Improved β-cell function after standardized weight reduction in severely obese subjects

MARIE GULDSTRAND,1 BO AHRÉN,2 AND ULF ADAMSON1
1Division of Internal Medicine, Karolinska Institute, Danderyd Hospital, SE-182 88 Danderyd; and 2Department of Medicine, Lund University, SE-221 84 Lund, Sweden
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Guldstrand, Marie, Bo Ahren, and Ulf Adamson. Improved β-cell function after standardized weight reduction in severely obese subjects. Am J Physiol Endocrinol Metab 284: E557–E565, 2003; 10.1152/ajpendo.00325.2002.—Islet function was examined in 13 severely obese women [body mass index 46.4 ± 5.5 (SD) kg/m²] before and after standardized 15 and 25% weight reduction (WR) instituted by bariatric surgery. The insulin response to arginine at fasting (AIR₁), at 14 mmol/l, and at >25 mmol/l glucose was reduced by 37–50% after 15 and 25% WR (P < 0.05). Insulin sensitivity was determined as the amount of glucose infused to reach 14 mmol/l divided by the insulin level (M/I), a measure showing a linear correlation with insulin sensitivity during euglycemic hyperinsulinemic clamps (r = 0.74, P < 0.001) and a hyperbolic relation to AIR₁ (r = −0.63, P < 0.001) in 169 healthy subjects. M/I was increased by 318 ± 182% after 15% (P = 0.004) and by 489 ± 276% after 25% WR (P = 0.007). The reduction in insulin secretion was not as large as anticipated from the increased insulin sensitivity, which resulted in an increased disposition index (DI; AIR₁ × M/I). Thus DI was increased by 95 ± 24% after 15% (P = 0.018) and by 176 ± 35% after 25% WR (P = 0.011). This improved β-cell function correlated independently with reduced glucose, triglycerides, and leptin and increased adiponectin levels and was associated with a reduced proinsulin-to-insulin ratio. In contrast, glucagon secretion was not significantly affected by WR. We conclude that WR results in improved β-cell function when related to insulin sensitivity.

insulin secretion; glucagon secretion; obesity; glucose tolerance

DUE TO THE CURVILINEAR RELATION between insulin sensitivity and insulin secretion, insulin resistance, as in obesity, is compensated for by increased insulin secretion, and, accordingly, the improved insulin sensitivity that follows weight reduction adaptively reduces insulin secretion (3, 7, 8, 15, 16). The relation between insulin sensitivity and insulin secretion has been shown to be hyperbolic in nature (16) and to be quantified by making a product of the two variables, namely the disposition index (DI; 8, 16). The relation is of clinical importance for glucose homeostasis because, if the islet compensation is inadequate in relation to insulin resistance, impaired glucose tolerance (IGT) or type 2 diabetes develops (3, 7, 15, 20, 21, 23, 45). The usually observed improvement of glucose homeostasis after weight reduction in obesity (9, 13, 35) therefore suggests that insulin secretion is not reduced as much as anticipated by the increased insulin sensitivity. However, it is not known whether insulin secretion is improved during weight reduction, despite its reduction per se because of the adaptation to the increased insulin sensitivity. Similarly, it is not clear by which mechanism β-cell function is altered during weight reduction in regard to signals affecting β-cell function or insulin-secretory mechanisms (3, 7, 15). In addition to insulin secretion, glucagon has an important role in glucose tolerance, as has previously been demonstrated in subjects with type 2 diabetes and IGT, who display increased circulating glucagon in relation to the ambient glucose level (2, 21, 31, 37, 42). However, the extent to which changes in glucagon secretion contribute to the improved glucose tolerance after weight reduction in obese subjects is not known.

In view of these fundamental questions on islet function after weight reduction in obesity, this study investigated how insulin and glucagon secretion adapts to improvement in insulin sensitivity after standardized massive weight reduction in severely obese subjects. To that end, 13 morbidly obese subjects [all with body mass index (BMI) >40 kg/m²] undergoing bariatric surgery were studied before operation and after standardized weight reduction by 15 and 25%. For detailed analyses of islet hormone secretion, we employed the glucose-dependent arginine stimulation test, which characterizes both acute insulin and glucagon secretion and the glucose sensitivity of β- and α-cell secretion (3, 22, 44). From this test, a measure of insulin sensitivity was also calculated, and in initial studies we observed a significant linear correlation with this measurement and insulin sensitivity as determined during a euglycemic hyperinsulinemic clamp test.

