Massive Weight Loss Decreases Corticosteroid-Binding Globulin Levels and Increases Free Cortisol in Healthy Obese Patients

An adaptive phenomenon?

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OBJECTIVE — Obesity, insulin resistance, and weight loss have been associated with changes in hypothalamic-pituitary-adrenal (HPA) axis. So far, no conclusive data relating to this association are available. In this study, we aim to investigate the effects of massive weight loss on cortisol suppressibility, cortisol-binding globulin (CBG), and free cortisol index (FCI) in formerly obese women.

RESEARCH DESIGN AND METHODS — Ten glucose-normotolerant, fertile, obese women (BMI >40 kg/m^2, aged 38.66 ± 13.35 years) were studied before and 2 years after biliopancreatic diversion (BPD) when stable weight was achieved and were compared with age-matched healthy volunteers. Cortisol suppression was evaluated by a 4-mg intravenous dexamethasone suppression test (DEX-ST). FCI was calculated as the cortisol-to-CBG ratio. Insulin sensitivity was measured by an euglycemic-hyperinsulinemic clamp, and insulin secretion was measured by a C-peptide deconvolution method.

RESULTS — No difference was found in cortisol suppression after DEX-ST before or after weight loss. A decrease in ACTH was significantly greater in control subjects than in obese ($P = 0.05$) and postobese women ($P \leq 0.01$) as was the decrease in dehydroepiandrosterone ($P \leq 0.05$ and $P \leq 0.01$, respectively). CBG decreased from 51.50 ± 12.76 to 34.33 ± 7.24 mg/l ($P \leq 0.01$) following BPD. FCI increased from 11.15 ± 2.85 to 18.16 ± 6.82 ($P \leq 0.05$). Insulin secretion decreased (52.04 ± 16.71 vs. 30.62 ± 16.32 nmol/m^{-2}; $P \leq 0.05$), and insulin sensitivity increased by 163% ($P \leq 0.0001$). Serum CBG was related to BMI ($r_0 = 0.708$; $P = 0.0001$), body weight ($r_0 = 0.643$; $P = 0.0001$), body fat percent ($r_0 = 0.462$; $P = 0.001$), C-reactive protein ($r_0 = 0.619$; $P = 0.004$), and leptin ($r_0 = 0.579$; $P = 0.007$) and negatively to *M* value ($r_0 = -0.603$; $P = 0.005$).

CONCLUSIONS — After massive weight loss in morbidly obese subjects, an increase of free cortisol was associated with a simultaneous decrease in CBG levels, which might be an adaptive phenomenon relating to environmental changes. This topic, not addressed before, adds new insight into the complex mechanisms linking HPA activity to obesity.

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Nutritional status and activity of the hypothalamic-pituitary-adrenal (HPA) axis are strictly associated (1,2). However, little is known about the effect of starvation and intentional calorie deprivation on the HPA axis (3,4). The HPA axis controls the secretion of cortisol with excessive secretion being inhibited by negative feedback. Several studies suggest that abnormalities in cortisol action and HPA axis control may be a factor that links disturbances of carbohydrate metabolism (which characterize insulin resistance) and obesity (5–6). In obesity, estimation of plasma cortisol does not reflect the function of the HPA axis (7), and levels of cortisol in obese patients have been reported to be normal (8), low (9), or increased (10). On the contrary, response to different stimuli (high secretion of cortisol after laboratory stress tests or after different exogenous neuropeptides) has been found to be altered (11). Compared with these tests, the dexamethasone suppression test (DEX-ST), mainly used in the diagnosis of Cushing's disease, appears to be inadequate. Nevertheless, it has been widely used to evaluate the HPA axis in obesity, and the outcomes are contradictory (12–14). The free cortisol index (FCI)— the total cortisol-to-cortisol–binding globulin ratio (CBG) — has also been proposed to be able to evaluate HPA axis activity (7,15) since it reflects the biologically active fraction of total cortisol (7). Moreover, FCI is significantly associated with several features of insulin resistance syndrome (15). CBG levels are generally decreased in obese and diabetic subjects, although genetic factors are also known to play a role in the interindividual variation in CBG levels (16,17). Reduced

