Alteration of the Counterregulatory Responses to Insulin-Induced Hypoglycemia and of Cognitive Function After Massive Weight Reduction in Severely Obese Subjects

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The autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis are reported as activated in excess in the morbidly obese state and, therefore, changes after weight loss can be anticipated. The aim of this study was to investigate the impact of a massive (~30%) weight reduction on the activation of the HPA axis and the ANS following bariatric surgery. Eight (7 women, 1 man) severely obese (125 ± 12 kg; body mass index [BMI], 45 ± 4 kg/m^2) nondiabetic subjects, underwent a 3-hour hyperinsulinemic (1,034 pmol/kg/h) glucose clamp study at hypoglycemia of arterial B-glucose concentration of 3.4 mmol/L. Cognitive function was evaluated by a visuospatial computerized problem-solving test, the Perceptual Maze Test (PMT). The mean weight loss was 40 ± 9 kg approximately 12 months postsurgery when their weight was stabilized (85 ± 6 kg; BMI, 31 ± 3 kg/m^2), and insulin sensitivity improved to an average increase of 376% ± 250% ($P < .01$) of initial value. Before weight reduction, all patients demonstrated brisk peak responses in glucagon, epinephrine, pancreatic polypeptide (PP), norepinephrine, and cortisol, indicative of preserved or exaggerated activation of ANS and HPA axis. In the reduced-obese state, all these responses were attenuated and most markedly so for glucagon, which was totally abolished. In contrast, the growth hormone (GH) response was increased after weight reduction. The cognitive function was clearly modified by weight reduction both during normoglycemia and hypoglycemia and was changed preferentially to a speed-preferring strategy in the reduced-obese state compared with a more accuracy preferred problem-solving process of PMT test presurgery. These results demonstrate a reduction of the glucose counterregulatory hormonal responses, increased insulin sensitivity, and perturbed cognitive function after massive weight reduction. It may be speculated on if the increased insulin sensitivity and reduced counterregulation to hypoglycemia could predispose to low plasma glucose concentrations.

PREVIOUS STUDIES indicate that parts of the autonomic nervous system (ANS) are activated in the obese state, thus the basal tonus in sympathetic and parasympathetic systems are changed, and the hypothalamic-pituitary-adrenal (HPA) axis is exaggerated compared with the non-obese situation.[1-5] It has been proposed that these changes are of importance for the metabolic adaptation to obesity mainly by contributing to insulin resistance and hyperinsulinemia.[6] Thus, increased parasympathetic drive to the pancreas, as demonstrated in a study by Weyer et al,[7] may have important pathophysiologic implications with regard to understanding the hypersecretion of insulin in insulin resistance.

In contrast, the role of glucagon in this context has given conflicting results. Thus, some but not all, studies show elevated glucagon levels in obesity,[8,9] and one study showed that altered glucagon secretion after weight reduction regulates energy expenditure both in the fasting state and after a meal and thereby contributing to the outcome of treatment for obesity.[10] Furthermore, other studies have observed that postprandial reactive hypoglycemia is prevalent after weight reduction,[11-13] but in these studies, the counterregulatory machinery to hypoglycemia, including the glucagon response, was not studied. However, to what extent the changes in the HPA axis and ANS are responsible for the perturbed glucagon secretion after weight reduction is not known.

The aim of the present study was, therefore, to investigate the impact of a massive weight reduction on the activation of the HPA axis and the ANS following bariatric surgery in a group of severely obese nondiabetic patients. To activate the HPA axis[14] and the ANS,[15] we applied a hypoglycemic clamp technique[16] measuring the responses of glucagon, epinephrine, pancreatic polypeptide (PP), cortisol, norepinephrine, and growth hormone (GH) before and after weight reduction. It is known that to achieve a correct threshold for cortisol activation, a > 60-minute steady state cortisol level is required during a single-level hypoglycemia,[14] which is consistent with the present study in which we used a single hypoglycemic level of 3 hours. In addition, we studied the responses of blood pressure and pulse rate, and cognitive functions during neuropsychologic stress tasks, according to previously standardized procedures.[17,18]

SUBJECTS AND METHODS

Subjects

The study was conducted in collaboration between the Division of Internal Medicine and the Division of Surgery at Danderyd Hospital and Department of Medicine, Lund University. Eight subjects from the waiting list for vertical banded gastroplasty (VBG) were recruited by one of the authors (Backman L.). The study group consisted of 8 (7 women, 1 man) severely obese nondiabetic patients (mean age, 40 years; range, 26 to 55) with body mass index (BMI) >40 kg/m^2. All participants were of Caucasian origin. All except 1 of the subjects had a family history of diabetes, and all subjects were selected to have a

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Submitted September 24, 2002; accepted February 14, 2003.

