Hyperthermia impairs liver mitochondrial function in vitro

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Willis, W. T., M. R. Jackman, M. E. Bizeau, M. J. Pagliassotti, and J. R. Hazel. Hyperthermia impairs liver mitochondrial function in vitro. Am J Physiol Regulatory Integrative Comp Physiol 278: R1240–R1246, 2000.—The effects of temperature on the relationships among the rates of pyruvate carboxylation, O₂ uptake (J₋), oxidative phosphorylation (Jₒ), and the free energy of ATP hydrolysis (Gᵢ) were studied in liver mitochondria isolated from 250-g female rats. Pyruvate carboxylation was evaluated at 37, 40, and 43°C. In disrupted mitochondria, pyruvate carboxylase maximal reaction velocity increased from 37 to 43°C with an apparent Q₁₀ of 2.25. A reduction in ATP/ADP ratio decreased enzyme activity at all three temperatures. In contrast, in intact mitochondria, increasing temperature failed to increase pyruvate carboxylation (malate + citrate accumulation) but did result in increased Jₒ and decreased extramitochondrial Gᵢ. Jₒ was studied in respiring mitochondria at 37 and 43°C at various fractions of state 3 respiration, elicited with a glucose + hexokinase-ADP-regenerating system. The relationship between Jₒ and Gᵢ was similar at both temperatures. However, hyperthermia (43°C) reduced the Jₒ/Gᵢ ratio, resulting in lower Gᵢ for a given Jₒ. Fluorescent measurements of membrane phospholipid polarization revealed a transition in membrane order between 40 and 43°C, a finding consistent with increased membrane protein conductance. It is concluded that hyperthermia augments nonspecific proton leaking across the inner mitochondrial membrane, and the resultant degraded energy state offsets temperature stimulation of pyruvate carboxylase. As a consequence, at high temperatures approaching 43°C, the pyruvate carboxylation rate of intact liver mitochondria may fail to exhibit a Q₁₀ effect.

Gluconeogenesis; exercise; glucose homeostasis; bioenergetics

DURING EXERCISE AT ROOM TEMPERATURE (~25°C), rat liver is subjected to temperatures as high as 43°C (5). Rowell and co-workers (29) reported that the temperature of blood draining the human liver may reach 42°C during exercise at ~50% maximal O₂ consumption in an environmental temperature of 48.9°C. We were interested in whether such high tissue temperatures might disturb the energy transduction of the inner mitochondrial membrane.

Previous studies indicate that temperature approaching 43°C impairs the coupling of mitochondrial oxidative phosphorylation. Hinkle and Yu (20) reported no effect of temperature on the ADP/O ratio of liver mitochondria oxidizing β-hydroxybutyrate or succinate over a range from 15 to 40°C. However, Brooks et al. (6), studying liver mitochondria oxidizing pyruvate + malate, observed a 7% fall in the ADP/O ratio over the range 37–43°C. Over this same temperature range, state 4 (nonphosphorylating or “resting”) respiration was elevated by 100%. Moreover, very little of the resting respiration was responsive to inhibition by oligomycin (6), strongly suggesting that the elevated temperature exacerbated the proton leak of the inner mitochondrial membrane (3). Mitochondrial uncoupling degrades the cytosolic free energy of ATP hydrolysis (Gᵢ) (9), and hepatic gluconeogenic flux has been shown to vary linearly with cellular energy state (2).

Pyruvate carboxylase of the mitochondrial matrix is a putative control point in gluconeogenesis from lactate (17). Pyruvate carboxylase activity is modulated by a number of effectors, including the concentration of acetyl-CoA and the ATP/ADP ratio of the mitochondrial matrix (4, 11). In addition, it would be expected that pyruvate carboxylase and other enzymes of gluconeogenesis would be stimulated by increased temperature, according to the Q₁₀ effect. It is, therefore, noteworthy that the data of Rowell et al. (29) suggest decreased, rather than increased, lactate-to-glucose recycling, in the face of elevated splanchnic O₂ consumption rate. Thus, although high temperature may stimulate an individual gluconeogenic enzyme, the stimulation may be offset by an opposing modulating influence, for example, a decrease in energy state.

