ATP and ADP levels are critical regulators of glucose-stimulated insulin secretion. In many aerobic cell types, the phosphorylation potential (ATP/ADP/Pi) is controlled by sensing mechanisms inherent in mitochondrial metabolism that feed back and induce compensatory changes in electron transport. To determine whether such regulation may contribute to stimulus-secretion coupling in islet cells, we used a recently developed flow culture system to continuously and noninvasively measure cytochrome c redox state and oxygen consumption as indexes of electron transport in perfused isolated rat islets. Increasing substrate availability by increasing glucose increased cytochrome c reduction and oxygen consumption, whereas increasing metabolic demand with glibenclamide increased oxygen consumption but not cytochrome c reduction. The data were analyzed using a kinetic model of the dual control of electron transport and oxygen consumption by substrate availability and energy demand, and ATP/ADP/Pi was estimated as a function of time. ATP/ADP/Pi increased in response to glucose and decreased in response to glibenclamide, consistent with what is known about the effects of these agents on energy state. Therefore, a simple model representing the hypothesized role of mitochondrial coupling in governing phosphorylation potential correctly predicted the directional changes in ATP/ADP/Pi. Thus, the data support the notion that mitochondrial-coupling mechanisms, by virtue of their role in establishing ATP and ADP levels, may play a role in mediating nutrient-stimulated insulin secretion. Our results also offer a new method for continuous noninvasive measures of islet cell phosphorylation potential, a critical metabolic variable that controls insulin secretion by ATP-sensitive K+–dependent and –independent mechanisms. Diabetes 53:401–409, 2004

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ATP/ADP/Pi, phosphorylation potential; FBS, fetal bovine serum; KATP, ATP-sensitive K+; KRB, Krebs-Ringer buffer; MEM, minimum essential medium; PCR, phosphocreatine; Pi, inorganic phosphate.

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Free ATP and ADP levels are understood to couple glucose metabolism with the closing of ATP-sensitive K+ (KATP) channels and the ionic events leading to the exocytosis of insulin (1,2). Furthermore, the phosphorylation potential (ATP/ADP/Pi) may augment insulin secretion beyond that mediated by KATP channels (3). Although it is well established that the critical ATP and ADP levels depend on islet substrate metabolism and mitochondrial electron transport (4–6), it is less broadly appreciated that ATP stimulates electron transport and ATP production (7,8) as a feedback regulator of ATP/ADP/Pi (9,10). Thus, mitochondrial ATP production, the ATP/ADP/Pi, and ultimately the insulin secretory rate depend dually on both substrate supply as well as energy demand.

Although the mechanisms by which mitochondria sense ADP levels and control ATP/ADP/Pi are not fully established, a likely candidate is cytochrome c oxidase, the rate-limiting step in the electron transport chain (11). Wilson et al. (11,12) validated a mathematical model correlating substrate supply and energy demand (i.e., as phosphorylation potential) to the redox state and oxygen consumption rate of cytochrome c oxidase for several cell types, including cultured kidney cells (13), perfused rat heart (14), neuroblastoma cells (12), and heart mitochondria (11). To determine whether such dual control of electron transport exists in β-cells, we measured islet oxygen consumption and cytochrome c reduction in response to an increased and decreased energy state (glucose vs. cell activation by glibenclamide [15]) using a previously developed flow culture system (16,17). Our results support the dual control of electron transport in islet cells and suggest that changes of β-cell phosphorylation potential may be estimated noninvasively using the Wilson’s model (11).

