Maximum oxidative phosphorylation capacity of the mammalian heart

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Mootha, Vamsi K., Andrew E. Arai, and Robert S. Balaban. Maximum oxidative phosphorylation capacity of the mammalian heart. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H769–H775, 1997.—It is difficult to estimate the maximum in vivo aerobic ATP production rate of the intact heart independent of limitations imposed by blood flow, oxygen delivery, and maximum mechanical power. This value is critical for establishing the kinetic parameters that control oxidative phosphorylation, as well as for providing insights into the limits of myocardial performance. In this study, the maximum ADP-P_i-driven heart mitochondrial respiratory rate ($M\dot{V}O_{2mito}$) was determined with saturating levels of oxygen, substrates, and cofactors at 37°C. These rates were normalized to cytochrome a_1 a_3 (cytochrome oxidase; Cyt *a*) content. To extrapolate this rate to the intact heart, the Cyt *a* content of the myocardium (nmol Cyt *a*/g wet wt myocardium) was determined in the same hearts. The maximum ADP-P_i-driven mitochondrial respiratory rates were 676 ± 31 and 665 ± 65 nmol $O_2 \cdot min^{-1} \cdot$nmol Cyt a^{-1} in the dog and pig, respectively. The Cyt *a* content in the two species was 43.6 ± 2.4 and 36.6 ± 3.1 nmol Cyt *a*/g wet wt, respectively. With these values, the $M\dot{V}O_{2mito}$ was calculated to be 29.5 (dog) and 24.3 (pig) μmol $O_2 \cdot min^{-1} \cdot$g wet wt myocardium^{-1}. Comparison with in vivo studies shows that the exercising heart can utilize 80–90% of its maximum oxidative capacity, implying there is little aerobic ATP production reserve in the mammalian heart.

canine; swine; adenosine diphosphate; inorganic phosphate; myocardial oxygen consumption; oxidative phosphorylation; mitochondria; cytochrome *a*; symmorphosis

THE MAJORITY OF myocardial ATP is generated in the mitochondria by oxidative phosphorylation. Thus myocardial O_2 consumption ($M\dot{V}O_2$) provides a good estimate of the myocardial ATP production rate. A dog's resting $M\dot{V}O_2$ is in the range of 4.0–5.5 μmol $O_2 \cdot min^{-1} \cdot$g wet wt^{-1}, and the oxygen extraction fraction is ~75%. Near-maximal whole body exercise can elicit a five- to sixfold increase in $M\dot{V}O_2$ and an increase in oxygen extraction to 96% (32). It is unclear what aspect of cardiac energetics is contributing to the performance limits at maximum workloads. Potential limiting parameters include blood flow and oxygen delivery, inherent mitochondrial ATP production capacity, and/or limits in actomyosin adenosinetriphosphatase (ATPase) activity or power. The inherent mitochondrial ATP production capacity refers to the maximum $M\dot{V}O_2$ ($M\dot{V}O_{2mito}$) of the heart in the absence of oxygen or blood flow limitations. The purpose of this study was to estimate the $M\dot{V}O_{2mito}$ of the dog myocardium where maximum estimates of in vivo $M\dot{V}O_2$ are available. Data were also collected in the pig for comparison purposes.

$M\dot{V}O_{2mito}$ is a critical parameter in establishing the kinetic impact of parameters controlling oxidative phosphorylation, as well as in determining the potential role of metabolism in limiting myocardial mechanical performance. However, the parameter has been difficult to estimate in vivo or in perfused hearts since substrate delivery, oxygen diffusion, and actomyosin ATPase are potentially limiting. Other studies have extrapolated $M\dot{V}O_{2mito}$ from mitochondrial enzyme activities or from uncoupled myocyte respiratory rates, but these estimates have fallen far short of observed exercise $M\dot{V}O_2$ values (8).

In the present study, we have utilized intact heart tissue and isolated heart mitochondria to estimate $M\dot{V}O_{2mito}$. We determined mitochondrial respiratory rates of pig and dog heart mitochondria, normalized to cytochrome a_1 a_3 (cytochrome oxidase; Cyt *a*) content, under conditions optimized to yield maximum ADP-P_i-driven ATP production rates. We simultaneously determined the Cyt *a* content of the myocardium utilizing an optical technique that corrects for contaminating myoglobin and hemoglobin. The maximum mitochondrial respiratory rate and myocardial Cyt *a* content, taken together, provide an estimate of $M\dot{V}O_{2mito}$. Comparison with in vivo exercise studies (1, 15, 18, 32) suggests that $M\dot{V}O_2$ approaches $M\dot{V}O_{2mito}$ during near-maximal whole body exercise.

