J. Endocrinol. Invest. 28: 509-515, 2005

Role of dehydroepiandrosterone sulfate levels on body composition after laparoscopic adjustable gastric banding in pre-menopausal morbidly obese women

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ABSTRACT. In humans, dehydroepiandrosterone (DHEAS) has been postulated to have anabolic and lipolytic properties that could potentially counteract the catabolic effect of cortisol. DHEAS secretion is reduced in morbid obesity, likely due to hyperinsulinemia, and laparoscopic adjustable gastric banding (LASGB), by inducing considerable and rapid weight loss, reduces insulin levels. To investigate the role of decreased insulin levels after LASGB-induced weight loss on DHEAS levels and on body composition changes, we studied 30 pre-menopausal morbidly obese women (BMI ranging 37-62 kg/m^2) before, 6, 12 and 24 months after LASGB. Total body water (TBW), fat-free mass (FFM) and fat mass (FM) were measured by bioelectrical impedance analysis; tissue hydration was also assessed by impedance vector analysis. At study ending, the subjects had a total weight loss of 28% of baseline body weight (15% after 6 months). After LASGB, weight loss was mainly due to decreased FM, and TBW, FFM, and body hydration were not significantly reduced. Weight loss was associated with an 82% rise in serum DHEAS already after 6 months while cortisol, cortisol/DHEAS molar ratio, and insulin levels fell by 5.5, 62 and 50%, respectively, after 6, 12 and 24 months ($p<0.05$). Conclusions: LASGB associated with a well balanced low-calorie diet permits a satisfactory 2-yr weight loss, sparing FFM and without body fluid alterations. As the result of a stable weight reduction program weight loss is associated to decrease in cortisol, cortisol/DHEAS molar ratio, and insulin plasma levels with marked rise in DHEAS. Higher cortisol/DHEAS molar ratio values at baseline are also associated to lower weight loss after LASGB, with lower decrease in FM and higher reduction in FFM and body cell mass, in spite of no differences in dietary regimes. Cortisol/DHEAS molar ratio is likely to represent a reliable marker of favourable modifications in body composition.
(J. Endocrinol. Invest. 28: 509-515, 2005)
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INTRODUCTION

Dehydroepiandrosterone (DHEAS) is the steroid secreted by the human adrenal glands in the highest amounts but its physiological relevance is so far controversial (1-3). Specific anti-obesity and anti-atherogenic effects of DHEAS have been reported, so linking DHEAS to hyperinsulinemia in obesity (4, 5). In fact, insulin levels may regulate DHEAS secretion (6). Both in aging and weight loss, two conditions known to modify circulating insulin levels and insulin sensitivity, disparate effects on DHEAS secretion in men and women have been observed. In fact, DHEAS levels are negatively correlated with age both in men and women but significantly lower in women, in obese women body mass index (BMI) is not correlated with DHEAS levels and DHEAS levels remarkably increase during weight loss only in obese men (7-9). DHEAS also exerts anti-glucocorticoid properties (10). Due to the weak androgenic effects of DHEAS in females, cortisol and DHEAS were proposed as agonist and antagonist pairs in the control of body composition (10-12).

Key-words: DHEAS, cortisol, morbid obesity, gastric banding, body composition, bioelectrical impedance analysis.

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Accepted January 17, 2005.

J. Endocrinol. Invest. 28: 509-515, 2005

Weight loss is associated with consistent changes in body composition. The ideal goal of weight reduction therapy in obesity is to obtain and maintain a reduction of fat mass (FM), without inducing significant loss of fat free mass (FFM) (13). Maintenance of FFM is of particular concern in obesity surgery, when large amounts of body weight (BW) are lost in morbidly obese patients because of the serious complications associated with rapid and prolonged weight loss (14-16). It is of general interest to increase the understanding of the mechanisms and consequences of significant weight loss after gastric restrictive procedures. The effect that prolonged sustained weight loss after gastric restrictive procedures displays on body composition in obese patients, and the long-term changes in body composition associated to DHEAS changes are still not well understood.
The aim of this study was to evaluate the effect of weight loss achieved by gastric restrictive procedures on circulating cortisol, DHEAS and insulin levels, and on body composition. This first aim is based on the evidence that gastric restrictive procedures do not induce malabsorption while inducing a less severe weight loss. Therefore, this open 24-month prospective study was designed to evaluate DHEAS/cortisol ratio, insulin levels and body composition in a group of morbidly obese pre-menopausal women undergoing laparoscopic silicone adjustable gastric banding (LASGB). This pilot study enrolled only pre-menopausal women to avoid gender and age differences in body composition, nutritional status and hormone concentrations.

