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Reduced Metabolic Efficiency of Skeletal Muscle Energetics in Hyperthyroid Patients Evidenced Quantitatively by In Vivo Phosphorus-31 Magnetic Resonance Spectroscopy

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Skeletal muscle energetics of seven hyperthyroid patients were investigated throughout a rest-exercise-recovery protocol using phosphorus-31 magnetic resonance spectroscopy (^{31}P MRS) to quantitatively document in vivo the metabolic bases of impaired muscle performance in hyperthyroidism. The contributions of the main pathways of adenosine triphosphate (ATP) synthesis to energy production and proton efflux were measured and compared with results from normal muscle. At rest, a reduced concentration of phosphocreatine (PCr) was calculated for hyperthyroid patients when compared with controls, whereas pH and concentrations of inorganic phosphate (Pi) and phosphomonoesters (PME) were not different from controls. During exercise, the analysis of changes in pH and PCr concentration demonstrated that (1) at the onset of exercise, the magnitude of glycolysis activation is significantly larger for patients, resulting in a marked pH decrease; (2) the energy cost of exercise is higher for patients as compared with controls performing the same amount of work; and (3) both anaerobic and aerobic pathways are significantly more activated in the hyperthyroid group throughout the 3 minutes of exercise. During recovery, the rates of proton efflux and PCr resynthesis were similar in both groups, excluding any alteration in oxidative function and proton handling as a cause of initial glycolytic hyperactivation. The increased energy cost measured for patients during exercise evidences an increased need for energy, which is (1) probably linked to the existence of additional ATP-consuming mechanism(s), and (2) supported by hyperactivation of both aerobic and anaerobic pathways. These findings imply that, all things equal, a hyperthyroid muscle requires more energy to function than normal, and as a result is potentially more fatiguable.

HYPERTHYROID PATIENTS frequently complain of exertional fatigue and muscle weakness. Although the mechanisms of action of thyroid hormones on muscle cells have been extensively studied, it is not clear which of the structural and biochemical abnormalities are responsible for the impairment of muscle performance. It was originally proposed that muscle weakness could be accounted for by an impaired energy supply for contraction.[1] This impairment contrasts with most of biochemical evidence indicating the presence of a hypermetabolic state in muscle cell in the thyrotoxic state.[2] In vitro studies have shown increased enzymatic activities in all main energy-producing pathways of skeletal muscle cells,[2-7] while increased glucose uptake and augmented fluxes of glucose through the oxidative and nonoxidative pathways have been measured in humans during experimental hyperthyroidism.[8] Abnormalities in blood circulation and/or oxygen supply have been eliminated as possible causes of intolerance to muscle exercise, since in hyperthyroid patients and animals, increased regional blood flow and overperfusion of muscle during exercise have been observed,[9,10] as well as increased basal oxygen uptake at rest and during recovery.[8,11]

A decrease in muscle mass is observed in hyperthyroidism,[1,12] but this is unlikely to be responsible for all of the abnormalities observed in muscle-intrinsic metabolism. Atrophy of type I and type II fibers has been documented histochemically in humans, but the relationship to metabolic abnormalities remains uncertain.[13]

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In relation to altered membrane permeability or to a direct effect of thyroid hormone, increased activities of ionic pumps such as sarcoplasmic Ca^{2+}-adenosine triphosphatase (ATPase) and Na^+/K^+-ATPase have been demonstrated in vitro on animal models.[14-17] This increased ionic pump activity and possible uncoupling of oxidative phosphorylation[5,17] could be the mechanism responsible for impaired muscle performance, since they increase the consumption of ATP elsewhere than in myofibrils. Therefore, it could be suspected that in hyperthyroidism, skeletal muscle energy production is less efficient, rather than impaired.

A few in vivo studies of muscle performed on hyperthyroid patients and animals using phosphorus-31 magnetic resonance spectroscopy (^{31}P MRS) have provided only qualitative and conflicting results. Taking into account the larger changes for pH and phosphocreatine (PCr) recorded throughout exercise in humans, Kaminsky et al concluded that hyperthyroid muscle should have a larger dependence on glycolytic metabolism.[18] Meanwhile, Argov et al[19] did not observe any abnormalities throughout the exercise period, whereas they measured a faster PCr recovery for hyperthyroid patients. This provides no reasonable explanation for muscular symptoms.

The aim of this study was to clarify which abnormalities of muscle bioenergetics in hyperthyroid patients are detectable by ^{31}P MRS, and how they can be related to the available evidence of biochemical changes. To address this issue, we have used the framework recently reported by Kemp et al[20] to quantitatively analyze the MRS data recorded throughout a rest-exercise-recovery protocol.

MATERIALS AND METHODS

Subjects

Seven patients (six females and one male) with untreated hyperthyroidism were included in the study. The mean age of the patient group was 40.7 years (range, 14 to 60) and the mean (±SE) weight, height, and body mass index were 60.1 ± 4.5 kg, 166 ± 4 cm, and 21.6 ± 1 kg/m^2, respectively. At the time of hospitalization, symptoms of hyperthyroidism had persisted from 2 to 4 months for six patients. For one of them, symptoms seemed to have begun 1 year earlier, but were ignored by the patient. Determination of the duration of symptoms was based on a clinical questionnaire administered by an experienced clinician from the Endocrinology Department. Once the hyperthyroid status was biologically confirmed, ^{31}P MRS examination of muscle metabolism was performed after informed consent was obtained. Patients had received symptomatic medication or mild sedatives for a maximum of 1 week before MRS was performed. At this time, no antithyroid drugs were used. Just after the MRS protocol, all patients received specific treatment (carbimazole) and for some of them surgery was performed. All patients had clearly elevated blood free levels of thyroxine (T_4; 55.54 ± 8.12 pmol/L, radioimmunologic assay [FT_4 Kit, CisBio Laboratory, Gif-sur-Yvette, France]; normal range, 7 to 17 pmol/L) and triiodothyronine (T_3; 24.0 ± 4.20 pmol/L, radioimmunologic assay [Techno Genetics, CisBio Laboratory]; normal range, (3.8 to 5.8 pmol/L), as well as a low level of thyroid-stimulating hormone (TSH; radioimmunologic assay [hTSH Kit, CisBio Laboratory]; normal range, 0.1 to 0.001 mU/L). None of the patients exhibited increased creatine phosphokinase levels. Hyperthyroidism was induced by Basedow's disease (n = 6) or by a toxic adenoma (n = 1). Four patients complained of myasthenia (n = 3), muscle cramps (n = 2), or myalgia (n = 2), and four patients presented brisked tendon reflexes.

