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To cite this version:

Jeoffrey Zoll, Jamal Bouitbir, Pascal Sirvent, Alexis Klein, Antoine Charton, et al.. Apparent Km of mitochondria for oxygen computed from Vmax measured in permeabilized muscle fibers is lower in water enriched in oxygen by electrolysis than injection. Drug Design, Development and Therapy, 2015, 9, pp.3589-3597. 10.2147/dddt.s81891 . hal-02633200

HAL Id: hal-02633200

https://hal.inrae.fr/hal-02633200

Submitted on 27 May 2020

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Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Apparent Km of mitochondria for oxygen computed from Vmax measured in permeabilized muscle fibers is lower in water enriched in oxygen by electrolysis than injection

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This article was published in the following Dove Press journal:
Drug Design, Development and Therapy
13 July 2015
Number of times this article has been viewed

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Background: It has been suggested that oxygen (O_2) diffusion could be favored in water enriched in O_2 by a new electrolytic process because of O_2 trapping in water superstructures (clathrates), which could reduce the local pressure/content relationships for O_2 and facilitate O_2 diffusion along PO_2 gradients.

Materials and methods: Mitochondrial respiration was compared in situ in saponin-skinned fibers isolated from the soleus muscles of Wistar rats, in solution enriched in O_2 by injection or the electrolytic process 1) at an O_2 concentration decreasing from 240 µmol/L to 10 µmol/L (132 mmHg to 5 mmHg), with glutamate–malate or N, N, N′, N′-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD)–ascorbate (with antimycin A) as substrates; and 2) at increasing adenosine diphosphate (ADP) concentration with glutamate–malate as substrate.

Results: As expected, maximal respiration decreased with O_2 concentration and, when compared to glutamate–malate, the apparent Km O_2 of mitochondria for O_2 was significantly lower with TMPD–ascorbate with both waters. However, when compared to the water enriched in O_2 by injection, the Km O_2 was significantly lower with both electron donors in water enriched in O_2 by electrolysis. This was not associated with any increase in the sensitivity of mitochondria to ADP; no significant difference was observed for the Km ADP between the two waters.

Conclusion: In this experiment, a higher affinity of the mitochondria for O_2 was observed in water enriched in O_2 by electrolysis than by injection. This observation is consistent with the hypothesis that O_2 diffusion can be facilitated in water enriched in O_2 by the electrolytic process.

Keywords: saponin-skinned fibers, mitochondrial respiration, glutamate–malate, TMPD–ascorbate, Km O_2

Introduction

In the course of studying the possible biological effects of a water enriched in oxygen (O_2) by a new electrolytic process,[1] we have recently shown that, when compared to water enriched in O_2 by injection, intragastric administration of water enriched in O_2 by electrolysis lessened the slow decline in peripheral tissue oxygenation observed in anesthetized pigs.[2] This observation is consistent with the hypothesis that O_2 diffusion can be facilitated in waters enriched in O_2 by electrolysis. It has been hypothesized that this phenomenon could be due to the fact that the electrolytic process could generate water susperstructures similar to clathrates,[3–5] which could trap O_2 molecules and reduce local pressure/content relationships for O_2, thus facilitating O_2 diffusion along PO_2 gradients. Interestingly, a series of studies conducted in vitro and in animal

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models suggests that isotonic saline submitted to a process involving Taylor–Couette–Poiseuille flow in the presence of O_2 (electrokinetically modified water [EMW]) can interfere with cell signaling pathways involved in inflammation, cell death, and survival, and could have beneficial effects in various situations where inflammation is present.[6–11] In human, EMW tends to increase VO_2max and reduces the rate of perceived exertion during aerobic exercise,[7] significantly reduces muscle fatigue during resistance exercise,[12] and prevents or attenuates muscle damage and inflammation in both types of exercise.[12–14] The authors hypothesized that these biological effects of EMW can also be due to the fact that this water could contain "charge-stabilized nanostructures".

