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Characterisation of oxidative phosphorylation in skeletal muscle mitochondria subpopulations in pig: a study using top-down elasticity analysis

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Abstract In skeletal muscle, two mitochondrial populations are present which, on the basis of their localisation, are termed intermyofibrillar and subsarcolemmal mitochondria (IMF and SS, respectively). These two populations have different biochemical characteristics and show different responses to physiological stimuli. In this paper, we characterise the oxidative phosphorylation of SS and IMF using 'top-down' elasticity analysis. We excluded the possibility that their different characteristics can be attributed to a different degree of breakage of the two types of mitochondria due to the different isolation procedures used in their preparation. The higher respiration rate and higher respiratory control ratio shown by IMF compared with those shown by SS are principally due to the higher activities of the reactions involved in substrate oxidation as confirmed by the measurement of cytochrome oxidase activity. There is no difference in the leak of protons across the inner mitochondrial membrane between IMF and SS; a faster rate of ATP synthesis and turnover is driven by the lower membrane potential in SS compared with in IMF.

Key words: Skeletal muscle mitochondrion; Top-down elasticity analysis; Oxidative phosphorylation; Respiratory chain

1. Introduction

Skeletal muscle is one of the major oxygen-consuming tissues and its contribution to the body's metabolic rate is significant. In fact, it has been reported that skeletal muscle mitochondria show a higher respiration rate than those in the liver, kidney and brain [1]. In skeletal muscle two different mitochondrial populations have been described. One is located between the myofibrils and are known as intermyofibrillar mitochondria (IMF). The other type, subsarcolemmal mitochondria (SS), are located just beneath the sarcolemma.

Different characteristics have been described for these two populations in both newborn and growing animals: they show differences in lipid content [2,3], enzyme activity [2,3], respiration rate [2–9], protein import [10] and protein synthesis [2]. Most reports have shown that SS exhibit both basal (state 4) and ADP-stimulated (state 3) respiration rates which are much slower than those of IMF [2–9]. Moreover, the ratio between state 4 and state 3 (respiratory control ratio, RCR, a parameter often used as an index of mitochondrial preparation integrity) is lower in SS than in IMF. In addition, the two populations show different responses to some stimuli such as cold exposure [5–9], exercise and immobilisation [4].

The actual physiological significance of the observed differences in the functional properties of SS and IMF is still a matter of debate because (1) the isolation procedures used for the two populations are different and hence the variation observed could be due to differences in the damage suffered by the two populations and (2) the existence of partial mitochondrial connectivity in skeletal muscle has been proposed [11]. In fact, as a result of their localisation SS are easily released during homogenisation of the tissue, while IMF are only released after enzymatic digestion of the tissue homogenate. Moreover, the RCR value is a good index of mitochondrial integrity only if it is used to compare the same kind of mitochondrial populations (or subpopulations) obtained from the same kind of tissue and from animals in the same physiological status. Recent publications have started to address this issue [2,9] and evidence is accumulating to suggest that differences between the two populations are not due to the isolation procedures used. A reduced RCR, on the other hand, could be due not only to damage to a particular mitochondrial fraction but to an increased inner membrane permeability to protons (which principally affects state 4), to a reduced activity of the respiratory chain (which principally affects state 3 and to a lesser extent state 4) or to a reduction in the activity of the phosphorylating system (which only affects state 3) [12]. Consequently the large difference in RCR value between the two mitochondrial subpopulations can also be attributed to their biochemical characteristics and to their different functions in the cell.

