Circulating Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor Binding Protein-3 Are Associated With Early Carotid Atherosclerosis

Shin-ichi Kawachi, Noriyuki Takeda, Akihiko Sasaki, Yoshiaki Kokubo, Kazuhisa Takami, Hiroshi Sarui, Makoto Hayashi, Noriyoshi Yamakita, Keigo Yasuda

Objective—Growth hormone (GH)–insulin-like growth factor (IGF)-1 axis regulates growth and survival of vascular cells and cardiomyocytes. The role of GH–IGF-1 axis in cardiovascular disease is controversial.

Methods and Results—We assessed the association of circulating levels of IGF-1 and IGF binding protein-3 (IGFBP-3) with early carotid atherosclerosis and atherosclerotic risk factors in 330 Japanese men (age 51.6±8.6 years, range 29 to 77, body mass index [BMI] 23.6±2.9 kg/m²). Intima-media thickness (IMT) of the common carotid artery was measured by ultrasound. Abdominal visceral adipose and subcutaneous adipose tissue area by computer-assisted tomographic scan were determined. Correlation coefficients were calculated by partial correlation analysis. BMI and plasma insulin showed positive associations with circulating IGF-1 and IGFBP-3. Subcutaneous adipose tissue was correlated with IGF-1. High-density lipoprotein cholesterol was inversely associated with IGF-1. Blood pressure, total cholesterol, triglyceride, and visceral adipose tissue were positively associated with IGFBP-3. IGF-1 and IGFBP-3 were associated with carotid IMT independent of age, BMI, blood pressure, and insulin. Insulin was associated with carotid IMT in univariate analysis. However, it was not correlated with carotid IMT in the multivariate analyses which included IGF-1 or IGFBP-3 as a covariate.

Conclusion—Increased circulating IGF-1 and IGFBP-3 may be stimulators of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2005;25:617-621.)

Key Words: insulin-like growth factor-1 ■ insulin-like growth factor binding protein-3 ■ atherosclerosis ■ cardiovascular disease ■ metabolic syndrome

The growth hormone (GH)–insulin-like growth factor (IGF)-1 axis plays an important role in the regulation of structure and function of the cardiovascular system.1,2 IGF-1 acts on vascular cells as a potent stimulator of cell proliferation and also as an inhibitor of apoptosis. IGF-1 is also capable of improving cardiac muscle survival, growth, and cellular calcium signaling. The role of GH–IGF-1 axis in cardiovascular disease has long attracted attention,3 prompted by the fact that patients with both GH excess4 and deficiency5 have an increased risk of developing cardiovascular disease.

Patients with GH deficiency are known to share many if not all features of insulin resistance syndrome, which is a constellation of potentially atherogenic abnormalities, such as hyperinsulinemia, glucose intolerance, hypertension, dyslipidemia, and coagulation abnormalities.6 The syndrome is a well-established precursor of cardiovascular disease. Recently, recombinant human GH administration has been shown to improve metabolic abnormalities and reverse early atherosclerotic changes in patients with GH deficiency.7 It may well be hypothesized that decreased activity of the GH–IGF-1 axis promotes atherosclerosis either by decreased effects of these hormones on vascular cells or by mediating atherogenic metabolic abnormalities. In fact, most8–11 but not all12,13 previous studies demonstrated that circulating levels of IGF-1 and/or IGF binding proteins (IGFBP) were decreased in patients with manifest coronary artery disease. This study was designed to examine the role of the IGF-1 axis in early atherosclerosis before overt clinical disease. To this end we measured circulating IGF-1 and IGFBP-3 in 330 Japanese men and assessed their associations with carotid intima-media thickness (IMT), which is known to predict future incidence of coronary artery disease.14 We included IGFBP-3 in our study, because it has been reported not only to modulate IGF-1 effects but also to possess IGF-1 independent growth inhibitory action.15 Moreover, epidemiological studies have demonstrated that circulating levels of IGF-1

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and IGFBP-3 have opposite predictive roles in the risk of several types of cancer. It would be interesting to see whether this is the case also for atherosclerosis.

