Thermogenic response and leptin levels rise after recovery of the euthyroid state

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ABSTRACT. Objective: The aims of the study were to compare: a) the thermogenic responses in subclinical hypothyroidism (SH) and euthyroid state; b) the relationship between thermogenic response and leptin level. Methods: Thirty women diagnosed with SH (mean age 39.9±4.1 yr; body mass index 23.2±2.5 kg/m2) were enrolled in the study. Thyroid function tests, leptin, and lipid profiles were measured during SH and after stable euthyroidism was recovered. Thermogenic response was measured by Water Immersion Calorimetry during SH and after the euthyroid state was attained. Results: The mean level of thermogenic response was found to be 1.45±0.43 kcal/kg.h in women with SH. It changed to 1.54±0.77 kcal/kg.h (p=0.01) in the euthyroid state; the change was statistically significant. Mean level of leptin was found to be 7.22±2.6 ng/ml in SH; and 15.8±8.0 ng/ml in the euthyroid state. There was a positive correlation between leptin and free T3 (r=0.460, p=0.009) levels in SH. There were positive correlations between leptin level and fat mass in SH (r=0.820, p=0.01) and in the euthyroid state (r=0.700, p=0.03). Conclusions: No correlations were found between thermogenic response and leptin levels in SH and in the euthyroid state. Thermogenic response and leptin levels rose after the euthyroid state was recovered.

INTRODUCTION
Subclinical hypothyroidism (SH) is defined as the increase of serum TSH concentration in the presence of normal serum thyroid levels. Subclinical thyroid failure is often asymptomatic; nearly 30% of patients with this condition may have symptoms suggestive of thyroid hormone deficiency (1-3). The balance between energy intake and expenditure regulates body weight and body fat. Leptin, the 146 amino acid protein hormone encoded by the ob gene and secreted by adipocytes regulates body weight by suppressing food intake and increasing energy expenditure (4, 5). The thyroid axis might influence leptin secretion and metabolism. Thyroid hormones increase the basal metabolic rate and thermogenesis by various mechanisms including uncoupling of oxidative phosphorylation (6). Hypothyroid patients have been reported to have low serum leptin levels (7). On the other hand, short-term hyperthyroidism in humans has no apparent effect on serum leptin concentrations (8). Semsroth et al. (9) have found that in humans an abnormal thyroid state has no independent major effect on serum leptin. No difference was found between leptin levels determined during thyroid disease and after normal thyroid function was restored. There were no major changes in the percentage body fat either.

Many studies were conducted on leptin levels in the hypothyroid, hyperthyroid, and euthyroid states. However, to our knowledge, no study has been done on leptin levels in normal weight patients with SH. Also, studies concerned with thermogenesis in SH are scarce. Therefore, the aims of the present study have been a) to compare thermogenic responses of patients with SH and euthyroidism; b) to compare the relationship between thermogenic response and leptin levels in these patients.

SUBJECTS AND METHODS
Thirty women diagnosed with SH [mean age 39.9±4.1 yr; body mass index (BMI) 23.2±2.5 kg/m2] were enrolled in the study. Exclusion criteria were overweight or obesity, any major organ or systemic disease, use of alcohol, smoking or any medication known to interfere with thyroid function. Women with a history of psychiatric disorder or pregnancy were also excluded. The cause of hypothyroidism was chronic autoimmune thyroiditis with positive anti-thyroidperoxidase (TPO) antibody. The diagnosis of SH was based on basal serum TSH values in the 5-20 μIU/ml range and normal free T3 (fT3) and free T4 (fT4) levels. In patients with SH, euthyroid state was reached after 2-4 months of levo-T4 (L-T4) supplement. Mean level of final L-T4 dosage to obtain euthyroidism was 67.1±4.1 μg/d. The study protocol was approved by the Ethics Committee of the Ege University Medical School. Written informed consent were obtained from all patients included in the the study.

