Circulating Betatrophin Concentrations Are Decreased in Human Obesity and Type 2 Diabetes

Javier Gómez-Ambrosi, Eider Pascual, Victoria Catalán, Amaia Rodríguez, Beatriz Ramírez, Camilo Silva, Maria J. Gil, Javier Salvador, and Gema Frühbeck


Context: Betatrophin is a secreted protein recently involved in β-cell replication with a potential role in type 2 diabetes mellitus (T2D).

Objective: The aim of the present study was to compare the circulating concentrations of betatrophin in human obesity and T2D.

Design, Setting, and Participants: Serum concentrations of betatrophin were measured by ELISA in 153 subjects: 75 obese normoglycemic subjects (OB-NG), 30 obese subjects with impaired glucose tolerance (OB-IGT), and 15 obese subjects with T2D (OB-T2D) matched by sex, age, and body adiposity, in comparison with 33 lean normoglycemic individuals (LN-NG).

Results: Circulating levels of betatrophin were significantly decreased in obese individuals and further diminished in IGT and T2D participants (LN-NG, 45.1 ± 24.4 ng/mL; OB-NG, 26.9 ± 15.4 ng/mL; OB-IGT, 18.3 ± 10.7 ng/mL; OB-T2D, 13.5 ± 8.8 ng/mL; P < .001). A marked sexual dimorphism was found, with betatrophin levels being significantly higher in women than in men (males, 21.1 ± 16.0 ng/mL; females, 34.1 ± 20.1 ng/mL; P < .001). Interestingly, betatrophin levels were positively correlated with the quantitative insulin sensitivity check index (r = 0.46; P < .001) and with high-density lipoprotein-cholesterol concentrations (r = 0.51; P < .001).

Conclusions: We conclude that serum betatrophin is decreased in human obesity, being further reduced in obesity-associated insulin resistance. Betatrophin levels are closely related to obesity-associated cardiometabolic risk factors, emerging as a potential biomarker of insulin resistance and T2D. (J Clin Endocrinol Metab 99: E2004–E2009, 2014)

Obesity prevalence has increased alarmingly, threatening the health advances achieved in the last decades (1). Importantly, obesity favors the clustering of cardiometabolic alterations such as type 2 diabetes mellitus (T2D) and dyslipidemia, leading to an increase in morbidity and mortality in relation to excess adiposity (2–4).

Betatrophin, also known as lipasin (5), refeeding-induced fat and liver (6), hepatocellular carcinoma-associated protein TD26 (7), and angiopoietin-like protein 8 (ANGPTL8) (8), is a protein encoded by the Gm6484 gene in mice and C19orf80 in humans. Betatrophin is a 22-kDa hormone primarily expressed in liver and adipose tissue that has been shown to promote pancreatic β-cell proliferation, expand β-cell mass, and improve glucose tolerance in a mice model of insulin resistance (9). Liver betatrophin expression has been reported to increase after the administration of the insulin receptor antagonist S961 in mice, leading to a compensatory increase in β-cell replication (9). Thus, betatrophin has emerged as a signaling molecule favoring a hepato-adipose-pancreatic cross talk

Abbreviations: BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; LN-NG, normoglycemic lean; LSD, least significant difference; NG, normoglycemic; OB-IGT, obese with IGT; OB-NG, obese normoglycemic; OB-T2D, obese with T2D; OGTT, oral glucose tolerance test; QUICKI, quantitative insulin sensitivity check index; T2D, type 2 diabetes mellitus.
implicated in the inter-organ compensatory response of β-cells in the setting of insulin resistance. However, despite the betatrophin effects reported in mice, whether similar effects take place in humans needs to be confirmed after the recent observations where human β-cells were unresponsive to mouse betatrophin (10). To investigate the possible involvement of betatrophin in the development of obesity-associated T2D, we analyzed the serum concentrations of betatrophin in human obesity and obesity-associated T2D. Our hypothesis was that betatrophin levels might be increased in obese individuals with T2D in response to insulin resistance and to compensate the loss of β-cell that characteristically takes place in T2D.