Supported in part by Grants No. 6589 and 6834 from the Swedish Research Council, Bert von Kantzow Foundation, Ernhold Lundström, Albert Påhlsson Foundation, Swedish Diabetes Association, Swedish Society for Medical Research, Lund University Hospital Research Funds, and the Faculty of Medicine, Lund University.

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0026-0495/03/5207-0015$30.00/0

doi:10.1016/S0026-9495(03)00103-3

fasting venous blood glucose level below 6.1 mmol/L. None of the subjects were taking any drugs known to affect blood pressure or carbohydrate metabolism, and none were using tranquilizers or antidepressive drugs. No evidence of impaired kidney or liver function was present in any individual. To induce a marked weight reduction in morbid obesity, within a rather short period of time, we used VBG, introduced by Mason[19] in 1982. In VBG, a small upper gastric pouch is created along the lesser curvature of the stomach that is separated from the rest of the stomach by a vertical staple line and a small outlet. All participating subjects had received written and oral information before giving their consent. The local ethics committee of the Karolinska Hospital, Stockholm, Sweden, approved the study.

Procedures

The hypoglycemic clamp studies were performed on an ambulatory basis a week before surgery and after a stable body weight was achieved approximately 12 months after the surgical intervention. Dietary changes and changes in body composition following VBG in relation to such a weight reduction as in our experiments have previously been published elsewhere.[20,21]

All subjects reported to the hospital at 7:30 AM after an overnight fast. All measurements were performed with the subjects in light clothing without shoes. Body weight was measured to the nearest 0.1 kg in the morning before breakfast. Height was measured to the nearest centimeter. BMI was then calculated as the weight (kg divided by height squared (m^2). The waist circumference was measured at the level of the umbilicus with the subject standing. Intravenous (IV) catheters were inserted into an antecubital vein on each side; 1 on the right forearm was used for infusions, and the other for blood sampling for the analyses of blood glucose and hormones. A third catheter was inserted into the radial artery of the left forearm under local anesthesia and connected to an automatic glucose analyzer (Gambro Instrumenta AB, Lund, Sweden) for the monitoring of arterial blood glucose every 1.5 minutes, whereas venous blood glucose was determined every 15 minutes by a glucose analyzer (Yellow Spring Instruments [YSI], Yellow Springs, OH) and were placed in a comfortable semirecumbent position for a 30-minute period before the experiment. Blood samples were drawn for the determination of glucose, insulin, C-peptide, glucagon, PP, cortisol, epinephrine, norepinephrine, and GH in the fasting basal state.

The hypoglycemic clamp was initiated with a high-dose IV infusion of short-acting insulin at the fixed rate of 1,034 pmol/kg/h, for 3 hours with a volumetric infusion pump. A total of 10 mL of a human albumin solution (20 mg/mL; Albumin, Kabi Vitrum, Stockholm, Sweden) was added to 490 mL of the insulin infusion solution in all experiments. The arterial blood glucose was clamped by a variable IV infusion of glucose (20%) adjusted at 1.5-minute intervals to achieve a blood glucose between 3.3 to 3.4 mmol/L. Venous blood samples for the analyses of insulin, C-peptide, glucagon, PP, cortisol, epinephrine, norepinephrine, and GH were obtained every 15 to 60 minutes. Pulse rate (beats per minute) and blood pressure, the latter checked with a wide cuff in the right upper arm, were manually recorded at start, after 30 minutes rest, and then at 15-minute intervals during the clamp.

Cognitive function tests. Cognitive functions were assessed by the perceptual maze test (PMT), which examines visuospatial skill, general intelligence, visually guided motor planning, and the ability to obey rules. This standardized test covers computerized neuropsychological tasks, and more detailed information of the test is reported elsewhere.[17,18] All parameters were assessed before and after weight reduction, once in the basal state (fasting conditions, euglycemia, without insulin infusion) and once during hypoglycemia at 90 minutes. The patients were informed that the time required to complete the tasks was being registered. After an introductory maze session, the subjects were given total of 16 mazes to solve 8 with target information and 8 with no target information. The following parameters, based on skill and strategy aspects,[22] were calculated for each maze: processing rate (nodes per second), inspection rate (time in seconds from presentation of maze until first key press, divided by number of nodes of maze), check time (time after completing the pathway until pressing the confirm button), left/right hand preference (arbitrary units for preference for left-*v* right-sided solutions), motor time (typical time for the most rapid key-pressing in milliseconds [ms]), largest (rows) correctly solved maze pattern during the test procedure, percentage of correctly solved mazes, and number of rub outs.