MATERIALS AND METHODS

Animal heart isolation and perfusion. Pig and dog hearts were harvested from chloralose (100 mg/kg iv) -anesthetized and heparinized (10,000 units iv) animals after a midline thoracotomy. The freshly isolated heart was weighed and then perfused via the aorta in a retrograde fashion with 400 ml of ice-cold isolation buffer [0.28 M sucrose, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 0.2 mM EDTA, pH 7.2] to remove blood and reduce free calcium for mitochondrial isolation. The perfused heart was also weighed, and the left ventricle was dissected free of fat, large vessels, and the right ventricular free wall. Approximately 5 g of myocardium were used for tissue homogenization and Cyt *a* assay. An additional 5 g were used for wet weight-to-dry weight ratio determination, while the remaining tissue was used for mitochondria isolation.

Isolation of cardiac mitochondria. The sections of the left ventricle (4–5 g) were minced in 20 ml isolation buffer. Trypsin (2.5 mg) was then added, and the tissue was incubated for 15 min at 4°C. The digestion was stopped by adding 20 ml of isolation buffer with 1 mg/ml bovine serum albumin (BSA) and 13 mg trypsin inhibitor. The suspension was decanted, and the remaining tissue was resuspended in 20 ml of ice-cold isolation buffer with 1 mg/ml BSA. The tissue was homogenized with a loose-fitting Teflon homogenizer (2 times) followed by a tight-fitting Teflon pestle (5 times). The homog-

enate was centrifuged at 600 *g* for 10 min at 4°C, and the supernatant was decanted and centrifuged at 8,000 *g* for 15 min. The buffy coat was removed, and the pellet was resuspended in 20 ml of ice-cold isolation buffer with 1 mg/ml BSA. The wash-and-centrifugation step was repeated twice, once in the presence of 1 mg/ml BSA and the final time in the absence of BSA. The final pellet was resuspended in 137 mM KCl, 10 mM HEPES, and 2.5 mM $MgCl_2$ at pH 7.2 (experimental buffer) and stored on ice. Mitochondrial protein concentration was determined with a Bio-Rad reagent assay, with BSA serving as the standard.

Respiratory rate and NADH fluorescence measurements. Experimental buffer containing 0.2 mg mitochondria, 6 mM K_2HPO_4, and substrate (1.2 ml total volume) was added to an oxygen chamber temperature controlled at 37°C. State 3 respiration was elicited by the addition of a known amount of ADP to the chamber. The rates of O_2 consumption following the addition of ADP (state 3) and the subsequent state 4 were monitored with a Clark-type electrode, which was calibrated to room air using a solubility coefficient of 199 nmol O_2/ml at 37°C and 230 nmol O_2/ml at 27°C. For uncoupled rates, 250 nM carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) was titrated to state 4 mitochondria to obtain a maximum rate. The state 3 respiratory rate per milligram mitochondrial protein was constant as a function of mitochondrial protein content in the chamber below concentrations of 0.6 mg/ml at 37°C. These data suggest that no oxygen diffusion limitations in the chamber or electrode occurred below 0.6 mg/ml.

The redox state of the pyridine dinucleotide was simultaneously monitored using a Perkin-Elmer LS50B fluorimeter (excitation 360 nm, emission 460 nm) coupled to the chamber using the Perkin-Elmer external fiber-optic system. The inner portion of the chamber was optically black. The fluorimeter was calibrated daily using known additions of free NADH to buffer solutions. Data from the Clark-type electrode and the fluorimeter were directed to an analog-to-digital converter and recorded using Analog Connection WorkBench (Strawberry Tree, Sunnyvale, CA) software on an Apple Macintosh system.

Mitochondrial and tissue Cyt a assay. Mitochondrial Cyt *a* content was determined spectrophotometrically using the method of Williams (34) as recently modified (3). Briefly, isolated mitochondria were solubilized in a 2% solution of Triton X-100, and an oxidized-reduced spectrum was obtained by reducing one sample with sodium hydrosulfite. With this isolation procedure, Cyt *a* was oxidized fully in the absence of any oxidizing agents (3). The Cyt *a* content was determined using the 605- to 630-nm wavelength pair, using a millimolar extinction coefficient of 12 (34).