A full description of SS and IMF oxidative phosphorylation may enable us to increase our understanding of their different roles in the cell. To try to achieve this, we applied a particular kinetic approach, the top-down elasticity analysis, to analyse the metabolic control of energy metabolism in IMF and SS (for review see [13]). To further characterise the oxidative and phosphorylative potential of these two populations, cytochrome oxidase (COX) and F1-ATPase activities were also measured. So that we could attribute the results

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Abbreviations: SS, subsarcolemmal mitochondria; IMF, intermyofibrillar mitochondria; RCR, respiratory control ratio; COX, cytochrome oxidase; $\Delta\Psi$, membrane potential; Ph_3MeP^+, triphenylmethylphosphonium

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obtained to the different biochemical characteristics of SS and IMF rather than to differences in the damage inflicted on them (due to the different isolation procedures used) or to extramitochondrial contamination, we measured the activity of citrate synthase (a mitochondrial matrix enzyme) in the presence and absence of detergent and we also measured non-mitochondrial ATPase activity.

2. Materials and methods

2.1. Theory

A multi-enzymatic complex of reactions that constitute a system such as oxidative phosphorylation can be conceptually divided into blocks of reactions connected by a common intermediate: one block contains the reactions that produce the intermediate and the other block those that consume it. Using this approach, we considered oxidative phosphorylation to consist of three blocks of reactions connected by the mitochondrial inner membrane potential ($\Delta\Psi$): the substrate oxidation block (which consists of all the steps from external substrate to $\Delta\Psi$ and includes the respiratory chain, substrate dehydrogenase and substrate transport) which produces $\Delta\Psi$, the proton leak block (representing the cation cycles and the leak of protons across the inner membrane) and the phosphorylating system (which consists of all the reactions involved in the synthesis and export of ATP and includes ATPase, the Pi transporter and the adenosine nucleotide carrier) (for further details see [13,14]). Using top-down elasticity analysis we can evaluate differences in the overall kinetic responses shown by substrate oxidation, the phosphorylating system and the proton leak to a change in $\Delta\Psi$ and we can determine the distribution of control of oxidative phosphorylation.

2.2. Mitochondrial isolation procedure

SS and IMF were isolated from the longissimus thoracis skeletal muscle of 1-week-old piglets, as previously described in Herpin et al. [15]. Immediately post mortem, muscle samples were homogenised in buffer A (100 mM sucrose, 180 mM KCl, 50 mM Tris base, 10 mM EDTA, 5 mM $MgCl_2$ and 1 mM ATP, pH 7.4) in an ice-cold Teflon pestle–glass Potter–Elvehjem homogeniser. Mitochondria were then obtained by differential centrifugation and suspended in storage buffer B (250 mM sucrose, 2 mM EDTA and 20 mM Tris base, pH 7.4). Gentle use of Nagarse (2 mg/g tissue) (Serva, Heidelberg, Germany) was necessary to digest myofibrils and to liberate IMF.

The mitochondrial protein content was determined by the Hartree method, with bovine serum albumin (BSA) as standard [16].

Mitochondrial integrity, $\Delta\Psi$ and the respiration rate were evaluated using fresh mitochondria, with some aliquots being frozen at −80°C for further analysis of COX and F1-ATPase activity. Mitochondrial integrity was evaluated by measuring the citrate synthase activity in the presence and absence of detergent, following the method of Robinson et al. [17]

2.3. COX activity

Measurements of COX activity were performed using a modification of the method described by Errede and Kamen [18]. Frozen-thawed mitochondria were diluted 10-fold and the oxidation of ferrocytochrome *c* (final concentration 56 μM) was followed using a Shimadzu Graphicord UV-240 spectrophotometer for 1 min at 550 nm and 25°C in a 35 mM KH_2PO_4 buffer, pH 7.4, containing 1 mM EDTA. Results were expressed as nmol cytochrome *c*/min/mg mitochondrial proteins.