RESEARCH DESIGN AND METHODS

Oxygen consumption and insulin secretion were measured using RPMI media 1640 (glucose-free; Gibco, Grand Island, NY) supplemented with 10% (vol/vol) dialyzed fetal bovine serum (FBS), antibiotic-antimycotic (100 units/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B; all from Gibco), and glucose (Sigma, St. Louis, MO). For cytochrome c measurements, a phenol red-free minimum essential medium (MEM) was prepared (0.2 g/ml CaCl2·9 H2O, 89 g/l MgSO4·6H2O, 0.14 g/ml NaH2PO4·H2O, 0.4 g/l KC1, 0.4 g/ml NaCl, 2.2 g/ml NaHCO3 without glucose; all from Sigma); 20 ml/l essential amino acids solution, 10 ml/l nonessential amino acids solution, 10 ml/l MEM vitamin solution (all from Gibco) supplemented with 10% (vol/vol) heat-inactivated FBS, 2 mM d-glutamine, antibiotic-antimycotic, and varying amounts of glucose. Antimycin A and KCN were purchased from Sigma.

Islet isolation. Rat islets were harvested from male Fisher rats (weighing ~250 g), anesthetized by intraperitoneal injection of sodium pentobarbital (35 mg/230 g rat). Islets were prepared by injecting collagenase (10 ml of 0.23
mg/ml Liberase; Roche Molecular Biochemicals, Indianapolis, IN) into the pancreatic duct and surgically removing the pancreas. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. The pancreata were placed into 15-ml conical tubes containing 5 ml of 0.23 mg/ml Liberase and incubated at 37°C for 30 min. The digestate was filtered (400 µm stainless steel screen), rinsed (Hank’s buffered salt solution), and purifited in a gradient solution of Optiprep (Nycosmed, Oslo, Norway [18]). Islets were cultured for 18–24 h before the experiments in RPMI media 1640 supplemented with 10% heat inactivated FBS.

**Flow culture system.** The islet flow culture system (16,17) includes a pump, gas equilibrator, a glass islet perfusion chamber, detectors for oxygen and cytochromes, and a fraction collector. To measure cytochromes by light absorption, islets were stabilized with Cytopore beads (Amersham Biosciences, Piscataway, NJ) that were layered into the chamber using a P200 pipette as follows (Fig. 1). First, 0.4 mg of beads in 20 µl media were allowed to settle onto the porous polyethylene frit at the chamber’s bottom. A mixture of 600 islets and Cytopore beads (0.12 mg; Amersham Biosciences) was added followed by another 0.4 mg Cytopore beads and a top frit. Porous frits were cored (0.3 cm) from polyethylene sheets (Small Parts, Miami Lakes, FL).

**Oxygen consumption measurements.** Oxygen consumption was calculated as the flow rate multiplied by the difference between inflow and outflow oxygen tension, measured using phosphorescence lifetimes of an oxygen-sensitive polymerized platinum porphyrin (17). The dye, consisting of dimethylsiloxane-bisphenol A-polycarbonate blockcopolymer (5 g; GE, Waterford, NY) that were layered into the chamber using a P200 pipette (NRC; Newport, Irvine, CA), dissolved in 100 ml dichloromethane, was painted inside the glass chamber

**RESULTS**

**Absorption spectra of rat islets.** Absorption by cytochrome c in islets was relatively small, but the second derivative revealed significant valleys at 551 nm (Fig. 2) that are most pronounced in the presence of cyanide, a blocker of cytochrome c oxidase subsequent to cytochrome a3 (Fig. 2A). The valley corresponding to cytochrome c was only detectable at a highly reduced state and could not be used in our system to assess the effects of glucose. Antimycin A, a blocker of electron transfer upstream of cytochrome c, fully oxidizes cytochrome c and the valley disappears (Fig. 2B). The magnitude of the valley at 551 nm was sensitive to glucose concentration (Fig. 2C and D), consistent with an increase in electron supply to the electron transfer chain. Thus, the state of cytochrome c made a major contribution to the absorption spectra of islets, and the depth of the valley at 551 nm reflected the extent of reduction.

