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Models of muscle contraction and energetics

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Abstract

How does skeletal muscle manage to regulate the pathways of ATP synthesis during large-scale changes in work rate while maintaining metabolic homeostasis remains unknown. The classic model of metabolic regulation during muscle contraction states that accelerating ATP utilization leads to increasing concentrations of ADP and Pi, which serve as substrates for oxidative phosphorylation and thus accelerate ATP synthesis. An alternative model states that both the ATP demand and ATP supply pathways are simultaneously activated. Here, we review experimental and computational models of muscle contraction and energetics at various organizational levels and compare them with respect to their pros and cons in facilitating understanding of the regulation of energy metabolism during exercise in the intact organism.

Keywords

computer models; energy metabolism; regulation; exercise; in vivo models; single fiber

Skeletal muscle has the extraordinary capacity to adjust to an immediate increase in energy demand. The dynamic response of the energetic system of a muscle cell to increased work or exercise is one of the most important aspects of cell functioning. At rest, both the concentration of adenosine triphosphate (ATP) in skeletal muscle and the ATP turnover rate are relatively low [1]. During muscle contraction, however, the rate of ATP utilization can increase by up to two orders of magnitude, while muscle ATP concentration remains near resting levels [2]. Maintenance of ATP homeostasis in working muscle during large-scale changes in ATP turnover rates requires close coupling of ATPase flux with ATP synthase flux [3]. Moreover, it requires precise coordination of the ATP-supply pathways (phosphagenic, glycolytic, and oxidative), each with distinct flux and energy capacities [4], to match the dynamics of ATP utilization. Surprisingly, while the flux through these energetic pathways increases in proportion to the energy demand, the concentrations of

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glycolytic, Krebs cycle, and respiratory chain intermediates remain relatively stable. Only the concentrations of a few energy-related metabolites, such as phosphocreatine (PC), adenosine diphosphate (ADP), and inorganic phosphate (P_i), change significantly during muscle contraction [5].

The classic model of metabolic regulation during muscle contraction states that upon arrival of activation signals at the myocyte, the increase in ATP demand accelerates cell ATPases leading to increased concentrations of their products (ADP, P_i , and H^+), which in turn serve as substrates for the ATP-supply pathways. In particular, ADP and P_i are considered to play a critical role in the control of mitochondrial oxidative phosphorylation and glycolysis. However, this simple model based on the study of mitochondria *in vitro* is not readily applicable to either blood-perfused tissue or intact vertebrates, especially in the transient state of contractions or at the onset of exercise [5].

Contractions in intact muscle or whole body dynamic exercise demand not only proportional increases in flux rates for almost all enzymatic reactions in the ATP supply and demand pathways at the cellular level, but also stimulation of the routes of convective and diffusive oxygen delivery, and mobilization of exogenous fuels towards the contracting muscles. However, the large changes in ATP turnover rate observed during rest-to-work transitions do not seem to be mediated by equally large changes in pool size of the numerous intermediates. Indeed, the concentrations of most intermediates do not vary by more than 0.5- to 3-fold over their resting values. Consequently, a key question remains: How does skeletal muscle manage to regulate the pathways of ATP synthesis during sustained exercise while maintaining metabolic homeostasis? This process involves the interaction of intracellular compartments, many metabolites and enzymes, reactions and pathways, as well as feedback and feedforward control loops at various biological scales. Here, we review experimental and computational models of muscle contraction and energetics at various organizational levels and compare them with respect to the insights provided and limitations in gaining quantitative understanding of the regulation of energy metabolism during muscle contraction in the intact organism *in vivo*. In Table 1, a comparison of experimental and computational models is reported with their best use, advantages and disadvantages.

Experimental models of muscle contraction and energetics

The regulation of skeletal muscle ATP concentration during exercise involves processes of ATP utilization (myosin ATPase, the sarco-endoplasmic reticulum Ca^{2+} -ATPases, and Na^+ - K^+ ATPase) to provide energy for muscle contraction and processes of ATP synthesis (substrate-level and oxidative phosphorylation) in order to maintain [ATP] constant. The entire physiological process is a multilevel whole-organism phenomenon that is highly regulated and involves not only the neuroendocrine, cardiovascular, respiratory, and neuromuscular systems, but also networks of reactions comprising the main pathways of energy metabolism, as well as their interaction. Consequently, information gathered at a specific scale assumes that the inputs to the subsystem investigated are not regulated by a higher-scale system. Nevertheless, critical mechanistic and phenomenological information can be gathered in these subsystems to determine the extent to which specific factors contribute to the control of transport and reaction fluxes linking the various physiological subsystems. Thus, information gathered at multiple scales needs to be integrated, analyzed, and interpreted in the context of the entire system investigated.

Isolated single skeletal muscle fiber model

The isolated single muscle fiber model has several advantages for the study of metabolism [6, 7]. Significantly, individual muscle fiber types can be evaluated, and O_2 supply to those fibers can be precisely controlled, thus avoiding the confounding effects of having fiber type

mixtures, extracellular gradients of metabolites, and oxygen delivery heterogeneity that characterizes whole tissue/organ preparations. It is also relatively easy to perform fluorescent studies in single fibers thus allowing measures of intracellular constituents including NAD(P)H, H^+ , Ca^{2+} , and O_2 which are of particular importance in the present discussion. In the single muscle fiber preparation, individual fibers are discriminated according to fiber type, and a single fiber is surrounded by a homogeneous medium [6]. Here we focus on a series of studies conducted in the last 10 years on single living muscle fibers microdissected from muscles removed from adult *Xenopus laevis*; these fibers lack myoglobin. After dissection, platinum clips are attached to the tendons and the muscle fibers are mounted in a glass chamber where they are continually superfused with Ringer solution at 20°C and 7.0 pH. One tendon end is attached to a force transducer system to monitor isometric force development during tetanic contractions induced via electrical stimulation. By using various stimulation protocols and by controlling the chemical composition of the medium while monitoring force generation by the fiber, and intracellular pH and PO_2 , the effect of putative factors affecting muscle contraction and energetics in this model can be investigated.

Using this model, some of the potential mechanisms regulating the dynamics of oxidative metabolism, ATP hydrolysis, and force development in response to square-wave or ramp-like changes in energy demand have been investigated over the last decade. A series of studies were initially conducted under conditions in which oxygen availability in the superfusate was varied from anoxic to hyperoxic levels [8,9,10,11,12]. Then, another series of studies was performed while some of the energetic pathways were altered by administering specific pharmacological agents [13,14,15].

Specifically, the differential contribution of oxidative and substrate-level phosphorylation to force production during repetitive, maximal tetanic contractions was investigated in fast-twitch (non-oxidative) fibers under conditions of high-oxygen availability versus anoxia [16]. Results showed that during the early phase (< 60 s) of low intensity contractions (0.25 Hz tetanic contractions), substrate-level phosphorylation had the capacity to maintain force and ATP hydrolysis in the absence of oxidative phosphorylation. This period was followed by an oxygen-dependent phase in which force generation was maintained only during the high PO_2 condition. As contraction frequency was increased, fatigue occurred even in the presence of O_2 , although the fatigue rate was much more rapid in the anoxic condition. The authors concluded that anoxia does not impair muscle contractility at low intensities until non-oxidative energetic pathways are compromised. More recently, the speed of glycolytic activation was assessed in the frog single muscle fiber [15]. Single myofibers were stimulated for 15 s at 0.67 Hz in anoxia either in the presence (IAA) or absence (Control) of the glycolytic inhibitor, iodoacetic acid while intracellular pH (pHi) was measured via a fluorescent technique employing injection of 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester into the fibers. Under both conditions, Control and IAA, pHi increased similarly over the first 6 contractions (first nine seconds), presumably due to phosphocreatine (PC) breakdown. However, beyond nine seconds, pHi decreased in the Control condition whereas it continued to increase slowly in the IAA condition. These results indicated that glycolysis is activated early in contractions, certainly no later than nine seconds under the stimulation pattern used in this study.