Analyses

The same calibration solution was used to calibrate both the YSI glucose analyzer and the Gambro glucose analyzer several times throughout the tests to make sure that they were operating accurately. Blood samples for the measurements of hormones were collected in plastic tubes containing 0.084 mL EDTA (0.34 mol/L). After gently mixing, the tubes were immediately placed on ice and afterwards centrifuged at +4°C. The samples were then stored at −70°C, pending concurrent analyses of specimens from both clamp occasions. All

Table 1. Characteristics of the 8 Obese Subjects Before Surgery and After 32% Weight Reduction

Characteristic	Before WR	After WR	*P* (before *v* after)
Body weight (kg)	124.9 ± 12.2	85.1 ± 6.0	<.01
BMI (kg/m^2)	45.0 ± 4.5	30.7 ± 2.7	<.01
Waist circumference (cm)	116 ± 4	102 ± 10	<.05
Arterial blood glucose (mmol/L)	5.8 ± 0.7	5.0 ± 0.5	<.01
Venous blood glucose (mmol/L)	5.3 ± 0.8	4.7 ± 0.4	NS
Plasma C-peptide (nmol/L)	1.5 ± 0.2	0.9 ± 0.2	<.05
Plasma insulin (pmol/L)	172 ± 48	74 ± 32	<.01
Plasma glucagon (ng/L)	67.0 ± 9.4	52.9 ± 10.7	<.01
Plasma epinephrine (nmol/L)	0.07 ± 0.03	0.07 ± 0.01	NS
Plasma norepinephrine (nmol/L)	1.8 ± 1.0	1.2 ± 0.4	NS($P = 0.20$)
Plasma PP (pmol/L)	120 ± 69	80 ± 29	NS($P = 0.22$)
Serum cortisol (nmol/L)	321 ± 88	254 ± 70	NS($P = 0.20$)
Serum GH (μg/L)	0.5(0.2, 1.4)	0.6(0.2, 1.2)	NS
Systolic blood pressure (mm Hg)	121 ± 14	114 ± 12	NS
Diastolic blood pressure (mm Hg)	83±10	72±9	<.01
Pulse rate (beats/min)	73±6	66±6	<.05

NOTE. Means ± SD or medians (interquartile range) are shown. The specimens are fasting values (normoglycemia).
Abbreviations: WR, weight reduction; NS, not significant ($P > .05$).

Table 2. Metabolic, Blood Pressure, and Pulse Rate Results From the Clamp Studies (hypoglycemia) Before and After Weight Reduction

Variable	Before WR	After WR	*P* (before *v* after)
Glucose infusion rate (μmol · kg^{-1} · min^{-1})	10.6 ± 2.8	23.9 ± 5.7	< .01
Insulin steady state concentration (90–180 min, pmol/L)	5,717 ± 1,495	3,150 ± 863	< .01
MCR of insulin (mL · kg^{-1} · min^{-1})	3.2 ± 0.6	5.8 ± 1.5	< .01
Insulin sensitivity (nmol glucose · kg^{-1} · min^{-1}/pmol insulin · L^{-1})	2.0 ± 0.8	8.3 ± 3.6	< .01
Systolic blood pressure (mm Hg)	121 ± 10	112 ± 9	< .05
Diastolic blood pressure (mm Hg)	75 ± 8	66 ± 6	< .05
Pulse pressure (mm Hg)	46 ± 6	46 ± 9	NS
Pulse rate (beats/min)	74 ± 7	71 ± 7	NS

NOTE. Means ± SD are shown.
Abbreviation: MCR, metabolic clearance rate.

samples from each patient were analyzed in the same determination. Plasma insulin and glucagon concentrations were analyzed with double-antibody radioimmunoassay (RIA) techniques (Linco Research, St Charles, MO), using guinea pig antihuman insulin antibodies, human insulin standard, ^{125}I-labeled human insulin, guinea pig antiglucagon antibodies specific for pancreatic glucagon, ^{125}I-labeled glucagon, and glucagon standard. Plasma C-peptide concentrations were analyzed with double-antibody RIA technique (Euro-Diagnostica, Malmö, Sweden) using a rabbit antiserum to synthetic, human C-peptide. PP was determined with double-antibody RIA using rabbit antihuman PP antibodies, ^{125}I-labeled human PP, and human PP standard with reagents from Linco Research. Plasma epinephrine and norepinephrine were analyzed by high-performance liquid chromatography (HPLC) with electrochemical detection.[23] RIAs were used for the serum determinations of cortisol (Cortisol [125I] RIA Kit; Orion Diagnostica, Espoo, Finland). GH was analyzed using a fluoroimmunoassay kit, (Wallac Oy, Turku, Finland).[24]

Calculations

For calculations of insulin sensitivity, a steady state condition was assumed during the last 90 minutes of the clamp. Calculations were performed according to DeFronzo et al.[25] Thus, insulin sensitivity (nmol glucose · kg^{-1} · min^{-1}/pmol insulin · L^{-1}) was expressed as the glucose infusion rate during the last 90 minutes divided by the measured mean insulin concentration of the 90- and 180-minute samples. The metabolic clearance rate (MCR) of insulin, defined as the volume of plasma completely and irreversibly cleared of the hormone in unit time (mL · kg^{-1} · min^{-1}), was calculated for each patient by introducing the infusion rate and steady state insulin level in the following formula: MCR (mL · kg^{-1} · min^{-1}) = infusion rate (pmol · kg^{-1} · min^{-1}/steady state plasma insulin concentration (pmol · mL^{-1}). Baseline values of hormones were calculated as the average of the double measurements at the start of the experiments. The area under the curve (AUC) of plasma hormones was calculated by the trapezoidal rule.

