT, which converts alanine to pyruvic acid and pyruvic acid to alanine, is a rate-controlling enzyme for gluconeogenesis in the liver. It is also known that the expression of genes related to gluconeogenesis, such as phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6P) and fructose-1,6-bisphosphatase, are repressed by insulin (36). Indeed, insulin secretion insufficiency and hepatic insulin resistance enhance gluconeogenesis and the expression of hepatic PEPCK and G6P genes because of decreased insulin activity (37), resulting in enhanced hepatic glucose release. A recent study in mice showed that the ALT2 gene, a subtype of ALT, is predominantly expressed in the liver, and its expression is enhanced in insulin-resistant fatty liver (38). Thus, the negative association between ALT activity and adiponectin in the current study may be related to enhanced gluconeogenesis as a result of hepatic insulin resistance. Thus, future studies should investigate the mechanisms underlying ALT expression and whether the changes in serum ALT activities are affected by changes in hepatic protein expression.

γ-GTP is a rate-controlling enzyme for re-synthesis of GSH from GSSG, and it is speculated that increased oxidative stress upregulates γ-GTP activity (39, 40). Upregulation of γ-GTP may also further enhance oxidative stress through increased ROS generation because γ-GTP produces ROS such as superoxide and hydrogen peroxide during the synthesis of GSH (10, 11). Furthermore, the mRNA levels of inflammatory cytokines, including IL-6, are increased by oxidative stress, such as enhanced ROS production (27–29). Future studies should investigate the associations among IL-6 and markers for the production of ROS, such as 8-hydroxydeoxyguanosine (a marker for DNA), carboxylated proteins (markers for proteins) and lipid peroxides (markers for lipids) and GSSG, in blood.

In summary, this study showed that circulating ALT activity is negatively associated with adiponectin concentrations, and γ-GTP activity is positively associated with increased IL-6 concentrations, while AST activity was not associated with these cytokines in middle-aged Japanese men not being treated for metabolic diseases.
Acknowledgments

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, Culture, Sports, Science and Technology of Japan, and by a grant from Suzuken Memorial Foundation. We thank Drs. S. Sasaki and K. Murakami for providing the BDHQ and for helpful discussions. The authors declare no conflict of interest.

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