Mitochondrial oxidative phosphorylation thermodynamic efficiencies reflect physiological organ roles

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Mitochondria cannot maximize energy production, efficiency, and the cellular ATP phosphorylation potential all at the same time. The theoretical and observed determinations of coupling of oxidative phosphorylation in mitochondria from rat liver, heart, and brain were compared using classical and nonequilibrium thermodynamic measures. Additionally, the optimal thermodynamic efficiency and flow ratios were determined for control of the two energy-converting complexes of the respiratory chain: complex I (NADH), which reflects the integrated control of the two energy-converting complexes of the respiratory chain, and complex II (FADH2), the predominantly chain: complex I (NADH), which reflects the integrated control of the two energy-converting complexes of the respiratory chain, and complex II (FADH2), the predominantly exert strong control on the rate of oxidation (18). Thus mitochondria do not simply consume oxygen and make ATP at a maximal rate limited only by substrate availability (23). In addition to responding to the level of cellular energy, the second law of thermodynamics dictates that mitochondria must consume energy during the process of oxidative phosphorylation. Physiologically, this is the energy prerequisite for supporting mitochondrial architecture and viability.

Mitochondria have been described as being capable of different missions for economizing energy production against energy demands of oxidative phosphorylation (23). Additionally, mitochondria from different organ systems demonstrate morphological and functional differences (9). Therefore, the measured ATP production for oxygen consumption (P:O ratio) can vary in different mitochondrial populations. These differences can reflect one of four physiological missions: maximizing either the ATP production or the cellular phosphate potential, minimizing the cost of production, or a combination of these three (22). Delineating these coupling relationships using mechanistically based methods is difficult. This difficulty is clearly demonstrated by the continued controversy over the stoichiometry of the chemiosmotic mechanism of oxidative phosphorylation (1).

The ideal values described by chemiosmotic theory linking proton pumping by mitochondria subcomplexes to proton-dependent ATP synthesis in molecular studies of isolated factors F1 and F0 of mitochondrial ATPase can only be realized if there is no intramitochondrial cost of converting substrate into energy. According to the second law of thermodynamics, this cannot be the case. Therefore, the efficiency of substrate conversion into energy must be less than 100% and the P:O ratio must be less than idealized stoichiometry (typically taken as 3:1). Thus the in vivo measured P:O ratios are typically submaximal and may be specific to oxygen-dependent organs (21) and predictably altered in pathologic states (2, 3, 8, 9, 24, 25).

It is uncertain how physiological conditions can regulate these different mitochondrial missions or whether these coupling changes are advantageous. The nonequilibrium thermodynamics approach to the problem of coupling may prove insightful (6, 7, 14). These thermodynamic parameters have been observed to change depending on the nutritional state of the animal (starved versus fed). For example, liver mitochondria have been described as operating at a thermodynamic set point that maximizes both ATP production and the cellular phosphate potential while minimizing the cost of ATP production (21). In the starved state, liver mitochondria were observed to change toward a mission of maximizing ATP production with minimal cost without regard to the cellular phosphate potential (8). However, direct measurements of mitochondrial energy transduction at either optimal efficiency or at maximal ATP outflow or power output have not been described. In addition, the thermodynamic status of the mitochondrial electron transport chain has not been delineated at varying levels of tissue energy demand.

Mitochondria have two different complexes for converting substrate to energy (9). Complex I reflects the global cellular NADH production from fatty acid oxidation, the tricarboxylic acid (TCA) cycle, and glycolysis.
Complex II receives FADH₂ directly from succinate dehydrogenase and is only dependent on the mitochondrial TCA cycle. We hypothesize that the thermodynamic missions for regulatory control of the two mitochondrial energy-converting complexes of the respiratory chain are different for mitochondria from liver, heart, and brain.

The purposes of the series of studies in mitochondria isolated from liver, heart, and brain were as follows: 1) to delineate the efficiency of mitochondrial respiration for the integrated cellular substrate response through NADH (complex I), 2) to examine the degree of thermodynamic coupling of mitochondrial respiration through the TCA cycle (complex II), and 3) to compare these response data to the theoretical degrees of coupling, optimal efficiencies, and optimal flow ratios using nonequilibrium thermodynamic models.