Statistical Analyses

Statistical analyses were performed with the JMP package version 4.0 (SAS Institute, Cary, NC). Paired means comparison was performed by the Wilcoxon signed- rank test. Spearman's product moment correlation coefficients (r_s) were obtained to estimate correlation between variables. The results from the cognitive tests were analyzed by Student's paired *t* test. Two-sided tests were used, and a *P*-value less than .05 was considered statistically significant. Data in the text are expressed as means ± SD unless otherwise stated. Data in figures are presented as means ± SEM. GH level was expressed as median (interquartile range) for skewed data.

RESULTS

Body Weights

Eight severely obese patients who underwent VBG lost 39.8 ± 9.1 kg by the end of 12.8 ± 5.6 months, which corresponds to an average decrease of 32% ± 5% of initial body weight (Table 1).

Insulin

During the experiments, the steady level of plasma free insulin was 1.8 times higher before than after weight reduction (Table 2). The insulin infusion, given in relation to body weight, yielded much higher circulating insulin levels before surgery, partly due to a markedly lower MCR of insulin in this situation. More glucose was required after weight reduction to clamp the arterial blood glucose at the desired hypoglycemic level (Fig 1A), indicating a pronounced improvement in insulin sensitivity after weight reduction (Table 2).

Glucose

The arterial blood glucose level was significantly higher before than after weight reduction (Table 1). During the clamp, glucose, decreased to a nadir at 60 minutes and was thereafter maintained at identical steady state levels of 3.4 ± 0.1 and 3.4 ± 0.1 mmol/L, respectively (Fig 1A). The corresponding venous blood glucose levels were significantly higher before

Table 3. Results of Peak Hormonal Measurements (60–180 min) During Insulin-Induced Hypoglycemia Before and After 32% Weight Reduction

Variable	Before WR	After WR	*P* (before *v* after)
Glucagon (ng/L)	81.0 ± 17.2	56.0 ± 13.9	<.01
Epinephrine (nmol/L)	2.1 ± 1.3	1.3 ± 1.0	<.01
Norepinephrine (nmol/L)	3.1 ± 0.8	2.4 ± 0.8	<.05
PP (pmol/L)	611 ± 232	390 ± 272	<.05
Cortisol (nmol/L)	651 ± 122	447 ± 87	<.05
GH (μg/L)	1.0 (0.1, 4.0)	7.7 (1.4, 13.2)	<.05

NOTE. Means ± SD or medians (interquartile range) are shown.

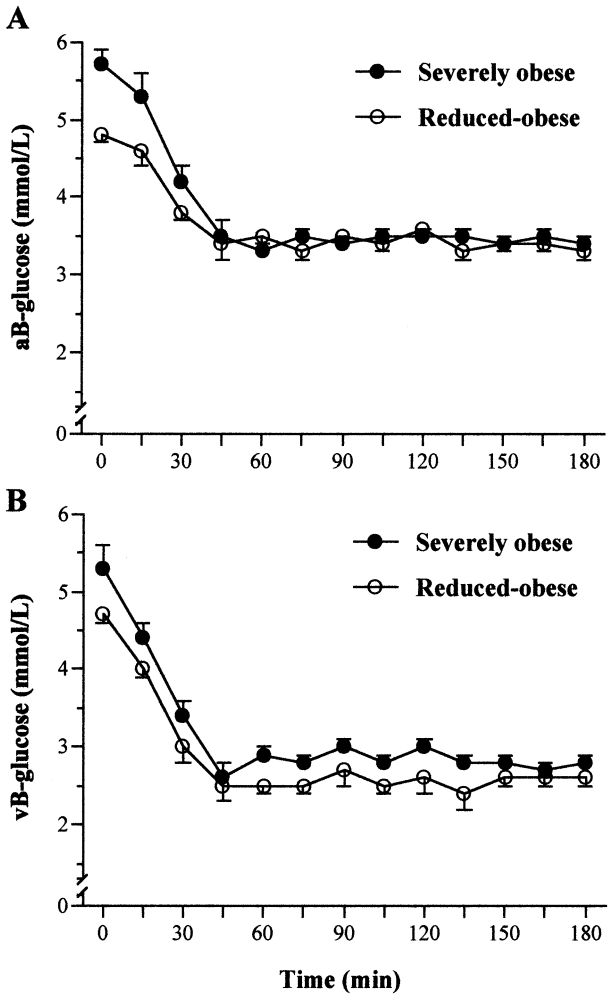

Fig 1. (A) Arterial blood glucose concentrations and (B) venous blood glucose concentrations during insulin-induced hypoglycemia, before (●—●) and after (○—○) ~32% weight reduction.

than after weight reduction 2.8 ± 0.1 *v* 2.6 ± 0.1 mmol/L; $P <$ .001) (Fig 1B).

