

## PAPER

# Restored insulin inhibition on insulin secretion in nondiabetic severely obese patients after weight loss induced by bariatric surgery

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**OBJECTIVE:** To examine the impact of important weight loss on insulin inhibition of its own secretion during experimentally induced hyperinsulinemia under euglycemic conditions.

**DESIGN:** Longitudinal, clinical intervention study—bariatric surgery (vertical banded gastroplasty—gastric bypass—Capella technique), re-evaluation after 4 and 14 months.

**SUBJECTS:** Nine obese patients class III (BMI =  $54.6 \pm 2.6$  kg/m<sup>2</sup>) and nine lean subjects (BMI =  $22.7 \pm 0.7$  kg/m<sup>2</sup>).

**MEASUREMENTS:** Euglycemic hyperinsulinemic clamp (insulin infusion: 40 mU/min m<sup>2</sup>), C-peptide plasma levels, electrical bioimpedance methodology, and oral glucose tolerance test (OGTT).

**RESULTS:** BMI was reduced in the follow-up:  $44.5 \pm 2.2$  and  $33.9 \pm 1.5$  kg/m<sup>2</sup> at 4 and 14 months. Insulin-induced glucose uptake was markedly reduced in obese patients ( $19.5 \pm 1.9$  μmol/min kg FFM) and improved with weight loss, but in the third study, it was still lower than that observed in controls ( $35.9 \pm 4.0$  vs  $52.9 \pm 2.2$  μmol/min kg FFM). Insulin-induced inhibition of its own secretion was blunted in obese patients ( $19.9 \pm 5.7\%$ , relative to fasting values), and completely reversed to values similar to that of lean ones in the second and third studies ( $-60.8 \pm 4.2$  and  $-54.0 \pm 6.1\%$ , respectively).

**CONCLUSION:** Weight loss in severe obesity improved insulin-induced glucose uptake, and completely normalized the insulin inhibition on its own secretion.

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**Keywords:** insulin resistance; insulin secretion; hyperinsulinemia; C-peptide; weight loss; bariatric surgery

## Introduction

Hyperinsulinemia is an early important metabolic change in obese patients,<sup>1–3</sup> and has been reported as an independent cardiovascular risk factor.<sup>4</sup> The pancreas is able to increase its secretion precisely to compensate for the defect in insulin action to maintain normal glucose tolerance,<sup>1,5</sup> and hyperinsulinemia is usually considered to result from increased insulin secretion produced as a compensatory response of the B cell. However, the European Group for the Study of Insulin Resistance (EGIR) reported a more prevalent fasting

posthepatic insulin delivery rate than insulin resistance in the obese subjects evaluated.<sup>6</sup> Insulin levels are a result of the rate of insulin secretion and of its metabolic clearance rate. In obese individuals, the posthepatic metabolic clearance rate is thought to be normal<sup>7</sup> and insulin secretion is enhanced.<sup>3,8</sup>

Insulin secretion is, in turn, determined by complex stimulatory and inhibitory influences on the β cell. Among the inhibitory influences is a direct or indirect negative feedback of circulating insulin on β-cell secretion, whose role is still controversial. In human obesity, some studies have reported a failure of insulin feedback and others have observed an inhibition similar to that in lean subjects.<sup>9–12</sup>

The obesity degree and/or the magnitude of insulin resistance may be important factors to justify these differences. In a previous study, we observed a failure of insulin

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feedback on insulin secretion in extreme obesity (BMI > 40 kg/m<sup>2</sup>), while in lean subjects the insulin inhibition was directly related to insulin sensitivity.<sup>7</sup> Since severe obesity (obese class III<sup>13</sup>) usually displays serious metabolic alterations<sup>13,14</sup> that can be changed by weight loss,<sup>14–17</sup> and the effect of weight loss on the insulin inhibition on insulin secretion was not yet investigated, the present study was designed to examine the impact of experimentally induced hyperinsulinemia under euglycemic conditions on its own secretion in these patients, before and after surgically (vertical banded gastroplasty—gastric bypass—Capella technique) induced weight loss.<sup>18,19</sup> C-peptide, which is the connecting peptide of proinsulin, is secreted from  $\beta$  cells in equimolar amounts with insulin, and its concentrations have been used to determine insulin production *in vivo*. In this study, insulin secretion was evaluated by C-peptide plasma concentrations.