The objective of the present study was to further investigate the biological effects of water enriched in O_2 by electrolysis by comparing mitochondrial respiration (Vmax) studied in situ in permeabilized muscle fibers[15] in waters enriched by injection and by the electrolytic process. The observations were made over a wide range of PO_2 and O_2 concentrations in order to compute the apparent Km of mitochondria for O_2. Since the rate of O_2 consumption by mitochondria depends on the flux of both O_2 and electrons,[16] the experiments were performed with glutamate–malate, as well as with tetramethyl-*p*-phenylenediamine (TMPD)-ascorbate, an artificial substrate that provides electrons to complex IV of the respiratory chain at a much larger rate than glutamate–malate.[17] Under the hypothesis that O_2 diffusion is facilitated in water enriched in O_2 by the electrolytic process, when compared to the water enriched in O_2 by injection, Km will be lower with both electron donors and the difference will be larger with TMPD–ascorbate than with glutamate–malate because of the higher flux of electrons. We also verified that the possible differences in mitochondrial respiration between the two waters and the two electron donors were not due to differences in the affinity of mitochondria to adenosine diphosphate (ADP) by measuring the apparent Km of mitochondria for ADP.

Materials and methods

Animal procedures were conducted in accordance with the Declaration of Helsinki and were approved by our local ethics committee Comité Régional d'Éthique en Matière d'Expérimentation Animale, (CREMEAS).

Mitochondrial respiration was studied in situ in muscle fibers permeabilized with saponin.[15,18,19] As reviewed in detail by Kuznetsov et al,[15] saponin is a detergent with a large affinity for cholesterol, which specifically destroys the cholesterol-rich sarcolemma (0.5 mmol cholesterol/mmol phospholipids) without altering the cholesterol-poor mitochondrial membrane (0.07 and 0.01 cholesterol/mmol phospholipids for the outer and inner mitochondrial membranes, respectively). The end result is a preparation with intracellular structures that are intact (mitochondrion, endoplasmic reticulum, myofilaments, and cytoskelton) within an intracellular space devoid of all solutes, which have been washed out, and in equilibrium with the incubation milieu.

Male Wistar rats (body mass ~300 g) were anesthetized (sodium pentobarbitate: 0.2 g/100 g body mass, intraperitoneally) and the soleus muscles were removed and placed in solution S (as will be discussed). Muscle fibers were immediately separated under binocular microscope and permeabilized with 50 µg/mL of saponin for 30 minutes at 4°C. After being placed for 10 minutes in solution R (as will be discussed) to wash out adenine nucleotides and creatine, skinned separated fibers were transferred in a 3 mL water-jacketed oxygraphic cell (Strathkelvin Instruments Limited, North Lanarkshire, Scotland) equipped with a Clark electrode, as previously described.[20]

Solutions R and S (prepared from tap water demineralized by reverse osmosis and then remineralized with Na_2SO_4 + Na_3PO_4, $12H_2O$) both contained 2.77 mM of CaK_2EGTA, 7.23 mM of K_2EGTA (100 nM of free Ca^{2+}), 6.56 mM of $MgCl_2$ (1 mM of free Mg^{2+}), 20 mM of taurine, 0.5 mM of dithiothreitol, 50 mM of potassium methanesulfonate (160 mM ionic strength), and 20 mM of imidazole (pH 7.1). Solution S also contained 5.7 mM of Na_2 ATP and 15 mM of creatine phosphate, while solution R contained 5 mM of glutamate, 2 mM of malate, 3 mM of phosphate, and 2 mg/mL of fatty acid-free bovine serum.

Mitochondrial respiration was measured in situ in solution R at 22°C starting with an initial O_2 concentration of 240 µmol/L (PO_2=133 mmHg).[21] In the solution used as control, this concentration was obtained by injection. In the experimental solution, this concentration was obtained by electrolysis.[1] In this process, remineralized water is pumped between two electrodes separated by a membrane permeable to electrical charges but not to gases, and the water enriched in O_2, which is recovered on the negative electrode (pH =7.1–7.2; conductivity =750–770 µS/cm; 4,375–5,000 µmol O_2/L), is used to prepare the final product. Both solutions, which were supplied by Danone Research, (Palaiseau, France), were prepared from demineralized water, which was remineralized with Na^+ (200 mg/L), SO_4^{2-} (250 mg/L), and PO_4^{2-} (240 mg/L). It was suspected that the electrolytic process could produce ozone which, in turn, could interfere with mitochondrial function through the generation of reactive oxygen species. However, in line with the observation that