Whole myocardium Cyt *a* content was determined from tissue homogenates using a spectrophotometric method that eliminates optical artifacts from myoglobin and hemoglobin using cyanide as a reducing agent (3).

RESULTS

Mitochondria characterization. Our isolation procedure resulted in a high yield of tightly coupled mitochondria. Typically, we obtained 1–2 mg mitochondrial protein/g wet wt myocardium. Respiratory control ratios (state 3 to state 4) ranged between 8 and 15 at 37°C, and the ADP-to-O ratio for mitochondria respiring on 5 mM glutamate-malate was 2.9 ± 0.2 (n = 7). Electron microscopy of the isolated mitochondria showed a very pure isolation, with little or no evidence of contamination by other organelles (data not shown).

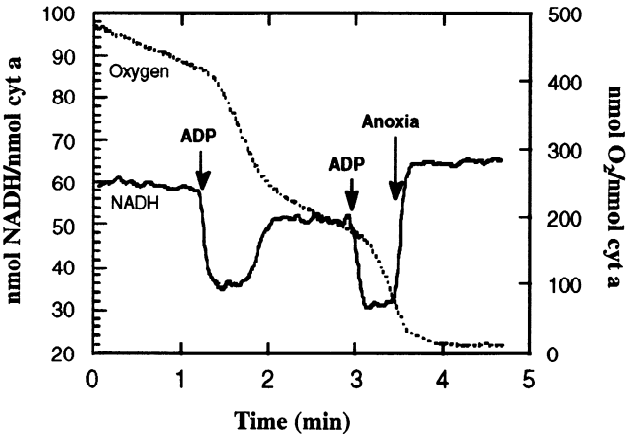

Fig. 1. Example of O_2 consumption and NADH fluorescence tracing. Isolated pig heart mitochondria (0.2 mg/ml) were allowed to respire on 6 mM glutamate-malate in the presence 6 mM P_i. Arrows show additions of 1 μmol ADP, which leads to a decline in NADH fluorescence and an increase in respiratory rate to the maximal attainable value. We assumed an oxygen solubility of 199 nmol O_2/ml at 37°C; Cyt *a*, cytochrome a_1 a_3 (cytochrome oxidase).

There was no increase in O_2 consumption on the addition of NADH or ascorbate, providing further evidence that the mitochondria were intact. A typical O_2 consumption and NADH fluorescence trace is presented in Fig. 1.

Maximum mitochondrial respiratory rate. To achieve the maximum ADP-P_i-driven respiratory rate of these mitochondria, it was necessary to optimize the incubation conditions. First, we evaluated the role of carbon fuel sources on the rate of state 3 respiration and pyridine nucleotide reduction. We screened a variety of substrates (glutamate, glutamate-malate, pyruvate, pyruvate-malate, acetoacetate, β-hydroxybutyrate, citrate, citrate-malate, isocitrate, isocitrate-malate), as well as multiple combinations and all these substrates together. We found that glutamate-malate yielded respiratory rates as high as any substrate combination in both pig and dog heart mitochondria. Figure 2 shows a

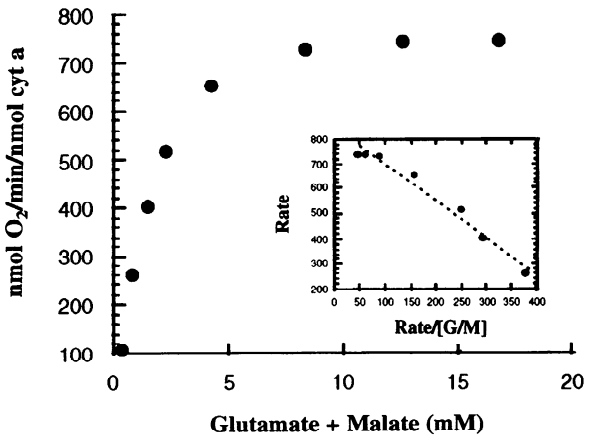

Fig. 2. Glutamate-malate (G/M) dose-response curve. Isolated mitochondria (0.2 mg/ml) were allowed to respire on varying concentrations of substrate in the presence of 6 mM P_i. State 3 rate, elicited by the addition of 500 μmol ADP, is plotted against G/M concentration ([G/M]). *Inset*, Eadie-Hofstee plot (r^2 = 0.96) of the same data. Michaelis constant (K_m) for G/M is calculated to be 1.5 mM.