As indicated above, the single muscle fiber model has been heavily used in the investigation of the oxidative response to contractions. In almost all of these studies, intracellular PO_2 (PiO_2) was assessed by observing the phosphorescence quenching of a palladium porphine that was microinjected into the muscle fibers. In the first study [8] of the series, muscle fibers were subjected to two 3-min periods of stimulation, separated by a 5-min rest period. The most important result was a lengthy delay (≈ 5 –13 s) before PiO_2 began to decline

following the onset of contractions. This suggested that the dynamics of oxidative phosphorylation is controlled by factors other than O_2 availability at the submaximal contraction intensity investigated. The other significant result was that prior contractions (the first 3-min stimulation period) speeded the on-kinetics of the PiO_2 decline; the half-time of the PiO_2 response was 18 ± 4 s in the second contraction bout as compared to 28 ± 5 s in the first bout. In a subsequent study [10], muscle fibers were subjected to incremental changes in stimulation frequency (energy demand) with 3-min rest periods between stages. In response to this protocol, steady state PiO_2 declined linearly with increasing stimulation frequency, suggesting that PiO_2 and oxygen consumption (VO_2) are also linearly related in these cells [10]. A later study [11] demonstrated that the mean response time for the decrease in PiO_2 was progressively faster at higher contraction intensities (increasing energy demand), suggesting that a faster accumulation of stimulators of oxidative phosphorylation initiated a more rapid acceleration of mitochondrial respiration.

Later, the effect of oxygen concentration on the dynamics of oxidative metabolism during exercise was studied by investigating the effect of varying extracellular PO_2 (PeO_2) on PiO_2 dynamics in muscle fibers during the transition from rest to tetanic contractions of high intensity [17]. As might be expected, the change in PiO_2 steady state values from rest to contractions was greater at each progressively greater PeO_2 because of the correspondingly higher initial PiO_2 . Perhaps surprisingly, the mean response time for PiO_2 slowed as the PeO_2 was increased. However, when the initial increase in metabolic rate was calculated, it did not differ with PeO_2 , suggesting again that oxygen concentration does not play a key role in determining the initial activation of oxidative metabolism in response to tetanic contractions in single skeletal muscle fibers exposed to PeO_2 ranging from 20 to 60 mmHg [17]. However, these data did not provide any information with respect to the mitochondrial reduction level during contraction. This led to an investigation of the effect of extracellular oxygen availability on the intracellular distribution of reduced nicotinamide adenine dinucleotide (NADH) during contractions [9]. Results showed that the rate of NADH oxidation within the cell is influenced by PeO_2 even at high levels (30 Torr) during the contraction cycle. In addition, this study demonstrated that although there was adequate oxygenation to support NADH utilization during the rest-to-work transition, there were regional differences involving the influence of oxygen on modulating mitochondrial respiration.

Finally, enzymatic reactions in the pathways of energy metabolism were manipulated in order to investigate their effect on the time profiles of PiO_2 during step-changes in energy demand. Howlett and Hogan [13] employed dichloroacetate (DCA) to investigate the effect of augmented substrate delivery on intracellular PO_2 dynamics at contractions onset. DCA enhances activity of the enzyme pyruvate dehydrogenase (PDH) thus facilitating conversion of pyruvate to AcetylCoA for entry into the Krebs cycle. In response to DCA, the time constant (τ) was speeded from 39.7 ± 4.8 s to 22.1 ± 3.6 s. These results indicated that prior turn-on of PDH leads to a faster overall activation of oxidative metabolism, arguing for an important role of intrinsic factors in addition to and/or in concert with O_2 supply. In another study [18], creatine kinase (CK), the enzyme that catalyzes PC breakdown, was inhibited by iodoacetamide. In comparison to control conditions, creatine kinase inhibition (CK_i) caused a speeding of the fall in PiO_2 at contractions onset; mean response time for $CK_i \approx 32$ s vs Control ≈ 49 s (see Fig. 1). These results support the notion that CK activity and its corresponding PC breakdown buffers ATP and ADP concentrations at contractions onset such that energetic control signals are dampened. This action of the PC system may be a major source of the “lag” in increased oxidative metabolism at the onset of changes in energy demand.

In 2006, *N*-benzyl-*p*-toluene sulfonamide (BTS) was used to inhibit cross-bridge cycling (actomyosin interaction) in isolated *Xenopus laevis* muscle fibers [19]. BTS attenuated developed tension by about 95% but did not affect either resting or peak intracellular $[Ca^{2+}]$ during repeated isometric contractions. Estimated energy cost of isometric tetanic contractions was reduced by more than 50% and extrapolation led to the calculation that actomyosin ATP requirement is about 58% of overall ATP consumption during isometric contractions in mixed fiber types. Interestingly, BTS also slowed the fall in PiO_2 (time to 63% of overall ΔPiO_2 from 75s to 101s suggesting an important role for the products of ATP hydrolysis in determining the VO_2 on-kinetics.

In summary, these studies support the following conclusions for isolated single myocytes: 1) glycolysis is rapidly activated at contractions onset and can support the maintenance of muscle performance for a limited period of anoxia during low intensity activity; 2) O_2 supply, over a wide range, is not a critical factor in the activation of mitochondrial metabolism at contractions onset; 3) in the face of constant extracellular PO_2 (30 Torr), PiO_2 declined linearly with increasing stimulus frequency; 4) the initial decline in PiO_2 at contractions onset is faster at higher contraction intensities; 5) although O_2 supply does influence the rate of NAD(P)H oxidation, it does not limit NAD(P)H oxidation at the onset of contractions; 6) prior turn-on of PDH to increase substrate supply speeds PiO_2 on-kinetics; 7) inhibition of CK speeds the fall of PiO_2 at contractions onset, probably because of a faster increase in [ADP] and decrease in [ATP]; 8) activation energy (Ca^{2+} handling) accounts for a substantial proportion of total ATP cost during isometric contractions; and 9) abolishing ATP consumption via actomyosin ATPase results in slower VO_2 onset kinetics, suggesting that Ca^{2+} is not the primary controller of mitochondrial oxidative phosphorylation onset kinetics.

Isolated whole muscle in situ model

While isolated single myocytes have significant advantages as noted above, there are also disadvantages to that model. For example, single fibers do not thrive as well at or near 37 °C, and therefore metabolism has often been studied at unphysiological temperatures. Furthermore, the extracellular levels of O_2 as well as other gases, nutrients, and waste products remain constant whereas they are known to change in the intact exercising human *in vivo* [7]. Finally, it is obvious that the accompanying complementary adjustments in the vascular system with metabolic transitions cannot be investigated and are not considered in single fiber experiments. In this regard, isolated, perfused whole muscle preparations offer some advantages, particularly in that they can be studied during spontaneous self-perfusion as well as under conditions of controlled pump perfusion.