SUBJECTS AND METHODS

Subjects. The core study was performed in 13 morbidly obese women 26–39 yr of age (mean ± SD: 33.5 ± 4.2 yr) and without cardiovascular diseases or impaired kidney or liver function. None of these subjects was taking any drugs known...
to affect carbohydrate metabolism; five had a family history of diabetes. All women were Caucasian except for one with African origin. The patients were recruited from the Division of Surgery at Danderyd Hospital to undergo a modified Mason vertical banded gastroplasty (8 patients) (27) or jejunoileal bypass (5 patients) (5) with the purpose of reducing weight. The studies were performed before and after 15 and 25% weight loss. These usually occur after 3–5 mo and ~1 yr, respectively, after bariatric surgery (35). One patient was lost to follow-up at 25% weight reduction. The study validating the use of the glucose-dependent arginine stimulation test vs. the euglycemic hyperinsulinemic clamp technique was performed in 169 healthy females aged 42–61 yr who were recruited from a larger cohort of women in the city of Malmo, Sweden, and who had undergone both a glucose-dependent arginine stimulation test and a euglycemic hyperinsulinemic clamp test (3, 19, 21, 25). These were healthy subjects without diabetes or any known history of cardiovascular, liver, or kidney diseases, and they were not taking any medication known to affect carbohydrate metabolism. Both tests were performed after an overnight fast and with ~2 wk in between. The local ethics committee of the Karolinska Hospital, Stockholm, and Lund University, Lund, Sweden, approved the study. All subjects gave written, informed consent before entrance in the study.

**Anthropometric measurements.** Body weight and height were measured with the subjects in light clothing and without shoes for calculation of BMI. Waist circumference was measured at the level of the umbilicus and hip circumference at the level of the greater trochanters. Body composition was determined with near-infrared spectrophotometry using a Futrex 5000 (Futrex, Gaithersburg, MD) (11).

**Oral glucose tolerance test.** An intravenous catheter was inserted into an antecubital vein, and a 75-g glucose load was given. Blood samples were taken at 0, 15, 30, 60, 90, and 120 min.

**Glucose-dependent arginine stimulation test.** Insulin and glucagon secretion was determined with intravenous arginine stimulation at three glucose levels (fasting, 14 mmol/l, and >25 mmol/l), as previously described (22, 44). Intravenous catheters were inserted into antecubital veins in both arms, and baseline samples were taken at ~5 and ~2 min. A maximally stimulating dose of arginine hydrochloride (5 g) was then injected intravenously over 45 s. Samples were taken at +2, +3, +4, and +5 min. Variable-rate 20% glucose infusions were then performed sequentially to raise and maintain blood glucose at 13–15 mmol/l and >25 mmol/l, respectively. New baseline samples were taken at these blood glucose levels, whereas arginine (5 g) was again injected and new samples taken.

**Euglycemic hyperinsulinemic clamp test.** Insulin sensitivity was determined with the euglycemic hyperinsulinemic clamp, performed according to DeFronzo et al. (12). Intravenous catheters were inserted into antecubital veins in both arms. After baseline samples were obtained, a primed constant infusion of insulin (Actrapid 100 U/m; Novo Nordisk, Bagsvaerd, Denmark) with a constant infusion rate of 0.28 nmol·m body surface area~−2·min−1 was started. After 4 min, a variable-rate 20% glucose infusion was added, and its infusion rate was adjusted manually throughout the clamp procedure to maintain the blood glucose level at 5.0 mmol/l, as determined bedside every 5 min. Samples for analysis of insulin were taken at 60 and 120 min.

**Analyses.** Blood glucose samples from the oral glucose tolerance test (OGTT) were chilled at 4°C and analyzed with an automatic glucose oxidase method at the hospital central laboratory, whereas blood glucose from the glucose-dependent arginine stimulation test was analyzed with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Blood samples for analysis of insulin and glucagon were immediately centrifuged at 5°C, and plasma was frozen at ~20°C until analysis in duplicate. Plasma insulin and glucagon concentrations were analyzed with double-antibody radioimmunoassay techniques (Linco Research, St. Charles, MO), using guinea pig anti-human insulin antibodies, human insulin standard, 125I-labeled human insulin, guinea pig antiguclagon antibodies specific for pancreatic glucagon, 125I-labeled glucagon, and glucagon standard. Total proinsulin was measured using goat antibodies raised against human proinsulin, human proinsulin standard, and 125I-human proinsulin as tracer (Linco). The assay detects intact proinsulin (100%) and des-31,32 proinsulin (95%) but does not cross-react with insulin, C-peptide, or des-64,65 proinsulin (<0.1%). Leptin was analyzed with a double-antibody radioimmunoassay using rabbit anti-human leptin antibodies, 125I-labeled human leptin as tracer, and human leptin as standard (Linco). Adiponectin was analyzed with a double-antibody radioimmunoassay using rabbit anti-human adiponectin antibodies, as well as the expectance tracer, and human adiponectin as standard (Linco). Cholesterol and triglycerides were analyzed using routine, standardized methods (Roche Diagnostics), and free fatty acids (FFA) were determined spectrophotometrically (Wako Chemicals, Neuss, Germany).