**Dynamic response of cytochrome c to glucose and oxygen.** Glucose stimulation (20 mmol/l) affected the

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![ISLET ATP/ADP CONTROL](image)

**FIG. 1.** Flow chamber housing the islets. Media equilibrated with gas entered the bottom of the chamber (2.5 mm internal diameter) and percolated up through the porous frit, continuously supplying the islets with fresh media. The total volume of the chamber was ~200 µl. Oxygen consumption was measured by lifetime detection of an oxygen-sensitive phosphorescent dye that was painted on the interior of the glass chamber ~1 cm above the islets. Measurement of cytochrome reduction was done by detecting the absorption of light by the islets in the chamber. Excitation and detection of light was transmitted to the sources and detection systems via optical fibers.
cytochrome c redox state within 1 min (less than the temporal resolution of our system [Fig. 3A]) and required about 30 min to reach steady state (Fig. 3B). The kinetics of the increase and decay to baseline were nearly identical and demonstrated the precise relation between glucose and cytochrome c reduction. After 150 min at high glucose, the absorbance returned to baseline. Increasing media oxygen content from 20 to 95% only slightly altered the cytochrome c peak, suggesting that islets were well perfused by 20% oxygen and were not hypoxic. Equilibration with 0% oxygen dramatically reduced cytochrome c. We observed slow oscillations (period of ~10 min) of cytochrome c reduction of an uncertain origin.

Absorbance data were normalized to spectra from cytochromes fully oxidized with antimycin A as a percentage of total cytochrome c (i.e., with KCN [Fig 4]). The resulting ratio of $[\text{Cyt}_2^{2+}]/([\text{Cyt}_2^{2+}] + [\text{Cyt}_2^{4+}])$, where $[\text{cyt}^{2+}]$ or $[\text{cyt}^{4+}]$ is the concentration of cytochrome C in the reduced oxidized state, is the fraction of total cytochrome in the reduced state and therefore represents a thermodynamic state variable that reflects one of two physiologically relevant driving forces of oxygen consumption.

Effect of glucose and glibenclamide. Perfused islets were subjected to a 60-min pulse of either 20 mmol/l glucose or 1 μmol/l glibenclamide. As expected, increasing glucose increased cytochrome c reduction, oxygen consumption, and insulin secretion (Fig. 5), all of which decreased toward the baseline after stimulation. In Krebs-Ringer buffer (KRB), both the baseline rate of oxygen consumption and cytochrome c reduction were lower than that observed in RPMI. Oxygen consumption reached a plateau 45–60 min after the switch to high glucose, whereas cytochrome c response reached a new steady state after only 15 min. When islets were perfused with a salt solution (KRB) containing 10 mmol/l glucose, glibenclamide increased oxygen consumption (0.26 ± 0.11 to 0.37 ± 0.16 nmol · min$^{-1}$ · 100 islets at peak$^{-1}$) and produced a 17.5-fold increase in insulin secretion but did not affect cytochrome c reduction (Fig. 6B). In substrate-rich media, glibenclamide increased insulin secretion threefold but had no effect on either cytochrome c reduction or oxygen consumption (Fig. 6A).

Calculating phosphorylation potential. Using the model derived by Wilson et al. (11) (see Appendix, Eq. 3), ATP/ADP/Pi was calculated from the oxygen consumption and cytochrome c data (Figs. 5 and 6). High glucose reversibly increased the phosphorylation potential (Fig. 7A and C). Equation 4 predicts that, in the absence of changes in cytochrome c reduction, increased oxygen consumption should decrease phosphorylation potential. In KRB containing 10 mmol/l glucose, glibenclamide decreased the phosphorylation potential by 40% (Fig. 7D). In culture media where oxygen consumption was threefold higher than in KRB, glibenclamide changed neither cytochrome c reduction nor oxygen consumption; hence, phosphorylation potential was unchanged (Fig. 7B). Thus, a metabolizable substrate increased the energy state, whereas a nonmetabolizable secretagogue decreased the energy state, but only in KRB.

DISCUSSION
To understand how insulin secretion depends on cytoplasmic ATP and ADP by $K_{\text{ATP}}$-dependent and -independent
Glucose increases cytochrome c reduction. The reduction of cytochrome c increased robustly in response to glucose, reflecting an increase in the glucose-stimulated production of NADH. At 3 mmol/l glucose, the reduction of cytochrome c was less in KRB than in RPMI (28 vs. 40%; Fig. 5), possibly reflecting reduced substrate availability in KRB. However, at 20 mmol/l glucose, cytochrome c reduction was similar in KRB and RPMI (~60%). The reduction at high glucose exceeded that reported for liver and kidney cells (21), possibly due to the unique effect of glucose to push the metabolic rate in islet cells. The tight relationship between glucose and cytochrome c is consistent with a mechanism involving transduction of the extracellular glucose signal by metabolism.