C-Peptide

Preoperative fasting concentration of C-peptide decreased significantly after weight reduction (Table 1). In both experiments, C-peptide was reduced according to similar kinetics and reached a concentration close to the detection limit of the assay after 120 minutes, indicating a similar suppressive effect in the 2 experiments during the last 60 minutes (Fig 2).

Hormonal Counterregulatory Responses

There were no marked reductions of mean preoperative fasting concentrations of the counterregulatory hormones versus after weight reduction except for glucagon (Table 1). Before weight reduction the hormonal counterregulation to hypoglycemia was characterized by brisk responses in glucagon, PP, cortisol (Fig 3A) and epinephrine, norepinephrine (Fig 3B). After weight reduction GH (data not shown) was increased while, epinephrine, norepinephrine, PP, and cortisol responses were reduced and the glucagon response was abolished (Fig 3A and B). Furthermore, the change in peak values of glucagon (Δ peak glucagon) was highly associated with Δ peak PP, (r_s = .93; P = .002), but not with Δ peak epinephrine (r_s = −.10; P = not significant [NS]; Table 3).

The results expressed as total AUCs during the experiment (0 to 180 minutes) (Table 4) show that the AUC_{PP} was significantly reduced by 46% ± 21% (P = .02), $AUC_{epinephrine}$ by 40% ± 34% ($P <$ .01), $AUC_{cortisol}$ by 33% ± 17% ($P <$ .01), in the reduced-obese state compared with the severely obese state.

Correlation analyses were performed to show if changes in peripheral peak levels of PP, glucagon, epinephrine, and cortisol during prolonged hypoglycemia were related to weight loss. The outcome of the analyses showed a strong association between weight loss and Δ peak PP (a marker for vagal activation) (r_s = .96; $P <$ .001), Δ peak glucagon (r_s = .93; $P <$ .001), Δ peak cortisol (a marker for HPA axis activation) (r_s = .78; $<$ .05), but not for weight loss and Δ peak epinephrine (a marker for adrenal medullary activation) (r_s = .19; P = NS), Δ peak norepinephrine (a marker for sympathetic activation) (r_s = -.24; P = NS).

Blood Pressure and Pulse Rate

Before weight reduction, the subjects had higher diastolic blood pressure and pulse rates than after weight reduction (Table 1). During the prolonged hypoglycemia both systolic and diastolic blood pressures were reduced after weight reduction, whereas pulse pressure and pulse rate did not change (Table 2).

Cognitive Function Tests

Cognitive functions were assessed with the PMT to detect differences in general problem-solving ability before and after weight reduction (Table 5). Before weight reduction, in the

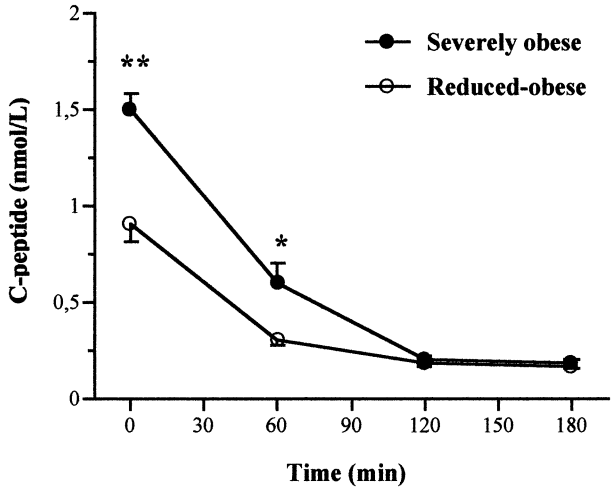

Fig 2. Comparison of C-peptide concentrations at 0 times and during the hypoglycemic glucose clamp in severely obese patients before (●—●) and after (○—○) ~32% weight reduction. Asterisks indicate significant differences before *v* after weight reduction. (P* < .05 and ***P* < .01). All measurements of C-peptide concentrations (60 to 180 minutes) are reduced compared with 0 times (*P* < .05).**

