Serum Insulin-Like Growth Factor-I Level Is Independently Associated With Coronary Artery Disease Progression in Young Male Survivors of Myocardial Infarction: Beneficial Effects of Bezafibrate Treatment

Giacomo Ruotolo, MD, PhD,* Peter Båvenholm, MD, PhD,† Kerstin Brismar, MD, PhD,‡ Suad Efendic, MD, PhD,§ Carl-Göran Ericsson, MD, PhD,§ Ulf de Faire, MD, PhD,† Jan Nilsson, MD, PhD,‡ Anders Hamsten, MD, PhD†

Stockholm, Sweden

OBJECTIVES We investigated whether the effect of bezafibrate on progression of coronary atherosclerosis in the BEzafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) was related to insulin-like growth factor (IGF)-I and glucose-insulin homeostasis.

BACKGROUND BECAIT, the first double-blind, placebo-controlled, randomized, serial angiographic trial of a fibrate compound, demonstrated that progression of focal coronary atherosclerosis in young patients after infarction could be retarded by bezafibrate treatment.

METHODS The treatment effects on serum concentrations of IGF-I and insulin-like growth factor binding protein (IGFBP)-1, as well as on basal and postload glucose and insulin levels, were examined, and on-trial determinations were related to the angiographic outcome measures.

RESULTS Bezafibrate treatment resulted in a significant reduction of serum IGF-I levels, both at two and five years, and on-trial serum IGF-I levels were directly related to changes in both minimal lumen diameter ($r = 0.25$, $p < 0.05$) and mean segment diameter ($r = 0.29$, $p < 0.05$). In contrast, none of the available indexes of insulin resistance (homeostasis model assessment estimate, basal and postload plasma insulin concentrations and serum IGFBP-1 levels) were related to the angiographic changes, nor were they significantly affected by bezafibrate treatment. Multiple stepwise regression analysis showed that the relation between on-trial serum IGF-I level and coronary artery disease (CAD) progression was independent of baseline angiographic score, age, body mass index, serum lipoprotein and plasma fibrinogen concentrations and measures of glucose–insulin homeostasis.

CONCLUSIONS IGF-I could be implicated in the progression of premature CAD, and a reduction of serum IGF-I concentration could account partly for the effect of bezafibrate on progression of focal coronary atherosclerosis. (J Am Coll Cardiol 2000;35:647–54) © 2000 by the American College of Cardiology

Most of the features of insulin resistance syndrome can be found in patients who have survived a myocardial infarction (1) and have been related to the progression of coronary artery disease (CAD) (2). Insulin resistance syndrome is characterized by clustering of hyperinsulinemia, hypertriglyceridemia, hypertension, obesity and a low high density lipoprotein (HDL) cholesterol level (3), but other factors have been suggested as components of insulin resistance syndrome as well. Insulin-like growth factor binding protein (IGFBP)-1, which is the best characterized of the binding proteins for insulin-like growth factor (IGF)-I, has recently been included among the components of insulin resistance syndrome (4). Its hepatic synthesis is mainly under insulin control, and a decreased concentration of IGFBP-1 is considered a very good marker of insulin sensitivity. However, six different proteins (IGFBP-1 through IGFBP-6)
have been shown to bind exclusively to IGF-I and IGF-II, with no cross-linking to insulin. IGFBP-3 binds 70% to 80% and IGFBP-1 20% to 30% of both IGFs, whereas only ~10% of IGF-I is free. The main function of IGFBPs seems to be the regulation of IGF-I availability (5).

IGF-I has insulin-like metabolic effects and, predominantly, growth-promoting actions. Because it is important for tissue repair and cell proliferation, it has been implicated in the pathogenesis of atherosclerosis (6). However, clinical studies have produced conflicting results regarding the relation between circulating IGF-I and CAD (7–9), and longitudinal data linking circulating IGF-I to progression of CAD are still lacking.

