Increased uncoupling proteins and decreased efficiency in palmitate-perfused hyperthyroid rat heart

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Received 28 April 2000; accepted in final form 13 October 2000

Boehm, Ernest A., Barney E. Jones, George K. Radda, Richard L. Veech, and Kieran Clarke. Increased uncoupling proteins and decreased efficiency in palmitate-perfused hyperthyroid rat heart. Am J Physiol Heart Circ Physiol 280: H977–H983, 2001.—The physiological role of mitochondrial uncoupling proteins (UCPs) in heart and skeletal muscle is unknown, as is whether mitochondrial uncoupling of oxidative phosphorylation by fatty acids occurs in vivo. In this study, we found that UCP2 and UCP3 protein content, determined using Western blotting, was increased by 32 and 48%, respectively, in hyperthyroid rat heart mitochondria. Oligomycin-insensitive respiration rate, a measure of mitochondrial uncoupling, was increased in all mitochondria in the presence of palmitate: 36% in controls and 71 and 100% with 0.8 and 0.9 mM palmitate, respectively, in hyperthyroid rat heart mitochondria. In the isolated working heart, 0.4 mM palmitate significantly lowered cardiac output by 36% and cardiac efficiency by 38% in the hyperthyroid rat heart. Thus increased mitochondrial UCPs in the hyperthyroid rat heart were associated with increased uncoupling and decreased myocardial efficiency in the presence of palmitate. In conclusion, a physiological effect of UCPs on fatty acid oxidation has been found in heart at the mitochondrial and whole organ level.

isolated mitochondria; cardiac efficiency

THE BIOCHEMICAL MECHANISMS responsible for the regulation of energy expenditure and the efficiency of energy usage are poorly understood. Possible ways to increase energy expenditure include increasing physical activity and energy dissipation as heat by futile metabolic cycles. Thyroid hormones are the primary regulators of basal metabolic rate in the body, increasing oxygen uptake in an animal after 24 h (26). They regulate growth and metabolism, affecting almost every cell in the body in an organ-specific manner. The heart is unique, in that it is affected in two ways by thyroid hormones: 1) directly and 2) indirectly, because the workload of the heart is increased to accommodate the increased basal metabolic rate (28). The overall effect is to cause the heart to hypertrophy and to upregulate the enzymes involved in metabolism, such as the proteins in the electron transport chain (11).

A group of enzymes that are upregulated by thyroid hormones in striated muscles are the uncoupling proteins (UCPs) (22, 25), proteins that exist in the inner mitochondrial membrane and appear to have no function other than to dissipate the proton gradient across the membrane (19). Various mechanisms have been proposed for free fatty acid-activated $H^+$ transport by the UCPs (see Ref. 3 for review), the net result being the exothermic movement of protons from the outside to the inside of the inner mitochondrial membrane, down their electrochemical gradient and uncoupled from ATP synthesis. Whichever transport mechanism is involved, the movement of protons via UCPs is stimulated by free fatty acids and is inhibited by albumin, which binds free fatty acids (3).

UCP1 has been shown to have a role in nonshivering thermogenesis in brown adipose tissue (31). UCP2, cloned recently as a second member of the UCP family, is ubiquitously expressed in human and rodent tissues including heart (12). Another novel member of the UCP family, UCP3, is preferentially expressed in skeletal muscle and brown adipose tissue (4). UCP4 is the most recent addition to the family and has been found solely in brain (24). Thus the new members, UCP2, UCP3, and UCP4, are well suited for regulated thermogenesis and energy metabolism in large mammals, including humans. In contrast to rapidly increasing information on their synthesis and distribution, there have been few studies on the physiological implications of changes in expression of UCP2 and UCP3. In particular, although the UCP2 gene is expressed abundantly in heart, UCP2 physiological function has yet to be defined but may involve control of mitochondrial reactive oxygen species, regulation of ATP synthesis, or regulation of fatty acid oxidation (see Ref. 3 for review).

