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Whole body and forearm substrate metabolism in hyperthyroidism: Evidence of increased basal muscle protein breakdown.

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Abstract

Thyroid hormones have significant metabolic effects and muscle wasting and weakness are prominent clinical features of chronic hyperthyroidism. To assess the underlying mechanisms we examined seven hyperthyroid women with Graves’ disease before (Ht.) and after (Eut.) medical treatment and 7 control subjects (Ctr.). All subjects underwent a 3-h study in the post-absorptive state. After regional catheterization protein dynamics of the whole body and of the forearm muscles were measured by amino acid tracer dilution technique using $^{15}$N-phenylalanine and $^2$H$_4$-tyrosine. Before treatment $T_3$ was elevated (6.6 nmol/l) and whole body protein breakdown was 40% increased. The net forearm release of phenylalanine was increased in hyperthyroidism ($\mu g/100 ml/min$): $-7.0 \pm 1.2$ Ht. vs. $-3.8 \pm 0.8$ Eut. ($p = 0.04$), $-4.2 \pm 0.3$ Ctr. ($p = 0.048$). Muscle protein breakdown, assessed by phenylalanine rate of appearance, was increased ($\mu g/100 ml/min$): $15.5 \pm 2.0$ Ht. vs. $9.6 \pm 1.4$ Eut. ($p = 0.03$), $9.9 \pm 0.6$ Ctr. ($p = 0.02$). Muscle protein synthesis rate (Rd) did not differ significantly. Muscle mass and muscle function were 10-20% decreased before treatment. All abnormalities were normalized after therapy.

In conclusion our results show that hyperthyroidism is associated with increased muscle amino acid release due to increased muscle protein breakdown. These abnormalities can explain the clinical manifestations of sarcopenia and myopathy.

Introduction

Thyroid hormones have profound metabolic effects and chronic hyperthyroidism is characterized by increased energy expenditure with increased oxidation of protein, glucose and lipids (19; 28). Loss of muscle mass and subsequent sarcopenia are prominent clinical features of hyperthyroidism (27) and recovery of muscle mass and function is prolonged lasting several months (24). Accelerated whole body protein catabolism has been demonstrated in experimental hyperthyroidism (16), but studies of whole body leucine kinetics in clinical and experimental hyperthyroidism have yielded inconsistent results. Studies of protein metabolism in hyperthyroid patients before and after treatment have suggested that the net protein catabolism is mainly due to depressed rates of whole body protein synthesis (7; 20) with low or normal rates of proteolysis. In experimental hyperthyroidism increased rates of proteolysis with no change in protein synthesis rates have been reported (6; 16), while Tauveron et al. (35) found both increased proteolysis and synthesis. Thyroid hormones have both anabolic and catabolic effects, and therefore the net effect on protein metabolism may vary and the above inconsistencies may relate to heterogeneity both of the hyperthyroid subjects, in terms of severity and duration of hyperthyroidism, and of the methods employed.

The metabolism of muscle protein in hyperthyroid subjects has previously been described measuring urinary excretion or arterio-venous differences of 3-methyl-histidine to estimate myofibrillar degradation, giving conflicting results, as some report normal values (6; 7; 20) and others report increased values (1; 30; 42) that were normalised after treatment. In addition increased net muscle release of certain amino acids has been reported in hyperthyroid subjects. To our knowledge no studies have hitherto addressed the issue of whether muscle loss and sarcopenia in hyperthyroidism is caused by defective muscle protein synthesis or breakdown employing tracer dilution techniques regionally across a muscle bed.
The current protocol was specifically designed to define the local mechanisms leading to loss of striated muscle mass and function in hyperthyroid patients. We examined 7 patients before and after therapy and 7 healthy control subjects, and we used infusion of phenylalanine and tyrosine tracers combined with catheterization across the forearm vascular bed to quantify muscle protein breakdown and synthesis.

Subjects

Seven hyperthyroid women, aged 26 – 49 years, with newly diagnosed diffuse toxic goitre (Graves’ disease, TSH receptor antibodies > 2 Iu/l) were consecutively recruited and studied before treatment and re-examined after 3 months of medical treatment with methimazole. An age-matched control group of seven healthy women using no medication were studied once. All participants gave their written informed consent after receiving oral and written information concerning the study according to the Declaration of Helsinki II. The Aarhus County Ethical Scientific Committee approved the study.