**Calculations.** The acute insulin (AIR), acute proinsulin (APIR), and acute glucagon responses (AGR) to arginine were calculated as the mean of the +2- to +5-min samples minus the mean prestimulus hormone concentration at fasting glucose (AIR1, APIR1, or AGR1), at 14 mmol/l glucose (AIR2, APIR2, or AGR2), and at >25 mmol/l glucose (AIR3, APIR3, or AGR3). Also, the ratio between the acute proinsulin-to-insulin (PI) response after the first arginine challenge was calculated (PI-secretory ratio). The slope between AIR2 and AIR3 [slopeAIR = (AIR3 − AIR1)/Δplasma glucose] was calculated as a measure of glucose potentiation of β-cell secretion (22, 44), and the plasma glucose level at which half-maximal insulin secretion is achieved (PG50), which is a measure of glucose sensitivity in the β-cells (44), was calculated from AIR3 and slopeAIR. Similarly, the slope between AGR1 and AGR3 [slopeAGR = (AGR3 − AGR1)/Δglucose] was calculated as the glucose inhibition of α-cell secretion. For estimation of insulin sensitivity from the arginine stimulation test, the amount of glucose infused to raise the glucose level to the target of 14 mmol/l (M value) was divided by the insulin concentration achieved immediately before the arginine injection (1 value) (M/I). For calculation of insulin sensitivity from the hyperinsulinemic euglycemic clamp test, the glucose infusion rate during the 2nd h of the clamp was divided by the mean of the measured mean insulin concentrations at 60 and 120 min (M/Iclamp). Previous studies estimating insulin sensitivity and insulin secretion with the use of data derived from the minimal model of results from an intravenous glucose tolerance test (IGTT) have shown that a hyperbolic function is the best predictor of the relation (16). To explore whether a hyperbolic function characterizes the relation between insulin secretion and insulin sensitivity with the use of data derived from the arginine stimulation test, we calculated the logarithmic transformation of AIR1 as a function of logarithmically transformed M/I, with the expectation that this relation is a linear function. We also plotted the residuals of log AIR1 and AIR3 by use of the hyperbolic and linear regressions, respectively, vs. log M/I or M/I, with the expectation that residuals of the best fit would be randomly distributed around zero. Because we found that the hyper-
Table 1. Characteristics of 13 obese subjects before surgery and after 15 and 25% weight reduction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>15% Weight Reduction</th>
<th>25% Weight Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>127.9 ± 20.1</td>
<td>107.4 ± 16.1‡</td>
<td>94.9 ± 18.7‡</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>46.4 ± 5.5</td>
<td>39.1 ± 4.7‡</td>
<td>35.0 ± 5.8‡</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>112.3 ± 7.2</td>
<td>103.7 ± 6.3‡</td>
<td>98.7 ± 9.2‡</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>137.6 ± 11.0</td>
<td>125.1 ± 14.3‡</td>
<td>117.9 ± 17.2‡</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.76 ± 0.21</td>
<td>0.84 ± 0.09 NS</td>
<td>0.78 ± 0.22 NS</td>
</tr>
<tr>
<td>Fat percentage, %</td>
<td>34.5 ± 5.2</td>
<td>28.1 ± 7.7</td>
<td>26.0 ± 3.1‡</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>44.2 ± 13.4</td>
<td>30.3 ± 7.4‡</td>
<td>25.0 ± 7.6‡</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>83.1 ± 9.4</td>
<td>77.2 ± 9.7</td>
<td>70.3 ± 11.8‡</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/l</td>
<td>336 ± 601</td>
<td>87 ± 59‡</td>
<td>70 ± 54‡</td>
</tr>
<tr>
<td>Fasting plasma glucagon, ng/l</td>
<td>47.7 ± 7.7</td>
<td>49.4 ± 8.9 NS</td>
<td>46.9 ± 9.5 NS</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>5.6 ± 1.4</td>
<td>4.8 ± 0.4‡</td>
<td>4.7 ± 0.3†</td>
</tr>
<tr>
<td>2-h Plasma glucose, mmol/l</td>
<td>7.4 ± 1.6</td>
<td>6.9 ± 1.3 NS</td>
<td>5.4 ± 0.8‡</td>
</tr>
<tr>
<td>AUCglucose, mmol·1⁻¹·120 min</td>
<td>309 ± 93</td>
<td>208 ± 66‡</td>
<td>130 ± 78‡</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/l</td>
<td>5.1 ± 1.7</td>
<td>4.4 ± 1.1</td>
<td>4.2 ± 1.3†</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/l</td>
<td>3.8 ± 1.4</td>
<td>1.5 ± 0.6‡</td>
<td>0.9 ± 0.4‡</td>
</tr>
<tr>
<td>Plasma FFA, mmol/l</td>
<td>1.3 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.3‡</td>
</tr>
<tr>
<td>Hb A₁c, %</td>
<td>4.6 ± 0.4</td>
<td>4.3 ± 0.4 NS (P = 0.059)</td>
<td>4.2 ± 0.4†</td>
</tr>
<tr>
<td>Plasma leptin, ng/ml</td>
<td>67.5 ± 17.2</td>
<td>27.9 ± 14.4‡</td>
<td>20.4 ± 11.0‡</td>
</tr>
<tr>
<td>Leptin/fat mass, ng·ml⁻¹·kg⁻¹</td>
<td>1.57 ± 0.44</td>
<td>0.87 ± 0.31‡</td>
<td>0.77 ± 0.27‡</td>
</tr>
<tr>
<td>Plasma adiponectin, µg/ml</td>
<td>7.2 ± 2.4</td>
<td>9.0 ± 2.4</td>
<td>11.1 ± 4.3‡</td>
</tr>
</tbody>
</table>