2.4. F1-ATPase activity and non-mitochondrial ATPase activity

The F1-ATPase activity was monitored spectrophotometrically through a linked-enzyme system following the oxidation of NADH at 340 nm and 38°C using a modification of the method described by Krieger et al. [4]. The activity was measured in 3 ml of reaction medium containing 100 mM KCl, 5 mM $MgCl_2$, 0.1 mM EGTA, 5 mM ATP, 1 mM phosphoenolpyruvate, 20 mM HEPES (pH 7.4), 15–20 U of pyruvate kinase–lactate dehydrogenase and 0.7 mM of NADH. After a 10 min temperature-equilibration period, the reaction was initiated by the addition of 10 μg of mitochondria previously solubilised in Chappell–Perry medium containing lubrol (100 mg/g of mitochondrial protein). The presence of contaminating non-mitochondrial material in the IMF and SS preparations was evaluated by the difference in the activity levels measured in the presence and absence of 3 μg oligomycin.

2.5. Measurement of proton-motive force and respiration rate

Proton-motive force is the sum of $\Delta\Psi$ and the pH gradient across the mitochondrial membrane. To simplify measurements, the pH gradient was abolished by the addition of nigericin so that the mitochondria increased $\Delta\Psi$ to compensate, and the whole of the proton-motive force was expressed as $\Delta\Psi$. $\Delta\Psi$ was determined from the distribution of the lipophilic cation triphenylmethylphosphonium (Ph_3MeP^+), which was measured using a Ph_3MeP^+-sensitive electrode, as described by Brown and Brand [19]. A Ph_3MeP^+-binding correction of 0.4 was applied for both IMF and SS, as suggested by Rolfe et al. [1]. The respiration rate was determined simultaneously using a Clark-type oxygen electrode. The mitochondrial respiration rate and $\Delta\Psi$ were measured in a mitochondrial suspension (0.5 and 1 mg/ml for IMF and SS, respectively) at 37°C in a standard incubation medium containing 80 mM KCl, 50 mM HEPES (pH 7), 1 mM EGTA, 5 mM K_2HPO_4, 4 μM rotenone, 80 ng/ml nigericin and 1% BSA using a saturating amount of succinate as substrate (5 mM).

State 4 and state 3 of respiration were initiated, respectively, by the addition of 5 mM succinate and, 3 min later, 100 μM ADP.

2.6. Evaluation of the kinetic responses of 'proton leak', 'phosphorylation system' and 'substrate oxidation' to a change in $\Delta\Psi$ in SS and IMF

For all determinations, the mitochondria were incubated in the incubation medium for 3 min before the addition of 4 μM Ph_3MeP^+, to allow us to calibrate the electrode. Respiration was then initiated by the addition of 5 mM succinate. For the evaluation of the kinetic response shown by the mitochondrial proton leak to a change in $\Delta\Psi$ in non-phosphorylating mitochondria, it is necessary to perform a titration with an inhibitor of substrate oxidation. To this end the incubation medium was supplemented with oligomycin (1 μg/ml) and the respiration rate was then inhibited by sequential addition of malonate up to 5 mM.

For evaluation of the kinetic response shown by the phosphorylating system to a change in $\Delta\Psi$ in phosphorylating mitochondria, it is necessary to perform a titration with an inhibitor of substrate oxidation. In this case, the incubation medium was supplemented with a saturating amount of ADP (500 μM). The state 3 respiration rate was inhibited by the sequential addition of malonate (up to 0.6 and 1.2 mM for SS and IMF, respectively) and, at any given $\Delta\Psi$, the value of the respiration rate for the proton leak was subtracted. Assuming that the H^+/O stoichiometry of the respiratory chain and the H^+/ATP ratio of the phosphorylating system both remain constant as $\Delta\Psi$ is varied, this method gives a measurement of mitochondrial ATP synthesis.

For evaluation of the kinetic response shown by substrate oxidation to a change in $\Delta\Psi$, a titration with oligomycin starting near state 3 has to be performed. For this, the incubation medium was supplemented with a saturating amount of ADP (500 μM) and state 3 of respiration was inhibited by the sequential addition of oligomycin (up to 1 μg/ml).

Flux control coefficients were obtained as reported by Hafner et al. [12].

