TRANSLATIONAL PHYSIOLOGY

Hyperthyroidism and cation pumps in human skeletal muscle

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Riis, Anne Lene Dalkjær, Jens Otto Lunde Jørgensen, Niels Møller, Jørgen Weeke, and Torben Clausen. Hyperthyroidism and cation pumps in human skeletal muscle. Am J Physiol Endocrinol Metab 288:E1265–E1269, 2005. First published December 14, 2004; doi:10.1152/ajpendo.00533.2004.—Skeletal muscle constitutes the major target organ for the thermogenic action of thyroid hormone. We examined the possible relation between energy expenditure (EE), thyroid status, and the contents of Ca2+-ATPase and Na+-K+-ATPase in human skeletal muscle. Eleven hyperthyroid patients with Graves’ disease were studied before and after medical treatment with methimazole and compared with eight healthy subjects. Muscle biopsies were taken from the vastus lateralis muscle, and EE was determined by indirect calorimetry. Before treatment, the patients had two- to five-fold elevated total plasma T3 and 41% elevated EE compared with when euthyroidism had been achieved. In hyperthyroidism, the content of Ca2+-ATPase was increased: (mean ± SD) 6,555 ± 604 vs. 5,212 ± 1,580 pmol/g in euthyroidism (P = 0.04) and 4,523 ± 1,311 pmol/g in healthy controls (P = 0.0005). The content of Na+-K+-ATPase showed 89% increase in hyperthyroidism: 558 ± 101 vs. 296 ± 34 pmol/g (P = 0.0001) in euthyroidism and 278 ± 52 pmol/g in healthy controls (P < 0.0001). In euthyroidism, the contents of both cation pumps did not differ from those of healthy controls. The Ca2+-ATPase content was significantly correlated to plasma T3 and resting EE. This provides the first evidence that, in human skeletal muscle, the capacity for Ca2+ recycling and active Na+-K+ transport are correlated to EE and thyroid status.

Ca2+-activated adenosine triphosphatase; Na+-K+-activated adenosine triphosphatase; energy expenditure

LEAN BODY MASS IS A MAJOR DETERMINANT of resting energy expenditure (REE) in humans, and skeletal muscle constitutes the major target organ for the thermogenic action of thyroid hormones. Skeletal muscle is by far the most abundant tissue of the human body and accounts for more than 25% of the total resting O2 consumption (4, 23, 31). Thus even a modest change in heat production in skeletal muscles leads to a significant modification of total body heat production (6). Animal studies show that thyroid hormones upregulate the content of Ca2+-ATPase in the sarcoplasmic reticulum (SR) and the maximum rate of Ca2+ uptake in SR from rat soleus increases over a fourfold range with thyroid status (14, 26). More recently, the amount of SR protein recovered from hyperthyroid rabbit muscle was found to be fourfold larger than that obtained from control animals (6). In intact soleus muscles prepared from hyperthyroid rats, the accumulation of 45Ca as measured over 6 h was fourfold larger than in soleus from euthyroid rats (11). Thus thyroid hormones markedly upregulate the capacity for Ca2+ uptake, allowing increased recycling of Ca2+ and ATP turnover. The stimulation of energy expenditure (EE) induced by thyroid hormones is for a significant part mediated by an increase in the capacity and activity of the Ca2+-ATPase in SR (4, 6, 25). The effects of thyroid hormones on the content and activity of Na+-K+-ATPase in skeletal muscle, however, accounts at most for only 15% of their thermogenic action (4).

We have previously demonstrated that the content of Na+-K+-ATPase in human skeletal muscle is closely correlated to plasma 3,5,3’-triiodothyronine (T3) and thyroxine (T4) (17), but the relations between thyroid status and the total content of Ca2+-ATPase in human skeletal muscle have not been characterized. In a controlled design, we examined the effect of hyperthyroidism on the contents of Ca2+-ATPase and Na+-K+-ATPase in human skeletal muscle in hyperthyroid patients with Graves’ disease before and after treatment and compared with healthy control subjects. We are testing the following hypotheses.

1) In hyperthyroid subjects, the contents of Ca2+-ATPase and Na+-K+-ATPase in skeletal muscle are increased and restored toward the normal level following standard treatment of hyperthyroidism.

2) The contents of Ca2+-ATPase and Na+-K+-ATPase in skeletal muscle are correlated to REE and plasma thyroid hormones.

METHODS

Participants. Eleven hyperthyroid patients (9 women and 2 men) with newly diagnosed Graves’ disease were studied before and after 3 mo of medical treatment with methimazole. The clinical diagnosis of diffuse toxic goiter was confirmed by measurement of thyroid-stimulating hormone (TSH) receptor antibodies >2 IU/L. In two cases, muscle biopsies could not be obtained: in one female hyperthyroid patient (before treatment) and in one female euthyroid patient (after treatment). These two patients did not differ from the rest of the patients, and the data from those patients are included in the mean values and in the unpaired analysis comparing with healthy control subjects. In the paired comparisons before and after treatment, however, only nine patients could be included. An age-matched control group of eight healthy women using no medication was 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, approval number 1998/4372.