Subjects and Methods

Subjects

To analyze the effect of obesity and insulin resistance on betatrophin concentrations, 153 Caucasian subjects (33 lean normoglycemic [LN-NG], 75 obese normoglycemic [OB-NG], 30 obese with impaired glucose tolerance [OB-IGT], and 15 obese with T2D [OB-T2D]) were recruited from healthy volunteers and patients attending the Department of Endocrinology and Nutrition at the Clínica Universidad de Navarra. Subjects were classified according to body mass index (BMI) (lean, <25 kg/m²; obese, ≥30 kg/m²). All participants were weight-stable (±2 kg) for the previous 3 months. Participants underwent a clinical assessment including medical history, physical examination, body composition analysis, and comorbidity evaluation performed by a multidisciplinary consultation team. All subjects were non-smokers. Individuals with signs of infection were excluded. Glucose intolerance or T2D of obese subjects was of recent-onset being diagnosed at the baseline visit. Participants were not on antidiabetic medication or insulin therapy, and therefore, did not have adequate glycemic control. Normoglycemia, IGT, and T2D were defined following the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus as previously described (11), based on both fasting plasma glucose concentrations and plasma glucose 2 hours after an oral glucose tolerance test (OGTT). The experimental design was approved, from an ethical and scientific standpoint, by the Hospital’s Ethical Committee responsible for research, and volunteers gave their informed consent to participate in the study.

Anthropometric measurements

The anthropometric and body composition determinations as well as the blood extraction were performed on a single day. Height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain Ltd), whereas body weight was measured with a calibrated electronic scale to the nearest 0.1 kg with subjects wearing a swimming suit and cap. Waist circumference was measured at the midpoint between the iliac crest and the rib cage on the midaxillary line. Blood pressure was measured after a 5-minute rest in the semi-sitting position with a sphygmomanometer. Blood pressure was determined at least three times at the right upper arm, and the mean was used in the analyses. Body density was estimated by air displacement plethysmography (Bod-Pod; Life Measurement, Inc). Percentage of body fat was estimated from body density using the Siri equation as previously described (2, 12).

Serum biochemistry

Blood samples were collected in the morning, after an overnight fast, in order to avoid potential confounding influences due to hormonal rhythmicity. Plasma glucose was analyzed by an automated analyzer (Roche/Hitachi Modular P800; Roche Diagnostics) as previously described (13). Insulin and C-peptide were measured by means of enzyme-amplified chemiluminescence assays (Immumite 2000; Siemens AG). Total cholesterol and triglyceride concentrations were determined by enzymatic spectrophotometric methods (Roche). High-density lipoprotein (HDL)-cholesterol was quantified by a colorimetric method in a Beckman Synchron CX analyzer (Beckman Instruments, Ltd). Low-density lipoprotein (LDL)-cholesterol was calculated by the Friedewald formula. Uric acid, alanine aminotransferase, γ-glutamyltransferase, and creatinine were measured by enzymatic tests (Roche) in an automated analyzer (Roche/Hitachi Modular P800). High-sensitivity C-reactive protein (CRP) was measured using the Tina-quant CRP (Latex) ultrasensitive assay (Roche). Leptin was quantified by a double-antibody RIA method (Linco Research, Inc) as previously described (14); intra- and interassay coefficients of variation were 5.0 and 4.5%, respectively. Serum betatrophin concentrations were determined using a validated ELISA kit (Human ANGPTL8 ELISA kit, CSB-EL028107HU; Cusabio) with intra- and interassay coefficients of variation being <8% and <10%, respectively. Betatrophin levels were confirmed by using another ELISA kit (SK00528-02; Aviscera Bioscience Inc) and Western blot (Supplemental Data).

Statistical analysis

Data are presented as mean ± SD. Differences in gender distribution were analyzed by χ² analysis. Differences between groups were analyzed by ANOVA, followed by Fisher’s least significant difference (LSD) tests. CRP concentrations were logarithmically transformed because of their non-normal distribution. Correlations between two variables were computed by Pearson’s correlation coefficients (r). Multivariate stepwise linear regression analysis was conducted for the dependent variable betatrophin, including the variables that showed a significant correlation with betatrophin as independent variables. The calculations were performed using the SPSS version 15.0.1 (SPSS Inc). A P value lower than .05 was considered statistically significant.

