Very-low-calorie diet: a quick therapeutic tool to improve $\beta$ cell function in morbidly obese patients with type 2 diabetes$^{1-3}$

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ABSTRACT

Background: Caloric restriction in obese diabetic patients quickly improves glucose control, independently from weight loss. However, the early effects of a very-low-calorie diet (VLCD) on insulin sensitivity and insulin secretion in morbidly obese patients with type 2 diabetes are still unclear.

Objective: The objective was to study the relative contributions of insulin sensitivity, insulin secretion, or both to improvement in glucose metabolism, after 1 wk of caloric restriction, in severely obese diabetic patients.

Design: Hyperglycemic clamps were performed in 14 severely obese (BMI, in kg/m$^2$: >40) patients with type 2 diabetes in good glucose control (glycated hemoglobin $<7.5\%$) before and after 7 d of a VLCD (400 kcal/d).

Results: The VLCD caused a 3.22 $\pm$ 0.56% weight loss ($P < 0.001$), 42.0% of which was fat loss, accompanied by decreases in fasting plasma glucose ($P < 0.05$) and triglycerides ($P < 0.01$). In parallel, the Disposition Index, which measures the body’s capability to dispose of a glucose load, increased from 59.0 $\pm$ 6.3 to 75.5 $\pm$ 6.3 mL·min$^{-1}$·m$^{-2}$ body surface area ($P < 0.01$), because of improvements in indexes of both first- and second-phase insulin secretion ($P < 0.02$), but with no changes in insulin sensitivity ($P = 0.33$).

Conclusion: The marked improvement in metabolic profile, observed in severely obese patients with type 2 diabetes after a 7-d VLCD, was primarily due to the amelioration of $\beta$ cell function, whereas no contribution of insulin sensitivity was shown. This trial was registered at www.clinicaltrials.gov as NCT01447524.

INTRODUCTION

In obese patients with type 2 diabetes, lifestyle modifications resulting in weight loss improve (1) or even normalize (2) blood glucose. This beneficial effect on glucose control is accounted for by improvements in both insulin secretion and SI$^2$ (3). However, the metabolic effects of caloric restriction per se may be, at least in part, independent of body weight reduction. Better glucose control and insulin sensitivity were reported in patients losing $\sim12\%$ of weight with a 400-kcal/d diet, compared with patients who received a 1000-kcal/d diet, when studied at a time when the same weight loss ($\sim12\%$) had occurred in both groups (4). Furthermore, improved control of blood glucose in type 2 diabetes with a VLCD for 40 d was documented during the first 10 d of caloric restriction, when weight loss was still trivial (2).

When caloric intake was increased after weight reduction, plasma glucose increased, although no weight was regained (2, 4). The mechanisms underlying these early improvements caused by a VLCD in patients with type 2 diabetes have been assessed in only a few studies. Thus, a fall in hepatic glucose production (5) and a modest increase in SI (5, 6) were reported as early as 7 d after a VLCD (5). A subsequent study replicated the effects of short-term VLCD on hepatic glucose production, but not on whole-body SI (7).

As for $\beta$ cell function, earlier studies reported an apparent improvement in the insulin secretion rate during the oral-glucose-tolerance test, after short-term application of a VLCD (5). However, no formal investigation of $\beta$ cell sensitivity to glucose was performed, and, because glucose was given orally, other factors (eg, incretins, ghrelin) might have been involved. One study reported an improvement in $\beta$ cell response during hyperglycemic clamps (ie, with exclusion of gut-related factors) after 8 wk of a VLCD and during a weight-stabilization period (ie, in the absence of the negative energy balance signal) (8). Furthermore, by study design, it did not explore the first-phase secretory response to glucose.

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4 Abbreviations used: AIR, acute insulin response; BSA, body surface area; DI, Disposition Index; HbA$_{1c}$, glycated hemoglobin; SI, insulin sensitivity; VLCD, very-low-calorie diet; 1stISR, total amount of insulin released during first-phase secretion; 2ndISR, second-phase insulin response; 2ndISR, second-phase insulin secretion rate (ie, total amount of insulin released during second-phase secretion).

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Both at baseline and at the end of VLCD, a hyperglycemic insulin clamp study was performed in all patients, as previously described (18). All studies were carried out at 0800 after a 12-h overnight fast, while the subjects were lying in bed, and lasted 180 min. In all subjects, 2 intravenous catheters were inserted into an antecubital vein (retrogradely) and into a wrist vein for substance infusion and sampling of arterialized blood, respectively, according to the hot box technique (19). After a 60-min period to establish baseline (−60 to 0 min), a hyperglycemic glucose clamp was carried out at zero time for the following 120 min (18). Plasma glucose was measured (glucometer Ascensia Breeze 2; Bayer) at bedside every 2–5 min as needed and was clamped at +7.0 mmol/L (+126 mg/dL). Under these conditions of constant hyperglycemia, the normal β cell secretory response is biphasic with an early burst of insulin release within the first 10 min (first phase), followed by a later monotonically increasing hormone release (second phase) (18). Blood samples for glucose, C-peptide, and insulin measurements were drawn every 2.5 min from 0 to 15 min and every 15 min from 15 to 120 min.