NADH and cytochrome c reduction are in equilibrium (23–25) so that kinetic responses of NADH can be related to changes of cytochrome c reduction. However, standard measures of NADH autofluorescence at 460 nm are confounded by contributions from NADPH and other intracellular constituents in multiple compartments. In addition, fluorescence measures of either NAD(P)H or mitochondrial membrane potential yield only relative change, whereas our cytochrome c reduction method yields a calibrated signal that represents a thermodynamic driving force for oxygen consumption.

Kinetics of oxygen consumption in response to glucose. Rates of oxygen consumption obtained depend on the method used. Whereas Cartesian divers record a nearly instantaneous response to glucose (26), continuous flow systems (22,27; the present study [Fig. 5]) record a slow (45- to 60-min) rise in oxygen consumption. To rule out an artifactual instrumentation delay, we determined that the oxygen sensor equilibrated in <10 min after an abrupt decrease in inflow oxygen tension; this must be an overestimate because metabolic poisons reduce islet oxygen consumption in <5 min (16). Thus, the gradual changes in oxygen consumption rate were observed 45–60 min after a glucose change. Although we cannot completely rule out baseline drifts after loading islets into our system, the characterization of our present system supports the validity of later more gradual changes in oxygen consumption and differences in the temporal response to glucose in KRB and RPMI.

Glibenclamide effects on oxygen consumption depend on media composition. As in other studies of oxygen consumption (15,22), glibenclamide increased oxygen consumption in KRB but not in RPMI. We suggest two explanations for this. When islets were perifused with a substrate-poor buffer (KRB) containing 10 mmol/l glucose, oxygen consumption was ~35% of that in substrate-rich RPMI media (Fig. 6), suggesting that glibenclamide-induced ATP consumption is a smaller fraction of the high ATP turnover seen in substrate-rich media. This would not be the case if glibenclamide also stimulates substrate usage as suggested (15). However, the inability of glibenclamide to enhance cytochrome c reduction or tolbutamide to change NADH levels (28,29) strongly argues against this. Because endoplasmic and membrane calcium transport consume ATP, differences in oxygen consumption may depend on the difference in KRB and RPMI calcium levels (2.6 vs. 0.42 mmol/l). Whatever its cellular effects, we simply used glibenclamide as a means to perturb and validate our model of the relationship between cytochrome c reduction, oxygen consumption, and phosphorylation potential.

Determining ATP/ADP/Pi. It is important to recognize that despite the central importance of adenine nucleotides in mediating insulin secretion, direct measures of the thermodynamically relevant free ATP and ADP are not available (6,30). The most direct approach has been to rapidly freeze isolated islets and measure their total cellu-

![Image](image-url)
lar ATP and ADP content. This provides good estimates of free ATP but not of free ADP because 95% of cellular ADP is bound or sequestered (30,31). Accordingly, only small increases in ATP/ADP have been seen in response to glucose (32,33). By identifying a large stable pool of nucleotides in islet secretory granules, an approximate fourfold increase in ATP/ADP was seen in response to glucose stimulation (34,35). However, even these estimates are ~10-fold higher than estimates of free ADP level based on calculations that assume that the creatine kinase reaction that buffers ATP and ADP levels is in equilibrium (30,31).