**Methods**

**Study Subjects**

The subjects were 330 Japanese men aged 29 to 77 years (mean ± SD, 51.6 ± 8.6 years) who participated in a health check-up program conducted in Matsunami General Hospital, an urban hospital located in Gifu Prefecture, Japan, in the year 2000. Those who had a previous history of acute myocardial infarction, angina, or stroke and those who had ischemic changes on the ECG taken in this study were excluded from the study. All subjects gave informed consent before entry. The study was approved by the ethics committee of Matsunami General Hospital and of Gifu University School of Medicine.

**Data Collection and Measurements**

The subjects came to the hospital in the morning and stayed there for 36 hours until they had completed all scheduled medical examinations. The health check program that they attended, as previously reported, included blood chemistry, a standard oral 75g glucose tolerance test, ECG, chest X-ray, barium examination of the upper gastrointestinal tract, and computer-assisted tomographic scan of the abdomen. Plasma glucose and insulin were measured by a glucose oxidase method and a double antibody radioimmunoassay, respectively. A parameter of insulin resistance was calculated from a tolerance test (AUC-PG) was calculated as a measure of glucose oxidase method and a double antibody radioimmunoassay, respectively. Area under the curve of plasma glucose in oral glucose tolerance test (AUC-PG) was calculated as a measure of glucose tolerance. A parameter of insulin resistance was calculated from a pair of values of fasting plasma glucose and insulin based on a homeostasis model (HOMA-R). Serum total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were measured by methods described elsewhere. Serum IGF-1 was measured by an immunoradiometric assay using a commercially available kit (Yuka Medias). The limit of detection was 0.3 ng/mL. Intra- and interassay coefficients of variation (CVs) were 5.2% and 7.7%, respectively. Serum IGFBP-3 was measured by an immunoradiometric assay kit (Diagnostic Systems Laboratories Inc; detection limit 1 ng/mL, intra-assay CV 2.6%, interassay CV 6.9%).

Body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m). Abdominal visceral (VAT) and subcutaneous adipose (SAT) tissue areas were measured by computer-assisted tomographic scan as described elsewhere. IMT of the common carotid artery was measured by B-mode ultrasound using a Logiq 500 (General Electric Yokogawa Medical System) according to the method of Pignoli et al modified by us. A longitudinal 2D ultrasound image of the common carotid artery was scanned with a 10-MHz linear array transducer while patients were in a supine position. The greatest IMT and those measured 1 cm upstream and downstream from the site of the greatest IMT were measured bilaterally. In total, 6 IMT values were obtained for each subject. The average of these measurements was calculated and used for the statistical analyses. The measurement of IMT was performed by a single physician throughout the study, so as to avoid interobserver variations. Smoking status was obtained by a self-administered questionnaire. Smoking status was expressed by the Brinkman index, which is calculated as the number of cigarettes per day multiplied by years of smoking.

**Statistical Analysis**

The results were expressed as mean ± SD. To improve normality of the distribution, triglyceride, insulin, and HOMA-R were transformed to their logarithms before statistical analysis. The median and range were also given for these variables. Statistical analyses were made using the Statistical Analysis System version 6.12 for Windows (SAS Institute Inc). Relations between variables were evaluated by partial correlation analysis. Because we tried to improve skewness in the distribution of the Brinkman index but we could not approximate normality by logarithmic, square root, and other transformations, we used Spearman partial rank correlation test for analyses of correlation between the Brinkman index and other variables after adjustment for age. Adjustment for age was done by regressing the Brinkman index and other variables separately on age. The Spearman correlation coefficients between these residuals were then calculated. P<0.05 was accepted as the significance level.