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ozone is quickly converted into O_2 ($2\ O_3 \rightarrow 3\ O_2$, with a half-life of only about 20 minutes), and because the solution enriched by electrolysis was prepared and conserved in sealed bottles several weeks before the experiment, the level of ozone was found to be very low (6.8±5.5 µg/L).

In the first series of experiments, after the determination of basal O_2 consumption (nonstimulated respiration, V_0), Vmax was measured under continuous stirring in the presence of saturating amounts of ADP (2 mM) as a phosphate acceptor and glutamate–malate as substrates (5 mM and 2 mM). The Vmax was monitored until the O_2 content decreased from the initial value of 240 µmol/L to 10 µmol/L, corresponding to a PO_2 of 5 mmHg[21] – ie, a value close to the range of PO_2 estimated in muscle cells (2–4 mmHg).[22] In the second series of experiments, the protocol was similar, but complex III of the respiratory chain was blocked with antimycin A (6.5 µM), and *N*, *N*, *N′*, *N′*-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD; 0.5 mM) and ascorbate (0.5 mM) were added as artificial electron donor to cytochrome c. In the third series of experiments, mitochondrial respiration was measured at increasing concentrations of ADP (from 2.5 µmol/L to 1.5 mmol/L) and decreasing PO_2 (from the initial value of 240 µmol/L to final values <10 µmol/L at the end of titration) using glutamate–malate as substrates (5 mM and 2 mM) and without creatine. Each of the first and second series of experiments was conducted on 12 preparations, while the third series of experiments was conducted on eleven preparations. Each preparation was obtained from a different animal.

In all the preparations, at the end of each experiment, cytochrome c was added to the oxygraphic cell. No increase in O_2 consumption was observed in any of the preparations, confirming the integrity of the outer mitochondrial membrane.[23] In addition, the acceptor control ratio (Vmax/V_0) was computed for each preparation in the first series of experiments (glutamate–malate as substrates) and was found to be 3.7±0.3, which is in good agreement with values reported in the literature.[24–26]

The fibers were then harvested and dried, and respiration rates were expressed as µmol O_2/min/g dry weight.

All reagents were purchased from Sigma-Aldrich Co. (St Louis, MO, USA), except ADP, which was obtained from Boehringer Ingelheim (Ingelheim, Germany).

Data are reported as the mean ± standard deviation. The apparent Km of mitochondria for O_2 (for the two types of water and with the two electron donors) and for ADP (for the two types of water and glutamate–malate) were calculated using nonlinear regression of the individual relationships between O_2 or ADP concentration and Vmax, using a Lineweaver–Burk plot. The apparent Km for O_2 (Km O_2) was compared using a two-way analysis of variance (ANOVA) (water × electron donor), while the apparent Km for ADP for the two waters was compared using one-way ANOVA. The level of significance was set at 0.05. All data are available in the Supplementary materials.

Results

Figure 1A shows the increase in Vmax with O_2 concentration measured for the two types of water and the two electron donors. As shown in Figure 1B, the Km O_2 computed from these curves was significantly lower in the solution enriched in O_2 by electrolysis than by injection, both with glutamate–malate (36.1±9.2 µmol/L versus 53.3±10.1 µmol/L, respectively; $P<0.001$) and TMPD–ascorbate as substrates (52.8±11.4 µmol/L and 90.3±10.7 µmol/L, respectively; $P<0.001$). The difference in Km O_2 between the two waters cannot be computed for each preparation and, consequently, no statistical comparisons can be made. However, the average difference was larger with TMPD–ascorbate (37.5 µmol/L or a 41% difference) than with glutamate–malate (17.2 µmol/L, or a 32% difference).