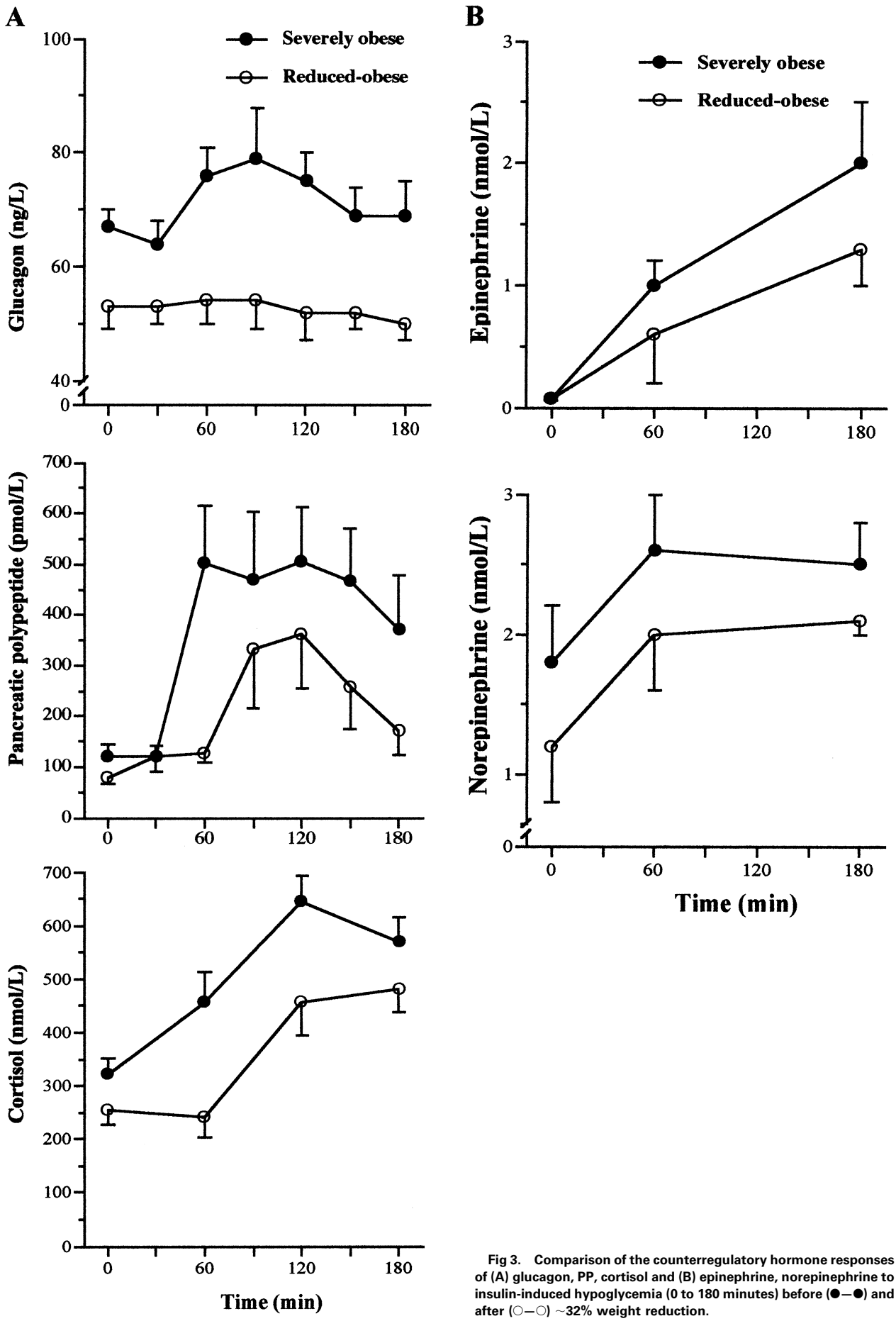

Fig 3. Comparison of the counterregulatory hormone responses of (A) glucagon, PP, cortisol and (B) epinephrine, norepinephrine to insulin-induced hypoglycemia (0 to 180 minutes) before (●—●) and after (○—○) ~32% weight reduction.

Table 4. Area Under the Curve for Glucagon, Epinephrine, Norepinephrine, PP, and Cortisol During Insulin-Induced Hypoglycemia (0–180 min) Before and After Weight Reduction

Variable	Before WR	After WR	*P* (before *v* after)
$AUC_{Glucagon\ (ng/L \cdot min)}$	217 ± 37	158 ± 32	<.01
$AUC_{Epinephrine\ (nmol/L \cdot min)}$	3.8 ± 1.6	2.3 ± 1.7	<.01
$AUC_{Norepinephrine\ (nmol/L \cdot min)}$	7.2 ± 1.9	5.8 ± 2.1	NS
$AUC_{PP\ (pmol/L \cdot min)}$	1,146 ± 498	668 ± 415	<.01
$AUC_{Cortisol\ (nmol/L \cdot min)}$	1,585 ± 213	1,049 ± 232	<.01

NOTE. Means ± SD are shown.

severely obese state, the patients used a more cautious strategy during both normo-and hypoglycemia by using a lower processing and inspection speed, more rub outs and, therefore, solved more mazes correctly (accuracy preferring). An analysis of separate phases of the maze-solution process suggested that the subjects in the reduced-obese state used a more impulsive-global cognitive strategy (speed preferring) by using a higher processing and inspection speed, but also activated a more sequential, left-hemisphere-type strategy in the solving process by using significantly fewer rub outs.