Calculation of thermodynamic coupling and efficiency. Mitochondria can vary the efficiency of oxidative phosphorylation by maximizing ATP production, the cellular phosphate potential, or the cost of ATP production. A schematic model of the thermodynamic elements involved in mitochondrial oxidative phosphorylation has been published by Stucki (23), and a modification is depicted in Fig. 1.

The input force for oxidative phosphorylation is the redox potential of oxidizable substrates (X₀), which is converted into the output force of the phosphate potential (Xₚ). This phosphate potential drives the ATP-utilizing reactions in the cell, represented as the ATP demand. The associated input flow (Jₒ) represents net oxygen consumption, and the outflow (Jₚ) represents the net rate of ATP production. Jₚ and Jₒ can be further expressed in the following equations, with Lₒ, Lₚ, Lₒp, and Lₚp, the phenomenological cross-coupling coefficients of the system:

\[ Jₒ = LₒX₀ + LₚXₚ \]

\[ Jₚ = LₒpX₀ + LₚpXₚ \]

The cross-coupling coefficient Lₒ represents the positive influence of substrate availability on oxygen consumption. Lₚ is the coefficient of negative feedback of the phosphate potential on ATP production. Lₒp is the positive coefficient of substrate-dependent ATP production, and Lₚp is the negative coefficient of phosphate inhibition on oxygen consumption.

The coupling of the energy conversion in the process is designated by the dimensionless parameter q, known as the degree of coupling of oxidative phosphorylation (6)

\[ q = \sqrt{\frac{Lₒp - Lₚ}{Lₚp - Lₒ}} \]

There exists experimental evidence that Onsager’s reciprocal relationship holds for oxidative phosphorylation such that Lₒp = Lₚp (19). Thus q is the ratio of the off-diagonal coefficients to the diagonal coefficients

\[ q = \frac{Lₒp}{\sqrt{LₚpLₒ}} \]

When the transmitochondrial proton gradient is collapsed with the uncoupler dinitrophenol (DNP), the mechanism for coupling energy output to energy production (Lₒp) vanishes. With the transmitochondrial proton gradient collapse, ATP production becomes nil and output force (Xₚ) also vanishes. Therefore, Eq. 1 is reduced to

\[ (Jₒ)unc = LₒX₀ \]

where (Jₒ)unc is the uncoupled rate of oxygen uptake.

The static head condition (i.e., no output) can be achieved in state 4 of oxidative phosphorylation. All coupling coefficients are maintained, but the net outflow of ATP (Jₚ) vanishes. The net oxygen consumption at static head [(Jₒ)sh] does not simultaneously vanish because of leakage and essential mitochondrial energy consumption. Hence, output force (Xₚ) is still expended to maintain static head force potential by compensating the dissipative leaks in the system. Setting Jₚ = 0 in Eq. 2

\[ LₒpX₀ + LₚXₚ = 0 \]

\[ Xₚ = -(Lₒp/Lₚ)X₀ = -(q²Lₒ/Lₚ)LₒX₀ \]

Substituting Eq. 7 into Eq. 1

\[ (Jₒ)sh = LₒX₀ - Lₚp(q²Lₒ/Lₚ)LₒX₀ = LₒX₀ - q²LₒX₀ \]

\[ (Jₒ)sh = LₒX₀(1 - q²) \]

In our experiments, state 3 is linear within the range of 95% saturation to 5% saturation. When the mitochondria are stimulated with an excess of ADP or DNP and followed until oxygen tension approaches zero, Jₒ is essentially linear. Thus Jₒ remains constant in the range studied, and, for any value of Jₒ X₀ is unique. Because Jₒ appears constant over the physiological range of oxygen tensions, X₀ may not be an important determinant of Jₒ. Per Eq. 5, (Jₒ)unc = LₒX₀. Therefore, Eq. 9 becomes

\[ (Jₒ)sh = (Jₒ)unc(1 - q²) \]
Given that ($J_{o_{\text{sh}}}$ (state 4) and $J_{o_{\text{unc}}}$ (DNP)) are experimentally obtainable, the solution of Eq. 10 allows for the direct calculation for $q$, the degree of thermodynamic coupling

$$q = \sqrt{1 - (J_{o_{\text{sh}}}/J_{o_{\text{unc}}})^2}$$  

(11)

The efficiency of the energy converter is defined as the output/input and is defined by Kedem and Caplan (7) as

$$\eta = -J_{pX_p}/J_{oX_o}$$  

(12)