Materials

We used L-$^{15}$N-phenylalanine, L-$^{15}$N-tyrosine and L-$^{2}$H$_{4}$-tyrosine from Cambridge Isotope Laboratories, Inc.. The chemical, isotopic and optical purity of the isotopes were tested before use. Solutions were prepared under sterile conditions and were shown to be free of bacteria and pyrogens before use.
Methods and study design

The participants were admitted to the Clinical Research Center the evening before the day of the examinations. The investigations were carried out in the post-absorptive state the morning after an overnight fast (10-12 h) without any caffeine consumption or cigarette smoking; only ingestion of tap water was allowed, and the participants were placed in the supine position under thermo neutral conditions. One intravenous catheter (Viggo AB, Helsingborg, Sweden) was placed in an antecubital vein for infusions, another in the contralateral antecubital vein for deep venous samples and a third in a superficial vein draining the ipsilateral hand, which was heated in a box with an air temperature 65°C to provide arterialized blood (17). Preceding every deep venous sampling forearm blood flow was determined by venous occlusion plethysmography.

After priming the amino acid pool with bolus injections of $^{15}$N-phenylalanine (0.7 mg/kg), $^{15}$N-tyrosine (0.3 mg/kg) and $^2$H$_4$-tyrosine (0.5 mg/kg), continuous infusions of $^{15}$N-phenylalanine (0.7 mg/kg/h) and $^2$H$_4$-tyrosine (0.5 mg/kg/h) were maintained for 3 hours. After 150 minutes with continuous infusions steady state is accomplished and blood samples were taken in triplicate during the last 30 minutes of each study. Enrichments of $^{15}$N-phenylalanine, $^{15}$N-tyrosine and $^2$H$_4$-tyrosine were measured by mass spectrometry as their t-butyldimethylsilyl ether derivates under electron ionization conditions and concentrations of phenylalanine and tyrosine were measured using $^1$-[${^2}$H$_8$]phenylalanine and $^1$-[${^{13}}$C$_6$]tyrosine as internal standards (22). Whole blood concentrations of amino acids was determined by HPLC technique (Biotek Kontron Instruments autosampler 465, System 265 and fluorescence detector SFM 25) with precolumn O-phthalaldehyde derivatization, after deproteinizing the blood samples with 10% 5-sulfosalicylic acid (12). Thyroid hormones (total T$_3$ and total T$_4$) and TSH were measured by immunofluorecent methods (Immulite, DPC, Los Angeles, USA). Free thyroid hormones thyroxin (fT$_4$) and 3,5,3’-triiodothyronine (fT$_3$) were measured by ultrafiltration and RIA (39; 40). The clinical diagnosis of diffuse toxic goiter

(Graves’ disease) was confirmed by measurements of thyrotropin receptor antibodies (Lumitest TRAK human, Brahms Diagnostica GMBH). We used a two-site immunoassay ELISA (2) to measure serum insulin. A double monoclonal immunofluorometric assay (Delfia, Wallac, Finland) was used to measure serum GH, while plasma glucagon (26), and serum C-peptide (Immunocheal, Stillwater, MN) was measured by radioimmunoassays. Serum IGF-I was measured with an in-house time resolved fluoro-immuno-assay and urea with a commercially available kit (COBAS, INTEGRA, Roche, Hvidovre, Denmark). Serum free fatty acids (FFA) were determined by a colorimetric method employing a commercial kit (Wako Chemicals, Neuss, Germany). Blood samples were deproteinized with perchloric acid for determination of glycerol, 3-hydroxybutyrate (B-OH), and lactate by an automated fluorometric method (14).

Respiratory exchange rates (RQ) and total energy expenditure (EE) was measured by indirect calorimetry (Deltatrac, Datex Instrumentarium Inc., Helsinki, Finland) and anthropometrical measurements and whole body DEXA scanning (Hologic QDR 1000/2000/W scanner) were performed to evaluate changes in body composition before and after treatment. Cross-sectional CT scanning of the femur was performed to assess muscle area, as described previously (24). The maximal voluntary isometric strength of the left quadriceps muscle as well as the right biceps muscle were assessed by means of an electronic dynamometer (Metitur Ltd., Finland), and the values reported are the highest of five attempts.

