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O2k-Protocols

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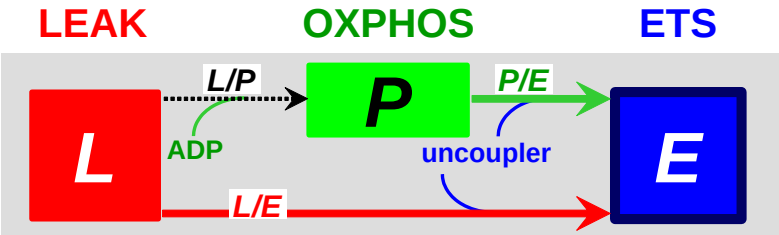
MitoPathways: Respiratory States and Coupling Control Ratios



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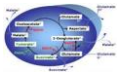
'*The growth of any discipline depends on the ability to communicate and develop ideas, and this in turn relies on a language which is sufficiently detailed and flexible.*'
Simon Singh (1997) Fermat's last theorem. Fourth Estate, London.

Section



1. Coupling in Oxidative Phosphorylation

In oxidative phosphorylation, the endergonic process of phosphorylation of ADP to ATP is coupled to the exergonic process of electron transfer to oxygen. Coupling is achieved through the proton pumps generating and utilizing the protonmotive force in a proton circuit across the inner

mitochondrial membrane. This proton circuit is partially uncoupled by proton leaks. Three different meanings of uncoupling (or coupling) are distinguished by defining intrinsically ***uncoupled***, pathologically ***dyscoupled***, and experimentally ***non-coupled*** respiration:

1. In partially uncoupled (or partially coupled) respiration, *intrinsic uncoupling* under physiological conditions is a property of the inner mt-membrane (proton leak), proton pumps (proton slip; decoupling), and molecular uncouplers (uncoupling protein, UCP1).
2. *Dyscoupled* respiration under pathological and toxicological conditions is related to mitochondrial dysfunction. An explicit distinction is made between physiologically regulated uncoupling and pathologically defective dyscoupling (analogous to distinguishing eustress versus dystress, function versus dysfunction).
3. *Non-coupled* respiration in the experimentally controlled fully uncoupled (non-coupled) state is induced by application of established uncouplers (protonophores, such as FCCP or DNP), with the aim of obtaining a reference state with collapsed mt-membrane potential, for evaluation of the respiratory capacity through the electron transfer system, or a defined state for measurement of mt-ROS production.

The coupling state (or uncoupling state) of mitochondria is a key component of mitochondrial respiratory control.

2. Respiratory Steady-States

Respiratory steady states have been defined by Chance and Williams (1955) according to a protocol for oxygraphic experiments with isolated mitochondria, summarized in Table 1.

$$e_N + D_{ROX} + S(Rot)_P \rightarrow T_L + D_P \rightarrow T_L \rightarrow anox \quad (1)$$

The classical titration protocol starts with State 1, e_N (endogenous substrates, no adenylates, with inorganic phosphate in the mitochondrial respiration medium), to State 2 after addition of ADP ($+D_{ROX}$), inducing a transient activation by ADP to effectively exhaust endogenous substrates, after which State 2 is a substrate-depleted state of residual oxygen consumption (ROX). ADP- and substrate activation is achieved in State 3 (*P*) by addition of substrate, e.g. succinate, +S(Rot), in the presence of rotenone to inhibit Complex I. In this State $S(Rot)_P$, respiration is high and ADP is gradually depleted by phosphorylation to ATP ($\rightarrow T_L$). Respiration drops in the transition to State 4, which is an ADP-limited resting state (LEAK state, *L*). A second ADP titration ($+D_P$) is followed by another State 3$\rightarrow$State 4 transition ($D_P \rightarrow T_L$). In the second State 3, ATP is present at a higher concentration, which may be indicated as state $S(Rot)_{TD}$. Finally, respiration becomes oxygen limited after the aerobic-anoxic transition ($\rightarrow$anox) in the closed oxygraph chamber (Tab. 1).