Means ± SD are shown. BMI, body mass index; AUC, area under the curve; FFA, free fatty acid. Probability level of random difference vs. baseline values as obtained by paired t-test. *P < 0.05, †P < 0.01, ‡P < 0.001; NS, not significant (P > 0.05).

bolic function predicts the relation, we estimated DI by multiplying AIR₃ times M/I.

Statistical analyses. Statistical analyses were performed with the SPSS for Windows system (SPSS, Chicago, IL). Differences in parameters before and after weight reduction were tested with Student’s paired t-test. Pearson’s product moment correlation coefficients were obtained to estimate linear correlations between variables. Linear stepwise forward multiple regression was used to assess the independent effect of several variables. Means ± SD or SE are shown as specified.

RESULTS

Body composition, baseline values, and glucose tolerance. Table 1 shows the characteristics of the 13 subjects before bariatric surgery and after 15.9 ± 2.1% (SD) and 25.6 ± 5.6% weight reduction, which was reached after 4.3 ± 1.2 (SD) and 11.5 ± 2.8 (SD) mo, respectively. Figure 1 shows that glucose tolerance was gradually improved after bariatric surgery. At baseline, two patients were classified as having type 2 diabetes and one as having IGT. After 15% weight reduction, all fasting glucose values were normalized while three patients had IGT, and after 25% weight reduction all patients had normal 2-h glucose.

Insulin and glucagon secretion. Table 2 and Figs. 2 and 3 show the results from the glucose-dependent arginine stimulation tests. The test identifies insulin secretion at baseline glucose (AIR₁), after rise of glucose to 14 mmol/l [AIR₂; being significantly higher (P < 0.001) than AIR₁ at all three study time points], and after rise of glucose to >25 mmol/l [AIR₃; being significantly higher than AIR₂ before surgery (P = 0.045), after 15% weight reduction (P = 0.05), and after 25% weight reduction (P = 0.006), AIR₁, AIR₂, AIR₃, and slopeAIR were significantly reduced after 15 and 25% weight reduction. These various measures of insulin secretion were altered in parallel. Thus, at 15% weight reduction, AIR₁ was reduced by 40 ± 33%, AIR₂ by 41 ± 36%, AIR₃ by 16 ± 26%, and slopeAIR by 37 ± 27%. The corresponding figures after 25% weight reduction were 39 ± 30% (AIR₁), 50 ± 23% (AIR₂), 39 ± 15% (AIR₃), and 42 ± 51% (slopeAIR). The PG₅₀,i.e., the glucose level at which half-maximal insulin secretion is achieved, was not significantly altered by the reduced body weight. Furthermore, in contrast to the reduced insulin secretion, AGR₁, AGR₂, AGR₃, and slopeAGR did not differ significantly after 15 or 25% weight reduction.