2.7. Statistics

The statistical significance of the differences between SS and IMF was determined using Student's *t*-test. Values were considered significant when $P < 0.05$.

3. Results

3.1. Respiratory parameters, oxidative capacity, F1-ATPase activity and mitochondrial integrity in SS and IMF subpopulations

The IMF subpopulation exhibited significantly higher values of the measured respiratory parameters than SS (see Table 1); in particular, in IMF, state 4, state 3 and RCR were higher than in SS by 53, 200 and 95%, respectively. Table 1 also shows COX and F1-ATPase activities representing the oxida-

tive capacity and phosphorylating potential of the mitochondria, respectively. The COX activity was about three times higher in IMF than SS, whereas the F1-ATPase activity did not differ between the two populations.

To assess if the aforementioned differences might be due to eventual mitochondrial damage or contamination, the percentage of broken mitochondria in each fraction was calculated and the non-mitochondrial ATPase activity was measured. The citrate synthase activity in the presence and absence of Triton is shown in Table 2. The activity measured in the presence of Triton is considered to reflect 100% breakage. From the ratio of the citrate synthase activities with or without Triton, we calculated that 77% SS and 87% IMF were intact. This slight difference in mitochondrial breakage cannot be responsible for the large metabolic differences between the two populations described here. Moreover, the levels of activity of non-mitochondrial ATPase (Table 1) indicate that there was no significant difference in terms of contamination between the two mitochondrial populations.

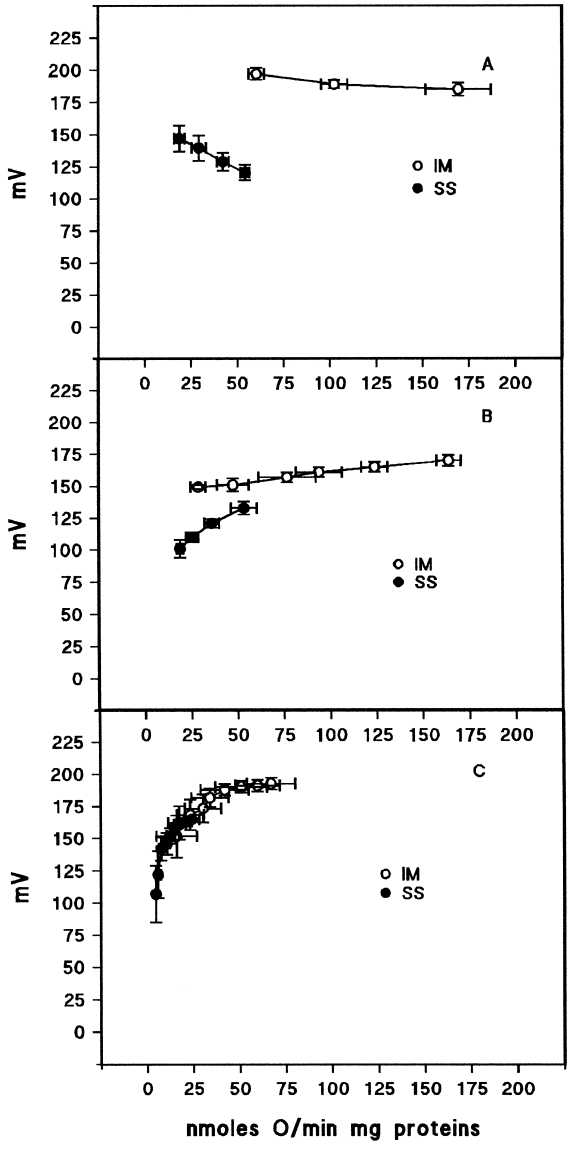

Fig. 1. Kinetic responses shown by substrate oxidation (A), the phosphorylating system (B) and proton leak (C) to a change in $\Delta\Psi$: comparison between IMF and SS.

