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Leptin, Thyrotropin, and Thyroid Hormones in Obese/Overweight Women Before and After Two Levels of Energy Deficit

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The aim of our study was to compare serum concentration of leptin and pituitary–thyroid axis hormones in obese/overweight women before and after two levels of energy deficit with those parameters in lean women on adequate energy intake. Additionally, we attempted to elucidate if the effect of weight reduction could be related to anthropometric and hormonal parameters before treatment. Anthropometric and hormonal parameters—serum leptin, TSH, T4, fT4, T3 and leptin to fat mass (Lep/fm), T3/T4, fT4/T4, T4/TSH, fT4/TSH —were compared in two groups of women (*n* = 18 each) —lean women (C: BMI 22.0 ± 1.2) and overweight/obese (Ov/Ob: BMI 29.9 ± 3.3). Ov/Ob women were subjected to weight-reducing treatment consisting of energy intake equal to 80% of calculated total energy expenditure for the first 4 wk and to 50% for subsequent 4 wk. All baseline hormone concentrations, Lep/fm, and fT4/T4 were higher in overweight/obese group. After 20% energy deficit decrease in BMI, percent body fat (fm%), leptin, T3, and TSH serum concentrations as well as in Lep/fm and T3/T4 was observed; T4/TSH increased, fT4, fT4/T4 and fT4/TSH did not change significantly. Increase in energy deficit from 20% to 50% resulted in normalization of Lep/fm, on the other hand, it provoked greater decline in thyroid hormone plasma concentration, which could hinder further mass reduction. Leptin and TSH levels were positively correlated after 50% energy deficit treatment. Changes in fm% were directly related to baseline T4/TSH, fT4/TSH, and log TSH. In conclusion, TSH serum concentration and its ratio to T4 and fT4 before weight reduction could be a good predictor of successful weight loss.

Key Words: Fat mass; energy deficit; leptin, triiodothyronine; thyroxine; thyrotropin.

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Introduction

Thyroid hormones and leptin play essential roles in regulating body energy balance. They exert similar, stimulating effect on energy expenditure using the sympathetic system as an amplifier *(1,2)*. Shortage of energy supply elicits adaptation in physiology of leptin and hormones of the hypothalamic–pituitary–thyroid (HPT) axis leading to diminution of energy expenditure. This adaptive process contributes to major obstacles in successful weight loss. Changes in HPT axis comprise decrease in TRH *(3,4)* and TSH synthesis *(3,5)*, decline in TSH *(5)*, as well as total and free T4 and T3 plasma concentrations *(4,6)*. Decrease in plasma leptin has been demonstrated beginning from the early phase of energy restriction, implying that it is not the result of fat mass reduction *(7,8)*. Declines in leptin receptor number and tissue responsiveness during fasting were also evidenced *(9)*.

The mutual relationship between leptin and HPT axis in humans has been the subject of numerous investigations, which have not brought unequivocal response. Several studies have shown that neither chronic hypo-, hyperthyroidism nor acute T3 treatment has affected serum leptin levels *(10,11)*. Others have shown a clear effect of thyroid hormone level on leptin secretion. However, the results of the later studies are contradictory with respect to the influence of thyroid hormones on leptin secretion *(12–15)*. Administration of TRH was found to evoke the decrease in plasma leptin level in women *(16)*. On the other hand, influence of leptin on HPT axis activity was shown to be stimulatory, because leptin injections reversed the effect of weight reduction on thyroid hormone level *(17)*. All these data suggest existence of hypothalamo–pituitary–thyroid–leptin axis with stimulatory influence of leptin on TRH and thyroid hormone production and the inhibitory effect of TRH and thyroid hormones on leptin secretion.

The aim of our study was an attempt to compare the effects of two levels of energy restriction on serum concentration of leptin and pituitary–thyroid axis hormones. Additionally, we intended to elucidate if the effect of weight reduction could be correlated with anthropometric and hormonal parameters before treatment.