Insulin sensitivity. The use of variables obtained from the glucose-dependent arginine stimulation test for the estimation of insulin sensitivity was validated against the gold standard technique, the euglycemic hyperinsulinemic clamp, by performing the two tests in 169 healthy female subjects aged 42–61 yr. The measure for insulin sensitivity in the glucose-dependent
The glucose-dependent arginine stimulation test was the amount of glucose infused to reach the target of 14 mmol/l divided by the achieved insulin concentration (M/I). Figure 4 shows that this value correlated linearly with the measure of insulin sensitivity during the euglycemic hyperinsulinemic clamps ($r = 0.67$, $P < 0.001$). This shows that the use of the glucose-dependent arginine stimulation test is possible for determination of insulin sensitivity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>15% Weight Reduction</th>
<th>25% Weight Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIR1 (insulin response at fasting BG), pmol/l</td>
<td>531 ± 600</td>
<td>263 ± 171$^\dagger$</td>
<td>234 ± 172$^\dagger$</td>
</tr>
<tr>
<td>AIR2 (insulin response at BG14), pmol/l</td>
<td>2,260 ± 1,171</td>
<td>1,421 ± 787$^\dagger$</td>
<td>1,082 ± 615$^\dagger$</td>
</tr>
<tr>
<td>AIR3 (insulin response at BG25), pmol/l</td>
<td>2,773 ± 1,151</td>
<td>2,334 ± 1,188$^*$</td>
<td>1,701 ± 814$^*$</td>
</tr>
<tr>
<td>SlopeAIR (pmol insulin/mmol glucose)</td>
<td>174 ± 95</td>
<td>115 ± 71$^*</td>
<td>$</td>
</tr>
<tr>
<td>PG50, mmol/l</td>
<td>10.1 ± 0.8</td>
<td>11.2 ± 1.1 NS</td>
<td>10.8 ± 1.0 NS</td>
</tr>
<tr>
<td>AGR1 (glucagon response at fasting BG), ng/l</td>
<td>18.8 ± 8.8</td>
<td>19.0 ± 11.9 NS</td>
<td>22.3 ± 7.2 NS</td>
</tr>
<tr>
<td>AGR2 (glucagon response at BG14), ng/l</td>
<td>16.7 ± 11.9 NS</td>
<td>17.9 ± 7.4 NS</td>
<td>17.3 ± 9.1 NS</td>
</tr>
<tr>
<td>AGR3 (glucagon response at BG25), ng/l</td>
<td>11.9 ± 7.5 NS</td>
<td>10.2 ± 5.1 NS</td>
<td>12.1 ± 8.4 NS</td>
</tr>
<tr>
<td>SlopeAGR (ng glucagon/mmol glucose)</td>
<td>−0.1 ± 0.6</td>
<td>−0.1 ± 0.6 NS</td>
<td>−0.6 ± 1.1 NS</td>
</tr>
<tr>
<td>M/I (insulin sensitivity at BG14), nmol glucose·kg$^{-1}$·min$^{-1}$/pmol insulin·l$^{-1}$</td>
<td>165 ± 137</td>
<td>446 ± 305$^\ddagger$</td>
<td>623 ± 351$^\dagger$</td>
</tr>
<tr>
<td>DI (disposition index), nmol·kg$^{-1}$·min$^{-1}$/pmol insulin·l$^{-1}$</td>
<td>56.2 ± 33.4</td>
<td>94.0 ± 93.6$^*$</td>
<td>135 ± 105$^*$</td>
</tr>
</tbody>
</table>

Means ± SD are shown. BG14, test condition at 14 mmol/l blood glucose; BG25, test condition at >25 mmol/l blood glucose. PG50, plasma glucose at half-maximal insulin secretion. Probability level of random difference vs. baseline values as obtained by paired t-test: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. 

Arginine stimulation test was the amount of glucose infused to reach the target of 14 mmol/l divided by the achieved insulin concentration (M/I). Figure 4 shows that this value correlated linearly with the measure of insulin sensitivity during the euglycemic hyperinsulinemic clamps ($r = 0.67$, $P < 0.001$). This shows that the use of the glucose-dependent arginine stimulation test is possible for determination of insulin sensitivity.

Fig. 2. Plasma levels of insulin (A) and glucagon (B) during the glucose-dependent arginine stimulation test in 13 morbidly obese subjects before bariatric surgery and after 15 and 25% weight reduction. Arginine (5 g; arrows) was injected iv at fasting glucose and after rise of glucose to 14 mmol/l or >25 mmol/l achieved by iv glucose infusion, as indicated by boxes. Means ± SE are shown.

Fig. 3. Insulin (A) and glucagon (B) responses to iv arginine (5 g) at fasting glucose and after rise of glucose to 14 mmol/l or >25 mmol/l achieved by iv glucose infusion, as indicated by boxes. Means ± SE are shown.
Fig. 4. Linear regression between insulin sensitivity (amount of glucose infused to reach 14 mmol/l divided by the insulin level [M/I]) as obtained by data derived from the euglycemic hyperinsulinemic clamp technique vs. insulin sensitivity as obtained by data derived from the glucose-dependent arginine stimulation test in 169 healthy female subjects, aged 42–61 yr. r, Linear regression coefficient; P, the probability level of random regression.

Table 2 shows that M/I as obtained during the glucose-dependent arginine stimulation test was gradually increased along with the reduction in body weight in the obese subjects, being increased by 318 ± 182% (SD) after 15% weight reduction (P = 0.004) and by 489 ± 276% after 25% weight reduction (P = 0.007).