**Results**

**Clinical and Laboratory Characteristics**

Table 1 shows the clinical and laboratory characteristics of the study subjects. They were relatively lean compared with black and white American men of a similar age range. Their mean BMI, however, accords well with the figure obtained from a survey of a large sample conducted in Japan. As shown in Table 2, after adjustment for age, BMI was correlated with circulating IGF-1 and IGFBP-3. SAT was correlated with IGF-1. VAT was correlated with IGFBP-3. Blood pressure was not correlated with IGF-1. However, there was a positive correlation between blood pressure and IGFBP-3. Total cholesterol and log triglyceride were correlated positively with IGFBP-3. HDL-cholesterol was inversely correlated with IGF-1. Both log fasting plasma insulin and log HOMA-R were correlated positively with IGF-1 and IGFBP-3. Fasting plasma glucose and AUC-PG were not associated with IGF-1 or IGFBP-3. Brinkman index was not

**Table 1. Clinical and Laboratory Characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD (Median, Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51.6</td>
<td>8.6</td>
</tr>
<tr>
<td>Brinkman index</td>
<td>553.1</td>
<td>561.2 (455, 0–2960)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>123.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77.9</td>
<td>10.5</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>110.0</td>
<td>49.1</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>129.6</td>
<td>56.7</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>197.1</td>
<td>31.4</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>140.0</td>
<td>93.3 (116.0, 30–803)</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>55.9</td>
<td>15.9</td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>101.1</td>
<td>13.0</td>
</tr>
<tr>
<td>Fasting plasma insulin, µU/ml</td>
<td>6.4</td>
<td>3.7 (5.5, 2.2–32.0)</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>1.63</td>
<td>1.02 (1.39, 0.50–8.05)</td>
</tr>
<tr>
<td>AUC-PG, mg/dl min</td>
<td>16 237</td>
<td>4323</td>
</tr>
<tr>
<td>IGF-1, ng/ml</td>
<td>160.7</td>
<td>36.9</td>
</tr>
<tr>
<td>IGFBP-3, ng/ml</td>
<td>3193.2</td>
<td>496.6</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.66</td>
<td>0.12</td>
</tr>
</tbody>
</table>

VAT indicates visceral abdominal adipose tissue; SAT, subcutaneous abdominal adipose tissue; HOMA-R, homeostasis model assessment of insulin resistance; AUC-PG, area under the curve of plasma glucose during 75g oral glucose tolerance test; IMT, common carotid arterial intima-media thickness.
associated with IGF-1 or IGFBP-3. Although not shown in Table 2, there was a close correlation between IGF-1 and IGFBP-3 ($r=0.489$, $P=0.0001$).

### The IGF-1 Axis and Carotid IMT

Both circulating IGF-1 and IGFBP-3 were associated with carotid IMT after adjustment for age (Table 3). Among the other variables tested, BMI, blood pressure, log fasting plasma insulin, and log HOMA-R showed significant correlations with carotid IMT. Correlation between IMT and Brinkman index was analyzed by Spearman rank correlation test after adjustment for age. Brinkman index was not significantly correlated with IMT.

To evaluate the confounding effects of these variables on the association between the IGF-1 axis and carotid IMT, 2 multivariate models were tested (Table 4). Both models contain all variables that showed a significant association with IMT in partial correlation analyses after adjustment for age. Then age, BMI, systolic blood pressure, and log fasting plasma insulin were included as independent variables. In addition, IGF-1 and IGFBP-3 were included in model 1 and 2, respectively. Because there were close correlations between systolic and diastolic blood pressure ($r=0.685$, $P=0.0001$), log fasting plasma insulin, and log HOMA-R ($r=0.976$, $P=0.0001$), we excluded diastolic blood pressure and log HOMA-R from our multivariate models. Both IGF-1 and IGFBP-3 were positively correlated with carotid IMT in the multivariate analyses. It should be noted that neither log fasting plasma insulin nor BMI was an independent correlate in these multivariate models.

### Discussion

We have found that serum IGF-1 and IGFBP-3 levels are associated with carotid IMT in Japanese men. The results are significant, because the prevailing view is that decreased activity of the GH–IGF-1 axis may predispose to cardiovascular disease.