This is a biphasic function, for which an optimal thermodynamic efficiency ($\eta_{\text{opt}}$) of the system can be discerned for any value of the coupling coefficient $q$ (22)

$$\eta_{\text{opt}} = \frac{q^2}{1 + \sqrt{1 - q^2}}$$  

(13)

Taking $\alpha = \arcsin(q)$ (22), Eq. 13 becomes

$$\eta_{\text{opt}} = \tan^2(\alpha/2)$$  

(14)

METHODS

Preparation of purified isolated mitochondria. Isolated mitochondria were prepared using a modification of standard techniques (15, 16). In brief, male Sprague-Dawley rats (250–300 g) were fasted overnight to deplete the levels of glycogen and fatty acids. The rats were then anticoagulated with heparin and subjected to decapitation. The livers, hearts, or brains were then removed and immediately immersed in cooled (2–4°C) mitochondria isolation buffer (MIB). The MIB buffer contained 0.3 M sucrose, 1.0 mM EGTA, 5.0 mM MOPS, 5.0 mM KH$_2$PO$_4$, and 0.1% bovine serum albumin (fatty acid free, Sigma). The pH was adjusted to 7.4 with KOH. The organs were minced into small (2 mm) cubes and washed in cold (2–4°C) MIB until the medium was free of blood.

For isolation of liver mitochondria, the minced liver was then homogenized using a tissue homogenizer (1,000 rpm for 20 s at 0–4°C), and the resulting homogenate was subjected to centrifugation.

Heart mitochondria isolation involved additional washing of the minced heart, which was then placed in 15 ml of isolation buffer into which 2.5 mg of Nagarse (Sigma) had been added. The suspension was then transferred into a cold glass homogenization vessel and homogenized six stokes up and down. The suspended homogenate is then centrifuged as described below. This technique has been described previously by Mela and Setz (13).

Liver and heart homogenates had red blood cells, unbroken cells, and nuclei, and other debris was removed by centrifugation at 1,000 g for 5 min at 5°C. The supernatant was carefully collected and centrifuged immediately at 10,000 g for 10 min. Crude mitochondria appeared as a solid light-brown pellet.

Preparation of isolated brain mitochondria involved additional washing as previously described (17). After micing, the brain tissue was placed in 10 ml of isolation buffer in which 5.0 mg of Nagarse (Sigma) has been added. The suspension was then transferred into a cold glass homogenization vessel and homogenized six stokes up and down. The homogenate was then centrifuged at 2,000 g for 3 min, and the pellet was resuspended in isolation buffer and centrifuged again at 2,000 g for 3 min. The supernatant was then centrifuged at 12,000 g for 10 min. The resulting pellet was then resuspended in 20 ml isolation media with 20 μl of 10% digitonin in DMSO added and subjected to centrifugation at 12,000 g for 10 min. The pellet, minus the light layer, was then resuspended in 10 ml isolation media without EGTA and centrifuged again with the resulting pellet resuspended in 0.1–0.2 ml isolation media without EGTA.

Purified mitochondria from all organs were obtained through modification of the technique of Sims (20). The pellet was resuspended in 12% Percoll solution (vol/vol in MIB), and the supernatant was discarded. The discontinuous density gradient was prepared in polycarbonate centrifuge tubes (16 × 76 mm) by layering 2.5-ml fractions of the resuspended pellet on two performed layers consisting of 3 ml of 26% Percoll and 40% Percoll. The tubes were then centrifuged at 31,000 g for 5 min (Beckman L-80 ultracentrifuge). Three major bands of material were obtained, and the band fraction near the interface of the lower two Percoll layers was isolated and diluted 1:4 by gently mixing with MIB. The resulting mixture was centrifuged at 10,000 g for 10 min to obtain the purified mitochondrial pellet. The purity of the mitochondrial isolation procedure was confirmed by electron microscopy. This pellet was resuspended in 1 ml MIB, and the protein concentration was determined by the method of Lowry et al. (12), with bovine serum albumin (Sigma) as the protein standard. A typical yield of mitochondrial protein was 12–16 mg/ml.