**Calculations of phenylalanine kinetics.**

The equations of Thompson et al. (37) were used for measurements of whole body phenylalanine kinetics. Phenylalanine flux ($Q_{phe}$) and tyrosine flux ($Q_{tyr}$) were calculated as follows:

$$Q = i^* \left( \frac{E_i}{E_p} - 1 \right),$$
in which $i$ is the rate of tracer infusion ($\mu$mol/kg/h), and $E_i$ and $E_p$ are enrichment of the tracer infused and plasma enrichment of the tracer at isotopic plateau, respectively. The rate of phenylalanine conversion by hydroxylation to tyrosine ($I_{pt}$) was calculated as follows:

$$I_{pt} = Q_{tyr} \times \frac{^{15}N-Tyr_{ei}}{^{15}N-Phe_{ei}} \times \left(\frac{Q_{phe}}{I_{phe} + Q_{phe}}\right),$$

where $^{15}N-Tyr_{ei}$ and $^{15}N-Phe_{ei}$ are the isotopic enrichments of the respective tracers in plasma and $I_{phe}$ is the infusion rate of $^{15}N$-phenylalanine ($\mu$mol/kg/h).

Phenylalanine incorporation into protein is calculated by subtracting $I_{pt}$ from $Q_{phe}$, since phenylalanine is irreversibly lost from the bloodstream either by its hydroxylation into tyrosine or by incorporation into protein.

In the forearm study phenylalanine balance ($PheBal$) was calculated as follows using Fick’s principle:

$$PheBal = (Phe_a - Phe_v) \times F,$$

in which $Phe_a$ and $Phe_v$ are arterial and deep venous phenylalanine concentrations and $F$ is blood flow in the forearm. Regional phenylalanine kinetics were calculated, using the equations described by Nair et al. (22). The forearm protein breakdown represented by phenylalanine rate of appearance ($Ra_{phe}$) was calculated as follows (4):

$$Ra_{phe} = Phe_a \times \left(\frac{Phe_{Ea}}{Phe_{Ev}} - 1\right) \times F,$$

in which $Phe_{Ea}$ and $Phe_{Ev}$ represent phenylalanine isotopic enrichment in arteries and veins. The local rate of disappearance, which represents the muscle protein synthesis rate, was calculated as:

$$Rd_{phe} = PheBal + Ra_{phe}.$$

comparisons. Results are expressed as mean ± standard error of the mean (SE). P-values less than 0.05 were considered significant.

Results

*Clinical characteristics: thyroid hormones, body composition, muscle strength and indirect calorimetry*

In the hyperthyroid state the patients had a 2-5-fold elevation of total and free T$_3$, compared to after treatment, when T$_3$ decreased to normal levels (Table 1). Patients and control subjects were of comparable age and height, and after treatment of the patients their body weight was not significantly different from the control subjects. All patients were treated with methimazole, which inhibits thyroid hormone synthesis in the thyroid gland. Methimazole has no known metabolic effects. The patients gained an average of 4 kg of body weight during treatment, and DEXA scans showed proportional increments in fat and lean body mass. The cross-sectional area of muscle at the mid-femoral level increased with treatment, while the fat area remained unchanged indicating that the increase of body fat mass with treatment was mainly in the upper body. Before treatment the maximum isometric contraction of both the upper or lower limps were decreased compared to after treatment, and the quadriceps contractility was decreased by 11% ± 4% (p = 0.04) in hyperthyroidism as compared to the euthyroid state. In the hyperthyroid state total energy expenditure was increased, EE (kcal/24h): 1989 ± 79 versus 1552 ± 87 after treatment and RQ was decreased indicating increased lipid oxidation (Table 2).

*Circulating metabolites and hormones*

In hyperthyroidism fasting levels of glucose (Table 2) tended to be higher, glucose a-v difference was lower, whereas circulating levels of insulin, c-peptide, glucagon, lactate, urea, GH, and IGF-I did not differ with thyroid state in our study. As for the metabolites of lipid metabolism,

glycerol levels were elevated during hyperthyroidism, and the concentrations of free fatty acids (FFA) and 3-hydroxybutyrate (3-OHB) tended to be elevated.