According to the chemiosmotic theory of mitochondrial oxidative phosphorylation, the electrochemical gradient for protons across the inner mitochondrial membrane, the protonmotive force (Δp), is utilized by the ATP synthase complex to drive ATP synthesis (27). Thus the magnitude of “static head” Gᵢ cannot exceed an upper limit defined by Δp and the proton/ATP stoichiometry. An alternative route that protons may take into the mitochondrial matrix is through a “leak” pathway (3, 19, 26). The mitochondrial proton leak dissipates Δp, rendering it less thermodynamically competent to support Gᵢ. The proton leak rate (J₇⁺) is given by Eq. 1.

\[ J_{7+} = \Delta p \cdot G_{M,H^+} \]  (1)

where G₉₆₇₆₇₆₇₆₇ is the apparent conductance of the proton leak pathway. Obviously, the proton leak short circuits oxidative phosphorylation, so that mitochondrial O₂ consumption (Jₒ) proceeds in the absence of ATP synthesis. This investigation was in part inspired by the...
following question: Do temperatures in the hyperthermic range [e.g., in the 42–43°C range observed by Brooks et al. (5, 6) in the exercising rat and by Rowell et al. (29) in the exercising human] disrupt the structure and function of the inner membrane sufficiently to increase the conductance of the proton leak pathway and, therefore, result in higher \( J_p \) and a degraded mitochondrial energy status?

Thus we hypothesized that hyperthermia would impair the ability of mitochondria to energetically support pyruvate carboxylation. On the basis of this hypothesis, we made the following predictions. 1) Intact liver mitochondria subjected to hyperthermia would fail to increase flux through pyruvate carboxylase, whereas \( J_p \) would rise and \( G_o \) fall. 2) The coupling of oxidative phosphorylation (\( J_o \)) to electron transport would be loosened by hyperthermia, altering the \( J_o \)-\( G_o \) relationship. 3) At the level of the inner mitochondrial membrane, the effects of hyperthermia would be revealed by a disturbance in the membrane order, which would be consistent with an increased conductance for proton leak across the inner membrane. To test these predictions, we have carried out three types of experiments. First, disrupted mitochondria were used to determine the Q\( _10 \) of pyruvate carboxylase, under conditions of optimal ATP and acetyl-CoA and also at various ATP/ADP ratios in the range observed in the matrix of intact mitochondria. Second, intact mitochondria were used to study the effect of temperature on the rate of pyruvate carboxylation, \( J_o \) and \( J_p \). Third, membrane fluidity was measured at various temperatures.

**METHODS**

Mitochondrial isolation. Female rats were fed Purina rat chow from weaning. When the rats weighed ~250 g, they were decapitated between 8 and 10 AM in a fed condition, and livers were excised. Liver mitochondria were prepared essentially as described by Johnson and Lardy (21). The final mitochondrial pellet was suspended in 220 mM mannitol + 70 mM sucrose at a concentration of 40–60 mg mitochondrial protein (biuret method) per milliliter.

