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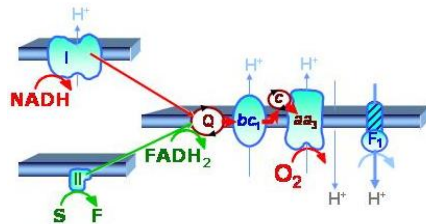
MitoPathways Compilation: Additive Effect of Substrates at the Q-Junction: Complexes I and II



Hélène Lemieux, Erich Gnaiger

Medical University of Innsbruck, Department of Transplant Surgery
D. Swarovski Research Laboratory
Innrain 66/6, 6020 Innsbruck, Austria
OROBOROS INSTRUMENTS Corp.
high-resolution respirometry
Schöpfstr. 18, A-6020 Innsbruck, Austria
Email: erich.gnaiger@oroboros.at
www.oroboros.at

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1. Compilation: A Quantitative Approach

The following compilation of respiratory flux in permeabilized muscle fibers and isolated mitochondria yields important insights into species- and tissue-specific adaptations. Mitochondrial respiratory flux per unit mass of tissue and flux per mitochondrial marker for control groups provide the basis for evaluation of mitochondrial defects (Renner et al 2003). Comparison of quantitative results presents major problems related to:

(*1*) differences of titration protocols, (*2*) different experimental temperatures (Table 1), (*3*) differences in pretreatment of the samples and variations in respiration media, (*4*) conversions of dry- to wet weight of the tisse, (*5*) quantification of mitochondrial density in the tissue, and (*6*) expression of oxygen flux in a variety of units (Section 3).

The present compilation is focussed on summarizing the additive effect on respiratory flux when substrates are combined for Complexes I+II, compared to substrates provided separately for either Complex I or II (convergent electron input into the respiratory system; MiPNet12.12). Important general conclusions can be derived from the present quantitative comparison (Table 1).

Table 1. Additive effect of succinate and substrate flux control ratio, *SCR,* with substrates for Complexes I/Complex I+II in muscle (J_{CI}/J_{CI+II}). Mitochondrial respiration measured with Complex I substrates (CI) and a combination of Complex I+II substrates (CI+II), in permeabilized muscle fibers (Pfi) and isolated mitochondria (Imt) from various source of muscle tissue. Experimental temperature, *T* [°C]. Respiratory flux, $JO_{2,37}$, for state CI+II was converted to 37 °C and is expressed as pmol $O_2.s^{-1}.mg^{-1}$ wet weight. *In vivo* data for comparison (*inv*). Adapted in part from Gnaiger (2009).

Tissue	Prep.	CI	CI+II	$J_{O2,37}$	*T*	CI/CI+II	Ref	Note
Mouse heart	Pfi	$PMGc_D$	$PMGSc_D$	**1,051**	**37**	0.53	Lemieux et al 2007	1
Mouse heart	Pfi	$PMGc_D$	$PMGSc_D$		30	0.62	Lemieux et al 2007	1
Mouse heart	Pfi	$PMGc_D$	$PMGSc_D$		25	0.65	Lemieux et al 2007	1
Mouse heart	Pfi		GMS_D	168[a]	25		Kuznetsov et al 1996	2
Rat heart	Pfi	$PMGc_D$	$PMGSc_D$	**472**	**37**	0.54	Lemieux et al unpubl.	3
Rat heart	Imt	GM_D	GS_D	356[a]	30	0.54	Costa et al 1988	4
Mouse soleus	Pfi	$PMGc_D$	$PMGSc_D$	**82**	**37**	0.74	Aragones et al 2008	
Rat P. major	Pfi	$PMGc_D$	$PMGSc_D$	**75**	**37**	0.69	Lemieux et al unpubl.	3
Rat quadriceps	Imt	GM_D	GMS_D		30	0.81	Garait et al 2005	5
Rat gastrocnemius	Imt	GM_D	GMS_D	80[a]	30	0.78	Capel et al 2005	10
Rat muscle	Imt	GM_D	GS_D		30	0.78	Llesuy et al 1994	4
Pigeon breast muscle	Imt	GM_D	GS_D		25	0.51	Rasmussen 1997	6
Horse skeletal	Pfi	GMc_D	$GMSc_D$	**82**	**37**	0.58	Votion et al. unpubl.	7
Human V. lateralis	Pfi	$GOcM_D$	$GOcMS_D$	90[a]	30	1.65	Gnaiger et al 2005	8
Human V. lateralis	Pfi	GMc_D	$GMSc_D$	**89**	**37**	0.49	Boushel et al 2007	9
Human V. lateralis	Pfi	GM_D	GMS_D	42[a]	25	0.74	Kunz et al 2000	11
Human V. lateralis	Imt	GM_D	GS_D	104[a]	25	0.71	Rasmussen 2000	12
Human V. lateralis	Imt	GM_D	GS_D	106[a]	25	0.71	Rasmussen et al 2001	13
Mouse heart	*Inv*			697	37		Boudina et al 2005	
Dog heart	*Inv*			423	37		Mootha et al 1997	
Human V. lateralis	*Inv*			289[b]	38		Rasmussen et al 2001	13