Thyroid function, leptin, and lipid profiles were measured during SH and after stable euthyroidism was attained. A brief clinical history, anthropometric and clinical profiles of all patients were recorded during SH and the euthyroid state. Blood pressure, heart rate, weight, height, BMI, waist circumference, and waist-to-hip ratio were also recorded. A two-point bioelectrical impedance apparatus calibrated for adults (Tanita TBF 300, TANITA Corp.) was used to measure the percentage body fat and fat mass.

Key-words: Leptin, subclinical hypothyroidism, thermogenic response.

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Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) from fasting blood glucose (FBG) and insulin concentrations using the following formula (10):

\[
\text{HOMA-IR}=\frac{(\text{fasting plasma insulin}[\mu\text{U/ml}])\times(\text{fasting plasma glucose}[\text{mmol/l}])}{22.5}
\]

Plasma glucose, triglyceride, total and HDL-cholesterol were determined by enzymatic procedures, serum insulin, FT₃, FT₄, and TSH levels were determined by the chemiluminescence method. Normal ranges adapted in our laboratory were as follows: TSH: 0.34-5.6 μIU/ml; FT₂: 2.5-3.9 pg/ml; FT₄: 0.61-1.12 ng/dl; anti-thyroglobulin antibody: 0-40 IU/ml; anti-TPO antibody: 0-35 IU/ml. Insulin levels were measured by enzymeimmunoassay; intra-assay and inter-assay coefficients of variation (CV) were both 2.4%. Serum leptin level was measured by enzyme-linked immunosorbent assays (BIOSOURCE, Leptin EASIA). Mean intra-assay and inter-assay CV were 3.6-5.2%.

Patients with SH received replacement therapy with 100 μg of L-T₄ daily. Dose titration was adjusted according to TSH results obtained at the end of the 2nd month. Patients took the medicine early in the morning on empty stomach. They were asked not to change their life style (diet or exercise) during the 6-month study period. All patients were available for follow-up.

**Water immersion calorimetry**

Thermogenesis was measured by Water Immersion Calorimetry in both SH and euthyroid state. The calorimeter used to measure thermogenesis in this study has been described in detail elsewhere (11-13). Briefly, it rests on measuring the heat exchange between the body and a known amount of water in which the body is immersed. Heat given off by the body raises the water temperature which is measured at short intervals for about 1 h. Heat (Q) transferred from the patient to the tank water during this period is calculated from the simple relation:

\[
Q = mc \Delta T
\]

where
- m = mass of water in the tank without the patient (g)
- c = heat capacity of water (cal/g)
- \( \Delta T \) = difference between final (measured) and initial (i.e. 34.3°C) water temperature.

The law of conservation of energy dictates that in a living system;

Food intake = Work done by the system + Energy stored + Heat loss

When food intake is 0 as in a fasting individual and work done is 0 as in a resting individual heat generated by the body comes from the stored energy only. Under these conditions, in a calorimeter such as the one described here, the heat lost by the patient is nearly equal to the heat gained by the tank water. Therefore, Q is a measure of heat production (thermogenic response) by the subject and can be expressed in kcal per h per unit mass of the patient (kg/h.kg). It should be noted that Q does not include heat loss from the patient by respiration and from the skin in the head region which is outside the water as explained in our previous reports (11-13).

**Statistical analyses**

Statistical analysis was performed with SPSS for Windows (13.0) software package. Numerical variables were entered as mean±SD. Pre-treatment and post-treatment values of patients were compared with Paired Samples Correlation test. Hypotheses were tested at α=0.05 significance level (p=0.05).