**Whole body amino acid kinetics**

Isotopic enrichments reached a plateau at the end of the study period (Fig 2). This was assessed based on the observation that when isotopic enrichment values for phenylalanine and tyrosine were plotted against time the ensuing slopes were not different from zero (p values between 0.20 and 0.29). In the hyperthyroid state whole body phenylalanine and tyrosine fluxes were increased (Table 3), compared to the euthyroid state. Phenylalanine conversion to tyrosine (reflecting amino acid degradation) was increased, and protein synthesis (phenylalanine disposal not accounted for by phenylalanine conversion to tyrosine) was increased.

**Amino acid levels**

Whole blood concentrations of amino acids are shown in table 4. The total amino acid concentrations did not differ between the study groups. Concentrations of phenylalanine, tyrosine and the branched chain amino acids (leucine, isoleucine and valine) were elevated in hyperthyroidism. By contrast the gluconeogenic amino acids: alanine, serine and glycine were decreased, although not significantly (p = 0.06).

**Regional muscle amino acid kinetics**

We saw a net forearm release of phenylalanine in all study groups, the release being increased in hyperthyroidism (Figure 1, p = 0.04 and 0.007 vs. euthyroid patients and controls, respectively). Muscle protein breakdown, assessed by phenylalanine rate of appearance (Ra), was increased (p = 0.03) in hyperthyroid patients, and became normalized with treatment. Muscle

protein synthesis rate (Rd) did not differ (p = 0.3). Forearm blood flow was 35% increased in hyperthyroidism and was normalized after treatment.

Discussion

The present study, which was designed to define the effects of thyroid hormone excess on muscle protein metabolism and the mechanisms leading to sarcopenia and myopathy in hyperthyroidism, shows a number of abnormalities in muscle protein metabolism. Sarcopenia was confirmed by DEXA and CT scans and we found decreased contractile force. A previous study has shown muscle fiber atrophy in muscle biopsies from hyperthyroid patients (41), supporting the concept that hyperthyroidism leads to decreased muscle protein and fiber content. We observed an increased net release of amino acids across the forearm muscular bed and application of phenylalanine tracer dilution technique revealed that the excessive loss of muscle protein was due to accelerated muscle protein breakdown rather than defective protein synthesis.

The hyperthyroid patients had increased whole body fluxes of both phenylalanine and tyrosine reflecting a high turnover state with concomitant increases of protein breakdown and synthesis. This is in keeping with the notion that thyroid hormones have both anabolic and catabolic actions as suggested by the reports of Rochon (29) and Tauveron (35) using a leucine tracer method. At the whole body level we observed a 40% increase in protein synthesis rate (calculated as total phenylalanine flux minus phenylalanine-to-tyrosine degradation) and in the forearm we saw a similar non-significant increase in regional muscle protein synthesis. The fact that the increase in muscle protein synthesis was non-significant could represent a type 2 error and the findings, in all probability, reflect that non-muscle and muscle protein synthesis is increased to a similar degree by thyroid hormones.
The results of our forearm studies showed that the changes in whole body fluxes to a large extent were due to increased muscle protein breakdown, as the forearm rate of appearance for phenylalanine was elevated by 60% in hyperthyroidism, whereas whole body protein breakdown (phenylalanine flux) was only elevated by 40%. This is supported by a previous observation of increased release of branched chain amino acids by the forearm (31) and by Morrison et al. (20) who reported a 6-fold increase in net tyrosine release across the leg in patients with untreated hyperthyroidism. The latter study however failed to observe any increase in 3-methylhistidine efflux from the leg, possibly because the analytical performance of 3-methylhistidine measurements is not sufficient to detect changes in muscle metabolism. Compared to other amino acids the concentrations of 3-methyl-histidine in the blood are very low and arterio-venous gradients of 3-methyl-histidine are more than a 1000-fold below that of certain other amino acids, making it very difficult to detect differences in regional muscle 3-methyl-histidine efflux.