No significant difference was observed for the apparent Km of mitochondria for ADP: 328±67 µmol/L and 303±70 µmol/L (P=0.4) between the water enriched in O_2 by injection and electrolysis, respectively (Figure 2).

Discussion

Results from the present experiment show that, when compared to a solution enriched in O_2 by injection, the apparent affinity for O_2 of the mitochondria was higher in a solution enriched in O_2 by the electrolytic process, the difference being larger with TMPD–ascorbate than glutamate–malate as electron donors. This observation is consistent with the hypothesis that O_2 diffusion can be facilitated in water enriched in O_2 by electrolysis.

As expected, Vmax increased with O_2 concentration in a curvilinear fashion toward a plateau and was significantly higher with TMPD–ascorbate than with glutamate–malate, the difference decreasing slightly with O_2 concentration (~14.3 µmol O_2/min/g versus 7.8 µmol O_2/min/g at 240 µmol/L, or a 75% difference; ~2.6 µmol O_2/min/g versus 1.8 µmol O_2/min/g at 10 µmol/L, or a 45% difference) (Figure 1A). The reduction in Vmax with O_2 concentration was larger than that reported by Gnaiger and Kuznetsov[27] with succinate and TMPD–ascorbate in isolated mitochondria because the apparent Km of cytochrome oxidase (COX) for O_2 has been shown to be higher in saponin-skinned muscle fibers. As for the

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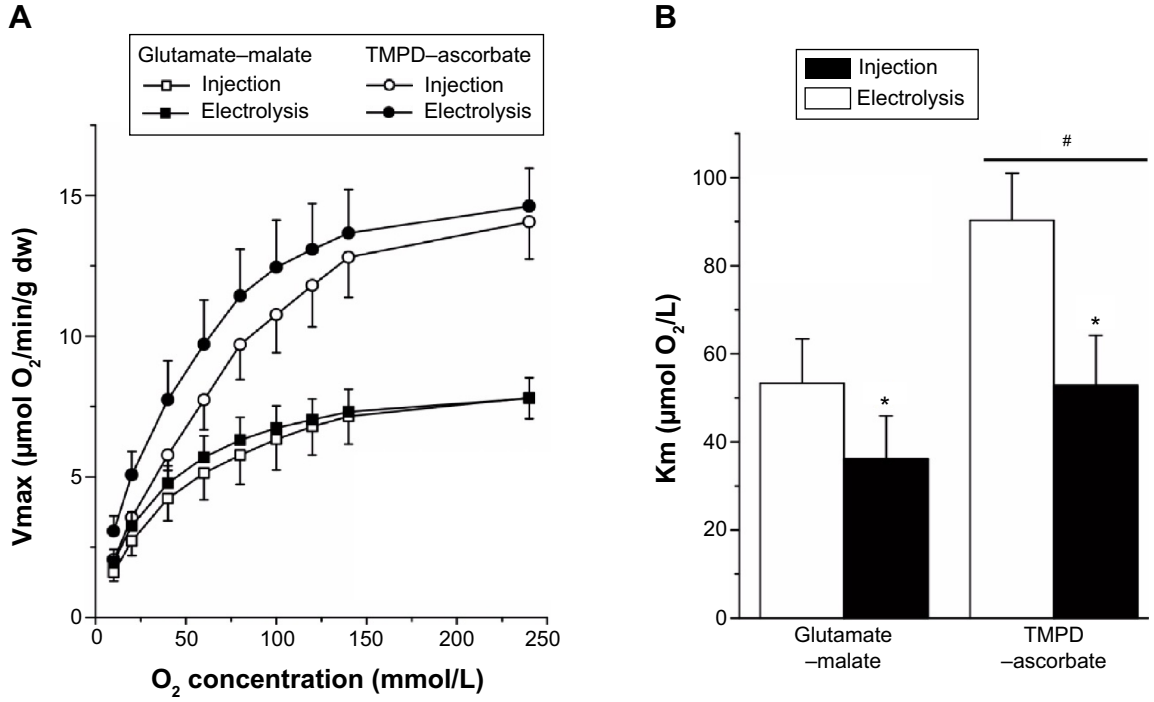

Figure 1 Vmax in solutions enriched in O_2 by injection and electrolysis for O_2 concentrations, as well as apparent Km of the mitochondria for O_2 with the two waters and two electron donors.