The BEZafibrate Coronary Atherosclerosis Intervention Trial (BECAIT), the first double-blind, placebo-controlled, randomized, serial angiographic trial of a fibrate compound, demonstrated that progression of focal coronary atherosclerosis in young survivors of myocardial infarction could be retarded by bezafibrate treatment (10). The angiographic effects observed in the bezafibrate-treated patients were accompanied by significant reductions in very low density lipoprotein (VLDL) cholesterol, triglycerides and plasma fibrinogen, as well as an increase in HDL cholesterol (10). In the present report from BECAIT, the treatment effects on IGF-I and IGFBP-1 levels, as well as on glucose–insulin homeostasis, are examined, and on-trial serum levels are related to the angiographic outcome measures. Our results demonstrate that serum IGF-I levels are reduced by bezafibrate treatment, and that on-trial IGF-I levels are independently associated with CAD progression in young survivors of myocardial infarction.

METHODS

Trial design. The rationale, design features and recruitment procedures of the study have been reported (10,11). In brief, 92 men (<45 years old) who survived a first myocardial infarction were included, all of whom had a serum cholesterol level of at least 5.2 mmol/liter and a mean serum triglyceride level of at least 1.6 mmol/liter, or both, as measured on two occasions after a three-month period of dietary and life-style intervention. The presence of visually detectable lesions in at least one coronary segment constituted a further inclusion criterion. Eligible patients were randomly assigned to double-blind treatment with bezafibrate (200 mg three times daily, n = 47) or placebo (n = 45), after having given written, informed consent to participate. All participants also continued the pretreatment diet throughout the study. Selective coronary angiography was repeated after two and five years. Patients with manifest diabetes mellitus were not included in the study.

Blood sampling was performed at baseline and then yearly during the entire treatment period for fasting serum lipoprotein determinations. IGF-I and IGFBP-1 were measured on serum samples drawn at baseline and two and five years after randomization, at which points oral glucose tolerance tests were also done.

The study protocol was approved by the Ethics Committee of the Karolinska Institute.

Oral glucose tolerance test. Glucose was ingested in a dose of 1.75 g/kg body weight in 150 to 200 ml of water flavored with lemon extract. Venous blood samples were collected through an indwelling catheter before and 15, 30, 45, 60, 90 and 120 min after glucose intake. Oral glucose tolerance was assessed according to criteria adopted from Reaven et al. (12). The plasma insulin response to glucose ingestion was evaluated by calculation of the insulin area above the fasting level throughout the 120 min of the test. The glucose area under the curve indicated the total glucose area during the oral glucose tolerance test. A relative insulin resistance index was calculated from the fasting blood glucose and plasma insulin concentrations using the computer-solved homeostasis model assessment (HOMA) (13).

Lipoprotein separation. The major serum lipoproteins (VLDL, low density lipoprotein [LDL] and HDL) were determined by a combination of preparative ultracentrifugation and precipitation of apolipoprotein B–containing lipoproteins, followed by lipid analyses (14). The HDL3 fraction was obtained as a bottom fraction in which cholesterol was determined after one preparative ultracentrifuge spin at a density of 1.125 kg/liter (15). For determination of the LDL particle size distribution, serum samples containing 1 μg of LDL cholesterol were subjected to 3% to 7.5% polyacrylamide gradient gel electrophoresis. All three samples from an individual patient (taken at baseline and two and five years after randomization) were applied to the same gel, along with three standard reference proteins (16). The intra-assay and interassay coefficients of variation for LDL peak particle size determinations were 1.3% and 2.3%, respectively.

Assays. Serum apolipoprotein B was determined by radioimmunoassay. Serum lipoprotein (Lp(a)) was measured using an enzyme immunoassay (TintElize Lp[a], Biopool, Umeå, Sweden). Glucose was measured in whole blood by the glucose oxidase method (17). Plasma insulin was mea-
Coronary angiography. Coronary angiography was performed using the percutaneous transfemoral technique at baseline and then repeated two and five years later. Quantitative computer-assisted evaluation was undertaken with the Cardiovascular Measurement System (Medis, Nuenen, The Netherlands) (22) by individuals who had no knowledge of the treatment assignment and the on-trial biochemical determinations, as described (10). Angiograms were routinely obtained before and within 10 min after intracoronary or sublingual nitroglycerin administration to eliminate differences in vascular constriction. The minimal lumen diameter (MLD), which reflects focal atherosclerosis, was measured at the site of the most severe atherosclerotic lesion in each segment that reduced the lumen diameter by at least 20%. The mean segment diameter (MSD) was calculated in all coronary segments, irrespective of the presence of visually detectable atherosclerosis, as an indicator of diffuse as well as focal atherosclerosis. Percent diameter stenosis was calculated from the most narrow lesion in each segment with a diameter reduction of at least 20%. Measurements of MSD were only undertaken in unoccluded segments and in segments proximal to an occlusion, whereas MLD and percent diameter stenosis were set to 0 mm and 100%, respectively, in occluded segments and distal segments not recorded. Patients who had coronary angioplasty for clinical reasons during the study were included in the analyses, but the segments involved in the procedure and all distal segments were excluded from subsequent analyses.

