Original Article

Liver Enzyme and Adipocytokine Profiles are Synergistically Associated with Insulin Resistance: the J-SHIPP Study

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Aim: Alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are associated with insulin resistance and arteriosclerotic disease. Since adiposity raises liver enzyme levels and causes insulin resistance, adipocytokines are thought to underlie the relationship between liver enzymes and insulin resistance. To clarify this hypothesis, we conducted a cross-sectional epidemiological study in a Japanese general population.

Methods: The study subjects were 903 middle-aged to elderly persons. Plasma levels of adiponectin and leptin were measured, while other clinical parameters were obtained from personal health records of medical check-ups. Insulin resistance was assessed by a homeostasis model assessment index (HOMA-IR).

Results: Plasma levels of ALT ($r = 0.379$, $p < 0.001$), GGT ($r = 0.225$, $p < 0.001$), adiponectin ($r = -0.346$, $p < 0.001$) and leptin ($r = 0.369$, $p < 0.001$) were significantly correlated with insulin resistance even on subgroup analysis by sex. Further, any combination of liver enzymes and adipocytokines was synergistically associated with insulin resistance ($p < 0.001$) after adjustment for possible covariates (ALT*adiponectin: $\beta = -0.098$, $p < 0.001$, ALT*leptin: $\beta = 0.129$, $p < 0.001$, GGT*adiponectin: $\beta = -0.054$, $p = 0.054$, GGT*leptin: $\beta = 0.126$, $p < 0.001$); however, in simple obese subjects with normal adipocytokine levels, liver enzymes were not associated with insulin resistance (mean HOMA-IR: worsened adipocytokine+/visceral obesity+, 2.01 ± 1.14; +/-, 1.39 ± 0.84; −/+, 1.23 ± 0.55; −/−, 1.03 ± 0.57; $p < 0.001$).

Conclusion: Plasma levels of ALT and GGT were independent determinants of insulin resistance only in subjects with a worsened adipocytokine profile. Use of liver enzyme levels as a marker of insulin resistance requires stratification by adipocytokine profile.


Key words; Alanine aminotransferase, γ-glutamyltransferase, Adiponectin, Leptin, Insulin resistance

Introduction

Metabolic syndrome is a major health burden in Western countries due to its deleterious effects on cardiovascular organs. Insulin resistance is a key concept linking metabolic syndrome, cardiovascular outcomes and risk profiles, including adiposity. The liver is also adversely affected by adiposity, and nonalcoholic fatty liver diseases (NAFLD), caused by lipid accumulation in hepatocytes, is an independent risk factor for future cardiovascular events1). NAFLD is accompanied by elevated liver enzymes such as γ-glutamyltransferase (GGT) and alanine aminotransferase (ALT). Several studies have reported that elevated plasma liver enzymes also predict the new onset of type 2 diabetes2- 3) and cardiovascular disease4), as well as cardiovascular, cancer and all-cause mortality4-6).

Visceral adipose tissue secretes several kinds of
adipocytokine. Among them, adiponectin is the most abundant adipocytokine in plasma, and levels correlate negatively with insulin resistance\(^7,8\). An association of lower adiponectin levels with type 2 diabetes and atherosclerosis has been clearly shown in both cross-sectional and longitudinal investigations\(^7\). At least three forms of circulating adiponectin have been identified, a lower molecular weight trimer (LMW), a middle molecular weigh hexamer (MMW), and a larger multimeric structure of high molecular weight (HMW) with 12 to 18 subunits\(^9\). Although previous biological and epidemiological analyses have indicated that the HMW complex is an active form of this protein\(^10-12\), the biological activity of adiponectin isoforms is controversial. A second major adipocytokine is leptin, which regulates food intake and energy homeostasis\(^13\). Leptin improves peripheral insulin sensitivity independent of its effects on energy expenditure\(^14\), and leptin resistance is accordingly considered to be another mediator of insulin resistance.

Adiposity is thus a common cause that worsens both liver enzyme profile and the adipocytokine profile. To date, however, it has remained unclear whether these markers independently reflect insulin resistance. Further, it is unclear whether the increased liver enzyme level is just a consequence of accumulated visceral fat or a complex phenotype affected by both adiposity and the adipocytokine profile.