Branched chain amino acids are known to stimulate protein synthesis and inhibit protein breakdown (15; 43) and the increase of blood concentrations of branched amino acids in hyperthyroidism may thus be a compensatory mechanism, whereby protein is preserved. The pattern of increased branched chain amino acid concentrations and decreased gluconeogenic amino acid concentrations in hyperthyroidism corresponds to what others have reported (38), and probably reflects increased splanchnic utilization of gluconeogenic amino acids and increased peripheral mobilization of branched chain amino acids (8; 9).

It is noteworthy that protein catabolism prevails in hyperthyroidism despite increased concentrations of a number of anabolic agents. Circulating levels of FFA, glycerol and ketone bodies are in general elevated and these lipid intermediates have been shown to stimulate protein anabolism (36); this may represent a protein sparing mechanism in hyperthyroidism (23; 28). Furthermore, basal insulin levels if anything tend to be elevated in hyperthyroid patients. Insulin

inhibits whole body and muscle protein breakdown (11; 13) and may stimulate protein synthesis in the presence of high amino acid concentrations. Previous studies have shown that GH exerts stimulation of protein synthesis and inhibition of breakdown (25) and even though we could not detect any difference in single GH measurements in the present study, it has earlier been shown that the 24-h production rate of GH in thyrotoxicosis is increased nearly 4-fold (10). It is possible that these anabolic fuels counteracted the increased catabolic drive in hyperthyroidism.

In the present study measurements of protein turnover were performed using phenylalanine and tyrosine tracers. The essential amino acid phenylalanine is an attractive tracer because it is not synthesized endogenously and has only two fates in the body: incorporation into protein or irreversible hydroxylation into tyrosine which occurs in the liver and the kidney (18). We therefore assume that the flux of phenylalanine measured across the forearm area represents skeletal muscle protein turnover (22). As with other tracer methods the major assumptions of the method are that phenylalanine truly reflects the turnover of protein in the body, that there is a single homogenous pool of free amino acids, that the infused tracer is uniformly mixed in the entire pool and that the tracer is in steady state, i.e. all fluxes are constant. In our study enrichment levels in arterialized and venous plasma were constant during the 30 minutes sampling period, indicating steady state conditions. Determination of blood flow represents another possible source of error; if for instance blood flow determinations are spuriously high, this would give rise to calculation of elevated values for net forearm amino acid release and muscle protein breakdown and protein synthesis. In the present study we recorded a 35 % increase in forearm blood flow. Previous studies have reported 50-280 % increases in basal resting forearm blood flow (5; 19) and more than 100 % increases in leg blood flow in hyperthyroid subjects (20). The validity of our data is further supported by the fact that only muscle protein breakdown was significantly increased in hyperthyroidism (p values between 1.8 and 3.1 %), whereas protein synthesis was not significantly
affected (p values between 25 and 29 %). It is possible that the increased blood flow may contribute to increased protein breakdown and relatively decreased synthesis via mass action by maintaining a lower amino acid concentration in the blood nourishing the muscle cells. Our data do not provide any answers to the issue of how hyperthyroidism affects the kinetics of specific muscle proteins, such as for instance myosin heavy chain. This question awaits answers from studies using a combination of tracer dilution and muscle biopsies techniques. Three previous studies (42) have however shown that urinary excretion of 3-methyl-histidine, a marker of muscle myofibrillar protein degradation, is increased in hyperthyroidism. This strongly supports the view that myofibrillar degradation is increased in hyperthyroidism. The findings of normal 3-methyl-histidine release across a muscle bed (20) and normal urinary 3-methyl-histidine excretion (7) in other studies may relate to methodological insensitivity of the arterio-venous technique in the former and possibly intake of food items containing 3-methyl-histidine in the latter. Our observations of 10-20% decrements in both muscle strength and area also support, that the myofibrillar content of muscle is decreased in hyperthyroidism.

We found that both protein breakdown and synthesis at the whole body level are increased in hyperthyroidism supporting the concept that thyroid hormone are both anabolic and catabolic and indicating that some of the excessive energy expenditure in hyperthyroidism, apart from glucose and FFA, is due to futile substrate cycling of protein and amino acids (21; 32; 33). Other energy consuming processes could be increased activity of Na⁺/K⁺-ATP-ase and Ca²⁺-ATP-ase (3). Spontaneous physical activity is increased in hyperthyroidism and non-exercise activity thermogenesis (34) may contribute.