Given the importance of free ATP and ADP to islet cells and the difficulty of measuring free ADP, we have suggested a new, albeit indirect, method for a continuous noninvasive measure of these key metabolic variables. Our analysis relies on the kinetic model derived by Wilson et al. (11) that describes oxygen consumption in terms of sub-
strate pressure (cytochrome c reduction) and energy demand as reflected by ATP/ADP/Pi. The system includes electron transfer from NADH to oxygen via complexes I, III, and IV in the electron transport chain, and the creation of the transmembrane proton gradient that ultimately phosphorylates ADP by F0F1 ATPase. The complexity of these biochemical and biophysical processes was reduced according to three observations. First, the catalytic capacities of the electron transport chain reactions from NADH to cytochrome c (sites 1–3) are nearly at equilibrium (23–25) and therefore are unlikely to regulate pathway flux. Similarly, the capacity of the F0F1 ATPases is sufficiently high that the conversion of biophysical energy in the proton gradient to chemical energy in ATP also operates at near-equilibrium. In contrast, the cytochrome c oxidase reaction that transfers electrons to O2 to produce H2O is nearly irreversible and not at equilibrium and thus is a candidate control site. Second, the flux of electrons

FIG. 6. Effect of glibenclamide on oxygen consumption rate (OCR), cytochrome c reduction, and insulin secretion rate (ISR). A: Islets were perifused with substrate-rich media as in Fig. 5A, except that glucose was 10 mmol/l throughout the experiment and 1 μmol/l glibenclamide was added for 60 min. A robust increase in insulin secretion was observed, but no significant change in oxygen consumption or cytochrome c reduction occurred. Each point represents the average of three separate experiments; the SE averaged for all data points for cytochrome c reduction was 3.3% and for oxygen consumption rate was 0.079 mmol · min⁻¹ · 100 islets⁻¹. B: Islets were perifused with a salt solution (KRB) as in Fig. 5B containing 10 mmol/l glucose as the only nutrient, and the effects of a pulse of 1 μmol/l glibenclamide was observed. Oxygen consumption (about one-third of that in the presence of substrate-rich media) increased by 60%, whereas little effect of glibenclamide on cytochrome c reduction was observed. Each point represents the average of four separate experiments; the SE averaged for all data points for cytochrome c reduction was 10.1% and for oxygen consumption rate was 0.095 mmol · min⁻¹ · 100 islets⁻¹. ISR, insulin secretion rate.
pushes the cytochrome oxidase reaction farther from the equilibrium so that spectroscopic monitoring of the ratio of reduced to oxidized cytochrome c provides a noninvasive measure of this driving force. Third, Wilson's steady-state (but nonequilibrium) kinetic model of the cytochrome oxidase reaction yields an algebraic expression (see appendix) that can predict the ATP/ADP/P_i ratio from the measured oxygen consumption rate and reductive state of cytochrome c.

**Effect of glucose and glibenclamide on ATP/ADP/P_i.** Increasing glucose resulted in increased cytochrome c reduction and oxygen consumption (Fig. 5) and calculated ATP/ADP/P_i levels (Fig. 7). In contrast, a non-nutrient stimulator of insulin secretion, glibenclamide, increased oxygen consumption in islets perifused in KRB ~50%, similarly to Panten et al. (22), but had little effect on cytochrome c reduction (Fig. 6A). Calculated ATP/ADP/P_i levels decreased in response to glibenclamide and presumably provided the driving force for the increased oxygen consumption. Thus, increased substrate pressure elicited by glucose resulted in an increased energy state, and an agent that induced the increased work of ion transport and insulin secretion in the absence of fuel decreased the energy state. Comparison of the magnitude of the calculated changes with direct measurements of phosphorylation potential are not possible because of the lack of a direct method to measure free ADP (6). However, 31P–nuclear magnetic resonance provides noninvasive estimates of phosphocreatine (PCr) and inorganic phosphate (P_i) in live cells (ADP levels are below the detection limit), and PCr/P_i is a measure of energy state that is correlated with the ATP/ADP/P_i. In studies of β-HC9 cells loaded with creatine, the ratio of PCr/P_i increased fourfold in response to glucose and decreased by 50% in response to gliben-
clamide (15), and studies measuring the effect of glibenclamide on total ATP/ADP in islets have reported 20–40% decreases (32,33). The directionality of the changes in energy state is consistent with the calculations in our study. The implication of this scenario is that phosphorylation potential is determined by substrate pressure and the mitochondrial coupling mechanisms rather than by the multifarious processes that use ATP.