Analytic methods

Plasma glucose concentrations were measured by the hexokinase method (MODULAR P Analyzer; Roche) (20). The intra-assay CV was 1.1% and interassay CV was 1.9%. The sensitivity of the method was 2 mg/dL (0.11 mmol/L).

C-peptide was analyzed with a chemiluminescence-based immunoassay (CLIA DiaSorin Analyzer; LIAISON), and the intra- and interassay CVs were 4.0% and 6.8%, respectively. The sensitivity of this assay was 0.01 mg/mL (3.31 pmol/L) (21). Plasma insulin concentrations were measured with a chemiluminescence-based immunoassay (ECLIA; Cobas Roche Diagnostics). The intraassay CV was 2.0%, and the interassay CV was 2.8%. The sensitivity of the method was 0.20 μU/mL (1.39 pmol/L) (22). Hb A1C was analyzed by HPLC (VARIANT 2; BIORAD Laboratories), and the intra- and interassay CVs were 0.77% and 3.19%, respectively. The sensitivity of the method was 1.6% (23).

Plasma total cholesterol was analyzed with a colorimetric-enzymatic method (CHOD-PAP, Roche Diagnostics). The intra-assay CV was 1%, and the interassay CV was 2.7%. The sensitivity of the method was 0.08 mmol/L (15). Plasma triglycerides were analyzed with a colorimetric-enzymatic method (GPO-PAP, Roche Diagnostics). The intraassay CV was 1.5%, and the interassay CV was 2.4%. The sensitivity of the method was 0.05 mmol/L (16).

Standard calculations

The AIR was calculated as the average incremental plasma insulin concentration at 2.5, 5.0, 7.5, and 10 min of the hyperglycemic clamp (18, 24). The 2ndIR was computed as the average incremental insulin concentration between 60 and 120 min of the hyperglycemic clamp (18). Glucose disposal during the clamp was computed as the rate of exogenous glucose infusion corrected for the (minimal) changes in the glucose pool (M value; in μmol · min⁻¹ · m⁻² BSA) (18).

The metabolic clearance rate of glucose during the clamp was computed as the ratio between the M value and the prevalent glucose concentration (18). Under these experimental conditions, the metabolic clearance rate of glucose fulfills the criteria of a direct experimental measurement of the DI of second-phase

SUBJECTS AND METHODS

Fourteen type 2 diabetic patients (7 men and 7 women) aged 60.3 ± 3.02 y with a diabetes duration of 4.8 ± 1.68 y were consecutively recruited from the Diabetes Division of Fatebenefratelli Hospital, Rome, Italy. The study protocol was approved by the Bioethics Committee of Fatebenefratelli Hospital, and all participants gave their written informed consent. The diagnosis of diabetes was established according to the criteria of the American Diabetes Association (13).

Inclusion criteria were as follows: treatment with diet alone or plus oral hypoglycemic agents, morbid obesity (BMI >40), and good metabolic control (Hb A1C <7.5%). Participants were excluded if they were treated with glucagon like peptide-1 agonists, dipeptidyl peptidase 4 inhibitors, or insulin or had a serum creatinine concentration >150 μmol/L.

All patients were studied during an inpatient stay at the Diabetes Division of Fatebenefratelli Hospital. One week before hospital admission, all hypoglycemic and antihypertensive medications were discontinued. To ensure patients’ safety, a blood sample for routine blood chemistry was collected and glucose and blood pressure profiles were assessed daily.

Participants were studied at baseline and then after 7 d of caloric restriction (VLCD). The VLCD consisted of a 400-kcal/d diet; the percentage distribution of lipids, proteins, and carbohydrates was in accordance with Italian Standards of Care (14). Urinary ketones (Auton Sticks, Arkray; Menarini Diagnostics) were assessed every morning as a biomarker of dietary adherence.

Plasma total cholesterol and triglycerides and Hb A1C were measured at baseline and at the end of the VLCD period (15, 16). Body composition was measured by dual-energy X-ray absorptiometry (Hologic QDR 4500; Hologic) in all subjects before and after the VLCD (17).
insulin secretion (DI; mL min\(^{-1}\) m\(^{-2}\) BSA) (25), in that it is the whole-body use of glucose promoted by the \(\beta\) cell at the same experimentally fixed glucose concentration. It measures whole-body capability to dispose an intravenous glucose load, and it reflects \(\beta\) cell adequacy to adjust to prevailing insulin resistance and insulin clearance. This DI has 2 advantages: 1) it is a direct experimental measure, not the product of 2 different experimental assessments, and 2) at variance with all other DIs, it requires no assumptions regarding the mathematical relation linking SI to \(\beta\) cell secretory response or glucose-stimulated insulin concentrations (25). SI during the hyperglycemic clamp was calculated as the ratio of the average insulin secretion rate to a step increase in glucose concentration of 1 mmol/L above baseline, in (pmol \cdot min\(^{-1}\) \cdot m\(^{-2}\) BSA)/(nmol/L).

