Limit to steady-state aerobic power of skeletal muscles

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Abstract

Like any other kind of cell, muscle cells produce energy by oxidizing the fuel substrate that they absorb together with the needed oxygen from the surroundings. Oxidation occurs entirely within the cell. It means that the reactants and products of reaction must at some time be dissolved in the cell's cytosol. If a cell operates at steady state, its cytosol composition remains constant. Therefore, the cytosol in a muscle that produces work at steady state must contain a constant amount of fuel, oxygen, and product of reaction dissolved in it. The greater the power produced, the higher the concentration of these solutes. There is a limit, however, to the maximum amount of solutes that the cytosol can contain without damaging the cell. General thermodynamic arguments, which are reviewed in this paper, help relate this limit to the dehydration and overhydration limits of the cell. The present analysis shows that the same limits entail a limit to the maximum power that a muscle can produce at steady state. This limit depends on the composition of the fuel mixture used by the muscle. The analysis also determines the number of fuel carbon atoms that must be oxidized in parallel within a cell to produce a given power. It may well happen that a muscle cannot reach the maximum attainable power because it cannot activate all the parallel oxidation paths that are needed to produce it. This may be due to a series of reasons ranging from health issues to a lack of training. The paper shows how the methods of indirect calorimetry can provide all the experimental data needed to determine the actual number of parallel oxidation paths that at steady state must be active in a muscle in a given exercise. A diagram relating muscle power to the number of parallel oxidation paths and fuel composition is finally presented. It provides a means to assess the power capacity of animal muscles and can be applied to evaluate their fitness, stamina, margins for improvement, and athletic potential.

Keywords Muscle power · Aerobic power · Steady-state animal power · Animal power limit · Cell energy production

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1 Introduction

Muscle cells produce energy aerobically by burning carbon. The oxidation process takes carbon from the fuel substrate (carbohydrates and lipids) within the cells and produces carbon dioxide and water. Under normal conditions, proteins provide a negligible contribution to muscle power and, for simplicity, will be ignored in what follows.

Apart from the fuel adopted, the overall chemistry of the process is the same as that occurring in internal combustion engines. In both cases, energy is taken from carbon oxidation; the end products are the same; and the power supplied in a given time interval is greater the greater the number of carbon atoms oxidized in that interval. In the cell, as in the engine, oxidation occurs entirely inside the system. This poses a limit on the maximum amount of power that the system can supply, since the system has a finite volume and the oxidation velocity, though large, is finite.

At a given pressure and temperature, the amount of fuel that can be burned in the combustion chamber of an internal combustion engine is limited. The value of this limit can be calculated from the thermodynamics of the gases and the stoichiometry of the oxidation. The determination of the analogous limit for a muscle cell is more complicated. It involves determining the maximum concentration of cytosol solutes that is compatible with the cell's life. In this case, too, however, the maximum amount of fuel that can be oxidized within a cell is limited by the general laws of classical thermodynamics. The theoretical basis for the derivation of this limit was originally proposed in [1]. The same concepts were subsequently applied in [2] to determine the maximum anaerobic power of skeletal muscles.

Section 2 presents a review of the main concepts involved in the establishment of the thermodynamic limit to oxidizable fuel. It also reports the results from [2] to be used in the present study. Though somewhat theoretical, this section may help give a better understanding of the thermodynamic approach adopted here and makes the present analysis reasonably self-contained. The reader more interested in the physiological aspects of this study may go directly to Section 3.

Muscles are made to produce mechanical work. Their mechanical power is, accordingly, of primary interest in many branches of muscle physiology and sports sciences. However, the main concern of the present paper is the muscle's *total power*. This is the ratio between the energy released from fuel oxidation (coinciding in practice with Gibbs free energy of reaction) and the time needed to release that energy. Total power is the sum of mechanical power and thermal power. The latter is the heat that the muscle liberates per unit time during its operation. By referring to the power produced by 1 kg of wet muscle tissue, we shall denote the mechanical power by p_m , thermal power by $\dot{q}$, and total power by p_t . Thus,

$$p_t = p_m + \dot{q} \quad (1)$$

The amount p_m of mechanical power that a muscle produces in a given exercise is to a good approximation proportional to the total power that it liberates. That is,

$$p_m = \eta p_t \quad (2)$$

The proportionality factor η , introduced here, is called the muscle efficiency or efficiency coefficient. It depends on the muscle type, muscle condition, and kind of exercise. It is defined as

$$\eta = \frac{W}{\Delta G_{fuel}} \quad (3)$$

where W indicates the work produced by the muscle and ΔG_{fuel} is the Gibbs free energy from the fuel oxidized to produce W . Different definitions of muscle efficiency are in use. They depend on the experiment considered, on the energy assumed as a source of work, on whether account is taken of basal energy expenditure, and so forth [3, 4]. Equation (2) is intended to refer to whole muscle tissue *in vivo*. As such, it implicitly accounts for the interactions of the contracting muscle with non-muscular tissues such as tendons (which may contribute to muscle efficiency [5]). The value of η may vary greatly in different animals, different muscle groups and different exercises. It ranges from less than 20% to about 68% and possibly more [4, 6–9].

Because muscle fuel oxidation occurs, to a good approximation, at constant volume, constant pressure and constant temperature, the values assumed by ΔG_{fuel} turn out to be very near to the enthalpy and internal energy changes produced by the oxidation. For this reason, the efficiency η given by Eq. (3) will almost coincide with some other muscle efficiencies introduced in the literature, such as the *gross* or *exercise efficiency* [7, 10, 11] and the *mechanical efficiency* [3, 12]. It should be stressed, moreover, that the efficiency defined in Eq. (3) refers to the whole transformation process from fuel oxidation to work production. As such, it should not be confused with other efficiency definitions that refer to particular steps of the whole fuel oxidation process. Reviews of this topic can be found in [3, 7, 13].

All of the above efficiencies are quite another thing than the thermodynamic efficiency introduced in classical thermodynamics and referring to the capacity of thermal machines to transform heat into work. A thermal machine produces work by taking heat from a hot source and transferring it to a cold sink. A muscle is not a thermal machine. It merely transforms chemical energy available from food (fuel) into other forms of energy, namely work and heat. No conversion of heat into work is involved here, nor could any such transformation occur, because living systems operate under almost isothermal conditions and the temperature jumps they witness are far too low for any significant transformation of heat into work to take place. For these reasons, the coefficient η appearing in Eq. (2) can in principle assume any value between 0 and 1, irrespective of temperature.

Because total power does not depend on as many factors as mechanical power, reference to p_t simplifies the analysis while still providing useful information on p_m . For a given muscle and a given exercise, all qualitative results concerning p_t (such as points of maximum or minimum power) also apply to p_m , since the latter is proportional to p_t , as apparent from Eq. (2). Thanks to the same equation, moreover, any quantitative result for p_t translates into a quantitative result for p_m , if the value of η relevant to the considered exercise is known.

This paper concerns the capacity of muscle cells to produce power in steady state conditions. In these conditions, the cytosol composition of the muscle cells remains constant in time. Because fuel oxidation occurs within the cells, it follows that, at steady state, the amount of reactants and products dissolved in the cytosol is constant. The overall number of moles of these cytosol solutes depends on the fuel composition and amount of fuel used per unit time. Section 4 provides some formulae for its calculation. They are based on the stoichiometry of the oxidation reactions, which are reviewed in Section 3 together with the relevant energetics.

There is a limit to the maximum amount of solutes that the cytosol can contain without harming the cell. This implies, in particular, a limit to the number κ_C of moles of carbon atoms that a muscle cell can oxidize simultaneously, through parallel oxidation paths within the cell. This limit depends on how long a carbon atom remains in the cell before leaving it as CO_2 . The problem is considered in Section 5, where the relation between κ_C and the muscle's total power

is obtained. The analysis of Section 6 then shows how the maximum value that κ_C can attain in a muscle cell is related to the limits of dehydration and overhydration of the cell. This result is used in Section 7 to calculate the maximum steady state power that a muscle can produce. The same section provides explicit formulae to relate this maximum power to the composition of the fuel mixture used and to the maximum number of parallel oxidation paths that the muscle can activate.

These results are represented graphically in Fig. 4, which also summarizes the main results of the present paper. The same figure offers a tool to evaluate the state of health, fitness, and training of a muscle and its steady-state power capacity. It also provides a means to assess the maximum amount of fat that a muscle can burn while working at steady state at a given level of power.

To facilitate their practical applications, the main formulae of the present paper are also expressed in terms of the volume of oxygen consumed by the muscle ($\dot{V}_{O_2}$) and the muscle's respiratory quotient (RQ), since these quantities are usually experimentally measured when assessing muscle metabolism by the methods of indirect calorimetry.

2 Thermodynamic preliminaries

At finite temperature, a finite system can only store a finite amount of energy. This statement sounds like a truism to most people. It is a direct consequence of the energy conservation principle: if a finite system stored an infinite amount of energy, it could supply/absorb any finite amount of energy to/from its surroundings without changing its energy content—which is impossible. The same statement implies that, under isothermal conditions, the values of the state variables of a system must fall within a definite domain for the energy limit of the system not to be exceeded. The analysis leading to this conclusion is discussed below.

For simplicity, reference will be made to homogeneous systems at uniform, but generally time-varying, temperature. For these systems, the first law of thermodynamics can, quite generally, be expressed in the form

$$\dot{U} = \dot{W}_{in} + \dot{Q} \quad (4)$$

where U , W_{in} and Q are, respectively, the system's internal energy, the work done *on* the system, and the heat absorbed *by* the system. A dot over a symbol denotes, as usual, time rate or time derivative. The time rate at which a system can absorb heat is limited by the second law of thermodynamics:

$$\dot{Q} \leq T \dot{S} \quad (5)$$

Here, T denotes absolute temperature and S is the entropy of the system. Both U and S are constitutive properties of the system. They are specified by appropriate functions of the system's state variables. That is, $U = U(\xi, T)$ and $S = S(\xi, T)$, where the symbol ξ collectively denotes all the system's state variables other than T . The equal sign in relation (5) applies if the process is reversible.