## Material and methods

### Study population

Nine obese patients (seven women and two men), aged  $39 \pm 4$  y, were studied three times: Study I (SI)—before bariatric surgery; Study II (SII)—after weight loss of 15–20% (~4 months after surgery); Study III (SIII)—after weight stabilization (38% weight loss relative to the initial body weight; ~14 months after surgery). Nine lean subjects (seven women and two male) aged  $34.4 \pm 4$  y were used as a control group (C). The anthropometric characteristics are given in Table 1. All volunteers had normal resting arterial blood pressure levels, as defined by JNC V<sup>20</sup> (systolic < 140 mmHg and diastolic < 90 mmHg), and normal fasting glucose according to the American Diabetes Association criteria.<sup>21</sup> None of them were on any medication, which could influence insulin secretion and sensitivity. All subjects had normal liver and renal function tests and no cardiovascular or respiratory disease that could contraindicate the surgery. Class III obesity was defined as body mass index (BMI)  $\geq 40$  kg/m<sup>2</sup>.<sup>13</sup> After the initial investigation, the obese patients were submitted to a vertical banded gastroplasty—gastric bypass—Capella technique.<sup>19</sup> The Institutional Review Board of the School of Medicine (Campinas State University) approved the investigation,

and all subjects gave informed consent before the protocol began.

### Experimental protocol

Body composition was evaluated by electrical bioimpedance<sup>22</sup> using a Biodynamic monitor. Each subject underwent an OGTT and a euglycemic hyperinsulinemic clamp on different days, approximately 1 week apart. Arterial blood pressure was measured by a mercury sphygmomanometer (a large cuff was used). For the OGTT, 40 g/m<sup>2</sup> of glucose was ingested over 5 min, and venous blood was sampled at 30 min intervals over 2 h for plasma glucose and insulin measurements. The American Diabetes Association criteria<sup>21</sup> were used to define glucose tolerance during OGTT. The clamp study, which was done after an overnight fast (12–14 h), consisted of 2 h of euglycemic insulin infusion at a rate of 40 mU/min m<sup>2</sup> of body surface area.<sup>23</sup> A polyethylene, 20-gauge catheter was inserted into an antecubital vein for the infusion of insulin and glucose. Another catheter was inserted retrogradely into a wrist vein, and the hand placed in a heated box (~60°C) to allow sampling of arterialized blood.<sup>24</sup> The 2-h period before the insulin infusion was started constituted the basal period. During insulin infusion glucose was measured at 5 min intervals and plasma glucose was maintained nearly constant with a variable glucose infusion. Arterialized venous samples for C-peptide and insulin measurements were obtained at 20 min intervals, from 20-min before until 2 h after starting the insulin infusion.

After an initial set of clinical and metabolic investigations, patients were submitted to a vertical banded gastroplasty—gastric bypass—Capella technique.<sup>19</sup> The nutritional guidelines after surgery consisted of a liquid hypocaloric diet (around 60% carbohydrate, 20% lipid and 20% protein),<sup>18</sup> with gradual reintroduction of solid foods and caloric increase, according to patient acceptance and postoperative period. Vitamin supplementation was started 1 month later.

The second and third metabolic studies carried out only in obese patients were similar to the first, except for OGTT, which was impossible because of gastric restriction and impaired digestive absorption.

**Table 1** Anthropometric characteristics in the studies

	Control	Study I	Study II	Study III	P1
Body weight (kg)	60 $\pm$ 3	145 $\pm$ 9***	118 $\pm$ 7***	90 $\pm$ 5***	0.0003
BMI (kg/m <sup>2</sup> )	22.7 $\pm$ 0.7	54.6 $\pm$ 2.6***	44.5 $\pm$ 2.2***	33.9 $\pm$ 1.5***	0.0003
Waist (cm)	75 $\pm$ 3	131 $\pm$ 6***	111 $\pm$ 7**	111 $\pm$ 6**	0.007
Free fat mass (kg)	46.8 $\pm$ 3.3	78.7 $\pm$ 3.7***	68.4 $\pm$ 3.5***	61.1 $\pm$ 2.3**	0.0008
Fat mass (%)	22.1 $\pm$ 2.5	45.4 $\pm$ 1.5***	41.8 $\pm$ 1.5***	31.8 $\pm$ 1.5*	0.0003

BMI body mass index; P1, P-value—Friedman analyses for the three studies in obese patients.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ —Studies I, II or III vs control group—Mann-Whitney analyses.