Statistical analysis. Continuous variables are presented as the least squares mean value ± SEM or median value (interquartile range); categoric variables are presented as numbers (percentages). The individual values of skewed variables were transformed to their natural logarithms before statistical tests. Per-patient means of MLD, MSD and percent diameter stenosis were calculated for baseline and follow-up angiograms. The angiographic outcome variables were median change (Δ) between the baseline and last follow-up angiogram for MLD and MSD, and between the last follow-up and baseline angiogram for percent diameter stenosis. Only corresponding segments from the baseline and follow-up angiograms were used in the assessment of angiographic change. The chi-square test was used for comparing the distribution of patients according to glucose tolerance category during the five-year follow-up. The responses of IGF-I, IGFBP-1 and glucose–insulin variables to the study drug over time in the bezafibrate and placebo groups were compared by two-way repeated measures analysis of variance (ANOVA). The disease progression data (ΔMLD, ΔMSD and Δ% diameter stenosis) were either used as continuous variables or divided according to tertiles. The association between baseline clinical characteristics and tertiles of angiographic outcome measures was evaluated by ANOVA. The Tukey-Kramer test was used for post-hoc analysis when differences between the groups were significant according to simple or repeated measures ANOVA. The association between IGF-I, IGFBP-1 and glucose–insulin variables and disease progression was first assessed by calculation of univariate Pearson and partial correlation coefficients. Baseline angiographic measurement, age, on-trial body mass index, serum Lp(a) level and LDL peak particle size were controlled for when calculating partial correlation coefficients. A multivariate model was then generated by multiple stepwise linear regression analysis using a forward approach to identify variables independently correlating with the angiographic outcome variables. Baseline angiographic measurement, age and on-trial body mass index, serum Lp(a) level and LDL peak particle size were selected as covariates.

RESULTS

Basic characteristics of the patients. The baseline characteristics of the 92 randomized patients have been described in detail (10). The present report is based on the 81 patients (42 treated with bezafibrate and 39 with placebo) who had a baseline angiogram and at least one post-

| Table 1. General Characteristics of Patients at Baseline (n = 81) |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Age (yrs) | 42 (39–44) |
| Body mass index (kg/m²) | 26.5 (24.7–29.6) |
| Hypertension | 15 (19%) |
| Smoking habits | | | | |
| Never smoked | 12 (15%) |
| Previous smokers | 51 (63%) |
| Current smokers | 18 (22%) |
| Tobacco consumption (g/day) | 20 (0–30) |
| Cholesterol (mmol/liter) | | | | | |
| Serum | 6.82 (5.98–7.83) |
| VLDL | 0.98 (0.72–1.45) |
| LDL | 4.63 (3.87–5.36) |
| HDL | 1.02 (0.93–1.27) |
| Triglycerides (mmol/liter) | | | | | |
| Serum | 2.18 (1.62–3.16) |
| VLDL | 1.65 (1.10–2.23) |
| LDL | 0.41 (0.35–0.51) |
| HDL | 0.16 (0.14–0.18) |

Data are presented as median value and interquartile range or number (%) of subjects. HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein.
Two-way repeated measures analysis of variance. Data are presented as the least squares mean value.