The canine *Gastrocnemius muscle complex* (*Gastrocnemius plus superficial digital flexor*; GS) muscle preparation was developed to investigate factors regulating energy metabolism in contracting muscle *in situ*. In this preparation, muscle contraction can be induced by stimulation of the branch of the sciatic nerve directly supplying the GS. In addition, the vasculature of the GS is isolated such that there is only one arterial supply and one venous drainage. This allows collection of blood samples directly across the muscle, as well as blood flow measurement for a specific, known muscle mass. These values can then be used to calculate muscle uptake or output of substrates and metabolites of interest, including O_2 . Two major advantages of this muscle preparation are the ability to control blood flow and substrate delivery (including O_2) to the muscle at any desired level and with the desired dynamics, and the ability to infuse metabolites or pharmacological agents directly into the muscle arterial supply, to the exclusion of the systemic circulation if necessary. The muscle in this preparation is also exposed for biopsy samples, thus allowing assessment of intramuscular parameters. This canine GS muscle preparation *in situ* is ideal for

investigations of whole muscle energy metabolism with special emphasis on oxidative metabolism.

With this isolated whole muscle model of muscle contraction, experiments were conducted to investigate whether the lag in oxygen uptake (VO_2) dynamics observed in humans at the onset of exercise results from a slow rate of adjustment in O_2 delivery, or a delay in the activation of mitochondrial oxidative phosphorylation. To determine if increased O_2 delivery to skeletal muscle could lead to faster muscle VO_2 dynamics, blood flow as well as arterial and venous oxygen content were measured during transitions from rest to 3 min of electrically stimulated isometric tetanic contractions (at $\sim 60\%$ peak VO_2). Specifically, the stimulated GS was either self-perfused (spontaneous) or pump-perfused at a constant elevated blood flow (fast O_2 delivery) corresponding to the steady-state value reached during the spontaneous contractions. In the latter perfusion method, any delay in the adjustment of convective O_2 delivery was eliminated by controlling muscle blood flow with a pump [20]. Regardless of perfusion method, similar time courses {Mean response time (MRT) = ~ 23 sec} of muscle VO_2 were obtained indicating that convective O_2 delivery does not limit VO_2 on-kinetics at moderate exercise intensities.

From these results, it was concluded that a sluggish adjustment of O_2 delivery at the onset of exercise was not the cause for the delay in VO_2 adjustment at submaximal intensities. Instead, the source of the delay resides within the muscle itself, perhaps in the evolution of stimuli that control mitochondrial oxidative metabolism. However, a limitation of O_2 delivery to mitochondria by diffusion remained possible. Accordingly, the rate of peripheral O_2 diffusion was enhanced by increasing the “driving pressure” for O_2 from muscle capillaries to mitochondria [21]. This was achieved by ventilating the anesthetized dogs with hyperoxic gas (100% O_2) and by administering a drug that induced a rightward shift of the HbO_2 dissociation curve; this was then combined with fast O_2 delivery as described above. Once again, no significant effect on muscle VO_2 on-kinetics (MRT = ~ 25 sec) was observed [21]. Taken together, these two studies indicate that, in highly oxidative skeletal muscle, during the transition from rest to contractions eliciting $\sim 60\%$ of peak VO_2 , neither convective nor diffusive O_2 delivery greater than normal, affects muscle VO_2 on-kinetics. This supports the hypothesis that the dynamics of muscle VO_2 at the onset of exercise, under normal conditions, is mainly set by intracellular factors that turn on muscle oxidative metabolism. However, how much “reserve”, if any, in terms of either convective O_2 delivery or diffusive O_2 delivery is available to muscle is unknown. This question of a “reserve” has direct relevance to disease states (e.g., peripheral arterial disease and diabetes) as well as aging and chronic conditions, which can reduce large artery flow and perhaps impair capillary function as well.

Next, the effect of convective O_2 delivery on VO_2 on-kinetics was investigated at higher metabolic intensities in this whole muscle model of contraction [22]. Accordingly, the same methods as in the previous studies were employed except that the transitions were made from rest to tetanic contractions corresponding to very high-intensity exercise ($\sim$ peak VO_2). Under these conditions, the results showed that there was a significant speeding of VO_2 on-kinetics (MRT from ≈ 25 down to ≈ 18 s) in response to the abolishment of delays in convective O_2 delivery (see Fig. 2). These results suggest that, for transitions involving contractions of a high metabolic intensity, convective O_2 delivery does play a role as a limiting factor for VO_2 on-kinetics. Thus, at higher intensities, impairments in arterial flow and capillary exchange would certainly be expected to influence the turn-on of oxidative metabolism. Nevertheless, the majority of the delay in VO_2 adjustment remains after elevating bulk O_2 delivery, insinuating that intrinsic metabolic factors are of great importance.

The isolated canine GS preparation was also used to investigate potential “bottlenecks” within the pathways of oxidative metabolism; such limiting reactions might be the loci of delayed metabolic activation of oxidative phosphorylation. Accordingly, experiments were conducted in which the activities of key enzymes were modified pharmacologically: (1) pyruvate dehydrogenase was activated with DCA infusion; (2) cytochrome c oxidase was activated by reducing the production of nitric oxide (NO) via inhibition of NO synthase; and (3) creatine kinase was blocked by iodoacetamide (IAA). Dichloroacetate infusion resulted in a significant activation of PDH, but it did not significantly affect PC breakdown, muscle lactate accumulation, estimated substrate-level phosphorylation, or VO_2 on-kinetics significantly [23]. Contrary to data from isolated myocytes [13], these results suggest that the speed of PDH activation is not responsible for the delayed activation of oxidative phosphorylation. Nevertheless, PDH activation with DCA resulted in increased metabolic efficiency. Next, the effects of NO synthase inhibition on muscle oxidative metabolism (i.e., VO_2 on-kinetics) were also evaluated using the isolated canine GS muscle *in situ* with muscle biopsies taken at rest and during contractions to assess effects on tissue ATP, PC, C, and lactate concentrations. Unexpectedly, no significant differences were observed for muscle metabolites and parameters of VO_2 on-kinetics during muscle contractions onset, except for reduced muscle fatigue [24]. This study suggested that relieving the inhibitory effect of NO on mitochondrial respiration does not accelerate the dynamics of muscle oxygen uptake at exercise onset. In agreement with results from the single muscle fiber described earlier, faster VO_2 kinetics after CK blocking by IAA was observed in a preliminary study using this dog model [25].