Seven healthy volunteers matched for sex (six females and one male), mean age (37.9 years; range, 22 to 61), weight (58 ± 3 kg), height (167 ± 3 cm), and body mass index (20.6 ± 1 kg/m^2) were included in the control group. None of them were engaged in regular physical activity. The study was approved by the local ethics committee.

MRS

Investigations were performed as previously described[21] using a Bruker 47/30 Biospec spectrometer (Bruker, Wissembourg, France) interfaced with a 30-cm bore, 4.7-T superconducting magnet. Subjects remained sitting on a chair by the magnet with their dominant arm resting in the magnet bore. To ensure good venous return, the forearm was placed approximately at the same height as the shoulder. It was restrained with velcro straps to prevent forearm movements. Magnetic field homogeneity was optimized by monitoring the signal from the water and lipid protons at 200.14 MHz. Pulsing conditions (2-second interpulse delay, 55-μs pulse length) were chosen to optimize the ^{31}P signal obtained with a 50-mm diameter double-tuned surface coil positioned over the belly of the flexor digitorum superficialis muscle. Spectra were time-averaged over 1 minute (32 scans) and sequentially recorded during 3 minutes of rest, 3 minutes of exercise, and 20 minutes of recovery.

Exercise Protocol

After three spectra recorded at rest, each subject performed finger flexions at 1.5-second intervals for 3 minutes, with women lifting a 4-kg and men a 6-kg weight. This exercise was chosen to ensure PCr consumption above 50% and a significant pH decrease.[21] We have previously determined that the intersubject variability for those parameters can be explained by the variability of power output.[22] Therefore, to normalize the extent of PCr hydrolysis and intracellular acidosis, both parameters were scaled to power output for patients and controls. All subjects were able to complete the exercise. The sliding amplitude of the weight was recorded using a displacement transducer connected to a personal computer. The amplitude of each flexion and the frequency of exercise were measured using ATS software (SYSMA, Avignon, France). Results were expressed as power output (W) for each minute of exercise.

Data Analysis

Raw MRS signals were transferred to an IBM RISC 6000 workstation (IBM, Paris, France) and processed using the NMR1 spectroscopy processing software (New Methods Research, Syracuse, NY). After deconvolution of free induction decay (FID; corresponding to a line broadening of 15 Hz) and Fourier transformation, baseline correction was performed as previously described.[23] Metabolite peak areas were measured by curve fitting of the spectrum signals to a Lorentzian shape function.[23] After correction for differential magnetic saturation effects,[21] absolute concentrations were expressed relative to ATP measured at rest (8.2 mmol/L of intracellular water). For calculating the concentration of metabolites in hyperthyroid muscle, the ATP concentration and total creatine content were taken as 75% and 88% of normal muscle.[13] Intracellular pH was calculated from the chemical shift of inorganic phosphate (Pi) relative to PCr,[24] and free cytosolic ADP concentration was calculated from pH and PCr using the creatine kinase (CK) equilibrium constant (K = 1.66 10^9 mol/L).[20] Values at rest were averaged over the three rest spectra. The PCr recovery profile was fitted to a single exponential curve described by the equation $PCr(t) = PCr_{rest} + PCr_{cons} * e^{-kt}$, where PCr_{rest} refers to the PCr calculated at rest and PCr_{cons} indicates the amount of PCr consumed at end of the exercise period. The initial rate of PCr recovery (Vi rec) was calculated from k and PCr_{cons}: $V_i = k * PCr_{cons}$ (mmol/L/min).[25]

For each minute of exercise, rates of ATP production from PCr hydrolysis, glycogenolysis, and oxidative processes were calculated according to the method developed in the Appendix.

Statistics

Results are expressed as the mean ± SEM. Differences between hyperthyroid patients and control subjects were examined using a nonparametric Mann-Whitney U test. Statistical differences were considered as significant at $P < .05$.

RESULTS

Figure 1 illustrates a typical stacked plot of serial ^{31}P MR spectra recorded throughout the rest-exercise-recovery protocol.

Resting Muscle

Analysis of ^{31}P MR spectra recorded at rest did demonstrate a reduced PCr for hyperthyroid patients as compared with controls (Table 1). Phosphomonoesters (PME) and Pi values were similar for both groups. The mean pH value of resting skeletal muscle was 6.99 ± .01 for the hyperthyroid group and 6.97 ± .01 for controls.

Exercising Muscle

Throughout the exercise period, power output remained constant, with no sign of fatigue, and no significant differences were observed between the groups (Table 1 and Fig 2A). The rates of consumption of energy stores (PCr) were similar for both groups, indicating that the alkalinizing effect of PCr breakdown was the same (Fig 2B). At end of exercise, the amounts of PCr consumed were similar for both groups (Table 1).

