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Review

Functional investigations of exercising muscle: a noninvasive magnetic resonance spectroscopy-magnetic resonance imaging approach

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Abstract. Muscle fatigue, which is defined as the decline in muscle performance during exercise, may occur at different sites along the pathway from the central nervous system through to the intramuscular contractile machinery. Historically, both impairment of neuromuscular transmission and peripheral alterations within the muscle have been proposed as causative factors of fatigue development. However, according to more recent studies, muscle energetics play a key role in this process. Intramyoplasmic accumulation of inorganic phosphate (P_i) and limitation in ATP availability have been frequently evoked as the main mechanisms leading to fatigue. Although attractive, these hypotheses have been elaborated on the basis of experimental results obtained in vitro, and their physiological relevance has never been clearly demonstrated in vivo. In that context, noninvasive methods such as 31-phosphorus magnetic resonance spectroscopy and surface electromyography have been employed to understand both metabolic and electrical aspects of muscle fatigue under physiological conditions. Mapping of muscles activated during exercise is another interesting issue which can be addressed using magnetic resonance imaging (MRI). Exercise-induced T2 changes have been used in order to locate activated muscles and also as a quantitative index of exercise intensity. The main results related to both issues, i.e. the metabolic and electrical aspects of fatigue and the MRI functional investigation of exercising muscle, are discussed in the present review.

Key words. Skeletal muscle; fatigue; recruitment; MRS; MRI; energetics.

Introduction

Functional noninvasive investigations of exercising muscle actually started in 1974 when Hoult et al. reported that high-energy phosphate compounds could be detected in vivo using 31-phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) [1]. Since that time, many studies have been devoted to the investigation of several metabolic aspects of exercising muscle, under a variety of conditions ranging from muscular diseases to a high level of training (for review see [2–4]). MRI is another powerful tool to investigate muscle function noninvasively. The acute effects of exercise on muscle magnetic resonance imaging (MRI) contrast were first reported in 1988 by Fleckenstein et al. [5]. From that time, it has been recognized that MRI could be used to distinguish between active and nonactive muscle groups, thereby providing functional assessment of exercising muscle.

In the present review, we will report more particularly on muscle fatigue investigated either with MRS or with a combination of noninvasive methods, including surface electromyography (EMG). The final section covers the

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issue of functional investigation of muscle using MRI. For each section, a short subsection will be devoted to technological issues.

Muscle fatigue

Muscle fatigue is defined as the decline in muscle performance during exercise [6]. Although this phenomenon has been analyzed throughout the last 2 centuries, the exact causes are still debated [7–10]. Basically, factors involved in muscle fatigue could be characterized as either central or peripheral; central factors would cause fatigue by disturbing neuromuscular transmission between the central nervous system and muscle membrane, whereas peripheral factors would lead to alteration within muscle [7, 8]. The relative contributions of these factors in the development of fatigue are still discussed, although it now seems established that fatigue has, at least for a significant part, a peripheral origin [9–11], central factors contributing modestly at ~20% [11]. Obviously, these respective contributions are highly dependent on the type of exercise. In the next sections, we propose an overview of peripheral factors leading to fatigue, central fatigue being beyond of the scope of our survey.

Peripheral fatigue: definition

From a peripheral point of view, failure in excitation-contraction (E-C) coupling is considered as the major mechanism leading to the development of fatigue. E-C coupling is defined as the sequence of events occurring from action potential generation at the surface of muscle fiber to the sliding of myofilaments. Different components such as action potential propagation along the sarcolemma, activation of myofilaments contraction by Ca^{2+} release from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR) and muscle relaxation mediated by Ca^{2+} reuptake throughout ATPase SR pumps take part in this sequence. Main factors that might be involved in force decline during muscle activity are the intramyoplasmic accumulation of inorganic phosphate (P_i) due to phosphocreatine (PCr) breakdown and the limitation in ATP availability [9–14].