Table 1
Comparison of Normal-Weight and Obese/Overweight Women (Mean ± SD)

Variable	Normal-weight women	Obese/overweight women		
		Before weight loss	After 20% energy deficit	After 50% energy deficit
BMI (kg/m^2)	22.0 ± 1.2	29.9 ± 3.3*	29.5 ± 3.1*	28.8 ± 3.1*
Weight (kg)	60.4 ± 4.4	78.7 ± 11.0*	77.6 ± 10.5*	75.9 ± 9.9*
Percentage body fat	26.8 ± 3.2	39.4 ± 3.5*	38.0 ± 3.9*	36.6 ± 4.5*
Percentage fat free mass	73.2 ± 3.2	60.6 ± 3.5*	62.0 ± 3.9*	63.4 ± 4.5*
T3 (nmol/L)	1.53 ± 0.26	2.43 ± 0.65*	2.08 ± 0.50*	1.66 ± 0.45
T4 (nmol/L)	112.61 ± 19.18	162.23 ± 14.04*	155.51 ± 13.79*	150.39 ± 13.24*
FT4 (pmol/L)	11.70 ± 2.68	20.93 ± 4.23*	19.42 ± 4.29*	18.85 ± 4.00*
TSH (mIU/L)	1.04 ± 0.39	1.86 ± 1.18*	1.39 ± 0.59*	1.30 ± 0.66
T3 /T4	0.014 ± 0.002	0.015 ± 0.004	0.013 ± 0.003	0.011 ± 0.003*
T4/TSH	119.56 ± 36.67	121.63 ± 71.73	143.61 ± 94.53	156.73 ± 113.41
FT4/TSH	12.67 ± 5.04	16.34 ± 11.71	18.64 ± 15.33	20.38 ± 16.27
FT4/T4	0.105 ± 0.025	0.129 ± 0.021*	0.125 ± 0.025*	0.125 ± 0.024*
Leptin (ng/mL)	9.91 ± 3.18	28.44 ± 11.22*	24.60 ± 9.76*	19.84 ± 8.04*
Leptin/fat mass	0.617 ± 0.199	0.931 ± 0.398*	0.837 ± 0.313*	0.717 ± 0.259

*Difference between normal-weight and obese/overweight subjects, independent samples *t*-test, $p < 0.05$.

Table 2
Changes in Anthropometric and Biochemical Variables in Obese/Overweight Women (Mean ± SD)

Changes in	Week 4 to baseline	Week 8 to week 4	Week 8 to baseline
BMI (kg/m^2)	–0.39 ± 0.62*	–0.66 ± 0.53**	–1.05 ± 0.94*
Weight (kg)	–1.09 ± 1.63*	–1.72 ± 1.46**	–2.81 ± 2.61**
Percentage body fat	–1.38 ± 0.97**	–1.41 ± 1.18**	–2.79 ± 1.49**
Percentage fat free mass	1.38 ± 0.97**	1.42 ± 1.18**	2.79 ± 1.49**
T3 (nmol/L)	–0.34 ± 0.49*	–0.51 ± 0.63*	–0.77 ± 0.60**
T4 (nmol/L)	–6.72 ± 11.70*	–5.12 ± 15.75	–11.84 ± 17.36*
FT4 (pmol/L)	–1.51 ± 4.92	–0.57 ± 2.86	–2.08 ± 5.55
TSH (mIU/L)	–0.469 ± 0.712*	–0.092 ± 0.328	–0.561 ± 0.647*
T3 /T4	–0.0016 ± 0.0033	–0.0029 ± 0.0041*	–0.0039 ± 0.0039**
T4/TSH	21.99 ± 46.94	13.12 ± 33.94	35.10 ± 64.87*
FT4/TSH	2.30 ± 8.44	1.75 ± 5.06	4.04 ± 9.71
FT4/T4	–0.0037 ± 0.0257	0.0005 ± 0.0251	–0.0033 ± 0.0347
Leptin (ng/mL)	–3.84 ± 5.08*	–4.76 ± 4.83**	–8.61 ± 6.78**
Leptin/fat mass	–0.0942 ± 0.18*	–0.1204 ± 0.16*	–0.2146 ± 0.24*

*Change is significantly different from zero (paired samples *t*-test), at $p < 0.05$.
**Change is significantly different from zero (paired samples *t*-test), at $p = 0.001$.