At the onset of exercise, control subjects did not display marked intracellular acidosis (0.08 ± .02 pH unit) as a sign of equilibrium between proton production and proton efflux (Fig 2C). Conversely, a significantly larger pH decrease (0.25 ± 0.07) was recorded for the hyperthyroid group, indicating a faster initial rate of acidosis (Table 1 and Fig 2C). During the second minute of exercise, a larger acidosis was measured for controls (0.31 ± 0.05 *v* 0.12 ± 0.03 pH units; $P < .05$), resulting in an equal end-of-exercise acidosis (Table 1). The mean buffering power of the muscle cytosol calculated from Pi and pH values did not vary between groups (31.6 ± 1.2 and 31.4 ± .7 slykes for patients and controls, respectively) and was similar to the value usually reported, ie, 30 slykes. The quantitative analysis of ATP production during exercise was performed according to the method reported by Kemp et al,[20] taking into account PCr and pH time-dependent changes throughout the rest-exercise-recovery period. The contribution of different metabolic pathways to ATP synthesis scaled to power output is illustrated in Figs 3 and 4. The energy cost, calculated at the beginning of exercise considering that oxidative participation to ATP synthesis is negligible, was significantly larger for the hyperthyroid group (47 ± 6 mmol/L/min/W) as compared with controls (37 ± 4 mmol/L/min/W). This means that with identical power output, the ATP cost of exercise was higher for hyperthyroid subjects than for controls. At the end of the first minute of exercise, the amount of ATP produced from PCr hydrolysis was similar for both groups (Fig 3A), whereas a significantly faster ATP production from glycogenolysis was calculated for patients (29 ± 5 mmol/L/min/W) compared with controls (14 ± 2 mmol/L/min/W) (Fig 3B). During the remaining period of exercise,

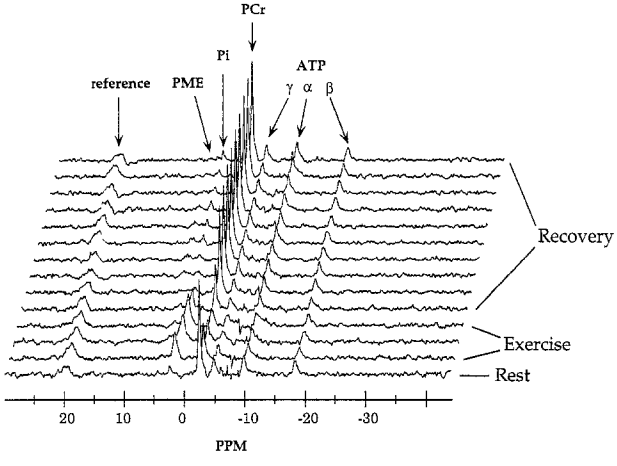

Fig 1. Typical series of ^{31}P MR spectra recorded from normal flexor muscles throughout the rest-exercise-recovery protocol. Y axis represents the signal intensity and X axis represents the frequency of the signals recorded on the phosphorus nuclei with respect to the PCr signal (−2.45 ppm) expressed in parts per million (PPM). Reference, reference compound (phenyl phosphonic acid) contained in a glass capillary and positioned at the center of the surface coil; PME, phosphomonoesters; Pi, inorganic phosphate; PCr, phosphocreatine; ATP (α, β, γ), α, β, and γ phosphate groups of ATP. The acquisition time of each spectrum (time resolution) is 1 minute.

Table 1. Metabolic and Mechanical Parameters Recorded and Calculated Throughout the Rest-Exercise-Recovery Protocol

	Patients	Controls
Rest		
PCr (mmol/L)	31.00 ± .57*	38.01 ± .96
pH	6.99 ± 0.01	6.97 ± 0.01
PME (mmol/L)	1.79 ± .23	1.22 ± .23
ADP (μmol/L)	7.36 ± .93	5.71 ± 1.37
First minute of exercise		
pH	6.74 ± .06*	6.89 ± .02
PCr_{cons} (mmol/L)	15.3 ± 1.5	15.3 ± .92
Mean power (W)	0.89 ± 0.05	0.99 ± 0.09
ADP (μmol/L)	20.4 ± 7.8	23.9 ± 3.3
Vi PCr_{cons} (mmol/L/min)	19.5 ± 3.3	20.93 ± 6.0
End of exercise		
pH	6.47 ± 0.09	6.47 ± 0.07
PCr_{cons} (mmol/L)	20.63 ± 2.4	21.39 ± 2.6
Mean power (W)	0.92 ± 0.07	0.99 ± 0.10
ADP (μmol/L)	18.13 ± 5.25	16.62 ± 4.50
Rate of ATP production (mmol/L/min/W)		
From PCr hydolysis	2.7 ± 1.4	3.9 ± 2.5
From glycogenolysis	16.2 ± 4.1	13.3 ± 3.7
From oxidative phosphorylation	31.24 ± 6.3*	17.5 ± 3.71
Recovery		
k_{rec}	0.9 ± 0.2	0.4 ± 0.1
Vi $PCr_{resynth}$ (mmol/L/min)	16.70 ± 3.2	11.25 ± 2.4
Rate of proton efflux (mmol/L/min)	2.99 ± 0.56	3.32 ± 0.5

NOTE. Results are presented as means ± SEM.

Abbreviations: PCr_{cons}, magnitude of PCr hydrolyzed; ADP, free adenosine diphosphate concentration; Vi PCr_{cons}, initial rate of PCr consumption calculated at the onset of exercise; k_{rec}, kinetics constant of the exponential recovery profile of PCr; Vi $PCr_{resynth}$, initial rate of PCr resynthesis calculated at the onset of recovery, which is equal to k_{rec} PCr_{cons}.

*Significant difference ($P < .05$).