The effect of IGF-1 axis on vascular cells is complex.\(^1\)\(^2\) IGF-1 stimulates vascular smooth muscle cell (VSMC) pro-

### TABLE 2. Correlation Between the IGF-1 Axis and Body Adiposity, Blood Pressure, and Metabolic Variables (Partial Correlation Coefficient After Adjustment for Age)

<table>
<thead>
<tr>
<th>Variables</th>
<th>IGF-1</th>
<th></th>
<th>IGFBP-3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>0.206</td>
<td>0.0002</td>
<td>0.154</td>
<td>0.0052</td>
</tr>
<tr>
<td>VAT</td>
<td>0.098</td>
<td>0.0748</td>
<td>0.197</td>
<td>0.0003</td>
</tr>
<tr>
<td>SAT</td>
<td>0.147</td>
<td>0.0075</td>
<td>0.083</td>
<td>0.1345</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.105</td>
<td>0.0560</td>
<td>0.161</td>
<td>0.0034</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.084</td>
<td>0.1295</td>
<td>0.128</td>
<td>0.0201</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.002</td>
<td>0.9728</td>
<td>0.211</td>
<td>0.0001</td>
</tr>
<tr>
<td>log triglyceride</td>
<td>0.082</td>
<td>0.1361</td>
<td>0.208</td>
<td>0.0002</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>−0.154</td>
<td>0.0052</td>
<td>−0.037</td>
<td>0.5013</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.084</td>
<td>0.1301</td>
<td>0.101</td>
<td>0.0674</td>
</tr>
<tr>
<td>Log fasting plasma insulin</td>
<td>0.226</td>
<td>0.0001</td>
<td>0.113</td>
<td>0.0401</td>
</tr>
<tr>
<td>Log HOMA-R</td>
<td>0.228</td>
<td>0.0001</td>
<td>0.126</td>
<td>0.0222</td>
</tr>
<tr>
<td>AUC-PG</td>
<td>−0.040</td>
<td>0.4661</td>
<td>0.053</td>
<td>0.3381</td>
</tr>
<tr>
<td>Brinkman index*</td>
<td>−0.005</td>
<td>0.9263</td>
<td>0.047</td>
<td>0.3911</td>
</tr>
</tbody>
</table>

**Note:** VAT indicates visceral abdominal adipose tissue; SAT, subcutaneous abdominal adipose tissue; HOMA-R, homeostasis model assessment of insulin resistance; AUC-PG, area under the curve of plasma glucose during 75g oral glucose tolerance test.

* Spearman rank correlation test was used to calculate correlation coefficient.
liferation and migration to promote neointimal formation. On the other hand, IGF-1 may serve to protect against plaque instability and rupture by suppressing VSMC apoptosis and increasing VSMC elastogenesis.

Previous studies demonstrated decreased circulating levels of total or free IGF-1 or IGFBP-3 in patients with coronary artery disease. In contrast, we found that carotid IMT increased with the levels of IGF-1 and IGFBP-3 in Japanese men. However, our results are not contradictory to the previous studies. The difference in study populations, that is, patients with manifest heart disease versus healthy men, seems to account largely for the discordance between ours and the above-mentioned studies.

Systemic circulatory IGF-1 levels are regulated by complex mechanisms. Nutritional status and physical activity are important determinants of circulating IGF-1 levels. There is evidence suggesting that IGF-1 levels can be downregulated by cytokines. It is possible that IGF-1 levels in patients with manifest coronary artery disease may be affected by increased production of cytokines which was observed in acute myocardial infarction. Self-restriction of physical activity to prevent anginal episodes, and poor nutrition during acute illness. In this regard, it is notable that several investigators have demonstrated time-dependent changes in circulating IGF-1 levels in patients with acute myocardial infarction. Conti reported that circulating IGF-1 levels were markedly reduced in the acute phase of myocardial infarction but were increased production of cytokines which was observed in acute myocardial infarction. Self-restriction of physical activity to prevent anginal episodes, and poor nutrition during acute illness. In this regard, it is notable that several investigators have demonstrated time-dependent changes in circulating IGF-1 levels in patients with acute myocardial infarction. Conti reported that circulating IGF-1 levels were markedly reduced in the acute phase of myocardial infarction but were normalized after 1 year. Furthermore, Lee et al. found that patients with acute myocardial infarction had higher circulating levels of IGF-1 and IGFBP-3 on day 1 of admission to hospital, with the levels decreasing through day 2 and day 3 and then bouncing back to levels higher than control levels from day 7 to day 21.