Pyruvate carboxylation. Mitochondria disrupted in Triton X-100 detergent were used for the assay of pyruvate carboxylase activity at selected temperatures and ATP/ADP ratios. Mitochondrial suspension was diluted 10-fold in 0.1% Triton X-100 and a 10-µl aliquot of the diluted suspension was promptly added to a cuvette previously equilibrated at 37, 40, or 43°C and a predetermined ATP/ADP ratio. Pyruvate carboxylase activity was measured according to the method of Crabtree et al. (8). The reaction medium contained 100 mM Tris-HCl, 5 mM MgCl\( _2 \), 10 mM pyruvate, 0.75 mM acetyl-CoA, 2.5 mM adenine nucleotide (ATP + ADP), 0.2 mM 5,5’-dithiobis(2-nitrobenzoic acid), and 1.0 U of citrate synthase, pH 7.3. After background deacylase activity was followed for 1–2 min, the reaction was initiated with the addition of 25 mM HCO\( _3^- \), and absorbance was followed at 412 nm. After appropriate background subtraction, activity was calculated using a millimolar extinction coefficient of 13.6 for the mercaptide ion formed by the condensation of 5,5’-dithiobis(2-nitrobenzoic acid) with coenzyme A generated in the citrate synthase reaction.

The carboxylation of pyruvate by intact mitochondria was estimated according to the method of Walter and Stucki (31). A 100-µl aliquot of mitochondrial suspension, containing 4–6 mg of protein, was added to a respiration chamber equipped with a Clark-type O\( _2 \) electrode (Rank Brothers, Cambridge, UK) equilibrated at 37, 40, or 43°C. The chamber contained 1.9 ml of a medium comprised of 220 mM sucrose, 10 mM MgSO\( _4 \), 6.6 mM KPO\( _4 \), 6.6 mM triethanolamine, 2.0 mM ATP, 10 mM pyruvate, and 10 mM KHCO\( _3 \), pH 7.40. O\( _2 \) content of the medium at the various temperatures was calibrated using sonicated heart mitochondria and spectrophotometrically determined quantities of NADH (13). O\( _2 \) consumption was continuously monitored. Samples (1.0 ml) were aspirated immediately after the addition of mitochondria or after a 6-min incubation and added to 25.0% HClO\( _4 \). Extracts, neutralized with 2 N NaOH + 0.3 M MOPS, were used to measure pyruvate, citrate, malate, ATP, and ADP. The sum of accumulated malate and citrate was found to be linear with time and mitochondrial addition over the ranges employed in this study.

\( J_p \). Submaximal \( J_o \) and \( J_p \) were studied at 37 and 43°C. Mitochondria, 1.0–1.5 mg of protein, were added to the respiration chamber containing a total volume of 2.0 ml of respiration medium. The medium contained 100 mM KCl, 50 mM Tris, 10 mM MgCl\( _2 \), 5 mM KPO\( _4 \), 1 mM EGTA, and 20 mM glucose. Potassium hydroxide (1.0 N) was used to adjust the pH of the medium to 7.40 at each temperature. This pH adjustment resulted in a 2 meq/l difference in medium K\( ^+ \) concentration between 37 and 43°C. Glutamate (10 mM final concentration) and malate (2.5 mM) were used as oxidative substrates.

Submaximal, steady-state rates of respiration were induced using hexokinase + glucose as an ADP-regenerating system (10). Mitochondria, 1.0–1.5 mg of protein, were added to the respiration chamber and allowed to equilibrate for 1 min. At 1.0-min intervals, glutamate + malate, ATP (2 mM final concentration), and finally hexokinase were added to the chamber. The rate of O\( _2 \) consumption was continually followed on a chart recorder. Exactly 3 min after hexokinase addition, 1.0 ml of the medium was sampled, acidified, and neutralized as described above. Samples were analyzed for ATP, ADP, and glucose 6-phosphate (G-6-P).

Maximal (state 3) respiration rate and the ADP/O ratio were also assessed using bolus additions (1 µmol) of ADP (13). Resting (state 4) respiration rate, the rate of respiration in the presence of ATP, was also measured, and the respiratory control ratio (RCR), defined as the state 3 respiration divided by state 4, was calculated.

Metabolite assays and calculation of \( G_p \). Malate, citrate, G-6-P, ATP, and ADP were assayed using enzyme reactions directly or indirectly coupled to the oxidation or reduction of NAD/NADH. These standard spectrophotometric assays are described by Bergmeyer (1).