In an alternative, conventional protocol (Estabrook 1967; Table 2) for measuring respiratory control ratios and ADP:O ratios (e.g. with succinate+rotenone), the sequence is from State 1, e_N (endogenous substrates, no adenylates), to a LEAK state 2' after addition of rotenone

and succinate, $S(Rot)_L$, State 3 after addition of ADP ($+D_P$), and State 4 after phosphorylation of ADP to ATP ($\rightarrow T_L$):

$$e_N + S(Rot)_L + D_P \rightarrow T_L$$

Table 1. Metabolic states of mitochondria (Chance and Williams, 1956; TABLE V).

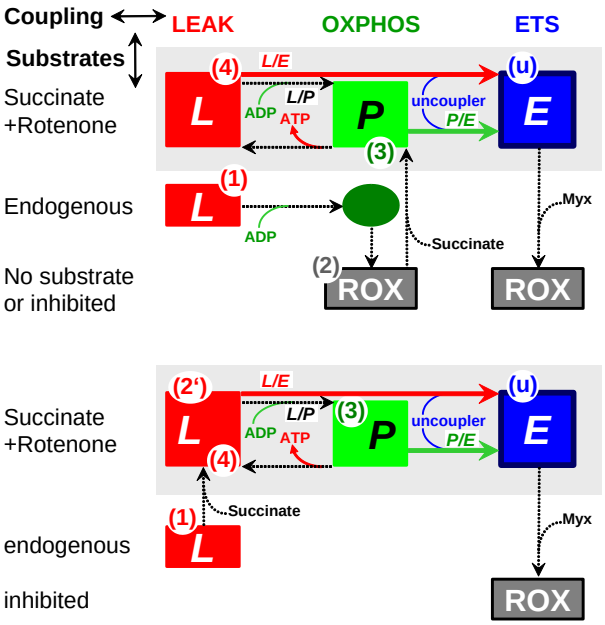
State	$[O_2]$	ADP level	Substrate level	Respiration rate	Rate-limiting substance	New abbreviation, example with S(Rot)	Coupling state
1	>0	Low	Low	Slow	ADP	e_N	
2	>0	High	~0	Slow	Substrate	e_D	ROX
3	>0	High	High	Fast	respiratory chain	$S(Rot)_D$ or $S(Rot)_{TD}$	OXPHOS
4	>0	Low	High	Slow	ADP	$S(Rot)_T$	LEAK
5	<0	High	High	0	Oxygen	$S(Rot)anox_T$	

Table 2. Definition of mitochondrial respiratory states. "*Chance and Williams (1956) proposed a convention following the typical order of addition of agents during an experiment:*" (Nicholls and Ferguson, 1992).

		New abbreviation, example with S(Rot)	Coupling state
State 1:	mitochondria alone (in the presence of Pi)	e_N	
State 2:	substrate added, respiration low due to lack of ADP	$S(Rot)_N$	LEAK
State 3:	a limited amount of ADP added, allowing rapid respiration	$S(Rot)_D$	OXPHOS
State 4:	All ADP converted to ATP, respiration slows	$S(Rot)_T$	LEAK
State 5:	Anoxia	$S(Rot)anox_T$	

The original definition of State 2 (Tab. 1; Chance and Williams 1955; 1956) is opposite to the state 2' obtained in the absence of ADP but presence of substrate (Tab. 2). States 2 (Tab. 1) and 2' (Tab. 2) are functionally different states of ROX and LEAK respiration. '*We have sought independent controls on whether State 2 corresponds to complete oxidation of the system. It is logical that this be so, for respiration is zero in State 2 because substrate, not phosphate acceptor, is limiting.*' (Chance and Williams 1955). State 2' and 4 are LEAK states, which yield significantly different oxygen kinetics of isolated mitochondria (Gnaiger et al 1998). The State 2, 2' and 4 terminology has become confusing: '.. *the controlled respiration prior to addition of ADP, which is strictly termed "state 2", is functionally the same as state 4, and the latter term is usually used for both states'* (Nicholls and Ferguson 1992). Alchemy has a tradition of using the same term for multiple meanings and different terms for the same. A termonological extension from integers to the fraction 3½ has been suggested to indicate an intermediate mitochondrial energy state somewhere between States 3 and 4 (König et al 1969). Paradoxically, a fractional numbering system (real numbers of mathematics) would suggest that ADP-activated hypoxia were intermediate between States 3.0 and 5.0, i.e. State 4.0. This state of terminology requires fundamental reconsideration for clarification, particularly for extending bioenergetics to mitochondrial respiratory physiology of the living cell.