Results

Anthropometric and biochemical characteristics of the individuals included in the study are shown in Table 1. Obese patients exhibited insulin resistance evidenced by significantly increased insulinemia and homeostatic model assessment (HOMA) and reduced quantitative insulin sensitivity check index (QUICKI), which were further altered in OB-IGT and OB-T2D. It is noteworthy that the β-cell secretory capacity/reserve was apparently preserved, as suggested by the increased C-peptide production in obese
patients, which was further increased in the OB-IGT and OB-T2D groups. Circulating concentrations of betatrophin were significantly lower in OB-NG individuals (40%) and further decreased in OB-IGT (59%) and OB-T2D (70%) participants (LN-NG, 45.1 ± 24.4 ng/mL; OB-NG, 26.9 ± 15.4 ng/mL; OB-IGT 18.3 ± 10.7 ng/mL; OB-T2D, 13.5 ± 8.8 ng/mL; P < .001) as depicted in Figure 1A. Striking differences (P < .001) in serum betatrophin levels between males (21.1 ± 16.0 ng/mL) and females (34.1 ± 20.1 ng/mL) were observed (Figure 1B). The validity of the ELISA was confirmed by correlation with a different ELISA kit as well as by Western-blot analysis (Supplemental Data).

A highly significant correlation (P < .001 for all) was found between betatrophin and BMI (r = −0.38), waist circumference (r = −0.47), insulin levels (r = −0.34), C-peptide concentrations (r = −0.40), QUICKI (r = 0.46), HOMA (r = −0.34), triglyceride concentrations (r = −0.36), and HDL-cholesterol levels (r = 0.51) (Figure 1, C—F, and Supplemental Table 1). Furthermore, circulating betatrophin levels were associated with most of the cardiometabolic risk markers, even after adjusting for body fat (Supplemental Table 1). When segregated by gender, the correlation between betatrophin levels and BMI (r = −0.38) was higher in women (r = −0.53; P < .001) than in men (r = −0.27; P = .021), whereas the association of betatrophin with any marker of glucose metabolism was very similar among genders. In the multiple linear regression analysis (Supplemental Table 2), the model that best predicted betatrophin levels included HDL-cholesterol levels, CRP, sex, and systolic blood pressure as predictive variables. This model explained 47% of the total variability of betatrophin concentrations (P < .001).

### Discussion

Betatrophin has been shown to promote pancreatic β-cell replication in the setting of insulin resistance in mice (9). Despite putting forward a potential cause-effect relationship between betatrophin and an increase in pancreatic β-cell proliferation, Yi et al (9) did not demonstrate the correlation between circulating betatrophin concentrations and the increase in β-cell mass, with most of their