The actual \( G_p \) was calculated according to Eq. 2

\[
G_p = \Delta G'_{ATP} - RT \ln \frac{[ATP]}{[ADP][P_i]}
\]

where \( R \) is the gas constant (1.987 cal·deg\(^{-1}\)·mol\(^{-1}\)), \( T \) is degrees Kelvin, and [ATP] and [ADP] are concentrations of ATP and ADP. Because the standard free energy of ATP hydrolysis (\( \Delta G'_{ATP} \)) varies with temperature, values at the respective temperatures were calculated using the Van’t Hoff equation according to the procedure provided by Teague et al. (30).

Fluorescence polarizability measurements. Isolated mitochondria were suspended in 20 mM potassium phosphate buffer (pH 7.4) to give a final protein concentration of 100 µg/ml. The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) in tetrahydrofuran (4 mM) was added to the mitochondrial suspen-
sion in a ratio of 3 μl of probe to 5 ml of mitochondrial suspension and was incubated for 1 h at 25°C.

Steady-state polarization measurements were performed using a luminescence spectrophotometer (model LS 50B, Perkin-Elmer, Norwalk, CT) fitted with a constant-temperature cuvette holder. A digitally thermostated circulating water bath enabled the temperature of the cuvette contents to be monitored to within ±0.1°C. Excitation and emission monochromators were set at 358 and 430 nm, respectively. Polarization was calculated from the fluorescence values according to Eq. 3

$$P = \frac{IV_v - GIV_h}{IV_h + GIV_v}$$

where P is polarization, IV_v is emission intensity of vertically polarized light parallel to the plane of excitation, IV_h is emission intensity of horizontally polarized light perpendicular to the plane of excitation, and G is a configuration-specific correction factor. Measurements were performed at 37–45°C in 1°C increments. Because DPH is an asymmetric molecule and does not undergo isotropic rotation within a membrane, the average extent of acyl chain motion, or membrane order, contributes more to the observed polarization than acyl chain dynamics, i.e., fluidity (24). Thus the results of steady-state polarization measurements in this study are discussed in terms of membrane order.

Statistical analysis. One-way ANOVA with repeated measures where appropriate was used to statistically evaluate temperature effects. When a significant F statistic was obtained, the Newman-Keuls multiple range test was employed to locate differences between two means.

RESULTS

Functional integrity of isolated mitochondria. The isolated liver mitochondria (n = 7 preparations) exhibited high functional integrity, as evaluated by conventional polarographic measurements at 37°C. With glutamate + malate as substrate, the state 3 (maximal) respiration rate, state 4 (resting) respiration rate, RCR, and ADP/O ratio were 80.4 ± 2.3 nmol O_2·min^(-1)·mg protein^(-1), 8.97 ± 0.29 nmol O_2·min^(-1)·mg protein^(-1), 9.17 ± 0.19, and 2.54 ± 0.07, respectively. These indexes of mitochondrial integrity indicate that the isolated mitochondria used in the experiments were capable of a broad metabolic scope (RCR > 9) and exhibited tight coupling of electron transport to ATP synthesis (ADP/O ratio approaching 3). The values indicate structural and functional integrity similar to mitochondria used in many excellent reports in the literature (5, 13, 20).

Pyruvate carboxylase activity. Maximal pyruvate carboxylase activity was assayed spectrophotometrically in detergent-treated mitochondria (0.04 mg of mitochondrial protein) provided with saturating substrates and optimal ATP. The measured rates were 3.75 ± 0.37, 5.66 ± 0.37, and 6.03 ± 0.15 nmol/min at 37, 40, and 43°C, respectively. Thus pyruvate carboxylase activity increased with a Q_10 of 2.25 over the temperature range 37–43°C. Decreasing ATP/ADP ratios, over the range reported to occur in the mitochondrial matrix (4, 32), decreased pyruvate carboxylase activity, but temperature stimulation of activity persisted at a given ATP/ADP ratio (Fig. 1). Thus the data of Fig. 1 clearly demonstrate that temperature and the matrix ATP/ADP ratio are important determinants of pyruvate carboxylase activity.