In what follows, we shall confine our attention to systems undergoing isothermal processes. An isothermal process will in particular be referred to as a *loading*, *unloading*, or *neutral* process if, at any time of the process, the system absorbs work ($\dot{W}_{in} > 0$), releases work ($\dot{W}_{in} <$

0), or does not exchange work at all ($\dot{W}_{in}=0$), respectively. The following function defines the system's specific Helmholtz free energy:

$$\Psi = \Psi(\xi, T) = U - TS \quad (6)$$

For isothermal processes, we have that

$$\dot{\Psi} = \dot{U} - T\dot{S} \leq \dot{W}_{in} \quad (7)$$

as immediately follows from Eqs. (4)–(6). From Eq. (7), we infer that

$$\dot{\Psi} \leq 0 \quad (8)$$

for every isothermal unloading or neutral process.

At a given temperature T , let us consider all the states that a system can reach while complying with its constitutive equations for U and S . These states are the *admissible* states of the system at the considered temperature. The set of all these states is the *admissible range* of the system. The system can always move between any two states of the admissible range through a reversible or an irreversible process. This possibility follows from relation (7), which at constant T is equivalent to

$$d\Psi = dU - T dS \leq dW_{in} \quad (9)$$

Since U and S are state functions, their increments depend on the initial and final state, but not on the process joining them. Thus, by controlling the external actions acting upon the system, we can always reduce dW_{in} to meet the equality in relation (9). (Often in practice, this means executing the process slowly enough to avoid the occurrence of thermal gradients.) However, when the equal sign holds true in relation (9), it also holds true in relation (5); which means that the process is reversible. At least in principle, therefore, any two states in the admissible range can be joined by at least one reversible process.

Let $\Psi_{\max}$ be the maximum value of Ψ in the admissible range. $\Psi_{\max}$ must be finite, since both U and S are finite, and T is a finite constant. Ψ may attain the value $\Psi_{\max}$ in one or more states of the system's admissible range. Clearly, relation

$$\Psi(\xi, T) \leq \Psi_{\max} \quad (10)$$

must apply to all admissible states. Obvious as it is, this relation introduces a restriction on the admissible states of the system. The same relation can be taken as representing the analytical definition of the system's admissible range in the space of variables ξ , generally a hyper-space.

The equal sign in relation (10) applies, of course, to states where the system's free energy reaches its maximum. No reversible loading process can start, however, from a state where $\Psi = \Psi_{\max}$. The reason is that such a process would produce a positive value of $\dot{W}_{in}$, and hence an increase in Ψ , because in this case relation (7) would hold with equality, the process being reversible. This would make Ψ exceed $\Psi_{\max}$, which is not possible. It must be concluded that the system can only leave a state where $\Psi = \Psi_{\max}$ through an unloading or neutral processes. Such a state must, therefore, belong to the boundary of the system's admissible range. Accordingly, in the space of state variables ξ , the analytical expression of this boundary must be given by

$$\Psi(\xi, T) = \Psi_{\max} \quad (11)$$

in agreement with relation (10).

In many systems of practical interest, functions U and S are continuous and differentiable. For these systems, the admissible range (10) is represented by a continuous volume in the space of the state variables ξ . The boundary to this range, that is Eq. (11), is then represented by a continuous surface—generally a hyper-surface—which will be referred to as the *limit surface* of the system at the considered temperature T . This surface sets a limit to the values of the state variables that the system can reach while complying with its constitutive equations for U and S . Equation (11) states that the limit surface is equipotential for Ψ . This means that, for these systems, the value of Ψ at a point of the limit surface suffices to determine the whole surface and, thus, the entire admissible range.

2.1 Non-thermodynamic and pure thermo-entropic components

In applying relations (10) and (11) to determine the admissible range and limit surface of a system, care must be exercised to exclude from Ψ any contribution that comes from any non-thermodynamic components of the system. As discussed at length in [1], a system contains a non-thermodynamic component if its internal energy can be decomposed into the sum of two functions, U' and U'' , according to the relation

$$U(\xi, T) = U'(\xi') + U''(\xi'', T) \quad (12)$$

Here, ξ' and ξ'' denote two appropriate subsets of ξ , which may, in particular, coincide with ξ . Function $U'(\xi')$ is independent of T . This means that $U'(\xi')$ cannot contribute to the specific heat c of the system (remember that c and U are related to each other by the well-known relation $c = \partial U / \partial T$). It also means that the specific heat c' of a hypothetical system with internal energy $U'(\xi')$ would vanish. As a consequence, such a system could not absorb any heat, no matter the temperature changes it undergoes. Since heat absorbed and entropy changes are related by the classical formula $dS = T dQ$, the entropy of such a hypothetical system is bound to remain constant, no matter the process ($dS = 0$ since $dQ = 0$ because the system cannot absorb heat). All these features are typical of the non-thermodynamic systems routinely considered in various branches of physics, ranging from classical mechanics to electricity. A term like $U'(\xi')$ in Eq. (12) can accordingly be considered as indicating the presence of a *non-thermodynamic component* in the system at hand. The contribution to Ψ coming from this component should be ignored when seeking the thermodynamic limit to the maximum free energy that the system can store because thermodynamics does not entail any bound to the internal energy of a non-thermodynamic system.

An analogous situation occurs when the entropy of the system has the following form:

$$S(\xi, T) = S'(T) + S''(\xi, T) \quad (13)$$

A non-vanishing function $S'(T)$ at the right-hand side of this equation represents a *pure thermo-entropic component* of the system. According to definition (6), the contribution of $S'(T)$ to Ψ is given by $-TS'(T)$. Under the isothermal conditions considered in the present analysis, this contribution reduces to a constant. For this reason, no restriction arises if the term $S'(T)$ is omitted from the expression of Ψ to be introduced in relations (10) and (11), because this is tantamount to omitting the same constant term from both sides of these relations.

Thus, when Eqs. (12) or (13) apply, the admissible range and limit surface of the system should be sought through the following relations:

$$\Psi''(\xi, T) \leq \Psi''_{\max} \quad (14)$$

and

$$\Psi''(\xi, T) = \Psi''_{\max} \quad (15)$$

rather than relations (10) and (11), respectively. Here, $\Psi''(\xi, T)$ is the part of Ψ , defined by

$$\Psi'' = \Psi'(\xi, T) - U''(\xi, T) - TS''(\xi, T) \quad (16)$$

Quite often in practice, Ψ'' is only a small portion of the total free energy Ψ of the system. Yet, it is this portion that determines the range of admissible states for systems that contain non-thermodynamic or pure thermo-entropic components. Also, as apparent from formulae (14)–(16), the limit surface of these systems is equipotential for Ψ'' , but generally not for Ψ , as defined by Eq. (6). Moreover, the states where Ψ reaches its maximum and minimum values are usually different from the analogous states for Ψ'' . In these systems, therefore, Ψ'' determines the admissible range and limit surface, while any consideration concerning the state(s) of maximum or minimum of the total free energy of the system should refer to Ψ .

2.2 Open and chemically reacting systems

The above analysis implicitly assumed that the material making up the system does not change in amount or kind during the process. This need not be true for open or chemically reacting systems, as the composition of these systems may vary due to the transport of material or as a result of chemical reactions. Of course, because entropy is an extensive quantity, a change in the amount of a substance within a system implies a proportional change in the contribution of the same substance to the entropy of the system. As a consequence, if substance i carries a pure thermo-entropic component, the pure thermo-entropic contribution of this component to S will be proportional to the amount of the substance i within the system. In open and chemically reacting systems, therefore, a purely thermo-entropic component of the system will show itself in the expression of S as one or more terms of the form $\xi_i S'_i(T)$, rather than merely as $S'_i(T)$. Here, ξ_i indicates the variable that measures the amount of substance i in the system, while $S'_i(T)$ represents the purely thermo-entropic contribution of the same substance per unit value of ξ_i .

From definition (6) it follows that, in the expression of the Helmholtz free energy of open or chemically reacting systems, any pure thermo-entropic component will appear as a linear term in ξ_i of the form $\Psi'_i = \xi_i \bar{\Psi}_i(T)$, where $\bar{\Psi}_i(T) = -TS'_i(T)$. Terms like this do not represent a contribution to Ψ due to a change in the state of the material making up the system, but they are, instead, due to a change in the system composition, which can in principle be fixed arbitrary and thus it is not a priori limited by the laws of thermodynamics. For this reason, when applying thermodynamic limitations (14) and (15), any component of Ψ of the form $\Psi'_i = \xi_i \bar{\Psi}_i(T)$ should be excluded from $\Psi''(\xi, T)$.

In many applications, open and chemically reacting systems are made up of homogeneous mixtures or solutions of different substances. In this case, the variable ξ_i may be identified with the moles, n_i , of substances i in the solution ($i = 1, 2, \dots, r$, where r is the number of solutes). The Helmholtz free energy of these systems can be written in the form (cf. e.g., [14] or [15] for further details)

$$\Psi = \Psi(n_1, n_2, \dots, n_r, p, T) = n_1 \mu_1 + n_2 \mu_2 + \dots + n_r \mu_r - p^o V + C \quad (17)$$

where V is the volume of the solution, p denotes pressure, p^o is a reference pressure, and C indicates an arbitrary constant. Functions μ_i introduced in the above equation are the *molar* Gibbs free energies of substances 1, 2, ..., r , respectively:

$$\mu_i = \mu_i(n_1, n_2, \dots, n_r, p, T) \quad (i = 1, 2, \dots, r) \quad (18)$$

They are also referred to as chemical potentials. In terms of them, the system's Gibbs free energy is given by:

$$G = G(n_1, n_2, \dots, n_r, p, T) = n_1 \mu_1 + n_2 \mu_2 + \dots + n_r \mu_r + (p - p^o) V + C \quad (19)$$

As is well known, Gibbs free energy and Helmholtz free energy are related to each other by the equation

$$\Psi = G - pV \quad (20)$$

As apparent from Eq. (17), functions μ_i represent the contribution to the free energy of the system coming from a fixed amount (one mole) of substance i in the solution. Therefore, any pure thermo-entropic contribution to the system free energy coming from substance i will appear in the expression of μ_i as an additive term which is a function of T only (the dependence on ξ_i or, equivalently, on n_i does not appear in these additive terms, simply because functions μ_i refers to a fixed amount—one mole—of solute). Terms of this kind in the expression of μ_i can easily be spotted and then ignored when considering the expression of Ψ'' to be used in relations (14) and (15).