In summary, we find that hyperthyroidism is characterized by elevated basal whole body protein breakdown and synthesis. The mechanisms maintaining sarcopenia and myopathy in

hyperthyroid subjects include an inappropriately high rate of net muscle amino acid release due to increased muscle protein breakdown.

Acknowledgements

Lone Svendsen, Lene Ring Kristensen and Iben Christensen are thanked for excellent technical assistance. The study was supported by The Aarhus University Research Fund and Musikforlæggerne Agnes og Knut Mørks fund.

Reference List


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Table 1

Clinical characteristics and thyroid hormones in hyperthyroid patients before (Ht.) and after treatment (Eut.), and in control subjects (Cont.)

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>P-value</th>
<th>Control subjects</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>39 ± 4 (26-49)</td>
<td></td>
<td>39 ± 3 (24-46)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>59.6 ± 3.0</td>
<td>63.6 ± 3.4</td>
<td>0.01</td>
<td>67.7 ± 3.4</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.67 ± 0.03</td>
<td></td>
<td></td>
<td>1.70 ± 0.03</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>21.4 ± 0.9</td>
<td>22.8 ± 1.1</td>
<td>0.01</td>
<td>23.6 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Dexa total fat (kg)</strong></td>
<td>18.2 ± 2.4</td>
<td>19.4 ± 2.4</td>
<td>0.08</td>
<td>19.5 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Dexa lean body mass (kg)</strong></td>
<td>38.4 ± 1.4</td>
<td>40.1 ± 1.4</td>
<td>0.2</td>
<td>44.1 ± 1.1</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Femoral fat area (cm^2)</strong></td>
<td>83 ± 9</td>
<td>82 ± 8</td>
<td>NS</td>
<td>91 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Femoral muscle area (cm^2)</strong></td>
<td>89 ± 5</td>
<td>101 ± 4</td>
<td>0.01</td>
<td>111 ± 4</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Muscle strength right arm (Nm)</strong></td>
<td>16.1 ± 1.4</td>
<td>19.1 ± 2.0</td>
<td>0.07</td>
<td>19.4 ± 1.1</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Muscle strength left thigh (Nm)</strong></td>
<td>28 ± 2</td>
<td>35 ± 2</td>
<td>0.009</td>
<td>38 ± 3</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Total T₃ (1.1-2.6 nmol/l)</strong></td>
<td>6.6 ± 0.9</td>
<td>1.8 ± 0.2</td>
<td>0.001</td>
<td>1.27 ± 0.08</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Total T₄ (58-161 nmol/l)</strong></td>
<td>234 ± 17</td>
<td>87 ± 9</td>
<td>&lt;0.001</td>
<td>77 ± 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Free T₃ (3.7-9.5 pmol/l)</strong></td>
<td>39.7 ± 5.4</td>
<td>8.7 ± 1.2</td>
<td>&lt;0.001</td>
<td>6.1 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Free T₄ (12-33 pmol/l)</strong></td>
<td>148 ± 17</td>
<td>27.8 ± 2.9</td>
<td>&lt;0.001</td>
<td>22.4 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>TSH (µmol/ml)</strong></td>
<td>&lt;0.002</td>
<td>0.01 ± 0.01</td>
<td>0.2</td>
<td>1.6 ± 0.4</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Normal reference values are given for thyroid hormones.

Table 2

Fasting circulating hormones and metabolites in hyperthyroid patients before (Ht.) and after treatment (Eut.), and in control subjects (Cont.).

