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Insulin resistance predicts the cardiovascular biomarker GDF-15 in obese patients

Abbreviated title: GDF-15 and insulin resistance

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], Anton Luger[1]

[1] Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

[2] Division of Nephrology, Department of Medicine III, Medical University of Vienna, Vienna, Austria

[3] Department of Surgery, Medical University of Vienna, Vienna, Austria

Corresponding author:

Anton Luger M.D.

Division of Endocrinology and Metabolism, Department of Internal Medicine III,

Medical University of Vienna, Vienna, Austria

Phone: + 43 140400 4310, Fax: + 43 140400 7790

Email: anton.luger@meduniwien.ac.at

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Abbreviations:

BMI, body mass index; CLIX, clamp-like insulin resistance index; CRP, C-reactive protein HbA1c, haemoglobin A1c; HOMA, Homeostatic Model Assessment; GDF-15, growth differentiation factor-15; MAP, mean arterial pressure; OGIS, Oral Glucose Insulin Sensitivity; RYGB, Roux-en-Y gastric bypass

ABSTRACT

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

Methods: Here we evaluate the relationship of plasma GDF-15 concentrations with parameters of obesity, inflammation, glucose and lipid metabolism in a cohort of 118 morbidly obese patients (37.2 +/– 12 years, 89 females, 29 males) and 30 age- and gender-matched healthy lean subjects. A 75g oral glucose tolerance test was performed in all subjects. 28 patients were studied before and one year after Roux-en-Y gastric bypass (RYGB) surgery.

Results: Obese subjects displayed increased plasma GDF-15 concentrations ($P<0.001$), with highest levels observed in patients with accompanying type 2 diabetes. GDF-15 correlated with age, waist-to-height ratio, mean arterial blood pressure, triglycerides, creatinine, glucose, insulin, C-peptide, haemoglobin A1c, Homeostatic Model Assessment (HOMA) insulin resistance index and negatively correlated to the Oral Glucose Insulin Sensitivity (OGIS). Age, HOMA index, OGIS and creatinine were independent predictors of GDF-15 concentrations. RYGB led to a significant reduction in weight, leptin, insulin and insulin resistance, but further increased GDF-15 levels ($P<0.001$).

Conclusions: Age, insulin resistance and creatinine predict plasma levels of GDF-15 in obesity. These relationships might account for the additional cardiovascular predictive information of GDF-15 when compared to traditional risk factors. Caution should be taken while interpreting increased GDF-15 levels following bariatric surgery, as the underlying pathophysiological mechanisms remain unknown.

INTRODUCTION

The global expansion of obesity counts among the paramount health care 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), also known as macrophage inhibitory cytokine-1, is a new promising cardiovascular biomarker (3, 4). GDF-15 is a product of macrophages, cardiomyocytes and endothelial cells, released in response to tissue injury, anoxia and proinflammatory cytokines like TNF-alpha, exerting thereby antiapoptotic effects (5, 6, 7). Several studies have revealed a strong prognostic value of GDF-15 in patients with coronary heart disease, heart failure, and also in apparently healthy women (8, 9, 10, 11). GDF-15 is directly associated to 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.* described that GDF-15 is also expressed in and released from adipocytes, and contributes to increasing adiponectin production (12). In women, circulating GDF-15 levels 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 co-morbidities 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 versus 30 age- and sex-matched healthy subjects, and in 28 patients undergoing laparoscopic Roux-en-Y gastric bypass surgery (RYGB).