2.3 Cytosol's free energy and admissible range

The cytosol is a rather particular aqueous solution in that it contains thousands of different solutes, although most of them in quite small amounts. Its Helmholtz free energy can, to a good approximation, be expressed as [2].

$$\Psi = n_{H_2O} \mu_{H_2O}^o(p^o, T) + \sum_{j=1}^s n_j \mu_j^o(p^o, T) + RT \sum_{j=1}^s n_j \ln \frac{\gamma_j n_j}{m_{H_2O}} - p^o V + C \quad (21)$$

Here, n_{H_2O} are the moles of solvent (water), s indicates the number of solutes, n_j is the number of moles of solute j , the quantities $\mu_{H_2O}^o$ and μ_j^o are the reference values of the chemical potentials of solvent and solutes at pressure p^o and temperature T , and γ_j denotes the activity coefficient of solute j . In general, the activity coefficients are functions of the state variables of the system. However, they can often be treated as constants if the state variables are confined in a comparatively small range. In the above equation, R is the universal gas constant, V is the volume of the cytosol, and m_{H_2O} is the mass of solvent.

Because p^o is a constant, the first two terms in the right-hand side of Eq. (21) represent two pure thermo-entropic components of Ψ , according to what we discussed above. Thus, the part of the Helmholtz free energy to be introduced into Eqs. (14) and (15) to determine the cytosol's admissible range and limit surface is the following:

$$\Psi'' = \Psi''(n_1, n_2, \dots, n_s, p, T) = RT \sum_{j=1}^s n_j \ln \frac{\gamma_j n_j}{m_{H_2O}} - p^o V \quad (22)$$

This expression is of little practical use, due to the enormous number of terms entering the summation at its right-hand side (in the cytosol, s has the order of magnitude of 10^4). However, it was shown in [2] that, for isothermal processes, the contribution of all terms in this summation can be calculated as coming from n_{eq} moles of just one single *equivalent solute* of activity coefficient γ_{eq} . The moles n_{eq} of the equivalent solute can be taken as coinciding with the overall moles of solute contained in volume V of cytosol. The value of γ_{eq} is determined experimentally from the limits of dehydration and overhydration of the cell.

When expressed in terms of n_{eq} and γ_{eq} , function Ψ'' assumes the much simpler form

$$\Psi'' = \Psi''(n_{eq}, p, T) = RT n_{eq} \ln \frac{\gamma_{eq} n_{eq}}{m_{H_2O}} - p^o V \quad (23)$$

Rather than referring directly to this function, it is sometimes more convenient to consider its specific value $\bar{\psi}''$ per mole of solvent. This is easily obtained by dividing both sides of (23) by n_{H_2O} . We thus obtain

$$\bar{\psi}'' = RT \frac{n_{eq}}{n_{H_2O}} \ln \frac{\gamma_{eq} n_{eq}}{n_{H_2O} M_{H_2O}} - p^o \bar{V} \quad (24)$$

where $M_{H_2O} = 18.015 \cdot 10^{-3}$ kg/mol is the molar mass of water and $\bar{V}$ is the cytosol volume per mole of solvent, i.e.,

$$\bar{V} = \frac{V}{n_{H_2O}} \quad (25)$$

By setting $\bar{\psi}''_{\max} = \Psi''_{\max}/n_{H_2O}$, from relations (24), (14) and (15) we obtain

$$RT \frac{n_{eq}}{n_{H_2O}} \ln \frac{\gamma_{eq} n_{eq}}{n_{H_2O} M_{H_2O}} - p^o \bar{V} \leq \bar{\psi}''_{\max} \quad (26)$$

and

$$RT \frac{n_{eq}}{n_{H_2O}} \ln \frac{\gamma_{eq} n_{eq}}{n_{H_2O} M_{H_2O}} - p^o \bar{V} = \bar{\psi}''_{\max} \quad (27)$$

For isothermal processes at constant pressure, these relations define the cytosol's admissible range and limit surface in the one-dimensional space of the variable $\bar{n}_{eq}$, which is defined by

$$\bar{n}_{eq} = \frac{n_{eq}}{n_{H_2O}} \quad (28)$$

and gives the total number of moles of solute dissolved per mole of cytosolic water. This one-dimensional space may be represented as a line, which will be referred to as the $\bar{n}_{eq}$ -axis. On this axis, the cytosol's admissible range (26) is represented by a segment, while the limit surface (27) reduces to the two points at the ends of this segment.

3 The energy content of aerobic muscle fuel

Muscle cells produce power aerobically by oxidizing a fuel mixture of carbohydrates (CHO) and fats. Proteins provide a negligible contribution to muscle power and, for simplicity, will be ignored. Most carbohydrates reach the muscle cells as glucose, while fats are absorbed as free fatty acids (FFA) of various chain lengths. As often done in muscle energetics, we assume that the carbohydrate component of muscle fuel is represented by glucose ($C_6H_{12}O_6$), while the lipid component is represented by palmitic acid ($C_{16}H_{32}O_2$).

Fuel oxidation occurs entirely within the muscle cells. It involves a complex series of enzyme-catalyzed subreactions, most of which take place in the cell's mitochondria. Eventually, all fuel is oxidized into carbon dioxide and water according to the following overall reactions:

The energy liberated by these reactions is in part turned into heat and in part used to produce adenosine triphosphate (ATP) for muscular contraction and, thus, mechanical work. The standard values of reaction energy, $\Delta G^{\circ'}$, reported in (29) and (30), refer to one mole of reactant— $C_6H_{12}O_6$ and $C_{16}H_{32}O_2$, respectively—in biological standard state, that is at 298 K (25 °C), 1 atm pressure, pH 7.0, and initial concentrations of 1 M for all reactants and products except protons. These values of $\Delta G^{\circ'}$ are widely used in the literature to calculate the energy available to a muscle cell from fuel oxidation [16]. To take into account the actual concentrations of the reacting component within the cell would result in comparatively small changes in the values of $\Delta G^{\circ'}$. This may not be worth the effort, owing to the difficulties in determining the actual values of the concentrations (or, better, the activities) to be used in the calculations, and the uncertainties affecting the variables that define the state of a working muscle.

The fat/carbohydrate ratio of the fuel mixture used by a muscle is different for different animals, different kinds of muscle, and different exercise conditions. It generally varies in time due to a host of factors such as power output, duration and kind of exercise, fuel availability, muscle training, blood transfer capacity, cellular uptake capacity, and even substrate selection at the mitochondrial level [17, 18]. Accordingly, by referring to a group of similar muscle cells, the grams of carbohydrate (glucose) and the grams of fat (palmitic acid) oxidized per unit time and unit mass of wet muscular tissue will be denoted as $\dot{m}_{CHO}$ and $\dot{m}_{FFA}$, respectively,

As apparent from reactions (29) and (30), fuel oxidation consumes O_2 and produces CO_2 . Let $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ denote, respectively, the *oxygen consumption rate* and the *carbon dioxide production rate* of a group of muscle cells. The quantity $\dot{V}_{O_2}$ measures the volume of oxygen consumed by the muscle cells per unit time and per unit mass. Likewise, $\dot{V}_{CO_2}$ gives the volume of carbon dioxide produced by the muscle cells per unit time and unit mass. By a procedure that is better described in [19], it can be calculated that

$$\dot{m}_{CHO} = 4.40 \dot{V}_{CO_2} - 3.06 \dot{V}_{O_2} \quad \text{g min}^{-1} \text{kg}^{-1} \quad (31)$$

and

$$\dot{m}_{FFA} = 1.64 \dot{V}_{O_2} - 1.64 \dot{V}_{CO_2} \quad \text{g min}^{-1} \text{kg}^{-1} \quad (32)$$

($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in liters per minute per kilogram of wet muscle).

In deriving Eqs. (31) and (32), the molar mass of glucose and that of palmitic acid were taken to be $M(C_6H_{12}O_6) = 180 \text{ g/mol}$ and $M(C_{16}H_{32}O_2) = 256.4 \text{ g/mol}$, respectively. Moreover, the molar volume of O_2 and CO_2 was conventionally taken as equal to 22.4 l/mol , which is the molar volume of an ideal gas at 0°C and 1 atm pressure. The quantities $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ introduced above can be determined from measurements of the arteriovenous difference in oxygen and carbon dioxide content in the blood flowing through the muscle (see, e.g., [20–23]). Accordingly, they should not be confused with the analogous quantities, usually denoted by the same symbols, referring to the whole body and usually determined from systemic respirometry.