Table 3. Baseline and On-Trial Body Mass Index, Glucose and Insulin Responses to the Oral Glucose Tolerance Test and Homeostasis Model Assessment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study Group</th>
<th>Baseline</th>
<th>2 Years</th>
<th>5 Years</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>Bezafibrate</td>
<td>27.6 ± 0.6</td>
<td>28.7 ± 0.6</td>
<td>29.0 ± 0.6</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>26.5 ± 0.6</td>
<td>27.2 ± 0.6</td>
<td>27.5 ± 0.6</td>
<td>0.15</td>
</tr>
<tr>
<td>GlucoseAUC (mmol/liter × min)</td>
<td>Bezafibrate</td>
<td>9,279 ± 403</td>
<td>10,162 ± 398</td>
<td>10,263 ± 450</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>9,109 ± 413</td>
<td>9,736 ± 413</td>
<td>10,583 ± 445</td>
<td>0.02</td>
</tr>
<tr>
<td>InsulinAUC (µg/ml × min)</td>
<td>Bezafibrate</td>
<td>9,878 ± 1,175</td>
<td>12,496 ± 1,191</td>
<td>15,325 ± 1,329</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>9,746 ± 1,220</td>
<td>11,039 ± 1,220</td>
<td>15,448 ± 1,313</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA index</td>
<td>Bezafibrate</td>
<td>3.9 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.3 ± 0.5</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3.8 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>4.5 ± 0.5</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Two-way repeated measures analysis of variance. Data are presented as the least squares mean value ± SEM.

BMI indicates body mass index; glucoseAUC, total glucose area under curve throughout the oral glucose tolerance test (OGTT); HOMA = relative insulin resistance by homeostasis model assessment; insulinAUC, insulin area under curve above fasting level throughout the OGTT.
show any significant relations to the angiographic outcome measurements. Significant relations of serum IGF-I levels to the progression of coronary lesions during the trial were also observed when patients were grouped according to tertiles of the angiographic outcome measurements (Fig. 2). The low tertile group comprises patients who progressed the least; the high tertile group those who progressed the most. Serum IGF-I levels were significantly related to changes in both MLD and MSD (i.e., patients who progressed less had lower, whereas those who progressed more had higher serum IGF-I levels) (ANOVA: p = 0.05 for ΔMLD, p = 0.02 for ΔMSD; p < 0.05 high vs. both low and medium tertiles).

Multiple stepwise regression analysis was also applied to study the independent relations of on-trial IGF-I, IGFBP-1 and glucose–insulin responses to the oral glucose tolerance test to the angiographic outcome measurements (Table 4). Baseline angiographic score, age and on-trial body mass index were entered as forced variables in the model. On-trial serum IGF-I levels were found to be independently and directly related to both ΔMLD and ΔMSD in this model, accounting for 5% and 7% of the multiple $R^2$, respectively. The relations of on-trial serum IGF-I levels to CAD progression were independent of on-trial serum lipoprotein (HDL₃ cholesterol, apolipoprotein B, Lp[a] and LDL size) and plasma fibrinogen levels, as well as glucose–insulin responses (glucose and insulin areas under the curve).

**DISCUSSION**

The present study shows an independent association between on-trial serum total IGF-I levels and CAD progression in young male survivors of myocardial infarction and suggests that the reduced progression of focal coronary atherosclerosis seen in bezafibrate-treated patients could be at least partly due to the significant reduction of serum IGF-I levels obtained with bezafibrate treatment. In contrast, none of the available indexes of insulin resistance (HOMA or plasma insulin and serum IGFBP-1 levels) showed any significant relation to the angiographic outcome measurements.

**IGF-I and CAD.** It has recently been suggested by many studies that circulating IGF-I might be implicated in the pathogenesis of CAD. IGF-I is the mediator of most of the mitogenic effects of growth hormone. It is known to be a potent regulator of vascular smooth muscle cell proliferation in vitro (23,24), and its expression is significantly increased in smooth muscle cells contained in atherectomy specimens from human plaques causing restenosis after percutaneous transluminal coronary angioplasty (25). Frystyk et al. (26) have suggested that the decrease of circulating total IGF-I after administration of the somatostatin analogue lanreotide could be responsible for the improvement in the long-term outcome after percutaneous transluminal coronary angioplasty. In addition, exposure of vascular allografts to IGF-I increases the vascular expression of IGF-I ligand and receptor proteins and accelerates atherosclerosis in rats by inducing myointimal proliferation and intimal thickening (27). Therefore, IGF-I's effects on the proliferation and migration of vascular smooth muscle cells into the intima could potentially be involved in the progression of coronary atherosclerosis in BECAIT.