respiratory rate-response curve for increasing glutamate-malate, which resembles a Michaelis-Menten relationship. An Eadie-Hofstee plot (Fig. 2, *inset*) shows that the Michaelis-Menten constant (K_m) for glutamate-malate is 1.5 mM. The pattern of substrate utilization (rapid utilization of glutamate and much slower oxidation of ketone bodies) was similar to previous reports (25). A basic linear relationship between the level of NADH and the state 3 respiratory rate with different substrates was observed (Fig. 3), as previously reported in liver mitochondria (19).

Next, we optimized the buffer conditions for ADP and P_i concentrations. We performed ADP- and P_i-response curves (Fig. 4) in the presence of 6 mM glutarate-malate to ensure that, in ensuing experiments, the state 3 rate was not limited by ADP or P_i. The K_m for P_i, determined by performing an Eadie-Hofstee plot (r^2 = 0.98) of the data shown in Fig. 4*B*, was 1.0 mM. The affinity of ADP was not calculated, since the consumption of ADP in these experiments significantly altered the ADP concentration ([ADP]) in the bath. However, a maximum rate was obtained with [ADP] values >0.4 mM. Based on these results, we conducted all our mitochondrial respiratory rate measurements in the presence of 6 mM glutarate-malate, 6 mM P_i, and 0.5 mM ADP.

We made further efforts to ensure that the isolated mitochondria were respiring at maximal rates. The adenine nucleotide translocase has been reported to preferentially transport ADP produced by creatine kinase over exogenously added ADP (22). Addition of 20 mM creatine and 6 mM ATP to our experimental buffer augmented state 4 respiration without a significant increase in the state 3 rate (data not shown). We also attempted to activate respiration with extramitochondrial calcium (23) but found that the residual calcium concentration prevailing in our incubation medium resulted in maximum calcium stimulation (data not shown). Because cofactor nonesterified CoA (CoASH) could be limiting in the trichloroacetic acid cycle, we added pantothenic acid, cysteine, and carnitine in an effort to regenerate CoASH, according to Russell and Taegtmeyer (28). However, these additions, too, had no effect on the state 3 respiratory rate. Davis and Lumeng (6) reported that aspartate aminotransferase and malic dehydrogenase, when added to isolated liver mitochondria, could shift the pyridine nucleotides to a more reduced state. Because we have noted a linear relationship between the state 3 respiratory rate and NADH fluorescence (Fig. 3), we reasoned that the addition of these two enzymes could augment respiration. However, addition of these enzymes to the mitochondria respiring on glutarate-malate led to a decreased state 3 rate and NADH level (data not shown).

We used the optimal buffer conditions to determine the resting state 4, coupled state 3, and uncoupled respiratory rates at 37°C. Maximum respiratory rates were normalized to mitochondrial Cyt *a* content (Table 1). Data were also collected at 27°C to determine the effect of temperature on this maximum rate, as well as aid in the comparison with other studies. The effect of a

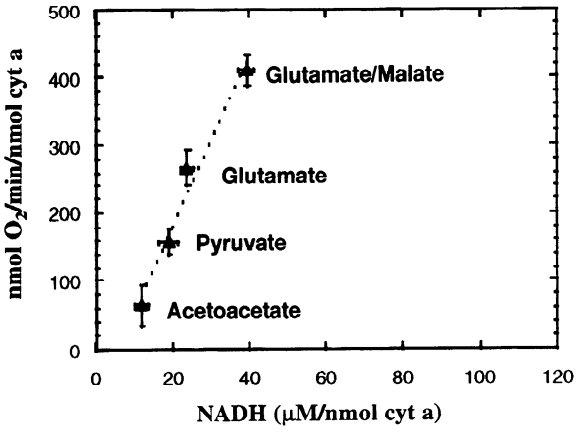

Fig. 3. State 3 respiratory rate vs. NADH fluorescence for dog heart mitochondria respiring on 4 different substrates. Results are means ± SE of 3 separate experiments. Correlation coefficient is r = 0.96.