Using the same muscle preparation, several aspects related to the regulation of muscle lactate metabolism and exchange were investigated. First the highly oxidative canine GS *in situ* exhibited net lactate (La^-) uptake when perfused with an elevated $[\text{La}^-]$ (≈ 9 mM) [23]. Additionally, when metabolic rate was increased by stimulating the muscle to contract, the rate of net La^- uptake increased along with the steady state VO_2 [23]. This study, along with results from other whole muscle models, illustrates that when blood $[\text{La}^-]$ increases to the range of 2.5–4.0 mM and greater, both resting and exercising muscles can become sites of net La^- uptake. A later study [26] found that net La^- uptake by this muscle increased in proportion to arterial plasma $[\text{La}^-]$ at different metabolic rates ranging from rest to the highest level for twitch contractions. There was a tendency for net La^- uptake to approach a plateau at higher plasma La^- concentrations (≈ 20 –30 mM) with the highest asymptote occurring at the highest metabolic rate. Taken together, these results suggest that skeletal muscle produces and consumes La^- simultaneously on an ongoing basis and that during intense contractions at a high metabolic rate it can be a significant consumer of La^- . Furthermore, when both contraction frequency and plasma La^- concentration values are increased systematically from rest, La^- output by skeletal muscle approaches a saturation limit in an asymptotic manner as plasma La^- increases, and this relationship is shifted upwards with increased metabolic rate [26]. Later studies on the same muscle preparation indicate that the ability of skeletal muscle to remove La^- from the blood can be reduced with physiologically high levels of epinephrine concentration in the circulation [27], and that the principal fate of the increased muscle La^- uptake during muscle contraction is oxidation [28].

In summary, the papers employing the GS *in situ* discussed above offer the following conclusions: 1) there appears to be no convective or diffusive limitation of the adjustment of O_2 supply at the onset of contractions under control, spontaneous conditions at submaximal metabolic rates; 2) similarly, there do not appear to be any O_2 diffusion limitations that restrict VO_2 at contractions onset; 3) convective O_2 delivery does retard VO_2 on-kinetics to some degree in the transition from rest to contractions eliciting $\text{VO}_{2\text{peak}}$; 4) PDH and supply of fuel to the TCA cycle in the form of acetyl-CoA does not contribute to the intramuscular

delay in adjustment of oxidative phosphorylation at the onset of contractions, 5) NO does not appear to slow VO_2 on-kinetics by way of its proposed inhibitory effect on cytochrome c oxidase, and thereby mitochondrial respiration; 6) preliminary results suggest that inhibition of CK and thereby removal of the buffering effects of PC does speed VO_2 on-kinetics, 7) oxidative skeletal muscle is a ready consumer of La^- during steady state submaximal contractions; 8) epinephrine reduces this La^- uptake; and 9) the vast majority of La^- taken up is oxidized as a fuel.

Intact organism in vivo human model

In human studies, the changes in cellular respiration needed to increase ATP synthesis during muscle contraction are mechanistically linked to the corresponding external respiration. Factors that link these dynamic responses are the circulation, ventilation, O_2 stores in blood and muscle, and O_2 exchange across membranes. Consequently, pulmonary oxygen uptake (VO_{2p}) dynamics measured at the mouth by indirect calorimetry (IC) has been used frequently to investigate the regulation of cellular respiration in working skeletal muscles, noninvasively. A close, but invasive, estimation of oxidative phosphorylation dynamics (i.e., muscle oxygen uptake, VO_{2m}) can be obtained using thermodilution and catheterization techniques to determine muscle perfusion, as well as oxygen and substrate delivery and extraction by contracting muscle. Previous findings comparing VO_{2p} and VO_{2m} responses during muscle contractions, showed that VO_{2p} dynamics is close to that of VO_{2m} after correcting for transport lags [29] and that VO_{2p} and VO_{2m} changes from their respective baselines are similar during exercise [30]. Other noninvasive methods used to obtain semi-quantitative information regarding concentration changes of metabolites involved in muscle energetics include Nuclear Magnetic Resonance (NMR) and Near Infrared Spectroscopy (NIRS). The same information can be acquired via a needle biopsy, but this technique does not allow continuous monitoring of metabolites and metabolic fluxes in humans.

The NMR techniques, imaging and spectroscopy, play a central role in the investigation of skeletal muscle energetics non-invasively *in vivo*. For more than twenty years, ^{31}P NMR spectroscopy has extensively contributed to our understanding of how the energetic machinery operates in exercising muscles [31,32]. With relatively simple technology, such as pulse-acquire sequences and surface coils facing the muscle of interest, it is possible to identify and quantify, at least in relative concentrations, key metabolites: ATP, PC, and P_i (see Fig. 3). Through some adaptations and specific developments, it is now possible to monitor a series of physiological and biochemical variables dynamically in the skeletal muscle of humans and small animals as well. The protocol illustrated here realized simultaneous NMR acquisitions of ASL imaging, ^1H myoglobin spectroscopy and ^{31}P spectroscopy, allowing parallel assessment of muscle perfusion, reoxygenation and ATP production during exercise recovery. Such a multiparametric, dynamic and non-invasive approach matches well the requirements for multiple-level computer modeling of skeletal muscle energetics, both at the development and at the validation steps. Intramyocellular pH is calculated from the relative positions on the spectrum, named chemical shifts, of the P_i and PC resonances and, when ATP consumption and the associated H^+ release cannot be matched anymore by H^+ pumping by mitochondria, the acidosis threshold can be precisely detected. The ratio of P_i/PC allows the determination of free [ADP], the main metabolite controlling mitochondrial oxidative phosphorylation. Mitochondrial ATP production can be estimated from the initial re-phosphorylation rate of creatine post-exercise. In steady-state conditions, ATP synthesis can also be measured by magnetization transfer protocols. Creatine kinase activity is similarly accessible, as well as quantification of La^- concentration changes in exercising muscles [33,34]. Skeletal muscle cellular oxygen content can be probed by ^1H NMR spectroscopy of deoxymyoglobin [35], even at rest in normoxic muscles

[36,37]. In dynamic protocols, muscle de- or reoxygenation rate can be measured from deoxymyoglobin kinetic data [38,39]. Several NMR techniques can be combined to obtain simultaneous determinations of muscle perfusion, muscle reoxygenation, and mitochondrial ATP production during exercise. This allows detailed investigation of regulatory mechanisms *in vivo*, such as the interactions between oxygen supply, uptake and utilization. Because of the simultaneity of data acquisition, additional variables can then be computed, such as muscle O₂ extraction [39].

Functional MRI imaging comprises several approaches [40]. The most popular one, the blood oxygen level dependent (BOLD) imaging, was initially proposed for brain studies [41] and conveys information primarily on capillary-venous oxygenation but BOLD contrast is a complex function of hemoglobin concentration and saturation, tissue fractional vascular space, microvascular architecture and orientation relative to the main magnetic field [42,43]. It is often unduly taken as a surrogate marker of tissue perfusion, which can be measured more adequately otherwise. Arterial spin labelling consists of applying radio-frequency pulses to tag magnetically the blood flowing in feeding arteries. The arrival of the magnetically labeled blood in the target organs determines a modulation of tissue magnetization, in direct proportion to perfusion [44, 45, 46]. With this NMR technique, tissue blood flow can be quantified non-invasively and with the high-temporal resolution that is required to monitor adequately the rapid and ample adaptations of perfusion in skeletal muscles. Post-exercise T2-weighted NMR imaging also detects muscle activation based on the T2 changes associated with glycogen consumption and intracellular fluid accumulation during exercise [47].

Much less spectacular, but extremely useful, especially for the development of computational models, is the determination of muscle mass and composition. Relative percentages of muscle tissue, fat and connective components, can be very precisely determined by high-resolution anatomical NMR imaging. These data provide valuable reference for normalization and calibration, in particular for adjusting mechanical load during exercise bouts.