Intact mitochondria must provide not only enzymatic catalysis for pyruvate carboxylation but also the ATP required in the pyruvate carboxylase reaction. Therefore, unlike disrupted mitochondria, where the enzyme maximal reaction velocity increased 61% from 37 to 43°C, intact mitochondria exhibited a numerical decrease in malate + citrate accumulation at 43°C compared with 37°C (Table 1). Furthermore, J_o was significantly greater in the hyperthermic condition, associated with lower (less-negative) G_p (Table 1).

Studies of J_o during graded respiration. Liver mitochondria support gluconeogenesis also by delivering ATP to the cytosolic ATP-utilizing reactions of the gluconeogenic pathway. To evaluate the effects of temperature on the control and economy of J_o, mitochondria were suspended in a medium with 2 mM ATP + 20 mM glucose, and graded rates of ATP turnover were established with additions of hexokinase.

In Fig. 2A the relationship between steady-state J_o and the G_p is shown at 37 and 43°C. At each temperature, J_o ranged from resting respiration in the presence of 2 mM ATP without added hexokinase to ~90% of state 3 respiration, elicited with hexokinase. Figure 2 shows a tendency for J_o to be higher at higher energy states (more-negative G_p) in the hyperthermic condi-

Table 1. Pyruvate carboxylation in intact mitochondria

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>n</th>
<th>Pyruvate Carboxylated</th>
<th>J_o</th>
<th>G_p</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>8</td>
<td>20.14 ± 2.30</td>
<td>10.17 ± 0.67</td>
<td>−13.64 ± 0.09</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>20.34 ± 1.70</td>
<td>11.00 ± 0.75</td>
<td>−13.47 ± 0.11</td>
</tr>
<tr>
<td>43</td>
<td>7</td>
<td>18.28 ± 1.63</td>
<td>12.78 ± 0.68</td>
<td>−13.26 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of mitochondrial preparations evaluated. Pyruvate carboxylation rate, sum of malate + citrate accumulation (nmol min^(-1)·mg mitochondrial protein^(-1)) during a 6-min incubation; J_o, O_2 consumption rate (nmol O_2·mg mitochondrial protein^(-1)·min^(-1)); G_p, free energy of ATP hydrolysis (kcal/mmol), primarily energetic state of extramitochondrial space (see METHODS AND DISCUSSION). *P < 0.05, 43 vs. 37°C.
tion, whereas the slope of the $J_o - G_p$ relationship tends (but $P > 0.05$) to be decreased by hyperthermia.

The output of useful chemical work by mitochondria requires the coupling of electron transport to the synthesis of ATP. Thus the effect of temperature on the relationship between $J_p$, assessed as accumulated G-6-P, and the steady-state $G_p$ was evaluated. The results of these experiments are shown in Fig. 2B. The value of $G_p$ at zero $J_p$, i.e., $G_p$ at static head, was essentially identical at both temperatures. However, compared with $37^\circ C$, the gain in $J_p$ for a given fall in $G_p$ tended to be less at $43^\circ C$. Because of experimental variability, the slopes at the two temperatures were not significantly different. However, for every mitochondrial preparation examined, higher $J_p$ values were observed at $37^\circ C$ than at $43^\circ C$ across the entire range of $G_p$ (data not shown). These data, in combination with the lack of a similar temperature effect on the $J_o - G_p$ relationship shown in Fig. 2A, suggest decreased coupling between electron transport and ATP synthesis at $43^\circ C$. This is shown in Fig. 2C. At both temperatures, elevated ATP turnover rate, and thus decreasing $G_p$, improved the $J_p / J_o$ ratio. However, compared with $37^\circ C$, $43^\circ C$ resulted in lower coupling ratios throughout the range of energy states examined, representing a range of $J_o$ from state 4 to 90% of state 3.