Notes: (**A**) Vmax in solutions enriched in O_2 by injection and electrolysis for O_2 concentrations ranging between 10 µmol/L and 240 µmol/L in rat permeabilized soleus muscle fibers with glutamate–malate and TMPD–ascorbate as substrates. (**B**) Apparent Km of the mitochondria for O_2 with the two waters and the two electron donors. Mean ± standard deviation; n=12; #statistically different from glutamate–malate and *from injection, $P<0.05$.

Abbreviations: dw, dry weight; TMPD, tetramethyl-*p*-phenylenediamine; n, number.

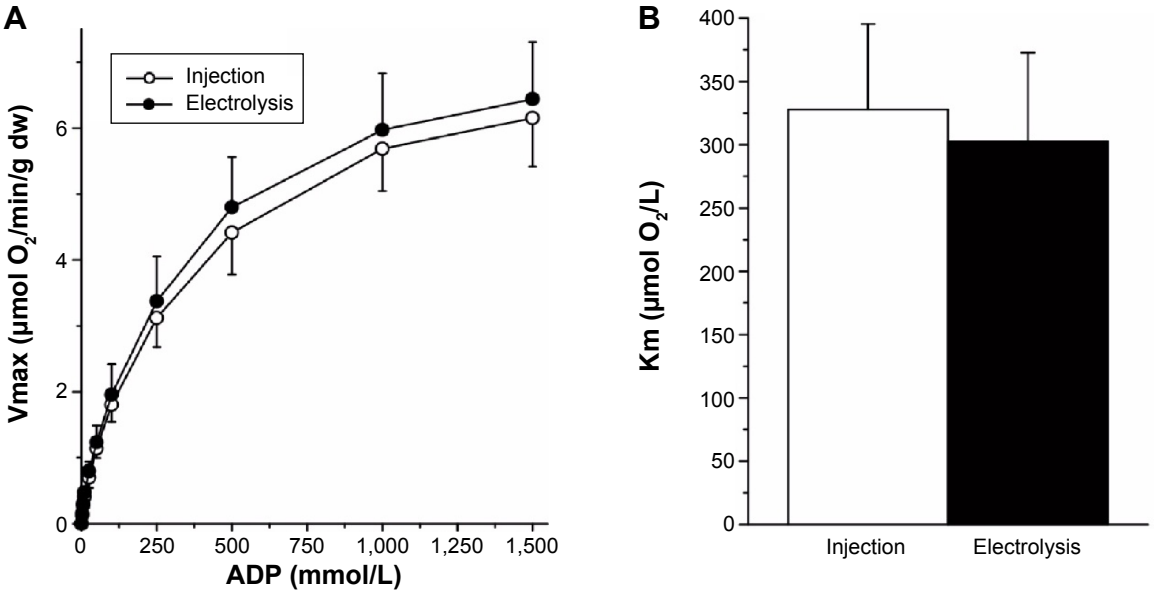

Figure 2 Vmax in solutions enriched in O_2 by injection and electrolysis at increasing ADP concentrations, and apparent Km of the mitochondria for ADP with the two waters.

Notes: (**A**) Vmax in solutions enriched in O_2 by injection and electrolysis at increasing ADP concentrations in rat permeabilized soleus muscle fibers with glutamate–malate as substrates. (**B**) Apparent Km of the mitochondria for ADP with the two waters. Mean ± standard deviation; n=11; no significant difference was observed between the two waters.

Abbreviations: dw, dry weight; ADP, adenosine diphosphate; n, number.

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difference observed between glutamate–malate and TMPD–ascorbate, this phenomenon is known as the "cytochrome oxidase excess"[16] and indicates that when O_2 availability is very high, electron supply from glutamate–malate through the respiratory chain is much lower than could be processed by COX: when electrons are directly supplied to COX at a larger rate from TMPD–ascorbate, Vmax reaches higher values. However, the difference between the two electron donors decreased with O_2 concentration. This observation is due to the fact that the control of O_2 consumption in complex IV of the respiratory chain at any O_2 concentration and over the range of electron supply by glutamate–malate and TMDP–ascorbate is shared by the availability of both O_2 and electrons; when compared to glutamate–malate, in spite of the much larger electron flux from TMPD–ascorbate, the difference in Vmax between the two substrates decreases with O_2 supply.