In recent years, the use of NIRS as a tool to investigate metabolic regulation during exercise has increased tremendously. With this technique, blood-tissue oxygenation can be measured within a local region of the muscle investigated and when combined with IC, imbalance between delivery and consumption of O₂ in peripheral sites can be assessed [48]. This illustrates the potential of NIRS to detect abnormal oxygenation adjustments in subjects with altered energy metabolism. Indeed, NIRS is currently used to detect and quantify abnormalities in muscle oxygenation in patients with peripheral arterial disease (PAD) and Spinal Cord Injury (SCI), and to detect metabolic impairment of subjects with a variety of genetic diseases, such as Friedreich's Ataxia (FA) and Mitochondria Myopathies (MM) [49]. However, several limitations need to be overcome before NIRS can be fully exploited clinically in the functional evaluation of muscle metabolism in health and disease [50,51,52].

Using the above-mentioned techniques several aspects of metabolic regulation during exercise have been investigated *in vivo*. The time profiles of muscle oxygenation and pulmonary VO₂ were monitored continuously to determine whether there is sufficient O₂ delivery to contracting muscle at the onset of exercise. Muscle oxygenation was unchanged during the first 10 s of exercise demonstrating a tight coupling between O₂ delivery and VO_{2m}. These results suggest that during volitional exercise in an intact organism, the cardiovascular, respiratory, and neuromuscular processes controlling muscle contraction are well coordinated to provide the necessary fuel and O₂ supply to the working muscles to maintain ATP and oxygen homeostasis.

The potential role of PDH in regulating the dynamics of oxidative and substrate-level phosphorylation was investigated by using DCA infusion to increase the active form of PDH while performing knee-extensor exercise. In one case, metabolite concentrations were obtained from biopsy samples and muscle VO_2 was determined simultaneously via thermodilution and A-V differences across the femoral bed [53]. In another case, dynamic changes in intramuscular ATP, PC, Pi, and pH were obtained using ^{31}P -NMR, while muscle VO_2 kinetics was obtained simultaneously using IC. Although DCA infusion resulted in increased PDH activation at rest and at the onset of exercise, no significant changes were observed in the dynamics of PC breakdown, La^- production, or VO_2 . However, a small reduction in the extent of PC breakdown, La^- formation, and VO_2 was observed [54]. In both cases, it was suggested that the activation state of PDH is not limiting for muscle O_2 utilization during the early phase of dynamic exercise in the intact organism *in vivo*. These results are similar to those determined in the isolated whole muscle *in situ* [23], but differ from those reported for isolated single muscle fibers [13].

Computational Models of Muscle Contraction and Energetics

Computational models of energy metabolism ranging from whole-body to mitochondrial level have been developed using data collected from models *in vivo* vs. *in vitro*, phenomenological vs. mechanistic approaches, and minimal vs. detailed descriptions. Several models of mitochondrial oxidative phosphorylation derived from data collected *in vitro*, have been developed with a degree of description of reaction and transport processes that has ranged from minimal to detailed. In most cases, these models are aimed at describing steady-state relationships consistent with experimental behavior observed in isolated mitochondria under a variety of perturbations. Since this review focuses on models of muscle contraction and energetics, only computational models that involve metabolic responses to large-scale changes in ATP turnover rate will be included.

Cellular-level model of increased energy demand

Three decades ago, Wilson et al. [55] presented a model of mitochondrial oxidative phosphorylation aimed at evaluating its O_2 dependence *in vivo* and *in vitro*. Even though this model was developed using experimental data from rat liver mitochondria at high and low O_2 partial pressures (PO_2), it provided quantitative predictions of changes in cellular energy metabolism under altered PO_2 , which have been instrumental in gaining understanding of the interactions among energy-related metabolites (ATP, ADP, Pi, NADH, NAD^+) and O_2 concentration during elevated ATP utilization rate [56].

A model of glycogenolysis coupled to ATPase in skeletal muscle was constructed with mammalian kinetic parameters obtained from the literature [57]. The model incorporated stoichiometric constraints, mass balance, and reversible thermodynamics and was tested under steady-state conditions. Analysis of this glycogenolytic-ATPase coupled system indicated that its control is almost entirely dominated by the external ATPase activity, and that ATP, ADP, and P_i concentrations are the main contributors to changes in flux rates. This model was augmented with pH-dependent enzyme kinetics and reaction equilibria to compute time profiles of pH changes and pH-dependent parameters, such as apparent equilibrium constants and maximum enzyme velocities. This enhanced model of glycogenolysis, coupled to creatine kinase and adenylate kinase required minimal adjustments of parameters to be able to simulate published results of experiments *in vitro* [58]. These results suggest that incorporation of appropriate physical chemistry of the reactions with accurate kinetic modeling in “skeleton” or modular models of subsystems of a large-scale metabolic system may give a reasonable simulation of experimental data *in vivo*, as long as interacting components of the system investigated are taken into account [59].

A more recent biophysical model of the mitochondrial respiratory system and oxidative phosphorylation was developed by Beard [60,61]. This model was mainly developed as a quantitative consensus of our current knowledge of the workings of the chemiosmotic theory of the respiratory chain and ATP synthesis and as a key building block towards the development of a complete energetic model of the cell. In a follow up study, this model was integrated with an axially distributed model of O₂ transport and exchange with tissue, specifically cardiac myocytes. The enhanced model predicts that inorganic phosphate is a key regulator of oxidative phosphorylation in the heart, activating oxidative phosphorylation and allowing ATP and PC concentrations to remain relatively unchanged over a large range of cardiac work rates. This model was then adapted to skeletal muscle by setting intracellular concentrations of creatine and phosphate to appropriate physiological values and adjusting the cellular mitochondrial content to match available data [61]. From the analysis of model simulations and comparison with ADP and P_i data from exercise studies in various groups of subjects with specific anomalies, the authors asserted that the mechanism of control of oxidative phosphorylation is through feedback of substrate for ATP synthesis [61]. Then, they concluded that no additional regulatory mechanisms, such as feed-forward control of certain enzymes via cytosolic Ca²⁺ or functional coupling between mitochondrial CK and adenine nucleotide translocase are necessary to explain the majority of the observed data. While this approach is appropriate to study the regulation of oxidative phosphorylation in isolated mitochondria, the model assumes that the supply of reducing equivalents and O₂ is never limiting. Consequently, model predictions need to be interpreted considering the limitations imposed by the model assumptions. Other studies with different assumptions have concluded that simple feedback alone cannot explain the regulation of oxidative phosphorylation in cardiac muscle *in vivo* [62] nor in intact skeletal muscle [63].

Whole-muscle model of muscle contraction and energetics

In working intact muscle, ATP is mostly used by actomyosin-ATPase and Ca²⁺-ATPase, and under most physiological conditions, the main source of ATP is provided by mitochondrial oxidative phosphorylation, except during the transition from rest to exercise when cytosolic substrate-level phosphorylation supplements the oxidative contribution. From a metabolic control systems perspective, most models of muscle contraction and energetics fit into two schemes: (a) feedback models and (b) combined feedback-feedforward models.