Oxygraph determinations. To determine the rates of oxidative phosphorylation, oxygen consumption studies were conducted in a 0.6-ml Clark oxygen electrode chamber (Yellow Springs Instruments) at 37°C. Data were recorded via a Macintosh Quadra 650 computer via LabView 2.0 converter software. The oxygraph medium consisted of 130 mM KCl, 2 mM KH$_2$PO$_4$, 1 mM MgCl$_2$, 0.5 mM K-EGTA, and 5 mM HEPES. Either 10 mM glutamate plus 10 mM malate or 5 mM succinate with 5 μM rotenone were added as respiratory substrate. The oxygraph medium was adjusted to pH 7.20 and 37°C. The rates of resting and ADP-stimulated oxygen consumption of 2.0 mg protein with glutamate/malate substrate and 2.0 mg protein with succinate as substrate were measured with standard techniques.

The rate of endogenous respiration (state 2) was measured in air-saturated oxygraph media before the addition of ADP (200 mM) to the oxygraph chamber. The maximum ADP stimulation rate (state 3) was then measured. After the resting rate (state 4) was recorded, the fully energized resting state (state 4 at static head) was induced by the addition of atracyside (0.5 mg/ml), and the uncoupled respiratory rate induced by the addition of an optimal amount of DNP (100 nM) was measured. Oxygen consumption rates were determined by the least-squares behavior, with operator set points and recorded as nanogram atoms oxygen per minute per milligram mitochondrial protein.

To quantify the relationship of ADP stimulation to oxygen consumption, the acceptor control ratio (state 3/state 2), the respiratory control ratio (state 3/state 4), and the ADP:O ratio were calculated [modified from Chance and Williams (4)].

Measurement of ATP production rates. The ADP:O ratio describes the end-point input capacity of oxidative phosphorylation. To describe the outflow capacity rate of oxidative phosphorylation, we developed a spectrophotometric assay to determine the ATP production rate. ADP-stimulated mitochondrial ATP production rates were measured by exploiting a coupled-enzyme system linked to NADPH production.
Table 1. Standard measures of oxidative phosphorylation coupling for liver, heart, and brain mitochondria

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<th>Brain</th>
<th>Liver</th>
<th>Heart</th>
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<tr>
<td></td>
<td>Complex I</td>
<td>Complex II</td>
<td>Complex I</td>
</tr>
<tr>
<td>RCR</td>
<td>5.74 ± 0.646</td>
<td>4.27 ± 0.25</td>
<td>6.5 ± 0.67</td>
</tr>
<tr>
<td>ACR</td>
<td>9.07 ± 1.73*</td>
<td>5.08 ± 0.68</td>
<td>18.89 ± 2.78</td>
</tr>
<tr>
<td>P/O</td>
<td>2.11 ± 0.11†</td>
<td>1.77 ± 0.13</td>
<td>2.26 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD. RCR, respiratory control ratio; ACR, acceptor control ratio; P/O, ratio of ATP production to oxygen consumption.

*P = 0.001 vs. liver complex I. †P < 0.001 vs. heart complex I. ‡P = 0.001 vs. liver complex I.

The following optimal concentrations for the assay reagents were determined by generating sequential dose-response curves: glucose (1.0 mM), glucose-6-phosphate dehydrogenase (G6PDH; 1.125 IU/ml), NADP (0.5 mM), and hexokinase (HK; 0.75 IU/ml). The coupled enzyme detection system reagents were then combined with the glutamate/malate and complex II (succinate plus rotenone) pathways. The rate of ATP production specifically from oxidative phosphorylation is then determined by subtracting the inhibitor-insensitive rate from the total rate. The rates are reported as millimoles of ATP produced per minute per milligram mitochondrial protein.

Parallel sample runs were made both with and without the addition of specific inhibitors of oxidative phosphorylation: oligomycin (0.05 mg/ml), which inhibits ATP synthetase (complex V); atracylods (0.05 mg/ml), which inhibits ADP/ATP translocase; or the uncoupler DNP (0.04 mM). The rate of ATP production specifically from oxidative phosphorylation is then determined by subtracting the inhibitor-insensitive rate from the total rate. The rates are reported as millimoles of ATP produced per minute per milligram mitochondrial protein.

The conventional measures of the coupling of mitochondrial oxidative phosphorylation are shown in Table 1. The respiratory control ratios (RCR) were similar for liver, heart, and brain mitochondria for both the integrated cellular pathway and the TCA cycle pathway. These RCR values are consistent with those of intact, functional isolated mitochondria (23). The acceptor control ratio for the complex I pathway was higher for the liver than for the heart or brain. The P:O ratio for the complex I pathway was higher than that of the complex II pathway in both liver and heart. The P:O ratio for complex I was lower in the brain than the liver or the heart.