In vitro studies of peripheral fatigue

In vitro studies on isolated whole muscle and skinned muscle fiber preparations have demonstrated that intramyoplasmic accumulation of P_i could cause force decline by inhibiting contractile protein sliding [15–17]. More recently, it has been suggested that this inhibitory mechanism would be indirectly caused by disturbance of the Ca^{2+} release from the SR during E-C coupling [10, 18–20]. Regarding the effect of limitation in ATP availability on fatigue development, major results obtained in isolated fibers and muscle membrane extracts have shown that ATP limitation could impair activity of ionic pumps involved in both action potential propagation and Ca^{2+} fluxes throughout the SR membrane [21–23], thereby causing force depression and slowing of muscular relaxation. According to all these results, intramuscular energy metabolism would have a key role in the failure of E-C coupling and consequently in the development of peripheral fatigue. However, although attractive, these hypotheses have been elaborated on the basis of experimental results obtained in vitro, and their physiological relevance has never been clearly demonstrated in vivo. The validation of these hypotheses under physiological conditions was necessary in the understanding muscle fatigue. In that context, ^{31}P MRS provides a noninvasive alternative for investigation of muscle energetics and its relationship to fatigue.

Noninvasive investigations of peripheral fatigue in vivo

MR techniques

MR spectra and images are generated by placing samples in a powerful magnetic field and then exciting them with radiofrequency energy. Susceptible nuclei (in the present case ^{1}H in water molecules or ^{31}P in high-energy phosphate compounds) will be boosted to a higher energy state, producing a detectable signal when the excitation stops. This signal declines over time via two concurrent processes described as relaxation. T1 relaxation (longitudinal or spin-spin relaxation) involves the loss of energy to surrounding nuclei with similar resonance frequencies and roughly determines signal intensity for a given repetition time. T2 relaxation (transverse or spin-lattice relaxation) results from interaction between the excited nuclei and any perturbing magnetic field with no transfer of energy and will roughly determine signal width. The nondiscriminatory nature of T2 relaxation mechanisms greatly increases the probability of such interactions, thus making T2 much shorter than T1 in heterogeneous solutions. Signals obtained in ^{31}P MR spectra and ^{1}H MR images are highly dependent on both T1 and T2 values together with nuclear density, i.e. the number of free rotational nuclei of the tissue investigated. For a given experimental time, compounds with shorter T1 will end up with higher intensities in MR spectra, while signal width in spectra is inversely proportional to T2 values. Regarding MR images, T1, T2 and nuclear density will determine the contrast of cellular and subcellular structures.

^{31}P MRS

Measurement of phosphorylated compounds concentrations in living cells is not easy. Traditional methods such as percutaneous needle biopsy and freeze clamping ex-

hibit limitations, especially related to alteration of anatomic integrity and partial degradation of phosphorylated metabolites during extraction and analysis. In addition, repeated measurements cannot be performed on the same muscle, making it impossible to achieve high-time resolution kinetics.

Compared with analytical methods, ^{31}P MRS offers the opportunity of measuring noninvasively and continuously with high-time resolution the concentration of phosphorylated compounds involved in muscle energetics. Typical ^{31}P MRS spectra exhibit six peaks, corresponding to PCr, P_i) the three phosphate groups of ATP (in positions, α, β et γ) and phosphomonoesters (PMEs) (fig. 1). Intracellular pH can also be measured noninvasively from the chemical shift of the P_i peak [24, 25]. Indeed, at physiological pH, myoplasmic P_i (pK of 6.75) exists as mono- and diprotonated form (respectively $H_2PO_4^-$ and HPO_4^{2-}). These two forms are exchanging so fast that only a single P_i signal is detected. However, the chemical shift of this single signal is weighted by the contribution of each form, making it sensitive pH. A signal assigned to phosphodiesters and located between the PCr and P_i signals is occasionally observed in quadriceps muscle. Changes in phosphorylated compound concentrations and intracellular pH during transition from rest to exercise and exercise to rest have been investigated in order to characterize muscle bioenergetics in both humans and animals [26–31]. Another immediate application of ^{31}P MRS is the calculation of ADP and AMP concentrations using creatine kinase and adenylate kinase equilibria, respectively [32, 33]. This has been useful to study metabolic control of ATP regeneration during and after muscular exercise [34–36].