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In the two cases where muscle biopsies could not be obtained both assay, CaCl₂ (0.55 mM) was added to the medium so as to establish \([^{32}\text{P}]\text{ATP}\) (0.3 Ci/ml) was added as a tracer (pH 7.4). In a parallel measurement, the content of Ca²⁺-ATPase was determined as described in detail elsewhere (10, 21). For the measurement of Ca²⁺-ATPase, ~30 mg of frozen muscle tissue were homogenized at 0°C in a HEPES-sucrose buffer (pH 7.4) with an Ultra-Turrax homogenizer. To obtain a smaller particle size, the suspension was further homogenized at 0°C using a glass homogenizer with a tight-fitting Teflon pestle rotating at 1,000 rpm. Aliquots (0.2 ml) of the homogenate were reacted for 30 s at 0°C in 3 ml of a buffer containing (in mM): 100 imidazole, 100 KCl, 5 MgCl₂, 0.5 EGTA, 0.05 ATP. [γ-\(^32\text{P}\)]ATP (0.3 μCi/ml) was added as a tracer (pH 7.4). In a parallel assay, CaCl₂ (0.55 mM) was added to the medium so as to establish a free Ca²⁺ content of ~50 μM. The phosphorylation reaction was started by addition of the homogenate (0.2 to 2.8 ml of medium) and 30 s later quenched with trichloroacetic acid and centrifuged. The pellet was resuspended and recentrifuged, and the final pellet was prepared for counting of \(^{32}\text{P}\) bound to the protein. The increase in \(^{32}\text{P}\) uptake induced by the presence of Ca²⁺ was expressed in picomoles per gram tissue wet weight. Na⁺-K⁺-ATPase was measured as the [\(^{3}\text{H}\)ouabain binding capacity of muscle tissue specimens weighing ~5 mg (21). The tissue samples were equilibrated for 2 h at 37°C in a Tris-vanadate buffer containing [\(^{3}\text{H}\)ouabain (10⁻⁶ M and 0.6 μCi/ml) and henceforth washed for 4 × 30 min in ice-cold Tris-vanadate buffer to remove the [\(^{3}\text{H}\)ouabain not bound to the tissue, blotted, weighed, and taken for counting. After correction for unspecific uptake, isotopic purity (95%), loss of \(^{3}\text{H}\) activity during the washout in the cold, and incomplete saturation, the [\(^{3}\text{H}\)ouabain bound was expressed in picomoles per gram tissue wet weight. The immunoblotting methods generally yield only relative values for tissue contents of cation ATPasases. In the present study, therefore, the above-mentioned methods were preferred to obtain values that could be expressed in molar units per gram tissue wet weight. (5) Plasma thyroid hormones (total T₃ and total T₄) and TSH were measured by immunofluorescence methods (Immulite; DPC, Los Angeles, CA). Free T₃ (fT₃) and free T₄ (fT₄) were measured by RIA (28, 29).

**Table 1. Clinical characteristics and thyroid hormones in 11 hyperthyroid patients before and after treatment and in 8 control subjects**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hyperthyroid</th>
<th>Euthyroid</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr (range)</td>
<td>36 ±9 (26–49)</td>
<td>40 ±8 (24–46)</td>
<td>0.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.8 ±2.3</td>
<td>22.4 ±2.6</td>
<td>0.0007</td>
</tr>
<tr>
<td>EE, kcal/24 h</td>
<td>2091 ±384</td>
<td>1485 ±239</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total T₃ (1.1–2.6 nmol/l)</td>
<td>7.7 ±2.5</td>
<td>1.7 ±0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total T₄ (58–161) nmol/l</td>
<td>253 ±49</td>
<td>94 ±33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Free T₃ (3.7–9.5) pmol/l</td>
<td>43 ±18</td>
<td>29 ±10</td>
<td>0.0045</td>
</tr>
<tr>
<td>Free T₄ (12–33) pmol/l</td>
<td>140 ±51</td>
<td>26 ±7</td>
<td>0.0001</td>
</tr>
<tr>
<td>TSH, μmol/ml (median range)</td>
<td>&lt;0.002</td>
<td>0.0019 (&lt;0.002–0.07)</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD, except for thyroid-stimulating hormone (TSH) values, which are presented as medians and range. Normal ranges for thyroid hormones in our laboratory are stated in parentheses. Ht., hyperthyroid patients before treatment; Eut., hyperthyroid patients after treatment; Cont., controls; BMI, body mass index; EE, energy expenditure; T₃, 3,5,3'-triiodothyronine; T₄, thyroxine. P values represent comparisons between patients in the hyperthyroid and euthyroid states by paired t-test and comparisons of healthy controls with patients in the hyperthyroid or euthyroid state by unpaired t-test.