MATERIALS AND METHODS

Patients

Thirty pre-menopausal morbidly obese patients (BMI ranging 37-62 kg/m^2) aged 36.7±6.4 yr (range 25-46 yr) agreed to participate in this pilot open prospective study. They were recruited a large series of morbidly obese women referring to the Endocrinology Unit from 2001 to 2003 and selection was based on the following criteria: 1) absence of thyroid diseases, diabetes mellitus, liver or renal failure, cancer, and acute or chronic inflammatory diseases based on a complete medical examination and laboratory investigations; 2) none of the patients was chronically treated with any type of medications; 3) normal menstrual pattern (i.e. at least 10 menstrual periods in the previous year, the last less than 60 days before the 1st examination); 4) eligibility for gastric banding so that patients who meet the diagnostic criteria for bulimia of the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DMS IV) were excluded; 5) absence of ulcers or malignancy at esophago-gastro-duodenoscopy performed before surgery. All subjects gave their informed consent to the study that was approved by the Ethics Committee of the Medical School of the "Federico II" University of Naples, Italy. The study was carried out in line with the ethical standards laid down in the appropriate version of the 1964 Declaration of Helsinki. Patients were operated with LASGB according to criteria of National Institutes of Health (NIH) (17). All patients underwent a comprehensive pre- and post operative psychiatric evaluation conducted by a psychotherapist who has expertise in bariatric patient management. This assessment included personal and social history, history of psychiatric problems, current living situation, and support system. None had any evidence of psychiatric diseases.

Study protocol

Patients were investigated before and 6, 12 and 24 months after LASGB (T0, T6, T12 and T24) by anthropometry evaluation, endocrine assessment and body composition analysis performed by conventional bioelectrical impedance analysis (BIA) and impedance vector analysis (BIVA). Daily calorie intake and diet composition were calculated during a personal interview using a detailed food-frequency questionnaire of 130 foods and beverages (18). Physical activity was encouraged. This consisted primarily of 60 to 90 min/day of moderate- intensity activity (e.g., brisk walking). Patients were also asked to record daily the amount of physical activity.
During follow-up, the diet was arranged to fit an energy intake of 5.6 MJ/day (55% carbohydrate, 25% fat, 20% protein, 30 g fiber). Individual requirements were estimated from resting metabolic rate and physical activity pattern. Resting energy expenditure was indirectly measured.
LAGB was performed with the Lap-Band™ System (Inamed Health, Santa Barbara, CA) according to Kuzmak (19) and Angrisani et al. (20). To minimize post-operative vomiting, the band was left completely unfilled at surgery (21). At discharge, patients were instructed to follow a solid diet containing an inventory of the foods permitted and a list of rules specifically developed for patients with gastric restriction (22). The band was tightened in case of weight stabilization (<4 kg of weight lost in the last month), providing that a solid food item per meal was ingested and a low vomiting frequency was observed (22). No more than one adjustment per month was performed and no more than 1.5 ml of sterile saline was added in each step. Band competence was always controlled with a barium swallow before and after the adjustment.

Anthropometry

All anthropometric measurements were made at the same time of the day with the subjects on a non-fasting day and wearing only light clothes without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BW was determined to the nearest 0.1 kg using a calibrated balance beam scale. BMI was calculated from weight and height (kg/m^2). The ideal body weight (IBW) was calculated according to Lorenz's formula:

$$IBW=height-100-(height-150/2).$$

Overweight was calculated as the difference between BW and IBW. The weight loss was expressed both in absolute (kg of the BW lost) and in relative (percentage of overweight lost: %OWL) terms.