The main result from the present experiment is that for both electron donors, when compared to the water enriched in O_2 by injection, a left shift in O_2 consumption by the mitochondria was observed with the water enriched in O_2 by electrolysis. Vmax plateau values were similar at the highest O_2 concentration for a given electron donor (7.8 µmol O_2/min/g for both water with glutamate–malate; 14.2 µmol O_2/min/g and 14.7 µmol O_2/min/g for water enriched in O_2 by electrolysis and injection, respectively). However, for any given Vmax, consistently lower O_2 concentrations were needed with the water enriched in O_2 by electrolysis in the steepest portion of the curve, the difference between the two waters being slightly larger with TMPD–ascorbate than glutamate–malate as substrates. As a consequence, when compared to the water enriched in O_2 by injection, the shift in the apparent Km O_2 of the mitochondria with the water enriched in O_2 by electrolysis was also larger.

The higher affinity of the mitochondria for O_2 in water enriched in O_2 by electrolysis was not associated with any increase in the sensitivity of the mitochondrial respiration to ADP since the apparent Km for ADP measured with glutamate–malate remained unaffected by the type of water used to prepare the solution. In addition, this phenomenon was observed with glutamate–malate, which provides electrons to COX through complex I to III of the respiratory chain complex, but also with TMDP–ascorbate, which directly provides electrons to COX. The reduction in the apparent Km of mitochondria in water enriched in O_2 by injection thus suggests that for a given O_2 concentration, O_2 flux to COX is facilitated in the solution enriched in O_2 with the electrolytic process. This phenomenon is not observed with any of the two electron donors at high O_2 content because in these situations, the control of O_2 consumption by COX depends mainly on the availability of electrons. It is only observed at lower O_2 concentrations where the rate of O_2 consumption by COX mainly depends on the availability of O_2, and it was higher with TMPD–ascorbate, which supplies a larger flux of electrons than does glutamate–malate.

Conclusion

These findings are in line with our recent observation in pigs using intragastric administration of water enriched in O_2 that, when compared to the water enriched in O_2 by injection, the decrease in transcutaneous O_2 pressure, which develops during anesthesia, was slower with the water enriched in O_2 by electrolysis.[2] Although the mechanisms behind these observations have to be elucidated, they are consistent with the hypothesis that O_2 diffusion could be facilitated in water enriched in O_2 by electrolysis.

Disclosure

François Péronnet and Ruddy Richard are occasional consultants for Danone Research. Alexis Klein and Liliana Jimenez are employees of Danone Research. The authors report no further conflicts of interest in this work.

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Supplementary materials

Table S1 Individual values of oxygen consumption, in µmol O_2/min/g dw, and apparent affinity for oxygen (Km O_2) of mitochondria in permeabilized muscle fiber preparations at decreasing O_2 content (from 240 µmol/L to 10 µmol/L) in solution enriched in O_2 by injection with glutamate–malate as an electron donor