The aim of this work was to test the hypothesis that increased UCPs would have physiological effects in isolated mitochondria and in the intact heart. Expression of UCP2 and UCP3 was increased in rat heart...
mitochondria by administration of triiodothyronine (T₃), which also increased mitochondrial uncoupling in the presence of the long-chain fatty acid palmitate. The increased uncoupling was associated with decreased efficiency (work or oxygen consumed) in working rat hearts during perfusion with palmitate. To our knowledge, this is the first time a possible physiological effect of UCPs has been shown in heart.

**METHODS**

*Induction of the hyperthyroid state.* T₃ (0.2 mg/kg body wt ip) was administered daily for 7 days to male Wistar rats. Weight-matched control rats received daily injections of 0.9% saline solution. Less than 24 h after the final injection, the rats were anesthetized and the hearts were removed.

*Mitochondrial isolation.* Mitochondria were isolated from rat hearts using a trypsin digestion procedure (35). Briefly, ventricular tissue from a single heart (1–1.5 g) was minced, washed, and suspended in 10 ml of isolation medium (0.3 M sucrose, 10 mM sodium HEPES, pH 7.2, and 0.2 mM EDTA). The tissue was subjected to mild trypsin digestion (1.25 mg) for 15 min at 4°C and then diluted with 10 ml of isolation medium (pH 7.4) containing 1 mg/ml BSA (Intergen, Oxford, UK) and 6.5 mg of trypsin inhibitor. The suspension was stirred, and the supernatant was discarded. The partially digested tissue was resuspended in 10 ml of isolation medium containing 1 mg/ml albumin and homogenized briefly with a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 600 g (4°C). The supernatant solution was decanted and centrifuged for 15 min at 8,000 g (4°C). The supernatant was discarded, and the pellet was twice resuspended in 10 ml of isolation medium containing 1 mg/ml albumin and each time centrifuged for 15 min at 8,000 g (4°C). The final washed pellet was suspended in 1 ml of isolation medium containing 1 mg/ml albumin. Protein was determined by the Lowry method (27).

*Western blotting.* Western blots were performed on cardiac mitochondria isolated as described above. Goat anti-UCP and rabbit anti-goat IgG peroxidase conjugate polyclonal antibodies were obtained from Autogen Bioclear-Santa Cruz Biotechnology. Briefly, after an SDS-polyacrylamide gel was run, the gel was incubated in transfer buffer [48 mM Tris, 39 mM glycine, 20% (vol/vol) methanol, and 0.1% (wt/vol) SDS] for 30 min. A piece of Immobilon-P membrane (Millipore) was soaked in methanol for 15 s and then rinsed with distilled water. The membrane and eight sheets of 3MM chromatography paper (Whatman, Maidstone, UK) were cut to the same size as the membrane, equilibrated in transfer buffer for 30 min, and layered onto semidyblotting apparatus (Trans-Blot SD, Bio-Rad). The apparatus was assembled, and the gel was transferred at 10 V, 0.18 A for 30 min. The membrane was removed and washed in Tris-buffered saline (TBS; 20 mM Tris-HCl, pH 7.5, and 0.5 M NaCl) for 10–15 min, added to 50 ml of 5% (wt/vol) milk powder in TBS, and left on a rotator at room temperature for 1 h. The membrane was washed in TBS several times over 20 min, and the primary antibody was then added [1:500 dilution in 5% (wt/vol) milk powder in TBS, total volume 50 ml] and left on a rotator at room temperature for 1 h. The membrane was washed three times with TBS + Tween 20 (TTBS) for 20 min before addition of the secondary antibody [1:1,000 in 5% (wt/vol) milk powder in TTBS, total volume 50 ml]. The membrane was left rotating at room temperature for 1 h before it was washed three times with TTBS for 20 min. The membrane was then covered in enhanced chemiluminescence detection solution (Amersham) and exposed to X-ray film for 5–15 min for visualization of protein bands.

Respiratory parameters. Respiratory experiments were carried out by using a Clarke oxygen electrode assembly (Strathkelvin, Glasgow, UK) in a medium containing 0.25 M sucrose, 20 mM HEPES, pH 7.4, 4 mM glutamate, 2 mM malate, 3 mM magnesium acetate, 5 mM potassium phosphate, 0.4 mM EGTA, 1 mg/ml albumin, and 0.3 mM dithiothreitol. Oxygen solubility was 230 nmol/ml in this medium at 30°C.