Endocrine assessment

Cortisol, DHEAS and insulin levels were assayed during the early follicular phase, 5-7 days after spontaneous menses. Blood samples were obtained between 08:00 and 09:00 h from antecubital vein after an overnight fast, with the patient in the resting position. Blood samples for hormonal determinations were promptly centrifuged, and serum separated and stored at –20 C until assay. Serum insulin was measured by a solid-phase chemiluminescent enzyme immunoassay using commercially available kits (Immunolite Diagnostic Products Co, Los Angeles, CA, USA). Cortisol and DHEAS were measured by coated-tube radioimmunoassay (Diagnostic Systems Laboratories, Frankfurt, Germany). Hormone measurements were all performed in triplicate. The intra- and interassay coefficient of variations were less than 5.5%. When required, the conventional overnight dexamethasone suppression test was performed to exclude Cushing's syndrome.

Conventional BIA

Body composition was determined by conventional BIA and BIVA. BIA is a widely used, non-invasive, simple and inexpensive technique for estimating body composition in humans. Resistance (R) and reactance (Xc) were measured by a single investigator with a single-frequency 50 kHz BIA (BIA 101 RJL, Akern Bioresearch, Firenze, Italy) according to the standard tetrapolar technique, with the subject in supine position and the electrodes placed on the dorsal surface of right foot and ankle, and right wrist and hand. Body composition was calculated from bioelectrical measurements and anthropometric data by applying the software provided by the manufacturer, which incorporated validated predictive equations for total body water (TBW), fat mass (FM), fat free mass (FFM), and extra-cellular water (23, 24). Soft tissue hydration of individual subjects was evaluated by BIVA by using the BIVA software (Piccoli A, Pastori G, 2002, Department of Medical and Surgical Sciences, University of Padova, Italy - available on request at: apiccoli@unipd.it). R and Xc were normalized by the height of subjects (R/H and Xc/H) and the resulting vectors were plotted on a graph reporting the gender-specific 50th, 75th, and 95th tolerance ellipses of similar vectors calculated from a healthy reference population (25). According to the RXc graph method, vectors falling within the reference gender-specific 75th tolerance ellipse indicated normal hydration, short vectors (below the lower pole of the 75th tolerance ellipse) indicated over-hydration and long vectors (above the upper pole of the 75th tolerance ellipse) indicated under-hydration (25, 26). Vector position was also compared with the fat-fluid linear threshold discriminating between short vectors from either edematous or obese subjects falling out of the lower pole of the reference 75% tolerance ellipse, with vectors from obese subjects without edema expected to fall above the fat-fluid threshold and vectors from edematous patients expected to fall below the fat-fluid threshold (27). Vector length was calculated as $|Z|=\sqrt{[(R/H)2+(Xc/H)2]}$ and vector phase angle as the arctan of Xc/R.

Statistical analysis

Values are given as mean±SD. Repeated analysis of variance (ANOVA) measures with Bonferroni test for multiple comparisons were used for paired data. Pearson bivariate correlation analysis was used to compute correlation between variables. Data were stored and analysed using SPSS program (Statistical Package for Social Science, release 11.01; SPSS Chicago, IL, USA). *p* values <0.05 were considered statistically significant.

RESULTS

Dietary intake based on an interview-administered questionnaire was reported in Table 1. After surgery, a significant reduction of food intake was observed and the composition of the diet well suited the dietary prescriptions. Conversely, although at every control each patient received specific recommendations to exercise regularly, only four patients complied with their recommendations and engaged in a regular physical activity. Anthropometry and body composition of the patients before and after surgery were reported in Table 2. No post-operative complications occurred in these patients. Weight loss observed after LASGB was mainly due to a decrease in FM, whereas TBW and FFM did not significantly decrease. In particular, at T_6 subjects lost 15% of their initial BW, of which 18.5±6.5 kg of FM and 0.8±1.5 kg of FFM. Between 6 and 12 months, OWL was 11%, with a contribution of 11.0±8.6 kg FM and 2.4±4.0 kg FFM. During the following 12 months, BW loss was 2.7% of initial BW, lost FM was 2.0±3.9 kg and FFM was 1.2±3.1 kg.