Equations (31) and (32) are a direct consequence of the stoichiometry of reactions (29) and (30), and are widely used in indirect calorimetry [19, 24]. Similar formulae can be found in the literature with slightly different coefficients, depending on the approximations made and the kind of fat molecule adopted to represent the average FFA. From Eqs. (31) and (32), the quantity $\dot{m}_{fuel}$ representing the overall time rate of the mass of substrate consumed per kilogram wet weight of muscle is immediately calculated:

$$\dot{m}_{fuel} = \dot{m}_{CHO} + \dot{m}_{FFA} = 2.76 \dot{V}_{CO_2} - 1.42 \dot{V}_{O_2} \quad \text{g min}^{-1} \text{kg}^{-1} \quad (33)$$

The muscle's respiratory quotient, RQ, is a dimensionless quantity defined as

$$RQ = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}} \quad (34)$$

Let w_{CHO} denote the mass fraction of carbohydrate contained in the fuel mixture consumed by the muscle. From Eqs. (32)–(34) we obtain, after some simple algebra,

$$w_{CHO} = \frac{\dot{m}_{CHO}}{\dot{m}_{fuel}} = \frac{4.40 RQ - 3.06}{2.76 RQ - 1.42} \quad (35)$$

Similarly, the mass fraction of fat (w_{FFA}) in the same mixture is calculated to be

$$w_{FFA} = \frac{\dot{m}_{FFA}}{\dot{m}_{fuel}} = 1 - w_{CHO} = \frac{1.64(1 - RQ)}{2.76 RQ - 1.42} \quad (36)$$

Thus, RQ defines completely the fuel mixture used by the muscle at any given time.

In particular, $RQ = 1$ indicates that a 100% carbohydrate fuel is being used ($w_{CHO} = 1$ and $w_{FFA} = 0$), while a 100% fat fuel ($w_{CHO} = 0$ and $w_{FFA} = 1$) produces $RQ = 0.695$. The latter value of RQ is actually a theoretical limit, because fat oxidation requires some concomitant carbohydrate breakdown to produce the intermediates that are needed for mitochondrial oxidation—as summarized by the aphorism “fats burn in a carbohydrate flame” (cf., e.g., [25, 26] and [27] p.159).

From Eqs. (35) and (36), the ratio between moles of fat (n_{FFA}) and moles of glucose (n_{CHO}) of the fuel mixture is easily obtained:

$$\frac{n_{FFA}}{n_{CHO}} = \frac{M(C_6H_{12}O_6)}{M(C_{16}H_{32}O_2)} \frac{w_{FFA}}{w_{CHO}} = \frac{180}{256.4} \frac{1.64(1 - RQ)}{4.40 RQ - 3.06} = \frac{1.15(1 - RQ)}{4.40 RQ - 3.06} \quad (37)$$

For the same mixture, the mole fraction of glucose (x_{CHO}) and the mole fraction of fat (x_{FFA}) can then be expressed as functions of RQ according to the following relations:

$$x_{CHO} = \frac{n_{CHO}}{n_{CHO} + n_{FFA}} = \frac{4.40 \text{ RQ} - 3.06}{3.25 \text{ RQ} - 1.91} \quad (38)$$

and

$$x_{FFA} = \frac{n_{FFA}}{n_{CHO} + n_{FFA}} = 1 - x_{CHO} = \frac{1.15 (1 - \text{RQ})}{3.25 \text{ RQ} - 1.91} \quad (39)$$

It is not difficult to verify that relations (38) and (39) are invertible for all values of RQ in the interval (0.695, 1], i.e., in the whole range of variability of RQ. Thus variables x_{CHO} or x_{FFA} can be used instead of RQ to characterize the composition of the fuel mixture. RQ is easier to determine experimentally. However, x_{FFA} is a better variable to use for graphic representations, because it varies in the interval [0, 1).

The fuel free energy $\Delta G_{\text{fuel}}(n_{CHO}, n_{FFA})$ liberated by the oxidation of n_{CHO} moles of glucose and n_{FFA} moles of fat is immediately obtained from the molar energies of reactions (29) and (30):

$$\Delta G_{\text{fuel}}(n_{CHO}, n_{FFA}) = 2840 n_{CHO} + 9770 n_{FFA} \quad \text{kJ} \quad (40)$$

Using Eq. (37)₃, this energy can be expressed as a function of n_{CHO} and RQ as follows:

$$\Delta G_{\text{fuel}}(n_{CHO}, \text{RQ}) = \frac{630.25 \text{ RQ} + 1272.55}{2.2 \text{ RQ} - 1.53} n_{CHO} \quad \text{kJ} \quad (41)$$

The same energy can also be expressed as a function of n_{CHO} and x_{FFA} . To do this, we may observe that from Eq. (39)₁ we get

$$n_{FFA} = x_{FFA} n_{CHO} + x_{FFA} n_{FFA} \quad (42)$$

That is,

$$n_{FFA} = \frac{x_{FFA}}{1 - x_{FFA}} n_{CHO} \quad (43)$$

or

$$\frac{n_{FFA}}{n_{CHO}} = \frac{x_{FFA}}{1 - x_{FFA}} \quad (44)$$

By introducing Eq. (43) into Eq. (40), we finally obtain

$$\Delta G_{\text{fuel}}(n_{CHO}, x_{FFA}) = \frac{6930 x_{FFA} + 2840}{1 - x_{FFA}} n_{CHO} \quad \text{kJ} \quad (45)$$

As is apparent from Eqs. (40), (41), or (45), different fuel mixtures (i.e., different values of RQ or x_{FFA}) produce different energies per mole of carbohydrate oxidized.

4 Steady-state concentration of fuel and oxidation products in muscle cytosol

Living cells do not take from the outside world more material than they need to perform their functions. Any surplus material would cause unnecessary burden because the cell has to get rid

of any excess material to maintain homeostasis. Thus, apart from the fuel that goes to replenish the internal reserves of fat and glucose, all the fuel that a muscle cell absorbs from the outside to meet its immediate demands for energy production is entirely burned within the cell.

In steady-state conditions, in particular, the number of molecules of carbohydrates, fats, and oxygen that a muscle cell absorbs from the surroundings must be proportional to the corresponding stoichiometric coefficients of reactions (29) and (30), namely, 6 mol of O₂ for each mole of glucose oxidized, and 23 mol of O₂ for each mole of fat oxidized (average value). Different proportions of absorbed reactants would mean unused residues, which the cell would have to expel because no material can accumulate inside it in steady-state conditions.

The steady state also requires that at any time of the energy-producing process, the mass of the reaction products released by a muscle cell should equal the mass of the absorbed reactants. As apparent from reactions (29) and (30), this means that the cell must release 6 mol of CO₂ for each mole of glucose oxidized, and 16 mol of CO₂ for each mole of fat oxidized. Water molecules resulting from the process can safely be ignored in the arguments that follow because they can freely cross the cellular membrane to keep the cell volume constant [28].

Oxygen and fuel must first be dissolved in the cell's cytosol to be processed by the mitochondria. Likewise, all CO₂ resulting from fuel oxidation must be dissolved in the cytosol before it can be released from the cell. This means that, when operating in a steady state, the muscle cytosol must accommodate, beside the solutes that it normally contains, all molecules taking part in reactions (29) and (30). The overall number of moles of these additional cytosol solutes, per kilogram of wet muscular tissue, will be denoted by $\Delta\bar{n}$ and depends on the fuel consumed per unit time or, equivalently, on the exercise intensity.

In steady-state conditions, the cytosol's composition does not change. Thus, $\Delta\bar{n}$ is constant at steady state. We decompose $\Delta\bar{n}$ into two parts as follows:

$$\Delta\bar{n} = \left(\Delta\bar{n}\right)_{CHO} + \left(\Delta\bar{n}\right)_{FFA} \quad \text{mol/kg} \quad (46)$$

where $\left(\Delta\bar{n}\right)_{CHO}$ and $\left(\Delta\bar{n}\right)_{FFA}$ indicate the contribution to $\Delta\bar{n}$ coming from glucose oxidation and fat oxidation, respectively.

4.1 Evaluation of $\left(\Delta\bar{n}\right)_{CHO}$

According to overall reaction (29), each molecule of glucose undergoing oxidation in steady state conditions requires an increase of six molecules of O₂ and six molecules of CO₂ in the cytosol solute content. This means a total of 13 extra molecules of solute per molecule of glucose that oxidizes because the glucose molecule itself must be accounted as a solute. These extra solutes, however, are only a small part of $\left(\Delta\bar{n}\right)_{CHO}$. To be fully oxidized in the mitochondria, glucose must first undergo glycolysis. This is a ten-step process that occurs entirely in the cytosol and transforms each molecule of glucose into two molecules of pyruvate, according to the overall reaction

(see, e.g., [16]). In steady state, this means a further increase of eight molecules of solute (two of pyruvate, two of NAD⁺, two of NADH and two of H⁺) to be accommodated in the cytosol for each molecule of glucose undergoing oxidation.

Still, this accounts for only a part of the extra load of cytosol solutes produced by glycolysis at steady state. Each of the ten glycolysis steps adds further molecules of solutes to those

accounted for with Eq. (47), thus producing a further increase in the cytosol solute content. The chemical reactions involved in these steps are well known; the reader is referred to one of the many excellent textbooks on the subject for details (see, e.g., [16, 29]). An inspection of these reactions shows that they produce 26 more molecules of cytosol solutes for every glucose molecule that glycolyses, which add to the eight molecules already accounted for from Eq. (47).

Moreover, the two pyruvate molecules resulting from glycolysis leave the cytosol to enter the cell's mitochondria. Here, they are processed in the Krebs cycle, which completes glucose oxidation. This part of the glucose oxidation process occurs entirely within the mitochondria and therefore it does not affect the solute content of the cytosol. It results, however, in some 32 molecules of ATP, which the mitochondrion liberates into the cytosol, where they hydrolyze according to the reaction

In steady-state conditions, the molecules of ATP, ADP, and P_i appearing in this reaction are continuously consumed and replaced, so that their content in the cytosol remains constant. Accordingly, reaction (48) introduces in the cytosol three additional molecules of solute for each ATP molecule (itself in solution) hydrolyzed. Thus, this final part of the energy-producing process of glucose oxidation adds another $3 \times 32 = 96$ molecules of cytosol solute per molecule of glucose oxidized.