2.1. LEAK Respiration, *L*



States $S(Rot)_N$ and $S(Rot)_T$ (State 4 and State 2') provide different states for estimating LEAK respiration (state *L*). If ATPase activity causes any recycling of ATP to ADP, then some activation of respiration by phosphorylation occurs in state $S(Rot)_T$. The aim is the induction of a *L* state for proper assessment of respiration merely compensating for LEAK (proton leak, electron slip and cation cycling in contrast to ATP synthase activity), in ***uncoupled respiration of mitochondria in a partially coupled state***. The increase of membrane potential must be considered in comparison to the ADP-activated OXPHOS state (State 3), hence determination of LEAK respiration (State 4) may be considered as a maximum estimate (Fig. 1).

The above protocol is varied in order to apply physiological high ATP concentrations (in the range of 1-5 mM; Gnaiger et al 2000; Gnaiger 2001),

$$S(Rot)_N + T_L + D_P \rightarrow S(Rot)_T \rightarrow anox \qquad (3)$$

When State 1 is not measured, the protocol starts with the LEAK state $S(Rot)_N$. After addition of ATP ($+T_L$), the system remains in a LEAK state; the ATPase effect is evaluated in the transition from $S(Rot)_N$ (LEAK state; Table 2) to $S(Rot)_T$ ($+T_L$, State 4; now the numerical sequence of states does not make sense).

2.2. OXPHOS Capacity, *P*

Stimulation by ADP (+D) yields State $S(Rot)_{TD}$ for assessment of the capacity of oxidative phosphorylation (OXPHOS, state *P*). The 'state TD' is different from the conventional State 3 (Tab. 2), due to the high ATP concentration, simulting physiological intracellular conditions. This protocol requires careful isolation of mitochondria without ATPase activity, and therefore cannot be applied to permeabilized fibres or permeabilized cells. In general, ADP-activated respiration in the presence of substrates yields the OXPHOS capacity, $S(Rot)_P$. Mitochondria respiring at OXPHOS capacity generate a proton gradient by proton pumping (CI, CIII, CIV) which is partially utilized by the ATP synthase to drive phosphorylation (coupled respiration) and is partially dissipated due to proton leaks (uncoupled respiration). In the OXPHOS state, therefore, mitochondria are in a ***partially coupled (or partially uncoupled)*** state.

2.3. Electron Transfer System Capacity, *E*

The capacity of the electron transfer system (ETS) is evaluated in an open-circuit operation of the transmembrane proton circuit. The open-circuit state is established experimentally by complete uncoupling (***non-coupled state***) using protonophores (uncouplers, such as FCCP or DNP) at optimum concentration for stimulation of maximum flux. ETS potentially exceeds the OXPHOS capacity. The important difference between ETS (state *E;* non-coupled) and OXPHOS capacity (state *P*; partially coupled) is somehow obscured when referring to the non-coupled state as State 3u (u for uncoupled).