SUBJECTS AND METHODS

Study subjects and design

The study protocol was approved by the institutional review board of the Medical University of Vienna. Thirty healthy subjects and 118 obese subjects were evaluated in a cross-sectional study. Inclusion criteria for the healthy subjects were BMI < 25 kg/m^2 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/m^2 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 gramm. 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 after 10 minutes sitting, in the left arm, using a sphygmomanometer and cuff appropriate for the arm circumference. 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, high-density lipoprotein (HDL)-cholesterol, CRP, creatinine, albumin and haemoglobin A1c (HbA1c) at baseline. Blood samples for the measurement of GDF-15 were collected in tubes containing EDTA, centrifuged at 3,500 rpm for 10 min and immediately frozen at -20° Celsius. Then, an oral glucose tolerance test (OGTT) was performed using 75g glucose. The Homeostatic Model Assessment (HOMA) insulin resistance index was calculated as the product of fasting glucose (in mg/dl) and insulin (in µU/ml) divided by the constant 405. 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 explained (15). Clinical, biochemical and metabolic characteristics of participants of the cross-sectional study are given in Table 1.

In an interventional study, 28 obese patients scheduled to undergo RYGB surgery, were studied at two time-points: before and one 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 following the protocol explained in the cross-sectional study.

Assays

Human GDF-15 was determined using a quantitative sandwich ELISA kit (# DGD150, R&D Systems, Minneapolis, MN) with intra- and inter-assay CV of < 2.8% and < 6% respectively. Insulin and C peptide were determined using commercially available RIAs (LINCO Research, St. Charles, MO). Leptin was measured 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, albumine, CRP, creatinine and HbA1c were quantified using routine tests in a certified clinical laboratory.

Statistical analysis

All data are expressed as mean ± SE. Distribution was tested for normality using histograms. Differences between the groups were tested using the Bonferroni-Holm corrected two-sided independent-samples t test for parametric data and the Mann-Whitney *U* test for non-parametric data (such as GDF-15). Spearman's 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. Non-normally distributed parameters were logarithmically transformed before regression analyses. Differences between baseline and post-RYGB values were tested using Bonferroni-Holm corrected paired Student's *t* test. The statistical software package SPSS release 15.0.1 (SPSS, Inc., Chicago, IL) was used. *P* values less than 0.05 were considered statistically significant.

RESULTS

Clinical, biochemical and metabolic characteristics of participants of the cross-sectional study are given in Table 1. Plasma GDF-15 concentrations were 339 ± 18 pg/ml in healthy subjects and 524 ± 25 pg/ml in obese patients ($P < 0.001$). Medians and interquartile ranges are presented in Fig. 1.

In the obese cohort, GDF-15 levels significantly correlated to age, waist circumference (and waist-to-height ratio), MAP, fasting glucose, fasting insulin, fasting C-peptide, HbA1c, HOMA insulin resistance index, fasting triglycerides and creatinine, and negatively correlated to OGIS (Table 2, Fig. 2 A-B). There was no association between GDF-15 and CRP (Table 2). Multiple regression analysis revealed that age, HOMA insulin resistance index, OGIS and creatinine were independent predictors of circulating GDF-15 levels (all models < 0.001). The correlations between GDF-15 and MAP, fasting triglycerides and fasting glucose disappeared when GDF-15 was adjusted for age. The correlations between GDF-15 and waist circumference, fasting insulin and fasting C-peptide remained significant also after adjusting GDF-15 for age and creatinine, but disappeared after an additional adjustment for HOMA insulin resistance index. 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 subjects) were taken together, all 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).

According to OGTT results, obese patients were divided in: 69 patients with normal glucose tolerance (NGT), 35 patients with impaired glucose tolerance (IGT) and 14 patients with newly diagnosed type 2 diabetes (DM) (Fig. 2C). GDF-15 was significantly increased in all these subgroups when compared to the healthy control group ($P = 0.001$ for comparison between healthy and NGT; $P < 0.001$ 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 patients and NGT, IGT and DM groups. Within the obese cohort, patients with DM had significantly higher GDF-15 levels (P=0.016) and were significantly older (P=0.028) when compared to patients with NGT (Fig. 2D). Differences in age and GDF-15 between other obese subgroups were insignificant.