Mitochondrial preparations in state 2 respiration (resting) were stimulated by addition of a saturating concentration of MgADP (350 μM) to give state 3 respiration. After a steady state had been reached, mitochondrial respiration was inhibited by 1 μg/ml oligomycin. In the absence of ADP, the mitochondrial (uncoupled) respiration rate occurs via the leak of protons across the inner membrane and through the Fₐ portion of the FₐFₐ, ATPase. In the presence of 1 μg/ml oligomycin, the respiration rate is due to proton leak across the inner membrane only. Inasmuch as uncoupling through the inner mitochondrial membrane is thought to be partly due to the action of UCPs in the presence of fatty acids, the above protocol was performed in the presence of albumin (1 mg/ml) or palmitate bound to albumin at 0.4 and 0.9 mM.

*Working heart perfusions.* Each heart was initially cannulated and perfused in the Langendorff mode. The left atrium was cannulated, the aortic line was clamped, and the heart was switched to a working mode using 250 ml of recirculating, modified Krebs-Henseleit buffer. The buffers contained 11 mM glucose and a combination of 4.5 mM pyruvate + 0.5 mM lactate or 0.4 mM palmitate prepound to 1% (wt/vol) BSA. Arterial and venous oxygenation levels were measured using a blood-gas analyzer (model ABL3, Radiometer, Copenhagen, Denmark). Arterial oxygenation was taken to be that in the reservoir at the base of the oxygenator. Cardiac venous oxygenation was determined using a fine needle and syringe to collect the effluent from the pulmonary artery.

Peak systolic pressure (PSP) and heart rate were recorded using an AD Instruments Maclab (Hastings, E. Sussex, UK) connected to an Apple Macintosh 6200. Pressure was recorded via a sidearm. Aortic and coronary flow rates were measured by the time taken for the flows to fill a 10-ml cylinder. The cardiac output (CO) was the sum of the coronary and aortic flows. The preload was set at a pressure of 15 cmH₂O, and the afterload was set at a pressure of 80 cmH₂O.

During Langendorff perfusion, all hearts were perfused with buffer containing 10 mM glucose as the sole substrate. On change to working mode, the hearts were perfused with the buffers described above in different orders to avoid possible experimental bias due to depletion of the endogenous substrates glycogen and triglycerides. A minimum of 10 min was used between each change of substrate before function and oxygenation measurements were taken.

**Calculations.** Cardiac hydraulic work was calculated as follows (37):

\[
\text{cardiac hydraulic work} (J \cdot \text{min}^{-1} \cdot \text{g wet wt}^{-1}) = \frac{\text{CO (ml/min)} \times 10^{-6} (\text{m}^3/\text{ml}) \times \text{PSP (mmHg)}}{\text{Heart wet wt} (g)} \times \frac{101,325 (\text{N/m}^2)}{760 (\text{mmHg})}
\]

where 1 atm (760 mmHg) is 101,325 N/m². Heart wet weights were calculated from the body weight using 3.72 g heart weight/kg body wt for controls (37) and 5.32 g heart weight/kg body wt for hyperthyroid animals (8).
Oxygen consumption was calculated as follows

\[ \text{O}_2 \text{ consumption (} \mu \text{mol} \cdot \text{min}^{-1} \text{g wet wt}^{-1}) = \frac{(P_{\text{ao}_2} - P_{V_{\text{O}_2}}) \times \text{coronary flow (ml/min)} \times \alpha_{\text{O}_2}}{\text{heart wet wt (g)} \times (P_{\text{atm}} - P_{\text{H}_2\text{O}}) \times V_{\text{O}_2}} \times 10^3 \]  

where \( P_{\text{ao}_2} \) is the arterial partial pressure of oxygen, \( P_{V_{\text{O}_2}} \) is the venous partial pressure of oxygen, \( \alpha_{\text{O}_2} \) is the solubility of oxygen, taken to be 0.0212 ml \( \text{O}_2 \)/ml plasma (7), \( P_{\text{atm}} \) is atmospheric pressure (760 mmHg), \( P_{\text{H}_2\text{O}} \) is the partial pressure of water (47.1 mmHg), and \( V_{\text{O}_2} \) is the molar oxygen gas (25.5 dm\(^3\) \( \text{O}_2 \)/mol).