OXPHOS and ETS capacity, as well as the difference between these states, depend on the types of substrates and substrate combinations applied in a respiratory protocol. Multiple substrate-uncoupler-inhibitor titration (SUIT) protocols are designed to evaluate the effects of substrate combinations on OXPHOS and ETS capacity.

$$GM_N + D_P + Omy_L + F_E + c + S_E + (Rot)_E + (Myx)_{ROX} \quad (4)$$

In protocol (4), two LEAK states are compared: GM_L, in terms of GM_N and GM_{Omy}. See Appendix for abbreviations, such as glutamate and malate (G and M) and oligomycin (Omy). And three *E* states are compared, in terms of non-coupled flux with CI, CI+II and CII substrates: GM_E, GMS_E and $S(Rot)_E$; all in the non-coupled state supplemented with cytochrome *c*.

2.4. Respiratory States of the Intact Cell

Cell respiration *in vivo* is regulated according to physiological activity, at intracellular non-saturating ADP levels in ROUTINE states of activity (*R*), and increases under various conditions of activation. When inubated in culture medium, cells maintain a ROUTINE level of activity, C_R (C, intact cells; *R*, ROUTINE mitochondrial respiration; corrected for residual oxygen consumption due to oxidative side reactions). When incubated for short experimental periods in a medium devoid of organic substrates, the cells respire solely on endogenous substrates at the corresponding state of ROUTINE activity Ce_R (e, endogenous substrate supply; Renner et al 2003).

ROUTINE cell respiration can be inhibited by oligomycin or (carboxy)atractyloside to a resting state, corresponding mainly to LEAK respiration (*L*), comparable to isolated mitochondria but without disturbing intracellular substrate conditions by cell membrane permeabilzation (Hütter et al 2004; Gnaiger 2008). It is difficult to stimulate intact cells to maximum OXPHOS activity, whereas uncouplers of OXPHOS are cell membrane permeable, and cells with standard or endogenous substrate supply can be activated by uncoupling to reveal the ETS capacity (state *E*; Steinlechner-Maran et al 1996; Stadlmann et al 2002). Respiratory control ratios (RCR) and uncoupling control ratios (UCR), therefore, can be studied in cells with intact plasma membranes. Clarification of the

concepts on RCR and UCR is required for comparison with bioenergetic paradigms derived from isolated mitochondria or permeabilized cells.

3. Coupling Control Ratios (*CCR*), Respiratory Control Ratio (RCR), Uncoupling Control Ratio (UCR)

Consider the following protocol in isolated mitochondria:

$$S(Rot)_N + T_L + D_P \rightarrow S(Rot)_T + Omy_L + F_E + (Myx)_{ROX} \quad (5)$$

where the example discussed above (protocol 3) is extended by addition of oligomycin (+Omy), titration of uncoupler (+F) and inhibitor myxothiazol (+Myx [MiPNet12.11]). The respiratory adenylate control ratio, RCR, is conventionally defined as the ADP-activated flux to measure coupled OXPHOS capacity (*P*; State 3) divided by LEAK flux (*L*; no adenylates, or State 4, or oligomycin-inhibited). An important aim in respirometric assays is the evaluation of the state of coupling or uncoupling. Unrelated to uncoupling, OXPHOS capacity and hence the RCR are lowered if a low capacity of the phosphorylation system limits OXPHOS capacity. This is the case when uncoupled flux as a measure of capacity of the electron transfer system (*E*) is higher than coupled flux (*P*) and there is an ETS excess capacity over OXPHOS capacity [MiPNet12.12]. The reference state appropriate for defining an index of coupling, therefore, is not coupled OXPHOS capacity (*P*) but the state yielding non-coupled electron transfer capacity (*E*). The RCR has the mathematically inconvenient property of increasing to infinity from a minimum of 1.0. In contrast, coupling control ratios (*CCR*) are defined between limits of 0.0 and 1.0, as derived from flux control analysis. Coupling control ratios are flux control ratios at constant substrate supply (Gnaiger 2009). The *CCR* expressing the 'leakiness' of mitochondria in the partially coupled state, is the **LEAK control ratio**, *L/E*,

$$L/E = S(Rot)_L / S(Rot)_E \quad (6)$$

(using the same abbreviation for a metabolic state and for oxygen flux in this metabolic state).

