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Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers

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Summary

OBJECTIVE Recent findings demonstrating important effects of the adipokines on metabolism, energy homeostasis and body weight regulation have prompted research on the possible role of negative energy balance in altering adipocytokine regulation. The goal of this study was to evaluate the effects of a hypocaloric diet in healthy normal-weight volunteers. An additional goal was to help clarify the contribution of restricted caloric intake to altered plasma adipokine levels in the eating disorders anorexia nervosa and bulimia nervosa.

DESIGN Participants were studied before and after a 4-week reduced-calorie diet (1000–12000 kcal/day).

PATIENTS Subjects included 15 healthy, normal-weight women (age 22 ± 3 years).

MEASUREMENTS Plasma concentrations of leptin, soluble leptin receptor protein (sOB-R), adiponectin, resistin, thyroid hormones and β-hydroxybutyrate were determined following overnight fast before and after the 4-week reduced-calorie diet.

RESULTS Subjects lost a mean of 3·4 ± 2·1 kg in response to the reduced-calorie diet. The weight loss phase was associated with a 60·3% decrease in plasma leptin levels ($P < 0·001$), a 43·5% increase in sOB-R levels ($P < 0·002$) and a 16·2% decrease in plasma adiponectin levels ($P < 0·04$). There was no significant change in plasma resistin levels.

CONCLUSIONS These results demonstrate that a modest decrease in energy intake sustained over several weeks may play an important role in altering levels of plasma leptin and sOB-R. The findings also provide preliminary evidence that, in contrast to previous results in obese subjects, caloric restriction with accompanying weight loss in healthy, normal-weight volunteers may lead to decreased circulating adiponectin levels. Additional studies will be needed to clarify the contribution of altered energy intake to abnormalities in cytokine levels in the eating disorders.

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The goal of this study was to assess the extent to which a 4-week reduced-calorie diet in healthy, normal-weight women is associated with alteration in circulating concentrations of leptin, the soluble leptin binding protein (the short form of the leptin receptor, sOB-R), adiponectin and resistin. Studies in normal-weight volunteers are of particular relevance in understanding physiological responses to dieting, and in interpreting studies of these metabolic regulators in patients with eating disorders, given that changes in food intake and body weight are common in these patient groups (American Psychiatric Association Workgroup on Eating Disorders, 2000).

Initial studies in obese individuals demonstrated that restriction of energy intake and weight loss is associated with decreased serum leptin levels (Maffei *et al.*, 1995). In healthy volunteers who observed a short-term fast, there was a decrease in circulating leptin levels that was disproportionately large relative to the extent of weight loss (Boden *et al.*, 1996; Kolaczynski *et al.*, 1996). Additionally, moderate calorie restriction resulted in a significant decrease in leptin levels in healthy volunteers (Dubuc *et al.*, 1998; Chin-Chance *et al.*, 2000), although the effect of more extended dieting has been studied less extensively. The bioactivity of circulating leptin may be affected by changes in the serum concentration of sOB-R (Brabant *et al.*, 2000; Lammert *et al.*, 2001). Currently, there is little information available on the effect of modest caloric restriction on sOB-R levels in healthy volunteers.

Adiponectin, a 244 amino acid protein derived from adipose tissue, is released into the circulation and appears to enhance insulin sensitivity and improve lipid metabolism (Diez & Iglesias, 2003). Plasma adiponectin levels are inversely correlated with body mass index (BMI) and body fat in healthy volunteers (Arita *et al.*, 1999; Hotta *et al.*, 2000), and with central fat distribution/intra-abdominal fat mass (Cnop *et al.*, 2003; Gavrila *et al.*, 2003a). Decreased adiponectin levels have been found in obesity (Arita *et al.*, 1999) and in type-2 diabetes (Hotta *et al.*, 2000; Weyer *et al.*, 2001). In obese and diabetic patients, weight loss is associated with an increase in plasma adiponectin levels (Hotta *et al.*,

2000; Yang *et al.*, 2001; Monzillo *et al.*, 2003). In a recently published study, however, caloric deprivation for 2 days was not associated with changes in serum adiponectin levels (Gavrila *et al.*, 2003a).

Resistin, another recently identified adipose-tissue-derived hormone, is correlated with body fat mass (Yannakoulia *et al.*, 2003), and has been linked to insulin resistance in animals (Kim *et al.*, 2001; Way *et al.*, 2001), although not in humans (Lee *et al.*, 2003). No previous study has reported the effects of caloric restriction and/or weight loss on circulating resistin levels in healthy, normal-weight humans.