Cardiac efficiency was calculated as follows

\[ \text{cardiac efficiency (} \%) = \frac{\text{hydraulic work (J min}^{-1} \cdot \text{g wet wt}^{-1})}{\text{O}_2 \text{ consumption (} \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g wet wt}^{-1})} \times 100 \]  

where the respiratory chain energy was taken to be 0.421 J/\( \mu \)mol (37).

Data analysis. Values are means ± SE. Differences, tested by ANOVA, were considered significant at \( P < 0.05 \) using a Scheffé’s post hoc test.

RESULTS

Rats injected daily with \( T_3 \) for 7 days had lower \( P < 0.01 \) body weights and cardiac systolic pressures with higher \( P < 0.01 \) heart rates and rate-pressure products than the control animals (Table 1).

UCPs and respiration in isolated mitochondria. Den- sitometric analyses of Western blots showed that UCP2 and UCP3 increased \( P < 0.05 \) by 32 ± 5 and 48 ± 13%, respectively, in hyperthyroid rat heart mitochondria (Fig. 1).

A representative trace showing mitochondrial respiration is shown in Fig. 2, with values of the respiratory parameters from control and hyperthyroid rat heart mitochondria shown in Table 2. Mitochondria were only used if the acceptor control ratio \( (V_{\text{max}}/V_{\text{oligo}}) \), where \( V_{\text{max}} \) is maximal respiration rate and \( V_{\text{oligo}} \) is oligomycin-insensitive respiration rate), a measure of the quality of the mitochondrial preparation, was >6. In the presence of 1 mg/ml albumin, there were no significant differences in respiratory parameters between control and hyperthyroid heart mitochondria (Table 2). The proton leak through the \( F_0 \) \( F_1 \) ATPase was eliminated by oligomycin, an inhibitor of \( F_0 \), leaving any respiration via the UCPs in the inner mitochondrial membrane. All mitochondria had the same \( V_{\text{oligo}} \) of 17.6 ± 1.8 nmol \( \text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1} \) and, hence, uncoupling, in the absence of palmitate (Fig. 3). High concentrations of palmitate caused a significant increase in \( V_{\text{oligo}} \) in all mitochondria: 36% in control and 71 and 100% with 0.8 and 0.9 mM palmitate, respectively, in hyperthyroid rat heart mitochondria. \( P < 0.05 \). There was no increase in uncoupling in the presence of 0.4 mM hexanoate (19.7 ± 2.2 vs. 20.1 ± 4.5 nmol \( \text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1} \) or 0.9 mM hexanoate (20.4 ± 0.7 vs. 19.7 ± 2.8 nmol \( \text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1} \) in hyperthyroid vs. control rat heart mitochondria.

Cardiac work, oxygen consumption, and efficiency. Perfusion of working hyperthyroid rat hearts with 0.4 mM palmitate decreased aortic flow rates by 33% \( P < 0.05 \); Fig. 4, left) with no change in coronary flow rates (Fig. 4, middle). Consequently, the CO was 36% lower \( P < 0.01 \); Fig. 4, right) and cardiac work was 44% lower \( P < 0.05 \); Fig. 5, top) in hyperthyroid rat hearts than in control rat hearts perfused with 0.4 mM palmitate. However, oxygen consumption was not lower in the palmitate-perfused hyperthyroid hearts (Fig. 5, middle). Palmitate decreased cardiac efficiency, the amount of work performed per unit of oxygen consumed, by 38% \( P < 0.05 \) in the hyperthyroid rat heart (Fig. 5, bottom).

DISCUSSION

We have found that hyperthyroidism resulted in a 32–48% increase in mitochondrial UCP2 and UCP3, respectively, associated with a 71–100% increase in mitochondrial \( V_{\text{oligo}} \) caused by the long-chain fatty acid palmitate. The intact, hyperthyroid rat heart had 38% decreased efficiency when perfused with palmitate. To our knowledge, this work is the first to demonstrate a possible physiological effect of UCPs in tissue other than brown adipose tissue and at a level higher than the isolated mitochondrion.