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**Table 1.** Demographic and Biochemical Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>LN-NG</th>
<th>OB-NG</th>
<th>OB-IGT</th>
<th>OB-T2D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>33</td>
<td>75</td>
<td>30</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Sex (males/females), n</td>
<td>14/19</td>
<td>38/37</td>
<td>15/15</td>
<td>7/8</td>
<td>.879</td>
</tr>
<tr>
<td>Age, y</td>
<td>46.9 ± 11.0</td>
<td>47.3 ± 4.5</td>
<td>48.9 ± 4.5</td>
<td>49.2 ± 5.8</td>
<td>.458</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67 ± 10</td>
<td>111 ± 19a</td>
<td>123 ± 26a,b</td>
<td>106 ± 10c</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.6 ± 1.2</td>
<td>39.4 ± 6.7a</td>
<td>43.7 ± 7.5a,b</td>
<td>39.0 ± 3.6a,c</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>25.3 ± 7.7</td>
<td>46.6 ± 9.4a</td>
<td>50.0 ± 7.8a,b</td>
<td>47.5 ± 6.3a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82 ± 6</td>
<td>118 ± 14a</td>
<td>129 ± 16a,b</td>
<td>120 ± 5a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>108 ± 11</td>
<td>119 ± 12a</td>
<td>125 ± 11a,b</td>
<td>125 ± 6a</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>67 ± 7</td>
<td>77 ± 9a</td>
<td>80 ± 8a,b,b</td>
<td>78 ± 5a</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>48,87</td>
<td>93 ± 9a</td>
<td>100 ± 10a,b,b</td>
<td>111 ± 20a,b,c</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>4.3 ± 2.3</td>
<td>12.9 ± 13.0a</td>
<td>19.2 ± 11.5a,b</td>
<td>23.7 ± 29.7a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C-peptide, ng/mL</td>
<td>1.11 ± 0.34</td>
<td>2.23 ± 1.15a</td>
<td>2.88 ± 1.63a,b</td>
<td>3.60 ± 2.86a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glucose 2-h OGTT, mg/dL</td>
<td>106 ± 39</td>
<td>113 ± 18</td>
<td>164 ± 19a,b</td>
<td>232 ± 29a,b,c</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insulin 2-h OGTT, mg/dL</td>
<td>66 ± 61</td>
<td>91 ± 55</td>
<td>153 ± 63a,b</td>
<td>154 ± 96a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.0 ± 0.5</td>
<td>3.0 ± 3.2a,b</td>
<td>4.8 ± 3.1a,b</td>
<td>6.3 ± 7.5a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.40 ± 0.04</td>
<td>0.35 ± 0.05a</td>
<td>0.31 ± 0.02a,b</td>
<td>0.31 ± 0.04a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>75 ± 29</td>
<td>107 ± 49</td>
<td>122 ± 55a</td>
<td>212 ± 291a,b,c</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>173 ± 34</td>
<td>195 ± 38a</td>
<td>191 ± 36</td>
<td>202 ± 54a</td>
<td>.034</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>94 ± 30</td>
<td>119 ± 37a</td>
<td>115 ± 28a</td>
<td>107 ± 35</td>
<td>.004</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>64 ± 14</td>
<td>54 ± 12a</td>
<td>50 ± 8a</td>
<td>53 ± 10a</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>4.1 ± 1.1</td>
<td>5.5 ± 1.3a</td>
<td>5.9 ± 1.2a</td>
<td>6.6 ± 1.4a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.1 ± 1.1</td>
<td>5.8 ± 8.2a,b</td>
<td>9.0 ± 9.6a,b</td>
<td>11.7 ± 14.4a,b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>21 ± 17</td>
<td>23 ± 15</td>
<td>24 ± 13</td>
<td>23 ± 10</td>
<td>.894</td>
</tr>
<tr>
<td>γ-GT, IU/L</td>
<td>12 ± 8</td>
<td>23 ± 20a</td>
<td>32 ± 33a</td>
<td>34 ± 28a</td>
<td>.001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.78 ± 0.18</td>
<td>0.80 ± 0.16</td>
<td>0.80 ± 0.18</td>
<td>0.83 ± 0.16</td>
<td>.843</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>7.7 ± 9.0</td>
<td>38.8 ± 28.9a</td>
<td>44.3 ± 22.6a</td>
<td>33.5 ± 18.0a</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase. Data are presented as mean ± SD. Individuals were matched by age and sex in the whole sample and by body fat in the obese groups. Differences between groups were analyzed by ANOVA, followed by LSD tests. Differences in gender distribution were analyzed by χ² analysis. CRP concentrations were logarithmically transformed for statistical analysis.