Changes in insulin sensitivity vs. insulin secretion. Figure 5 shows the relation between AIR1 and M/I in the 169 healthy female subjects. Visual inspection of the relation suggests a nonlinear function, which is also supported by a hyperbolic function having a higher regression coefficient (r = -0.63, P < 0.001) than a linear function (r = -0.42, P < 0.001). When data were logarithmically transformed, a linear relation between the two variables was evident, and the function was \[ \text{log AIR}_1 = 4.95 - 0.88 \log \text{M/I} \] (95% confidence interval of slope 0.75–1.01). Because the best fit of the slope for the regression coefficient would be near -1.0, we conclude that a hyperbolic function results in the best fit of the relation. This was also evident by plotting the residuals in AIR1 vs. M/I after logarithmic transformation of the data or without any transformation (Fig. 5). It is seen that the residuals were randomly distributed around zero after logarithmic transformation of the data, whereas increases in residuals in AIR1 were evident at low and high M/I without any transformation. The hyperbolic relation between AIR1 and M/I justifies the calculation of DI as AIR1 times M/I, as previously established (16). Table 2 shows that DI was significantly increased after weight reduction. The reason for this is that there was a disproportionately lower reduction in insulin secretion in relation to the increased insulin sensitivity. Hence, although insulin secretion per se was reduced after weight reduction, \( \beta \)-cell function was improved.

Proinsulin-to-insulin ratio. Fasting plasma proinsulin levels were reduced by a larger degree than fasting plasma insulin, resulting in a reduced fasting proinsulin-to-insulin ratio after weight reduction (Table 3). Also, the reduction in the proinsulin response to arginine after weight reduction was more pronounced than the reduction in the insulin response to arginine, resulting in a significantly decreased proinsulin-to-insulin ratio after arginine challenge after both 15 and 25% weight reduction. Thus, whereas the insulin response to arginine at fasting glucose was reduced by 40 ± 33% (SD) after 15% weight reduction (P = 0.008) and by 39 ± 30% after 25% weight reduction (P = 0.006), the proinsulin response to arginine was reduced by 72 ± 26% after 15% weight reduction (P = 0.009) and by 85 ± 18% after 25% weight reduction (P = 0.002), which explains the increased PI-secretory ratio (P < 0.001; Table 3).

Relation between improved DI and changes in body composition. There were significant correlations between DI on the one hand and body weight, body fat mass, and waist circumference on the other hand as well as between the increase in DI after weight reduction on the one hand and the reduction in body weight, body fat mass, and waist circumference after weight reduction on the other hand (all P < 0.001). Because these measures of body composition are interrelated, a multivariate regression analysis was undertaken in which the change in DI after 15% weight reduction (\( \Delta \text{DI} \)) was used as the dependent variable and the

Fig. 5. A: nonlinear regression between insulin sensitivity (M/I) and insulin secretion [acute insulin response (AIR1)] as obtained by data derived from the glucose-dependent arginine stimulation test in 169 healthy female subjects aged 42–61 yr. B and C: relation between residuals of AIR1 vs. M/I as obtained by data derived from the glucose-dependent arginine stimulation test in 169 healthy female subjects aged 42–61 yr when a linear relation is assumed between logarithmically (lg) transformed data (B) and nontransformed data (C).
corresponding changes in body weight, waist circumference, and body fat mass were used as independent variables. The regression showed that changes in waist, but not in the other variables of body composition, was independently related to changes in DI ($r^2 = 0.55, P = 0.008$).

**Factors mediating improved β-cell function.** Several factors might mediate the improved β-cell function during weight reduction in obesity. Five potential factors were evaluated in this study: circulating glucose, triglycerides, and FFA and circulating levels of the adipocyte-derived hormones leptin and adiponectin, which were all altered by the weight reduction (Table 1). A multivariate analysis with the change in DI between baseline and after 15% weight reduction ($\Delta$DI) as the dependent variable and $\Delta$fasting glucose, $\Delta$fasting triglycerides, $\Delta$fasting FFA, $\Delta$fasting leptin, and $\Delta$fasting adiponectin as independent variables showed that fasting glucose ($r = -0.69$), triglycerides ($r = -0.72$), leptin ($r = -0.62$), and adiponectin ($r = 0.45$) all significantly and independently contributed to $\Delta$DI ($P < 0.05$ or less). $R^2$ for the model was 0.79 ($P < 0.001$). In contrast, changes in FFA did not correlate with improved β-cell function.

**DISCUSSION**

Massive weight reduction by bariatric surgery improves glucose intolerance and type 2 diabetes in subjects with severe obesity (9, 13) as a result of the combination of improved insulin resistance that follows weight reduction (30) and improved islet function (7, 15). In this study, we aimed at disclosing the mechanisms underlying the improved islet function in morbidly obese subjects undergoing standardized 15 and 25% weight reduction. Because we aimed at relating the changes in insulin secretion to those in insulin sensitivity, which is required for an accurate understanding of islet function, we initially explored the use of data derived from the glucose-dependent arginine stimulation test for calculating insulin sensitivity. We therefore compared M/I data from the arginine test with the measurement of insulin sensitivity during euglycemic hyperinsulinemic clamps in 169 healthy female subjects. We found that insulin sensitivity determined by these two techniques was linearly related, with an $r$ value of 0.67. This shows that, although a true steady state is not reached during the 20- to 25-min glucose infusion to the target of 14 mmol/l glucose and, furthermore, that although insulin is not kept constant during the test, there is a good relation between the two tests. As expected, weight reduction increased insulin sensitivity in the obese subjects because the M/I value increased by ~300% after 15% weight reduction and by ~500% after 25% weight reduction and, similarly, fasting insulin, which correlates negatively with insulin sensitivity, was reduced from >300 pmol/l before weight reduction to ~80 and 70 pmol/l after weight reduction.

