The Relationship between Insulin Resistance and the Cardiovascular Biomarker Growth Differentiation Factor-15 in Obese Patients

Greisa Vila,1 Michaela Riedl,1 Christian Anderwald,1 Michael Resl,1 Ammon Handisurya,2 Martin Clodi,1 Gerhard Prager,3 Bernhard Ludvik,1 Michael Krebs,1 and Anton Luger1*

BACKGROUND: Growth differentiation factor-15 (GDF-15) is a stress-responsive cytokine linked to obesity co-morbidities such as cardiovascular disease, inflammation, and cancer. GDF-15 also has adipokine properties and recently emerged as a prognostic biomarker for cardiovascular events.

METHODS: We evaluated the relationship of plasma GDF-15 concentrations with parameters of obesity, inflammation, and glucose and lipid metabolism in a cohort of 118 morbidly obese patients [mean (SD) age 37.2 (12) years, 89 females, 29 males] and 30 age- and sex-matched healthy lean individuals. All study participants underwent a 75-g oral glucose tolerance test; 28 patients were studied before and 1 year after Roux-en-Y gastric bypass surgery.

RESULTS: Obese individuals displayed increased plasma GDF-15 concentrations ($P < 0.001$), with highest concentrations observed in patients with type 2 diabetes. GDF-15 was positively correlated with age, waist-to-height ratio, mean arterial blood pressure, triglycerides, creatinine, glucose, insulin, C-peptide, hemoglobin A1c, and homeostatic model assessment insulin resistance index and negatively correlated with oral glucose insulin sensitivity. Age, homeostatic model assessment index, oral glucose insulin sensitivity, and creatinine were independent predictors of GDF-15 concentrations. Roux-en-Y gastric bypass led to a significant reduction in weight, leptin, insulin, and insulin resistance, but further increased GDF-15 concentrations ($P < 0.001$).

CONCLUSIONS: The associations between circulating GDF-15 concentrations and age, insulin resistance, and creatinine might account for the additional cardiovascular predictive information of GDF-15 compared to traditional risk factors. Nevertheless, GDF-15 changes following bariatric surgery suggest an indirect relationship between GDF-15 and insulin resistance. The clinical utility of GDF-15 as a biomarker might be limited until the pathways directly controlling GDF-15 concentrations are better understood.

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The global expansion of obesity counts among the paramount healthcare concerns of this century (1). Excess weight is associated with increased health risks and especially with significantly increased cardiovascular mortality (2). Therefore, individual cardiovascular risk stratification and respective therapy are important tasks in the management of obese patients. Growth differentiation factor-15 (GDF-15),4 also known as macrophage inhibitory cytokine-1, is a promising new cardiovascular biomarker (3, 4). GDF-15, a product of macrophages, cardiomyocytes, and endothelial cells, is released in response to tissue injury, anoxia, and proinflammatory cytokines, and which exerts antiapoptotic effects (5, 6, 7). Several studies have revealed a strong prognostic value of GDF-15 in patients with coronary heart disease and heart failure, and also in apparently healthy women (8–11). GDF-15 is directly associated with measurements of endothelial and cardiovascular dysfunction and is proposed to carry predictive information that outranks that of traditional cardiovascular risk factors (3). Recently Ding et al. found that GDF-15 is also expressed in and released from adipocytes, and contributes to increasing adiponectin production (12).
In women, circulating GDF-15 concentrations are increased with type 2 diabetes and correlate with body mass index (BMI), body fat, glucose, and C-reactive protein (CRP) (13).

The relation of GDF-15 to BMI, and to obesity comorbidities such as diabetes, inflammation, endothelial dysfunction, and cardiovascular disease, highlight the importance of characterizing GDF-15 in obese patients. Here we studied the relationship of GDF-15 with anthropometrical measurements of obesity, blood pressure, parameters of glucose and lipid metabolism, inflammation, and renal function in a cohort of 118 morbidly obese patients vs 30 age- and sex-matched healthy individuals, and in 28 patients who underwent laparoscopic Roux-en-Y gastric bypass surgery (RYGB).