### Analytical procedures

Plasma glucose was measured by the glucose oxidase technique in a Beckman glucose analyzer during the clamp study (Beckman, Fullerton, CA, USA). Plasma concentrations of insulin and C-peptide were measured by radioimmunoassay using a specific kit for human insulin (less than 0.2% cross reactivity with proinsulin) and for C-peptide (Linco Research Inc., St Louis, MO, USA). Plasma uric acid, total cholesterol, HDL cholesterol and triglycerides were assayed spectrophotometrically on an automated colorimetric system (Cobas Miras, Roche).

### Data analysis

Whole-body glucose utilization (or M value) was calculated from the infusion rate of exogenous glucose (GIR) during the second hour of the insulin clamp period, after correction for changes in glucose levels in a distribution volume of 250 ml/kg. The M value was normalized per kilogram of fat-free mass ( $\mu\text{mol}/\text{min kg}$  Free fat mass (FFM)) and divided by the prevailing steady-state insulin plasma levels (log transformed). Areas under OGTT time-concentration curves were calculated by the trapezoidal rule.

### Statistical analysis

All data are given as the mean  $\pm$  s.e.m. Comparison of the means in the three studies from obese patients was done using the nonparametric Friedman analysis, and ANOVA for repeated measures was carried out to compare variables curves of obese and control groups. Comparison of means between obese and control group was done using the nonparametric Mann-Whitney *U*-test. Simple linear and stepwise regression analyses were calculated, using the *StatView* computerized program. A *P*-value  $\leq 0.05$  indicated significance.

## Results

### Anthropometric characteristics

The patients were extremely obese, with a BMI of  $54.6 \pm 2.6 \text{ kg/m}^2$ , with very high fat mass (about 45% of

body weight). The weight loss induced by the surgery was very important, reaching  $-18.4 \pm 0.5\%$  in SII and  $-37.6 \pm 0.5\%$  in SIII. So, the anthropometric measures were quite improved together with a reduction in the body fat percentage, but were still significantly higher than control values (Table 1).

### Metabolic characteristics

Seven of the obese patients had a normal glucose tolerance<sup>21</sup> and two had impaired glucose tolerance and normal fasting plasma glucose. The area under the glucose curve during the OGTT was similar to control ( $920 \pm 46$  vs  $814 \pm 37 \text{ mmol/l min}$ ). Fasting plasma insulin and the area under the insulin curve were significantly higher in the patients. The weight loss induced significant decreases in the fasting plasma insulin and glucose levels (Table 2). Decreases in serum uric acid ( $494 \pm 42$  vs  $327 \pm 30 \mu\text{mol/l}$ , *P* = 0.009) and in plasma triglycerides ( $1.67 \pm 0.09$  vs  $1.10 \pm 0.07 \text{ mmol/l}$ , *P* = 0.002) were observed, while there was no significant change in the fasting total cholesterol (from  $4.60 \pm 0.25$  to  $4.26 \pm 0.15 \text{ mmol/l}$ ) and HDL cholesterol (from  $0.81 \pm 0.05$  to  $1.00 \pm 0.08 \text{ mmol/l}$ ).

### Clamp data

The plasma glucose levels during insulin infusion varied  $-2.9 \pm 0.4$ ;  $3.6 \pm 1.0$ ;  $0.5 \pm 1.2$  and  $-0.7 \pm 1.3\%$  from the basal values in the control and in the studies I, II and III, respectively. Thus, plasma glucose did not change significantly during the clamp period compared to the fasting state in each group study. Although the insulin infusion was the same in the studies, the steady-state plasma insulin levels were similar in the control and the obese and decreased after weight reduction (*P* = 0.01). Insulin sensitivity (M value or M/LBM) was significantly lower in the obese group and improved from  $19.5 \pm 1.9$  to  $35.9 \pm 4.0 \mu\text{mol}/\text{min kg FFM}$ , *P* = 0.0003 14 months after the surgery (Table 2).