**Results**

**Thyroid status and cation pumps.** In the hyperthyroid state, the patients had between two- and fivefold increased plasma total T₃ and fT₃ compared with posttreatment, when T₃ decreased to normal levels (Table 1). Upon admission, the patients were clinically hyperthyroid with tachycardia and 41 ± 18% increase in REE. Body weight increased 4.7 ± 2.8 kg during treatment. As shown in Fig. 1, in hyperthyroid patients the content of Ca²⁺-ATPase in vastus lateralis was increased: 6,555 ± 604 vs. 5,212 ± 1,580 pmol/g after treatment (P = 0.04, paired comparison, n = 9) and 4,523 ± 1,311 pmol/g in healthy controls (P = 0.0005). The mean relative increase in Ca²⁺-ATPase in hyperthyroidism makes up 26% of the values measured after euthyroidism had been restored and 45% of the Ca²⁺-ATPase content in healthy controls. As shown in Fig. 2, the content of Na⁺-K⁺-ATPase showed a 89% increase in before and after treatment, the data are included in the mean values and in the unpaired comparison with healthy control subjects. The paired comparisons before and after treatment comprise nine patients as regards Ca²⁺-ATPase and eight patients as regards Na⁺-K⁺-ATPase. Pearson’s product moment correlation with two-tailed probability values was used to measure the strength of association between the variables.

**Fig. 1.** Skeletal muscle Ca²⁺-ATPase in 11 hyperthyroid patients before (n = 10) and after treatment (euthyroid, n = 10) and in 8 healthy controls. Mean values with bars showing SD. P values represent comparison between patients in the hyperthyroid and euthyroid states by use of paired t-test (n = 9) and comparisons of healthy controls with patients in the hyperthyroid or euthyroid state by use of unpaired t-test. NS, not significant.
hyperthyroidism: 558 ± 101 vs. 296 ± 34 pmol/g in euthyroidism (P = 0.0001, paired comparison, n = 8) and 278 ± 52 pmol/g in healthy controls (P < 0.0001). After restoration of euthyroidism, the contents of both cation pumps were not significantly different from those of healthy controls.

In hyperthyroid patients and healthy controls, Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase contents were positively correlated to circulating free and total thyroid hormone levels and to EE per kilogram of lean body mass, and the two cation pumps were intercorrelated (Fig. 3). Ca\textsuperscript{2+}-ATPase correlations to total T\textsubscript{3}: r = 0.74, P = 0.001, T\textsubscript{3}; r = 0.69, P = 0.006, T\textsubscript{4}; r = 0.61, P = 0.022, total T\textsubscript{4}; r = 0.68, P = 0.004, EE/kg body wt: r = 0.54, P = 0.03 Na\textsuperscript{+}, K\textsuperscript{+}-ATPase: r = 0.63, P = 0.02. Na\textsuperscript{+}, K\textsuperscript{+}-ATPase correlations to total T\textsubscript{3}; r = 0.92, P < 0.0001, T\textsubscript{3}; r = 0.91, P < 0.0001, T\textsubscript{4}; r = 0.87, P < 0.0001, total T\textsubscript{4}; r = 0.84, P < 0.0001, EE/kg body wt: r = 0.79, P = 0.0003.

**DISCUSSION**

Probably the most important new information gained from the present study is that in hyperthyroidism the content of Ca\textsuperscript{2+}-ATPase in human skeletal muscle is increased. Moreover, this increase is normalized by achievement of euthyroidism by standard methimazole treatment and shows significant correlation to plasma thyroid hormone levels as well as to energy expenditure. Our values for the contents of Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in skeletal muscle of healthy control subjects correspond closely to those previously published by our laboratory and others (5, 10).

In all our patients, we confirm our previous finding of elevated Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content in human skeletal muscle in hyperthyroidism and its normalization after treatment (17). Hyperthyroid patients suffer from muscle weakness, fatigue, and decreased contractility, which are normalized after treatment (22). The gross increase in skeletal muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in hyperthyroidism is thus unlike the upregulation induced by physical training, not associated with increased muscle strength. This is probably due to a concomitant increase in the content of Na\textsuperscript{+} channels in muscle, as demonstrated in animal studies (15), allowing increased passive flux of Na\textsuperscript{+} into the muscle cells dissipating the membrane potential and reducing excitability and contractility (5). In the rat, the increase in the content of Na\textsuperscript{+} channels and passive Na\textsuperscript{+}-K\textsuperscript{+} fluxes induced by thyroid hormone treatment precedes the
upregulation of Na\(^+\)-K\(^-\)-ATPase, indicating that the latter is a compensatory mechanism to maintain transmembrane Na\(^-\)-K\(^+\) distribution and cell membrane potential (12, 15).