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control subjects</th>
<th>P-value Ht. vs. Eut.</th>
<th>P-value Ht. vs. Cont.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Expenditure</td>
<td>1989 ± 79</td>
<td>1470 ± 59</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(kcal/24h)</td>
<td>1552 ± 87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-protein RQ</td>
<td>0.81 ± 0.01</td>
<td>0.87 ± 0.02</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.3 ± 0.1</td>
<td>4.98 ± 0.06</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Glucose a-v diff.</td>
<td>-0.07 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>&lt;0.001</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>838 ± 104</td>
<td>587 ± 61</td>
<td>0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>FFA (µmol/l)</td>
<td>103 ± 14</td>
<td>46 ± 7</td>
<td>0.02</td>
<td>0.004</td>
</tr>
<tr>
<td>Glycerol (µmol/l)</td>
<td>374 ± 118</td>
<td>129 ± 30</td>
<td>0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Butyrate-OH (µmol/l)</td>
<td>532 ± 28</td>
<td>547 ± 37</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate (µmol/l)</td>
<td>3.8 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>32.8 ± 5.1</td>
<td>32.4 ± 3.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>516 ± 67</td>
<td>433 ± 48</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide (pmol/l)</td>
<td>1.4 ± 0.4</td>
<td>1.9 ± 1.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>200 ± 13</td>
<td>198 ± 9</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Comparisons between euthyroid patients and healthy controls are not significant.

Table 3

Fasting amino acid fluxes and forearm blood flow in hyperthyroid patients before (Ht.) and after treatment (Eut.), and in control subjects (Cont.).

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>P-value</th>
<th>Control subjects</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyperthyroid</td>
<td>Euthyroid</td>
<td>Ht. vs. Eut.</td>
<td>Control subjects</td>
</tr>
<tr>
<td>Total $Q_{phe}$ ($\mu$mol/kg/h)</td>
<td>61.3 ± 2.3</td>
<td>43.4 ± 2.2</td>
<td>&lt;0.001</td>
<td>39.1 ± 1.1</td>
</tr>
<tr>
<td>Total $Q_{tyr}$ ($\mu$mol/kg/h)</td>
<td>59.9 ± 4.3</td>
<td>37.7 ± 1.8</td>
<td>0.002</td>
<td>33.0 ± 2.2</td>
</tr>
<tr>
<td>$Q_{phe\rightarrow tyr}$ ($\mu$mol/kg/h)</td>
<td>7.6 ± 1.3</td>
<td>4.9 ± 0.6</td>
<td>0.04</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>Protein synthesis rate ($\mu$mol/kg/h)</td>
<td>53.7 ± 2.3</td>
<td>38.5 ± 2.1</td>
<td>0.001</td>
<td>34.7 ± 1.0</td>
</tr>
<tr>
<td>Forearm blood flow (ml/100ml/min)</td>
<td>3.8 ± 0.3</td>
<td>2.8 ± 0.2</td>
<td>0.04</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Muscle balance$_{phe}$ ($\mu$g/100ml/min)</td>
<td>-7.0 ± 1.2</td>
<td>-3.8 ± 0.8</td>
<td>0.04</td>
<td>-4.2 ± 0.3</td>
</tr>
<tr>
<td>Muscle $R_{phe}$ ($\mu$g/100ml/min)</td>
<td>15.5 ± 2.0</td>
<td>9.6 ± 1.4</td>
<td>0.03</td>
<td>9.9 ± 0.6</td>
</tr>
<tr>
<td>Muscle $R_{d_{phe}}$ ($\mu$g/100ml/min)</td>
<td>8.5 ± 2.2</td>
<td>5.8 ± 1.1</td>
<td>0.29</td>
<td>5.7 ± 0.9</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE.

The amino acid fluxes and forearm blood flow of euthyroid patients were not significantly different from those of healthy controls.
Table 4

Whole blood concentrations of amino acids in hyperthyroid patients before (Ht.) and after treatment (Eut.), and in control subjects (Cont.).