**Study Participants and Methods**

**STUDY PARTICIPANTS AND DESIGN**

The study protocol was approved by the institutional review board of the Medical University of Vienna. Thirty healthy individuals and 118 obese individuals were evaluated in a cross-sectional study. Inclusion criteria for the healthy individuals were BMI <25 kg/m² and no previous medical history. The obese patients were recruited from the obesity outpatient clinic of the Division of Endocrinology and Metabolism, and inclusion criteria were BMI >35 kg/m² and no previously diagnosed diabetes mellitus. Exclusion criteria were positive medical history for coronary heart disease, heart failure, peripheral artery disease, stroke, malignancy, and chronic liver, renal, or endocrine disease. During the study day, participants underwent a thorough medical examination. Weight was measured to the nearest 100 g. Height, waist, and hip circumference were measured to the nearest centimeter. BMI was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured on the left arm by use of a sphygmomanometer and a cuff appropriate for the arm circumference, after the study participant had been sitting for 10 min. Mean arterial pressure (MAP) was calculated as (2 × diastolic blood pressure + systolic blood pressure)/3. Blood samples were withdrawn for the measurement of triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, CRP, creatinine, albumin, and hemoglobin A1c (Hb A1c) at baseline. Blood samples for the measurement of GDF-15 were collected in tubes containing EDTA, centrifuged at 1500g for 10 min, and immediately frozen at −20 °C. Then, an oral glucose tolerance test (OGTT) was performed using 75 g glucose. The homeostasis model assessment (HOMA) insulin resistance index was calculated as the product of fasting glucose (in mg/dL) and insulin (in mU/L) divided by the constant 405. The oral glucose insulin sensitivity (OGIS) was calculated as explained in http://webmet.pd.cnr.it/ogis (14). The clamp-like insulin resistance index (CLIX) was calculated as previously reported (15). We calculated the glomerular filtration rate (GFR) using the Modification of Diet in Renal Disease formula (16).

In an interventional study, 28 obese patients scheduled to undergo RYGB surgery were studied at 2 time points: before and 1 year after the intervention. At both study days, a clinical examination was performed; weight, height, and waist circumference were measured; and blood samples were withdrawn according to the same protocol used in the cross-sectional study and explained above.

**ASSAYS**

We measured human GDF-15 using a quantitative sandwich ELISA kit (# DGD150, R&D Systems) with intrand interassay CVs of <2.8% and <6%, respectively. Insulin and C peptide were determined by using commercially available RIAs (LINCO Research). Leptin was measured by using the Human Fluorokine MAP Base Kit (Obesity Panel) and the Leptin Fluorokine MAP (R&D Systems). Fasting glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, albumin, CRP, creatinine, and Hb A1c were quantified by using routine tests in a certified clinical laboratory.

**STATISTICAL ANALYSIS**

Data distributions were tested for normality by using histograms. Normally distributed data are expressed as mean (SE), nonnormally distributed data are presented as median and interquartile range (IQR). Differences between the groups were tested by using the Bonferroni-Holm–corrected 2-sided independent-samples t-test for parametric data and the Mann–Whitney U-test for nonnormally distributed data (such as GDF-15). Spearman rank correlations were computed to assess the relationship between variables. Multiple regression analyses were performed for identifying independent relationships and adjusting the effects of covariates. Nonnormally distributed parameters (GDF-15, creatinine, insulin, C-peptide, HOMA insulin resistance index, and triglycerides) were logarithmically transformed before regression analyses. Differences in GDF-15 between the 4 subgroups [healthy, obese with normal glucose tolerance (NGT), obese with impaired glucose tolerance (IGT), and obese with type 2 diabetes mellitus (DM)] were tested by use of one-way ANOVA followed by post hoc t-tests with Bonferroni correction for multiple testing. In the interventional study, differences between baseline and post-RYGB values were tested using a Bonferroni–Holm–
corrected paired Student t-test. The statistical software package SPSS release 15.0.1 (SPSS) was used. P values < 0.05 were considered statistically significant.

Results

Clinical, biochemical, and metabolic characteristics of participants of the cross-sectional study are given in Table 1. Median (IQR) plasma GDF-15 concentrations were 309 (275–411) ng/L in healthy individuals and 427 (344–626) ng/L in obese patients (P < 0.001) (Fig. 1).

In the obese cohort, GDF-15 concentrations were significantly correlated with age, waist circumference (and waist-to-height ratio), MAP, fasting glucose, fasting insulin, fasting C-peptide, Hb A1c, HOMA insulin resistance index, and fasting triglycerides and creatinine and negatively correlated with OGIS (Table 2, Fig. 2 A-B). GDF-15 was not associated with renal function (GFR) or CRP (Table 2). Multiple regression analysis revealed that age, HOMA insulin resistance index, OGIS, and creatinine were independent predictors of circulating GDF-15 concentrations (Table 3). The correlations between GDF-15 and MAP and fasting triglycerides and fasting glucose disappeared when GDF-15 was adjusted for age. The correlations between GDF-15 and waist circumference and fasting insulin and fasting C-peptide remained significant after we adjusted GDF-15 for age and creatinine, but disappeared after an additional adjustment for HOMA insulin resistance index and OGIS. Arterial hypertension was present in 49 patients (41%). There were no significant differences in plasma GDF-15 between patients with and without hypertension.