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Abbreviations: BPD, biliopancreatic diversion; CBG, cortisol-binding globulin; CRP, C-reactive protein; DEX, dexamethasone; DEX-ST, DEX suppression test; FCI, free cortisol index; FFM, fat-free mass; HPA, hypothalamic-pituitary-adrenal; OGTT, oral glucose tolerance test; RIA, radioimmunoassay.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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levels of CBG favor locally increased cortisol disposal with no change in circulating cortisol; thus, fat accumulatates. Therefore, in obesity, tissues may be exposed to a relative excess of cortisol without any increase in circulating cortisol.

Biliopancreatic diversion (BPD), a malabsorptive bariatric surgical technique, causes massive weight loss (18,19), restoration of insulin sensitivity, and amelioration of insulin secretion (18). It restores the regular diurnal rhythm and pulsatility of insulin, leptin, and adiponectin but does not influence those of cortisol (19).

Thus far, no data are reported in literature concerning the effects of surgically induced massive and stable weight and fat mass loss on FCI and CBG concentrations and response to DEX-ST. The present study aimed to investigate 10 glucose-normotolerant morbidly obese (BMI >40 kg/m^2) women before and 2 years after BPD for *1*) changes of FCI and CBG levels and *2*) suppression of morning cortisol, ACTH, and DHEA levels after intravenous dexamethasone (DEX).

RESEARCH DESIGN AND METHODS

— The study group included 10 normotensive obese women (BMI >40 kg/m^2) evaluated before and 2 years after BPD. Postobese subjects were admitted monthly to our unit in the first 3 months after surgery and were controlled every 3 months thereafter. During the second year, they were admitted every 6 months. Patients were required to record their weight monthly. A stable weight (fluctuation $<5\%$) is generally achieved by 12–15 months. Ten normal weight (BMI ≤ 24 kg/m^2) volunteers recruited among nurses and students in the hospital staff served as healthy control subjects to evaluate suppressibility of the HPA axis after DEX-ST. Subjects were nonsmokers and were 20–35 years of age. Glucose tolerance was evaluated by a 75-g oral glucose tolerance test (OGTT) (20). They were evaluated in the early follicular phase by undergoing the OGTT and the euglycemic-hyperinsulinemic clamp study before the DEX-ST. None of the study participants had endocrine or nonendocrine diseases. They were not taking any medications except subjects after BPD, who were prescribed oral supplementation of sulfate iron (525 mg daily) calcium carbonate (1 g daily), multivitamins (Supradyn Roche, Milan, Italy) (1 tablet a day), and ergocalciferol (400,000 UI intramuscular) (Ostelin fl, Teofarma, Italy) every 2 weeks. Medical histories, physical examinations, electrocardiogram, and blood screening showed that patients were in good health. None were taking anticonvulsant medications or corticosteroids. Depression was excluded by the Italian version of the Epidemiological Studies Depression Rating Scale (21).

The ethical committee of Catholic University approved the study, and subjects signed an informed consent document before participation. Body composition was estimated by the isotopic dilution method (22). Fat-free mass (FFM) was measured in kilograms and obtained by dividing total body water by 0.73 (22). Subjects underwent BPD, which is a malabsorptive surgical procedure (23).

Insulin sensitivity was estimated by a euglycemic-hyperinsulinemic clamp as previously described (24). Whole-body glucose uptake, normalized by FFM (*M* value in mmol $\cdot$ kg$_{FFM}^{-1}$ $\cdot$ min^{-1}), was determined during a primed constant infusion of insulin (at the rate of 6 pmol $\cdot$ min^{-1} $\cdot$ kg^{-1}).