**Summary and conclusions.** We have combined measures of cytochrome c reduction and oxygen consumption to calculate changes in phosphorylation potential based on a kinetic model developed by Wilson et al. (11). Our results support the dual control of islet mitochondrial respiration by substrate availability and energy demand and offer a new method for continuous noninvasive measures of islet cell phosphorylation potential, a critical metabolic variable that controls insulin secretion by K\textsubscript{ATP}-dependent and -independent mechanisms.

**ACKNOWLEDGMENTS**

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Rats used in this study were kindly provided by Dr. Åke Lernmark.

**APPENDIX**

Minimal model of coupled electron transport and oxidative phosphorylation based on a five-step mechanistic description of the kinetic properties of cytochrome c oxidase

**Reduction of model complexity by focusing on rate-limiting steps.** Wilson et al. (11) derived a minimal model that describes oxygen consumption in terms of substrate pressure (cytochrome c reduction) and energy demand as reflected by ATP/ADP/P\textsubscript{i}. The system transfers electrons from NADH to oxygen via complexes of the electron transport chain, the proton gradient, and ultimately the phosphorylation of ADP by F\textsubscript{0}F\textsubscript{1} ATPase. However, the remarkable reduction in complexity of the model that was achieved was based on the experimental findings that the reactions in the electron transport chain from NADH to cytochrome c (sites 1–3) are in near-equilibrium (23–25), where the overall equation is

\[
\text{NADH} + 2\text{cyt}^3 + 2\text{ADP} + 2\text{P}, \leftrightarrow \text{NAD}^+ + 2\text{cyt}^2 + 2\text{ATP}
\]

(1)

Thus, phosphorylation of ADP, generation of proton gradients, and transport of adenine nucleotides across the mitochondrial membrane are fast, as is the transfer of electrons from NADH to cytochrome c, so these steps cannot be rate-controlling. The near-equilibrium status of Eq. 1 implies that respiratory control must reside in cytochrome oxidase as the rate-limiting step in oxygen consumption (11). Therefore the kinetics of coupled mitochondrial electron transport and oxidative phosphorylation can be represented by the factors controlling cytochrome oxidase. The thermodynamic driving force for cytochrome oxidase is determined by the reduced to oxidized ratio of any intermediates from NADH to cytochrome c. Because cytochrome c is the only intermediate where this ratio can be readily and noninvasively measure, the rate of oxygen consumption was modeled in a five-step mechanism describing the transfer of electrons from cytochrome c to oxygen via cytochrome oxidase.

The uncoupling effects of uncoupling protein 2, which may govern the levels of ATP/ADP/P\textsubscript{i} (36,37), is not considered in this analysis. Neglecting to consider the dissipation of energy as heat will cause the prediction of ATP/ADP/P\textsubscript{i} from the model to be overestimated. However, the fact that the directionality and order of magnitude of the changes in the phosphorylation potential measured in this study (see DISCUSSION) were similar to those found in other studies indicates that uncoupling protein 2 may modify but not override the basic mitochondrial coupling mechanisms and justifies this simplification for this initial test of the hypothesis in islets. It is also assumed that all oxygen consumption is due to respiration, which is supported by studies showing that inhibitors of electron transport resulted in a 70–100% inhibition of oxygen consumption (27).

**Derivation of the mathematical model.** In Wilson’s reaction scheme (11), electrons are first transferred to a copper molecule that is bound to the cytochrome a\textsubscript{3} subunit of cytochrome c oxidase (reaction 1). The reduction of the Cu atom “opens” the oxygen binding site, allowing oxygen to diffuse in and complex with the Cu (reaction 2). The oxygen complex can rearrange and bind to reduced cytochrome a\textsubscript{3} (reaction 3). The two partially reduced states can accept one reducing equivalent from cytochrome c and then transfer two equivalents to the oxygen moiety to create bound-peroxide intermediate (reactions 4a and 4b). The cycle is completed by the acceptance of two more electrons and release by water (reaction 5).