Fasting and steady-state plasma C-peptide levels were higher in the obese before surgery than in the control and decreased after weight reduction (Table 3 and Figure 1). The percentage decrease throughout all the insulin infusion period was significantly lower in study I compared to the

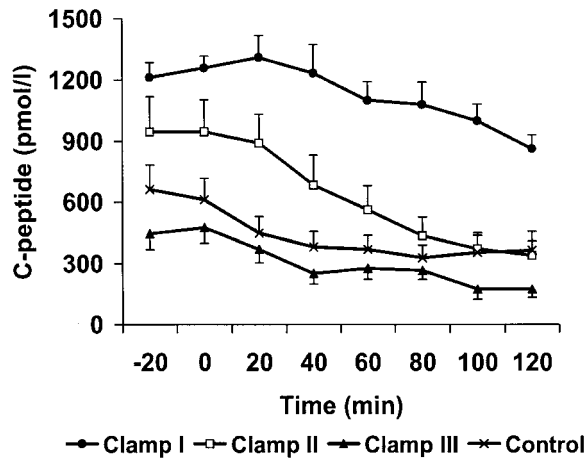
**Table 2** Clamp data

	Control	Study I	Study II	Study III	P1
Fasting plasma glucose (mmol/l)	$5.2 \pm 0.1$	$4.9 \pm 0.1$	$4.6 \pm 0.1^{**}$	$4.6 \pm 0.1^{**}$	0.02
Steady-state plasma glucose (mmol/l)	$5.0 \pm 0.1$	$5.1 \pm 0.1$	$4.7 \pm 0.1^*$	$4.6 \pm 0.1^*$	0.002
Fasting plasma insulin (pmol/l)	$86 \pm 5$	$231 \pm 18^{***}$	$109 \pm 9^*$	$58 \pm 5^{**}$	0.0009
Steady-state plasma insulin (pmol/l)	$852 \pm 71$	$889 \pm 73$	$841 \pm 57$	$657 \pm 35^*$	0.01
M/FFM ( $\mu\text{mol}/\text{min kg FFM}$ )	$52.9 \pm 2.2$	$19.5 \pm 1.9^{***}$	$24.9 \pm 2.2^{***}$	$35.9 \pm 4.0^{**}$	0.0003
M/FFM/l ( $\mu\text{mol}/\text{min kg FFM}/\text{ins ln}$ )	$7.7 \pm 0.4$	$2.8 \pm 0.3^{***}$	$3.4 \pm 0.2^{***}$	$5.4 \pm 0.5^{***}$	0.0001

Steady-state period, from 60 to 120 min of insulin infusion; M/FFM ( $\mu\text{mol}/\text{min kg}/\text{FFM}/\text{pmol/l}$ ), M value from the clamp normalized by kilogram of fat-free mass; M/FFM/l, M value from the clamp normalized by kilogram of fat-free mass divided by the natural logarithm of steady-state plasma insulin (pmol/l).

P1, *P*-value—Friedman analyses for the three studies in obese patients;

\**P*  $\leq 0.05$ ; \*\**P*  $\leq 0.01$ ; \*\*\**P*  $\leq 0.001$ —Studies I, II or III vs control group—Mann-Whitney analyses.



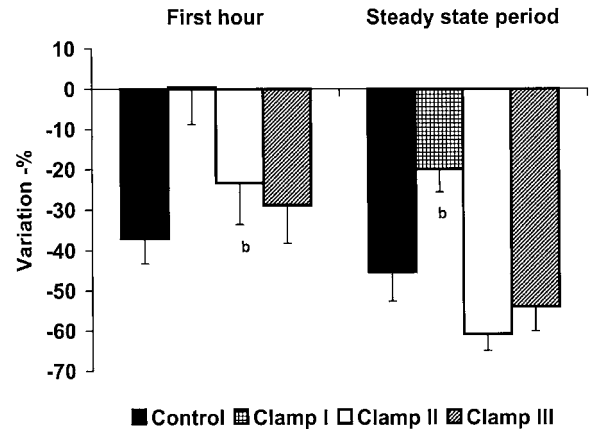
**Figure 1** Plasma C-peptide levels during the euglycemic insulin clamp.  $P \leq 0.0001$ ; ANOVA for repeated measures.

control group and to studies II and III (Figure 2). The fasting C-peptide-to-insulin molar ratio was similar in all studies (Table 3).