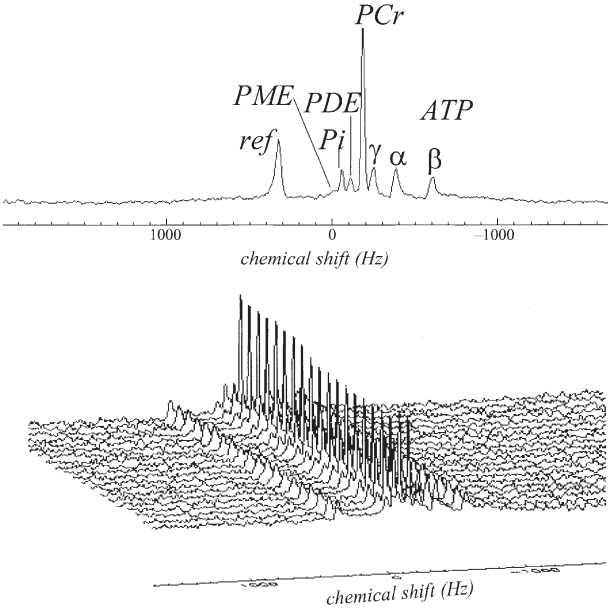

Figure 1. Typical series of ^{31}P MR spectra recorded with a time resolution of 2 s at end of exercise (first spectrum, bottom) and during the following recovery period. Assignments of signals are specified on the top spectrum: ref, reference compound; PME, phosphomonoesters; PDE, phosphodiesters; P_i, inorganic phosphate; PCr, phosphocreatine; α, β, γ, phosphate groups of ATP. Horizontal axis represents the chemical shifts expressed in Hz. Spectra are recorded from the thigh muscles.

More recently, the contribution of the different metabolic pathways involved in energy production, i.e. ATP production from (i) PCr degradation, (ii) anaerobic glycolysis and (iii) mitochondrial phosphorylation, has been quantified due to advances in ^{31}P MRS data analysis [27, 37–41]. This quantitative analysis represents a real advance in the exploration of muscle energetics. Indeed, classical methods used to estimate muscle energy production are either invasive or do not allow simultaneous investigations of aerobic and anaerobic pathways. For example, ATP utilization during exercise can be estimated from the changes in muscle temperature [42–46] given that energy production from ATP hydrolysis is converted into work and heat. However, given the slight changes in muscle temperature, these thermodynamic studies cannot be easily done in situ, and investigations are generally performed on isolated muscle or fibers bathing in solution. Estimation of ATP production from oxidative and anaerobic pathways can be done from O_2 uptake (VO_2) measurements and analytical methods, respectively. VO_2 measurements provide an estimate of mitochondrial function in relation to muscle force production [47]. VO_2 can be measured from expired gases [48, 49] or from blood samples [50–52]. The major limitation of this method is the inability to estimate ATP production from nonoxydative metabolic pathways, e.g. PCr degradation and anaerobic glycolysis, which are usually referred to as a single parameter known as oxygen deficit. On the other hand, energy production from anaerobic pathways can be calculated using analytical methods on muscle extracts [53–57].

Therefore, when compared with classical methods, ^{31}P MRS presents several advantages for the measurement of phosphorylated compound content and the quantification of ATP production from the different metabolic pathways. However, one has to keep in mind that due to its low sensitivity, MRS does not work at the level of a single fiber, and metabolic changes are recorded from a mixture of fibers with different characteristics, i.e. high-energy phosphate content, resistance to fatigue and bioenergetics. Also, metabolite concentrations less than 0.5 mM are hardly detectable using ^{31}P MRS. Magnetic field strength used for energetics investigations ranges from 1.5 to 9.4 T with the higher field providing the higher time resolution.

Contribution of ^{31}P MRS to the study of peripheral fatigue

Our aim in this section is to review the various hypotheses put forward in order to explain peripheral fatigue, i.e. intramyoplasmic accumulation of P_i and limitation in ATP availability, and to show the contribution of ^{31}P MRS in understanding their mechanisms under physiological conditions.