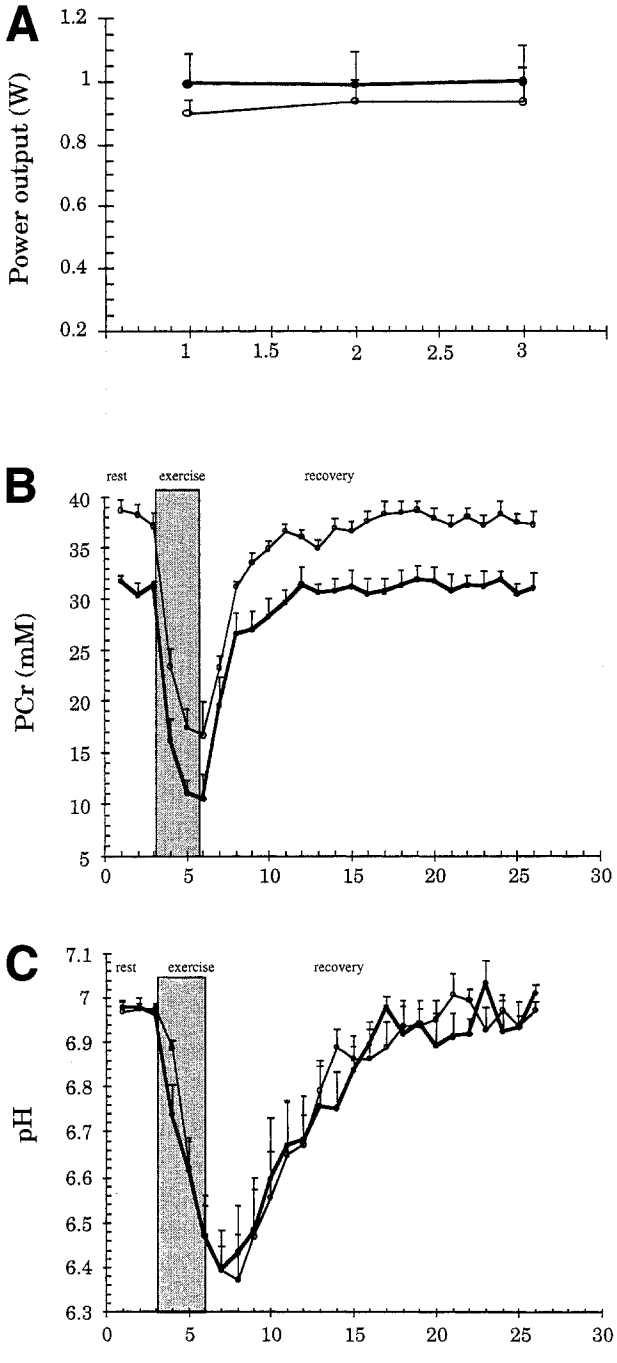

Fig 2. Time-dependent changes of power output throughout the exercise period (A), and PCr (B) and pH (C) throughout the experimental protocol. R, Ex, and Rec refer respectively to the resting, exercise, and recovery periods. (●) Hyperthyroid patients; (○) controls. Results are presented as means ± SEM.

rates of anaerobic ATP synthesis were similar for both groups (Fig 3B). The indirectly estimated aerobic ATP production was significantly higher for patients during the second and third minute of exercise than for controls (Table 1 and Fig 4B). The average final value was approximately twice as high in patients as compared with controls.

Recovery From Exercise

As shown in Table 1 and in Fig 2, at the onset of recovery, the initial rate of PCr resynthesis tended to be faster in the hyperthyroid group, but the difference was not statistically significant. The return kinetics of pH to the resting value were similar in the two groups, as evidenced by similar rates of proton efflux (Table 1). At the end of recovery, all of the metabolic parameters reached their respective preexercise values.

DISCUSSION

Validity of the Study

We have hypothesized that both ATP and total creatine are reduced in patients according to the biochemical assays reported previously.[13] Values of PCr/ATP measured in patients are identical to control values, suggesting that PCr and ATP might both be reduced in the same proportion in patients. The major consequence of this assumption is an underestimation of both energy cost and extent of PCr consumption for patients. Ignoring this hypothesis would have led to increased values of

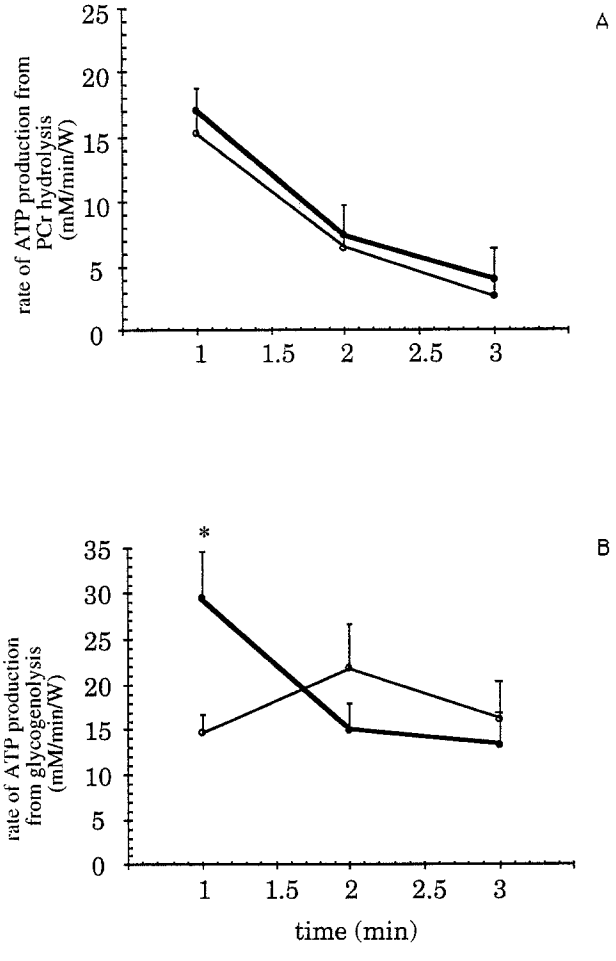

Fig 3. Time-dependent changes of the rate of ATP production (mmol/L/min/W) from PCr hydrolysis (A) and glycogenolysis (B). (●) Hyperthyroid patients; (○) controls. Results are presented as means ± SEM. *Significant differences ($P < .05$).