Results

Before treatment concentrations of TSH, T4, T3, fT4, and leptin in serum as well as Lep/fm and fT4/T4 were higher in Ov/Ob women (Table 1).

In the Ov/Ob group fm% correlated positively with leptin ($r = 0.60$, $p = 0.008$). Correlation between serum leptin concentration and fm in lean women did not reach statistical significance.

After 4 wk of 20% energy deficit, BMI and fm% declined significantly, whereas percentage fat free mass (ffm%) was increased. Significant decrease also occurred in serum T3, T4, TSH, and leptin concentrations, as well as in Lep/fm ratio; however, all those values remained significantly different from those observed in normal weight women (Table 2). Percentage body fat correlated positively with leptin ($r = 0.64$; $p = 0.004$).

When the periods of 20% and 50% energy deficit were compared, significant declines in weight, BMI, fm%, and serum T3 and leptin concentrations as well as T3/T4 and Lep/fm ratios were observed (Table 2).

After 50% energy deficit, serum T3, T4, TSH, and leptin concentrations as well as Lep/fm and T3/T4 were signifi-

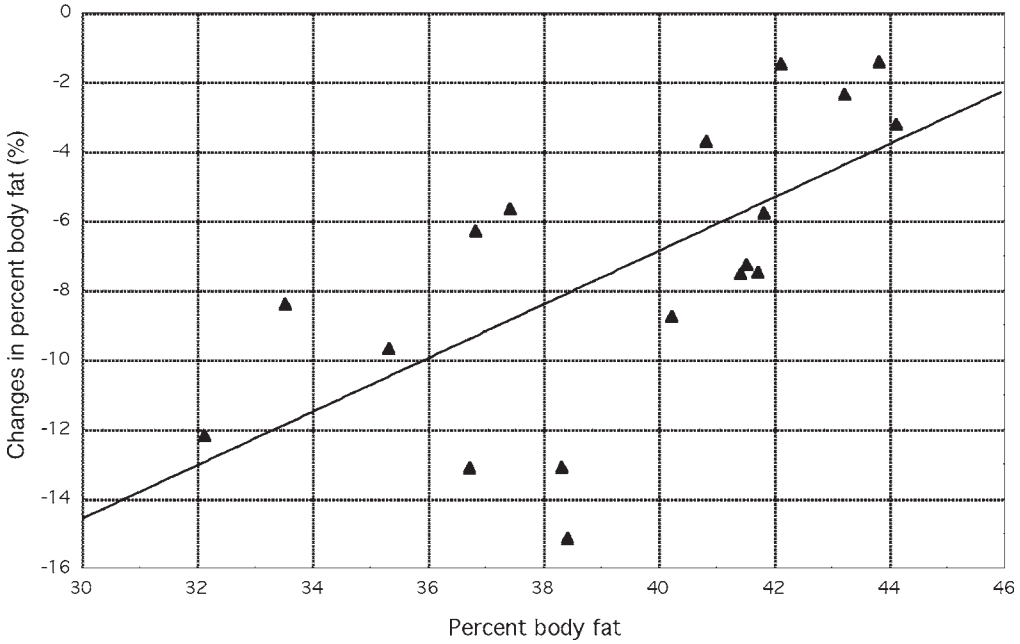

Fig. 1. Correlation of pretreatment percentage body fat and percentage body fat change after 4 wk of 50% energy deficit ($r = 0.66$; $p = 0.003$).

cantly lower as compared to the pretreatment values, whereas significant increase in T4/TSH was observed (Table 2). Serum T3 and TSH levels and Lep/fm after 50% energy deficit reached values that did not differ from those observed in women with ideal body weight, whereas T4, fT4, and leptin concentrations as well as fT4/T4 ratio remained higher in Ov/Ob women. Ratio T3/T4 became significantly lower after 50% deficit in Ov/Ob group (Table 1). Percent body fat correlated positively with leptin ($r = 0.56$; $p = 0.016$). Positive correlation was found also between TSH and leptin ($r = 0.52$; $p = 0.027$).