The soft tissue hydration was evaluated according to the RXc graph method in Figure 1. Before surgery, no patients' vectors were below the boundary line threshold discriminating between the obese and the edematous, indicating a normal hydration. All vectors fell in the lower left quadrant, out of the boundary line of 75th tolerance ellipse, as expected in morbidly obese patients with normal hydration. The bioelectrical measurements (R, Xc, phase angle and length of the vectors) before and after surgery were reported in Table 2. Phase angle and Xc were

Table 1 - *Dietary intake based on interviewer-administered questionnaire in 30 morbidly obese patients before (T_0) and 6 (T_6), 12 (T_{12}) and 24 months (T_{24}) after laparoscopic adjustable gastric banding.*

	Baseline	T_6	T_{12}	T_{24}
Energy intake (MJ/day)	13.3±1.9	5.6±0.9*	5.7±1.2*	5.6±1.2*
Total carbohydrate (% energy)	45.6±9.4	55.2±9.1*	55.9±8.3*	55.3±7.8*
Total fat (% energy)	37.8±7.5	28.8±2.5*	25.2.±7.8*	25.4±6.3*
Total protein (% energy)	17.5±2.1	18.5±4.1	19.1±3.6	18.9±3.2

*p<0.05 *vs* T_0.

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Table 2 - *Anthropometry, body composition, by bioelectrical impedance analysis (BIA), electrical variables, by bioelectrical impedance vector analysis (BIVA), and endocrine parameters in 30 morbidly obese patients before (T_0) and 6 (T_6), 12 (T_{12}) and 24 months (T_{24}) after laparoscopic adjustable gastric banding (LASGB). Data are expressed as mean±SD.*

		After LAGB		
	T_0	T_6	T_{12}	T_{24}
Body weight (kg)	126.3±17.3	107.2±13.7*	93.8±7.3*	90.4±6.0*
Body mass index (kg/m^2)	48.4±7.1	40.9±5.3*	35.9±3.6*°	34.3±3.0*°
Fat mass (kg)	64.3±13.4	45.8±10.3*	34.8±4.9*°	32.3±3.3*°
Free fat mass (kg)	62.0±5.2	61.0±4.7	59.0±5.1	58.1±4.5
Body cell mass (kg)	34.3±6.8	32.2±4.4	30.6±8.2	30.3±3.6
Total body water (l)	42.3±3.5	43.1±4.5	42.8±3.8	41.9±3.3
Weight loss (%)	\	15.0±3.7	25.1±5.2°	27.6±6.4°
Resistance (Ohm)	443.3±54.1	439.9±53.5	446.8±69.7	451.3±55.4
Reactance (Ohm)	56.7±9.5	47.5±8.4*	47.2±5.9*	48.3±5.1*
Phase angle (°)	6.7±0.5	6.1±0.8*	6.2±0.2*	6.2±0.4*
Vector length (Ohm/m)	275±36	278±40	276±43	280±28
Cortisol (nmol/l)	506.4±34.1	478.5±32.8*	480.5±37.3*	480.0±31.6*
DHEAS (μmol/l)	2.7±0.8	5.0 ±0.6*	5.0 ± 0.5*	5.0±0.4*
Cortisol/DHEAS ratio	198.5±59.5	95.7±13.1*	94.4±9.7*	95.6±7.9*
Insulin (pmol/l)	279.8±89	107.4±29.8°	85.6±12.5*	82.3±13.0*

*$p<0.05$ *vs* T0; °$p<0.05$ *vs* T6; DHEAS: dehydroepiandrosterone.

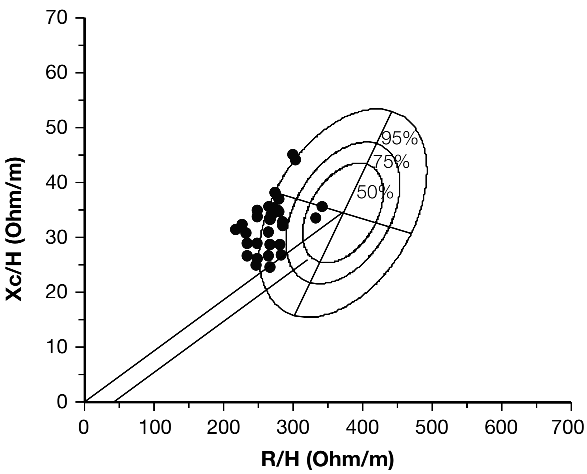
significantly reduced after surgery. However, no significant differences in R and in the vector length were observed, indicating no significant changes in the hydration status of the patients. None of the treated subjects dropped out of the study.