We studied subclinical early carotid atherosclerosis by measuring carotid IMT. The study subjects were healthy men without active illness. Those who had a previous history of cardiovascular disease or ischemic ECG changes were excluded. The features of the study probably allowed us to evaluate the association between circulating IGF-1 and IGFBP-3 and carotid atherosclerosis with minimal, if any, effects of cardiovascular disease on levels of IGF-1 and IGFBP-3. This study suggests that increased circulating IGF-1 and IGFBP-3 may promote atherosclerosis.

The role of the GH–IGF-1 axis in atherosclerosis was initially implicated in patients with pituitary disorders. Increase in carotid IMT was reported in patients with GH deficiency and also in those with GH excess. This study extended these early studies to subjects without pituitary disorders. It is interesting to note that there may be a U-shaped curve between GH–IGF-1 axis activity and atherosclerosis. Although metabolic abnormalities associated with both GH deficiency and excess and the direct effect of GH–IGF-1 on vascular cells probably contribute to form such a U-shaped curve, the mechanisms underlying such a relationship need to be elucidated.

IGFBP-3 is a member of 6 IGFBPs, which associate with IGF-1 and IGF-II with high affinity. IGFBP-3 is the most abundant IGFBP in the circulation. For years, the role of IGFBPs was thought to be confined to preventing IGFs from binding the receptor and activating the cellular signaling pathways. In recent years, however, accumulating evidence indicates that IGFBPs are also able to modulate IGF actions positively. Furthermore, they may exert IGF-independent effects. In this study, circulating IGFBP-3 levels were correlated with carotid IMT. Because there was a close correlation between circulating levels of IGF-1 and IGFBP-3, it is difficult to statistically determine whether either possesses real linkage with carotid IMT. In contrast with negative associations between IGFBP-3 and cancer risk after adjustment for IGF-1, the association between IGFBP-3 and carotid IMT was eliminated after adjustment for IGF-1 in our study. The role of IGFBP-3 in atherosclerosis seems different from its role in certain cancers.

Recently, Juul demonstrated that the low IGF-1 and high IGFBP-3 predicted increased risk of ischemic heart disease with a case control study which was conducted in a large prospective study on cardiovascular epidemiology. Their results are opposite to ours in terms of IGF-1 but similar for the role of IGFBP-3. However, if we look at their data closely, there were no differences in the levels of IGF-1 and IGFBP-3 between their cases with ischemic heart disease and controls before statistical adjustment. The difference in the levels of IGF-1 emerged after adjustment for IGFBP-3 and vice versa. Interpretation of these analyses is difficult because of colinearity between IGF-1 and IGFBP-3.

Although the correlation coefficients of IGF-1 and IGFBP-3 with carotid IMT in our study were rather weak compared with that between age and carotid IMT, they were comparable to that of blood pressure with carotid IMT. Aging influence on carotid IMT was noted in a number of previous studies and is generally attributed to exposure of arterial wall over time to atherogenic effects of various risk factors. However, even in subjects without major cardiovascular risk factors, carotid IMT increased with age. Thus it may be possible that increasing IMT with aging may reflect a specific effect of aging on arterial wall other than atherosclerosis.

In our study, it should be noted that circulating IGF-1 levels were correlated with insulin levels. The association between IGF-1 and insulin may reflect the effect of insulin to increase hepatic IGF-1 production, or circulating levels of both hormones may be determined by common nutritional and other causal factors. For example, nutritional surfeit which upregulates circulating IGF-1 can lead to increased adiposity, which in turn results in hyperinsulinemia. The association between IGF-1 and insulin raises another important issue. Hyperinsulinemia is an important feature of the insulin resistance syndrome and has been noted to be a predictor of coronary artery disease in several prospective studies. In our multivariate models, IGF-1 and IGFBP-3 were independent correlates with carotid IMT, whereas insulin was not. Because IGF-1 is a much more potent mitogenic factor than insulin itself, there is a possibility that IGF-1 mediates at least partly the link between hyperinsulinemia and atherosclerosis.

Because this study is cross-sectional in nature, a causal relationship cannot be established. However, the association between carotid IMT and circulating IGF-1 and IGFBP-3 in
an apparently healthy population has important implications for the pathogenesis of early atherosclerosis.

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