Table 1. Effect of seven daily \( T_3 \) injections on rat body weight and cardiac function

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Systolic Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Rate-Pressure Product, mmHg min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>397 ± 7</td>
<td>95 ± 3</td>
<td>250 ± 7</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>12</td>
<td>358 ± 5*</td>
<td>85 ± 3*</td>
<td>309 ± 14*</td>
</tr>
</tbody>
</table>

Values are means ± SE of all measurements taken throughout the perfusion periods. \( T_3 \), triiodothyronine. *\( P < 0.01 \).
Myocardial UCP2 and UCP3. It has been shown, using Northern blot analysis, that UCP2 is expressed in heart to a much greater extent than UCP3 (16). The increased levels of UCP2 and UCP3 reported here were not as great as the increased UCP mRNA in the hyperthyroid rat heart (22, 25), but changes in mRNA do not necessarily equate to a greater amount of translated protein. In addition, the 32–48% increase in UCPs shown by Western blotting was paralleled by the 71–100% higher uncoupled mitochondrial respiration rates and the 38% decreased efficiency in the palmi-
tate-perfused hyperthyroid rat hearts.

Uncoupling of isolated mitochondria by fatty acids has been shown to interfere with mitochondrial ATPase activity and increase \( V_{\text{oligo}} \) with a concomitant decrease in the phosphorus-to-oxygen ratio (for review see Ref 43). In contrast to the well-known uncoupling effects of fatty acids in isolated mitochondria, the uncoupling effect of fatty acids in vivo has not been shown because of their dual role as substrates for oxidation and as genuine uncouplers of oxidative phosphoryla-

### Table 2. Respiratory parameters of mitochondria isolated from control and hyperthyroid rat hearts in the presence of albumin (1 mg/ml)

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>( V_o ) (State 2)</th>
<th>( V_{\text{max}} ) (State 3)</th>
<th>( V_{\text{oligo}} )</th>
<th>Acceptor Control Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>25.4 ± 1.6</td>
<td>124 ± 7</td>
<td>17.6 ± 1.8</td>
<td>8.0 ± 1.0</td>
</tr>
<tr>
<td>T3</td>
<td>13</td>
<td>24.2 ± 2.1</td>
<td>123 ± 11</td>
<td>18.1 ± 2.2</td>
<td>7.5 ± 0.9</td>
</tr>
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</table>

Values are means ± SE expressed as nmol O\(_2\)·min\(^{-1}\)·mg mitochondrial protein\(^{-1}\). Acceptor control ratio is calculated as \( V_{\text{max}} / V_{\text{oligo}} \), where \( V_{\text{max}} \) is maximal respiration rate and \( V_{\text{oligo}} \) is oligomycin-insensitive respiration rate.

Fig. 2. Representative mitochondrial respiration trace. Points at which additions were made to the respiration buffer are shown.

Fig. 3. Oligomycin-insensitive respiration (\( V_{\text{oligo}} \)) in control and hyperthyroid rat heart mitochondria in the absence or presence of increasing concentrations of palmitate ([palmitate]). Values are means ± SE. *\( P < 0.05 \) vs. control and hyperthyroid without palmitate; #\( P < 0.05 \) vs. control without palmitate.

tion. In the absence of palmitate and in the presence of 1 mg/ml albumin, we found no difference in mitochondrial respiratory parameters, in that the state 2 and state 3 respiration rates and $V_{\text{oligo}}$ were the same in control and hyperthyroid rat heart mitochondria. In the presence of increasing concentrations of palmitate, $V_{\text{oligo}}$ increased significantly in both groups of mitochondria but was higher than control in the hyperthyroid rat mitochondria at 0.8 and 0.9 mM palmitate. Even higher concentrations of palmitate did not further stimulate respiration because of the detergent effects of the fatty acids (data not shown). The increase in respiration rate (uncoupling) did not occur in the presence of hexanoate, a short-chain fatty acid, showing that the uncoupling was mediated specifically by long-chain fatty acids. The increase in $V_{\text{oligo}}$ in the presence of palmitate has been shown in hyperthyroid rat skeletal muscle mitochondria in the absence, but not presence, of fatty acid-free albumin (21). This finding and the mitochondrial uncoupling in the presence of a nonoxidizable analog (14) support the proposal that uncoupling is mediated by increased $\beta$-oxidation of palmitate, causing increased proton transport via the UCPs.