In the absence of ATPase activity, LEAK flux is equal in states $S(Rot)_N$, $S(Rot)_T$ and $S(Rot)_{Omy}$, all providing an estimate of LEAK respiration, $S(Rot)_L$. The LEAK control ratio, *L/E*, can be measured in intact cells [MiPNet08.09; MiPNet10.04],

$$L/E = C_L / C_E \quad (7)$$

If ATPase activity is responsible for $S(Rot)_T > S(Rot)_{Omy}$, the increased $S(Rot)_T$ increases the apparent *L/E* ratio, and $S(Rot)_{Omy}$ provides a more accurate estimate of LEAK. C_L and C_E are LEAK respiration and ETS capacity measured in coupled oligomycin-inhibited and non-coupled cells, corrected for residual oxygen consumption. In cells, the **ROUTINE control ratio**, *R/E*, is,

$$R/E = C_R / C_E \quad (8)$$

where C_R is ROUTINE respiration, which is partially coupled and reflects the state of activation of cellular respiration according to routine ATP demand and degree of coupling. The (inverse) $UCR_{E/R}$ is an expression of the capacity of the electron transfer system relative to ROUTINE respiration (Steinlechner-Maran et al 1996; Renner et al 2003; Hütter et al 2004).

Analogous to the ROUTINE control ratio in cells, the **phosphorylation system control ratio**, *P/E*, relates OXPHOS capacity (*P*; State 3 at saturating [ADP]) to non-coupled respiration. In the present example, the OXPHOS/ETS control ratio, $P/E = UCR_{E/P}^{-1}$ is,

$$P/E = S(Rot)_P / S(Rot)_E \quad (9)$$

The *P/E* control ratio is an expression of how close the capacity of partially coupled oxidative phosphorylation (*P*) approaches the capacity for non-coupled electron transfer (*E*). If *P/E* < 1.0, then the phosphorylation system exerts control over OXPHOS capacity.

Comparable to ROUTINE respiration in intact cells, various intermediary ADP controlled states, $S(Rot)_{T[ADP]}$, can be established by varying ADP supply over a wide range (Gnaiger et al 2000). Corresponding steady-state ADP concentrations regulate respiratory flux at graded levels between the minimum of LEAK flux and the maximum of OXPHOS capacity. OXPHOS capacity at state TD can be calculated as J_{max} from hyperbolic ADP kinetics, or directly measured at saturating ADP concentration.

4. Summary of Respiratory States and Coupling Control Ratios in Isolated Mitochondria and Cells

Mitochondrial respiratory states and coupling control ratios are defined in isolated mitochondria or permeabilzed cells and tissues, at a given substrate (and inhibitor) combination (X), and in intact cells (C). In a medium without energy substrates, cells respire on endogenous substrate (Ce), whereas culture medium or medium of varied composition m provides substrates for respiration and growth (Cm). Isolated mitochondria (Imt) are distinguished from intact cells (C), permeabilized cells (PC) or permeabilized tissue (PT).

4.1. Residual Oxygen Consumption - ROX

Imt_{ROX} Oxygen uptake due to residual oxidative side reactions in isolated mitochondria, estimated by inhibiting various respiratory complexes after uncoupling, is used to correct mitochondrial respiratory states. Correction is controversial due to the possible induction of electron leak from the electron transfer system by application of specific inhibitors.

PC_{ROX} or PT_{ROX}, probably higher than Imt_{ROX}, where isolation eliminates organelles and non-mitochondrial membranes

with oxygen-consuming activity.

C_{ROX} Residual oxygen consumption in intact cells, higher than PC_{ROX}, where permeabilization eliminates specific substrates for ROX.

4.2. ETS Capacity - State *E*

X_E = X_E'-Imt_{ROX} (in Imt; X_E'-PC_{ROX} ín PC); ETS capacity (non-coupled respiration) in the presence of substrate X.

C_E = C_E'-C_{ROX}; non-coupled respiration, measure of ETS capacity at optimum uncoupler concentration. Apparent ETS capacity, C_E' (non-coupled respiration, not corrected for C_{ROX}). Level flow in the terminology of thermodynamics of irreversible processes.