Analysis of relationships between baseline values and changes evoked by treatment revealed significant dependence of alterations of both body mass and hormone concentrations on initial values. Percentage body fat before treatment was directly related to the decline in percentage body fat after 50% energy deficit ($r = 0.66$; $p = 0.003$) (Fig. 1). We also observed direct relations between initial log TSH and decline in fat mass after 50% energy restriction ($r = 0.73$; $p = 0.001$) (Fig. 2). Baseline T4/TSH and fT4/TSH were negatively correlated with the decrease in fat mass after 20% and 50% energy deficit periods (for T4/TSH $r = –0.59$; $p = 0.010$ and $r = –0.76$; $p = 0.000$; for fT4/TSH $r = –0.60$; $p = 0.009$ and $r = –0.73$; $p = 0.001$, respectively) (Figs. 3 and 4). Similarly, changes in pituitary–thyroid axis hormones after dietary treatment were inversely related to their levels before treatment. Initial TSH level was inversely related to its alteration after 20% energy restriction ($r = –0.60$; $p = 0.009$). Baseline T3 plasma concentration was negatively correlated with changes in T3 concentration after 20% and 50% energy restriction ($r = –0.62$; $p = 0.007$ and $r = –0.55$; $p = 0.023$). Total T4 level before treatment correlated negatively with its relative value after 50% energy deficit ($r = –0.62$; $p = 0.006$). Initial free thyroxine values were inversely related to its changes in concentration both after 20% ($r = –0.49$; $p = 0.040$) and 50% energy deficit ($r = –0.63$; $p = 0.006$). Leptin was the only hormone decrease that was not related to its level before treatment.

Discussion

Before the weight-reducing treatment, leptin, thyrotropin, and thyroid hormone serum concentrations in overweight/obese women exceeded those found in normal weight women. In human and animal studies increase in circulating T3 concentration concomitantly with augmentation of TSH response to TRH stimulation have been found to be the adaptation to overfeeding *(18,19)* through the increase in metabolic rate with TSH response to TRH stimulation being directly related to metabolic rate *(18)*.

Energy restriction affected TSH and thyroid hormone levels in different degrees. Greater decrease in TSH and T3 serum concentrations than in T4 serum level resulting in diminishing of T3/T4 ratio, which became significantly lower than in normal weight women, may reflect the decline in TSH secretion concomitantly with diminution in peripheral deiodination. Such pattern of changes in thyroid activity induced by energy restriction is consistent with findings reported by others *(20–22)*. Apart from lowering of energy