Experiment number	Oxygen content in µmol/L									Km
	240	140	120	100	80	60	40	20	10	
1	8.97	8.81	8.43	8.10	7.41	6.78	5.53	3.71	2.20	36.77
2	7.04	6.19	5.84	5.38	4.84	4.27	3.49	2.18	1.31	63.00
3	7.50	6.55	6.18	5.33	4.99	4.66	3.83	2.50	1.50	58.06
4	7.18	6.44	5.89	5.48	4.87	4.28	3.57	2.34	1.43	62.01
5	7.59	6.75	6.39	5.88	5.29	4.69	3.81	2.49	1.49	59.00
6	7.75	7.34	6.98	6.54	5.76	4.88	3.97	2.58	1.36	58.93
7	8.45	8.19	7.96	7.56	7.16	6.05	5.12	3.18	1.75	42.11
8	7.13	6.41	5.95	5.44	4.98	4.54	3.95	2.35	1.34	53.72
9	7.95	7.21	6.78	6.27	5.68	5.13	4.22	2.75	1.66	52.24
10	8.24	7.34	7.02	6.63	6.02	5.46	4.56	2.94	1.85	46.49
11	6.88	5.79	5.54	5.17	4.76	4.07	3.15	2.06	1.21	68.28
12	9.05	8.72	8.47	8.12	7.51	6.79	5.52	3.51	2.04	39.18
Mean	7.81	7.15	6.79	6.33	5.77	5.13	4.23	2.72	1.60	53.32
SD	0.74	0.99	1.02	1.09	1.04	0.95	0.79	0.52	0.31	10.12

Abbreviations: dw, dry weight; SD, standard deviation.

Table S2 Individual values of oxygen consumption, in µmol O_2/min/g dw, and apparent affinity for oxygen (*Km* O_2) *of* mitochondria in permeabilized muscle fiber preparations at decreasing O_2 content (from 240 µmol/L to 10 µmol/L) in solution enriched in O_2 by injection with TMPD–ascorbate as an electron donor

Experiment number	Oxygen content in µmol/L									Km
	240	140	120	100	80	60	40	20	10	
1	13.29	12.28	11.18	10.38	9.38	7.95	6.05	3.42	1.72	78.52
2	14.11	12.40	10.48	9.45	8.40	6.25	5.15	3.87	2.51	121.00
3	13.83	11.46	10.60	9.96	9.01	7.03	5.76	3.87	2.03	90.75
4	12.09	11.50	10.15	9.00	8.46	6.45	5.02	3.63	1.98	85.00
5	13.48	12.41	12.05	11.26	10.07	8.27	5.67	3.07	1.88	79.92
6	16.51	15.51	14.38	13.15	12.02	9.41	6.68	3.81	2.01	89.64
7	15.22	14.13	12.98	11.98	10.54	8.97	6.15	3.66	2.25	86.99
8	13.36	11.72	10.99	10.01	8.88	7.44	5.48	3.22	1.99	90.74
9	14.55	13.22	12.88	11.12	10.03	7.93	6.12	3.75	2.36	86.84
10	13.02	11.82	10.75	9.94	8.99	6.87	5.26	3.21	1.79	94.04
11	16.12	15.24	14.18	12.97	11.85	9.07	6.54	3.78	2.24	88.14
12	13.15	11.86	10.95	9.89	8.75	7.12	5.37	3.35	1.97	92.03
Mean	14.06	12.80	11.80	10.76	9.70	7.73	5.77	3.55	2.06	90.30
SD	1.32	1.42	1.47	1.35	1.24	1.05	0.54	0.29	0.23	10.71

Abbreviations: dw, dry weight; TMPD, tetramethyl-*p*-phenylenediamine; SD, standard deviation.

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Table S3 Individual values of oxygen consumption, in µmol O_2/min/g dw, and apparent affinity for oxygen (Km O_2) of mitochondria in permeabilized muscle fiber preparations at decreasing O_2 content (from 240 µmol/L to 10 µmol/L) in solution enriched in O_2 by electrolysis with glutamate–malate as electron donor