In the interventional study, we measured GDF-15 levels in 28 subjects undergoing laparoscopic RYGB surgery, at baseline and one year after the intervention. RYGB-induced changes in clinical, biochemical and metabolic parameters are presented in Table 3. GDF-15 significantly increased from 474 ± 31 to 637 ± 52 pg/ml after bariatric surgery (Fig. 2E). One year after RYGB, the correlation between GDF-15 and age remained significant (R=0.495, P=0.009), while all other associations did not. Postoperative GDF-15 correlated to postoperative osteocalcin values (R=0.465, P=0.017). The RYGB-induced increase in GDF-15 was positively associated to the decreases in BMI (R=0.541, P=0.004) and in HOMA insulin resistance index (R=0.622, P=0.003) (Figure 2F).

DISCUSSION

GDF-15 is known as a stress-induced cytokine that increases in response to cardiovascular dysfunction and carries prognostic information on cardiovascular mortality in healthy people and in patients with known cardiovascular-disease (3, 11). The main finding of this study is that GDF-15 is related to all parameters characterising glucose metabolism positively correlating to glucose, insulin, C-peptide, HbA1c and HOMA-insulin resistance index, and negatively correlating to the oral glucose insulin sensitivity (measured as OGIS). HOMA insulin resistance index and OGIS are both independent predictors of GDF-15 in obese patients. We included both HOMA and OGIS in the multiple regression analysis, as they estimate different processes. HOMA insulin resistance index is a parameter calculated using fasting glucose and insulin levels and reflects mainly hepatic, but not peripheral insulin

resistance (16). OGIS assesses insulin sensitivity in response to OGTT, thereby reflecting mainly peripheral insulin resistance (14). GDF-15 levels were also predicted by creatinine, a parameter known to reflect muscle mass in subjects with normal renal function (17). GDF-15 was higher in obese patients with newly diagnosed diabetes when compared to obese patients with normal glucose tolerance. Nevertheless, obese patients with diabetes presented a small and older subgroup of our cohort. Whether patients with diabetes really present increased GDF-15 levels when compared to age-and sex-matched healthy subjects, remains to be evaluated in further studies.

The strongest predictor of GDF-15 in obesity is age, a parameter that in reality outranks all modifiable cardiovascular risk factors in the cardiovascular risk stratification (18). In addition, GDF-15 is strongly associated to the waist-to-height ratio, but not to BMI in obese subjects (despite the wide BMI range: 37-62 kg/m^2). Recently, the measurements of abdominal obesity, and especially the waist-to-height ratio were identified to have a stronger cardiovascular predictor value when compared to BMI (19). In summary, the strong relationships between GDF-15 and age, insulin resistance, creatinine and waist-to-height ratio might altogether contribute to the increased prognostic information of GDF-15 when compared to other cardiovascular risk clinical and biochemical markers (3).

In addition to cardiovascular disease, GDF-15 has been linked to inflammation and cancer (20). Main sources of GDF-15 are macrophages, endothelial cells and cardiomyocytes (5, 6, 7). In vitro studies found increased GDF-15 release after tissue injury, anoxia and stimulation with proinflammatory cytokines like TNF-alpha, but not with lipopolysaccharide (5). Inflammation is implicated in the pathophysiology of atherosclerotic plaques and therefore in cardiovascular-events (21). 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 subjects. Nevertheless, this relationship disappeared

within the adipose 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 altered GDF-15 levels in obesity (12). Nevertheless, a recent study demonstrated increased circulating GDF-15 concentrations in obesity, but no differences at the level of gene expression within the adipose tissue (13). The pathophysiological mechanism underlying increased GDF-15 levels in obesity remains unknown and might not only be linked to the adipose tissue. Endothelial dysfunction, cardiac stress, beta 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 becomes important to identify the influence of GDF-15 on beta 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 therapeutical mean for achieving weight loss in severe obesity. Confirming previous studies, we present here that RYGB surgery significantly decreased body weight, leptin, CRP, insulin and HOMA insulin resistance index (22, 23). Nevertheless, GDF-15 increased further. A similar increase was observed following diet-induced weight loss (13). The RYGB-induced increase in GDF-15 correlated only to age and to the bone formation marker osteocalcin one year after surgery. Higher bone turnover and bone loss are known sequelae of bariatric surgery (24). During the last years, several publications have revealed the presence of bone-metabolic cross-talks (22, 25, 26). Despite a recent study showing that GDF-15 inhibits the formation of mature osteoclasts, little is known on the link between GDF-15 and bone metabolism (27). The mechanisms underlying the increased postoperative GDF-15 concentrations remain unknown. Nevertheless, the strong association with insulin resistance is noticeable even during the changes following bariatric surgery, as obese patients with larger reductions in weight and insulin resistance presented smaller increases in GDF-15 (Figure 2F). It is important to emphasize that the increase in