**Aim**

If an elevated liver enzyme profile is preceded by a change in adipocytokine profile, associations of plasma GGT/ALT levels with cardiovascular morbidity and mortality should not be blindly applied to anyone who shows elevated liver enzymes. To clarify this hypothesis, we conducted a cross-sectional epidemiological study in apparently healthy middle-aged to elderly Japanese subjects.

**Subjects and Methods**

**Study Subjects**

The study subjects consisted of 903 consecutive apparently healthy middle-aged to elderly participants in the medical check-up program at Ehime University Hospital Anti-aging Center. This check-up program is specifically designed to evaluate aging-related disorders, including atherosclerosis, cardiovascular disease, physical function, and mild cognitive impairment\(^15,16\). Participants who had a history of symptomatic cardiovascular disease, who had been diagnosed with type 2 diabetes (fasting plasma glucose \(\geq 126\) mg/mL, or HbA1c \(\geq 6.5\)%, or taking blood glucose-lowering treatment), or whose liver enzyme levels were more than two-fold higher than the upper limit of the reference range (ALT \(> 98\) IU/L, GGT \(> 142\) IU/L) were excluded from participation. All clinical data used in this study were obtained during the check-up process. This cross-sectional investigation was carried out as part of a longitudinal study, the Shimanami Health Promoting Program (J-SHIPP study). This series of studies was approved by the ethics committee of Ehime University Graduate School of Medicine. All study subjects provided informed consent.

**Clinical Measurements**

Basic clinical parameters were obtained from the personal health record evaluated at the medical check-up program. Plasma samples were obtained from each subject after overnight fasting of more than 11 hours and immediately frozen and stored at \(-80^\circ\)C until analysis. Plasma concentration of adiponectin was determined using a commercially available ELISA kit (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan)\(^17\). Inter- and intra-assay coefficients of variation of this assay were 4.1% and 4.0%, respectively. Leptin concentration was measured using a commercially available RIA assay (Human Leptin RIA Kit, Linco Research Inc; MO, USA). Inter- and intra-assay coefficients of variation in this assay were 5.3% and 8.1%, respectively. The homeostasis model assessment index for insulin resistance (HOMA-IR: fasting immunoreactive insulin (\(\mu\)U/mL) \(\times\) fasting glucose (mg/dL)/405) was used as an index of insulin resistance.

**Liver Enzyme Risk Score and Adipocytokine Risk Score**

Precise cut-off points of plasma liver enzyme and adipocytokine levels have not been defined. We therefore used a quartile as a cut-off value of the plasma markers\(^4,18,19\). Subjects with plasma ALT levels in the highest quartile (28 IU/L in males, 23 IU/L in females) were considered as having high ALT. High GGT was defined as more than 44 IU/L in males and 27 IU/L in females. Liver enzyme risk score based on ALT and GGT was calculated by adding the number of parameters which exceeded the cut-off value. Adipocytokine risk score was calculated based on plasma leptin and adiponectin levels, with high leptin defined as more than 5.0 ng/mL in males and 10.3 ng/mL in females. Adiponectin levels lower than 5.0 \(\mu\)g/mL in males and 7.5 \(\mu\)g/mL in females were considered a risk factor.

**Measurement of Abdominal Visceral Fat Area**

Visceral fat area (VFA) (cm\(^2\)) was measured from
Clinical characteristic of study subjects

<table>
<thead>
<tr>
<th></th>
<th>male (n = 324)</th>
<th>female (n = 579)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.1 ± 9.0</td>
<td>65.4 ± 8.6</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 2.7</td>
<td>22.7 ± 3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visceral fat (cm²)</td>
<td>125.6 ± 62.0</td>
<td>83.5 ± 48.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>21 ± 25</td>
<td>6 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>137 ± 20</td>
<td>134 ± 20</td>
<td>0.063</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80 ± 11</td>
<td>76 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>114 ± 61</td>
<td>103 ± 56</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>62 ± 18</td>
<td>73 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>102 ± 9</td>
<td>98 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.49 ± 1.00</td>
<td>1.27 ± 0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>24 ± 11</td>
<td>20 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>35 ± 21</td>
<td>25 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>8.3 ± 4.5</td>
<td>11.7 ± 5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>4.0 ± 2.3</td>
<td>7.9 ± 4.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation. Statistical significance was assessed by analysis of variance. BMI, body mass index; HOMA-IR, homeostasis model assessment index for insulin resistance; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

Results

Clinical characteristics of the study subjects are summarized in Table 1. Plasma levels of adiponectin and leptin were significantly higher in females, as previously reported. In contrast, male subjects showed higher ALT and GGT values.