In the pooled data from the three studies of the obese group, fasting plasma C-peptide levels were related to BMI ( $r = 0.59$ ,  $P = 0.001$ ), % fat mass ( $r = 0.58$ ,  $P = 0.002$ ), M/FFM ( $r = -0.73$ ,  $P < 0.0001$ ) and waist ( $r = 0.53$ ,  $P = 0.01$ ), but not to waist/hip ratio and to fasting plasma glucose ( $P = \text{NS}$ ). In a stepwise model (including BMI, age, M/FFM, % fat mass, sex and waist), M/FFM and percentage of fat mass remained significantly related to fasting plasma C-peptide (multiple  $r = 0.82$ ,  $P < 0.0001$ ;  $r^2 = 0.63$ ).

## Discussion

The obese subjects of this study had class III obesity<sup>13</sup> with very high BMI, fat mass and fasting plasma insulin levels and were markedly insulin resistant. The bariatric surgery induced marked weight reduction, but BMI after weight stabilization was around  $34 \text{ kg/m}^2$ . The weight reduction was accompanied by improvement in body composition, fat/free



**Figure 2** Percentage variation of plasma C-peptide levels during insulin infusion (first hour and steady-state periods) relative to fasting plasma levels. (b)  $P \leq 0.01$  vs control group;  $P = 0.0008$ —Friedman analyses for the percent variations of steady-state period values in the three studies of obese patients.

fat mass, because of a proportionally higher fat than FFM loss. The important metabolic abnormalities, such as severe peripheral insulin resistance, increased insulin secretion, high serum triglycerides and uric acid levels, all improved after weight loss.

Insulin secretion during the fasting state and under insulin infusion during the euglycemic clamp was assessed using the C-peptide plasma measurements. C-peptide was chosen to evaluate insulin secretion because of their equivalent molar ratio secretion, negligible hepatic extraction<sup>25</sup> and similar clearance in lean and obese subjects.<sup>8</sup> Regardless of the method used for calculation, insulin secretion was much higher in the obese in the first study, and the decrease after weight loss was important and statistically significant even in the second study, with further reduction in the third study. Fasting C-peptide levels were mainly related to insulin sensitivity and percentage of fat mass, as demonstrated by the stepwise model including BMI and M value. These results again demonstrate the well-known role of insulin resistance and obesity to determine fasting insulin secretion.

**Table 3** C-peptide results during the clamp studies

	Control	Study I	Study II	Study III	P1
Fasting plasma C-peptide (pmol/l)	$634 \pm 106$	$1235 \pm 60^{**}$	$946 \pm 164^*$	$471 \pm 85$	0.008
Plasma C-peptide 0–60 min (pmol/l)	$401 \pm 71$	$1227 \pm 105^{***}$	$713 \pm 129^*$	$313 \pm 52$	0.0008
Steady-state plasma C-peptide (pmol/l)	$350 \pm 77$	$980 \pm 67^{***}$	$384 \pm 79$	$207 \pm 43$	0.001
Clamp variation 0–60 min (%) <sup>a</sup>	$-37.0 \pm 6.3$	$0.4 \pm 9.2^{**}$	$-23.3 \pm 10.3$	$-28.8 \pm 9.4$	ns
Clamp variation 60–120 min (%) <sup>a</sup>	$-45.3 \pm 7.4$	$-19.9 \pm 5.7^{**}$	$-60.8 \pm 4.2$	$-54.0 \pm 6.1$	0.0008
Fasting C-peptide/insulin ratio	$7.5 \pm 1.2$	$5.5 \pm 0.4$	$8.0 \pm 1.1$	$8.6 \pm 1.6$	ns

Plasma C-peptide 0–60 min—mean C-peptide values from plasma samples collected at 20, 40 and 60 min of insulin infusion; Steady-state plasma C-peptide—mean plasma C-peptide values from plasma samples collected at 80, 100 and 120 min of insulin infusion;

<sup>a</sup>Percent variation of clamp to fasting values.

P1, P-value—Friedman analyses for the three studies in obese patients.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ —Studies I, II or III vs control group—Mann-Whitney analyses.