**RESULTS**

Rates of oxygen consumption (Jo) during the basal (state 2), maximal ADP-stimulated (state 3), and resting (state 4) states for both the mitochondrial complex I (glutamate/malate) and complex II (succinate plus rotenone) are shown in Table 2. These data were derived from separate animals (n = 8) for each organ group and are presented as means ± SD. For liver, heart, and brain, the TCA cycle pathway-linked electron transport (through FADH2; complex II) resulted in higher rates of state 2 basal oxygen consumption than the rate of electron transport from the integrated cellular respiratory pathway (carried by NADH; complex I). Complex I basal consumption was higher for heart (22.4 ± 4.3 nmol

Table 2. Measures of oxygen consumption for liver, heart, and brain mitochondria

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Liver</th>
<th>Heart</th>
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<tr>
<td></td>
<td>Complex I</td>
<td>Complex II</td>
<td>Complex I</td>
</tr>
<tr>
<td>Jo state 2</td>
<td>14.96 ± 1.83‡</td>
<td>31.68 ± 2.89</td>
<td>6.60 ± 0.96</td>
</tr>
<tr>
<td>Jo state 3</td>
<td>120.78 ± 9.05*</td>
<td>151.80 ± 8.07†</td>
<td>112.2 ± 1.96*</td>
</tr>
<tr>
<td>Jo unc</td>
<td>139.92 ± 8.91*</td>
<td>162.1 ± 10.12</td>
<td>72.38 ± 11.19</td>
</tr>
<tr>
<td>Jo state 4</td>
<td>22.44 ± 2.93*</td>
<td>35.64 ± 0.96</td>
<td>18.26 ± 1.97*</td>
</tr>
<tr>
<td>Jo static head</td>
<td>12.32 ± 2.04</td>
<td>24.20 ± 1.86‡</td>
<td>4.40 ± 1.31</td>
</tr>
</tbody>
</table>

Values are means ± SD in nanomoles O2 per minute per milligram mitochondrial protein. Jo, output flow; Jo unc, uncoupled rate of O2 uptake; CI, complex I; CII, complex II.
O$_2$·min$^{-1}$·mg$^{-1}$) than for liver (6.6 ± 2.3 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001). Complex II basal consumption was higher for both heart (35.4 ± 5.7 nmol O$_2$·min$^{-1}$·mg$^{-1}$) and brain (31.7 ± 7.1 nmol O$_2$·min$^{-1}$·mg$^{-1}$) compared with liver (15.0 ± 3.1 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001).

ADP stimulation resulted in uniformly higher state 3 than state 2 oxygen consumption rates for both pathways for mitochondria from liver, heart, and brain. Heart complex I state 3 consumption (158.9 ± 21.4 nmol O$_2$·min$^{-1}$·mg$^{-1}$) was higher than that of liver (112.2 ± 4.8 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001) and brain (151 ± 20 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.0005). In the heart, complex II state 3 oxygen consumption (120.8 ± 22.2 nmol O$_2$·min$^{-1}$·mg$^{-1}$) was significantly higher than for complex I (121 ± 22 nmol O$_2$·min$^{-1}$·mg$^{-1}$; P = 0.0022).

The resting state 4 oxygen consumption was higher overall for the heart (49.4 ± 29.9 nmol O$_2$·min$^{-1}$·mg$^{-1}$) versus the liver (20.7 ± 6.6 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.0002) or brain (29.0 ± 8.6 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.0002). Resting oxygen consumption for complex I was higher in the heart (49.4 ± 29.9 nmol O$_2$·min$^{-1}$·mg$^{-1}$) than in either liver (18.3 ± 20 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.0003) or brain (22.4 ± 7.2 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.0012).

The oxygen consumption at the fully energized resting condition at static head [(J$_{o}$)$_{sh}$] was obtained during state 4 after the addition of atractyloside. (J$_{o}$)$_{sh}$ was greater for complex II (24.9 ± 8.6 nmol O$_2$·min$^{-1}$·mg$^{-1}$) than for complex I (11.4 ± 7.5 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001). The (J$_{o}$)$_{sh}$ for heart complex I (18.7 ± 6.4 nmol O$_2$·min$^{-1}$·mg$^{-1}$) was greater than that of the liver (4.4 ± 3.2 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001). For complex II, the (J$_{o}$)$_{sh}$ for heart (34.1 ± 5.5 nmol O$_2$·min$^{-1}$·mg$^{-1}$) was greater than that of the brain (24.2 ± 4.5 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.0013) and liver (16.5 ± 4.0 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001).