Finally, an index of insulin clearance (in L \cdot min\(^{-1}\) \cdot m\(^{-2}\) BSA) was calculated as the ratio of the average insulin secretion rate divided by the average insulin concentration during the hyperglycemic clamp. Further details can be found in the online material under “Supplemental data” in the online issue.

**Model-based measurement of \(\beta\) cell function**

The analyses of the glucose and C-peptide curves during the hyperglycemic clamp follow the general strategy proposed by several laboratories (26), with some slight modifications, which were previously described in detail (27) and can be found in the online material under “Supplemental data” in the online issue. The main outputs of this model are as follows:

1) first-phase parameters—total amount of insulin secreted due to first phase (1stISR; in pmol/m\(^2\) BSA) and glucose sensitivity of first-phase secretion (\(\sigma_1\)), expressed as the amount of insulin secreted in response to a rate of increase in glucose concentration of 1 mmol/L between time 0 and 1 min of the study, in (pmol/m\(^2\) BSA)/(nmol/L \cdot min\(^{-1}\))

2) second-phase parameters—total amount of insulin secreted due to second phase (2ndISR; in pmol/m\(^2\) BSA) and glucose sensitivity of second-phase secretion (\(\sigma_2\)), expressed as the steady state insulin secretion rate in response to a step increase in glucose concentration of 1 mmol/L above baseline, in (pmol \cdot min\(^{-1}\) \cdot m\(^{-2}\) BSA)/(nmol/L).

**RESULTS**

After the VLCD, weight loss was 3.22 \(\pm\) 0.56%, namely 3.58 \(\pm\) 0.60 kg (\(P = 0.0000045\)), 42% of which was fat loss. Both BMI and waist circumference decreased significantly (\(P = 0.001\) and \(P = 0.001\), respectively) (Table 1). Plasma triglycerides (\(P = 0.0003\)), but not total cholesterol (\(P = 0.78\)), decreased after the VLCD (Table 1).

Fasting plasma glucose decreased significantly (\(P = 0.044\)), whereas no significant changes in either fasting insulin (\(P = 0.699\)) or fasting C-peptide occurred (\(P = 0.57\)) (Table 1). During the hyperglycemic clamp, both the M value (glucose disposal) and the DI increased significantly after VLCD (\(P = 0.008\) and \(P = 0.005\), respectively) (Figure 1), but SI did not change significantly (\(P = 0.33\)) (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Effects of a VLCD on anthropometric and metabolic variables(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before VLCD</td>
<td>After VLCD</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>114.29 (\pm) 4.98</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>44.8 (\pm) 1.64</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>132.32 (\pm) 3.36</td>
</tr>
<tr>
<td>Fasting glucose (nmol/L)</td>
<td>7.66 (\pm) 0.51</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>75.66 (\pm) 10.81</td>
</tr>
<tr>
<td>Fasting C-peptide (nmol/L)</td>
<td>1.32 (\pm) 0.15</td>
</tr>
<tr>
<td>M value ((pmol \cdot min(^{-1}) \cdot m(^{-2}) BSA))</td>
<td>979 (\pm) 107</td>
</tr>
<tr>
<td>AIR (pmol/L)</td>
<td>(-0.63 \pm 9.4)</td>
</tr>
<tr>
<td>Disposition Index (mL \cdot min(^{-1}) \cdot m(^{-2}) BSA)</td>
<td>59.0 (\pm) 6.3</td>
</tr>
<tr>
<td>SI [(mL \cdot min(^{-1}) \cdot m(^{-2}) BSA)/(pmol/L)]</td>
<td>0.28 (\pm) 0.07</td>
</tr>
<tr>
<td>1stISR (pmol/m(^2) BSA)</td>
<td>232 (\pm) 101</td>
</tr>
<tr>
<td>(\sigma_1) [(pmol/m(^2) BSA)/(nmol/L \cdot min(^{-1}))]</td>
<td>35.1 (\pm) 15.1</td>
</tr>
<tr>
<td>2ndIR (pmol/L)</td>
<td>260 (\pm) 81</td>
</tr>
<tr>
<td>2ndISR (pmol/m(^2) BSA)</td>
<td>23,872 (\pm) 4444</td>
</tr>
<tr>
<td>(\sigma_2) [(pmol \cdot min(^{-1}) \cdot m(^{-2}) BSA)/(nmol/L)]</td>
<td>24.6 (\pm) 4.0</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>6.77 (\pm) 0.14</td>
</tr>
<tr>
<td>Triglycerides (nmol/L)</td>
<td>2.51 (\pm) 0.26</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.78 (\pm) 0.30</td>
</tr>
</tbody>
</table>