However, previous clinical studies did not support the hypothesis of a positive relation between circulating IGF-I and the presence of CAD (7–9). Spallarossa et al. (7) have recently reported that serum concentrations of IGF-I are
reduced in patients with significant coronary stenosis, whereas Botker et al. (9) were unable to show any association between serum total or free IGF-I levels and the presence of CAD in a smaller number of patients. Moreover, Janssen et al. (8) have shown that high fasting serum free IGF-I levels are associated with less atherosclerotic plaques and a lower prevalence of CAD in an elderly group of patients. It is noteworthy that all of these reports (7–9) are based on cross-sectional studies and include mostly patients >45 years old. Some of these studies include both men and women, or only patients with normal glucose tolerance or moderately obese individuals. In contrast, BECAIT is a longitudinal, serial angiographic study of male patients <45 years old who have survived a myocardial infarction, with a mean body mass index of 27 kg/m² (range 20 to 36). BECAIT includes a substantial proportion of patients with abnormal glucose tolerance, many of whom developed manifest diabetes during the five-year follow-up period. Our data are in agreement with the hypothesis suggesting that increased IGF-I activity associated with insulin resistance and decreased IGFBP-1 levels may promote benign hyperplasia, such as atherosclerosis, and with the notion that hyperinsulinemia could stimulate intimal hyperplasia indirectly through IGF-I (28). However, stimulation of smooth muscle cell proliferation may not be the only mechanism by which IGF-I could promote progression of CAD in BECAIT, because the on-trial serum IGF-I level, although independently related to CAD progression, was directly associated with the serum Lp(a) concentration and indirectly related to the LDL peak particle size.

**Determinants of IGF-I.** Serum levels of IGF-I and IGFBP-1 are dependent on age, diet, physical activity, growth hormone levels (29–31) and genetic factors (32,33). The heritability of serum IGF-I levels has been reported to be higher than that of IGFBP-1 and insulin levels, as well as independent of the genetic influences on these two variables (33). The young survivors of myocardial infarction participating in BECAIT had normal serum total IGF-I concentrations, whereas their IGFBP-1 levels were markedly decreased (31), the latter secondary to insulin resistance with ensuing hyperinsulinemia (5,34). This means that the proportion of IGF-I not bound to IGFBP-1 was increased due to a rise in either the free, biologically active fraction or the fraction bound to IGFBP-3, or both. However, it should be taken into account that the in vivo production of IGFBP-3 is regulated primarily by IGF-I, whereas in vitro IGFBP-3 appears to be stimulated by various growth factors.

**Effects of bezafibrate.** The present study demonstrates a significant reduction of serum IGF-I levels by long-term bezafibrate treatment. The mechanisms by which fibrates lower serum IGF-I levels are unclear. Fibrates are known to
be potent activators of peroxisome proliferator-activated receptor-alpha, which modulates the expression of many different genes, such as those encoding different apolipoproteins and lipoprotein lipase (35). It is possible that activation of peroxisome proliferator-activated receptor-alpha binding to the promoter regions of either IGF-I or growth hormone genes (as IGF-I synthesis is under strict growth hormone regulation) may lead to a reduced synthesis of IGF-I. Another fibrate compound, fenofibrate, has already been reported to inhibit the growth of human vascular smooth muscle cells in vitro and has been suggested as a possible therapy for restenosis (36). Its antiproliferative effect does not seem to be specific for a single growth factor, because fenofibrate also seems to inhibit both platelet-derived growth factor and basic fibroblast growth factor (36).

Insulin resistance and disease progression. No relations were observed between the variables reflecting insulin resistance and progression of CAD. Therefore, no support was provided for the hypothesis that insulin resistance is causally related to progression of CAD (37). It is noteworthy that insulin sensitivity deteriorated dramatically in both study groups during the five-year trial, albeit to a slightly lesser extent in the bezafibrate-treated patients.

Implications and conclusions. The present study suggests that serum IGF-I could be implicated in the progression of premature CAD, independently of other coronary risk factors. In contrast, the serum concentration of IGFBP-1 and other markers of insulin resistance were unrelated to the progression of CAD. Therefore, no support was provided for the hypothesis that insulin resistance is causally related to progression of CAD (37). It is noteworthy that insulin sensitivity deteriorated dramatically in both study groups during the five-year trial, albeit to a slightly lesser extent in the bezafibrate-treated patients.

Reprint requests and correspondence: Dr. Anders Hamsten, King Gustaf V Research Institute, Karolinska Hospital, S-171 76, Stockholm, Sweden. E-mail: HAMSTEN@instmed.ks.se.

REFERENCES