(J$_{o}$)$_{unc}$ values were determined after the addition of DNP, and the results are summarized in Table 2. Overall the (J$_{o}$)$_{unc}$ was lower in the liver (105.5 ± 39.7 nmol O$_2$·min$^{-1}$·mg$^{-1}$) compared with the heart (161.0 ± 35.7 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001) and brain (151.0 ± 25.1 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P < 0.0001), and the (J$_{o}$)$_{unc}$ was higher for complex II (156.2 ± 27.4 nmol O$_2$·min$^{-1}$·mg$^{-1}$) versus complex I (122.2 ± 46.1 nmol O$_2$·min$^{-1}$·mg$^{-1}$; P = 0.0009).

The degrees of thermodynamic coupling (q) were calculated using Eq. 11 and the experimentally obtained values of (J$_{o}$)$_{sh}$ and (J$_{o}$)$_{unc}$. The overall degree of thermodynamic coupling is higher for liver mitochondria (0.955 ± 0.021) than for brain (0.937 ± 0.026, P = 0.0002) or heart (0.917 ± 0.037, P = 0.0002). Additionally, the coupling of complex I pathway respiration (0.957 ± 0.020) is greater than that for complex II (0.916 ± 0.028, P < 0.0001). As shown in Fig. 2, these coupling ratios approach the theoretical maximal coupling values of the thermodynamic set points corresponding to maximal net output power (q_p = 0.910) and economic net output flow (q^ec = 0.953).

The efficiency of substrate to energy conversion is related to the thermodynamic coupling coefficient by Eq. 12 (23). As shown in Fig. 3, complex I in all three tissues are intrinsically more efficient (P = 0.0001) than the complex II pathway for oxidative phosphorylation.

The maximal oxygen consumption data during level flow is shown in Fig. 4. Maximal oxygen consumption is greater in complex II than complex I (144.8 ± 3.6 vs. 131.8 ± 6.2 nmol O$_2$·min$^{-1}$·mg$^{-1}$, P = 0.032). Among the tissues are intrinsically more efficient (P = 0.0001) than the complex II pathway for oxidative phosphorylation.

Fig. 1. Calculated thermodynamic q values are shown for brain, heart, and liver mitochondria. Data are means ± SD for integrated cellular substrate pathway, mitochondrial complex I (CI), and tricarboxylic acid cycle pathway, mitochondrial complex II (CII). Also shown are maximal coupling values of the thermodynamic set points corresponding to maximal net output flow (ATP) at optimal efficiency (q^opt = 0.786), maximal net output power (q_p = 0.910), economic net output flow (q^ec = 0.953), and economic net output power at optimal efficiency (q^ec = 0.972). *P < 0.005 vs. brain CI; †P < 0.0001 vs. heart CII.

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substrate pathway, CI, and tricarboxylic acid cycle pathway, CII. *P < 0.003 vs. brain CI. †P < 0.0001 vs. liver CI.

There were no significant differences in the maximal rate of ATP production between the integrated cellular and TCA cycle pathways (Fig. 5). There were differences in maximal ATP production between the cellular pathways and TCA cycle pathways of the liver and those of the heart and brain.

DISCUSSION

It is well known that mitochondria from different organ systems demonstrate morphological and functional differences (9). This study utilizes thermodynamic measures to quantify oxidative phosphorylation capacity in mitochondria from liver, hearts, and brains. This is the first study to suggest that the TCA cycle and integrated cellular pathways of respiration are optimized for different degrees of thermodynamic coupling.

Overall, oxygen consumption was higher for the TCA cycle pathway (FADH$_2$ linked), compared with the integrated cellular pathway (NADH linked). Electron flow from the integrated cellular pathway extrudes protons from three sites (complexes I, III, and IV) compared with flow from the TCA cycle (FADH$_2$), which has proton extrusion from only two sites (complexes III and IV). Because oxidative phosphorylation is dependent on the utilization of the chemiosmotic gradient produced from extruded protons, the ADP-stimulated oxygen consumption should be higher for the TCA cycle pathway compared with the integrated cellular pathway.