Measurements of mitochondrial membrane order. The steady-state fluorescence polarization of DPH was used to study the effect of temperature on the physical properties of mitochondrial membrane lipids. As expected, membrane order (inversely related to polarization) decreases with rising temperature. However, Arrhenius plots of polarization values are biphasic, with the break point in the plot occurring between $42$ and $43^\circ C$ (Fig. 3). The slope of the regression line for points from $37$ to $42^\circ C$ is $-0.1147$ and is significantly different from the slope for points from $41$ to $45^\circ C$ ($-0.2958$, $P < 0.05$). The rationale for choosing $42^\circ C$ as the break point in the plot was based on the fact that the change in polarization from $42$ to $43^\circ C$ (i.e., 0.00986) was nearly identical to the total change observed from $37$ to $42^\circ C$ (i.e., 0.01086). Thus we interpret these results to indicate that an abrupt change in order of the mitochondrial membrane occurs when temperature rises above $42^\circ C$. 

![Fig. 2. Energetic response of liver mitochondria responding to extra-mitochondrial ATP demand (hexokinase additions) at 37 and 43°C. Glutamate (10 mM) and malate (2.5 mM) were provided as oxidative substrates. A: $O_2$ consumption rate ($J_o$, nmol $O_2 \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$) vs. free energy of ATP hydrolysis ($G_p$, kcal mol$^{-1}$). At $37^\circ C$, $y = 380.5 + 25.24x$ ($r = 0.97$); at $43^\circ C$, $y = 307.6 + 19.85x$ ($r = 0.98$). B: rate of oxidative phosphorylation ($J_p$, nmol glucose 6-phosphate min$^{-1} \cdot$mg mitochondrial protein$^{-1}$) vs. $G_p$. At $37^\circ C$, $y = 1,956 + 135x$ ($r = 0.96$); at $43^\circ C$, $y = 1,536 + 106x$ ($r = 0.94$). C: coupling of $O_2$ consumption to oxidative phosphorylation ($J_p / J_o$) vs. $G_p$. At $37^\circ C$, $y = 11.0 + 0.703x$ ($r = 0.89$); at $43^\circ C$, $y = 10.8 + 0.709x$ ($r = 0.89$). Data for both temperatures are based on 5 separate mitochondrial preparations, each titrated to 5 steady-state ATP turnover rates with additions of hexokinase. All points are individual data points without baseline subtraction.

![Fig. 3. Arrhenius plot of temperature ($T$) dependence of 1,6-diphenyl-1,3,5-hexatriene (DPH) polarization obtained in liver mitochondria. Break point in plot occurs at $42^\circ C$. DPH fluorescence polarization measurement was performed as described in METHODS. Values are means ± SE for 6 separate mitochondrial preparations. Slope of regression line for $37$–$42^\circ C$ is significantly different ($P < 0.05$) from slope of regression line for $42$–$45^\circ C$.](http://ajpregu.physiology.org/ by 10.220.33.2 on June 12, 2017)
DISCUSSION

Pyruvate carboxylase, liberated from detergent-disrupted mitochondria and provided optimal ATP, exhibited increased activity between 37 and 43°C with an apparent Q₁₀ of 2.25. In intact liver mitochondria, in contrast, hyperthermic temperature failed to increase flux through pyruvate carboxylase. In this case, hyperthermia was associated with decreased Gᵢ and higher J₀ (Table 1). Because the activity of pyruvate carboxylase is modulated by the ATP/ADP ratio of the mitochondrial matrix (Fig. 1) (4, 11), we interpret the results in the intact mitochondria to indicate that temperature stimulation of pyruvate carboxylase (Q₁₀ effect) was offset by hyperthermic degradation of mitochondrial energy coupling and energy state.