Circulating levels of adipokines in patients with eating disorders are likely to be influenced by frequent variations in meal patterns and fluctuations in body weight. Circulating levels of leptin in patients with anorexia nervosa are markedly reduced in comparison to healthy, normal-weight controls (Hebebrand *et al.*, 1995; Grinspoon *et al.*, 1996; Mantzoros *et al.*, 1997). In patients with bulimia nervosa, circulating leptin levels are also significantly lower than in controls (Brewerton *et al.*, 2000; Jimerson *et al.*, 2000; Monteleone *et al.*, 2000). Initial reports indicate that adiponectin levels are increased in anorexia nervosa (Delporte *et al.*, 2003; Iwahashi *et al.*, 2003; Pannacciulli *et al.*, 2003). While it is possible that this increase could be related to caloric restriction and/or weight loss *per se*, it is of note that little information has been published on the effects of weight loss in normal-weight subjects.

Methods and materials

Subjects

Subjects included 15 medication-free women with a mean age of 22 ± 3 years. Participants were in a normal-weight range (BMI 23·3 ± 1·3 kg/m^2) and denied recent dieting behaviours. Subjects were medically healthy as assessed by history and physical examination, and free of current or past history of obesity and major psychiatric illness (including eating disorders) as assessed by a semistructured research interview. The study was approved by the institutional human protections committee and written informed consent was obtained from subjects prior to study enrolment.

Procedures

Subjects participated in 3 outpatient assessment visits at 4-week intervals on an NIH-funded General Clinical Research Center. Following the baseline visit, subjects were instructed to follow their usual dietary intake on an *ad libitum* basis for the next 4 weeks. Subjects were then asked to follow a 4-week reduced-calorie diet. This diet provided 1000–1200 kcal/day (44% of calories as carbohydrate and 25% of calories as fat), with a projected weight loss of approximately 1 kg/week. Subjects were asked to maintain their usual daily activity level throughout the study. Study visits were scheduled to occur during the follicular phase of three consecutive menstrual cycles. For 2 subjects, post-dieting testing occurred during the luteal phase due to delay of their menstrual cycle.

Study procedures took place in the morning, with subjects having fasted since the previous evening. Height and body weight were measured, and blood samples were obtained for measurement of metabolic and related hormonal variables. Plasma samples were stored at –70 °C until assayed. The current report is a follow-up on a previous study demonstrating that modest weight loss in the healthy female volunteers was associated with a significant decrease in plasma tryptophan concentrations (Wolfe *et al.*, 1997).

Laboratory methods

Plasma leptin, adiponectin and insulin levels were measured by radioimmunoassay (RIA; Linco Research Inc., St Charles, MO, USA), as previously described (Jimerson *et al.*, 2000; Gavrila *et al.*, 2003b). Plasma concentrations of sOB-R and resistin were measured by an enzyme-linked immunosorbent assay (ELISA; Biovendor Laboratory Medicine Inc., Brno, Czech Republic), as described in a previous report (Yannakoulia *et al.*, 2003). Serum thyroid hormones were measured by fluoroimmunoassay [3,5,3′triiodothyronine (T3), free thyroxin (fT4), and thyroid stimulating hormone (TSH)] or by RIA (reverse T3), and plasma β-hydroxybutyrate (B-HBA) was analysed using a 3-hydroxybutyrate dehydrogenase enzymatic method.

Statistical analysis

Results are presented as mean ± SD in the text, and mean ± SEM in Figure 1. Variables not normally distributed (leptin, resistin, T3, reverse T3, fT4, B-HBA) based on the Kolmogorov–Smirnoff Test with Lilliefors correction were log- or square root-transformed to normalize distribution prior to parametric testing, or were analysed by nonparametric methods, as indicated. Data obtained from the initial baseline visit were used for comparisons with outcome measures following the study diet. Changes in outcome measures were assessed using paired *t*-test or Wilcoxon signed ranks test. Relationships between variables of interest were assessed by Pearson correlation coefficient, or by Spearman rank order correlation (rho) for variables not normally distributed. In assessing the relationship between the change in plasma leptin and change in plasma sOB-R following dieting, the correlation was adjusted to control for BMI. A two-tailed alpha level of 0·05 was used to determine significance following Bonferroni adjustment to control for comparison of both the reduced-calorie and ad libitum dietary phase measurements with baseline values. Data was analysed using SPSS Software for Windows^{TM} (Chicago, IL, USA).