Heart and brain mitochondrial systems apparently utilize more oxygen but can produce ATP at a faster rate than liver systems. However, liver mitochondria appear to be more efficient at producing ATP. The efficiency of ATP production for oxygen consumed is reflected not only in a higher P:O ratio but also in a higher thermodynamic coupling ratio for liver mitochondria.

In this study, we used purified mitochondria to ensure that a consistent population of mitochondria were identified for each organ. Additional studies of the RCR were performed to ensure the biochemical integrity of the purified mitochondria (5). The stimulation of static head respiration due to extraneous ATP hydrolysis (leading to artificially lower values for q) was eliminating by inhibiting the mitochondrial adenine nucleotide translocase. Finally, we chose to study the mitochondrial coupling at the physiologically relevant temperature of 37°C.

Comparison with previous studies. The thermodynamic coupling of oxidative phosphorylation could be influenced by the cellular energy state. Yet, the experimental mean (of the integrated cellular and TCA cycle pathways) degree of coupling obtained for isolated liver mitochondria (0.955) is similar to the value of previously obtained in normothermic isolated perfused whole liver from fasted rats (0.952) (21). In that study, Soboll and Stucki derived the degree of coupling using experimental measurements of the adenyl nucleotide pool constituents and the determination of mass action ratios. The effects of tissue energy demand on the regulation of the mitochondrial electron transport chain and variation in coupling were not addressed, nor were further determinations of specific thermodynamic flow characteristics.

The thermodynamic coupling obtained in this study differs from those based on free energy changes. Lennard (10) used experimentally measured ratios of ATP, ADP, and P, and found similar coupling (0.98) for all substrate conditions, including succinate, glutamate...
plus malate, 3-hydroxybutyrate, and 2-oxoglutarate. These calculations were based on a modification of Rottenberg's estimation of the degree of coupling. This approximation depends on the RCR and is considered a less sensitive approximation of \( q \) (18). This might explain the insensitivity in the estimation of \( q \) (similar values despite wide variations in the RCRs, 3.58 to 5.33, for the substrates). In addition, these approximated values for \( q \) are significantly greater than those obtained in other native membranes and organelles (13, 21).

Physiological implications. The cellular and TCA cycle respiratory pathways balance their rate and efficiency of ATP production against the energy needs of the cell. The rate of mitochondrial ATP production might better describe the utilization of ATP for cellular processes than cytoplasmic ATP levels. For example, cellular ATP utilization for muscular contraction and calcium handling would be much higher in a beating heart than an arrested heart even though the measured cytoplasmic ATP levels could be similar. ADP delivery to the mitochondria of the beating heart would lead to higher rates of oxidative phosphorylation and ATP production.

Stucki (22) has proposed physiological meanings for the degrees of mitochondrial oxidative coupling. This theory is based on the thermodynamic tradeoff of reducing efficiency to produce maximum output or increasing efficiency to economize the output. The specific thermodynamic degrees of coupling correspond to the following set points: \( q_f \) = maximal net output flow (ATP) at optimal efficiency, \( q_p \) = maximal net output power (\( X_p \)), \( q_f^{ec} \) = economic net output flow, and \( q_p^{ec} \) = economic net output power at optimal efficiency. For each function, there is an unique maximal value of \( q \) that corresponds to the optimal coupling (i.e., maximal net ATP outflow). Using these calculated output superfunctions, the corresponding maximal coupling values of each set point are \( q_f = 0.786, q_p = 0.910, q_f^{ec} = 0.953, \) and \( q_p^{ec} = 0.972 \).

In the liver, mitochondria are optimized for the maximal production of ATP at minimal cost without regard to the cellular phosphate potential. The cellular respiratory pathway \( (q = 0.968) \) is more tightly coupled than the TCA cycle pathway \( (q = 0.943) \). The experimentally derived value of \( q \) for cellular respiration is close to that of \( q_f^{ec} (0.972) \), suggesting that the integrated cellular respiratory pathway is optimized for the economic maintenance of the hepatocellular energy state.