GDF-15 is not in line with the significantly improved cardiovascular function following bariatric surgery (28). Therefore, the usefulness of GDF-15 as cardiovascular biomarker in patients who have undergone gastric bypass surgery is questionable.

Taken together, age, insulin resistance and creatinine are independent predictors of GDF-15 in obese patients. These parameters account among the major cardiovascular prognostic factors and might contribute to the recently found increased cardiovascular prediction value of GDF-15 when compared to classical predictors. The strong link with insulin resistance suggests the importance of a detailed evaluation of GDF-15 as cardiovascular biomarker in patients with obesity and/or type 2 diabetes.

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Table 1. Clinical, biochemical and metabolic characteristics of the study participants

	Control (n=30)	Obese (n=120)	*P* value
Sex (Male/Female)	9/21	30/90	
Age (years)	38.2 ± 1.6	37.3 ± 1.1	n.s.
Weight (kg)	67.7 ± 1.9	134.9 ± 2	< 0.001
BMI (kg/m^2)	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 (µU/ml)	8.02 ± 0.6	30.6 ± 1.6	< 0.001
Fasting C-peptide (ng/ml)	1.7 ± 0.0	4.9 ± 0.4	< 0.001
HOMA insulin resistance index	1.7 ± 0.1	7.6 ± 0.5	< 0.001
OGIS	471 ± 8	318 ± 6	< 0.001
HbA1c (%)	5.3 ± 0.06	5.6 ± 0.05	0.02
Triglycerides (mg/ml)	81.5 ± 3	161 ± 8	< 0.001
Total cholesterol (mg/dl)	188 ± 6	201 ± 4	n.s.
LDL cholesterol (mg/dl)	109 ± 5	123 ± 3	0.04
HDL cholesterol (mg/dl)	62.5 ± 2	47.6 ± 1	< 0.001
CRP (mg/dl)	0.14 ± 0.02	1.16 ± 0.08	< 0.001
Creatinine (mg/dl)	0.89 ± 0.02	0.9 ± 0.02	n.s.

Values are presented as mean ± SE. The P values correspond to the differences between control and obese subjects. BMI=Body Mass Index; MAP=Mean arterial pressure; HOMA= homeostatic model assessment; OGIS= Oral Glucose Insulin Sensitivity; LDL=low-density lipoprotein; HDL=high-density lipoprotein; CRP= C-reactive protein; n.s.= not significant.

Table 2. Spearman correlations of GDF-15

	Obese	Healthy + obese
Age	0.512***	0.369***
BMI	0.041	0.282***
Waist circumference	0.349***	0.449***
MAP	0.196*	0.343***
Fasting glucose	0.336***	0.370***
Fasting insulin	0.270**	0.387***
Fasting C-peptide	0.363***	0.455***
HbA1c	0.386***	0.394***
HOMA insulin resistance index	0.324***	0.421***
OGIS	-0.204*	-0.327***
CLIX	-0.128	-0.304***
CRP	-0.024	0.216*
Fasting triglycerides	0.187*	0.328***
Creatinine	0.401***	0.312***