Results

As previously reported (Wolfe *et al.*, 1997), mean BMI for the study subjects was 23·3 ± 1·2 kg/m^2 at baseline, 23·2 ± 1·0 kg/m^2 at the end of the *ad libitum* dietary phase, and 22·1 ± 1·2 kg/m^2 at the end of the reduced-calorie diet phase. Thus, there was a significant decrease in body weight (3·4 ± 2·1 kg, $P < 0·0001$) associated with the reduced-calorie diet, while weight did not change significantly during the *ad libitum* dietary phase.

Leptin and sOB-R

Plasma leptin levels were significantly correlated with BMI at the postdieting visit ($r = 0·63$, $P < 0·02$), although not at baseline ($r = 0·26$, $P =$ ns). Following the reduced-calorie diet, plasma leptin levels decreased significantly by 60·3% (Table 1; Fig. 1a). The decrease in leptin was correlated with the decrease in BMI ($r = 0·61$, $P < 0·02$).

Plasma sOB-R levels were significantly correlated with BMI at baseline ($r = −0·65$, $P < 0·01$) and following dieting ($r = −0·87$, $P < 0·001$). Dieting was associated with a significant 43·5% increase in sOB-R levels (Table 1; Fig. 1b). The increase in sOB-R was inversely correlated with change in BMI ($r = −0·72$, $P < 0·003$). The decrease in plasma leptin associated with dieting was correlated with the concurrent increase in plasma sOB-R levels ($r = −0·55$, $P < 0·04$). This relationship no longer reached significance, however, after covarying for change in BMI ($r = −0·20$, $P =$ ns).

Adiponectin and resistin

Plasma adiponectin concentrations obtained at baseline and following the 4-week reduced-calorie diet were not significantly correlated with the respective BMI measurements ($r = 0·18$, $P =$ ns; $r = 0·37$, $P =$ ns). Following the dieting phase, adiponectin levels significantly decreased by 16·2% from baseline values (Table 1; Fig. 1c). The decrease in adiponectin levels following dieting was not significantly correlated with change in BMI ($r = −0·16$, $P =$ ns).

Plasma concentrations of adiponectin were not significantly correlated with corresponding leptin values at baseline ($r = 0·12$, $P =$ ns) or at the postweight loss visit ($r = 0·28$, $P =$ ns). Similarly, plasma concentrations of adiponectin were not significantly correlated with the corresponding sOB-R-values at baseline ($r = −0·35$, $P =$ ns) or at the postweight loss visit ($r = −0·25$, $P =$ ns). The decrease in adiponectin levels following dieting was not significantly correlated with the decrease in leptin values ($r = −0·05$, $P =$ ns).

Plasma resistin concentration did not change significantly from baseline to postdieting testing (Fig. 1d).