Experiment number	Oxygen content in µmol/L									Km
	240	140	120	100	80	60	40	20	10	
1	7.13	6.61	6.41	6.10	5.70	5.21	4.53	3.50	2.45	25.52
2	8.98	8.82	8.01	7.84	7.39	6.67	5.48	3.67	2.11	37.51
3	7.39	6.72	6.54	6.31	5.79	5.28	4.59	3.57	2.42	27.02
4	6.87	6.35	5.99	5.64	5.12	4.42	3.54	2.22	1.21	57.53
5	7.49	6.91	6.59	6.25	5.77	5.21	4.55	3.54	2.39	29.24
6	8.34	8.02	7.78	7.44	7.04	6.37	5.18	3.57	2.13	34.21
7	9.18	8.55	8.38	8.13	7.69	6.99	5.75	3.68	2.34	34.63
8	6.99	6.46	6.22	5.86	5.41	4.76	3.95	2.38	1.25	49.43
9	7.61	7.08	6.88	6.58	6.13	5.52	4.65	3.22	1.85	35.74
10	7.75	7.31	7.11	6.77	6.40	5.83	4.89	3.36	2.01	33.02
11	7.88	7.39	7.19	6.95	6.55	6.05	5.19	3.45	2.06	30.99
12	7.81	7.46	7.28	7.05	6.68	5.95	4.91	3.01	1.60	38.48
Mean	7.79	7.31	7.03	6.74	6.31	5.69	4.77	3.26	1.99	36.11
SD	0.73	0.80	0.74	0.78	0.80	0.77	0.62	0.49	0.43	9.18

Abbreviations: dw, dry weight; SD, standard deviation.

Table S4 Individual values of oxygen consumption, in µmol O_2/min/g dw, and apparent affinity for oxygen (Km O_2) of mitochondria in permeabilized muscle fiber preparations at decreasing O_2 content (from 240 µmol/L to 10 µmol/L) in solution enriched in O_2 by electrolysis with TMPD–ascorbate as electron donor

Experiment number	Oxygen content in µmol/L									Km
	240	140	120	100	80	60	40	20	10	
1	14.84	14.42	13.12	13.02	12.05	8.90	6.83	4.54	3.11	63.00
2	12.26	10.63	10.10	9.45	8.67	7.45	5.94	3.89	2.27	62.40
3	14.86	13.85	12.96	11.65	10.28	8.47	6.58	4.28	2.46	78.19
4	15.15	14.50	13.88	13.25	12.28	10.76	8.66	5.63	3.58	42.84
5	14.15	13.78	13.23	12.33	11.20	10.01	7.88	4.99	3.18	46.99
6	16.78	15.99	15.87	15.75	14.68	13.00	10.69	6.79	4.07	34.97
7	16.54	15.55	15.01	14.15	13.21	11.55	9.45	6.22	3.54	43.89
8	12.33	11.07	10.26	9.92	8.97	7.59	6.02	4.35	2.29	57.52
9	14.48	13.40	13.02	12.26	11.44	9.61	7.68	4.95	2.98	50.8
10	14.31	13.24	12.91	12.08	11.01	9.25	7.36	4.77	2.94	54.27
11	15.00	13.78	13.44	12.82	11.84	10.05	7.96	5.34	3.25	48.45
12	14.72	13.76	13.19	12.66	11.55	9.84	7.79	5.09	3.13	50.28
Mean	14.62	13.66	13.08	12.45	11.43	9.71	7.74	5.07	3.07	52.80
SD	1.35	1.55	1.63	1.68	1.66	1.58	1.38	0.83	0.54	11.38

Abbreviations: dw, dry weight; TMPD, tetramethyl-*p*-phenylenediamine; SD, standard deviation.

Table S5 Individual values of Vmax, in µmol O_2/min/g dw, and apparent affinity of mitochondria for ADP (Km ADP, in µmol/L) in permeabilized muscle fiber preparations in solution enriched in O_2 by injection or electrolysis with glutamate–malate as electron donor

Experiment number	Injection		Experiment number	Electrolysis	
	Vmax	Km		Vmax	Km
1	5.85	321.5	1	6.64	285.8
2	7.92	298.0	2	9.68	230.7
3	6.80	449.0	3	7.94	220.3
4	8.32	407.8	4	6.76	231.6
5	9.08	418.4	5	7.60	299.5
6	7.94	295.6	6	6.90	233.6
7	6.56	275.6	7	9.11	384.5
8	7.28	328.8	8	7.64	322.5
9	6.95	289.7	9	8.11	316.3
10	6.92	237.8	10	6.67	413.0
11	8.38	284.8	11	7.58	391.0
Mean	7.45	327.9	Mean	7.69	302.6
SD	0.95	67.4	SD	0.99	70.2

Abbreviations: dw, dry weight; ADP, adenosine diphosphate; SD, standard deviation.

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