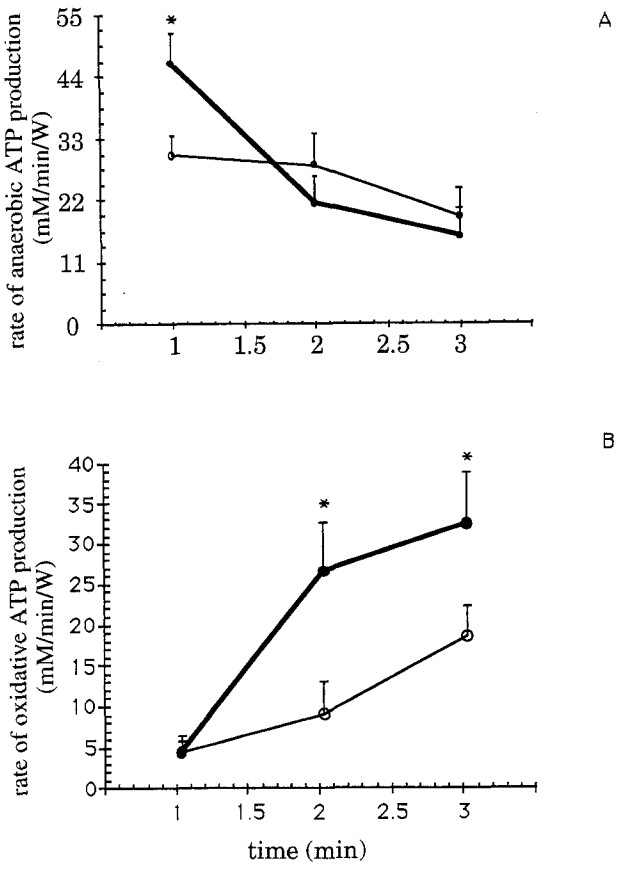

Fig 4. Time-dependent changes of the rate of ATP production (mmol/L/min/W) from anaerobic (A) and oxidative (B) pathways. (●) Hyperthyroid patients; (○) controls. Results are presented as means ± SEM. *Significant differences ($P < .05$).

this whole set of parameters, thereby enhancing differences for these parameters between patients and controls. So, the initial hypothesis cannot flaw the final result.

All patients exhibited clinically and biologically clear hyperthyroidism and received symptomatic medication, including beta-adrenergic blocking agents. These agents are known to impair the metabolic response of skeletal muscle for maximal exercise by inhibiting the increase in blood flow and decreasing the rate of glycogen breakdown.[26] Considering these effects, the medication could have lowered the magnitude of glycogenolysis and oxidative pathway activation. If such were the case, our results would be underestimated. Moreover, there exists evidence that beta-adrenergic blockers do not prevent the effects of thyroid hormone on changes in fiber types and mitochondrial enzyme activities in muscle cell.[27,28] Finally, the duration of treatment was at most 1 week at the time of the examination by MRS, eliminating the possible chronic effects of long-term treatment.[26]

Metabolic Changes Associated With Hyperthyroidism

At rest. The marked reduction of PCr observed for hyperthyroid subjects at rest agrees with previous results obtained from needle biopsy specimens.[13] This reduction could not be ascribed to changes in T_1 relaxation times or to an abnormal ratio of muscle to fat. Indeed, saturation factors, which directly reflect relaxation times, were unchanged in hyperthyroid patients. Also, water to fat ratios were not different between hyperthyroid patients (2.45 ± 0.14) and controls (2.26 ± 0.27). The underlying cause of reduction of PCr is unknown. It could be related to alteration in the rates of creatine uptake and breakdown and/or synthesis of adenine nucleotides and/or exchange of Pi. Normally, a lower PCr content exists in slow-twitch muscles, mainly composed of type I fibers, and could be associated, for the hyperthyroid group, with a modification of fiber type composition. However, previous histochemical analyses have shown that atrophy of both fiber types is associated with altered thyroid hormone level.[13] In addition, therapy-induced normalization of contractility and energy turnover rates has been reported for a hyperthyroid patient in spite of persisting atrophy of both fiber types.[13]

The mean pH values measured at rest were normal for both groups, in agreement with the few in vivo studies performed on patients.[18,19] Recently, Thompson et al[29] observed rat muscle cells to be more alkaline in the experimental hyperthyroid state. However, the time of exposure to excessive T_3 doses was short (5 days) and could explain this discrepancy.[5,30]

During exercise. During exercise, alterations in proton concentration are governed by creatine kinase activity, glycogenolysis, proton efflux, and buffering capacity. Taking into account that the amount of PCr breakdown, rate of proton efflux, and buffering capacity were identical in both groups, the larger pH decrease measured in patients during the first minute of exercise could have been associated with a dysregulation of glycolysis, as previously suggested.[18] However, it seems unlikely, since it should have been effective during the whole exercise and not only at the beginning. Finally, oxidative deficiency could have enhanced glycolysis activity. The analysis of the kinetics of PCr recovery, which are directly dependent on aerobic metabolism, clearly eliminates oxidative deficiency as a cause of the marked acidosis recorded at the onset of exercise.

The twofold increase in the rate of aerobic ATP production measured at end of exercise agrees with the results of Kimura et al.[31] They showed that hyperthyroid patients exercising on bicycle ergometer displayed a twofold increase in oxygen consumption scaled to power output. The quantitative analysis demonstrates that the energy cost of exercise, illustrated by the amount of ATP produced for a given power output, is significantly higher for hyperthyroid subjects. In other words, hyperthyroid muscles function less economically than normal and require more energy to perform a given exercise. Several mechanisms, including reduced ATP synthesis or increased ATP consumption, can account for this lower efficiency.

In the case of reduced synthesis, the reduction could originate from enzymatic deficiency affecting glycolytic or oxidative metabolism. Several animal studies have reported an increase in enzymatic activities, rather than a decrease,[2-7] although opposite results in experimental hyperthyroidism in humans have also been published.[12]

Hence, increased ATP consumption during exercise, rather than reduced synthesis, is more likely to be responsible for the

augmented energy cost. This is consistent with previous results of increased rate of ATP turnover during ischemic muscle contraction of a single hyperthyroid patient[13] and throughout force development in rats.[11] Even the clinically observed catabolic state, including an increase in basal metabolic rate, hyperthermia, and weight loss, matches this frame of hyperactivity.[1,2,32] Increased consumption could be supported by muscle atrophy,[1,32,33] alteration in fiber type composition,[34] increased activity of ATP-consuming ionic pumps, or uncoupling of oxidative phosphorylation.