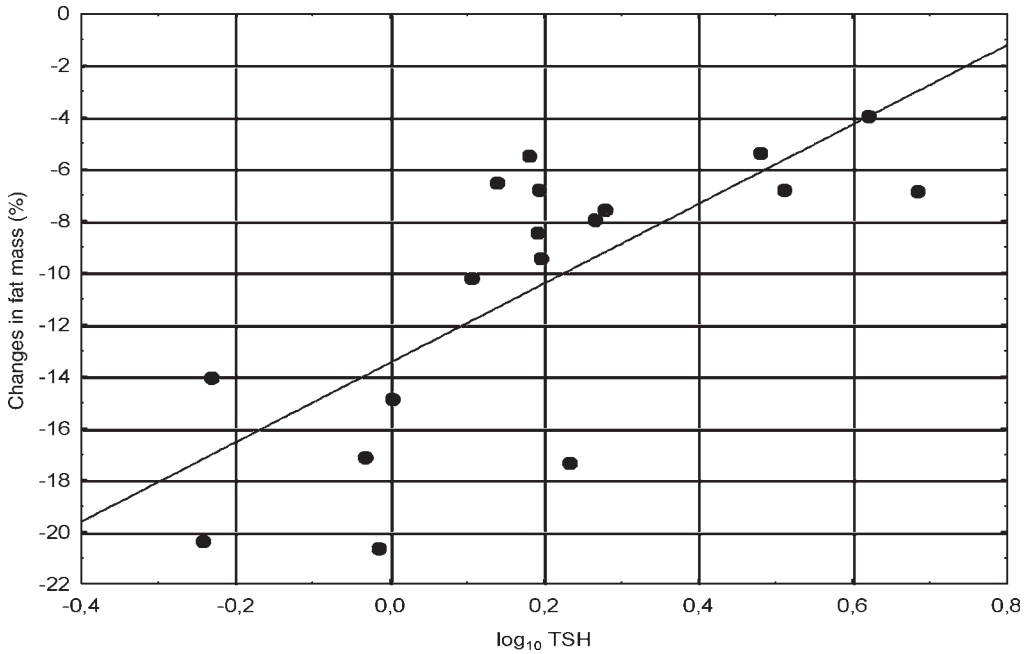

Fig. 2. Correlation of pretreatment serum $\log_{10}$ TSH and fat mass change after 4 wk of 50% energy deficit ($r = 0.73$; $p = 0.001$).

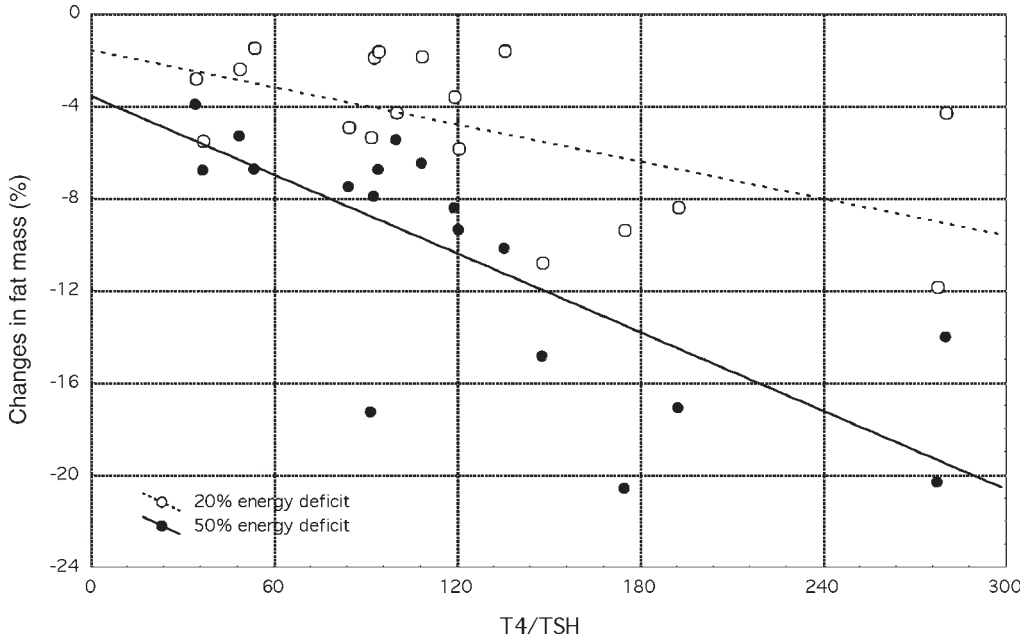

Fig. 3. Correlation of pretreatment serum T4 to TSH ratio and fat mass change after 4 wk of 20% energy deficit and subsequent 4 wk of 50% energy deficit ($r = -0.59$; $p = 0.010$ and $r = -0.76$; $p = 0.000$, respectively).

expenditure, the consequences of the decrease in thyroid hormone and TSH concentrations could comprise diminishing of their effects on adipose tissue metabolism's influence on beta-adrenergic receptor number, their susceptibility and responsiveness *(23,24)*, direct stimulation of lipolysis by TSH through binding to its own receptor *(25,26)*, and mediation of thyroid hormone action through induction of type II iodothyronine synthesis *(27)*. On the other hand, influence of the decrease in T3 on adipose tissue could also indicate diminishing in adipogenic potential, since T3 was demonstrated to be necessary in the first steps of adipose tissue differentiation *(28,29)*.