Summing up all the above contributions, we can express the contribution to $\Delta\bar{n}$ coming from glucose oxidation as

$$\left(\Delta\bar{n}\right)_{\text{CHO}} = (13 + 8 + 26 + 96) \kappa_{\text{CHO}} = 143 \kappa_{\text{CHO}} \quad \text{mol/kg} \quad (49)$$

Here, κ_{CHO} indicates the number of glucose (or carbohydrate) molecules, per kilogram of wet muscle, that must at any time be dissolved in the cytosol to keep energy production from glucose oxidation steady. Because in different exercises the muscle uses different fuel mixtures of fat and carbohydrates, κ_{CHO} assumes different values depending on RQ and on the kind and intensity of exercise. It may be worth stressing that κ_{CHO} strictly refers to the number of carbohydrate molecules of fuel dissolved in the cell's cytosol and therefore it is quite distinct from the total content of carbohydrate in the cytosol.

4.2 Evaluation of $(\Delta\bar{n})_{\text{FFA}}$

As is apparent from Eq. (30), oxidation of palmitic acid charges the cytosol with 40 extra molecules of solutes per molecule of palmitic acid oxidized, namely, 23 molecules of O_2 , 16 molecules of CO_2 , which add to the molecule of $\text{C}_{16}\text{H}_{32}\text{O}_2$ in solution as a reactant (these are average values, because $\text{C}_{16}\text{H}_{32}\text{O}_2$ represents the average FFA molecule oxidized by the muscle). This estimate does not, however, take into account the load of cytosol solutes coming from hydrolysis of the ATP supplied to the cytosol by the mitochondria, where the molecules of fat are oxidized. The process results in 106 molecules of ATP for each molecule of palmitic acid (cf., e.g., [16]). In steady state, these ATP molecules are continuously injected by the mitochondria into the cytosol, where they hydrolyze according to reaction (48). This results in a further $3 \times 106 = 318$ molecules of solute per molecule of palmitic acid, which add to the 23 molecules of solute coming from the overall reaction (30), as mentioned above. The contribution to $\Delta\bar{n}$ from fat oxidation can, accordingly, be expressed as

$$\left(\Delta\bar{n}\right)_{FFA} = (23 + 318) \kappa_{FFA} = 341 \kappa_{FFA} \quad \text{mol/kg} \quad (50)$$

where κ_{FFA} is the number of fuel molecules of fat that must be kept dissolved in the cytosol, per kilogram of wet muscular tissue, to maintain steady state oxidation. Again, κ_{FFA} depends on the fuel composition and kind and intensity of exercise.

In steady-state conditions, the ratio of glucose and fat molecules in the fuel undergoing oxidation inside a muscle cell coincides with the ratio of molecules of glucose and fat in the fuel absorbed by the cell. Thus, from Eq. (44) we infer that, at steady state,

$$\frac{\kappa_{FFA}}{\kappa_{CHO}} = \frac{n_{FFA}}{n_{CHO}} = \frac{x_{FFA}}{1-x_{FFA}} \quad (51)$$

From this and from Eq. (50), it follows that

$$\left(\Delta\bar{n}\right)_{FFA} = 341 \frac{x_{FFA}}{1-x_{FFA}} \kappa_{CHO} \quad \text{mol/kg} \quad (52)$$

By introducing this equation and Eq. (49) into Eq. (46), and by using Eq. (39)₃, we obtain after some simple algebra

$$\Delta\bar{n} = \frac{143 + 198x_{FFA}}{1-x_{FFA}} \kappa_{CHO} = \frac{237.05 \text{ RQ} - 45.43}{4.40 \text{ RQ} - 3.06} \kappa_{CHO} \quad \text{mol/kg} \quad (53)$$

This gives $\Delta\bar{n}$ as a function of the fuel mixture composition.

The present calculations refer to the textbook values of mitochondrial ATP yield from metabolic substrates. Although experiments on muscle energetics support these values [4], mitochondrial studies suggest that the same values may overestimate the actual ones [30]. If this is the case, Eqs. (49)–(50) and, thus, result (53) can readily be modified to accommodate for any different ATP yield one may want to consider.

5 Parallel carbon oxidation paths and carbon transit time

Muscle cells can operate close to their maximum power at almost no notice. This comes at a price though, as the cells must possess enough ready-made enzymes to oxidize the available fuel at the required rate. Producing enzymes on an as-needed basis is not a choice here, as this would take time and impair the cell response. It must be concluded that to be ready to produce work at a high rate, a muscle cell keeps the cytosolic concentration of oxidation enzymes at the highest expendable value, which may depend on previous training.

Similar remarks apply to enzymes in the cell's mitochondria, which are responsible for aerobic substrate oxidation. Thus, the number of mitochondria per cell may vary depending on training, with obvious implications for the amount of fuel that the cell can oxidize per unit time, and hence on the cell's aerobic power.

Once an enzyme is used by a molecule of reactant to speed up a reaction or a reaction step, the same enzyme is ready to be reused by another molecule of reactant. Thus, one single enzyme molecule can be reused over and over again to handle any number of molecules of a reactant. Clearly, if fuel oxidation took place in a sequence in which the molecules of reactant are processed one after the other, there would be no point in a muscle cell storing a large number of molecules of the same enzymes. On the contrary, the very fact that the cell stores so

many copies of the same enzyme indicates that fuel oxidation within the cell involves running in parallel a large number of identical reactions, thus processing many fuel molecules simultaneously.

The maximum power (energy per unit time) that a muscle cell can produce relates directly to the maximum number of fuel molecules that the cell can oxidize simultaneously. This number is calculated in the next section.

From a qualitative standpoint, the presence of many parallel oxidation paths within the cell is suggested by the fact that, in a given time interval, a single muscle cell (i.e., a single muscle fiber) can operate at different values of power. This means that in a given time interval, the cell can oxidize different amounts of fuel, which is only possible if in that interval the cell can activate a different number of simultaneous oxidation reactions depending on the power it must produce. Further evidence of the presence of parallel oxidation paths is provided by a muscle cell that operates in isometric contraction under different loads. In this case, the cell does not supply power to its surroundings. However, the amount of fuel that the cell oxidizes in a given time interval is different for different values of the applied load. Again, this indicates that, depending on the value of the applied load, a different number of fuel molecules are oxidized simultaneously, which is only possible through the activation of a different number of parallel oxidation reactions.

To be oxidized inside a cell, carbon atoms enter the cells as molecules of fat or carbohydrate. In this form, they are fed into one of the many oxidation paths available within the cell to leave the cell as carbon dioxide (CO_2). In steady-state conditions, the average time it takes a carbon atom to complete its journey through the cell—from its entrance as a fuel molecule to its exit as waste gas—will be denoted as $\Delta\tau$ and referred to as the *carbon transit time*. Fats and carbohydrates may take different times to oxidize. Thus, $\Delta\tau$ will in general depend on the fuel composition, which the muscle uses to operate.

When muscle cells produce work in steady state, the fuel molecules that are kept dissolved in the cell's cytosol to feed the oxidation process are continuously replaced as soon as they are consumed. Their number therefore remains constant in time. Let κ_C denote the overall moles of carbon atoms carried by the fuel molecules that are simultaneously processed per unit mass of wet muscle. As this definition should make clear, κ_C depends both on muscle power and on fuel composition.

From the above definition of $\Delta\tau$, it follows that the ratio $\kappa_C/\Delta\tau$ measures the moles of CO_2 produced per second by 1 kg wet weight of muscle working in steady state. On the other hand, as recalled in Section 3, $\dot{V}_{\text{CO}_2}$ measures the liters of CO_2 produced by 1 kg wet weight of muscle per minute. Because at ambient temperature and atmospheric pressure the volume of 1 mole of gas, considered as a perfect gas, is 25.4 l, it can be concluded that, at steady state,

$$\frac{\kappa_C}{\Delta\tau} = \frac{\dot{V}_{\text{CO}_2} [\text{L}/\text{min kg}]}{25.4 [\text{L}/\text{mol}] 60 [\text{sec}/\text{min}]} = \frac{1}{1524} \dot{V}_{\text{CO}_2} \quad \text{mol sec}^{-1} \text{kg}^{-1} \quad (54)$$

where both κ_C and $\dot{V}_{\text{CO}_2}$ depend on the exercise intensity.

From Eq. (54) it follows that, in steady-state conditions,

$$\Delta\tau = 1524 \frac{\kappa_C}{\dot{V}_{\text{CO}_2}} \quad \text{sec} \quad (55)$$

where $\dot{V}_{\text{CO}_2}$ is expressed in $[\text{L min}^{-1} \text{kg}^{-1}]$. The value of κ_C depends both on $\dot{V}_{\text{CO}_2}$ and on $\Delta\tau$. Thus, Eq. (55) does not express $\Delta\tau$ as an explicit function of $\dot{V}_{\text{CO}_2}$, unless we are referring to the very special—and possibly irrelevant—case in which κ_C is constant. The same equation,

however, implies that $\Delta\tau$ is independent of exercise intensity for a given fuel composition. The reason is that, in steady-state conditions, both κ_C and $\dot{V}_{CO_2}$ are proportional to muscle power, which makes the ratio $\kappa_C/\dot{V}_{CO_2}$ independent of muscle power and hence of exercise intensity (the same ratio, however, may generally vary with the compositions of the fuel used). Thus, to determine $\Delta\tau$ in a muscle burning a given fuel mixture, for example a full carbohydrate fuel ($x_{FFA}=0$), the intensity of exercise is immaterial, as long as in the exercise the muscle uses that mixture.

Let $\Delta\tau|_{CHO}$ be the value of $\Delta\tau$ for full carbohydrate fuel. This value can be estimated from the delay of the onset of aerobic oxidation in an intense exercise in which the muscle produces its maximal effort straightaway starting from rest. Such an all-out exercise is performed in certain sports activities, like the 100 m sprint run. Initially, the energy that the muscle produces is, in this case, prevalently anaerobic. The contribution from aerobic oxidation starts after about 5 sec from the beginning of the exercise [31]. It becomes significant if maximal effort continues beyond about 10 sec (cf., e.g., the data reported in [27], p. 170). The first fuel molecules that are oxidized aerobically are carbohydrates because fats take longer to be oxidized. Thus, assuming that at least some molecules of oxygen and carbohydrates start their oxidation process right from the start of the exercise, the time needed for a carbohydrate molecule to be oxidized within a muscle cell—from the beginning of the reaction chain to the release of CO_2 —is between 5 and 10 sec. In want of a better estimate, we shall accordingly assume that the carbon transit time for full carbohydrate fuel is $\Delta\tau|_{CHO}=7.5$ sec.