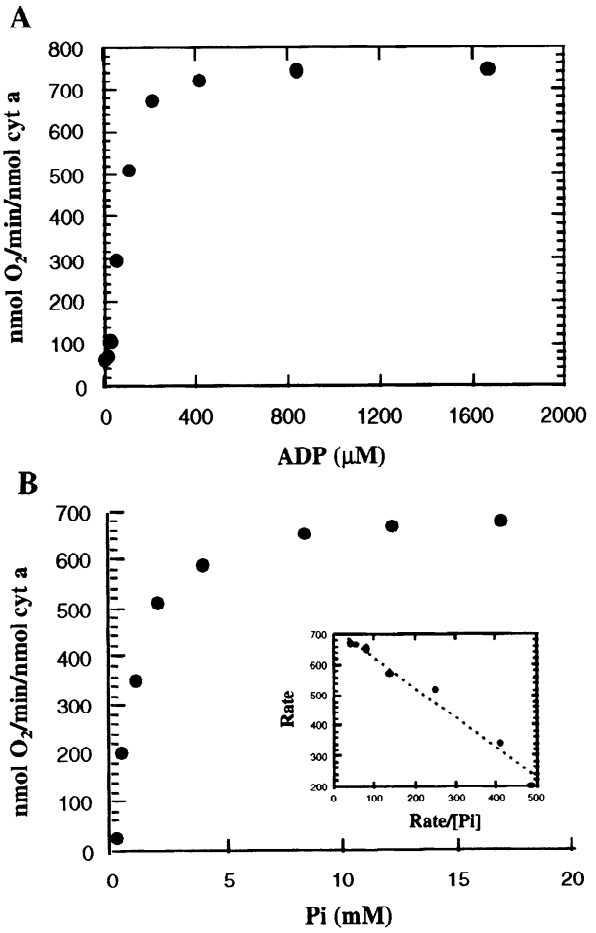

Fig. 4. ADP and P_i dose-response curves. Dose-response curves were performed to ensure that ADP-P_i is not limiting respiratory rate of mitochondria. State 3 rates are plotted vs. concentration of ADP or P_i ([P_i]). *A*: dog heart mitochondria (0.2 mg/ml) were allowed to respire in experimental buffer (see text) containing 6 mM glutamate-malate and 6 mM P_i. State 3 was elicited by addition of varying concentrations of ADP. *B*: pig heart mitochondria (0.2 mg/ml) were allowed to respire in experimental buffer containing 6 mM glutamate-malate and varying concentrations of P_i. State 3 respiration was elicited by addition of 500 μM ADP. *Inset*, Eadie-Hofstee plot (r^2 = 0.98) of the same data. K_m for P_i is calculated to be 1.0 mM. No significant difference was observed between dog and pig heart mitochondria.

Table 1. *Mitochondrial respiratory rates*

	n	State 4	State 3	Uncoupled	Q_{10}
Pig	5	46 ± 9	665 ± 65	685 ± 88	1.7 ± 0.2
Dog	6	74 ± 6	676 ± 31	705 ± 41	1.8 ± 0.2

Values are means ± SE; n, no. of animals. Respiratory rates are nmol $O_2 \cdot min^{-1} \cdot$ nmol cytochrome oxidase^{-1}. Q_{10}, effect of a 10° rise in temperature rate, was calculated from increase in state 3 rate observed in the same set of experiments between 27 and 37°C.

10° rise in temperature on rate was found to be similar in pig and dog heart mitochondria at ~1.7 (Table 1).

Myocardial Cyt a content. Myocardial Cyt *a* content was determined using an optical technique that corrects for contaminating myoglobin and hemoglobin. This method relies on the selective reduction of cytochrome oxidase by cyanide, which does not affect the myoglobin or hemoglobin absorbance spectra (3). Other techniques that measure Cyt *a* activity are much more prone to error, since enzymatic activity is dependent on buffer conditions (3, 4). Wet weight data were collected immediately after removal of the heart before perfusion and/or corrections were made for the increase in water volume (~10%) that occurred with the cold saline perfusion. Myocardial Cyt *a* content in the pig and dog heart are shown in Table 2, along with mitochondrial Cyt *a* content.