Plasma levels of liver enzymes, in particular ALT levels, were positively associated with HOMA-IR in both sexes (Table 2). Anthropometric parameters and adipocytokine levels were also significantly correlated with HOMA-IR. Multiple linear regression analysis adjusted for age, sex, VFA, alcohol consumption, and adipocytokines levels showed that the association between liver enzymes and HOMA-IR was independent of these confounding factors (Table 3, Model 1, 2).

Significant correlations were seen between ALT and adiponectin levels (men, \( r = -0.212, p < 0.001 \); women, \( r = -0.160, p < 0.001 \)), as well as leptin levels (\( r = 0.261, p < 0.001 \); \( r = 0.310, p < 0.001 \)). GGT was also significantly correlated with adiponectin (men, \( r = -0.251, p < 0.001 \); women, \( r = -0.111, p = 0.008 \)) and leptin levels (\( r = 0.209, p < 0.001 \); \( r = 0.189, p < 0.001 \)). We therefore conducted combined analyses of liver enzymes and adipocytokines to clarify the possible synergistic effect on insulin resistance. Results showed that the combination of any of the worsened liver enzyme and adipocytokine profiles was synergistically associated with HOMA-IR (Fig. 1). In addition to their direct effects, multiple regression analysis adjusted for possible covariates identified the adipocytokine-liver enzyme interaction terms as independent determinants of HOMA-IR (Table 3, Model 3 and 4).
GGT levels were independent markers of insulin resistance after adjustment for abdominal obesity. Further, liver enzyme and adipocytokine levels were synergistically associated with insulin resistance. In subjects with normal adipocytokine levels, however, liver enzyme levels did not reflect insulin resistance. A worsened adipocytokine profile may be required in the relation between liver enzyme levels and insulin resistance.

By facilitating fat infiltration in the liver, the accumulation of visceral fat results in metabolic disorders, including NAFLD, and fatty liver releases more liver enzymes into the peripheral blood. Westerbacka et al. reported a significant correlation between liver fat accumulation measured by proton spectroscopy and plasma ALT levels. Further, hepatic fat accumulation may play an independent role in the development of peripheral insulin resistance. Fabbrini et al. reported that intrahepatic fat levels, but not visceral fat accumulation, were a better marker of metabolic disorders. In the present study, we revealed

**Table 2.** Simple correlation analysis for HOMA-IR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male r</th>
<th>Male p</th>
<th>Female r</th>
<th>Female p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.034</td>
<td>0.537</td>
<td>0.115</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.442</td>
<td>&lt;0.001</td>
<td>0.464</td>
<td>&lt;0.001</td>
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<tr>
<td>Visceral fat (cm²)</td>
<td>0.482</td>
<td>&lt;0.001</td>
<td>0.479</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>0.023</td>
<td>0.686</td>
<td>0.091</td>
<td>0.029</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>0.321</td>
<td>&lt;0.001</td>
<td>0.403</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>0.214</td>
<td>&lt;0.001</td>
<td>0.195</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>−0.368</td>
<td>&lt;0.001</td>
<td>−0.315</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>0.505</td>
<td>&lt;0.001</td>
<td>0.509</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Regression analysis was further adjusted for age, sex and alcohol consumption. The following independent variables were log-transformed: ALT, GGT, adiponectin and leptin.

The combined association of the liver enzyme risk score and adipocytokine risk score with HOMA-IR is depicted in Fig. 2. Subjects with worsened risk scores for both showed markedly higher HOMA-IR (males 2.7 ± 0.6, females 3.0 ± 1.4) as compared with the normal control group (1.0 ± 0.6, 1.0 ± 0.5, p < 0.001); however, in subjects with a normal adipocytokine profile, no association was found between liver enzymes and HOMA-IR. Fig. 3 depicts stratified analysis by adipocytokine and visceral fat profiles. An association between liver enzymes and HOMA-IR was observed only in subjects with a worsened adipocytokine profile. Although visceral obesity exerted an additive effect in subjects with either a normal or worsened adipocytokine profile, this was not essential for an association between the liver enzyme profile and HOMA-IR.