It is noteworthy that although T3 serum concentration and T3/T4 diminished in the stepwise manner dependently on the magnitude of energy deficit, the drop in T4 and TSH occurred after smaller energy restriction and was not deepened by further diminution of energy intake. The apparent dependence of deiodination on the magnitude of energy deficit may point to application of smaller energy deficit in obesity treatment, which could be more effective in long-

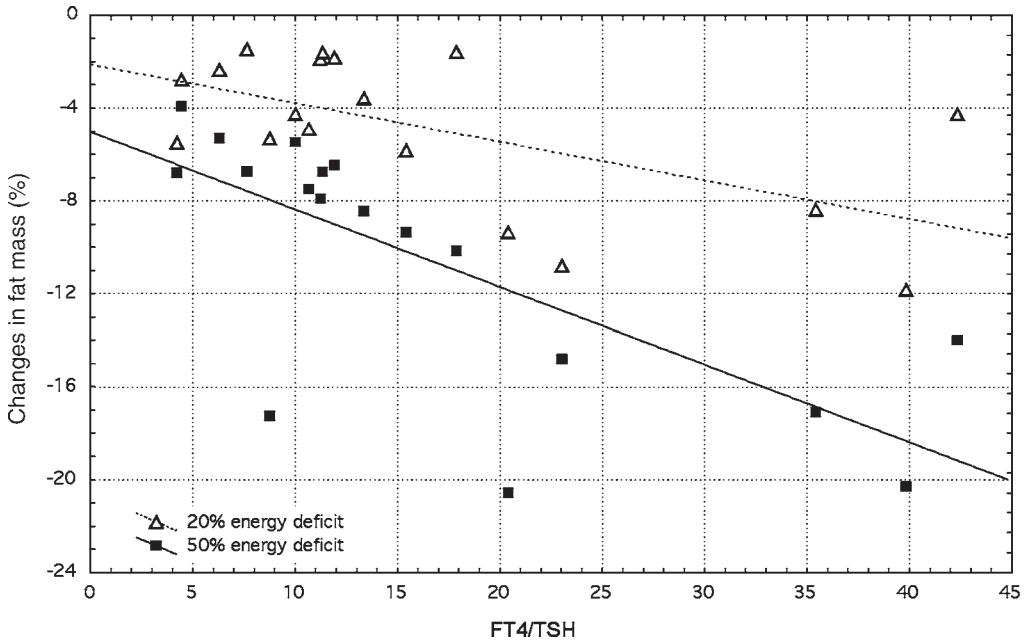

Fig. 4. Correlation of pretreatment serum fT4 to TSH ratio and fat mass change after 4 wk of 20% energy deficit and subsequent 4 wk of 50% energy deficit ($r = –0.60$; $p = 0.009$ and $r = –0.73$; $p = 0.001$, respectively).

term obesity treatment by diminishing the decline in metabolic rate.

The only hormonal parameter that was not significantly affected by the applied treatment was free T4. Moreover, despite a slight decrease in T4 concentration, we did not find changes in fT4/T4 either. This effect could be due, at least in part, to considerable interpersonal variability in fT4 serum concentration. However, the influence of plasma free fatty acids, elevated during energy restriction because of enhanced lipolysis, on dissociation of T4 from binding proteins cannot be excluded *(30)*. Unfortunately, plasma FFA concentration was not estimated in our study. Another explanation of unchanged fT4 level despite of the decrease in T4 could be the decrease in thyroid hormone binding globulin (TBG) and transthyretin (TTR) plasma concentrations. However, data concerning the effect of caloric restriction on TBG and TTR in humans are not consistent, reporting a decrease *(31)*, an increase *(32,33)* or a lack of change in their plasma concentrations *(34,35)*.