The corresponding differential equations for the chemical reactions were solved for steady-state conditions, and an algebraic relation between oxygen consumption rate (\(v_{\text{OCR}}\)), cytochrome c reduction, and phosphorylation potential was derived (Eq. 2, which is equivalent to Eqs. 3–5 in the study by Wilson et al. [11]). Consistent with classic observations of respiratory control, oxygen consumption is increased by both the supply of electrons traversing the electron transport chain as well as the demand for ATP mediated by decreased ATP/ADP/P\textsubscript{i} levels.

\[
v_{\text{OCR}} = \frac{k_1[\text{cyt}^2]^a_{\text{ST}}}{1 + \beta + \frac{1}{K_v} \left(\frac{[\text{cyt}^2]}{[\text{ATP}]}\right)^{0.5} \left(\frac{[\text{ADP}]}{[\text{P}]}\right)}
\]

where

\[
\beta = \frac{k_1(1 + K_v)}{k_4b + K_3k_{4a}}
\]

(2)

Small \(k\)’s represent rate constants, and large \(K\)’s represent thermodynamic equilibrium constants of the reactions shown. \([\text{cyt}^2]\) is the total concentration of cytochrome a\textsubscript{3}.

The model, rewritten as a function determining ATP/ADP/P\textsubscript{i} becomes

\[
\frac{[\text{ATP}]}{[\text{ADP}][\text{P}]} = \frac{k_a [\text{cyt}^2]^a_{\text{ST}}}{100v_{\text{OCR}} - 1 - \beta K_v \left(\frac{[\text{cyt}^2]}{100 - [\text{cyt}^2]}\right)^{0.5}}
\]

(3)

where \(c_{\text{P}}\) is the total concentration of cytochrome c and [cyt\textsubscript{red}] is the percentage of cytochrome c in the reduced
TABLE 1
Parameter values used to calculate ATP/ADP/Pi

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
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</tr>
<tr>
<td>E_{max} (V)</td>
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</tr>
<tr>
<td>E_{maxCu} (V)</td>
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</tr>
<tr>
<td>k_1 (µM⋅mol⁻¹⋅min⁻¹)</td>
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</tr>
<tr>
<td>k_2 (µM⋅mol⁻¹⋅min⁻¹)</td>
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<tr>
<td>k_3</td>
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<tr>
<td>k_{kappa} (µM⋅mol⁻¹⋅min⁻¹)</td>
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</tr>
<tr>
<td>k_{alpha} (µM⋅mol⁻¹⋅min⁻¹)</td>
<td>600</td>
</tr>
<tr>
<td>α_{cT} (µmol/l)</td>
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</tr>
<tr>
<td>c_{T} (µmol/l)</td>
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</tr>
</tbody>
</table>

Total concentrations of cytochrome c and α_{k} were estimated by fitting data as described in the APPENDIX. All other parameters were obtained by Wilson et al. (11) in studies of pigeon heart mitochondria.

state (see RESEARCH DESIGN AND METHODS). Here it is assumed that the magnitude of the second derivative of absorption is linearly proportional to the concentration of cytochromes such that cyt_{red} = 100 is linearly proportional to the concentration of cytochrome. That the magnitude of the second derivative of absorption state (see RESEARCH DESIGN AND METHODS). Here it is assumed that the magnitude of the second derivative of absorption is linearly proportional to the concentration of cytochromes such that cyt_{red} equals 100 times [cyt c^2]/[cyt c^2] + [cyt c^3]. Values of rate and equilibrium constants used in the calculations in this article were those estimated from experiments on pigeon heart mitochondria (11) and as was the case for pigeon heart mitochondria. A value of &cT was not known a priori. Using a value for ATP/ADP/Pi, (0.02) obtained in islets (30) and values for oxygen consumption rate and cytochrome c reduction, Eq. 3 was solved for c_{T} to yield 3.5 µmol/l, which was used for all calculations.

REFERENCES