Methodological aspects

To analyze the metabolic mechanisms underlying the development of peripheral fatigue, indices of energy metabolism and mechanical performance must be measured simultaneously during exercise. ^{31}P MRS investigations are performed in superconducting magnets, and ^{31}P MR signals are collected using surface coils dedicated to the muscle studied. Experimental protocols are generally composed of successive periods of rest, exercise and recovery. In humans, ^{31}P MRS investigations have been performed in lower (quadriceps, gastrocnemius) and upper limbs (forearm flexor muscles, wrist flexor muscles) using whole body or smaller horizontal magnet [58–60]. Regarding animal experiments, rat and mouse muscles have been investigated using either horizontal [27, 61–64] or vertical [60] magnets.

Mechanical performance during exercise can be measured using ergometers or force transducers. The main parameters that are calculated in order to characterize muscle performance are peak force, shortening velocity and relaxation time. Fatigue development is accompanied by a decrease in peak force and an increase in both shortening velocity and relaxation time. The time course of fatigue development depends on the experimental protocol and varies according to the type (isometric, shortening or lengthening contraction), intensity and duration of exercise. Fiber type composition of the muscle studied may also affect fatigue development. Typically, two kinds of fatiguing protocols are used: prolonged moderate exercise (>50% of maximum capacity) and short high-intensity exercise (<50% of maximum capacity). Both protocols, which lead respectively to moderate and high levels of fatigue, involve repeated or sustained contractions. In humans, muscle contractions can be voluntary or electrically induced using surface electrodes positioned on the skin [11, 39, 65, 66]. In animals, contractions are electrically induced, either directly using needle electrodes inserted in the muscle mass [63, 67] or indirectly via nerve stimulation [27, 64, 68]. Although less invasive, direct stimulation is less often used than nerve stimulation. Indeed, regarding direct stimulation, the stimulated muscular mass can vary among experiments according to the position of the electrodes. On the other hand, proper nerve stimulation allows activation of the whole mass of the corresponding innervated muscle.

Intramyoplasmic P_i accumulation and fatigue development

Time-dependent changes in P_i in exercising muscle

^{31}P MRS has been widely used to measure the intramuscular concentration of P_i in resting muscle and during exercise. In resting muscle, P_i concentration is near 5 mM in humans [37, 69, 70] and 3 mM in rats [62, 71]. During exercise, PCr degradation through creatine kinase (CK) reaction is the major source of P_i, and the time course of P_i changes is therefore almost stoichiometric with the breakdown in PCr [59]. In the case of high-intensity or prolonged exercise, a net degradation of ATP into ADP and AMP can occur, thereby contributing to additionnal P_i production. P_i produced during exercise can serve as a substrate of glycogen phosphorylase in order to produce glucose 1-P and can also be transported into mitochondria. However, as shown by MR measurements, most of the P_i accumulates in the myoplasm [72]. P_i accumulation during muscle contraction generally exhibits two phases: an initial rapid phase, within a minute, leading to a large accumulation in P_i, followed by a second phase during which P_i concentration reaches a steady state. Thus, during moderate exercise, P_i concentration can reach 10–20 mM in rat and human muscle [62, 69–71]. During high-intensity exercise, P_i concentration rapidly reaches 20 mM in rats [62, 69–71] and up to 40 mM in humans [69, 73]. It is thus noteworthy that intramyoplasmic P_i accumulation is twice larger in exhausting exercise leading to fatigue as compared with moderate exercise which is not linked at all to fatigue.

P_i accumulation: a causative factor of fatigue?

Myoplasmic accumulation of P_i during exercise has been frequently cited as a causative factor of peripheral fatigue. As an elegant illustration, the abnormally low P_i production in CK knockout mice (unable to utilize PCr) is linked to a higher resistance to fatigue as compared with wild-type muscle, suggesting a key role of P_i accumulation in muscle fatigue [74]. Historically, it was first proposed that fatigue development would not be due to P_i per se but to its diprotonated form ($H_2PO_4^-$). At physiological pH, myoplasmic P_i (pK of 6.75) exists as mono- and diprotonated forms in equilibrium [75]. $H_2PO_4^-$ concentration is low at pH 7 (36% of total P_i) but becomes progressively the major form as pH decreases (85% of total P_i at pH 6). On the basis of results obtained on skinned muscle fibers, it has been suggested that $H_2PO_4^-$ ions might directly affect the function of contractile proteins by disturbing ATP hydrolysis at the myosin ATPase level [15–17, 76]. This phenomenon would decrease the proportion of force-producing cross-bridges, leading to a decline in force production. Attempts to show the inhibitory effect of P_i on force under physiological conditions have mainly been performed using ^{31}P MRS [11, 61, 69, 76–78] and focused on P_i and $H_2PO_4^-$ time-dependent changes, one the one hand, and force reduction, on the other hand [75]. A strong correlation has been demonstrated between $H_2PO_4^-$ accumulation and force reduction in human muscle during different types of experimental protocols including sustained or repeated contractions with maximal or submaximal intensities [69, 76, 77]. These results favour the hypothesis of $H_2PO_4^-$ being an