The effects of temperature on mitochondrial state 3 and state 4 respiration rates, the RCR, and the ADP/O ratio have been studied in detail previously by Brooks et al. (6) and Hinkle and Yu (20). In general, their data indicate little (6) or no (20) decrement in the coupling of electron transport and J₀ at temperatures in the range examined in the present study. However, the disparity between these previous results and ours, which indicate consistent, if not always statistically significant, temperature effects, may be easily explained by the very different experimental conditions. Brooks et al. and Hinkle and Yu stimulated mitochondrial respiration by adding a bolus of ADP. Bolus addition of ADP drastically reduces Gᵢ (see Eq. 2), so that most of the O₂ consumption during state 3 respiration takes place outside the region of energetic control of flux (9, 27). In this situation, Δp falls (19, 26, 27) as protons flow toward the low Gᵢ at a maximal rate, which is kinetically limited by the ATP synthase complex. Thus the major portion of J₀ takes place when Gᵢ and Δp are low (9). Under these low-energy conditions, the proton leak of the inner membrane is essentially not operational for two reasons (see Eq. 1): 1) the driving force for proton backleak, Δp, is very low, and 2) the conductance term of Eq. 1 falls as Δp falls, a phenomenon termed “nonohmic” behavior of the leak (3, 26). Thus any temperature-induced insult to membrane integrity may be masked by the low-energy conditions imposed by bolus ADP addition.

In contrast, we evaluated the function of intact mitochondria during steady-state, submaximal respiration elicited with a hexokinase + glucose ADP-regenerating system. At these intermediate (“energized”) chemiosmotic forces and flows, J₀ is proportional to the disequilibrium between Δp and the extra mitochondrial Gᵢ (9, 27). We observed that elevating the temperature from 37 to 43°C resulted in lower J₀ at a given Gᵢ (Fig. 2B), suggesting a lower Δp in the hyperthermic condition. Dissipation of Δp by the proton leak in hyperthermia was further evidenced by the reduction in the J₀/J₀ coupling ratio (Fig. 2C). We interpret these data to indicate that hyperthermia degrades mitochondrial function by exacerbating the proton leak of the inner membrane. In support of this interpretation, it can be calculated from the data of Brooks et al. (6) on resting mitochondria that oligomycin-insensitive respiration rose 62% from 37 to 43°C. Our own experiments with liver mitochondria at rest in the presence of oligomycin (data not shown) and with skeletal muscle mitochondria at intermediate phosphorylation rates (34) corroborate these findings of Brooks et al. Respiration sustained by isolated mitochondria in the presence of oligomycin indicates the operation of the proton leak (19). Thus our present and previous (34) data, as well as those of Brooks et al. (5, 6), support the contention that hyperthermia degrades the energy state sustained by mitochondria during intermediate (energized conditions) rates of ATP turnover by augmenting the nonspecific leak of protons across the mitochondrial inner membrane.

Our measurements of DPH polarization indicate that a transition in membrane order occurred as temperature increased from 40 to 43°C and were consistent with temperature-induced alteration of the proton leak conductance. The break point in the Arrhenius plot of DPH polarization values indicated that at 42°C there was a marked decrease in order within the lipid bilayer of the mitochondrial membrane. This temperature-dependent decrease in order would allow water to intercalate into the lipid domain of the membrane, which would greatly increase proton conductance through the bilayer. Gutknecht (18) and Nichols et al. (28) demonstrated that increasing the water concentration within a pure phospholipid bilayer increased the proton conductance across the membrane. One possible alternative mechanism for increased proton conductance due to temperature-dependent changes in the lipid domain of the bilayer is a temperature-dependent activation of phospholipase A₂. Data from Cafiso and Hubbel (7) indicate that oxidation or degradation of <2% of the double bonds in vesicles formed from egg phosphatidylcholine resulted in a 15% increase in proton conductance across the membrane. Additionally, Frankel (16) showed that volatile products of lipid degradation by phospholipase A₂ act as weak protonophores, which would act as uncouplers of ATP synthesis to H⁺ transport.