Decrease in cardiac efficiency. It is important to note that the values of oxygen consumption reported in this study are comparable to those reported in other studies using the isolated perfused heart (37, 42). Discrepancies between literature values may be partly explained by the methods used to normalize to wet weight or dry weight. For example, Taegtmeyer et al. (41) obtained a range of values for oxygen consumption of $\sim$50–75 $\mu$mol·min$^{-1}$·g dry wt$^{-1}$. Conversion to units of wet weight (as used in this study) requires knowledge of wet weight-to-dry weight ratio. Although in vivo this is $\approx$5, the buffer-perfused heart is more edematous and wet weight-to-dry weight ratios are very dependent on the exact method of measurement. For example, if the heart has been freeze-clamped on the cannula, as in this study, relatively high values (up to 11) can be obtained compared with hearts that are blotted and dried “fresh.” The wet weight-to-dry weight ratio of the perfused hearts in this study was 7.5 ± 0.2. Use of this value to adjust the values of Taegtmeyer et al. leads to a range of 6.7–10 $\mu$mol·min$^{-1}$·g wet wt$^{-1}$, which compares favorably with our measurements (5.4–7 $\mu$mol·min$^{-1}$·g wet wt$^{-1}$). Furthermore, the experiments of Taegtmeyer et al. were performed at an increased workload (140 vs. 80 cmH$_2$O afterload), which probably explains their slightly higher values.

The decrease in cardiac efficiency in the palmitate-perfused hyperthyroid rat hearts suggests that it was due to increased uncoupling of the mitochondria. Although less work was performed for a similar level of oxygen consumed in all hearts, this may be explained by the fact that oxygen extraction in the working heart preparation is already thought to be near maximal under normal conditions. In this way, an increased oxygen demand in the hyperthyroid heart caused a decrease in cardiac work, rather than an increase in coronary flow (via nitric oxide or adenosine) and oxy-

![Graph](http://ajpheart.physiology.org/article-pdf/10.1152/ajpheart.00266.2017)

Fig. 4. Aortic flow, coronary flow, and cardiac output of control and hyperthyroid (H-thyroid) rat hearts. GPL, glucose (11 mM), pyruvate (4.5 mM), and lactate (0.5 mM); GF, glucose (11 mM) and palmitate (0.4 mM). Values are means ± SE. *P < 0.01 compared with control GF.

![Graph](http://ajpheart.physiology.org/article-pdf/10.1152/ajpheart.00266.2017)

Fig. 5. Cardiac work, oxygen consumption, and cardiac efficiency in control and hyperthyroid rat hearts, calculated using Eqs. 1–3, respectively. GPL, glucose (11 mM), pyruvate (4.5 mM), and lactate (0.5 mM); GF, glucose (11 mM) and palmitate (0.4 mM). Values are means ± SE. *P < 0.05 compared with control GF.
gen consumption, as might be expected. The decrease in efficiency only occurred in the presence of palmitate. Other than detergent effects, which would also have been observed in control hearts, the most plausible reason for the difference was uncoupling of the mitochondria.

The quantification of the efficiency of the contractile machinery could also be accomplished using a parameter such as pressure-volume area, which is an estimate of total mechanical energy, rather than hydraulic work, as reported here. In agreement with our data, however, Goto et al. (13) demonstrated that contractile efficiency, using pressure-volume area as the energy output term, was also reduced in the hyperthyroid rabbit.

Although there are other possible sources of reduced contractile efficiency in the hyperthyroid heart, these are unlikely to contribute to the decrease in efficiency in this model. For example, at the molecular level, one of the best-documented effects of thyroid hormones on cardiac tissue is that of a switch in the myosin ATPase isoform. The V₃ isoform predominates in the euthyroid heart of most species, whereas in the hyperthyroid heart the faster V₁ form predominates (15), which has profound physiological effects on the first derivative of left ventricular pressure. However, any myosin isoform change was avoided here by the use of the rat as an experimental model, because the V₁ form predominates in the euthyroid and the hyperthyroid heart (34). Changes in calcium handling are also unlikely to contribute to the change in efficiency in the presence of palmitate only, unless the energy sources for the various pumps and ATPases were derived from specific ATP pools.