Table 1
State 4, state 3, RCR, COX activity and F1-ATPase activity of IMF and SS

	SS	IMF
State 4	67 ± 5	102 ± 9*
State 3	149 ± 12	446 ± 21*
RCR	2.3 ± 0.2	4.5 ± 0.5*
COX	1021 ± 361	3020 ± 246*
F1-ATPase	0.25 ± 0.04	0.24 ± 0.04

Respiration is measured as nmol O/min/mg protein. F1-ATPase activity is measured as nmol Pi/min/mg protein. COX activity is measured as nmol cytochrome *c*/min/mg protein. Values are the mean ± S.E.M. of four different mitochondrial preparations for the value of states 4 and 3, and of three preparations for the COX and F1-ATPase activities. *$P < 0.05$ versus SS.

3.2. Kinetic responses of 'substrate oxidation', 'phosphorylation system' and 'proton leak' to changes in $\Delta\Psi$ in SS and IMF

Fig. 1A depicts the response of the substrate oxidation system at any value of $\Delta\Psi$ as the rate of oxygen consumption per mg of mitochondrial protein. It shows that the SS substrate oxidation system is less active than that of IMF, since it generates a lower $\Delta\Psi$. Fig. 1B shows the kinetic response of the phosphorylating system to $\Delta\Psi$: the two mitochondrial subpopulations show differences in the activity of their phosphorylating system, with SS being more active than IMF. In other words, a given value for the sum of ATP synthesis and export is driven by a lower $\Delta\Psi$ in SS than in IMF.

Fig. 1C shows the kinetic response of proton leak to a change in $\Delta\Psi$, i.e. the response to a change in $\Delta\Psi$ of the oxygen consumption used to drive the proton leak flux. The proton flux is the same in SS and IMF at any given $\Delta\Psi$.

3.3. Control of oxidative phosphorylation

Table 3 shows the control exerted by the proton leak, substrate oxidation and phosphorylating system over respiration and oxidative phosphorylation in SS and IMF. This was analysed both for non-phosphorylating mitochondria (state 4) and phosphorylating mitochondria (state 3). The control coefficients were calculated using the data given in Fig. 1 and by applying the equation reported by Hafner et al. [12]. The degree of control exerted by any block in our simplified system of oxidative phosphorylation (see Section 2.1) is expressed as a coefficient. These coefficients represent the fractional change in the flux through a given block resulting from an infinitesimally small change in the activity of the same or other block. Moreover, control coefficients give information on the relative importance, under a certain set of conditions, of each block of the system in controlling the flux. The control that the proton leak, substrate oxidation and phosphorylating

Table 2
Citrate synthase activity of SS and IMF measured in the absence (intact) and presence (broken) of Triton

	SS	IMF
Intact	0.080 ± 0.02	0.066 ± 0.01
Broken	0.340 ± 0.05	0.510 ± 0.03*
% breakage	23.5 ± 2.5	13.3 ± 1.7*
Non-mitochondrial ATPase	0.070 ± 0.052	0.138 ± 0.038

Citrate synthase activity is expressed in μmol CoA/min/mg protein. Non-mitochondrial ATPase is measured as nmol Pi/min/mg protein. Results are presented as mean ± S.E.M. of values from four pigs in each group, with the exception of non-mitochondrial ATPase, which was the mean of values from five pigs. *$P < 0.05$ versus SS.