β-Cell function was estimated on 2-h OGTT values of glucose, insulin, and C-peptide. Samples were withdrawn every 30 min. β-Cell function was assessed using a model describing the relationship between insulin secretion and glucose concentration, which has been previously described in detail (18). The model expresses insulin secretion as a sum of two components: β-cell glucose sensitivity and the rate sensitivity. Basal and total insulin secretions during the OGTT were calculated from the estimated model parameters. Total insulin secretion was calculated as the integral over the first 2 h of the OGTT.

Intravenous DEX-ST was performed as previously described (25). DEX phosphate (1 mg/h) (4 mg Soldesam; Lab Italiani Farmaceutici, Milan, Italy) was infused for 4 h beginning at 11.00 A.M. Blood samples were taken at 8:00 P.M. and 12:00, 8:00, and 11:00 A.M. before starting the test and after at 3:00 and 8:00 P.M. Samples were also taken at 12:00 and 8:00 A.M. the following day to measure cortisol, DHEA, and ACTH.

Analytical assays

Samples were collected in tubes with aproteinin (500 units/l) in an ice bath and frozen immediately at −80°C. Plasma glucose was measured by the glucose-oxidase method (Beckman, Fullerton, CA), while FFAs were quantified by spectrophotometric measurements. Hormones were assayed in duplicate. Plasma insulin was assayed by a microparticle enzyme immunoassay (Abbott, Pasadena, CA) (sensitivity 1 μU/ml, intra-assay coefficient of variation [CV] 6.6%). CBG was measured by radioimmunoassay (RIA) (Radim, KP31; Angleur, Liege, Belgium). Intra- and interassay CVs were 3.6 and 7.5%, respectively; SHBG was quantified by an RIA method (CIS Bio International, Gif-sur-Yvette, France) (intra- and interassay CVs 2.5 and 3.8%). Serum cortisol was determined by RIA (cort-CTK 125; Dia Sorin, Saluggia, Italy) (sensitivity 2.7 nmol/l), interassay CVs at concentration levels of 16.6, 40.8, and 198.8 nmol/l were 6.8, 14.6, and 4.3%, respectively, and intra-assay CVs at concentrations of 9.1, 45.9, and 135.3 nmol/l were 9.9, 5.7, and 6.1%. ACTH was determined with an immunoradiometric assay method (Nichols Institute Diagnostics, San Juan Capistrano, CA) (sensitivity 0.22 pmol/l). Interassay CV at concentrations of 1, 7.3, and 24.2 pmol/l were 2.4, 8.5, and 4.3%, and interassay CVs at concentrations of 1 and 24 pmol/l were 9.9 and 3.9%. Plasma DHEA was assayed by RIA method (Radim, Pomezia, Italy) (sensitivity 15 pg/ml; intra- and interassay CVs 3.1 and 6.9%). Serum leptin was assayed using an ELISA kit (Linco Research, St. Charles, MO) (sensitivity 0.5 ng/ml). Intra- and interassay CVs were 4.2 and 4.5%. C-reactive protein (CRP) was assessed by a routine laboratory test (Beckman Coulter, Fullerton, CA)

Statistical methods

Data are presented as means ± SD unless otherwise stated. Before statistical analysis, normal distribution and homogeneity of the variances were tested. All parameters fulfilled these conditions. Comparisons between groups were performed using paired *t* test while comparisons among groups were performed using one-way ANOVA followed by Bonferroni's post-hoc test for multiple comparisons whenever appropriate. Relationships between variables were sought by linear correlation analysis (Spearman's r_0). Levels of statistical significance were set at $P < 0.05$. Data analyses were performed with SPSS statistical software (SPSS V12.0; SPSS, Chicago, IL).