Muscle Atrophy and Increased Energy Cost

Faced with a given external load, muscle that is atrophied would develop reduced capacity of ATP synthesis due to smaller fibers. In this case, the atrophied muscle would develop larger ATP consumption, illustrated by larger PCr consumption, considering that all muscle fibers are recruited during exercise. In our opinion, the current results cannot be explained solely by atrophy. The muscle we have investigated is, according to other evidence, not considerably involved in hyperthyroidism.[1] We have no independent measure of muscle mass, but since the average body mass index of patients was not significantly lower than in controls, and since signs of muscle atrophy were not visible in the arms, it seems unlikely that muscle atrophy (decrease in muscle mass) could account for the observed reduction of metabolic efficiency (increase of energy cost). An important point to consider is whether the surface coil examined the same muscle mass in patients and controls. Failure to observe any significant difference for the water to fat ratio between patients (2.45 ± 0.14) and controls (2.26 ± 0.27) suggests that this was indeed the case.

Changes in Fiber Types and Increased Energy Cost

Changes in fiber types, which are well documented in hyperthyroidism, could also account for reduced metabolic efficiency.[34] Hyperthyroidism causes changes in fiber types in skeletal muscle towards increased proportions of type IIA fibers (fast glycolytic/oxidative).[2,4,35-38] Knowing that flexor muscles are composed of equivalent amounts of type I and type II fibers, the alteration of flexor muscle energetics exposed to high levels of thyroid hormone should have been minor if fiber type composition were the only mechanism responsible for changes observable by MRS.

Possible Causes of Increased Energy Cost

It has long been known that thyroid hormones control mitochondrial metabolism and the active transport of Na^+ and K^+ through the sarcolemma. An excess of thyroid hormones could dysregulate these mechanisms, thereby leading to an increased energy cost, with ATP consumption increasing elsewhere than in myofibrils. Mitochondria isolated from animals treated with sufficient doses of thyroid hormone display an increased rate of substrate oxidation,[39] while the activity of the electron transport chain and the membrane-bound α-glycerophosphate dehydrogenase are both augmented.[40,41] Treatment with T_3 also increased carrier-mediated adenosine diphosphate (ADP) uptake and rate of oxygen consumption by rat mitochondria, whereas respiratory control did not change.[42] These observations suggest this hypermetabolic status should be accompanied by the production of an equivalent amount of ATP, in agreement with our results. The overproduction of ATP must then be regulated by an increase in ATP-consuming processes as previously suggested.[43] An excess in thyroid hormone has been proposed to induce uncoupling of oxidative phosphorylation in muscle cells.[5,17,44] This phenomenon is known as a prevention of ADP phosphorylation to ATP, with the free energy released through electron transport appearing as heat, rather than as newly made ATP. According to a recent review,[5] the uncoupling process could result from a binding of thyroid hormone to adenine nucleotide translocase, thereby producing an imbalance between PCr synthesis and an increased rate of ATP production. The amount of ATP produced that cannot be used in myofibrils would be hydrolyzed throughout futile cycles both in the intramembrane space and outside the mitochondria.[5,44] Also, the regulation of this ATP overproduction could be ensured by increased activity of Na^+/K^+-ATPase, although this alteration might represent an independent action of thyroid hormones. Increased activities of ionic pumps, such as sarcoplasmic Ca^{2+}-ATPase and Na^+/K^+-ATPase, have been demonstrated in vitro on animal models.[14-17] Thyroid status stimulates active Na^+/K^+ transport,[45,46] alters the number of ouabain sites,[47] and increases the maximum Na^+/K^+-ATPase activity.[48] Evidences has been reported that increased energy expenditure for transmembrane active Na^+/K^+ transport mediates a significant fraction of the thermogenic response to thyroid hormones.[49] These results support the inference that altered thyroid status enhanced energy utilization linked to active Na^+ transport by hyperactivation of the Na^+/K^+ pump.

In conclusion, the present study shows that, using in vivo MRS, hyperthyroid subjects display a higher ATP cost during muscle exercise, which is compensated by activation of both aerobic and anaerobic energy-producing pathways. This observation could best be explained by increased activity of ATP-consuming mechanisms, such as ATPase pumps and/or increased mitochondrial oxidation and/or uncoupling of oxidative phosphorylation. Accordingly, the hyperthyroid muscle requires more energy to function, which is a possible explanation for exercise intolerance as a clinical manifestation of hyperthyroidism.

APPENDIX

For each minute of exercise, rates of ATP production from PCr hydrolysis (P_{ATP}), glycogenolysis (G_{ATP}), and oxidative processes (O_{ATP}) were calculated according to the method recently proposed by Kemp et al.[20]

Rate of ATP Production From PCr Hydrolysis: P_{ATP}

The rate of ATP production from PCr hydrolysis was calculated from the time-dependent changes of PCr for each minute of exercise. This rate was scaled to the power output for each minute of exercise.

Rate of ATP Production From Glycogenolysis: G_{ATP}

Throughout the exercise period, glycogen breakdown to pyruvic and lactic acid leads to a pH decrease, which is limited by proton efflux and the buffering power of cytosol. Considering that the glycogenolytic production of 1 mol of ATP, when coupled to ATP hydrolysis, yields 1.5 mol of protons, G_{ATP} can be simply deduced from the total amount of protons produced throughout exercise.[50] This metabolic proton generation can be calculated from pH changes (measured from ^{31}P MR spectra), the amount of protons passively buffered in the cytosol ($[H^+]\beta$, equation 1), the amount of protons consumed by net PCr hydrolysis ($[H^+]_{PCr}$, equation 2), and the amount of protons leaving the cell (proton efflux, equation 3).

equation (1): $[H^+]_\beta = \beta\Delta pH$, where

$$\beta = \beta_{nonbicarb,non\text{-}Pi} + \beta_{Pi} = (-22\,[pH] + 170)\frac{[2.3\ Pi]}{[1 + 10^{(pH-6.5)}]\,[1 + 10^{(6.75-pH)}]},$$

where $\beta_{nonbicarb,non\text{-}Pi}$ refers to the buffering power associated with nonbicarbonate, non-Pi systems, and bPi indicates the buffering power associated with Pi as previously described.[20] ΔpH refers to the extent of intracellular acidosis measured for each minute of exercise

$$\text{Equation (2): } [H^+]_{PCr} = \frac{d(PCr)/dt}{1 + 10^{(pH-6.75)}}$$

where d(PCr)/dt refers to the rate of PCr hydrolysis measured for each minute of exercise and pH is the pH value at the end of the corresponding minute of exercise.