Table 3
Control of oxidative phosphorylation in SS and IMF

	IMF		SS	
	state 4	state 3	state 4	state 3
Control over respiration by:				
Substrate oxidation	0.127	0.337	0.225	0.445
Phosphorylating system	–	0.541	–	0.508
Proton leak	0.873	0.083	0.774	0.078
Control over phosphorylation by:				
Substrate oxidation	–	0.420	–	0.450
Phosphorylating system	–	0.636	–	0.587
Proton leak	–	−0.056	–	−0.038
Control over proton leak by:				
Substrate oxidation	0.127	0.09	0.225	0.160
Phosphorylating system	–	−0.08	–	−0.146
Proton leak	0.873	0.91	0.775	0.986
Control over $\Delta\Psi$ by:				
Substrate oxidation	0.0929	0.0634	0.587	0.420
Phosphorylating system	–	−0.055	–	−0.384
Proton leak	−0.0929	−0.084	−0.587	−0.036

Data represent values for control coefficients calculated from the kinetic data shown in Fig. 1 using the equation reported by Hafner et al. [12].

system exert over $\Delta\Psi$ represents the fractional change in the value of $\Delta\Psi$ due to an infinitesimally small change in the activity of one block and it gives information on the homeostatic control of $\Delta\Psi$ (for more details see [1,12]). An eventual negative value means that the blocks compete with each other for the generation of $\Delta\Psi$; in other words, an increase in the flux through one block will result in a decrease in the flux through another.

For the simplified model used in this analysis, the pattern of control was similar for the two mitochondrial populations, with the exception of the control over $\Delta\Psi$. In particular, as already observed for other tissues and for the whole skeletal muscle population [1], we found that for both SS and IMF the control over state 4 respiration is predominantly due to the proton leak (87 and 77% for IMF and SS, respectively), with substrate oxidation exerting only a minor degree of control (13 and 23%, respectively). In the case of state 3, the control over respiration is shared between the substrate oxidation block and the phosphorylating system, with only a minor degree of control being due to the proton leak. In contrast, a difference between SS and IMF in the control of $\Delta\Psi$ was observed. In the case of SS, the control exerted over $\Delta\Psi$ by each block is greater than in the case of IMF. In other words, small variations in the activity of one block strongly affect the value of $\Delta\Psi$ in SS but not in IMF, suggesting that the homeostatic control of $\Delta\Psi$ is more efficient in IMF.

4. Discussion

In good agreement with previous observations in rats [2–4], ducklings [9] and piglets [6,7,20], our results show that SS and IMF have different biochemical characteristics and demonstrate that these differences are essentially due to the much smaller contribution made by the substrate oxidation reactions in SS than in IMF.

It was conceivable that the difference observed in respiratory activity and coupling between SS and IMF might be attributable either to the different isolation procedures used or to contamination. However, we excluded these possibilities for the following reasons:

1. The slight difference in biochemical breakage between SS and IMF (about 10%) cannot account for the large difference in RCR value (2.2 U) between these two populations. In addition, the insignificant difference in non-mitochondrial ATPase activity between SS and IMF allows us to exclude the possibility that the large differences in biochemical characteristics between the two mitochondrial subpopulations might be due to a difference in extramitochondrial ATPase contamination.
2. If SS had suffered more breakage than IMF, we should have expected a difference in the kinetic response shown by the proton leak to a change in $\Delta\Psi$, i.e. at the same given $\Delta\Psi$ the respiration rate should have been much higher in SS than in IMF. However, the kinetic response shown by the proton leak to a change in $\Delta\Psi$ did not differ between SS and IMF (see Fig. 1).
3. It might be thought that the reduced RCR in SS could be attributed to the different exposure times of SS and IMF to the free fatty acid (known to uncouple mitochondrial respiration) present in the homogenate during the isolation procedure. However, this possibility was excluded by measuring the respiration rate in the presence of BSA free of fatty acid. Moreover, other reports have shown that resuspension of IMF in 'subsarcolemmal supernatant' instead of in isolation medium does not affect the RCR and respiration of IMF [9].