One physiological consequence of a proton leak is, of course, that a given ATP turnover rate necessitates a higher rate of electron transport, i.e., O₂ consumption. However, perhaps an even more important outcome of the leak is that it may slow ATP turnover, even though such turnover is “submaximal” (33). Because the proton leak dissipates Δp, lower Gᵢ is required to elicit a given J₀. Thus, in the absence of compensatory mechanisms, if the ATP-utilizing site, e.g., pyruvate carboxylase, is inhibited by a fall in ATP/ADP ratio (Fig. 1) (4, 31), then lower Gᵢ would be predicted to exert a braking influence on the ATP-utilizing reaction, and the ATP turnover rate so established would be less than that in the absence of a proton leak. According to this explanation, pyruvate carboxylation rate in intact mitochondria is not increased with temperature, because the Q₁₀ stimulation of pyruvate carboxylase is offset by the inhibitory influence of energetic signals on the enzyme. Thus the present results are consistent with the view that the...
higher blood lactate concentrations commonly observed (15, 22, 25, 29) when thermal stress is superimposed on exercise stress result, in part, from a diminished capability of the liver to carry out gluconeogenesis from lactate (29). Furthermore, because Rowell et al. (29) observed higher rates of splanchnic O2 uptake during exercise in the heat, our data support the contention that the impairment in splanchnic lactate extraction they observed was more likely the result of a direct thermal insult to hepatocyte membrane bioenergetics (2) than to inadequate hepatic blood flow. Moreover, this concept may also be applicable to the energetics of skeletal muscle during exercise in the heat and is briefly discussed in Perspectives.

In the present study the ATP/ADP ratio of the mitochondrial matrix was not directly assessed; rather, analysis of the entire suspension medium indicated that increased temperature decreased the ATP/ADP ratio (and, therefore, Gp, since a nearly constant P1 concentration was obtained). It can be calculated that this measurement represents primarily the ATP/ADP ratio of the extramitochondrial space [~13 nmol adenine nucleotide/mg mitochondrial protein compared with 4,000 nmol adenine nucleotide added to the 2.0-ml reaction medium (4)]. Nevertheless, the ATP/ADP ratios of the mitochondrial matrix and the extramitochondrial space are thermodynamically linked according to membrane potential (ΔΨ) and the electrogenic adenine nucleotide translocase (27). Thus the two adenine nucleotide ratios tend to change in the same direction, whether the site of ATP utilization is inside or outside the mitochondrion (4, 23). Our results show similar temperature dependence of energetic responses to elevated ATP turnover, whether the ATP utilization was in the matrix (pyruvate carboxylation) or in the extramitochondrial space (G-6-P synthesis). In both cases, hyperthermia resulted in higher J0 and lower Gp.

Perspectives

The metabolic response to aerobic exercise is perturbed when environmental temperatures are sufficiently high to drive liver (29) and muscle (12, 22, 25) temperatures well above 40°C. Compared with cooler environmental conditions, exercise performed under such hot conditions results in greater decreases in blood glucose (22), higher blood lactate concentrations (15, 22, 25, 29), depressed cellular energy state in skeletal muscle (14, 22), and earlier onset of fatigue (12, 22). Results from the present study provide a possible explanation for these temperature-induced metabolic impairments, not only with regard to hepatic gluconeogenesis but, perhaps, also the energy metabolism of skeletal muscle. The steady-state phosphocreatine concentration, thus energy state, of skeletal muscle exercising at a given power output is reduced under hyperthermic conditions (14, 22). Reduced energy state in muscle would be predicted to impair contractile performance, leading to the requirement for greater recruitment of high-threshold (fast-twitch) motor units to satisfy an unchanging power output (33). Consistent with this view, exercise in the heat is associated with higher blood lactate concentration (15, 22, 25, 29) and earlier onset of fatigue (12, 22).

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