When data from all participants (healthy and obese individuals) were taken together, all the above relationships between GDF-15 and anthropometric or metabolic parameters remained significant. In addition, GDF-15 was weakly but significantly related to BMI, CRP, and CLIX (Table 2), but not to GFR.

Obese patients were subdivided according to OGTT results: 69 patients with NGT, 35 patients with IGT, and 14 patients with newly diagnosed DM (Fig. 2C). GDF-15 was significantly increased in all of these subgroups compared to the healthy control group (P = 0.001 for comparison between healthy and NGT; P < 0.001

| Table 1. Clinical, biochemical, and metabolic characteristics of the study participants. 
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<tbody>
<tr>
<td></td>
<td>Healthy (n = 30)</td>
<td>Obese (n = 120)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>9/21</td>
<td>30/90</td>
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</table>
| Age, years       | 38.2 (1.6)       | 37.3 (1.1)       | NS
| Weight, kg       | 67.7 (1.9)       | 134.9 (2)        | <0.001|
| BMI, kg/m²       | 22.6 (0.4)       | 47.1 (0.6)       | <0.001|
| Waist-to-height ratio | 0.46 (0.01) | 0.74 (0.01)       | <0.001|
| MAP, mmHg        | 97.1 (2.1)       | 120 (1.4)        | <0.001|
| Fasting glucose, mg/dL | 86.9 (1.1)   | 110 (1.6)        | <0.001|
| Fasting insulin, mU/L | 7.4 (5.7–8.6) | 26 (20–36)       | <0.001|
| Fasting C-peptide, µg/L | 1.6 (1.4–2)     | 4.1 (3.1–5.7)    | <0.001|
| HOMA insulin resistance index | 1.5 (1.2–1.9) | 6 (4.7–9.7)      | <0.001|
| OGIS             | 471 (8)          | 318 (6)          | <0.001|
| Hb A₁c, %        | 5.3 (0.06)       | 5.6 (0.05)       | 0.02 |
| Triglycerides, mg/dL | 81 (69–96)      | 137 (103–185)    | <0.001|
| Total cholesterol, mg/dL | 188 (6)       | 201 (4)          | NS
| LDL cholesterol, mg/dL | 109 (5)          | 123 (3)          | 0.04 |
| HDL cholesterol, mg/dL | 62.5 (2)        | 47.6 (1)         | <0.001|
| CRP, mg/L        | 1.4 (0.2)        | 11.6 (0.8)       | <0.001|
| Creatinine, mg/dL | 0.9 (0.83–0.95) | 0.85 (0.79–0.95) | NS

* Normally distributed data are expressed as mean (SE), nonnormally distributed data are presented as median (IQR). The P values correspond to the differences between healthy and obese individuals.

* NS not significant.

* To convert concentrations to millimoles per liter, multiply by 0.0555 for glucose; by 0.0113 for triglycerides; by 0.0259 for cholesterol, LDL cholesterol, and HDL cholesterol; and by 88.4 for creatinine.
for comparison between healthy and IGT; \( P < 0.001 \)
for comparison between healthy and DM; Fig. 2D).
There were no significant differences in age between
healthy study participants and those in the NGT, IGT,
and DM groups. Within the obese cohort, patients with
DM had significantly higher GDF-15 concentrations
(\( P < 0.016 \)) and were significantly older (\( P < 0.028 \))
compared to patients with NGT (Fig. 2D). Differences
in age and GDF-15 between other obese subgroups
were not found to be significant.

In the interventional study, we measured GDF-15
concentrations in 28 individuals undergoing laparoscopic
RYGB surgery, at baseline and 1 year after the interven-
tion. RYGB-induced changes in clinical, biochemical, and
metabolic parameters are presented in Table 4. GDF-15
significantly increased from 474 (31) to 637 (52) ng/L af-
after bariatric surgery, \( P < 0.001 \) both before and after ex-
clusion of the outlier value (173% increase in GDF-15
after bariatric surgery) (Fig. 2E). One year after RYGB, the
correlation between GDF-15 and age remained signifi-
cant (\( R = 0.495, P = 0.009 \)), whereas all other associations
did not. The RYGB-induced increase in GDF-15 was pos-
itively associated with the decreases in BMI (\( R = 0.541, \)
\( P = 0.004 \)) and in the HOMA insulin resistance index
(\( R = 0.622, P = 0.003 \)) (Fig. 2F).