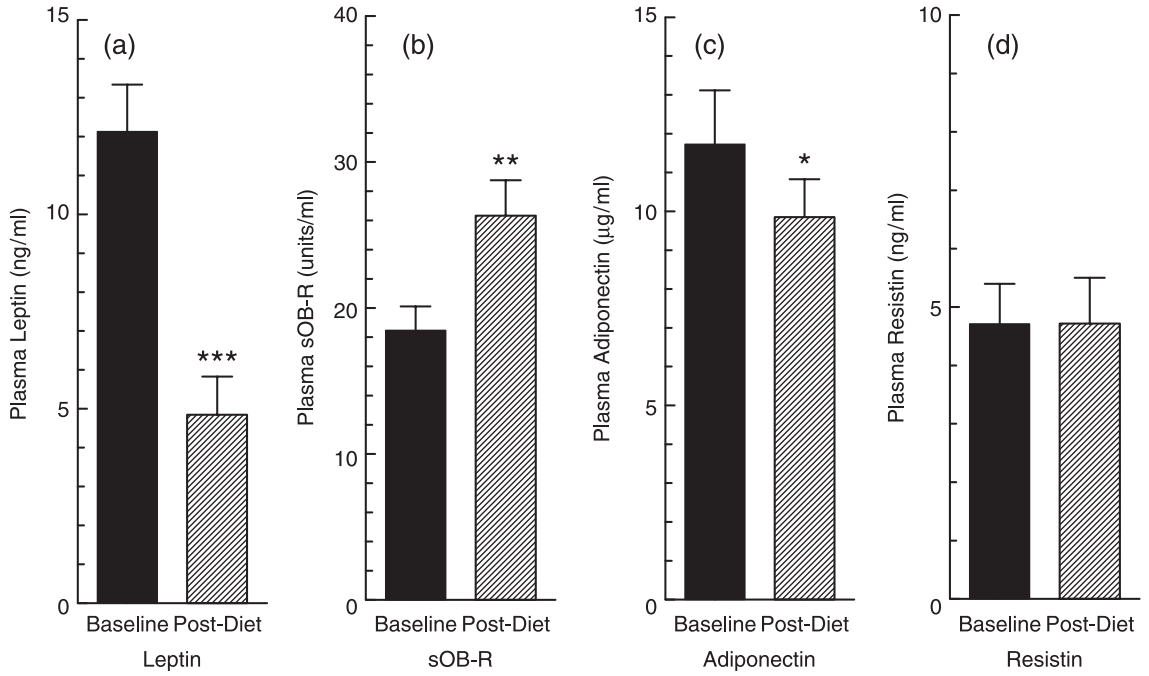

Fig. 1 Changes in leptin (a), sOB-R (b), adiponectin (c) and resistin (d) in healthy, normal-weight female volunteers following a 4-week hypocaloric diet. *$P < 0·04$, **$P < 0·002$, ***$P < 0·001$.

Table 1 Comparison of adipokine and hormone levels for baseline (BL), *ad libitum* diet and reduced-calorie diet (RCD) study phases

Hormone	Baseline	*Ad libitum* diet*	Reduced-calorie diet	BL *vs.* RCD	BL *vs.* RCD
Leptin (ng/ml)†	12·1 ± 4·7	10·5 ± 7·0	4·8 ± 3·8	$t = 5·78$	$P = 0·001$
sOB-R (units/ml)	18·5 ± 6·3	19·4 ± 5·7	26·3 ± 9·4	$t = 4·20$	$P = 0·002$
Adiponectin (μg/ml)	11·7 ± 5·4	10·6 ± 4·4	9·8 ± 3·8	$t = 2·64$	$P = 0·04$
Resistin (ng/ml)‡	4·7 ± 2·7	4·7 ± 2·3	4·7 ± 3·0	$z = 0·35$	P = ns
B-HBA (mg/dl)‡	1·0 ± 0·8	0·6 ± 0·4	3·4 ± 2·7	$z = 3·07$	$P = 0·004$
T3 (ng/dl)‡	141·7 ± 90·4	111·7 ± 64·0	127·3 ± 86·4	$z = 2·61$	$P = 0·02$
reverse T3 (ng/ml)‡	0·17 ± 0·03	0·16 ± 0·04	0·19 ± 0·05	$z = 1·97$	$P = 0·10$
fT4 (ng/dl)‡	0·83 ± 0·22	0·73 ± 0·17	0·75 ± 0·24	$z = 0·94$	P = ns
TSH (μU/ml)	1·79 ± 0·92	1·62 ± 0·93	1·75 ± 0·77	$t = 0·20$	P = ns
Cortisol (μg/dl)	16·9 ± 5·0	16·7 ± 6·1	19·4 ± 7·1	$t = 1·57$	P = ns
Insulin (μU/ml)	9·6 ± 3·7	10·0 ± 3·8	8·5 ± 3·8	$t = 1·21$	P = ns

*There were no significant differences between values for baseline and *ad libitum* diet phases. †Values were log-transformed prior to paired *t*-test. ‡Wilcoxon signed ranks test was used for statistical comparisons.

Other metabolic indices

In comparison to baseline values, the reduced-calorie weight loss phase was associated with a significant decrease in serum T3, and a significant increase in plasma B-HBA (Table 1). The dieting phase was not associated with significant changes in plasma insulin, fT4, reverse T3, TSH or serum cortisol concentrations.

The decrease in leptin levels associated with the reduced-calorie diet was not significantly correlated with the corresponding alterations in T3 levels (rho = 0·24, P = ns) or in B-HBA levels (rho = –0·15, P = ns). There were no significant changes in circulating concentrations of leptin, sOB-R, adiponectin, resistin, B-HBA, insulin, cortisol, or thyroid hormones following the 4-week *ad libitum* dietary intake phase (Table 1).

Discussion

Results of this study show that a 4-week reduced-calorie diet results in highly significant alterations in circulating concentrations of leptin and sOB-R, a modest decrease in adiponectin, and no significant change in resistin levels in healthy, normal-weight women.

With respect to the effect of modest caloric restriction on leptin levels, an initial study showed that a 3 day diet with a 30% restriction in energy intake in healthy male volunteers resulted in a 22% decrease in leptin levels (Chin-Chance *et al.*, 2000). The current study extends these findings by showing that a modest reduction in energy intake over 4 weeks in healthy women was associated with a marked 60·3% decrease in plasma leptin levels and that, in spite of the relatively narrow range of weight change, there was a highly significant positive correlation between change in leptin and change in body weight.