The increased insulin sensitivity after weight reduction was accompanied by reduced insulin secretion. This reduced insulin secretion is an adaptation to the improved insulin sensitivity, because insulin secretion is inversely related to insulin sensitivity (3, 7, 8, 15, 16). However, the main finding in the study is that the reduction in insulin secretion was quantitatively lower than was the increase in insulin sensitivity, resulting in an increased DI. We calculated the DI by multiplying AIR1 times M/I as obtained from the arginine test. Such a calculation of DI has been justified by the evidence that insulin secretion and insulin sensitivity display a hyperbolic function, which results in a constant value when the two parameters are multiplied. This has previously been demonstrated for insulin secretion and insulin sensitivity as derived from minimal-model analysis of data obtained in the IGTT (16). We show here that AIR1 and M/I are also related to each other in a nonlinear manner and that the relation is consistent with a hyperbolic function. By using this approach, we found that DI was significantly increased after both 15 and 25% weight reduction. This is equivalent to improved β-cell function, which resulted in improved glucose tolerance. Hence, weight reduction was accompanied by a reduced insulin secretion per se, as adjustment for the increased insulin sensitivity, in combination with improved islet function. The improved β-cell function was also verified by reduced proinsulin-to-insulin ratios after weight reduction. Both baseline proinsulin and arginine-stimulated proinsulin secretions were reduced by a larger degree than baseline insulin and the insulin response to arginine. This may be explained by more efficient proinsulin conversion in the β-cells, perhaps due to normalization of a pathogenic defect. An increased proinsulin-to-in-

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**Table 3. Results of proinsulin measurements in the glucose-dependent arginine stimulation test in obese subjects before surgery and after 15 and 25% weight reduction**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>15% Weight Reduction</th>
<th>25% Weight Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma proinsulin, pmol/l</td>
<td>37.9 ± 33.2</td>
<td>8.6 ± 2.9*</td>
<td>6.9 ± 3.1†</td>
</tr>
<tr>
<td>Fasting proinsulin/insulin ratio</td>
<td>0.17 ± 0.06</td>
<td>0.12 ± 0.05*</td>
<td>0.09 ± 0.08†</td>
</tr>
<tr>
<td>APIR1 (proinsulin response at fasting glucose), pmol/l</td>
<td>30.4 ± 46.1</td>
<td>6.2 ± 3.8†</td>
<td>3.4 ± 6.2‡</td>
</tr>
<tr>
<td>APIR2 (proinsulin response at BG 14), pmol/l</td>
<td>81.8 ± 45.9</td>
<td>34.8 ± 17.3‡</td>
<td>28.3 ± 21.3†</td>
</tr>
<tr>
<td>APIR3 (proinsulin response at BG 28), pmol/l</td>
<td>130.5 ± 64.8</td>
<td>50.4 ± 21.3‡</td>
<td>51.7 ± 55.9†</td>
</tr>
<tr>
<td>PI-secretory rate, %</td>
<td>5.1 ± 3.5</td>
<td>2.6 ± 1.3‡</td>
<td>0.6 ± 1.7‡</td>
</tr>
</tbody>
</table>

Means ± SD are shown. Fasting proinsulin levels and proinsulin responses to arginine (APIR) at the 3 different glucose levels (APIR1, APIR2, and APIR3, respectively) are shown as are the proinsulin-to-insulin ratios in the β-cell response to arginine at fasting glucose (PI-secretory rate). Probability level of random difference vs. baseline values as obtained by paired t-test: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. 

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sulin ratio is a sign of a defective proinsulin conversion, which is associated with β-cell dysfunction (24, 36, 40).