Discussion

GDF-15 is known as a stress-induced cytokine that in-
creases in response to cardiovascular dysfunction and
carries prognostic information on cardiovascular mor-
tality in healthy people and in patients with known car-
diovascular disease (3, 11). The main finding of this
study was that GDF-15 is related to all parameters char-
terizing glucose metabolism and is positively corre-
lated to glucose, insulin, C-peptide, Hb A1c, and
HOMA insulin resistance index, and negatively corre-
lated to the oral glucose insulin sensitivity (measured as
OGIS). HOMA insulin resistance index and OGIS were
both independent predictors of GDF-15 in obese pa-
tients. We included both HOMA and OGIS in the mul-
tiple regression analysis because they are used to esti-
mate different processes. The HOMA insulin resistance
index is a parameter that is calculated by using fasting
glucose and insulin concentrations and reflects mainly
hepatic, but not peripheral, insulin resistance (17).
OGIS is an indicator of insulin sensitivity in response to
OGTT and therefore reflects mainly glucose clearance
and muscle sensitivity to insulin (14).

GDF-15 concentrations were not related to renal
function (measured as GFR), but were predicted by
creatinine, a parameter known to reflect muscle mass
in individuals with normal renal function (18). GDF-15 was higher in obese patients with newly diag-
nosed DM compared to obese patients with NGT. Nev-
Fig. 2. Scatterplots representing the relationship between (A) GDF-15 and age and (B) GDF-15 and HOMA insulin resistance index in obese individuals. (C), Glucose concentrations in response to 75g–2h-OGTT in healthy individuals (white triangles), obese-NGT group (black triangles), obese-IGT group (white circles) and obese-DM patients (black circles). Data are presented as mean ± SE. To convert glucose concentrations to mmol/L, multiply by 0.0555. (D), GDF-15 plasma concentrations in healthy individuals, and obese patients with NGT, IGT and DM. Data are presented as mean (SE). *P < 0.05 versus healthy individuals, $P < 0.05 versus the obese-NGT group, and §P < 0.05 versus the obese-DM group. (E), Individual GDF-15 plasma concentrations before and after RYGB. (F), Scatterplot displaying RYGB-induced changes in GDF-15 and HOMA insulin resistance index.
theless, obese patients with diabetes were a small and older subgroup of our cohort. Whether GDF-15 concentrations are increased in patients with DM compared to age- and sex-matched healthy individuals remains to be evaluated in further studies.

The strongest predictor of GDF-15 in obese individuals was age, a parameter that outranks all modifiable cardiovascular risk factors in the cardiovascular risk stratification (19). In addition, GDF-15 was strongly associated with the waist-to-height ratio, but not to BMI in obese individuals (despite the wide BMI range: 37–62 kg/m²). Recently, the measurements of abdominal obesity, and especially the waist-to-height ratio, have been identified to have a better cardiovascular predictive value compared to BMI (20). In summary, the strong relationships between GDF-15 and age, insulin resistance, creatinine, and waist-to-height ratio taken together might contribute to the increased prognostic information of GDF-15 compared with other clinical and biochemical markers of cardiovascular risk (3).

In addition to cardiovascular disease, GDF-15 has been linked to inflammation and cancer (21). Macrophages, endothelial cells, and cardiomyocytes comprise the main sources of GDF-15 (5, 6, 7). In vitro studies have found increased GDF-15 release after tissue injury, anoxia, and stimulation with proinflammatory cytokines such as tumor necrosis factor-α, but not with lipopolysaccharide (5). Inflammation has been implicated in the pathophysiology of atherosclerotic plaques and therefore in cardiovascular events (22). Obesity is associated with a mild systemic inflammation and, as expected, we found a mild but significant relationship between GDF-15 and CRP in the whole cohort comprising healthy and obese individuals. Nevertheless, this relationship disappeared within the obese cohort, revealing the independence of GDF-15 concentrations from the degree of systemic inflammation in obesity. Given the fact that GDF-15 is secreted by adipocytes and therefore considered to be an adipokine, we assumed that GDF-15 concentrations are altered in obese individuals (12). Nevertheless, results of a recent study demonstrated increased circulating