In previous studies, plasma sOB-R concentrations did not change significantly in women studied after 22 h of fasting (Landt *et al.*, 2001), but did increase in male volunteers following a 72 h fast (Chan *et al.*, 2002), and in obese patients following weight loss (Laimer *et al.*, 2002). This study provides new data demonstrating that a more prolonged, less pronounced energy restricted diet increases circulating levels of sOB-R in healthy, normal-weight female volunteers. This finding suggests that the increase in sOB-R levels observed in anorexia nervosa may be a result of energy restriction and weight loss (Kratzsch *et al.*, 2002; Krizova *et al.*, 2002; Monteleone *et al.*, 2002).

It is of interest that in healthy, normal-weight women, a reduced-calorie diet was associated with a modest but significant decrease in plasma adiponectin values. These results contrast with previous studies in obese or diabetic subjects showing an increase in plasma adiponectin following weight loss. Hotta *et al.* (2000) studied the effect of weight loss on nondiabetic obese subjects (6 men and seven postmenopausal women, BMI 36·8 ± 1·2, age 45 ± 5 years). These subjects lost 10 ± 1% of BMI over 2 months of inpatient treatment on a low calorie diet. Plasma adiponectin levels increased significantly by 42 ± 13%. A subsequent study showed that a 21% weight-reduction resulting from gastric partition surgery (7 men and 15 females, BMI 39·6 ± 5·9, age 34·0 ± 11·4 years) was associated with a 46% increase in adiponectin levels (Yang *et al.*, 2001). An increase in adiponectin levels has also been reported following gastric bypass surgery in morbidly obese patients (Faraj *et al.*, 2003). It is of note, however, that adiponectin levels did not change with moderate dietary-induced weight loss over 6 months in overweight and obese subjects (Ryan *et al.*, 2003), including obese subjects with insulin resistance (although adiponectin did increase with weight loss in diabetic obese subjects; Monzillo *et al.*, 2003).

Differences in the effects of weight loss on plasma adiponectin levels may be mainly related to the nature of the subject groups (obese *vs.* lean), as well as amount and speed of weight loss. Recent

preclinical data suggest that macronutrient composition may also influence plasma adiponectin levels (Naderali *et al.*, 2003). Adiponectin levels are inversely correlated with central adipose tissue stores (Addy *et al.*, 2003; Cnop *et al.*, 2003; Gavrila *et al.*, 2003a; Staiger *et al.*, 2003) and positively correlated with subcutaneous adipose tissue (Addy *et al.*, 2003). Thus, it is possible that loss of visceral fat may result in increased adiponectin levels with weight loss in obese individuals. In contrast, predominant loss of subcutaneous adipose tissue, as would be expected in lean healthy subjects, may result in decreased adiponectin levels, as seen in this study. Additional studies incorporating measurements of regional body composition will be needed to confirm these data. In comparison to the findings of the current study in healthy volunteers, it may be that more severe and protracted restriction of energy intake and loss of body weight can contribute to the increased levels of adiponectin recently described in anorexia nervosa (Delporte *et al.*, 2003; Iwahashi *et al.*, 2003; Pannacciulli *et al.*, 2003).

Little is known about the effects of weight loss on resistin regulation. To our knowledge, this study provides the first evidence that modest energy restriction and weight loss is not associated with alteration in circulating resistin levels in healthy, normal-weight volunteers. These findings are similar to results in obese subjects with insulin resistance placed in a behaviour modification programme to induce weight loss (Monzillo *et al.*, 2003), and to results for healthy lean subjects exposed to caloric deprivation for 2 days (Lee *et al.*, 2003).

For comparison with other studies, plasma levels of thyroid hormones, B-HBA and insulin were also measured. The decrease in T3 and increase in B-HBA levels observed in this study reflect the anticipated effects of restricted energy intake and weight loss. Previous data have demonstrated that fasting-induced decreases in circulating leptin are likely to play a role in alterations in the hypothalamic–pituitary–gonadal axis and the hypothalamic–pituitary–thyroid axis associated with weight loss (Ahima *et al.*, 1996; Chan *et al.*, 2003). Based on these observations, it seems likely that low circulating leptin levels contribute to amenorrhea and thyroid hormone alterations observed in anorexia nervosa. This proposal, as well as the potential physiological significance of previously reported alterations in sOB-R and adiponectin levels, require additional evaluation in future studies.

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