Table 1—*Anthropometrical characteristics and blood pressure of the studied subjects*

	Obese NGT women*		
	Baseline	After BPD†	Control subjects*
Age (years)	38.66 ± 13.35	41.57 ± 12.21	39.24 ± 13.56
Body weight (kg)	114.56 ± 13.88	85.60 ± 14.25	60.23 ± 10.21
BMI (kg/m^2)	42.57 ± 5.99	31.78 ± 5.54	22.54 ± 5.89‡
FFM (kg)	70.93 ± 9.93	62.60 ± 8.55	55.67 ± 8.79‡
Fat mass (kg)	43.62 ± 9.77	23 ± 6.51	16.78 ± 4.87‡
Waist-to-hip ratio	0.96 ± 0.04	0.90 ± 0.03	0.83 ± 0.02‡
Systolic blood pressure (mmHg)	146.11 ± 13.18	114.44 ± 7.68	118.54 ± 6.98
Diastolic blood pressure (mmHg)	88.88 ± 1.76	80.0 ± 8.08	81.2 ± 7.10

Data are means ± SD. *n = 10. †$P \leq 0.0001$ (paired t test). ‡$P \leq 0.001$ (ANOVA). NGT, normal glucose tolerance.

RESULTS

Body composition and analytic parameters

A stable weight was achieved after 13 ± 2 months. Body weight ($P \leq 0.0001$) and fat mass ($P \leq 0.0001$) were significantly reduced 2 years after BPD (Table 1). FFM and fat mass decreased by 25 and 45%, respectively, and lipid profile significantly ameliorated (Table 2). FFAs were significantly lowered from 0.42 ± 0.10 to 0.20 ± 0.07 mmol/l ($P \leq 0.0001$), and circulating sex hormone–binding globulin increased (Table 2) ($P \leq 0.05$). Levels of CRP significantly decreased from 1.03 ± 0.24 to 0.22 ± 0.08 mg/l ($P \leq 0.0001$).

Insulin sensitivity and secretion

After BPD, fasting glucose and insulin decreased from 5.98 ± 1.90 to 3.86 ± 0.30 mmol/l ($P \leq 0.01$) and from 93.60 ± 56.49 to 41.16 ± 24.01 pmol/l ($P \leq 0.05$), respectively. Insulin sensitivity markedly increased by ~163% in the post-BPD group. The insulin-mediated glucose uptake (M value) improved from 14.92 ± 1.23 $\mu mol \cdot kg_{FFM}^{-1} \cdot min^{-1}$ to 39.13 ± 0.98 $\mu mol \cdot kg_{FFM}^{-1} \cdot min^{-1}$ after BPD ($P \leq 0.0001$).

Both fasting insulin secretion (152.87 ± 83.49 vs. 88.90 ± 46.00 pmol/min per m^2, $P \leq 0.05$) and total insulin output (52.04 ± 16.71 vs. 30.62 ± 16.32 nmol/m^{-2}, $P \leq 0.05$) decreased following BPD. β-Cell glucose sensitivity significantly increased (80.43 ± 4.89 to 97.98 ± 4.70 pmol/min per m^2 per mmol/l, $P \leq 0.05$), while the rate sensitivity (0.88 ± 0.27 vs. 0.32 ± 0.50 nmol/min per m^2 per mmol/l) and the potentiation factor (0.87 ± 0.10 vs. 0.83 ± 0.3–fold) did not change. In healthy subjects, mean glucose uptake was 42.34 ± 1.02 $\mu mol \cdot kg_{FFM}^{-1} \cdot min^{-1}$ ($P \leq 0.001$ vs. obese women). Both fasting insulin secretion (55.54 ± 23.45 pmol/min per m^2, $P \leq 0.001$) and total insulin output (29.44 ± 13.56 nmol/m^{-2}, $P \leq 0.001$) were significantly lower than in obese women. β-Cell glucose sensitivity was higher than in obese women (96.78 ± 3.28 pmol/min per m^2 per mmol/l , $P \leq 0.05$).