DISCUSSION

Our study clearly shows that the counterregulatory responses during hypoglycemia are preserved or even exaggerated in morbid visceral obesity compared with the reduced-obese state, a finding consistent with the hypothesis that the autonomic sympathoadrenal and parasympathetic systems, as well as the HPA axis, might be hyperactive.[2-5,7] However, as to the HPA axis, some controversy may exist. Yanovski et al[26] reported in obesity an enhanced increase in total cortisol, but not in the free cortisol fraction, indicating that in obesity an elevated corticosteroid-binding globulin-transcortin (CBG) has to be considered. On the other hand, the enhanced MCR of cortisol, known to take place in the morbidly obese state,[27] has also to be taken into consideration in this context. In line with the idea that the ANS and HPA axis are overactivated during obesity[4-7] and that weight reduction consequently should result in a lower level of activation, the main outcome of the study was the reduced glucose counterregulatory hormones in the ~ 32% reduced-obese subjects. The magnitude of the reduced ANS responses to weight loss appeared to be more closely associated to PP (a marker for vagal activation)[15,28] and cortisol (a marker for HPA axis activation),[14] than to epinephrine and norepinephrine (markers for sympathoadrenal activation),[15] Furthermore, the GH response to hypoglycemia in obesity was increased by weight reduction.[29] As to the changes of sympathoadrenal activation, our results including the change of systolic and diastolic blood pressure, are well in line with previous results.[30,31] Somewhat in contrast to the moderately diminished responses of PP, epinephrine, norepinephrine ,and cortisol, the glucagon response appeared to be totally abolished after the weight reduction, the latter being an unexpected observation.

Control of the glucagon secretion during insulin-induced hypoglycemia is complex. Stimulatory signals include decreases in intraislet insulin,[32] autonomic nervous input,[15] whereas insulin can inhibit glucagon secretion.[16] Therefore, it is difficult to determine whether it is reduced stimulatory or increased inhibitory mechanisms that are responsible for decreased glucagon secretion after weight reduction. A possibility is a reduced autonomic activation, because ANS is known to contribute to the glucagon response to insulin-induced hypoglycemia[15] and during the hyperinsulinemic clamp, the suppression of glucagon was closely associated with the reduced PP response, a surrogate measure for parasympathetic activation.[28,33]

The reduced PP response achieved here brings into focus the question whether the surgical technique used for VBG effects the vagal nerve or not. In the current study, no vagotomy was performed, consequently, there is no indication that any major surgical damage to autonomic nerves would affect the results in the present study design.

As indicated above, the prevailing insulin level can directly suppress glucagon secretion.[16] It is unlikely that differences in endogenous insulin secretion would matter, as during the clamp the C-peptide level was similarly suppressed to the detection limit before and after weight reduction. The circulating insulin

Table 5. Results of the PMT Before and After 32% Weight Reduction During Normoglycemia and During Hypoglycemia (at 90 min)

	Normoglycemia			Hypoglycemia		
	Before WR	After WR	*P**	Before WR	After WR	*P*†
Processing rate (nodes/s)	5.2 ± 1.0	6.2 ± 1.5	<.01	5.0 ± 0.7	5.7 ± 1.2	< .01
Inspection rate (nodes/s)	21.9 ± 6.7	29.5 ± 9.1	<.01	21.4 ± 7.3	40.4 ± 13.2	<.01
Check time (s)	0.8 ± 0.1	0.8 ± 0.2	NS	0.9 ± 0.1	0.9 ± 0.2	NS
L/R preference (au)	0.6 ± 0.11	0.6 ± 0.04	NS	0.5 ± 0.10	0.6 ± 0.07	<.01
Motor time (ms)	467 ± 32	417 ± 69	<.05	427 ± 56	514 ± 46	<.01
Max rows (rows)	13.6 ± 1.4	12.8 ± 1.5	<.01	12.8 ± 0.6	13.3 ± 1.0	<.05
Correctly solved mazes (%)	80 ± 10	68 ± 6	<.01	73 ± 10	68 ± 5	NS
Rub outs (no./maze)	1.5 ± 0.5	0.5 ± 0.3	<.01	0.8 ± 0.2	0.3 ± 0.1	<.01

NOTE. Means ± SEM are shown.