The SR is a specialized Ca\(^2+\) storage membrane system in muscle cells and plays a central role in the contractile function of the muscle by virtue of its ability to regulate cytosolic Ca\(^2+\) concentration (3, 20). Ca\(^2+\) release from the SR initiates muscle contraction, whereas Ca\(^2+\) reuptake into the SR membrane vesicles lowers cytosolic Ca\(^2+\), causing muscle relaxation. The events that regulate Ca\(^2+\) release and removal can affect cytosolic Ca\(^2+\) levels and, hence, the rate and extent of muscle contraction and relaxation. The Ca\(^2+\) reuptake function of the SR is mediated by the Ca\(^2+\) transport pump, the sarcoplasmic reticulum Ca\(^2+\)-ATPase (SERCA). Ca\(^2+\) release from the SR to trigger muscle contraction is mediated by Ca\(^2+\) channels, the ryanodine receptors. In animals, thyroid hormones increase the mRNA expression and protein of both the Ca\(^2+\)-ATPase and the ryanodine receptor in skeletal muscle (1, 16). This dual upregulation explains that, in rat muscle, both contraction rates and relaxation rates are increased in hyperthyroidism and decreased in hypothyroidism (9, 13). In keeping with this, one of the classical clinical features of thyroid disorders is the shortening of the Achilles tendon reflex time in hyperthyroidism and its prolongation in hypothyroidism (30).

The slow-to-fast transition in hyperthyroid red muscle is mainly due to increased SERCA1 isoform expression. SERCA1 seems to be the only SERCA isoform able to interconvert energy from ATP hydrolysis into heat without calcium transport, the uncoupled ATPase activity (7). Recently, an animal model described how the calorigenic action of thyroid hormone in hyperthyroidism might be due, at least in part, to the increased uncoupled ATPase activity in both red and white muscles (2).

The observed increase in skeletal muscle Ca\(^2+\)-ATPase in our study might in part be the result of a transition of muscle fiber types induced by hyperthyroidism from the type I muscle fibers with a low content of SR and Ca\(^2+\)-ATPase to the type II muscle fibers with a high content of SR and Ca\(^2+\)-ATPase (27). Moreover, because SERCA1 has a higher ATP turnover number (1,000–1,100/min) than that of SERCA2 (450/min) (8), an increase in the ratio between type II and type I fibers further augments the capacity for Ca\(^2+\) recycling and the ATP turnover associated with this transport process. Therefore, the 45% increase in Ca\(^2+\)-ATPase content demonstrated in the present study may be combined with an increased average ATP turnover number. This would allow for an even more pronounced relative rise in energy expenditure.

We chose to reexamine the patients after 3 mo of treatment, and at that time the patients were clinically euthyroid, and circulating thyroid hormones and REE were normalized. We might have obtained a more complete normalization of the cation pumps after a longer period of euthyroidism, allowing for further regeneration of the muscles (22).

Observations of correlations do not establish causality. The hypothesis that the calorigenic actions of thyroid hormones are in part mediated by increased Ca\(^2+\) recycling in the SR of skeletal muscle is generated from knowledge obtained from animal studies. The human data we present here cannot disprove this hypothesis; on the contrary, the hypothesis is supported. Moreover, the downregulation of Ca\(^2+\)-ATPase induced by the standard therapy was accompanied by normalization of REE. The increased content of Ca\(^2+\)-ATPase in skeletal muscle can only in part explain the increase in REE in hyperthyroidism. Thyroid hormone affects energy balance by several mechanisms (24). In hyperthyroidism, spontaneous physical activity is increased (19). Thyroid hormone regulates gene expression of a large number of proteins known to regulate energy expenditure, for example uncoupling protein-3 (18), and increased protein synthesis also consumes energy (2).

In summary, our data are consistent with the hypothesis that, in human subjects, the calorigenic actions of thyroid hormones are in part mediated by increased skeletal muscle content of Ca\(^2+\)-ATPase, allowing for increased energy expenditure by both active Ca\(^2+\) transport and uncoupled ATPase activity. We confirm our previous finding of elevated Na\(^+\)-K\(^-\)-ATPase content in skeletal muscle in hyperthyroidism, which to a minor extent adds to the increased energy expenditure by increased active Na\(^+\)-K\(^+\) transport. In a wider perspective, active Ca\(^2+\) transport in skeletal muscle may be a regulatory target for energy turnover and weight control.

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GRANTS

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