HPA axis suppressibility, CBG levels, and FCI

No differences were found in fasting cortisol, DHEA, and ACTH among control, obese, and post-BPD subjects. Effects of the DEX infusion on the three hormones are depicted in Fig. 1. Morning cortisol (Fig. 1*A*) was significantly suppressed in all subjects (89 ± 3% in pre-BPD, 89 ± 2% in post-BPD, and 89 ± 5% in control subjects) (P = NS), decreasing from 555 ± 115 nmol/l to 60 ± 20 ($P \leq 0.0001$) in women before surgery, from 590 ± 120 to 64 ± 22 nmol/l ($P \leq$

Table 2—*Biochemical parameters in the studied groups*

	Obese NGT women*		
	Baseline	After BPD	Control subjects*
Fasting glucose (mmol/l)	5.98 ± 1.90	3.86 ± 0.30†	4.01 ± 0.28‖
Fasting insulin (pmol/l)	93.60 ± 56.50	41.16 ± 24.01‡	40.78 ± 20.76‖
Total colesterol (mmol/l)	5.36 ± 1.19	3.34 ± 0.66§	4.01 ± 0.89
HDL cholesterol (mmol/l)	1.14 ± 0.27	1.42 ± 0.29‡	1.49 ± 0.31
Triglycerides (mmol/l)	1.45 ± 0.60	1.0 ± 0.45‡	1.20 ± 0.67
Albumin (g/dl)	4.06 ± 0.27	3.94 ± 0.37	4.01 ± 0.41
Proteins (g/dl)	7.29 ± 0.54	7.2 ± 0.58	7.31 ± 0.60
sGOT (UI/l)	20.1 ± 6.08	27.71 ± 6.60	25.89 ± 8.90
sGPT (UI/l)	30.1 ± 15.36	32.43 ± 13.78	30.98 ± 14.89
SBHG (nmol/l)	46.0 ± 12.8	85.6 ± 16.3‡	80.9 ± 21.7‖
CBG (mg/l)	51.5 ± 12.8	34.3 ± 7.2†	44.1 ± 6.0‖
GH (ng/ml)	0.84 ± 0.49	2.80 ± 1.85†	1.89 ± 1.42
Leptin (mg/ml)	63.3 ± 2.9	21.8 ± 4.3	19.9 ± 7.7‖

Data are means ± SD. *n = 10. †$P \leq 0.01$, ‡$P \leq 0.05$, and §$P \leq 0.001$ by paired t test. ‖$P \leq 0.001$ by ANOVA. ¶$P \leq 0.0001$. To convert glucose to mg/dl, divide by 0.05551. To convert insulin to μUI/ml, multiply by 7.175. To convert triglycerides to mg/dl,divide by 0.01129. To convert cholesterol to mg/dl, divide by 0.0258. GH, growth hormone; SBHG, sex hormone–binding globulin; sGOT, serum gluamic oxaloacetic transaminase; sGPT, serum glutamic pyruvic transaminase.