BMI=body mass index; MAP=Mean arterial pressure; HOMA= homeostatic model assessment; OGIS= Oral Glucose Insulin Sensitivity; CLIX=clamp-like insulin resistance index, CRP=C reactive protein. * for P<0.05, *** for P < 0.01 and *** for P < 0.001

Table 3. Clinical and biochemical parameters of morbidly obese subjects before and one year after RYGB

	Baseline	1 year after surgery	*P* value
Age (male/female)	42.9 ± 1.9 (3/25)		
GDF-15 (ng/ml)	474 ± 31	637 ± 52	< 0.001
Weight (kg)	128 ± 3	95 ± 3	< 0.001
Fasting insulin (µU/ml)	32.6 ± 4	12.5 ± 0.7	< 0.001
HOMA insulin resistance index	6.9 ± 0.9	2.7 ± 0.2	< 0.001
Fasting triglycerides (mg/dl)	166 ± 18	123 ± 16	n.s.
Total cholesterol (mg/dl)	190 ± 5	160 ± 6	< 0.001
LDL cholesterol (mg/dl)	121 ± 5	87 ± 5	< 0.001
CRP (mg/dl)	1.16 ± 0.1	0.45 ± 0.1	< 0.001
Creatinine (mg/dl)	0.8 ± 0.02	0.79 ± 0.02	n.s.
Albumine (g/l)	42.4 ± 0.4	40.8 ± 0.4	< 0.001
Leptin (ng/ml)	110 ± 7	36 ± 4	< 0.001

Data are presented as mean ± SE. P for comparison between preoperative and postoperative values (Bonferroni-Holm corrected paired t-tests). HOMA= homeostatic model assessment; LDL=low-density lipoprotein; CRP= C-reactive protein; n.s.= not significant.

Legends

Figure 1.

GDF-15 plasma concentrations in lean (n=30) and obese (n=120) individuals matched for age and sex. Bars represent interquartile ranges (IQR) and lines mark medians. Whiskers extend from the box up to the smallest/highest observations that lie within 1.5 IQR from the quartiles. Observations that lie further from the quartiles are marked by circles (1.5 – 3 IQR) or an asterisk (more than 3 IQR from the quartiles).

Figure 2.

Scatterplots representing the relationship between (A) GDF-15 and age and (B) GDF-15 and HOMA insulin resistance index in obese subjects. (C) Glucose levels in response to 75g-2h-OGTT in healthy subjects (white triangles), obese-NGT group (black triangles), obese-IGT group (white circles) and obese-DM patients (black circles). Data are presented as mean ± SE. (D) GDF-15 plasma concentrations in healthy subjects, and obese patients with NGT, IGT and DM. Data are presented as mean ± SE. * for $P<0.05$ versus healthy subjects, $ for $P<0.05$ versus obese-NGT group and § for $P<0.05$ versus obese-DM group. (E) Individual GDF-15 plasma concentrations before and after RYGB. (F) Scatterplot between RYGB-induced changes in GDF-15 and HOMA insulin resistance index.

Figure 1

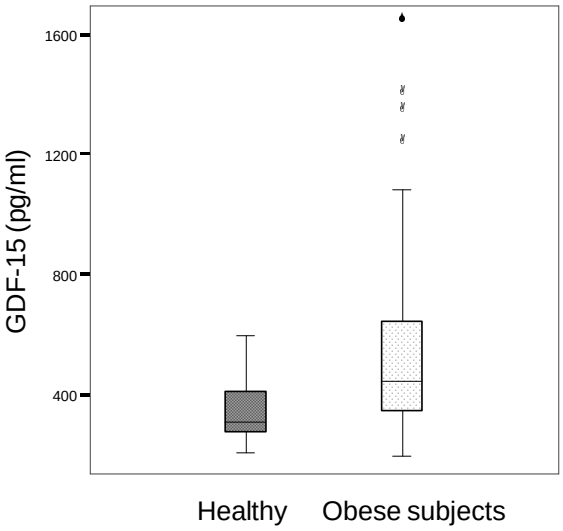

Figure 2