Insulin is secreted from the β-cells by a glucose recognition mechanism followed by an exocytotic mechanism releasing insulin-storing granules. The glucose-dependent arginine stimulation test discloses both of these mechanisms, because AIR1, AIR2, and AIR3 reflect the exocytosis due to arginine depolarizing the plasma membranes, resulting in massive inflow of calcium (41), whereas slope_AIR reflects the glucose potentiation of the β-cells (22). We found that the improved insulin sensitivity after weight reduction was accompanied by a parallel reduction in the two mechanisms. This suggests that the signals mediating the link between insulin sensitivity and insulin secretion target both the exocytotic and the glucose potentiation phases. This finding in our prospective study is similar to recent cross-sectional studies in obese women, in pregnant women, or in subjects with polycystic ovary, where differences between subjects exhibiting different insulin sensitivity rely on differences of both exocytotic mechanisms and β-cell glucose potentiation (3, 4, 7, 15). In contrast, we found that the glucose sensitivity of the β-cells, i.e., the glucose level at which a half-maximal insulin secretion is achieved, was not altered by the weight reduction. The measure representing glucose sensitivity, PG50, is dependent on both AIR3 and slope_AIR. Therefore, the finding that PG50 was not altered along with the improved β-cell function after weight reduction is probably explained by the parallel improvement in insulin-secretory capacity and glucose potentiation. However, because AIR2 and AIR3 were significantly different, we cannot be certain that AIR3 represents maximal insulin-secretory capacity. If AIR3 is not maximal, then it is also possible that PG50 is underestimated. Therefore, we cannot be entirely certain that PG50 may not also have changed with weight reduction.

Several factors might explain the improved β-cell function after weight reduction. One possibility is a reduction in circulating glucose, because hyperglycemia is known to affect β-cell function deleteriously (34). In the present study, the fasting glucose levels were reduced by −0.8 mmol/l after 15% weight reduction. Thus reducing the glucose load, i.e., reducing glucotoxicity (34), may be one mechanism underlying improved β-cell function. In addition, substrate-specific factors might contribute, such as circulating lipids. The subjects had high circulating triglycerides and FFA before surgery, and these levels were reduced by weight reduction, and, furthermore, the reduction of triglycerides correlated with increased DI. High lipids are toxic for the β-cells (28, 29); therefore, reducing the circulating lipids may be a second important factor underlying the improved β-cell function. This is also supported by a previous study showing that improved glucose metabolism after weight reduction in obese diabetic subjects is related to reduced lipids (30).

The multivariate regression analyses including various measures of body composition showed that reduced waist circumference was the best predictor for improved DI after weight reduction. This could indicate a role of the fatty acids released from intra-abdominal fat for improvement of insulin sensitivity and insulin secretion, because it has been reported that, in dogs, insulin resistance is associated with fatty acids derived from intra-abdominal fat (38, 39). However, the reduction in FFA did not correlate with improved DI. Instead, the association between reduction in waist circumference and improvement of insulin sensitivity and insulin secretion may be dependent on hormones produced and secreted by the abdominally located adipocytes. Two of these hormones were analyzed in the present study, leptin and adiponectin. We confirm that, whereas leptin levels are reduced after weight reduction, adiponectin levels increase, as has recently been reviewed (14). We also found that leptin levels were reduced after change in body adiposity was controlled for; i.e., leptin per unit fat mass was reduced, suggesting altered regulation of its secretion rate. Leptin has previously been shown to improve insulin resistance in rats (6), and recently an increase in insulin sensitivity has been reported after leptin administration to humans with lipodystrophy (32, 33). Furthermore, adiponectin has been shown to increase insulin sensitivity in mice (46), and in the present study we found a linear correlation between the increase in circulating adiponectin and insulin sensitivity after 15% weight reduction (r = 0.46, P = 0.012). Moreover, adiponectin-deficient mice have been shown to exhibit insulin resistance (18, 26). Regarding effects on insulin secretion, leptin in most studies inhibits insulin secretion (1, 10, 14, 17), whereas the influence of adiponectin has not yet been established. We found that changes in both of these hormones contributed independently to the improved DI after weight reduction. In fact, the multivariate regression analyses showed that reduction in fasting glucose, in fasting triglycerides, and in fasting leptin, together with the increase in fasting adiponectin, independently contributed to almost 80% of the increase in DI. This suggests that both circulating glucose and lipids as well as adipocyte-derived hormones are important for improved islet function. However, interventional studies are required to establish more direct relations between these variables and insulin secretion after weight reduction.

The glucose-dependent arginine stimulation test determines glucagon secretion (22). In subjects with diabetes, hyperglucagonemia is often prevalent, and this may underlie the hyperglycemia caused by increased hepatic glucose production (2, 21, 31, 37, 42). We found, however, that neither baseline glucagon levels nor the glucagon responses to arginine were significantly altered after 15 or 25% weight reduction. This suggests that, under these conditions, changes in glucagon secretion do not contribute to the improved glucose homeostasis.

In conclusion, this study revealed a reduced insulin secretion along with the increased insulin sensitivity after both 15 and 25% weight reduction but that insulin secretion was not reduced by as large a degree as would be expected from the increased insulin sensitivity.
Consequently, DI was increased along with an improved glucose tolerance. Weight reduction also reduced the pro-insulin-to-insulin ratio. The findings show that, although insulin secretion is adaptively reduced when insulin sensitivity is increased after weight reduction, β-cell function improves.

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