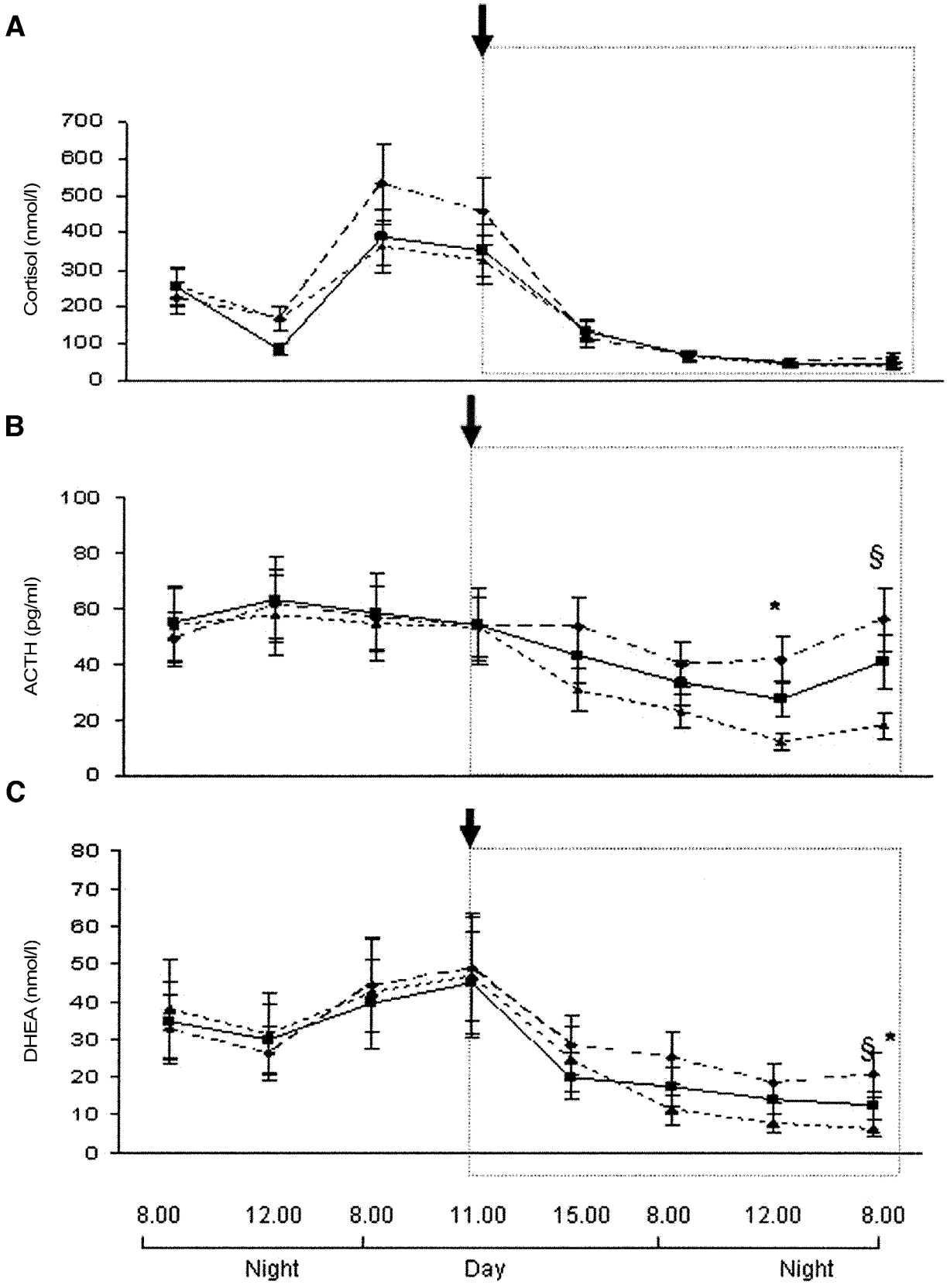

Figure 1—*Mean ± SEM of cortisol (A), ACTH (B), and DHEA (C) before and after dexamethasone infusion. DEX-ST is represented by the square. Start of infusion is indicated by the black arrow. Level of significance at the ANOVA test followed by the Bonferroni's post-hoc test was* *P = *0.05 between control subjects and obese women;* §P ≤ *0.01 between control subjects and women after BPD.* ▲, *control subjects;* ■, *obese women;* ◆, *post-BPD women.*

0.0001) in the same subjects after surgery, and from 459 ± 134 to 46 ± 12 ($P \leq 0.0001$) in control subjects.