**Discussion**

In this study, we showed that elevated ALT and

<table>
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<tr>
<th>Variable</th>
<th>β</th>
<th>p</th>
<th>β</th>
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<th>β</th>
<th>p</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>0.224</td>
<td>&lt;0.001</td>
<td>0.173</td>
<td>&lt;0.001</td>
<td>0.100</td>
<td>&lt;0.001</td>
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<tr>
<td>GGT (IU/L)</td>
<td></td>
<td></td>
<td>0.117</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td>0.162</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>−0.159</td>
<td>&lt;0.001</td>
<td>−0.158</td>
<td>&lt;0.001</td>
<td>−0.180</td>
<td>&lt;0.001</td>
<td>−0.318</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>0.295</td>
<td>&lt;0.001</td>
<td>0.322</td>
<td>&lt;0.001</td>
<td>0.295</td>
<td>&lt;0.001</td>
<td>0.318</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visceral fat area (cm²)</td>
<td>0.171</td>
<td>&lt;0.001</td>
<td>0.200</td>
<td>&lt;0.001</td>
<td>0.147</td>
<td>&lt;0.001</td>
<td>0.195</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT*adiponectin</td>
<td>−0.098</td>
<td>&lt;0.001</td>
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<tr>
<td>ALT*leptin</td>
<td>0.129</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>GGT*adiponectin</td>
<td>−0.054</td>
<td>0.054</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GGT*leptin</td>
<td>0.126</td>
<td>&lt;0.001</td>
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Regression analysis was further adjusted for age, sex and alcohol consumption. The following independent variables were log-transformed: ALT, GGT, adiponectin and leptin.

GGT levels were independent markers of insulin resistance after adjustment for abdominal obesity. Further, liver enzyme and adipocytokine levels were synergistically associated with insulin resistance. In subjects with normal adipocytokine levels, however, liver enzyme levels did not reflect insulin resistance. A worsened adipocytokine profile may be required in the relation between liver enzyme levels and insulin resistance.
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that changes in adipocytokine levels, but not simple visceral obesity, were required for the association between liver enzyme levels and insulin resistance. Hepatic adiposity may be the underlying mechanism for the association between the liver enzyme level and insulin resistance.

Another possible explanation for the association between liver enzymes and insulin resistance is oxidative stress caused by changes in adipocytokine levels. Kamada et al. 29) reported that oxidative stress in adiponectin knockout mice was significantly higher than in wild-type mice, and that these high levels cause hepatic DNA damage and eventually hepatocarcinogenesis. Canbakan et al. 30) examined the effects of plasma leptin levels on oxidative stress and the severity of histological changes in NAFLD. Results showed that leptin had a preventive effect on the progression of liver injury. Since GGT production is up-regulated in order to counteract free radicals, oxidative stress and consequent inflammation in fatty liver are thought to be other underlying mechanisms of the association between liver enzyme levels and insulin resistance. 31).

Accumulated evidence indicates that plasma liver

Fig. 1. Association between liver enzyme/adipocytokine profiles and HOMA-IR.

Values are the mean ± standard deviation. Study subjects were classified into four groups according to liver enzyme and adipocytokine profiles. Subject classification was performed within sex and then combined to avoid gender differences. Statistical significance was assessed by analysis of variance and post hoc analysis was performed using Dunnett’s test. Number of subjects in each category is shown in the column. Adpntn indicates adiponectin.

Fig. 2. Synergistic associations between liver enzyme risk score and adipocytokine risk score with HOMA-IR.

Study subjects were divided into nine groups according to risk scores for liver enzymes and adipocytokine. Number of subjects in each category is shown in the column. Statistical significance was assessed by analysis of covariance adjusted for age, sex, visceral fat area and alcohol consumption.
Conclusion

In conclusion, our study shows that plasma levels of ALT and GGT are independent determinants of insulin resistance only in subjects with a worsened adipocytokine profile. The use of the liver enzyme level as a marker of insulin resistance, metabolic disorders, and cardiovascular events requires stratification by adiposity status.

Acknowledgements

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Disclosures

The authors have nothing to disclose.

Conflicts of Interest

None declared.
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