ROX/*E*' = C_{ROX}/C_E'; flux control ratio of oxidative side-reactions, normalized for total uncoupled respiratory flux.

4.3. OXPHOS Capacity – State *P*

X_P OXPHOS capacity, measured after activation by saturating ADP concentration. X_P may be estimated in the coupled states X_D or X_{TD} (State 3), corrected for ROX.

P/E = X_P/X_E; OXPHOS control ratio, measures how close X_P approaches the upper limit of ETS capacity, X_E. Excess ETS capacity over the phosphorylation system yields *P/E*<1.0; weak coupling reduces the effect of ETS excess capacity and increases respiration (X_P) without increasing phosphorylation.

4.4. ROUTINE Respiration – State *R*

C_R = C_R'-C_{ROX}; ROUTINE respiration (ROX-corrected).

R/E = C_R/C_E; ROUTINE control ratio, measures how close ROUTINE activity of cells approaches the upper limit of C_E.

4.5. LEAK Respiration – State *L*

X_L = X_L'-X_{ROX}; LEAK respiration, in the partially coupled state after eliminating phosphorylation, e.g. after depletion of ADP in the presence or absence of ATP (N or T), or after inhibiting ANT (Cat, Atr) or ATP synthase (Omy). Static head in thermodynamics of irreversible processes.

C_L LEAK respiraton in the cell, measured after inhibiting the ANT (Cat, Atr) or ATP synthase (Omy).

L/E = C_L/C_E (or X_L/X_E); LEAK control ratio, measures how close C_L or X_L approaches the upper limit of C_E or X_E, which is reached in non-coupled mitochondria (*L/E*=1.0).

net*R/E* = (*R*-*L*)/*E*; net ROUTINE control ratio; fraction of ETS capacity directly utilized to drive phosphorylation of ADP to ATP.

Appendix

A1. Abbreviations

A1.1. Abbreviations for substrates of the TCA cycle and major entries (single capital letters for the most commonly used substrates)

P	Pyruvate
G	Glutamate
M	Malate
S	Succinate
F	Fumarate
Og	Oxoglutarate
Ce	Cellular substrates *in vivo,* endogenous
Cm	Cellular substrates *in vivo*, with exogenous substrate supply from culture medium or serum

A1.2. Other substrates and redox components of the respiratory system

Oca	Octanoate
Paa	Palmitate
Oct	Octanoyl carnitine
Pal	Palmitoyl carnitine
As	Ascorbate
Tm	TMPD
c	Cytochrome *c*
Gp	Glycerophosphate

A1.3. Phosphorylation system (adenylates, P_i, uncouplers, downstream inhibitors of ATP synthase, ANT, or phosphate) are denoted by subscripts. If P_i is always present at saturating concentration, it does not have to be indicated in the titration protocols.

P_i	Inorganic phosphate
N	no adenylates added (state *L*)
D	ADP at saturating concentration (state *P*: saturating [ADP])
D0.2	ADP at specified concentration (saturating versus non-saturating ADP is frequently not specified in State 3)
T	ATP (state L_T)
TD	ATP+ADP (state *P*, in the presence of physiological high (mM) ATP concentrations)
T[ADP]	High ATP and varying ADP concentrations, in the range between states T and TD.
0my	Oligomycin (state L_{Omy})
Atr	Atractyloside (state L_{Atr})
u	Uncoupler at optimum concentration for maximum non-coupled flux (state *E*).

A1.4. Inhibitors of respiratory complexes, dehydrogenases or transorters:

Ama	Antimycin A
Azd	Sodium azide
Hci	Hydroxycinnamate
Kcn	KCN
Mna	Malonate
Myx	Myxothiazol
Rot	Rotenone

A1.5. Respiratory states, flux control ratios and protocols

Coupling control states

E	Electron transfer system capacity state
L	LEAK state
P	OXPHOS capacity state
R	ROUTINE state of cell respiration

Coupling control ratios (*CCR*)

L/E	LEAK *CCR*
P/E	Phosphorylation system *CCR*
R/E	ROUTINE *CCR*

Protocols

(i) Letters in normal font are used for the substrates X;
(ii) Subscripts are used for effectors of the phosphorylation system and for indicating coupling control states.