a P < .05 vs LN-NG.
b P < .05 vs OB-NG.
c P < .05 vs OB-IGT.
experiments being in an artificial model of insulin resistance, based on the use of an insulin receptor antagonist, as opposed to the traditionally clinical models of obesity and/or insulin resistance addressed in the present study. Contrary to our hypothesis, serum betatrophin concentrations were markedly reduced in obesity and obesity-associated insulin resistance. This is, to our knowledge, the first study describing decreased serum betatrophin levels in human obesity. Obese subjects exhibited a 40% reduction in serum betatrophin concentrations as compared to lean individuals, and betatrophin concentrations were further reduced up to 70% in obese participants with T2D. Interestingly, circulating levels of betatrophin are increased in individuals with long-standing type 1 diabetes, a condition accompanied by very low or absent insulin and almost total destruction of the β-cells (15). In this regard, betatrophin levels have been suggested to be regulated by insulin resistance and not insulin deficiency per se (9). The decreased levels of betatrophin found in obesity in the present study may be related to hyperglycemia and to the degree of insulin resistance. Supporting this notion, betatrophin concentrations were strongly and negatively correlated with all markers of insulin resistance such as glycemia, insulinemia, HOMA, and glycemia and insulinemia 2 hours after the OGTT, even after adjusting for body fat. It is noteworthy that recent data by Jiao et al (10) show that elevated hepatic betatrophin expression increased the replication of mice β-cells but not human β-cells transplanted into mice. Importantly, in the work of Jiao et al (10), human β-cells were unresponsive to mouse betatrophin. Although mouse and human betatrophin have a 73% homology in their amino acidic sequences, whether the mice protein actually operates and fully activates the human betatrophin receptor needs to be elucidated (10). Our data are the first to report the dramatic gender dimorphism observed in betatrophin concentrations with circulating levels being significantly higher in women than in men and more closely associated to BMI. This finding may underlie the higher risk of developing T2D observed in men as compared to women (16).

During the review process of this manuscript, two studies in humans have been published finding no changes related to obesity and/or T2D (17) or increased levels in obesity and T2D (18), in contrast to our results. We have confirmed our results by using ELISA kits from two different providers and obtaining the same differences between groups and genders identified originally. The different results as regards the recently reported findings may be attributed to methodological differences between the
immunoassays or to dissimilarities between the subjects’ characteristics. In this sense, in our case obese subjects with T2D were diagnosed very recently, whereas in the mentioned studies patients had a longer history of diabetes. Furthermore, a potential diverse degree of inflammation may also exert a differential impact because it impinges on both glucose and lipid metabolism (14).

Previous research in mice has shown that betatrophin operates as a blood lipid regulator by modulating serum triglyceride levels and that its hepatic expression is reduced by fasting and restored by refeeding (5, 6, 8). Betatrophin induces triglyceride elevation through reduced triglyceride clearance by lipoprotein lipase inhibition (5). Moreover, betatrophin overexpression increases triglyceride levels (5, 8), whereas betatrophin deficiency reduces triglyceride concentrations associated with both a reduction in very low-density lipoprotein secretion and an increase in lipoprotein lipase activity, exerting no effect on glucose homeostasis (19). Mechanisms involving a betatrophin-activated autophagic process in human liver cell lines have been proposed (20). Moreover, a single-nucleotide polymorphism in the betatrophin gene that substitutes tryptophan for arginine at residue 59 is reportedly associated with lower circulating concentrations of LDL- and HDL-cholesterol, but not with BMI, triglyceride levels, or HOMA in humans (8). According to this, elevated blood levels of betatrophin might be expected in relation to the hypertriglyceridemia accompanying obesity (21), thereby suggesting betatrophin inhibition as a potential therapeutic strategy for reducing plasma lipoprotein levels (8). On the contrary, data from the present study show that betatrophin concentrations in obese humans are greatly reduced and strongly correlated with triglyceride levels (negatively) and with HDL-cholesterol (positively), with the latter emerging as the major determinant of betatrophin levels according to the regression analysis. Although correlation does not imply causality, it might be speculated that betatrophin levels may be decreased in response to the increased lipidemia as a compensatory mechanism aimed at reducing lipoprotein levels.

In conclusion, our results show that serum betatrophin concentrations are decreased in obese subjects and are further reduced in parallel to the increase in insulin resistance. Moreover, serum betatrophin levels are markedly increased in women as compared to men, which could help to explain the increased risk of diabetes found in men. Further studies modulating betatrophin levels and activity, determining which are the pathophysiological regulators of betatrophin, together with the identification of the betatrophin receptor will undoubtedly help to elucidate the exact role of betatrophin in obesity and obesity-associated comorbidities such as T2D and dyslipidemia.

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Disclosure Summary: The authors declare that no competing interests exist.

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