The application of 'top-down' elasticity analysis to SS and IMF gives a complete description of their oxidative phosphorylation. It shows that no differences in proton leak exist between the two subpopulations, although differences can be detected in the reactions involved in substrate oxidation and in the synthesis and export of ATP. In particular, the overall reactions involved in the production of $\Delta\Psi$ (i.e. those involved in the oxidation of the substrate) are less active in SS than in IMF. This is also confirmed by the low COX activity, indicating that SS have a low oxidative potential. When we examined the overall reactions involved in the synthesis and export of ATP, we found their activity levels to be greater in SS than in IMF and we found no difference in F1-ATPase activity. Despite this, state 3 of respiration proceeds at more than twice the rate in IMF than in SS; this phenomenon is due to the higher activity of the substrate oxidation block in IMF. In

other words, the system involved in the production of the proton-motive force is less active in SS than IMF, with the consequence that $\Delta\Psi$ is lower (both in state 4 and in state 3). In non-phosphorylating mitochondria, the oxygen consumption is used to balance the leak of protons across the membrane; in this case, a lower $\Delta\Psi$ will drive a lower proton flux and, consequently, in state 4 a lower oxygen consumption is detected in SS. Despite the fact that the phosphorylating system is more active in SS than in IMF, the maximal production of ATP and the oxygen consumption (state 3) will be lower in SS than in IMF because of the low value of the driving force in SS.

The RCR value depends on the balance of three factors that are able to affect states 4 and 3: (a) the permeability of the inner membrane, (b) substrate oxidation and (c) the phosphorylating system (see Table 3). As no difference in the kinetic response of the proton leak to a change in $\Delta\Psi$ was found between SS and IMF, it is possible to exclude the first factor as the origin of the difference in RCR value observed between the two mitochondrial subpopulations. On the other hand, the remaining two factors, both of which showed different activity levels between the two mitochondrial populations, may presumably make relevant contributions. The fact that a higher activity of the reactions involved in the synthesis and export of ATP was detected in SS than IMF, implying a considerable positive control over state 3 and no control over state 4, would lead us to expect a higher value of RCR in SS than in IMF. On the other hand the fact that a lower activity of the reactions involved in the oxidation of the substrate was detected in SS than in IMF, principally affecting state 3 and to a lesser extent state 4, would lead us to expect a lower RCR in SS than in IMF. The overall effect of balance between the above factors actually resulted in a lower RCR in SS than in IMF, indicating that the contribution made by the lower activity of the substrate oxidation exceeds that made by the higher activity of the phosphorylating system.

The physiological significance of the differences observed in the biochemical characteristics between the IMF and SS subpopulations is not yet clear. However, some hypotheses can be proposed. For example, they could represent two structurally distinct mitochondrial populations able to respond differently to various physiological stimuli or they could represent a single population at different states of maturation. Whatever the origins of SS and IMF, our results clearly show that they have distinct biochemical and functional properties. In the cell, they could well play complementary roles as already suggested by Schmidt and Herpin [20]. Since IMF have a high capacity for ATP production, they could supply the energy for muscle contraction and for the regulation of sarcoplasmic calcium during the contraction–relaxation cycle [21]. SS, which are nearer the capillaries, could be involved in providing the energy necessary for the transport of oxygen from the erythrocytes to the muscle cell, for the phosphorylation of some sarcolemmal proteins [22] and for the transport of ions [23] and metabolites [24] across the sarcolemma. At a more speculative level, some other hypotheses are proposed. The low level of respiration in SS could be important in preserving the available oxygen for IMF, which are localised further from the erythrocyte. Thereby, SS could play a crucial role in the antioxidant defences of the skeletal muscle cell, regulating both the diffusion of the oxygen and its concentration within the cell. A decoupled respiration, such as that present in SS can, in fact, be regarded as a useful mechanism for removing excess oxygen and preventing the accumulation of oxygen radicals, as suggested by Skulachev [25]. Moreover, the low $\Delta\Psi$ found in SS should allow these mitochondria to reduce the intracellular oxygen concentration with only a limited formation of reactive oxygen species. The last hypothesis is supported by the work of Korshunov et al. [26] showing that the generation of reactive oxygen species is a function of $\Delta\Psi$.