**RESULTS**

Anthropometric and biochemical data, leptin levels, and thermogenic response were evaluated in both SH and euthyroid states in all patients. In SH, mean levels of systolic and diastolic blood pressures were found to be 115.4±10.1 mmHg and 55.8±10.4 mmHg, respectively. After treatment, they were 115.1±9.4 mmHg and 50.1±12.3 mmHg, respectively. Mean level of thermogenic response in women with SH was found to be 1.45±0.43 kcal/kg.h; it increased to 1.54±0.77 kcal/kg.h (p=0.008) in the euthyroid state. Patient characteristics and their mean thermogenic responses are listed in Table 1.

Biochemical variables were determined before and after treatment (Table 2). In SH, mean levels of triglycerides (p=0.04), FBG (p=0.03), fasting insulin (p=0.04), and HOMA-IR (p=0.01) were statistically significantly higher than those in the euthyroid state. Mean levels of TSH, FT₃, and FT₄ were found to be 8.7±2.6 μIU/ml, 0.75±0.1 ng/dl, 2.84±0.2 pg/ml in SH; and 3.3±0.6 μIU/ml, 0.99±0.3 ng/dl, 3.40±0.9 pg/ml, respectively in the euthyroid state. Mean levels of leptin, fasting insulin, and HOMA-IR were 7.22±2.6 ng/ml, 10.3±2.3 μIU/ml, 2.23±0.7 in SH, while they were 15.8±8.0 ng/ml, 8.3±2.7 μIU/ml, 1.89±0.6, respectively, in the euthyroid state.

There were no correlations between fT₃ and waist circumference, insulin level, and blood pressure. And also, no correlation was found between leptin and TSH levels. However, we found a positive correlation between leptin and the fT₃ levels (r=0.460, p=0.009) in SH, but not in the euthyroid state. There were positive correlations between leptin and fat mass in SH (r=0.820, p=0.01) and in the euthyroid state (r=0.700, p=0.03). There was no correlation between thermogenic response and leptin levels in SH and in the euthyroid state and no correlations between thermogenic response and fat mass or fat free mass.

**DISCUSSION**

The only limitation of the Water Immersion Calorimetry method used to measure thermogenic response in this study is that heat loss from the patient by way of respiration and transcutaneous exchange between the body and a known amount of water in which the body is immersed is 0 as in a resting individual heat generated by the body comes from the stored energy only. Under these conditions, in a calorimeter such as the one described here, the heat lost by the patient is nearly equal to the heat gained by the tank water. Therefore, Q is a measure of heat production (thermogenic response) by the subject and can be expressed in kcal per h per unit mass of the patient (kg/h.kg). It should be noted that Q does not include heat loss from the patient by respiration and from the skin in the head region which is outside the water as explained in our previous reports (11-13).

### Table 1 - Anthropometric characteristics of patients in subclinical hypothyroid and euthyroid states.

<table>
<thead>
<tr>
<th></th>
<th>Subclinical hypothyroid</th>
<th>Euthyroid state</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>39.9±4.1</td>
<td>22.7±2.0</td>
<td>0.70</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2±2.5</td>
<td>22.7±2.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.8±7.7</td>
<td>57.0±4.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115.4±10.1</td>
<td>115.1±9.4</td>
<td>0.80</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>55.8±10.4</td>
<td>50.1±12.3</td>
<td>0.48</td>
</tr>
<tr>
<td>Heart rate</td>
<td>79.8±8.7</td>
<td>80.4±6.6</td>
<td>0.90</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>65.1±6.0</td>
<td>64.6±5.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Waist/hip</td>
<td>0.78±0.3</td>
<td>0.76±0.5</td>
<td>0.77</td>
</tr>
<tr>
<td>Percent body fat (BIA)</td>
<td>21.0±2.4</td>
<td>18.8±1.1</td>
<td>0.58</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>19.3±1.3</td>
<td>18.2±0.8</td>
<td>0.38</td>
</tr>
<tr>
<td>Thermogenic response (kcal/kg.h)</td>
<td>1.45±0.45</td>
<td>1.54±0.77</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD; **significant (p<0.05).** BMI: body mass index; BIA: bioelectrical impedance analysis.

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