The analogous quantity, $\Delta\tau|_{FFA}$, representing the value of $\Delta\tau$ for full fat fuel oxidation, can be estimated as follows. From the data reported on p. 814 of [32], we learn that the maximum time rates at which muscle mitochondria produce ATP are 16.7 mmol/sec for glycogen oxidation and 6.7 mmol/sec for fatty acids oxidation. In the absence of precise data on the oxidation velocities of carbon atoms within the muscle cell, we shall assume that these velocities are proportional to the respective ATP maximum production rates reported above. This is tantamount to assuming that $\Delta\tau|_{FFA}/\Delta\tau|_{CHO}=6.7/16.7=2.49$, which means $\Delta\tau|_{FFA}=18.7$ sec, because we assumed $\Delta\tau|_{CHO}=7.5$ sec.

For a fuel mixture of carbohydrates and fats, the value of $\Delta\tau$ varies linearly from $\Delta\tau|_{CHO}$ to $\Delta\tau|_{FFA}$, depending on the mole fraction of fat in the mixture (remember that $\Delta\tau$ is an average time). That is,

$$\Delta\tau = \Delta\tau|_{CHO} + (\Delta\tau|_{FFA} - \Delta\tau|_{CHO}) x_{FFA} \quad (56)$$

By inserting in this equation the values of $\Delta\tau|_{CHO}$ and $\Delta\tau|_{FFA}$ estimated above, we obtain

$$\Delta\tau = 7.5 + 11.2 x_{FFA} \quad \text{sec} \quad (57)$$

In a fuel mixture of n_{CHO} moles of carbohydrates and n_{FFA} moles of fat, the moles n_C of carbon atoms are given by

$$n_C = 6 n_{CHO} + 16 n_{FFA} \quad (58)$$

since we assumed that in muscle fuel all carbohydrates and fats could be treated as glucose ($C_6H_{12}O_6$) and palmitic acid ($C_{16}H_{32}O_2$), respectively. In view of Eq. (37)₃, this Eq. (58) can also be written as

$$n_C = \frac{4 RQ + 0.02}{2.20 RQ - 1.53} n_{CHO} \quad (59)$$

or, by introducing Eq. (43) into Eq. (58), as

$$n_C = \frac{6 + 10x_{FFA}}{1 - x_{FFA}} n_{CHO} \quad (60)$$

Once resolved for n_{CHO} , Eq. (60) can be introduced into Eq. (45) to yield

$$\Delta G_{\text{fuel}}(n_C, x_{FFA}) = \frac{6930 x_{FFA} + 2840}{6 + 10x_{FFA}} n_C \quad \text{kJ} \quad (61)$$

which gives the energy produced by oxidation of n_C moles of carbon atoms in a fuel mixture with a mole fraction of fats of x_{FFA} .

Let Δg_C denote the average value of the free energy liberated per mole of carbon atoms oxidized. That is,

$$\Delta g_C = \frac{\Delta G_{\text{fuel}}(n_C, x_{FFA})}{n_C} \quad (62)$$

In view of Eq. (61), we can then write

$$\Delta g_C = \Delta g_C(x_{FFA}) = \frac{6930 x_{FFA} + 2840}{6 + 10x_{FFA}} \quad \text{kJ mol}^{-1} \quad (63)$$

According to this formula, Δg_C ranges from 610.4 kJ mol⁻¹ for $x_{FFA} = 1$ (full fat fuel), to 474.0 kJ mol⁻¹ for $x_{FFA} = 0$ (full carbohydrate fuel). A graphic representation of Eq. (63) is contained in Fig. 1 below.

Because each mole of carbon atom liberates the energy Δg_C and each carbon atom takes an average time $\Delta\tau$ to oxidize, the total power produced at steady state from fuel oxidation per unit mass of wet muscle can be expressed as

$$p_t = \frac{\Delta g_C}{\Delta\tau} \kappa_C \quad (64)$$

This power is spent by the muscle in a number of ways, mainly to produce heat and mechanical work. The ratio $\Delta g_C/\Delta\tau$ appearing in Eq. (64) represents the power liberated at steady state per mole of carbon atoms oxidized. By introducing estimate (57), the same ratio can be expressed as

Fig. 1 Free energy, Δg_C , liberated upon oxidation of one mole of carbon atoms of fuel, cf. Eq. (63). Power, $\Delta g_C/\Delta\tau$, produced at steady state per mole of carbon atoms, as given by Eq. (65)

$$\frac{\Delta g_C}{\Delta \tau} = \frac{3465 x_{FFA} + 1420}{56 x_{FFA}^2 + 71.1 x_{FFA} + 22.5} \text{ kW/mol} \quad (65)$$

thanks to Eq. (63). A plot of Eq. (65) is given in Fig. 1.

Indirect calorimetry provides a comparatively simple means to determine p_t experimentally from the measurements of the muscle's $\dot{V}_{O_2}$ and RQ. A formula relating these quantities can be obtained from Eq. (64) by making use of Eqs. (55) and (34). We thus obtain

$$p_t = \frac{1}{1524} \Delta g_C \dot{V}_{CO_2} = \frac{1}{1524} \Delta g_C \text{RQ} \dot{V}_{O_2} \text{ kW kg}^{-1}, \quad (66)$$

which holds true provided that $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ are expressed in $[\text{L min}^{-1} \text{ kg}^{-1}]$ and Δg_C in $[\text{kJ mol}^{-1}]$; see Eq. (63). Using Eq. (39), this equation can also be expressed in terms of x_{FFA} as follows:

$$p_t = \frac{1}{1524} \Delta g_C \dot{V}_{O_2} \frac{1 - 1.661 x_{FFA}}{1 + 2.826 x_{FFA}} \text{ kW kg}^{-1} \quad (67)$$

For example, if we assume $\dot{V}_{O_2} = 0.3 \text{ l min}^{-1} \text{ kg}^{-1}$ (a reasonable value for a muscle in a submaximal exercise, cf., e.g., [33–35]) and we take $x_{FFA} = 0.2$, we obtain from Eq. (63) that $\Delta g_C = 528.25 \text{ kJ mol}^{-1}$. With this value of Δg_C , Eq. (67) yields $p_t = 0.044 \text{ kJ sec}^{-1} \text{ kg}^{-1} = 44 \text{ W kg}^{-1}$. The same calculations for $x_{FFA} = 0.1$ (i.e., for a fuel with less fat and more carbohydrate) yields $\Delta g_C = 504.71 \text{ kJ mol}^{-1}$ and $p_t = 0.065 \text{ kJ sec}^{-1} \text{ kg}^{-1} = 65 \text{ W kg}^{-1}$. Though possessing a smaller Δg_C , a fuel mixture richer in carbohydrates produces, as known, more power.

6 Limits to oxidable fuel in steady-state conditions

For given values of pressure and temperature, relation (26) gives the analytical expression of the admissible range of a muscle cell in terms of the single variable $\bar{n}_{eq}$ (moles of solutes per mole of cytosolic water). As already observed, this range is represented by a segment on the $\bar{n}_{eq}$ -axis (Fig. 2) and therefore it is fully determined by the segment's endpoints into which the limit surface degenerates in the present case. These endpoints are the overhydration and dehydration limits of the cell. They can be determined experimentally by dehydrating and overhydrating the cell [2].

In the absence of more specific data, we shall refer to atmospheric pressure and normal body temperature ($T = 310 \text{ K} = 37^\circ \text{C}$), and to the following values, typical of skeletal muscle cells of most animals (cf. [2, 36–39]):

- Water molecules at rest: about 99% of all molecules in a cell.
- Cytosolic water at rest: roughly 68–78% of cell weight, depending among other things on muscle type, age, state of health, and training.
- Total molecules of cytosol solutes in a healthy cell at rest: $n_{eq}^0 \approx 0.01 n_{H_2O}^0$, where $n_{H_2O}^0$ are the moles of cytosolic water at rest. This makes $\bar{n}_{eq} \approx 0.01$ for a cell at rest in normal healthy conditions (a rough estimate assumed here in the absence of a precise chemical analysis of the cytosol).
- Dehydration limit: $\bar{n}_{eq} = 12.7 \cdot 10^{-3}$ (corresponding to a loss of about 21% of cytosolic water from normal rest values).

Fig. 2 Representation of a typical muscle cell's admissible range in the $\bar{n}_{eq}$ -axis. Variable $\bar{n}_{eq}$ denotes the total moles of cytosol solutes per mole of cytosolic water; see Eq. (28). If the cell absorbs water, the rest state moves to the left of the figure, thus increasing $\Delta\bar{n}_{eq}|_{\max}$. The converse occurs if the cell loses water

- e) Overhydration limit: $\bar{n}_{eq} = 8.9 \cdot 10^{-3}$ (corresponding to a gain of about 12% of cytosolic water from normal rest values).

The muscle cell admissible range represented in Fig. 2 refers to the data reported above. A detailed discussion on its construction is given in [2]. Figure 2 shows that, in the considered case, the maximum amount of extra solutes that can be added, per mole of cytosolic water, to the solutes already dissolved in the cytosol in normal resting conditions is

$$\Delta\bar{n}_{eq}|_{\max} = 2.7 \cdot 10^{-3} \quad (68)$$

(mol of solutes per mol of cytosolic water). If this limit is exceeded, the cell leaves the admissible range. This is bound to produce irreversible changes in the cell, which may damage it permanently or require the expenditure of energy to be repaired, as already observed in Sect. 2.