Both cortisol and insulin levels significantly decreased at T_6, T_{12} and T_{24} ($p<0.05$) (Table 2). In particular, cortisol and insulin levels decreased by 5.5 and 62%, respectively. Conversely, DHEAS increased at T_6, T_{12} or T_{24} ($p<0.05$) by 82%. Therefore, the cortisol/DHEAS molar ratio was reduced by 50% of the baseline after weight loss ($p<0.05$). As expected, at T_0 insulin levels were positively related with body weight (r=0.74, $p<0.01$), BMI (r=0.64, $p<0.01$), and cortisol levels (r=0.38; $p<0.05$). These correlations were still present after weight loss at any time. At T_0 DHEAS levels were inversely correlated with insulin (r=−0.56; $p<0.01$), as well as with cortisol (r=−0.56, $p<0.05$). These correlations disappeared after weight loss at any time.

Endocrine and anthropometric variables were also significantly correlated: at T_0 a significant positive correlation was found between the cortisol/DHEAS molar ratio and BMI or FM (r=0.93 and r=0.70, respectively; $p<0.01$), associated to an inverse correlation with FFM, body cell mass (BCM), resistance and reactance (r=−0.59, r=−0.56, r=−0.72, r=−0.60, respectively; $p<0.01$). All these correlations disappeared at T_{24}. The cortisol/DHEAS molar ratio, both at T_0 and T_{24}, was inversely correlated with the difference in %OWL and FM (r=−0.51 and r=−0.59, respectively; $p<0.01$) and positively correlated with the difference in FFM and BCM (r=0.61 and r=0.66, respectively; $p<0.01$).

DISCUSSION

In a group of pre-menopausal morbidly obese women, we show that weight loss obtained after LASGB associated with a well balanced low-calorie diet induced consistent and stable modifications of endocrine variables along with changes in body composition, evaluated by conventional BIA and BIVA, during a 2-yr follow-up. In particular, we observed that the highly significant 28% reduction in BW was associated to a reduction in circulating insulin, cortisol, and cortisol/DHEAS molar ratio, along with an increase in DHEAS, and was due to a prevalent FM loss associated to a limited FFM and BCM mobilization, without relevant changes in body hydration, as also indicated by the reduction in phase angle and Xc. This evidence agrees with a recent study in patients



R/H: resistance normalized by the height of subjects;
Xc/H: reactance normalized by the height of subjects.

Fig. 1 - Individual impedance vectors in 30 morbidly obese women before laparoscopic adjustable gastric banding were plotted on the reference 50th, 75th, and 95th tolerance ellipses of a healthy reference population. The mean impedance vector of the reference population is shown (arrow). The fat-fluid threshold is drawn below the mean reference vector (27).