The lowest ACTH concentrations (Fig. 1*B*) were observed at midnight following DEX-ST. The decrease in ACTH levels was significantly greater in control subjects than in obese ($P = 0.05$) and postobese ($P \leq 0.01$) women. At the midnight measurement, the ACTH concentration decreased with respect to the value measured the day before the test by 52.47 ± 19.51% (57.68 ± 18.85 to 32.11 ± 11.27 pg/ml, $P \leq 0.01$) in obese women, by 32.59 ± 12.05% (50.24 ± 6.92 to 39.40 ± 19.62 pg/ml, $P = 0.01$) after weight loss, and by 63.52 ± 9.63% (59.19 ± 10.84 to 12.79 ± 4.14 pg/ml, $P \leq 0.0001$) in control subjects. Both before and after surgery, obese women had higher values of ACTH than control subjects at midnight ($P = 0.02$ and $P = 0.001$, respectively) and in the early morning following the DEX-ST ($P = 0.001$ and $P \leq 0.0001$). During the test, mean concentrations of cortisol and ACTH were 196 ± 60 nmol/l and 44.11 ± 15.08 pg/ml in obese women, 202 ± 60 nmol/l and 48.25 ± 13.34 pg/ml in post-BPD women, and 211.72 ± 45.25 nmo/l and 28.38 ± 3.04 pg/ml in control subjects.

DHEA (Fig. 1*C*) decreased from 39.79 ± 5.02 to 14.14 ± 3.71 nmol/l in obese women ($P \leq 0.0001$), 45.36 ± 8.80 to 22.63 ± 5.73 nmol/l in post-BPD women, and 43.54 ± 6.67 to 6.16 ± 1.05 nmol/l in control subjects ($P \leq 0.0001$). DHEA concentration was significantly lowered in control subjects compared with obese women both before ($P \leq 0.05$) and after ($P \leq 0.01$) weight loss. CBG decreased significantly (Table 2) ($P \leq 0.01$), and consequently, FCI significantly increased (from 11.15 ± 2.85 to 18.16 ± 6.82, $P \leq 0.05$) after surgery. The FCI-to-ACTH ratio decreased from 8.36 ± 3.22 to 10.08 ± 2.77 (P = NS).

In obese women, serum CBG was related to BMI ($r_0 = 0.708$, $P = 0.0001$), body weight ($r_0 = 0.643$, $P = 0.0001$), fat mass percentage ($r_0 = 0.462$, $P = 0.001$), CRP ($r_0 = 0.619$, $P = 0.004$), and leptin ($r_0 = 0.579$, $P = 0.007$) and negatively to *M* value ($r_0 = -0.603$, $P = 0.005$). Changes in sex hormone–binding globulin negatively correlated with changes in fasting ($r_0 = -0.673$, $P = 0.03$) and total ($r_0 = -0.634$, $P = 0.048$) insulin secretion.

CONCLUSIONS — In the present study, we observed a significant decrease in circulating CBG levels and a concomitant increase of the metabolically active free cortisol fraction in obese women after surgically induced weight loss (BPD). Interestingly, despite the complete suppression of cortisol secretion by DEX infusion in all subjects independent of their body weight, the midnight fall in ACTH levels was significantly greater and the suppression of DHEA more enhanced in normal weight subjects compared with obese and post-BPD women (Fig. 1). A subtle primary dysfunction of the HPA axis might be envisaged in morbid obesity and after BPD. In post-BPD women, the HPA axis may adapt to a changing environment leading to decreased CBG levels and an overall increase in FCI. Several hypotheses can be formulated to explain the decrease of CBG levels and the different response to the DEX-ST.

The decrease in CBG levels following BPD may alter the sensitivity of the pituitary gland to cortisol. The recently reported (26) CBG-deficient mouse model is in agreement with the crucial role of the binding globulin in the ACTH feedback regulation of glucocorticoids. Additionally, a decrease in CBG levels would act to amplify the availability of metabolically free cortisol at peripheral target organs. Increased FCI might be a counteracting mechanism to the risk of hypoglycemia (stressor). In fact, in healthy subjects, hypoglycemia is reversed by a small permissive amount of glucocorticoids (27). Thus, postobese women may be able to maintain an elevated level of free cortisol available to all tissues without increasing total cortisol as demonstrated in free-living animals (28). Increased free cortisol appears to promote food-seeking behavior in animals (28).