From ³¹P-MRS studies of arm muscles of humans during steady-state exercise, Chance et al. [64] concluded that the principal control of oxidative metabolism in skeletal muscle is by ADP (or Pi/PC), similar to the conclusions he had arrived at three decades earlier in isolated mitochondria [65]. The dependence of VO₂ on [ADP] measured by Chance and Williams followed a Michaelis-Menten hyperbolic relationship. Jeneson and co-workers [66] demonstrated that a second-order Hill equation better described the dependence between VO₂ and [ADP] during exercise. These two relationships incorporating ADP as the only substrate, and two additional bisubstrate relationships incorporating ADP and P_i in reversible and irreversible reactions were investigated using a minimal mathematical model of the skeletal muscle energetic system consisting of three ordinary nonlinear differential equations. After comparing model simulations with each of these four functions with corresponding experimental data from a rest-to-work transition followed by a recovery period, it was concluded that the second-order Hill equation model fit the data best. However, since there were only small differences among the results provided by the other feedback models, additional high quality data would be necessary to distinguish significant differences among these mechanisms. A feedforward only model was also tested, but simulations showed instantaneous changes incompatible with experimental observations.

In a further study, a similar expression for oxidative phosphorylation was combined to cellular ATP flux and creatine kinase kinetics by ordinary nonlinear differential equation to quantify human muscle ATPase rate and ATP synthase rate during muscle stimulation [67]. Although model validation was not possible, PC and Pi model simulations were in agreement with ^{31}P -MRS data obtained on forearm flexor muscle. This model provided a quantitative approach to study cellular energy metabolism in intact stimulated muscle and was successfully integrated to multi-level system models to investigate O_2 transport in whole-skeletal muscle at various increased levels of energy demand [69, 71, 75] and environmental conditions [74].

Using a previously developed model of mitochondrial oxidative phosphorylation, Wu et al [61] demonstrated good agreement between predictions of [ADP] and [Pi] and data from ^{31}P -MRS studies on human forearm. These results suggest that, since no additional control mechanism was incorporated into the model, the rate of oxidative phosphorylation is primarily controlled via feedback of the substrates for ATP (i.e., ADP, Pi).

A series of studies *in silico* with a computer model of oxidative phosphorylation in intact skeletal muscle led to the conclusion that some cytosolic mechanism activates directly all oxidative phosphorylation complexes in parallel with the activation of ATP utilization and substrate dehydrogenation. The mechanism was named the “each-step-activation mechanism” or “parallel activation mechanism” [63]. Within this mechanism, the feedback regulation via [ADP] and [Pi] plays only a minor, fine-tuning role in intact skeletal muscle *in vivo*. This mechanism of activation was able to account for several dynamic characteristics of the oxidative metabolic system in intact tissues, especially in skeletal muscle, such as the steep VO_2 -[ADP] phenomenological relationship at the onset of exercise, and the PC on/off asymmetry.

The concept that some enzymes in the ATP-producing system are ‘latent’ in resting skeletal muscle and are activated during muscle contraction [2] was incorporated in a recent model of skeletal muscle metabolism [68]. This model was developed to quantify interactions among cellular and skeletal muscle metabolic adaptations, and to predict metabolic responses to exercise after periods of altered loading states. The model can be applied to test complex hypotheses during exercise involving the integration and control of metabolic processes at various organizational levels in individuals who have undergone periods of chronic loading or unloading.

Multi-level system models of exercise

Several computational models at various scales and degrees of complexity have been developed and validated to investigate the regulation of skeletal muscle energetics during exercise [68,69,70,71,72]. The strategy for building these whole-body, multi-scale models has been to choose the level of detail and complexity of the processes of transport and metabolism that are required to test mechanisms of metabolic regulation and to make predictions of the responses to exercise in humans. Indeed, even though the models incorporate biochemical data on enzyme kinetics from cellular studies conducted *in vitro*, the main sources of physiological data for model development and identification have been experimental metabolic studies on skeletal muscle *in vivo* or *in situ* during occlusion or exercise in humans and dogs. Thus, physiologically based minimal models were used to test hypotheses concerning the characteristic O_2 uptake dynamics at a specific biological scale and to identify the limiting factors affecting these dynamics under distinct experimental conditions.

A model was developed that describes the dynamics of O_2 transport and utilization in contracting skeletal muscle during exercise [73]. The model takes into account the dynamics

of O_2 stores in blood and tissue and muscle O_2 consumption is estimated from measurements of StO_{2m} and VO_{2p} . This model was then enhanced to investigate factors affecting muscle O_2 utilization during exercise by linking ATP formation from oxidative phosphorylation and net PC breakdown to the rate of ATP hydrolysis [71]. Then, the dynamics of the metabolic flux rates of oxidative phosphorylation, ATP hydrolysis, and the forward and reverse rates of the creatine kinase reaction were simulated in the large muscle groups used in cycling. Results from these studies demonstrated that the response time of muscle O_2 utilization (UO_{2m}) was significantly faster than that of VO_{2p} at exercise onset, which contrasts with previous experimental findings using surrogate measurements of UO_{2m} . The implications of this finding affects our current understanding of metabolic control at exercise onset since a faster dynamics of O_2 consumption implies a significantly smaller contribution of non-oxidative sources of ATP synthesis for a given physical task. These results highlight the importance of linking phenomena at various scales with multi-scale mechanistic models.

To quantify the extent to which convection and diffusion processes limit the dynamics of UO_{2m} *in vivo*, a multi-scale model of O_2 transport and utilization [74] was developed to describe the O_2 dynamic responses in blood and tissue of the canine muscle model previously described. In this case, the availability of experimental data on the dynamics of A-V oxygen content difference and blood flow facilitated model validation and testing of predictions. Analysis of model simulations showed that the permeability surface area is a critical factor responsible for the observed O_2 gradient between blood and tissue, which may be rate limiting in delivering O_2 to muscle. In contrast to conclusions reached in a study of isolated whole muscle *in situ* [21], these results suggest that O_2 diffusion across the tissue-capillary membrane limits the dynamics of VO_2 during muscle contraction. This model was enhanced to account for changes in tissue and microvascular volume distribution during exercise to analyze muscle oxygenation measurements by NIRS where Hb and Mb contribution to the signal cannot be discriminated. Thus, the model of O_2 transport and metabolism in contracting muscle were used in combination with experimental data obtained by IC and NIRS to predict the extent of Hb and Mb contribution to the NIRS signal during exercise [75].

These models and methods of analysis developed previously serve as references for developing advanced computational models of muscle O_2 transport and metabolism that can be applied to investigate exercise responses in healthy and chronically ill individuals. Indeed, top-down and bottom-up approaches can be used interactively to develop multiscale models of O_2 transport and metabolism. This combined approach has the advantage that, while it preserves mass balances and thermodynamic feasibility, it incorporates experimentally derived mechanisms that operate *in vitro*, into a multi-scale model of an *in vivo* system, which maintains the integrity of regulatory mechanisms. For instance, models developed using a bottom-up approach to represent cellular metabolic systems (e.g., oxidative phosphorylation, glycolysis, and the citric acid cycle) can be combined with more comprehensive multi-scale models that integrate experimental information at various scales. Such integrative systems have been recently developed to provide insights into mechanisms of metabolic regulation that may not be evident from experimental studies alone. These models of O_2 transport and metabolism included extra- and intra-cellular domains, as well as detailed reaction and transport kinetic expressions within and between intracellular domains, respectively [70,76,77]. Using large-scale parameter estimation algorithms in its development, this model was able to simulate the dynamics of key intracellular metabolite concentrations and metabolic fluxes in response to reduced blood flow and O_2 supply to mitochondria. This model was recently enhanced to investigate the role of the dynamics of cytosolic and mitochondrial NADH/NAD⁺ as a regulatory factor of muscle energy metabolism during exercise and demonstrated that mitochondrial NADH/NAD⁺ shuttling

activity and muscle glycogen stores affect primarily the cytosolic redox state while changes in mitochondrial redox state are minimal.