treated by LASGB using absorptiometry and dilution methods (28), and is likely to be due to a satisfactory nutritional management in that a well balanced mildly hypocaloric diet was coupled to a more conservative post-operative band management (21, 22, 29, 30). Conversely, as the majority of our patients did not modify their sedentary lifestyle and remained persistently inactive, any expected role of physical activity on body composition or on hormonal secretion can be ruled out. Similarly, it is possible to rule out that subtle variations of the hydration of soft tissues can propagate errors in the prediction of body composition from the reference methods to the predictive equations used in conventional BIA. In fact, the unchanged length of the impedance vector indicated a normohydration status both at baseline and after surgery for all subjects in our study group (27, 30).
Obesity is associated with multiple endocrine and metabolic alterations, which have not only been considered as a consequence of obesity, but have also recently been shown to be strictly involved in the development of obesity, especially the abdominal phenotype (31, 32). Some endocrine abnormalities can be partially reversed after weight loss and many hormones are also involved in the complex network of the adaptive response to starvation (33). In particular, cortisol and DHEAS have been proposed to act as an agonist and antagonist pair in the control of body composition and FFM in severe pathologic conditions, the cortisol/DHEAS ratio being associated with increased protein catabolism (10, 12). On the other hand, DHEAS has recently been proposed as the possible missing link between hyperinsulinemia and obesity or atherosclerosis *via* the inhibitory effect of increased insulin on 17,20 lyase activity controlling DHEAS adrenal secretion (4, 6).
The endocrine changes observed in our study group are consistent with the favourable modifications in body composition after weight loss. In fact, significant reductions in cortisol, cortisol/DHEAS molar ratio, and insulin plasma levels and increase of DHEAS levels were obtained: these changes were already evident at T_6. In that, this confirms and extends our previous observation in a small cohort of severely obese premenopausal women, further suggesting a possible role of the obesity-related hyperinsulinemia in reducing DHEAS production *via* 17,20 lyase inhibition (34).
Taking into account the relationships of endocrine changes with body composition, at baseline we observed that higher cortisol/DHEAS molar ratio or lower DHEAS values were associated to higher body weight, BMI and FM and lower FFM or BCM, while after weight loss, only the correlations between DHEAS and body composition were still evident. This suggests that at baseline the cortisol effects on liposynthesis and protein catabolism are prevalent to the anabolic DHEAS action, such as those described in HIV-positive patients (12). After weight loss, endogenous DHEAS increases, likely linked to the decreased inhibition of insulin on 17,20 lyase activity (6, 35), and cortisol decreases, then the weak androgenic lipolytic and anabolic effect on protein synthesis of DHEAS could become more evident, making endogenous anti-glucocorticoid effects of DHEAS more likely to favorably act on body composition (10). In this context, it is of some interest that weight loss was also associated to the restoration of a positive correlation between cortisol and DHEAS. Higher cortisol/DHEAS molar ratio values at baseline are also associated to lower weight loss after LASGB, with lower decrease in FM and higher reduction in FFM and BCM, in spite of no differences in dietary regimes. This observation suggests that in our study group, cortisol/DHEAS molar ratio might represent a reliable marker of weight loss and of maintenance of FFM. In contrast, Hankey et al. (36) and Jakubowicz et al. (37) did not report any effect of weight loss on DHEAS metabolism. These discrepancies might been explained by the different characteristics of our study group, consisting of only severely obese women in pre-menopause, with a restricted age range, obtaining a sustained and prolonged reduction in body weight during a 2-yr follow-up. Conversely, the above-mentioned studies evaluated population groups of both women and men, with an older age and a lower BMI. In this con-

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text, again the strict age range of the study subjects might likely account also for the absence of the widely accepted influence of age on DHEAS (8, 9).

Although our study did not evaluate the early modification when the weight loss was more rapid and changes in fluid compartments were more likely to occur, one advantage of our study is, however, the length of the follow-up. At the best of our knowledge, there are no studies reporting hormonal changes and body composition modifications in surgically-induced weight loss in patients prospectively studied for as long as 24 months. In this context, the present study extends data obtained in a smaller cohort of morbidly obese patients of the same geographical area, treated with LASGB with a similar post-surgical nutritional management (30).

In conclusion, although weight loss results in multiple metabolic and endocrine alterations, our experience demonstrated that:

1) in morbidly, obese women treated with LASGB, with a 2-yr satisfactory weight loss due to a prevalent FM associated to a limited FFM and BCM mobilization, and without relevant changes in body hydration status, weight loss is associated to decrease in insulin that, in turn, is accompanied with marked rise in DHEAS. This gives rise to a more anabolic endocrine pattern as a marker of the favorable modifications in body composition observed in our study group;
2) weight loss in our group of obese patients, mainly due to FM loss, along with the endocrine changes observed, might enable them to maintain long-term reduced body weight.

The cortisol/DHEAS molar ratio, more than DHEAS levels, seems to represent a reliable marker of favorable modifications in body composition. Further studies, focusing on the immediate post-LASGB period in obese subjects could be helpful to clarify the possible relationships among endocrine variables, body composition and hydration status.

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