Abbreviations: au, arbitrary unit; PMT, Perceptual Maze Test.

*Before *v* after WR during normoglycemia; †before *v* after WR during hypoglycemia.

levels were, however, much higher before weight reduction, partly because a higher total dose of insulin was administered, as insulin was dosed in relation to body weight, and partly also due to a more reduced MCR, known to be present in the morbidly obese state.[34] Against this background one would expect the inhibition by insulin on the glucagon secretion to be more pronounced before than after weight reduction. The totally abolished glucagon response after weight reduction is however not compatible with the above idea. An alternative explanation could be that in the morbidly obese state the α cell is the subject of resistance to the inhibitory effect of insulin, perhaps in parallel to insulin resistance in liver and muscle tissue. We further hypothesize that the ~32% weight reduction at least partly restored this insulin inhibitory effect, thereby contributing to the blunted glucagon response to hypoglycemia.

A further possibility to the change of the glucagon responses would be that weight reduction in obesity changes the activation of glycemic thresholds of the hormonal responses. It is obvious that the glycemic stimulus was identical (3.4 mmol/L in arterial whole blood) in the 2 series of experiments in our study. Furthermore, this hypoglycemic level has been shown to be sufficient to activate the hormonal counterregulation in normal man, as well as in patients with type 1 diabetes using the same technique as in the present study.[16,35] It is important in this context to note that whole blood glucose was measured in arterial blood and that the arterial readings were used in the clamp. The corresponding venous whole blood glucose level was 2.8 and 2.6 mmol/L, respectively, indicating an AV-difference of 0.6 to 0.8 mmol/L. The arterialized venous blood glucose was not measured in this study, but from previous experience,[36] its level usually is at about 50% of this difference, ie, reaching an estimated level of 3.0 to 3.2 mmol/L (arterialized whole blood). A recalculation from whole blood to plasma glucose using a factor of 1.11 results in an estimation of arterialized plasma glucose between 3.33 to 3.55 mmol/L.[37] The activation of glycemic thresholds of hypoglycemia-induced glucagon, epinephrine, and PP responses in normal healthy individuals has been described to be 3.8 to 3.9 mmol/L in arterialized plasma.[14,38,39] Thus, the glycemic level obtained in the present study was below the limit for activation of the counterregulatory hormones.

Our observation that the activation of epinephrine and PP was obviously at exactly the same arterial blood glucose concentration in our experiments talks against the possibility that the threshold activation is different in the reduced obese state. This is consistent with a study by Vea et al[40]that showed there were no differences in thresholds for counterregulatory hypoglycemic responses, including that of glucagon, in subjects with severe obesity (BMI ~42) versus lean control subjects (BMI ~22). Many studies[14,38,39] demonstrate that epinephrine and glucagon are activated at the same glycemic threshold, and therefore it is not likely that a difference in activation of glycemic thresholds would explain the finding of an abolished glucagon response in the reduced-obese state in the present study.

Also changes in fasting blood glucose seem to be irrelevant, because the blood sugar level itself, as for example in diabetes, has not been shown to be important for the glucagon response.[41] We are aware that this study was performed mainly in females. As the counterregulatory response to hypoglycemia may show gender differences, a follow-up study on reduced-obese male individuals is certainly desirable.[14,42]

It is known that the cognitive function may deteriorate during hypoglycemia provided the arterialized venous plasma glucose is reduced below an arterialized venous plasma glucose concentration of 2.7 mmol/L (corresponding concentration in arterial whole blood is 3.1 mmol/L).[39] In the present study, the hypoglycemic level did not reach below this concentration. Therefore, to identify the effect of weight reduction on hypoglycemic modulation of cognitive function, another experimental design using more pronounced hypoglycemia should have been chosen. On the other hand, the cognitive function during normoglycemia appears to be clearly modified by weight reduction to be changed preferentially to a speed-preferring strategy compared with a more accuracy preferred problem-solving process of the PMT test preoperatively. These changes in cognitive function could be seen as reflecting differences in hemispheric activation patterns.[18] Interestingly, it has been suggested that behavioral and cognitive factors may determine the outcome of gastric restriction procedures for the surgical treatment of obesity.[43] These findings merit further attention.

In summary, the main observation of the present study was the reduction of the glucose counterregulatory hormonal responses during prolonged hypoglycemia in the reduced-obese state, most notably affecting the glucagon response, which was totally abolished. Irrespective of the mechanisms behind these modifications after massive weight reduction, it can be speculated that the physiologic impact of an increased insulin sensitivity and defects in counterregulatory hormone responses may be important, predisposing to low plasma glucose concentrations in the late postprandial state.[11,44]

ACKNOWLEDGMENT

The authors are grateful to Lilian Bengtsson and Ulrika Gustavsson for expert technical assistance.

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