Higher leptin concentration and leptin to fat mass ratio in Ov/Ob than in normal weight subjects found by us and others have been considered to be an indicator of leptin resistance *(36)*. Leptin resistance has been demonstrated to be due to alterations in leptin signaling in several points, i.e., leptin binding in plasma *(37)*, hypothalamic ob receptor density and sensitivity *(38)*, the increased expression of inhibitors of intracellular leptin signalling *(39)*, and decreased sensitivity to proopiomelanocortin products acting as efferent leptin effectors *(40)*.

The relative decline in leptin level after treatment considerably exceeded those of BMI and percentage fat mass, which resulted in the decrease in leptin to fat mass ratio. Greater decrease in serum leptin level than in body weight or fat mass during energy restriction has been demonstrated *(41–43)*. However, from the results of these studies, the connection between the magnitude of energy deficit and decrease in leptin concentration is not clear. This could be due to the use of a restrictive diet irrespective of personal requirements for maintenance. In our study the leptin level declined proportionally to the magnitude of the energy deficit. Because the magnitude of the decrease in leptin concentration has been found to be related to the increased sensation of hunger in women *(42)*, it could be suggested that greater energy deficit in our study caused stronger hunger sensation as a mechanism counteracting depletion of body energy stores. It is noteworthy that although leptin concentration after weight-reducing treatment remained higher in Ov/Ob than in control women, leptin to fat mass ratio did not differ between both groups. This can be regarded as a positive result of our treatment, because decrease in leptin to fat mass ratio during weight reduction is considered to prove amelioration in leptin tissue responsiveness *(8)*.

A positive correlation between TSH and leptin found by us in Ov/Ob women after 50% energy deficit treatment could corroborate stimulation of the HPT axis by leptin in humans found by others *(17)*. However, despite data suggesting stimulatory effects of leptin on thyroid activity in humans *(17)*, and parallelism between changes in leptin and hormones of pituitary axis concentrations evoked by weight-reducing treatment *(44)*, the only report demonstrating significant correlations between leptin and TSH in humans found by us in the literature concerned ultradian

fluctuations of these hormones in plasma *(45)*. On the other hand, lack of statistically significant relations between leptin and TSH in plasma was demonstrated several times: in cross-sectional studies in healthy adults of both sexes *(46)*, in morbidly obese subjects both before and after bariatric surgery *(47)*, as well as in healthy and obese children before and after weight loss *(44)*. It seems that absence of such correlations could result from specificity of TSH and leptin secretion and their possible interconnections in humans coexisting with influences of other factors. The range of normal values of TSH is wide and both nutritional manipulations and weight alterations do not shift them beyond these limits. Similarly, leptin level, although directly related to body fat mass, is subject to multiple hormonal and nervous influences, thus the same fat mass is connected with wide range of leptin concentrations *(48)*. Significant positive correlation between TSH and leptin concentrations found by us in Ov/Ob group after 50% energy deficit treatment could be due to the strong effect of this dietary regimen imposed on hormonal regulations.

Analysis of relations between hormone concentrations as well as their baseline reciprocal ratios and the effect of treatment on fat mass suggested predictive significance of initial TSH concentration and its ratio to T4 and fT4.

Positive correlation between baseline TSH level and negative between T4/TSH, fT4/TSH, and the decrease in fat mass after treatment suggest that TSH level could reflect thyroid resistance to TSH stimulation and/or decreased capacity for TSH action in adipose tissue in obese/overweight subjects. Obviously, the increase in T4, fT4 to TSH ratios evoked by treatment may be at least in part due to the greater decline in TSH than T4 level or due to unchanged fT4 in the response to energy restriction. However, tendency to negative correlation between percentage fat mass and T4/TSH seen after 50% energy deficit could suggest inhibitory effect of fat mass on stimulation of thyroid activity by TSH. It is tempting to speculate the existence of an adipose tissue–derived factor diminishing thyroid response to TSH stimulation. It could be suggested that a circulating factor secreted by adipose tissue, such as TNF-alpha, weakens thyroid response to TSH similarly to its effect on insulin resistance in different tissues *(49,50)*. Such a possibility exists because TNF-alpha has been found to reduce thyroid hormone plasma level and thyroid response to TSH after systemic administration *(51,52)* and to supress iodine uptake as well thyroid peroxidase and thyroglobulin synthesis in vitro *(52,53)*.