Throughout the recovery period, despite the intracellular proton load associated with PCr resynthesis ($[H^+]_{PCr}$), pH recovers back to its basal value as a result of proton efflux (Pe). Hence, as recently reported,[51] proton efflux can be calculated considering together proton production from PCr resynthesis and pH changes as follows:

$$\text{Equation (3): Pe (mmol/L/min)} = d(pH)/dt\beta + [H^+]_{PCr}$$

where β represents the buffer capacity of muscle cytosol, d(pH)/dt refers to the rate of pH changes upon the initial period of recovery, and $[H^+]_{PCr}$ represents the rate of proton production associated with PCr resynthesis. Taking into account pH and PCr changes throughout the initial period of recovery, we have previously determined a linear relationship between Pe and the extent of intracellular acidosis measured at end of exercise.[52]

$$\text{Equation (4): Pe} = 0.52 + 4.1\ \Delta pH.$$

Considering, in agreement with others,[20] that this pH dependence of proton efflux remains valid during exercise, proton efflux has been calculated for each minute of exercise based on the DpH calculated at the corresponding time of exercise. After G_{ATP} has been calculated, it was scaled to the power output for each minute of exercise.

Rate of Anaerobic ATP Production (An_{ATP}) and Energy Cost (EC)

The rate of anaerobic ATP production scaled to power output corresponds to the sum of $P_{ATP} + G_{ATP}$. It represents the amount of anaerobically produced ATP for a given unit of power. At the onset of exercise, it has been shown, from a comparative analysis of ischemic and aerobic exercise, that contribution of oxidative metabolism is minor as compared with anaerobic metabolism.[53] Under this condition, the rate of anaerobic ATP production scaled to power output calculated at the onset of exercise can be considered as a reliable estimation of the total rate of ATP production necessary for a given unit of power: the energy cost.

Rate of Oxidative ATP Production: O_{ATP}

Considering that the energy cost retains a constant proportionality to power output throughout the entire exercise period, any decrease of anaerobic ATP production must be compensated by an increase of oxidative ATP production (O_{ATP}). Therefore, O_{ATP} can be calculated at any time of exercise as the difference between EC and anaerobic ATP production scaled to power output.

REFERENCES

1. Ramsay ID: Muscle dysfunction in hyperthyroidism. Lancet 2:931-934, 1966
2. Kubista V, Kubistova J, Pette D: Thyroid hormone induced changes in the enzyme activity pattern of energy-supplying metabolism of fast (white), slow (red), and heart muscle of the rat. Eur J Biochem 18:553-560, 1971
3. Courtright JB, Fitts RH: Effects of thyrotoxicosis on mitochondrial enzymes of rat soleus. Horm Metab Res 11:304-306, 1979
4. Janssen JW, van Hardeveld C, Kassenaar AA: Evidence for a different response of red and white skeletal muscle of the rat in different thyroid states. Acta Endocrinol Copenh 87:768-775, 1978
5. Seppet EK, Saks VA: Thyroid hormones and the creatine kinase system in cardiac cells. Mol Cell Biochem 134:299-309, 1994
6. Winder WW, Holloszy JO: Response of mitochondria of different types of skeletal muscle to thyrotoxicosis. Am J Physiol 232:C180-C184, 1977
7. Winder WW: Time course of the T3- and T4-induced increase in rat soleus muscle mitochondria. Am J Physiol 236:C132-C138, 1979
8. Foss MC, Paccola GM, Saad MJ, et al: Peripheral glucose metabolism in human hyperthyroidism. J Clin Endocrinol Metab 70:1167-1172, 1990
9. McAllister RM, Sansone JC, Laughlin MH: Effects of hyperthyroidism on muscle blood flow during exercise in rats. Am J Physiol Heart Circ Physiol 37:H330-H335, 1995
10. Sandler MP, Robinson RP, Rabin D, et al: The effect of thyroid hormones on gluconeogenesis and forearm metabolism in man. J Clin Endocrinol Metab 56:479-485, 1983
11. Everts ME, van Hardeveld C, Ter Keurs HE, et al: Force development and metabolism in perfused skeletal muscle of euthyroid and hyperthyroid rats. Horm Metab Res 15:388-393, 1983
12. Martin WHd, Spina RJ, Korte E, et al: Mechanisms of impaired

exercise capacity in short duration experimental hyperthyroidism. J Clin Invest 88:2047-2053, 1991

13. Wiles CM, Young A, Jones DA, et al: Muscle relaxation rate, fibre-type composition and energy turnover in hyper and hypothyroid patients. Clin Sci 57:375-384, 1979

14. Azuma KK, Hensley CB, Tang MJ, et al: Thyroid hormone specifically regulates skeletal muscle Na(+)-K(+)-ATPase alpha 2- and beta 2-isoforms. Am J Physiol 265:C680-C687, 1993

15. Everts ME, Clausen T: Effects of thyroid hormone on Na^+-K^+ transport in resting and stimulated rat skeletal muscle. Am J Physiol 255:E604-E612, 1988

16. Kim DH: Effect of thyrotoxicosis on sarcoplasmic reticulum in rat skeletal muscle. Am J Physiol 243:C151-C155, 1982

17. Sterling K: Thyroid hormone action at the cell level (second of two parts). N Engl J Med 300:173-177, 1979

18. Kaminsky P, Robin Lherbier B, Walker P, et al: Muscle bioenergetic impairment in hyperthyroid man: A study by ^{31}P NMR spectroscopy. Acta Endocrinol Copenh 124:271-277, 1991

19. Argov Z, Renshaw PF, Boden B, et al: Effects of thyroid hormones on skeletal muscle bioenergetics: In vivo phosphorus-31 magnetic resonance spectroscopy study of human and rats. J Clin Invest 81:1695-1701, 1988

20. Kemp GJ, Radda GK: Quantitative interpretation of bioenergetic data from P-31 and H-1 magnetic resonance spectroscopic studies of skeletal muscle—An analytical review. Magn Reson Q 10:43-63, 1994