1. The additive effect of succinate is a general feature of mitochondrial respiratory control in muscle mitochondria. The corresponding Q-junction ratios range from 0.5 to 0.8, representing the OXPHOS flux ratio with Complex I substrates relative to the CI+II substrate combinatiojn. An important mechanism of increasing the Q-junction ratio is the limitation of OXPHOS capacity by the phosphorylation system (MiPNet12.12).
2. The additive effect applies equally to permeabilized muscle fibers and isolated mitochondria.
3. The Q-junction ratio increases with decreasing experimental temperature in the range of 37 °C to 25 °C, thus ruling out the application of a common temperature coefficient or single Q_{10} value for relating experimental data obtained <37 °C to the physiological temperature.

4. Mitochondrial respiratory capacity per tissue is higher in heart than skeletal muscle, depends on muscle type, body mass and species.
5. Large differences of respiratory capacity are reported by different groups. A variety of artefacts may lead to an underestimation of respiratory capacity. Fluxes in the higher ranges agree closely when measured in isolated mitochondria and permeabilized muscle fibers.
6. Respiratory capacity measured in permeabilized fibers (PF) and isolated mitochondria (IM) falls short of explaining the high respiratory capacity of human skeletal muscle *in vivo*.

2. Titration Protocols with Substrate Combinations

Numbers refer to Notes in Table 1; abbreviations see MiPNet12.15.

1 *Innsbruck protocol*: GM_N+D+*c*+P+S+u+Rot+*; and PM_N+D+*c*+G+S+u+(Rot)+*: The *L*/*P* ratio is obtained for GM_N/GM_D. The effect of P is measured after state GMc_D, and compared to the effect of G on state PMc_D in a separate protocol. Flux in the common state $PMGc_D$ was not different in the two protocols. No effect of *c*, added at the earliest ADP-activation state. The early addition of *c* ensures comparability of all states in case of any effect of *c*, which has to be considered for a diagnostic protocol (Gnaiger 2007). Results at 25 , 30 and 37 °C can be used to convert literature data reported at different temperatures to 37 °C. The Q_{10} depends on the temperature span and on the respiratory state with different substrates. The conversion factor from 25 °C (30 °C) to 37 °C was 2.00 (1.62) for J_{I+II}; (corresponding to Q_{10} of 1.78 and 1.99), but was significantly lower for J_I. Oxygen limitation of flux was prevented by maintaining oxygen levels in the respirometer above air saturation.

2 No comparison was made with the single substrate.

3 GM_N+D+*c*+P+S+u+Rot+*: No effect of *c*. Lemieux H, Gnaiger E, unpubl. Oxygen limitation of flux was prevented by maintaining oxygen levels in the respirometer above air saturation.