CBG may also act as a proper hormone (28,29), and its levels (4) or its binding capacity (28,30) decrease in condition of restrain, stress, and reduced food intake. Nevertheless, in anorectic women, we have previously found normal levels of CBG but rapid escape to the effect of DEX-ST (31). However, little is known about the transcriptional regulation of the CBG gene in response to FFA or insulin sensitivity, and this may represent a simplistic mechanism by which CBG levels are decreased. A reduced CBG liver synthesis might also occur after bariatric surgery, but in our series, liver enzymes and protein levels were unchanged after massive weight reduction. Nevertheless, changes in hepatic CBG expression at the gene level might take place after surgery. Changes in CBG and FCI levels might also be related to the improvement of the low-grade inflammatory status (28) occurring in post BPD-patients (32), as suggested by the decrease of CRP. The observed dissociation between cortisol and DHEA response to the DEX-ST in post-BPD women also supports this hypothesis, with higher concentrations of DHEA (the biologically active form of the hormone) in postobese women compared with obese and control subjects.

Finally, other pleiotropic effects of cortisol cannot be excluded. In fact, enterocytes experience an adaptational hyperplasia after jejunoileal bypass (33), and glucocorticoids represent an important factor in the modulation of intestinal physiological functions, contributing to a more functional cell morphology and production of a potent regulator of crypt cell proliferation (34). The increase in FCI might contribute to this long term-adaptational hyperplasia. In contrast with previous reports (17), we did not find any relation between fasting CBG and insulin secretion, likely because of the small sample size of patients or as a distinctive effect of the surgery.

Concerning the effect of the DEX-ST, the dissociation between cortisol (which was fully suppressed in all subjects) and DHEA in obese and postobese women compared with healthy control subjects, as well as differences in ACTH levels, suggest a different adrenal sensitivity and/or pituitary response as seen in the presence of a stress situation among the three groups. Previous studies, which have used an oral DEX-ST to indirectly test activity of the HPA axis in obesity, found no impairment of cortisol suppression (12–14). We used intravenous DEX infusion (25) to minimize the possible alteration in oral DEX absorption and/or liver metabolism since postobese women had severe lipid malabsorption. Any attempt to explain these results should take into account that in our series, data on cortisol clearance and/or distribution volume, which can affect results, are lacking.

Thus, we combined the DEX-ST with a single measurement of CBG and calculation of FCI. DEX-ST reliably explores the negative feedback regulation of cortisol on ACTH and relies on multiple time points. FCI is strongly associated with CBG-binding activity and with adrenal cortisol production, but one morning point alone for CBG and cortisol might

not be representative of the whole-day FCI rhythm. Moreover, estimation of free cortisol by equilibrium dialysis would be more accurate than a derivative method to assess the amount of free hormone. Additionally, we used an intravenous DEX-ST (25) to minimize the possible variations in DEX absorption in postobese women who had severe lipid malabsorption.

The wide variability in the HPA axis balance in weight loss subjects may be related to the stress of starvation and/or to specific metabolic alterations rather than to the weight loss per se. For instance, in starving subjects, Yanovski et al. (14) reported that a relatively high dietary carbohydrate intake prevents the disruption of the HPA axis by reducing the reliance of ketones (4,14).

We are aware that the present findings cannot be applied to all obese subjects as our patients were a subset of "relatively healthy" obese women who were minimally affected by their obesity. They were not affected by hypertension, diabetes, or severe dyslipidemia. Thus, they may be the ones who have the fewest abnormalities of their HPA axis. However, future studies addressing this issue will be important to our understanding of dysregulation of the HPA axis.

In conclusion, we observed that after weight loss in morbidly obese subjects, free cortisol increased with an associated decrease in CBG levels. From the DEX infusion data, it is suggested that pituitary response and/or adrenal sensitivity differ between massively obese post-BPD patients and normal weight subjects. The long-lasting CBG decreases after drastic weight loss might be an adaptive phenomenon relating to environmental changes. This topic, not addressed before, adds new insight to the complexity of the intricate mechanisms linking HPA activity to obesity. The challenge remains to understand why CBG decreases and which consequences it exerts on the bioactivity of cortisol.

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