In conclusion, our results clearly confirm that SS and IMF have distinct biochemical properties. In addition they show that this is due solely to a much lower activity level in oxidative pathways and a weaker ability to generate the $\Delta\Psi$ of SS. Further investigations, however, will be required to fully understand the significance of the lower respiratory potential of SS.

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References

[1] Rolfe, D.F.S., Hulbert, A.J. and Brand, M.D. (1994) Biochim. Biophys. Acta 1118, 405–411.
[2] Cogswell, A.M., Stevens, R.J. and Hood, D.A. (1993) Am. J. Physiol. 264, C383–C389.
[3] Palmer, J.W., Tandler, B. and Hoppels, C. (1977) J. Biol. Chem. 252, 8731–8739.
[4] Krieger, D.A., Tate, C.A., McMillin-Wood, J. and Booth, F.W. (1980) J. Appl. Physiol. 48, 23–28.
[5] Skulachev, V.P., Maslov, S.P., Sivkova, V.G., Kalinichenko, L.P. and Maslova, G.M. (1963) Biochemistry (Moscow) 25, 54–60.
[6] Herpin, P. and Barrè, H. (1989) Comp. Biochem. Physiol. 92B, 59–65.
[7] Herpin, P. and Lefaucheur, L. (1992) J. Therm. Biol. 17, 277–285.
[8] Goglia, F., Lanni, A., Duchamp, C., Rouanet, J.L. and Barrè, H., Comp. Biochem. Physiol. 106B, 95–101.
[9] Roussel, D., Rouanet, J.L., Duchamp, C. and Barrè, H. (1998) FEBS Lett. 439, 258–262.
[10] Takahashi, M. and Hood, D.A. (1996) J. Biol. Chem. 44, 27285–27291.
[11] Kirkwood, S.P., Munn, E.A. and Brooks, G.A. (1986) Am. J. Physiol. 251, C395–C402.
[12] Hafner, R.P., Brown, G.C. and Brand, M.D. (1990) Eur. J. Biochem. 188, 313–319.
[13] Brand, M.D. (1997) J. Exp. Biol. 200, 193–202.
[14] Lombardi, A., Lanni, A., Moreno, M., Brand, M.D. and Goglia, F. (1998) Biochem. J. 330, 521–526, C395–C402.
[15] Herpin, P., Berthon, D., Duchamp, C., Dauncey, M.J. and Le Dividich, J. (1996) Reprod. Fertil. Dev. 8, 147–155.
[16] Hartree, E.F. (1972) Anal. Biochem. 48, 422–427.
[17] Robinson, J.B., Brent, L.G., Sumegi, B. and Srere, P.A. (1987) in: Mitochondria: A Practical Approach (Darley-Usmar, V.M., Rickwood, D. and Wilson, M.I., Eds.), pp. 153–170, IRL Press, Oxford.
[18] Errede, B. and Kamen, M.D. (1978) Biochemistry 21, 17(6), 1015–1027.
[19] Brown, G.C. and Brand, M.D. (1985) Biochem. J. 234, 399–405.
[20] Schmidt, I. and Herpin, P. (1997) Comp. Biochem. Physiol. 118B, 639–647.
[21] Carafoli, E. (1975) J. Mol. Cell. Cardiol. 7, 83–89.
[22] Walaas, O., Walasa, E., Liptad, E., Altersen, A.R., Horn, R.S. and Fossum, S. (1972) FEBS Lett. 80, 417–422.
[23] Sjodin, R.A. and Beauge, L.A. (1973) J. Gen. Physiol. 61, 22–250.
[24] Muller, W. (1976) Cell Tissue Res. 174, 367–389.
[25] Skulachev, V.P. (1996) Q. Rev. Biophys. 29, 169–202.
[26] Korshunov, S.S., Skulachev, V.P. and Starkov, A.A. (1997) FEBS Lett. 416, 15–18.