21. Bendahan D, Confort-Gouny S, Kozak-Reiss G, et al: Heterogeneity of metabolic response to exercise in humans: New criteria of invariance defined by in vivo phosphorus NMR spectroscopy. FEBS Lett 272:155-158, 1990

22. Bendahan D, Confort-Gouny S, Kozak Ribbens G, et al: ^{31}-P MRS test to both determine aerobic and anaerobic functions in human muscle during exercise and recovery. Proceedings of the Second Annual Scientific Meeting of the Society for Magnetic Resonance (SMR) 3:1254, 1994

23. Mazzeo A, Levy G: An evaluation of new processing protocols for in vivo NMR spectroscopy. Magn Reson Med 17:483-495, 1991

24. Moon RB, Richards JH: Determination of intracellular pH by P-31 NMR. J Biol Chem 248:7276-7278, 1973

25. McCully KK, Iotti S, Kendrick K, et al: Simultaneous in vivo measurements of HbO_2 saturation and PCr kinetics after exercise in normal humans. J Appl Physiol 77:5-10, 1994

26. Opie LH: Effect of beta-adrenergic blockade on biochemical and metabolic response to exercise. Am J Cardiol 55:95D-100D, 1985

27. Angeras U, Oldfors A, Hasselgren PO: The effect of beta-adrenergic blockade on the myopathic changes in experimental hyperthyroidism in rats. Acta Pathol Microbiol Immunol Scand A 94:91-99, 1986

28. Winder WW, Baldwin KM, Terjung RL, et al: Effects of thyroid hormone administration on skeletal muscle mitochondria. Am J Physiol 228:1341-1345, 1975

29. Thompson CH, Anderson Y, Jagasia DH, et al: Effect of tri-iodothyronine administration on skeletal muscle intracellular pH in the rat. Clin Sci 84:645-649, 1993

30. Sterling K, Brenner MA, Sakurada T: Rapid effect of triiodothyronine on the mitochondrial pathway in rat liver in vivo. Science 210:340-342, 1980

31. Kimura H, Kawagoe Y, Kaneko N, et al: Low efficiency of oxygen utilization during exercise in hyperthyroidism. Chest 110:1264-1270, 1996

32. Kyle LH, Ball MF, Doolan PD: Effect of thyroid hormone on body composition in myxedema and obesity. N Engl J Med 275:12-17, 1966

33. Martin WHd: Triiodothyronine, beta-adrenergic receptors, agonist responses, and exercise capacity. Ann Thorac Surg 56:S24-34, 1993

34. Kemp GJ, Thompson CH, Taylor DJ, et al: ATP production and mechanical work in exercising skeletal muscle: A theoretical analysis applied to ^{31}P magnetic resonance spectroscopic studies of dialyzed uremic patients. Magn Reson Med 33:601-9, 1995

35. Caiozzo VJ, Swoap S, Tao M, et al: Single fiber analyses of type IIA myosin heavy chain distribution in hyper- and hypothyroid soleus. Am J Physiol 265:C842-C850, 1993

36. Lomax RB, Robertson WR: The effects of hypo- and hyperthyroidism on fibre composition and mitochondrial enzyme activities in rat skeletal muscle. J Endocrinol 133:375-80, 1992

37. Ianuzzo D, Patel P, Chen V, et al: Thyroidal trophic influence on skeletal muscle myosin. Nature 270:74-6, 1977

38. Fitts RH, Winder WW, Brooke MH, et al: Contractile, biochemical, and histochemical properties of thyrotoxic rat soleus muscle. Am J Physiol 238:C14-20, 1980

39. Hoch FL, Lipman F: Inhibition of mitochondria phosphorylation by thyroxine. Fed Proc 12:218-219, 1953

40. Lee YP, Lardy HA: Influence of thyroid hormones on L-alphaglycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat. J Biol Chem 240:1427-1436, 1965

41. Bronk JR: Thyroid hormone: Effects on electron transport. Science 153:638-639, 1966

42. Babior BM, Creagan S, Ingbar SH, et al: Stimulation of mitochondrial adenosine diphosphate uptake by throid hormones. Proc Natl Acad Sci USA 70:98-102, 1973

43. Atkinson DE: The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 7:4030-4034, 1968

44. Hoch FL: Thyrotoxicosis as a disease of mitochondria. N Engl J Med 266:498-505, 1962

45. Biron R, Burger A, Chinet A, et al: Thyroid hormones and the energetics of active sodium-potassium transport in mammalian skeletal muscles. J Physiol 297:47-60, 1979

46. Asano Y: Increased cell membrane permeability to Na+ and K+ induced by throid hormone in rat skeletal muscle. Experientia 32:199-203, 1978 (suppl)

47. Lin MH, Akera T: Increased (Na^+, K^+)-ATPase concentrations in various tissues of rats caused by thyroid hormone treatment. J Biol Chem 253:723-726, 1978

48. Asano Y, Liberman UA, Edelman IS: relationships between Na^+-dependent respiration and Na^+K^+ adenosine triphosphatase activity in rat skeletal muscle. J Clin Invest 57:368-379, 1976

49. Ismail-Beigi F, Edelman IS: The mechanism of the calorigenic action of thyroid hormone. Stimulation of Na^+-K^+-activated adenosine triphosphatase activity. J Gen Physiol 57:710-722, 1971

50. Hochachka PW, Mommsen TP: Protons and anaerobiosis. Science 219:1391-1397, 1983

51. Kemp GJ, Taylor DJ, Styles P, et al: The production, buffering and efflux of protons in human skeletal muscle during exercise and recovery. NMR Biomed 6:73-83, 1993

52. Vialettes B, Paquis-Fluckinger V, Silvestre-Aillaud P, et al: Extrapancreatic manifestations in diabetes secondary to mitochondrial DNA point mutation within the tRNA(Leu(UUR)) gene. Diabetes Care 18:1023-1028, 1995

53. Kemp GJ, Thompson CH, Barnes PRJ, et al: Comparisons of ATP turnover in human muscle during ischemic and aerobic exercise using P-31 magnetic resonance spectroscopy. Magn Reson Med 31:248-258, 1994