Physiological relevance. That UCPs are found in the inner mitochondrial membrane has been known for over a decade, with the theory of uncoupling proposed over a decade, with the theory of uncoupling proposed. The quantification of the efficiency of the contractile machinery could also be accomplished using a parameter such as pressure-volume area, which is an estimate of total mechanical energy, rather than hydraulic work, as reported here. In agreement with our data, however, Goto et al. (13) demonstrated that contractile efficiency, using pressure-volume area as the energy output term, was also reduced in the hyperthyroid rabbit.

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Physiological relevance. That UCPs are found in the inner mitochondrial membrane has been known for over a decade, with the theory of uncoupling proposed in 1956 (39). The role of UCPs has been well established in isolated mitochondria from brown adipose tissue, where they are the proteins that facilitate the heat production in nonshivering thermogenesis. The mechanism behind this has been worked out over the past decade using isolated mitochondrial preparations from brown adipose tissue and liver (for a review, see Ref. 19).

The physiological importance of UCP2 and UCP3 has been suggested for skeletal muscle, mainly from studies that have investigated the regulation of their respective expression to different stimuli. For example, cold exposure, thyroid hormone, elevated dietary fat composition, tumor necrosis factor-α, insulin-dependent diabetes, and specific peroxisome proliferator-activated receptor agonists have been shown to increase skeletal muscle UCP expression (4–6, 18, 23, 36). On the other hand, exercise training lowers skeletal muscle UCP expression (4). These results, with the fact that skeletal muscle contributes up to 30% to the basal metabolic rate under normal conditions (32), have implicated skeletal muscle UCPs in the process of heat generation, obesity, and perhaps maintenance of insulin sensitivity (20, 36, 38).

UCPs have been shown to increase in the presence of fatty acids (40). Studies of isolated mitochondria have shown that production of reactive oxygen species is greatly increased at times when the proton electrochemical gradient is high, for example, when ADP is limiting or unavailable. Addition of ADP or an uncoupling agent strongly suppresses superoxide formation (40). The hyperthyroid rat heart has a low rate of change of ATP free energy and a high free ADP concentration (8), making it unlikely that ADP is limiting oxidative phosphorylation. Thus an increase in UCPs to prevent the generation of reactive oxygen species is unlikely to occur in heart.

Regulation of ATP synthesis. The capacity for rapid, large increases in ATP synthesis during contraction in skeletal muscle necessitates significant rates of flux through metabolic pathways, such as mitochondrial respiration, in the resting or basal state. To maintain high rates of flux during periods of rest, an uncoupling of fuel utilization and work must take place (33), and a proton leak across the inner mitochondrial membrane via UCPs would be one such uncoupling mechanism. However, the heart is constantly working and has a high and comparatively constant rate of ATP turnover, suggesting that UCPs are not involved in the regulation of cardiac ATP synthesis.

Regulation of free fatty acid oxidation. Inefficiency of oxidative phosphorylation due to uncoupling will result in increased fuel utilization, primarily of fatty acids. An increase in the expression of UCP2 and UCP3 proteins under conditions in which fatty acid β-oxidation is increased strongly suggests that UCPs are required to control fatty acid metabolism and may protect cells from the detrimental consequences of excessive fatty acid metabolism or storage (1, 9, 10). Indeed, metabolic states associated with enhanced lipolysis, including hyperthyroidism (29, 30), are correlated with increased expression of UCP2 and UCP3 (5, 22, 25). Our finding of increased UCP2 and UCP3 in heart, associated with a decrease in efficiency in the presence of palmitate, suggests a role for UCPs in the regulation of fatty acid oxidation.

We thank Sharon Chan for excellent technical assistance. This work was supported by the British Heart Foundation. Parts of this work have been presented in abstract form (2, 17).

REFERENCES

5. Brun S, Carmona MC, Mampel T, Vinas O, Giralt M, Iglésias R, and Villarroya F. Activators of peroxisome proliferator-