Some of the recently described factors secreted by the adipocytes have actions on insulin secretion and/or insulin sensitivity, such as leptin, adiponectin, TNF $\alpha$ . Some authors suggest that leptin can inhibit insulin secretion<sup>27</sup> and its plasma levels markedly decrease with weight reduction. It is not possible to know if plasma leptin had some influence on the insulin secretion in the present study. In our surgery department, other obese patients submitted to the same bariatric surgery had reduced leptin and insulin plasma levels, but only BMI was related to the latter in a multivariate regression analysis.<sup>28</sup> In addition, some studies have reported that the interaction between leptin and insulin depends on factors such as leptin concentration, exposure time and glucose concentration. Thus, the functional role played by leptin on insulin secretion is still under discussion.

A suppressive effect of insulin on its own secretion has been seen in some studies<sup>26,29</sup> but not in others.<sup>30,31</sup> In a previous study, insulin infusion under euglycemic conditions at steady state inhibited its own secretion in lean subjects but this insulin action was blunted in the obese group.<sup>7</sup> In this study we observed that in severely obese patients insulin-induced inhibition of insulin secretion was blunted before surgery (SI) compared to the control group, suggesting insulin resistance to this effect. After weight loss, the decrease in C-peptide during insulin infusion improved. The percentage variation of C-peptide levels during steady state or in the first hour of the clamp, relative to fasting plasma levels, 14 months after the surgery were similar to those observed in lean subjects. It is interesting to note that 4 months after surgery or only after 15–20% reduction in BMI, although the basal C-peptide levels were still higher than controls, the percentage variation of C-peptide levels during steady-state insulin infusion relative to the fasting plasma levels was similar to control levels, showing an early recovery.

The results of the present study allowed us to compare two different effects of insulin in severely obese patients and after weight loss. Insulin-induced glucose uptake, which was reduced in these patients, improved after weight loss, but was still lower than that observed in controls. The lower steady-state insulin plasma levels, in Study III, can be explained by the decreased endogenous insulin secretion after weight loss as suggested by the C-peptide evaluation. Thus, the improvement of the insulin sensitivity could be underestimated but the correction of M value by the prevailing insulin plasma levels demonstrated a still lower sensitivity in the obese after weight reduction. On the other hand, insulin-induced inhibition of its own secretion was completely reversed to values very similar to those observed in lean controls after weight loss. These results demonstrate for the first time that weight loss restores the insulin action on its own secretion and suggest that it has an earlier recovery after weight loss than glucose utilization, or that the first effect is only disturbed in situations of severe insulin resistance.

In obesity, the mechanism of insulin resistance, insulin hypersecretion and impaired insulin inhibition on its own secretion are not completely understood. The inhibition of glucose-stimulated insulin secretion by somatostatin is maintained,<sup>32</sup> which suggests a specific defect in insulin feedback, even if there is evidence that functioning  $\beta$  cell mass is enhanced.<sup>33</sup> The effect of insulin on its own secretion may be indirect (neurally mediated) and/or direct, through an action of insulin on  $\beta$  cells via the insulin-signaling pathway. Previous data suggest that the inhibition of pancreatic insulin secretion by hyperinsulinemia is neurally mediated.<sup>34</sup> In lean individuals, insulin activates the sympathetic system<sup>35</sup> and causes a sympathetic shift in the autonomic balance, whereas in the obese sympathetic activation during the clamp is blunted,<sup>36</sup> despite its enhanced basal activity.<sup>37</sup> Accordingly, after BMI reduction in the obese there is also evidence of improvement in the sympathetic activation by insulin.<sup>38</sup>

Some components of the insulin-signaling pathway are present in normal islet  $\beta$  cells.<sup>39,40</sup> And, in knockout mice lacking the insulin receptor in pancreatic  $\beta$  cells there is an increase in basal insulin concentrations,<sup>41</sup> suggesting that insulin resistance in  $\beta$  cells can contribute to fasting hyperinsulinemia. The pattern of regulation of the insulin signaling proteins in  $\beta$  cells of obese patients is unknown, but in other tissues there is a widespread decrease in insulin receptor and insulin receptor substrates, and in their activation. Although these proteins have a tissue-specific regulation, it is reasonable to speculate that a downregulation of the insulin receptor and its substrates in the pancreas of severely obese patients may contribute to insulin resistance in  $\beta$  cells of these patients, and that weight loss may control these alterations.

We concluded that insulin inhibition on its own secretion is blunted in insulin-resistant severely obese patients, partially related to the degree of obesity, and weight loss completely normalizes the insulin effect on its own secretion.

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