### Table 3. Determinants of log GDF-15 (standardized β-coefficient and P value) in multiple linear regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β Coefficient</th>
<th>P</th>
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<tbody>
<tr>
<td>Age</td>
<td>0.437</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log creatinine</td>
<td>0.319</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log HOMA-insulin resistance</td>
<td>0.343</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OGIS</td>
<td>0.177</td>
<td>0.019</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.113</td>
<td>0.143</td>
</tr>
<tr>
<td>MAP</td>
<td>−0.072</td>
<td>0.332</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>−0.044</td>
<td>0.664</td>
</tr>
<tr>
<td>Log insulin</td>
<td>−0.220</td>
<td>0.544</td>
</tr>
<tr>
<td>Log C-peptide</td>
<td>0.190</td>
<td>0.078</td>
</tr>
<tr>
<td>Log triglycerides</td>
<td>−0.108</td>
<td>0.175</td>
</tr>
</tbody>
</table>

### Table 4. Clinical and biochemical parameters of morbidly obese individuals before and 1 year after RYGB.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 year after surgery</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age, male/female</td>
<td>42.9 (1.9)</td>
<td>474 (31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GDF-15, ng/L</td>
<td>128 (3)</td>
<td>32.6 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>128 (3)</td>
<td>95 (3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>6.9 (0.9)</td>
<td>12.5 (0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA insulin resistance index</td>
<td>166 (18)</td>
<td>123 (16)</td>
<td>NS³</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>190 (5)</td>
<td>160 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>121 (5)</td>
<td>87 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>11.6 (1)</td>
<td>4.5 (1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.8 (0.02)</td>
<td>0.79 (0.02)</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42.4 (0.4)</td>
<td>40.8 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin, µg/L</td>
<td>110 (7)</td>
<td>36 (4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Data are presented as mean (SE) P for comparison between preoperative and postoperative values (Bonferroni-Holm corrected paired t-tests).

To convert concentrations to millimoles per liter, multiply by 0.0113 for triglycerides; by 0.0259 for cholesterol, LDL cholesterol, and HDL cholesterol; and by 88.4 for creatinine.

NS, not significant.
GDF-15 concentrations in obese individuals, but no differences at the level of gene expression within the adipose tissue (13). The pathophysiological mechanism underlying increased GDF-15 concentrations in obesity remains unknown and may not be linked only to adipose tissue. Endothelial dysfunction, cardiac stress, β-cell function, and insulin resistance may all contribute to the changes in GDF-15. In the light of the strong association between GDF-15 and parameters of glucose metabolism, it is important to identify the influence of GDF-15 on β-cell function and glucose uptake and vice versa, an eventual effect of glucose and insulin on GDF-15 release.

Bariatric surgery is to date the only efficient therapeutic means for achieving weight loss in individuals with severe obesity. Our observation that RYGB surgery significantly decreased body weight, leptin, CRP, insulin, and HOMA insulin resistance index confirmed the results of previous studies (23, 24). Nevertheless, GDF-15 concentrations increased further. The RYGB-induced increase in GDF-15 was significantly correlated with age. The strong association with insulin resistance was noticeable even during the changes following bariatric surgery, because obese patients with larger reductions in weight and insulin resistance had smaller increases in GDF-15 (Fig. 2F). Nevertheless, these results suggest an indirect association between GDF-15 and insulin resistance, and the pathophysiological mechanisms that control postoperative GDF-15 concentrations remain unknown. It is interesting to note that GDF-15 concentrations also increase after diet-induced weight loss and in patients with anorexia nervosa (13, 25). To date, it is not known whether circulating GDF-15 concentrations depend on albumin or any carrier proteins. It is important to emphasize that the increase in GDF-15 is not in line with the significantly improvement in cardiovascular function that occurs following bariatric surgery (26). Therefore, GDF-15 is highly likely to be an unreliable cardiovascular biomarker in patients who have undergone gastric bypass surgery.

In summary, age, insulin resistance, and creatinine were independent predictors of GDF-15 in obese patients, and these associations might contribute to the recently found increased cardiovascular prediction value of GDF-15 compared with classical predictors. Nevertheless, the increase in GDF-15 concentrations following weight loss is not in line with a direct relationship between GDF-15 and insulin resistance and/or clinical measurements of obesity. The utility of GDF-15 as a biomarker might be limited until the pathways that directly control GDF-15 concentrations in humans are better understood.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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