The quantity $\Delta\bar{n}$, introduced in Sect. 4, represents the extra solutes that must stay dissolved in the cytosol during steady state exercise at a given power. This quantity cannot exceed the limit $\Delta\bar{n}|_{\max}$ implied by the value $\Delta\bar{n}_{eq}|_{\max}$ given by Eq. (68). The quantity $\Delta\bar{n}_{eq}|_{\max}$ refers to one mole of cytosolic water, while $\Delta\bar{n}$, as defined in Sect. 4, refers to 1 kg of wet muscular tissue. Therefore, in order to determine $\Delta\bar{n}|_{\max}$, we may recall that the water content of a skeletal muscle, inclusive of interstitial water, is about 64% of the muscle wet weight [40]. Thus, because the molar mass of water is $18.015 \cdot 10^{-3}$ kg/mol, from Eq. (68) we infer that

$$\Delta\bar{n} \leq \frac{0.64}{18.015 \cdot 10^{-3}} \Delta\bar{n}_{eq}|_{\max} = 35.52 \Delta\bar{n}_{eq}|_{\max} \quad \text{mol/kg} \quad (69)$$

where the coefficient 35.52 represents the moles of cytosolic water per kilogram of wet muscle. From relation (69), it follows that the maximum value that $\Delta\bar{n}$ can attain is, in the present case,

$$\Delta\bar{n}|_{\max} = 35.52 \Delta\bar{n}_{eq}|_{\max} \quad \text{mol/kg} \quad (70)$$

A limitation to the value of $\Delta\bar{n}$ implies a limitation to the maximum values of κ_{CHO} and κ_C of the fuel mixtures that the cell can use. These maxima will be indicated as $\kappa_{CHO}|_{\max}$ and $\kappa_C|_{\max}$, respectively. The value of $\kappa_{CHO}|_{\max}$ is readily obtained by solving Eq. (53) after setting $\Delta\bar{n} = \Delta\bar{n}|_{\max}$ into it. We thus obtain

$$\kappa_{CHO}|_{\max} = \frac{1-x_{FFA}}{143 + 198x_{FFA}} \Delta\bar{n}|_{\max} = \frac{4.40 \text{ RQ} - 3.06}{237.05 \text{ RQ} - 45.43} \Delta\bar{n}|_{\max} \quad \text{mol/kg} \quad (71)$$

If we refer, for example, to a muscle cell with the admissible range of Fig. 2, we have that

$$\Delta\bar{n}|_{\max} = 0.096 \quad \text{mol/kg} = 96 \text{ mmol/kg} \quad (72)$$

as immediately follows from Eqs. (68) and (70). In this case, Eq. (71) particularizes to

$$\kappa_{CHO}|_{\max} = 0.096 \frac{1-x_{FFA}}{143 + 198x_{FFA}} = 0.096 \frac{4.40 \text{ RQ} - 3.06}{237.05 \text{ RQ} - 45.43} \quad \text{mol/kg} \quad (73)$$

Similar calculations determine the maximum value of the quantity κ_C . In this case, we start by observing that from Eq. (58) we have that

$$\kappa_C = 6 \kappa_{CHO} + 16 \kappa_{FFA} \quad (74)$$

From this and from Eq. (51), we infer that, in general,

$$\kappa_C = 6 \kappa_{CHO} + 16 \frac{x_{FFA}}{1-x_{FFA}} \kappa_{CHO} = \frac{6 + 10x_{FFA}}{1-x_{FFA}} \kappa_{CHO} \quad (75)$$

From this equation, by substituting for κ_{CHO} the value of $\kappa_{CHO}|_{\max}$ given by Eq. (71), the maximum value of κ_C is finally obtained:

$$\kappa_C|_{\max} = \frac{6 + 10x_{FFA}}{143 + 198x_{FFA}} \Delta\bar{n}|_{\max} = \frac{8.00 \text{ RQ} + 0.04}{237.05 \text{ RQ} - 45.43} \Delta\bar{n}|_{\max} \quad \text{mol/kg} \quad (76)$$

The last equality is a consequence of Eq. (39)₃. Equation (76) shows that, for a given fuel mixture, the value of $\kappa_C|_{\max}$ —i.e., the maximum number of parallel oxidation paths that the cell can activate at steady state—is proportional to $\Delta\bar{n}|_{\max}$ and thus, in view of Eq. (70), also to $\Delta\bar{n}_{eq}|_{\max}$.

In the particular case of a muscle cell with the admissible range of Fig. 2, we obtain from Eqs. (72) and (76) that

$$\kappa_C|_{\max} = 0.096 \frac{6 + 10x_{FFA}}{143 + 198x_{FFA}} = 0.096 \frac{8.00 \text{ RQ} + 0.04}{237.05 \text{ RQ} - 45.43} \quad \text{mol/kg} \quad (77)$$

This is the maximum number of carbon atoms of fuel that, at steady state, can be oxidized simultaneously, by 1 kg of wet muscle made of cells with the admissible range and rest state of Fig. 2. The bold curve in Fig. 3 plots Eq. (77).

As apparent from Eq. (76), $\kappa_C|_{\max}$ depends on the fuel composition. However, the variation of $\kappa_C|_{\max}$ with fuel composition is modest (cf. Fig. 3). On the contrary, the values of $\kappa_C|_{\max}$ are quite sensitive to changes in the cytosolic water content. The reason is that the cell's admissible range and, in particular, its left and right limits are, by their very definition, independent of the changes in cytosolic water content. However, an increase δn_{H_2O} in the moles of cytosolic water at constant solute content (i.e., at $n_{eq} = \text{const.}$) makes the coordinate of the rest state decrease from its original value $\bar{n}_{eq} = n_{eq}^o / n_{H_2O}^o$ to the final value $\bar{n}_{eq} = n_{eq}^o / (n_{H_2O}^o + \delta n_{H_2O})$. As a result, the rest state moves to the left of the diagram of Fig. 2 by the amount $\delta(\Delta\bar{n}_{eq}|_{\max}) = n_{eq}^o / n_{H_2O}^o - [n_{eq}^o / (n_{H_2O}^o + \delta n_{H_2O})]$. Or, equivalently,

Fig. 3 Values of $\kappa_C|_{\max}$ vs. fuel composition (represented in the figure by x_{FFA} or x_{CHO}). All diagrams refer to a muscle cell with the admissible range of Fig. 2. The bold curve, marked “normal”, is a plot of Eq. (77). Absorption or loss of cytosolic water moves this curve into the light curves, as specified in the figure

$$\delta\left(\Delta\bar{n}_{eq}|_{\max}\right) = \frac{n_{eq}^o}{n_{H_2O}^o} \frac{\delta n_{H_2O}}{n_{H_2O}^o + \delta n_{H_2O}} \quad (78)$$

This means an equal increase in $\Delta\bar{n}_{eq}|_{\max}$ (cf. Fig. 2). The resulting increase in $\kappa_C|_{\max}$ may turn out to be comparatively large, as follows from Eqs. (70) and (76).

For example, the upper two curves of Fig. 3 show the effect on $\kappa_C|_{\max}$ of an increase in cytosolic water of 5 and 10%, respectively, in a cell with the admissible range of Fig. 2. The result is an increase in $\kappa_C|_{\max}$ ranging from 15 to 40% of its original values. The implications for muscle power capacity are obvious since $\kappa_C|_{\max}$ represents the maximum number of carbon atoms that can be oxidized simultaneously at steady state. This explains the observed increase of intracellular water in muscle cells at the onset of moderate to high exercise (see, e.g., [38, 41–43]). In order to meet the immediate demand for power, muscle cells absorb water from unused muscles and other fluid compartments, so that they can increase the value of $\kappa_C|_{\max}$ and thus burn more fuel per unit time.

On the contrary, if the cytosol loses water, then $\delta n_{H_2O} < 0$. In this case, Eq. (78) yields a negative value for $\delta(\Delta\bar{n}_{eq}|_{\max})$: a loss of cytosolic water reduces $\Delta\bar{n}_{eq}|_{\max}$ and thus the maximum amount of oxidable carbon atoms at steady state (c.f., e.g., the curves marked –5% and –10% in Fig. 3). This reduces the maximum power that the muscle cell can produce, which is consistent with a well-known effect of muscle dehydration [44, 45]. The same conclusion is also consistent with the observed decline of muscular power associated with progressive loss of intracellular water occurring with ageing [46].

7 Total power diagram

By introducing into Eq. (64) the value of κ_C given by Eq. (76)₁ and by using Eq. (65), we get, after some algebra,

$$p_t|_{\max} = \frac{693 x_{FFA} + 284}{221.76 x_{FFA}^2 + 308.66 x_{FFA} + 107.25} \Delta \bar{n}|_{\max} \quad \text{kW/kg} \quad (79)$$

where $p_t|_{\max}$ denotes the value that p_t assumes when $\Delta \bar{n} = \Delta \bar{n}|_{\max}$. This formula expresses the muscle's maximum total power per kilogram of wet tissue as a function of the mole fraction of fat in the fuel mixture burned by the muscle. For muscle cells possessing the admissible range and rest state of Fig. 2, the quantity $\Delta \bar{n}|_{\max}$ to be introduced in Eq. (79) is given by Eq. (72). In this case, Eq. (79) particularizes to

$$p_t|_{\max} = \frac{33.26 x_{FFA} + 13.63}{110.88 x_{FFA}^2 + 154.33 x_{FFA} + 53.63} \quad \text{kW/kg} \quad (80)$$

The top (bold) curve of Fig. 4 provides a plot of this equation. Whether the muscle can actually produce $p_t|_{\max}$ depends on many factors. These may be external factors (e.g., adequate supply of oxygen and fuel) or internal factors (e.g., the presence of enough mitochondrial mass in the cells). Many of these factors depend on training.

The maximum power of a muscle is strictly related to the number κ_C of carbon molecules that the muscle cells can oxidize simultaneously. If $\kappa_C < \kappa_C|_{\max}$, the number of parallel oxidation paths that can be activated within a cell is less than the maximum admissible. In these conditions, the muscle's maximum power will be less than $p_t|_{\max}$.