<table>
<thead>
<tr>
<th>Amino acids (µmol/l)</th>
<th>Patients</th>
<th>P value*</th>
<th>Controls</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyperthyroid</td>
<td>Euthyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>188 ± 26</td>
<td>201 ± 23</td>
<td>NS</td>
<td>299 ± 90</td>
</tr>
<tr>
<td>Glutamate</td>
<td>285 ± 25</td>
<td>295 ± 31</td>
<td>NS</td>
<td>303 ± 28</td>
</tr>
<tr>
<td>Serine</td>
<td>202 ± 16</td>
<td>255 ± 24</td>
<td>0.1</td>
<td>250 ± 9</td>
</tr>
<tr>
<td>Glutamine + Histidine</td>
<td>896 ± 79</td>
<td>815 ± 42</td>
<td>NS</td>
<td>845 ± 23</td>
</tr>
<tr>
<td>Glycine</td>
<td>527 ± 33</td>
<td>652 ± 76</td>
<td>0.06</td>
<td>584 ± 33</td>
</tr>
<tr>
<td>Threonine</td>
<td>264 ± 22</td>
<td>239 ± 24</td>
<td>NS</td>
<td>230 ± 40</td>
</tr>
<tr>
<td>Citrulline</td>
<td>56 ± 11</td>
<td>54 ± 7</td>
<td>NS</td>
<td>80 ± 16</td>
</tr>
<tr>
<td>Arginine</td>
<td>126 ± 15</td>
<td>126 ± 11</td>
<td>NS</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>Alanine</td>
<td>398 ± 29</td>
<td>520 ± 60</td>
<td>0.05</td>
<td>480 ± 42</td>
</tr>
<tr>
<td>Taurine</td>
<td>259 ± 29</td>
<td>272 ± 31</td>
<td>NS</td>
<td>330 ± 25</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>168 ± 14</td>
<td>123 ± 7</td>
<td>0.01</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>120 ± 6</td>
<td>100 ± 4</td>
<td>0.02</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>Valine + Methionine</td>
<td>433 ± 41</td>
<td>337 ± 19</td>
<td>0.04</td>
<td>304 ± 20</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>145 ± 14</td>
<td>95 ± 5</td>
<td>0.004</td>
<td>97 ± 7</td>
</tr>
<tr>
<td>Leucine</td>
<td>273 ± 19</td>
<td>196 ± 9</td>
<td>0.002</td>
<td>173 ± 12</td>
</tr>
<tr>
<td>Ornithine</td>
<td>161 ± 29</td>
<td>130 ± 33</td>
<td>NS</td>
<td>135 ± 18</td>
</tr>
<tr>
<td>Lysine</td>
<td>316 ± 31</td>
<td>254 ± 16</td>
<td>NS</td>
<td>257 ± 18</td>
</tr>
<tr>
<td>Branched chain amino acids</td>
<td>851 ± 73</td>
<td>628 ± 30</td>
<td>0.006</td>
<td>574 ± 37</td>
</tr>
<tr>
<td>Some gluconeogenic amino acids</td>
<td>1127 ± 75</td>
<td>1426 ± 135</td>
<td>0.04</td>
<td>1315 ± 58</td>
</tr>
<tr>
<td>Amino acids total</td>
<td>4818 ± 230</td>
<td>4682 ± 192</td>
<td>NS</td>
<td>4719 ± 120</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. * P-values indicate comparisons between patients before (hyperthyroid) and after treatment (euthyroid). ** P-values indicate comparisons between hyperthyroid patients and healthy controls. Comparisons between euthyroid patients and healthy controls are not significant. Where NS are stated the comparisons of the means are not significant. Branched chain amino acids: leucine, isoleucine, valine and methionine. Some gluconeogenic amino acids: alanine, glycine and serine.
Legend for Figure 1.

Forearm protein turnover in hyperthyroid patients before and after treatment and in healthy controls.

Data are presented as means ± SE.

a. Phenylalanine a-v-balance across the forearm
b. Rate of appearance of phenylalanine (muscle protein breakdown)
c. Rate of disappearance of phenylalanine (muscle protein synthesis)
Figure 1

a. Muscle balance \( \text{phe} \) (g/100 ml/min)  
\[ p = 0.04 \]  
\[ p = 0.02 \]

b. \( R_a(\text{phe}) \) (g/100 ml/min)  
\[ p = 0.02 \]  
\[ p = 0.03 \]

c. \( R_d(\text{phe}) \) (g/100 ml/min)
Figure 2

Legend Figure 2

Enrichment of phenylalanine during the last 30 minutes of the study with constant infusions of $^{15}$N-phenylalanine. Hyperthyroid patients, ○ Euthyroid patients and ▼ Healthy controls. P-values represent the probability of slope = zero.