4,6,12,13 Separate incubations, GM_N+D or GS_N+D, hence the *L*/*P* ratio is obtained for both, GM_N/GM_D and GS_N/GS_D. GS_N/GS_D is a complex function of coupling and of the relative contributions of G and S to total flux. The possible difference remains to be determined between GS_N/GS_D and GMS_N/GMS_D.

5,10,11 Separate incubations, GM_N+D or GMS_N+D, hence the *L*/*P* ratio is obtained for both, GM_N/GM_D and GMS_N/GMS_D.

7 Votion D, Lemieux H, Gnaiger E, unpubl. Oxygen limitation of flux was prevented by maintaining oxygen levels in the respirometer above air saturation.

8 *Greenland-Monte Rosa protocol*: OcM_N+D+G+S+Rot+Omy+u+*c*+*: *L*/*P* and *L*/*E* ratios were

obtained for three states in sequence, OcM_N/OcM_D; $S(Rot)_{Omy}/S(Rot)_D$; $S(Rot)_{Omy}/S(Rot)_u$. There was no difference between OXPHOS capacity with $S(Rot)_D$, and ETS capacity with $S(Rot)_u$, and no difference in *L/P* ratios with OcM and S(Rot). The cytochrome *c* effect was checked very late in the titration protocol. The absence of a significant *c*-effect not only showed integrity of the outer mitochondrial membrane after fiber preparation, but also preservation of integrity over a 90-100 min incubation in the O2k (MiR05). Involving healthy subjects only, no pathological injury was expected that might lead to a *c*-effect. Oxygen limitation of flux was prevented by maintaining oxygen levels in the respirometer above air saturation.

7,9 *Innsbruck-Copenhagen protocol*: GM_N+D+*c*+S+u+Rot+*: No effect of *c*. *L/P* and *P/E* flux control ratios are obtained under different conditions of flux: $L/P = GM_N/GM_D$; $P/E = GMSc_D/GMSc_u$. Oxygen limitation of flux was prevented by maintaining oxygen levels in the respirometer above air saturation.

11 Flux is low in comparison to the results reported by other authors. Oxygen limitation of flux in permeabilized fibers incubated at oxygen levels below air saturation may in part explain the low flux (Gnaiger 2003).

+* These protocols were continued with additional titration steps.

3. Conversion to *SI* Units and Temperature Correction

For conversion to *SI* units, see MiPNet12.15. Briefly, for the 'bioenergetic units' [ng.atom $O\cdot min^{-1}\cdot mg^{-1}$ = natom $O\cdot min^{-1}\cdot mg^{-1}$ = µmol $O\cdot min^{-1}\cdot g^{-1}$], the multiplication factor to obtain flux in *SI* units [nmol $O_2\cdot s^{-1}\cdot g^{-1}$ = pmol $O_2\cdot s^{-1}\cdot mg^{-1}$] is 1000/(2·60) = 8.33. For units [nmol $O_2\cdot min^{-1}\cdot mg^{-1}$] the corresponding multiplication factor is 1000/60 = 16.67.

[a] Measured at 25 °C or 30 °C, and converted to 37 °C on the basis of an extensive study on the temperature coefficient for mouse heart (Lemieux et al 2006 [1]). The validity of application of this temperature dependence to mitochondria from different tissues remains to be determined, but appears to be justified in the range 30 °C to 37 °C (compare $J_{O2,37}$ [8] and [9]; Brooks et al 1971).

2 [a] 50.5 ng atoms $O\cdot min^{-1}\cdot mg^{-1}$ Wd (per mg tissue dry weight; 25 °C) is equivalent to 421 nmol $O_2\cdot s^{-1}\cdot g^{-1}$ Wd or 84.2 nmol $O_2\cdot s^{-1}\cdot g^{-1}$ Ww, using a Ww/Wd ratio of 5.0 (Kuznetsov et al 2004). The temperature coefficient of 2.0 [1] was used to adjust to 37 °C. The flux is very low, even in comparison with rat heart.