Fig. 4 Total power, p_t , produced at steady state per kilogram of wet muscle as a function of fuel composition. The diagrams refer to muscle cells with the admissible range and rest state described in Fig. 2. The bold curve gives the muscle's total power when the maximum number of parallel oxidation paths are active, Eq. (79). The other curves correspond to the activation of different fractions of parallel oxidation paths

To describe the actual capacity of a muscle to produce power, it makes sense to consider other curves, analogous to curve $p_{l|\max}$, but relevant to a lesser number of parallel oxidation paths. Because for any given fuel composition the quantity $\kappa_{C|\max}$ is proportional to $\Delta\bar{n}_{|\max}$, these curves are readily obtained from Eq. (79) by substituting $r\Delta\bar{n}_{|\max}$ for $\Delta\bar{n}_{|\max}$, where r is any real number between 0 and 1. The resulting expression represents therefore the maximum power corresponding to fraction $r\kappa_{C|\max}$ of $\kappa_{C|\max}$; or, which is the same, the power produced by the muscle when only a fraction r of the maximum admissible number of parallel oxidation paths is active. A set of curves relevant to different values of r are plotted in Fig. 4. Their analytical expression is

$$p_t = \frac{693 x_{FFA} + 248}{221.76 x_{FFA}^2 + 308.66 x_{FFA} + 107.25} r \Delta\bar{n}_{|\max} \quad \text{kW/kg} \quad (81)$$

This equation is analogous to Eq. (79) and can be obtained in a similar way.

As recalled in Section 3, a minimum of carbohydrates must be contained in the muscle fuel for mitochondria to oxidize fat: the cell's physiology prevents muscles from burning full fat fuel ($x_{FFA} = 1$). For this reason, the grey zone in Fig. 4 is not actually reachable by a muscle, although the same zone is perfectly admissible from the energetic standpoint. From the data in the literature, we know that the left-hand limit of the grey zone should fall somewhere between $x_{FFA} = 0.82$ and $x_{FFA} = 0.87$. Its actual position is of little concern to the present analysis, which, being based on energetic arguments, applies to every point of Fig. 4. It should be kept in mind, however, that if a point falls in the grey zone of the diagram, that point may not be reachable for physiological—rather than energetic—reasons.

A point (x_{FFA}, p_t) of Fig. 4 defines a particular combination of fuel mixture and total muscle power in steady-state conditions. There is only one power curve $r\Delta\bar{n}_{|\max}$ passing through each point. The value of r of such a curve can be determined by solving Eq. (81) upon inserting into it the coordinates x_{FFA} and p_t of the considered point. The value of r can also be estimated directly from Fig. 4 by graphic interpolation. Take, for instance, point Q in that figure. A simple glance at that figure shows that the value r to be associated with point Q is about 0.79. From Fig. 4, we can therefore determine the fraction $r\kappa_{C|\max}$ of parallel oxidation paths that must be active at steady state in a muscle that produces a given total power from a given fuel mixture.

All the experimental information we need to enter a point in the diagram of Fig. 4 are the values of $\dot{V}_{O_2}$ and x_{FFA} (or RQ). The reason is that these quantities determine the value of p_t , as follows immediately from Eqs. (63), (66), (67), and (34).

To produce a given power, a muscle may use an entire range of fuel mixtures, corresponding to different fat/carbohydrate ratios. The choice of the particular mixture depends on many factors, among which the power level, carbohydrate availability, duration of exercise and muscle training are especially significant [47]. Line AB divides Fig. 4 into two regions. The values of power below this line can be produced from any fuel mixture, ranging from full carbohydrate ($x_{FFA} = 0$) to virtually full fat ($x_{FFA} = 1$). By contrast, for values of power above line AB, the fat content of the fuel used cannot exceed the limit imposed by curve $p_{l|\max}$. Take, for instance, the power corresponding to point P of Fig. 4. In this case, the fuel mixtures that the muscle can use to produce this power are limited to the abscissae of the points of segment PS. This means that the fat content of the allowable mixtures ranges from zero to a maximum value, determined by the abscissa of point S. Fuel mixtures still richer in fat cannot possibly be

used to produce the considered power because this would require the activation of a number of parallel oxidation paths greater than those that the muscle cells can accommodate.

The above diagrams may help assess the physiological state and fitness of a muscle. This is best illustrated by the following discussion on some typical cases. All of them refer to a muscle which is producing power in steady-state conditions. All the experimental information needed for the following discussion can be obtained from the measurement of the values of $\dot{V}_{O_2}$ and RQ for the considered muscle in the considered exercise.

Case 1. The muscle works at a point on curve $p_{l|max}$.

Let us refer for instance to point S of Fig. 4. The power produced in the exercise can be read at point P of the p_f -axis. The muscle can produce the same power at any other point of segment PS. If the point is different from S, however, the muscle would burn more carbohydrates than it does at point S. Point S represents, therefore, the point of maximum fat consumption at the considered power. This gives the muscle the maximum stamina in the exercise because the muscle reserves of fat are larger than those of carbohydrates. As a consequence, by operating at point S, the muscle has a greater capability of doing extra work at a higher power at the end of the exercise, as needed, for instance, in a final sprint. Generally speaking, in order to work at a point of curve $p_{l|max}$, the muscle must activate the maximum number of parallel oxidation paths that a muscle cell can possibly contain. Thus, the capability of working at or near curve $p_{l|max}$ denotes good muscle health and accomplished training.

Case 2. The muscle works at a point Q below curve $p_{l|max}$ but above line AB.

In this case, the number of active parallel oxidation paths is less than $\kappa_{C|max}$. Thus, to produce the same power as in case 1, the muscle must burn more carbohydrates and less fat. The incapacity of the muscle to activate the maximum number of parallel oxidation paths may result from various factors, such as a lack of adequate training, disease, ageing, etc. It may also simply be due to the abundance of carbohydrates following a recent meal. In the last case, point Q should move toward S as the carbohydrates reserves start to diminish. Apart from any matter concerning fuel mixture composition, the muscle may perform the exercise quite satisfactorily. However, if the muscle is incapable of activating additional parallel oxidation paths, it cannot perform a similar exercise at a power that exceeds that of point C at which curve $r\Delta\bar{n}|_{max}$ through Q (shown dashed in Fig. 4) crosses the p_f -axis. In the considered example, this point falls just above 0.2 kW/kg. By contrast, a healthy and well-trained muscle can increase the number of parallel oxidation path up to the value $\kappa_{C|max}$ (corresponding to point R of Fig. 4). The muscle could thus perform the considered exercise at a much higher power still using the same fuel composition.

Case 3. The muscle works at a point Q below line AB.

To produce power below line AB, a muscle need not activate all its admissible parallel oxidation paths. The actual number of parallel oxidation paths used is determined by the power curve through the point at which the muscle is working. For instance, to work at point H on curve $0.5 \Delta\bar{n}|_{max}$ the number of oxidation paths that a muscle needs to activate is $0.5 \kappa_{C|max}$. By working near the boundary of the grey area of Fig. 4, a muscle can produce any

power below line AB while burning the minimum amount of carbohydrates (cf., e.g., point K in Fig. 4). It may well happen, of course, that a muscle cannot work in that part of the diagram, because it can only activate a smaller number of parallel oxidation paths. In this case, to produce the same power it has to burn more carbohydrates and less fat (see, for instance, point H in Fig. 4). Even for low power steady-state exercises, therefore, the considered diagrams may provide useful information on the muscle's state of health and training, once the values of $\dot{V}_{O_2}$ and RQ during the exercise are known.

8 Conclusions

The maximal steady-state *total power* that can be produced by a muscle with the properties described in Fig. 2 is about 254 W per kilogram of wet muscle. This result follows from Eq. (79) and is represented in Fig. 4 by the point at which curve $p_{t|\max}$ crosses the p_r -axis. To generate this power, the muscle needs a full carbohydrate fuel ($x_{FFA} = 0$), consistent with the data reported in [48]. Man and most animals cannot supply this power for too long a time, due to their limited carbohydrate reserves. By burning a fuel mixture richer in fats, the muscle can work much longer. In this case, however, its maximal power is lower. For instance, for a fuel mixture with $x_{FFA} = 0.8$ the muscle's $p_{t|\max}$ turns out to be about 168 W per kilogram of wet muscle, as shown by Fig. 4.

By referring to the above values of $p_{t|\max}$ (namely, 254 W/kg and 168 W/kg) and assuming a muscle efficiency $\eta = 0.24$ (an acceptable value for human cycling [4, 49]), from Eq. (2) we calculate that the corresponding maximal values of mechanical power per kilogram of wet muscle are $p_m = 60$ W/kg and $p_m = 38$ W/kg for $x_{FFA} = 0$ and $x_{FFA} = 0.8$, respectively. These values appear to be quite realistic if compared to the experimental values reported in [33, 35]. The same values and any other value obtained in a similar way from curve $p_{t|\max}$ for different fuel mixture compositions represent the maximal steady state mechanical power that the muscle can produce from the considered fuel mixture.

Mechanical power is comparatively easy to measure experimentally. The determination of the composition of the fuel mixture used by a muscle is well within reach of current experiments, although it involves some more delicate procedures. From experiments, therefore, the point in the diagram of Fig. 4 at which a muscle works in a given steady-state exercise can readily be found. As discussed in Section 7, this provides some vital information concerning the state of health, ageing, and fitness of the muscle and its possible improvement as a result of training. It also provides a piece of objective evidence to estimate the athletic potential of an individual in sports activities that require prolonged aerobic work production.

The results of this paper may find application to other fields of biological physics, in addition to the ones explicitly mentioned above. This is the case, for instance, of the energetics of avian flight muscles — a field where much interesting experimental work is already available [9, 50–52]. More generally, the analysis presented here with reference to muscle cells appears to be extendible, with due changes, to any other kind of cell, whenever a thermodynamic limit to cell activity and consumed substrate is sought.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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