\(^1\) All values are means \(\pm\) SEMs. AIR, acute insulin response; BSA, body surface area; \(\sigma_1\), glucose sensitivity of \(\beta\) cell during first-phase insulin secretion; \(\sigma_2\), glucose sensitivity of \(\beta\) cell during second-phase insulin secretion; SI, insulin sensitivity; VLCD, very-low-calorie diet; 1stISR, total amount of insulin released during first-phase secretion; 2ndISR, second-phase insulin response; 2ndISR, second-phase insulin secretion rate, ie, total amount of insulin released during second-phase secretion.

\(^2\) Wilcoxon’s signed-rank test.
As for the parameters of first-phase insulin secretion, AIR increased significantly after the VLCD (P = 0.016) (Figure 2A). Neither model-derived parameter (ie, 1stISR or σ²) showed any significant change (P = 0.18 and P = 0.31, respectively) (Table 1).

As for the parameters of second-phase insulin secretion, 2ndIR showed a nonsignificant trend to increase (P = 0.096) after VLCD. As to the model derived parameters, 2ndISR increased significantly (P = 0.014) (Figure 2B), and σ² showed a nonsignificant (P = 0.068) trend to increase after the VLCD. The insulin clearance index did not change significantly (P = 0.39) after the VLCD (Table 1).

DISCUSSION

The major result of our study, ie, the amelioration of the DI in extremely obese type 2 diabetic patients after 1 wk of acute negative energy balance, could be due, on purely theoretical grounds, to one or more of 3 changes: 1) improved β cell function, 2) increased SI, and 3) decreased insulin clearance. We detected signals of improved β cell function after the VLCD, whereas the changes in SI and insulin clearance were minimal.

Note that VLCD caused detectable reductions in body weight, fat mass, and triglycerides. Nevertheless, SI was unaffected, apparently in contrast with the improvement of hepatic SI reported by Lim et al (9). Differences in study subjects (extremely obese compared with overweight/obese patients), in experimental design (hyperglycemic clamp compared with isoglycemic insulin clamp), and in the role played by glucose toxicity (28) might underlie this apparent discrepancy (9).

In our study, we explored first-phase and second-phase insulin secretory responses with both traditional indexes and model-based assessments, the latter of which measure insulin secretion rates, not concentrations, and β cell sensitivities to glucose, independently of changes in insulin clearance. Although statistical significance was achieved only for AIR and 2ndISR, the general scenario is consistent with improvements in both first- and second-phase insulin secretion brought about by the VLCD in only 7 d. In contrast, no effects were detected in fasting insulin secretion rates, as reflected by insulin and C-peptide concentrations before the hyperglycemic clamp.

In patients with type 2 diabetes, several mechanisms might be involved in the amelioration of β cell function, including, but not limited to, concomitant therapy, lower glucose excursion, and lower mean glucose. However, the careful selection of patients allows exclusion of these important confounding factors (glucose toxicity, glucose variability, and treatment) on the observed modification of metabolic parameters, because, in the current study, only severely obese diabetic patients in good glycemic control, treated with diet alone or plus oral hypoglycemic therapy (suspended at the beginning of the caloric restriction), were studied.

On the whole, our results are consistent with and extend to morbidly obese diabetic patients the recent findings of Lim et al (9), which were obtained in patients with a BMI of ~30, in whom the dramatic improvement in glucose control caused by the VLCD was primarily due to quick improvements in β cell function and liver SI, with no evident role of peripheral SI.

Among the mechanisms underlying remission of type 2 diabetes (12) after bariatric surgery, the potential role played by sudden negative energy balance per se has recently gained renewed attention (29, 30). Very recent data support the hypothesis that caloric restriction is a mediator of early metabolic improvements, after Roux-en-Y gastric bypass (31), via an improvement of hepatic SI, with a consequent reduction in hepatic glucose production, at least in diabetic patients. Our data support the idea that bariatric surgery may improve β cell function in patients with type 2 diabetes, also by inducing a negative caloric balance.

In summary, we have reported evidence that short-term caloric restriction per se improves glucose control and β cell function in morbidly obese patients with type 2 diabetes, ie, a class of potential candidates for bariatric surgery. Further research exploring the actual beneficial role of acute caloric restriction per se after bariatric surgery procedures is therefore warranted.

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The authors’ responsibilities were as follows—SF: designed the research; IM, IG, FDM, FA, AMS, FP, ADF, and SF: conducted the research; PP and
REFERENCES