4	*a*	At 30 °C, 527 ngatom $O.min^{-1}.mg^{-1}$ P_{mt} was reported (mitochondrial protein, P_{mt}), equivalent to 4.39 nmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ P_{mt}. The mitochondrial density in rat heart (per wet weight, Ww) is 50 mg P_{mt}/g Ww. This yields 220 nmol $O_2 \cdot s^{-1} \cdot g^{-1}$ Ww. The temperature coefficient of 1.62 was used to convert from 30 °C to 37 °C (Q_{10} of 2.0). The Q-junction ratio was calculated for the original data at 30 °C.
8	*a*	At 30 °C, 55.7 nmol $O_2 \cdot s^{-1} \cdot g^{-1}$ Ww was measured in state $GOcMS_D$ (glutamate, octanoylcarnitine, malate and succinate). The temperature coefficient of 1.62 was used to convert from 30 °C to 37 °C (Q_{10} of 2.0 [1]). The literature on temperature dependence of human skeletal muscle is limited, suggesting a Q_{10} of 2 in the temperature range of 25 °C to 35 °C from a single experiment with glutamate+malate (Byrne and Trounce 1985). While this appears to support the presently used temperature coefficient, the mouse heart data are very different, since the Q_{10} was only 1.3 for GMc_D in the range of 25 °C to 37 °C (Lemieux et al 2007).
10	*a*	295 nmol $O_2 \cdot min^{-1} \cdot mg^{-1}$ P_{mt} (30 °C) converts to 4.92 nmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ P_{mt}. To convert to tissue-specific flux, the mitochondrial content was taken from [12], and a temperature coefficient of 1.62 was used to convert from 30 °C to 37 °C (Q_{10} of 2.0 [1]).
11	*a*	8.8 nmol $O_2 \cdot min^{-1} \cdot mg^{-1}$ W_d (25 °C) is equivalent to 147 nmol $O_2 \cdot s^{-1} \cdot g^{-1}$ W_d or 21.3 nmol $O_2 \cdot s^{-1} \cdot g^{-1}$ W_w, using a W_w/W_d ratio of 6.9 given in this paper. The temperature coefficient of 1.78 [1] was used to adjust to 37 °C. The flux is about half or less compared to other studies.
12	*a*	622 µmol $O \cdot min^{-1} \cdot g^{-1}$ P_{mt} (25 °C) converts to 5.18 nmol $O_2 \cdot s^{-1}$·mg P_{mt}. At a mitochondrial protein content per wet weight of muscle of 10 mg $P_{mt} \cdot g^{-1}$ Ww, this yields 51.8 nmol $O_2 \cdot s^{-1} \cdot g^{-1}$ Ww. The temperature coefficient of 2.00 was used to convert from 25 °C to 37 °C (Q_{10} of 1.78).
13	*a*	Based on 10 mg $P_{mt} \cdot g^{-1}$ Ww and measurement at 25 °C, respiration per muscle mass of 7.8 mmol $O_2.min^{-1}.kg^{-1}$ Ww is reported after conversion to 38 °C, corresponding to 52.8 nmol $O_2 \cdot s^{-1} \cdot g^{-1}$ Ww based on the Q_{10} of 2.0 as assumed by these authors (corresponding to a temperature coefficient of 2.46 from 25 °C to 38 °C). We then applied the temperature coefficient of 2.00 to convert from 25 °C to 37 °C.
13	*b*	The authors assumed a Q_{10} of 2.0, with a corresponding correction factor of 0.933 from 38 °C to 37 °C.

Notes-Pitfalls-Corrections

In Table 1 (printed edition 2007), the results of Capel et al (2005) should refer to rat gastrocnemius muscle mitochondria, but not to human vastus lateralis mitochondria.