The experimentally observed degree of coupling for the TCA cycle respiratory pathway is between that of \( q_p \) (maximal net output power) and \( q_f^{ec} \) (economic net output flow). This suggests that in the liver, the TCA cycle pathway is set to the economic production of ATP for cellular processes in addition to maintaining the hepatocellular energy state. We interpret these data to indicate that in the presence of excess cellular substrates for the TCA cycle, liver mitochondrial oxidative phosphorylation is optimized to produce ATP for cellular processes (e.g., glycogen formation). In the heart and brain, the experimentally derived value of \( q \) for cellular respiration pathway is remarkably close to that of \( q_f^{ec} (0.953) \), suggesting that in these organs the integrated cellular respiratory pathway is optimized to economically produce ATP for cellular processes. In the brain, the coupling of the TCA cycle pathway approaches that of \( q_f \) (0.910), implying that this mitochondrial substrate pathway is geared toward maximizing the cellular energy state and the maintenance of cellular integrity. In contrast, the TCA cycle coupling in the heart is between \( q_p \) and \( q_f^{ec} (0.786) \), consistent with providing maximal ATP production for both cellular processes (i.e., contraction) and preserving the cellular energy state.

The relative difference in the thermodynamic efficiency of the two respiratory pathways appears to reflect these physiological roles. The cellular pathway may be more efficient because it must maintain integrated cellular survival pathways, whereas in the setting of relative substrate excess, the TCA cycle pathway may sacrifice efficiency to maximize the production of ATP.

Thermodynamic efficiency. The absolute values of the thermodynamic efficiency are similar to the result (\( \approx 50\% \)) calculated by Lehninger (9) of the generation of ATP from glucose using a totally different methodology. The optimal condition for biological energy conversion is not necessarily the state of maximal thermodynamic efficiency (1, 2, 14, 22). Rottenberg (18) proposes that in biological systems, the efficiency at maximal output must be less than 50%. By Eq. 14, high thermodynamic efficiency could be achieved at vanishingly low (non-physiological) respiratory rates. The rate of energy production or maintenance of the cellular phosphate potential may be more important than the efficiency of the energy conversion process.

The methodological approach of this study also allows for the calculation of the optimal thermodynamic flow ratio. The value of determining optimal flow ratios rather than optimal thermodynamic efficiency is illustrated by the fact that maximum flow ratios are characterized by states of zero thermodynamic efficiency (23). In addition, optimal flow ratios are a characteristic of oxidative phosphorylation and provide additional information into the respiratory response to energy demand stimulation by ADP. For example, the ADP:O ratio describes the state of the end-point capacity of oxidative phosphorylation based on the input of ADP, not the output of ATP. The ADP:O ratio therefore depends on the assumption that all the ADP is converted to ATP in a linear fashion and is insensitive to nonlinear variations in the rate of either ATP production or oxygen consumption. By directly measuring ATP production and oxygen consumption rates at physiological flows, these limitations are minimized. Ultimately, in fact, most metabolic processes in living cells are dynamic systems (14), and the characterization of flows better reflects complex system behavior than do models dependent on end-point measurements.

We conclude that, beyond defining the stoichiometry of substrate utilization to energy production of mito-
chondria, we have the potential to discern distinct physiological missions for rat mitochondria. In the liver, mitochondrial substrate pathways are set to economize either the cellular energy state or ATP production. In the brain, ATP production from the TCA cycle is maximized toward maintenance of the cellular energy state and cellular integrity. In the heart, the TCA cycle pathway is set toward maximizing the production of ATP for cellular processes. This study was performed in isolated rat mitochondria, and findings from this study may not apply to other species or organ systems. In addition, in vivo variations in substrate availability may alter the observed rates of mitochondrial respiration. The coupling of oxidative phosphorylation not only can be expected to change with the nutritional state of the cell but may also reflect an ontogenetic response of rat mitochondria to fit specific organ roles.

Perspectives

The coupling relationships of oxidative phosphorylation may change during development or pathophysiological states. Identification of these changes may allow for new insights into organ differentiation and metabolic-based therapeutic approaches.

This study was supported in part by a Center of Excellence Award from the Emergency Medicine Foundation, a grant from the American Heart Association (Colorado Chapter), and by National Institutes of Health Grants HL-43696 and GM-4922. Address for reprint requests: C. B. Cairns, Colorado Emergency Medicine Research Center B-211, UCHSC, 4200 E. Ninth Ave. Denver, CO 80262.

Received 12 February 1997; accepted in final form 27 January 1998.

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