Estimation of $\dot{M}VO_{2mito}$. $\dot{M}VO_{2mito}$ was estimated by taking the maximum mitochondrial respiratory data in Table 1 and multiplying this value by the Cyt *a* values in Table 2. This calculation resulted in the $\dot{M}VO_{2mito}$ values presented in Table 3 expressed as micromoles O_2 per minute per gram wet weight at 37°C. These values can be directly compared with the in vivo data from the literature as shown in Table 4. A comparison of the $\dot{M}VO_{2mito}$ data (Table 3) with the in vivo data reveals that, in dogs, the maximum in vivo rates approach 80–90% of the calculated $\dot{M}VO_{2mito}$. No in vivo exercise data are available in the pig, only apparently submaximal work induced by pharmacological stimuli and aortic clamping in the anesthetized animal (Table 4).

DISCUSSION

These data suggest that, when the canine heart is stressed to near maximum workloads, the mitochondrial ATP production rate approaches 90% of the maximum rate. These data suggest that there is little excess aerobic ATP synthesis capacity in the mammalian heart in vivo.

The maximum cardiac respiratory rate in vivo has been determined using several approaches. Studies of exercising dogs (Table 4) have measured coronary blood flow and arteriovenous O_2 differences to directly determine the $\dot{M}VO_2$ during maximal workloads. Elzinga and van der Laarse (8) estimated $\dot{M}VO_2$ by reviewing coronary blood flow studies and by using established oxygen extraction fractions to deduce a maximal value of 24–32.1 μmol $O_2 \cdot min^{-1} \cdot g^{-1}$. Karas et al. (16) assumed a fixed efficiency of 20% and used exercise cardiac output data to estimate the maximum $\dot{M}VO_2$ to be 25.4 μmol $O_2 \cdot min^{-1} \cdot g^{-1}$. However, these studies did not measure mitochondrial oxidative capacity since the oxygen delivery could be limited by coronary blood flow or the actual limits of ATPase activity in the myofilaments. Hence, these studies would be expected to underestimate the true oxidative capacity. Elzinga and van der Laarse (8) reviewed the use of uncoupled isolated myocytes in the estimation of maximum $\dot{M}VO_2$ and found that the technique results in no more than 14.3 μmol $O_2 \cdot min^{-1} \cdot g^{-1}$, much below measured in vivo values (Table 4) or the maximum rates of uncoupled or ADP-P_i-driven respiration in the current study. He attributed the low maximum rate to be due to myocyte damage during isolation. Bussemaker et al. (4) attempted to use succinate dehydrogenase activity as a measure of aerobic capacity; however, they found significant discrepancies in the activity of the enzyme depending on isolation conditions. Estimates of mitochondrial respiratory capacities have been made for the in vitro perfused rat heart based on cytochrome determinations or mitochondrial volume (20, 24). These studies came to similar conclusions with regard to the perfused rat heart respiratory rates approaching $\dot{M}VO_{2mito}$.

In the present study, we have used isolated mitochondria to estimate $\dot{M}VO_{2mito}$. This strategy provides an advantage over individual enzyme activities in that the ATP-producing machinery is still intact. Furthermore, the approach is appealing since substrate delivery to

Table 2. *Cytochrome a content*

	n	Mitochondria, nmol Cyt *a*/mg	Myocardium, nmol Cyt *a*/g wet
Pig	4	1.00 ± 0.10	36.6 ± 3.1
Dog	4	1.27 ± 0.14	43.6 ± 2.4

Values are means ± SE; n, no. of animals. Cyt *a*, cytochrome $a_1 a_3$. Wet weight-to-dry weight ratio for left ventricle was found to be 4.62 ± 0.45 (n = 8).

Table 3. *Estimates of myocardial and mitochondrial respiratory capacities*

	n	Coupled	Uncoupled
Pig	5	24.3	25.1
Dog	6	29.5	30.6

n, No. of animals. Values are in μmol $O_2 \cdot min^{-1} \cdot$ g wet wt^{-1}.

Table 4. *In vivo maximum $\dot{M}VO_2$*

Challenge	Resting $\dot{M}VO_2$, μmol $O_2 \cdot min^{-1} \cdot$ g wet wt^{-1}	Challenge $\dot{M}VO_2$, μmol $O_2 \cdot min^{-1} \cdot$ g wet wt^{-1}	Reference
Dog			
Exercise		24	18
Exercise	4.0	25.4	32
Exercise + α-blocker	5.44	24.9	15
Exercise (control)	6.7	19	1
Exercise (LVH)	9.2	28	1
Pig			
Constriction + dobutamine	5.0	15.0	21

Pig challenge was open-chest preparation; 42.9 μmol O_2/ml gas at 37°C was assumed where necessary. $\dot{M}VO_2$, myocardial O_2 consumption; LVH, left ventricular hypertrophy.

the mitochondria can be controlled and the viability of the mitochondria can be monitored using conventional indexes of coupling, such as the acceptor control ratio and the ADP-to-O ratio. The ADP-to-O ratio of ~3 found in this study is similar to that observed in the intact heart (30).