A multi-scale model of oxygen transport and metabolism linking whole-body to cellular processes was developed to distinguish responses of oxygen utilization at cellular, tissue and whole body level to exercise of moderate intensity. Simulation shows that the MRT of external and cellular respiration in normal subjects are similar even when a transit delay exists between tissue cells and the lungs [69]. This model can be integrated with experimental data obtained by NIRS and IC for comparative quantitative analysis of the regulation of respiration in subjects suffering from abnormal function associated with disease states (e.g., chronic obstructive pulmonary disease, metabolic myopathies and congenital heart disease).

A different, physiologically based whole-body model that distinguishes tissue-organ systems including hormonal control was used to simulate the coordinated responses of metabolic pathways leading to glucose homeostasis during exercise [72]. Model simulations showed the importance of the interaction among tissues demonstrating that external fuel supplies from other tissue-organs to skeletal muscle are significant for prolonged exercise. Thus, this model integrated with experimental data can be a useful tool to investigate the role of carbohydrate and lipids for fuel oxidation in skeletal muscle, in obesity and in subjects with insulin resistance.

In summary, comprehensive models of cellular energy metabolism have improved our understanding of the interaction among energy-related metabolite concentrations during large ATP demand transitions in which feedback and feedforward control models have been proposed to describe the regulation of cellular respiration. The integration of cellular energy metabolism models to multi-level system models with experimental information at various whole body-levels enabled researchers to, 1) quantify cellular and skeletal muscle adaptations to metabolic responses to exercise after periods of altered loading; 2) quantify O₂ transport and utilization in canine and human muscle under different physiological conditions concluding that O₂ diffusion may limit muscle O₂ utilization; 3) demonstrate the role of key metabolites in cytosol and mitochondria as regulatory factors of muscle energy metabolism; 4) demonstrate that O₂ transport processes from lungs to muscle are tightly coupled to provide enough O₂ for working skeletal muscle during exercise in normal subjects; 5) identify the role of external fuel supplies from other tissue/organ systems to skeletal muscle during prolonged exercise. Model simulations of these models provide the dynamic changes of metabolites and metabolic fluxes that are not practical to measure with the actual experimental techniques.

More rapid progress in field of exercise metabolism may be obtained by integrating experimental and computational models in a systematic manner to study biological system behavior. Indeed, an iterative process in defining alternative hypotheses, designing experimental methods and models, and carrying out experiments is essential to move forward in different field of science [78].

The efficacy of this iterative process can be improved using computational models of biological and physiological systems. In this process, computational models provide 1) quantitative analysis of biological systems function; 2) a method to refine hypotheses; 3) the basis for optimal design of experiments (see Fig. 4). The contribution of computational modeling to the iterative process depends on the experimental design method for integration of data and new knowledge derived from the experiments. Relationships between tissue-organ systems and whole body functions require integration of many perspectives and disciplines including biochemistry, biophysics, physiology, bioinformatics and

computational modeling. Investigation of a particular phenomenon can be effectively achieved with a multidisciplinary team of researchers capable of establishing a common ground to obtain research outcomes that go beyond single-discipline approaches.

Comparison of Models

Experimental approaches

Over the past decades, several experimental models have been employed to improve our understanding of the regulation of muscle energetics during exercise in health and disease. Nevertheless, the interaction of factors affecting muscle contraction and the dynamics of muscle oxidative metabolism *in vivo* are not completely understood. Each experimental model has its specific advantages inherent to the scale investigated, but suffers from the incompleteness of not considering the entire system.

The isolated single skeletal muscle fiber model has distinct advantages for the study of metabolism as indicated at the beginning of this section. However, this model also has limitations. In a sense, two of its strengths are also weaknesses: a) the absence of myoglobin removes a confounding factor yet mammalian muscles do have myoglobin, and b) blood flow is rendered irrelevant, but intact systems do require adaptations of both blood flow rate and distribution. There is also the possibility of an unstirred layer surrounding the single myofibers [12,17]; such a layer could affect the diffusion coefficient for the system and influence interpretation of PiO_2 changes. It should be noted that investigators [12,17] using this model consider the possibility of a significant unstirred layer to be minimal because the superfusate is flowing and/or stirred. Finally, oxygen uptake (VO_2) has only been directly measured in one [79] of the studies investigating on-kinetics in single muscle fibers; in all of the others, PiO_2 has been used as a surrogate for VO_2 .

The isolated whole muscle model *in situ* has unique advantages such as manipulation of O_2 delivery and direct measurement of VO_2 across the muscle, but there are also limitations [20,21,22,80], a few of which will be described here. First, the muscle is acutely denervated and often perfused by blood from the contralateral artery via a pump; therefore, it is possible that the flow pattern could be somewhat different from that obtained in whole animals *in vivo*. The pattern of muscle contraction is somewhat unusual in comparison to typical dynamic exercise in that all motor units are synchronously stimulated; however, this may be similar to repetitive maximal contractions *in vivo*. Also, the muscle fiber composition is heavily oxidative in nature so that the results may strictly apply only to muscles of high aerobic potential. Resting muscle blood flow is often elevated which may reflect some loss of vascular tone.

The intact organism *in vivo* (or human model) has the main advantage that it represents the “real” physiological system with all its complexity intact, i.e., it is the gold standard to be investigated. Nevertheless, this experimental model has several limitations when trying to quantify the extent to which the time profiles of substrate supply and flux rates, intermediary metabolites and H^+ concentrations change during the transition from rest to muscle contraction. These limitations are associated with the structural and functional physiological adjustments occurring at various scales of the organism and to the inadequate specificity of available measurements. In this experimental model, the regulation of energy metabolism during exercise is inferred from investigating the interplay between O_2 transport and utilization and the reactions involved in the energetic pathways. However, the inherent redundancy and coordination of the subsystems (cardiorespiratory, neuroendocrine, and neuromuscular) in eliciting the observed responses diminishes the ability to discriminate the extent of effects exerted by any individual system. In addition, actual measurements used by exercise physiologists to study energy metabolism, lack specificity. For example, muscle O_2

utilization, UO_{2m} , is a reaction process occurring in the mitochondria; a surrogate variable commonly used to estimate UO_{2m} is muscle O_2 uptake, VO_{2m} , which is calculated from the product of muscle blood flow and the arterio-venous O_2 content difference. On the other hand, muscle O_2 delivery is also determined using muscle blood flow. Thus, the use of blood flow to determine both O_2 delivery and uptake dynamics limits the ability to discern whether the dynamics of O_2 transport is faster than those of muscle O_2 utilization. Moreover, the dynamic response of VO_{2m} is referred to the whole muscle region under contraction and it does not take into account blood flow heterogeneities, O_2 consumption distribution, and fiber type recruitment along working muscle.