We did not find any relationship between fat mass reduction and baseline plasma leptin which has been demonstrated by some *(54,55)* but not all studies *(41,56)*. Analysis of experimental conditions does not allow any conclusions about the reasons of such discrepancies.

Clear inverse relations between TSH and thyroid hormone baseline concentrations and their changes after treatment could indicate that in the case of dietary-induced obesity, subjects adapting well to overnutrition with respect to thyroid activity also show good adaptation to undernutrition. This suggests that weight reduction could be more difficult to obtain in subjects with higher initial thyroid hormone and TSH levels, because of greater hormonal decline in response to energy shortage.

In summary: increase in energy deficit from 20% to 50% resulted in normalization of Lep/fm suggesting improvement in leptin sensitivity, but, on the other hand, it provoked greater decline in thyroid hormone plasma concentration which could hinder further mass reduction. Relating baseline values of hormonal parameters to changes in fat mass reduction we observed correlation between TSH serum concentration and its ratio to T4 and fT4 and the decrease in fat mass which could imply predictive significance of these parameters for successful weight loss. However, this possibility needs further examination.

Materials and Methods

The experiment was carried out in overweight/obese women (Ov/Ob) ($n = 18$) aged 35.5 ± 9.1 (mean ± SD). The BMI range was 25.9–30.0, 10 women had BMI within the range 25.9–30.0 and in 8 women the BMI was over 30. Body mass before treatment was 78.7 ± 11.0 kg (mean ± SD). Mean BMI was 29.9 ± 3.3 (mean ± SD). The control group consisted of 18 age-matched lean women with body mass 60.4 ± 4.4 kg (mean ± SD) and BMI 22.0 ± 1.2 (mean ± SD). All women were premenopausal. The women were recruited from hospital medical personal. The exclusion criteria included any endocrine disturbances and any medication, which could alter metabolism.

Obese/overweight women participated in a 2 mo weight-reduction trial using a hypocaloric, low fat diet. Total energy expenditure (TEE) of all subjects was calculated according to Frankenfield et al. *(57)*. Estimates of resting energy expenditure were multiplied by activity factor of 1.4. In the first month subjects were prescribed a diet that provided 80% of the TEE (about 0.8 g protein/kg of ideal body mass, 30% of calories from fat and 58% of calories from carbohydrates). Dietary recommendations during the second month of the study were as follows: 50% energy less than TEE, protein about 0.8 g/kg of ideal body mass, 30% of calories from fat, and 52% of calories from carbohydrates. Hormonal and metabolic parameters as well as weight and body composition were determined before and after the first and the second stage of dietary treatment. All measurements were performed after overnight fasting, during follicular phase of menstrual cycle. Procedures for this study were approved by institutional ethic authority and written informed consent was obtained from all subjects.

Body composition was determined by bioelectrical impedance method with BIA Akern 101/S apparatus. Plasma thyrotropin concentration was analyzed by an IRMA test and plasma T3, total, and fT4 by RIA kits delivered by POLATOM

(Warsaw, Poland), leptin by RIA kit produced by Linco Research (St. Charles, MO, USA). For TSH, the intraassay coefficient of variation (CV) was 1.5% and the interassay CV was 1.9%; for leptin, the CVs were 4.6 and 4.7%, respectively. The intraassay CV was 3.6% for T3, 4.0% for T4, and 4.6% for fT4; the interassay CV was 4.8% for T3, 3.6% for T4, and 4.0% for free T4.

Results were expressed as mean ± SD. Differences were considered significant at $p < 0.05$. Pearson's correlation coefficients were used to quantify the relations between variables. Comparisons between groups were made by paired or independent sample *t* test as appropriate. All analyzes were done using STATISTICA version 6.

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