Example: In the protocol *:PM_N +D +*c* +G +S +F +(Rot) +(Myx+..), the respiratory state after addition of rotenone is: PMGS*c*$(Rot)_E$. With reference to this protocol, it may be convenient to use an abbreviation, such as S$(Rot)_E$, e.g. if cytochrome *c* addition is used as a quality control and exerts no effect on respiratory capacity.
Suspended cells may be permeabilized in the oxygraph chamber with digitonin (Dig), after measurement of endogenous respiration in mitochondrial respiration medium. The initial protocol is indicated above as *; referring to the initial steps of endogenous respiration and permeabilization: Ce_R +PM +Dig.

A2. Conversion Factors for Units of Oxygen Flux

Comparability of quantitative results on respiratory fluxes is aided by using common units. Considering the variety of units used in various disciplines of respiratory physiology (Gnaiger 1983), a common basis may only be found with reference to proper *SI* units (Gnaiger 1983, 1993).

When respiratory activity is expressed per volume of the experimental oxygraph chamber as volume-specific oxygen flux, the base *SI* unit is mol·s^{-1}·m^{-3}. Since the oxygen concentration in pure water at equilibrium with air at standard barometric pressure of 100 kPa is 254.8 to 207.3 µmol·dm^{-3} (25 to 37 °C), it is most practical to express oxygen concentration in units µmol/litre [MiPNet10.07],

1 µmol O_2/litre = 1 µmol/dm^3 = 1 µM = 1 nmol/ml = 1 nmol/cm^3

The proper *SI* unit, µmol O_2·s^{-1}·dm^{-3}, is used for the corresponding respiratory flux in the classical bioenergetic literature (Chance and Williams 1956). In the bioenergetic context of H^+/2 e or H^+/O ratios (Mitchell and Moyle 1967) or P:O ratios, corresponding fluxes were then frequently expressed as J_P/J_O, where $J_{O2} = 2 \cdot J_O$, the latter in 'bioenergetic' units [natoms O·s^{-1}·ml^{-1}] (Slater, Roging, Mol 1973). In bioenergetics a variety of expressions is used for units of amount of oxygen (natoms oxygen; natoms O; ng.atom O; nmol O), with the identical meaning: 0.5 nmol O_2.

Table 3. Conversion of various units into *SI* units when expressing respiration as mass-specific oxygen flux.

J	[Unit$_i$]	x	Factor	=	J_{O2} [*SI*-Unit]
					nmol O_2·s^{-1}·g^{-1}
					pmol O_2·s^{-1}·mg^{-1}
12	ng.atom O·min^{-1}·mg^{-1}	x	8.33	=	100
12	µmol O·min^{-1}·g^{-1}	x	8.33	=	100
12,000	natom O·min^{-1}·g^{-1}	x	0.00833	=	100
6	nmol O_2·min^{-1}·mg^{-1}	x	16.67	=	100
6	µmol O_2·min^{-1}·g^{-1}	x	16.67	=	100
6	mmol O_2·min^{-1}·kg^{-1}	x	16.67	=	100

Mass-specific oxygen flux, J_{O2}, is obtained from volume-specific flux, $J_{V,O2}$, when the mass-concentration is known of the biological material (Table 3). When respiration is expressed per cell (or per million cells), then the extensive quantity (per system) is distinguished as oxygen flow, I_{O2} [pmol·s^{-1}·10^{-6} cells], from the size-specific expression, e.g. per mg cell protein, J_{O2} [pmol·s^{-1}·mg^{-1}] (Gnaiger 1993; Renner et al 2003).

References: Gnaiger_2012_MitoPathways_References.pdf