When dealing with NMR measurements, the specificity is better. However, other issues affect the ability to obtain perfusion, oxygenation, and metabolic measurements simultaneously to investigate muscle contraction and energetics. For instance, while BOLD and ASL functional NMR imaging provide oxygenation and perfusion information with high temporal and spatial resolution, data collection on metabolism is suffering from poor signal to noise ratio resulting from the low concentration of the metabolites in question and the lower gyro-magnetic ratio of ^{31}P . Consequently, there is always a compromise between high acquisition rate (typically 1–4s), with no localisation other than the volume covered by the coil- and spatial resolution, with relatively long acquisition times (several minutes). Because of frequent mismatch between tagging and reading slices, ASL perfusion imaging during exercise remains a challenge at the moment, with very few published reports [45].

Computational models

Existing models of muscle contraction and energetics have been developed at the cellular, whole-tissue, and whole-body levels. Cellular-level models are typically built using a bottom-up approach, while whole-body models are usually top down. Both approaches may be applied in the development of models of muscle contraction and energetics depending on the question addressed and data available for model characterization. In the bottom-up approach, each reaction participating in a metabolic pathway is considered individually and its kinetic behavior is modeled in detail, using available information on the enzyme catalytic mechanism and its regulation [81]. Kinetic information derived under non-physiological conditions *in vitro*, requires correction for pH, ionic strength, temperature, and metal ion concentrations in solution [58]. This approach has proven powerful in providing a mechanistic understanding of phenomena characterized by data sets from studies *in vitro* and *in vivo* [61]. In the top-down approach, mechanistic detail in specific pathways or groups of reactions may be traded for phenomenological relations that reproduce the overall biochemical kinetics, while remaining amenable to parameterization from available data observed *in vivo* [76]. The kinetics of the resulting lumped reactions are modeled by using phenomenological rate laws, such as mass action or single-step enzyme kinetics. A large number of parameters are typically introduced in the dynamic model, which must be estimated using robust large-scale parameter estimation methods coupled with physiological and thermodynamic constraints and experimental data collected *in vivo* [77]. These studies have proven expedient in predicting system behavior and useful in evaluating mechanisms of metabolic regulation *in vivo* under a variety of conditions, such as ischemia, hypoxia, and exercise [69,70,76]. As both top-down and bottom-up approaches mature, hybrid “middle-out” approaches may evolve to simulate complex systems spanning scales ranging from the subcellular to the whole body level. This approach may be effective and efficient to conduct multilevel modeling aimed at investigating the regulation of energy metabolism during exercise.

In the evaluation of different computational models dealing with functional interactions between cells, organs and whole body levels, it is important to select the level of model

details and the scope of the simulation according to the specific scientific question being investigated. Therefore, physiologically based models for studying muscle metabolism should offer the opportunity to compare model simulation and experimental data to evaluate the adequacy and accuracy of the computational model in matching experimental evidences. Physiological plausibility of the computational model has to be consistent with the current physiological knowledge of the experimental model. When a model requires calibration with experimental data, the analysis of parameter estimates can be used as an additional approach in comparing competing models, especially when the parameters have specific biochemical and/or physiological meaning. Ultimately, the capability of the model has to be examined by validation with experimental data. In particular, the predictive power of a model should be tested by simulating metabolic responses and predicting experimental data obtained under physiological and/or pathological conditions which are not used for model construction. Validated models can further be combined with other existing models to build an integrated system to investigate its sub-system interactions.

Conclusions

Successful analysis of the phenomena involved in the regulation of energy metabolism during muscle contraction and elucidation of the key mechanisms controlling the various components of this physiological system requires that we understand the functional interactions between the key components of cells, tissues/organs, and the entire organism. This information resides in the interactions within each biological scale and between scales, thus requiring integration using multiscale computational models. To develop these models, experimental information at each scale is critical, as well as linking the scales within the context of a theoretical physico-chemical framework supplemented with phenomenological control mechanisms.

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Teaser

Computational systems physiology is a way of integrating biological information at various scales into a theoretical framework to facilitate understanding of how function results from the interaction of system components.

In Memory of Dr. Marco Eugenio Cabrera (1954–2009)

We dedicate this work to the memory of Marco E. Cabrera, who was the senior author of this paper and who tragically died before this paper could be published. Marco was a dear friend, valued research collaborator, and esteemed mentor, whom we miss greatly. His ready smile, enthusiasm, and breadth of vision engaged many friends and collaborators. Marco's research scope encompassed a wide variety of fields including exercise physiology, human and animal performance evaluation, metabolic systems biology, mathematical modeling, and computer simulation. He had an exceptional ability to unite theoretical and experimental researchers from different fields in studying biological systems. Through his research, Marco developed many close collaborations locally at Case Western Reserve University, University Hospitals of Cleveland, and the Cleveland Clinic as well as nationally and internationally. Through continuation of his research thrusts by his collaborators including many students, his efforts will not be in vain.

Fig. 1.

Effect of CK inhibition (CK_i) with iodoacetamide on intracellular PO₂ kinetics. Note the faster decline in PO₂ for CK_i in the left hand gray area highlighting the onset of contractions and the faster rise of PO₂ for CK_i in the right side gray area highlighting the off-kinetics at the cessation of muscle contractions. Reproduced with permission from Kindig, C.A., et al. *J Appl Physiol* 98:541–549, 2005.

Fig. 2.

VO_2 increases more rapidly with fast convective O_2 delivery during contractions of intensity high enough to elicit $\text{VO}_{2\text{peak}}$. VO_2 values are per minute. Time is truncated at 48s to illustrate differences early in the 4-min protocol. Reproduced with permission from Grassi, B., et al. *J Appl Physiol* 89:1293–1301, 2000.

Fig. 3.
Multi-parametric functional NMR of skeletal muscle.

Fig. 4. Relation between experimental and computational models for optimal design of experiments and to generate hypotheses.

Table 1

Comparison of experimental and computational models.

	Best use of model	Advantages	Disadvantages	Reference
Experimental model				
Isolated single skeletal muscle fiber	Energy metabolism during individual muscle fiber isometric contraction	No heterogeneity of fiber types & O ₂ delivery	<p>a. Does not effect of myoglobin & blood flow;</p> <p>b. No physiological temperature used</p>	[6,8–19]
Isolated in situ whole-muscle	Energy metabolism and Drug delivery in self and pump perfusion whole muscle contraction	Control of O ₂ delivery & infusion of pharmacological agent	<p>a. Muscle fiber composition is mainly oxidative;</p> <p>b. Blood flow may differ from whole animal <i>in vivo</i></p>	[20–28]
Intact organism <i>in vivo</i> : Human model	Cellular energy metabolism linked to whole body respiration	Gold standard physiological systems with all its complexity intact	Not easy access to experimental information at different whole body-levels at the same time	[29–40,42,44–47,49–54]
Computational model				
Cellular-level	Regulation of cellular energy metabolism	Model validation with experimental data <i>in vitro</i>	Model validation is difficult with experimental data <i>in vivo</i>	[55–61,63,65]
Whole-muscle	Regulation of energy metabolism in intact skeletal muscle	Model validation with experimental data <i>in vivo</i>	No link between cell, muscle and whole body levels	[64, 66–68]
Multi-level system	Regulation of metabolic response to ischemia, hypoxia and exercise	<p>a. Integration of cellular and whole body data experimental data;</p> <p>b. Model validation with experimental data <i>in vivo</i></p>	Limited experimental data for estimation of model parameters	[68–77]