The maximum rate of mitochondrial respiration is dependent on carbon substrate source and the level of state 3 NADH concentration. The linear relation between NADH and respiratory rate suggests that NADH has not reached saturating levels, and any increase in state 3 NADH level will increase the respiratory rate. This result also suggests that other potential rate-limiting steps, such as the adenylate translocase and oligomycin-sensitive ATPase of inner mitochondrial membrane (F1-ATPase) activity, do not significantly contribute to this maximum rate since the state 3 rate seems to depend on the NADH level alone. Therefore, the capacity of intermediary metabolism to produce NADH is apparently the rate-limiting step near maximum ATP synthetic rates in the heart. This notion is supported by the modest increase in respiration observed with the uncoupler that effectively bypasses the translocase and F1-ATPase (Table 1). The importance of substrate oxidation in the rate limitation of oxidative phosphorylation has also been reported by others in heart mitochondria (7, 9).

The major weakness of this study is the possibility that in vivo mitochondria are capable of respiring at rates higher than those observed in our in vitro preparation. Efforts were made to optimize the buffer conditions so as to obtain maximal respiration. However, the strong dependence of the respiratory rate on NADH generation leaves open the possibility that a more efficient NADH delivery system exists in vivo than we can replicate in vitro. In addition, the presence of severely damaged mitochondria resulting in nonfunctional cytochrome chains could also contribute to a lower turnover number. However, no evidence for a significant pool of lysed or damaged mitochondria was found in this study using standard approaches. Because of the difficulties in obtaining a true $M\dot{V}O_{2mito}$ in intact hearts or cells in the absence of oxygen limitations or tissue damage (8), we believe that using isolated mitochondria rates is a reasonable estimate of $M\dot{V}O_{2mito}$.

Another limitation of this approach is the use of instrumented animal data to estimate intact heart maximum O_2 consumption rates. All of these in vivo data rely on heavily instrumented animals under controlled conditions, which may be subject to underestimates and experimental variability. For example, Huang and Feigl (15) had some animals that approached 43 μmol $O_2 \cdot min^{-1} \cdot g^{-1}$, well above the mean presented in Table 4. Whether these high values represented a true maximum respiratory rate, biological variability, or experimental variation is unknown. It is interesting to note that the SE associated with the in vivo work (<10%) (1, 15) are very similar to those obtained in the current determination of $M\dot{V}O_{2mito}$, suggesting that the range of results in either approach could be covered by experimental or biological variability. In addition, the means of maximum respiratory rates from several labs using different approaches are remarkably similar (see Table 4), suggesting that experimental procedures are not significantly impacting these values. Comparing these highly instrumented in vivo studies to minimally instrumented free-running animals chasing a truck at ~30 miles/h (31) reveals remarkably similar physiological limits. These free-running animals achieved a heart rate of ~290 beats/min and a cardiac output of ~480 $ml \cdot kg^{-1} \cdot min^{-1}$, which are very similar to the values obtained with the more severely instrumented animals under more controlled conditions (15, 32). Comparison of pressures is difficult between these studies, since different parameters were reported. These comparisons suggest that a reproducible maximum rate of cardiac respiration has been determined in the laboratory dog.

An apparent conflict with these in vivo heart data is the recent study by Richardson et al. (27) in the quadriceps femoris of trained athletes. Despite the fact that the mitochondrial content and blood flow capacity of this tissue is predictably much lower than those of the heart, these investigators found a maximum respiratory rate also approaching 26 μmol $O_2 \cdot min^{-1} \cdot g^{-1}$ with vigorous exercise. This value is higher than previous studies in model systems (for example, see Refs. 14, 29). The reason this value in human skeletal muscle approaches the estimated $M\dot{V}O_{2mito}$ of the heart is unclear and may be related to the uncertainties in human studies, as pointed out by these authors. No data on the maximum respiratory rate or cytochrome content are available from the subjects used in this study.

In skeletal muscle (29) and isolated renal tubules (12) $M\dot{V}O_{2mito}$ was also found to be only slightly higher than work-induced maximum respiratory rates. These data, together with the current findings, are consistent with the hypothesis that energy conversion and utilization pathways are matched on the cellular level where neither exists with a large excess capacity. This hypothesis is related to the general theory of symmorphosis, where a balance in structural design and functional demand has been proposed (33). This model suggests that excess capacity is energetically inefficient and that an economical design will result in a balance between capacity and function. With regard to mitochondria, any increase in mitochondrial content would leave less physical space for muscle fibers, resulting in reduced maximum power, or for blood vessels, resulting in reduced oxygen delivery and aerobic capacity. An efficient design would have a balance between mitochondrial capacity and metabolic needs, as well as permit the operation of oxidative phosphorylation at near-maximum velocities. To attain the maximum oxidative phosphorylation rate will require, based on our current understanding, severe alterations in cytosolic metabolite concentrations and thermodynamics. These cytosolic changes would include [ADP] and P_i concentration ($[P_i]$) approaching levels almost 5- to 10-fold higher (~500 μM and 5 mM, respectively) than the "resting

state" observed over lower workloads (17) to maximally stimulate oxidative phosphorylation. This increase in ADP and P_i will result in a drop in cytosolic ATP free energy for performing work. Furthermore, the allosteric effects of ADP and P_i on myosin-ATPase (5) and active transport (10) must also be compensated for. Finally, the delivery of NADH/FADH to the cytochrome chain will also have to be maximized via full activation of dehydrogenases and other associated reactions.

The issue of optimal mitochondria content is further complicated by the inherent inefficiency of mitochondrial function. The mitochondrial proton leak contributes to 20–40% of resting O_2 consumption in the liver, up to 52% in rat skeletal muscle, and ~38% of resting O_2 consumption in the whole rat (26). Studies have found a relationship between mitochondrial volume and basal metabolic rate (11), supporting this notion that mitochondrial volume significantly contributes to the basal metabolic rate. Thus excessive mitochondrial capacity would not only reduce space for other functional elements but significantly increase the basal metabolic load, reducing overall efficiency. Thus it is an advantage to attain a given metabolic rate with the minimal amount of mitochondria inner membrane, consistent with the results of this study, if maximum rates can be obtained without compromising other processes in the cell as outlined above.

What limits myocardial performance and O_2 consumption during whole body exercise in vivo is not known. Again, either blood flow and oxygen delivery, myofilament ATPase capacity, or $M\dot{V}O_{2mito}$ could, in part, contribute to the limitation. Recent studies using ^{31}P nuclear magnetic resonance have shown that during near-maximum workloads in open-chest preparations, cytosolic ADP and P_i levels increase, suggesting a supply-and-demand mismatch between energy conversion and utilization (13, 17, 21, 35). Zhang et al. (35) went on to show that with an exogenous vasodilator the respiratory rate and myocardial phosphorylation potential would increase at the maximum workload, suggesting an overall flow limitation. However, these apparent work limitations in the instrumented open-chest dog (17, 35) occurred at O_2 consumption levels roughly one-half of those obtained by exercising awake dogs (15, 32). This suggests that an oxygen delivery limitation, which occurs in the anesthetized open-chest dog, may not occur in the awake animal. Von Restorff et al. (32) further tested this hypothesis by showing that vasodilators increased coronary flow under awake exercising conditions but did not increase work or O_2 consumption. This latter result suggests that oxygen delivery is not rate limiting for myocardial work in vivo. Thus it is still unclear what ultimately limits myocardial performance under awake exercising conditions.

On the basis of the current results, $M\dot{V}O_{2mito}$ is approached at maximum exercising workloads in the heart in vivo. Thus it is not unreasonable to propose that all mechanisms of increasing oxidative phosphorylation, including increases in cytosolic [ADP] and [P_i], Ca^{2+} concentration, and carbon substrate delivery (see Ref. 2), will be required to meet the metabolic demands under these conditions. In addition, yet-undefined control pathways could also be contributing to the regulation of oxidative phosphorylation. Further experimentation will be required to establish how the heart can regulate oxidative phosphorylation to approach its apparent maximum respiratory rate in vivo.

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