important determinant of peripheral fatigue. However, the direct cause-effect relationship have been questioned on the basis of other noninvasive investigations focusing on the different time scales of both events [11, 61, 78]. Indeed, during a 4-min maximal voluntary contraction exercise, a continuous force decrease has been reported, whereas P_i and $H_2PO_4^-$ concentrations have reached a steady state after 2 min of exercise [11]. Similar results have been obtained in rat gastrocnemius muscle electrically stimulated with a continuous force decrease along the stimulation protocol, whereas $H_2PO_4^-$ concentration first increased and then decreased after 5 min of stimulation as a result of PCr resynthesis [78]. It must be pointed out that the discrepant results mentioned above may be related to different experimental protocols, thereby suggesting that the relationship between P_i and/or $H_2PO_4^-$ and force would be dependent on exercise intensity and duration. In that context, a comparative analysis of different stimulation protocols has shed light on this point [27]. Repeated isometric contractions were elicited during 6 min at different frequencies ranging from 0.8 to 7.6 Hz in order to obtain increasing levels of metabolic and mechanical changes. Interestingly, the relationship between P_i or $H_2PO_4^-$ accumulation and isometric force was analyzed when force was maximal, i.e. in the early stage of the stimulation period, and at end of the stimulation period, when force was reduced as a sign of fatigue. Both $[P_i]$ and $[H_2PO_4^-]$ were strongly correlated with isometric force at the end of the stimulation protocol but not in the early stage whereas $[P_i]$ and $[H_2PO_4^-]$ did not differ significantly between these two stages of the stimulation protocol. These results clearly demonstrated in vivo that the inhibitory effect of P_i and/or $H_2PO_4^-$ on force cannot be direct. Indeed, if such was the case, given that P_i accumulates close to the myofilaments in contracting muscle as the result of myosin ATPase activity, P_i and/or $H_2PO_4^-$ should affect muscle performance equally well when force was maximal and at end of the stimulation period. These results do not reject the involvement of myoplasmic P_i accumulation in the development of peripheral fatigue but suggest that another mechanism might be involved. Recent results obtained in vitro have suggested that rather than a direct effect on contractile protein function, Pi could affect force indirectly by disturbing Ca^{2+} fluxes throughout the SR membrane during E-C coupling [18–20, 79]. An original study has shown that microinjection of P_i in electrically stimulated single mouse muscle fibres caused a temporary decrease in Ca^{2+} concentration at rest and during tetanic contraction, and a decline in force output [79]. A possible explanation would be that P_i enters the SR and binds Ca^{2+} to form highly insoluble CaP_i species [10, 18–20]. These species would precipitate within the SR, thereby reducing the amount of releasable Ca^{2+}. This reduction of releasable Ca^{2+} would lead to the decline in force production, hence causing fatigue [10, 18–20]. According to recent results, this P_i transport into the SR would be time related, lasting several minutes for physiological P_i concentration [18, 79]. This delay would account for the relationship between P_i and/or $H_2PO_4^-$ concentration and fatigue during the final but not the the early stage of a muscle activity, as previously demonstrated [27].

Limitation in ATP availability and fatigue development

Limitation in intramuscular ATP availability has been frequently proposed as a causative factor of fatigue development [7, 13, 14]. As an illustration, biochemical analyses in rat and *Xenopus* muscles have demonstrated a strong correlation between the decline in ATP concentration and the extent of fatigue [14, 80]. ATP is indeed crucial for the contractile process, being directly involved in energy-consuming mechanisms such as myofilament sliding, action potential propagation and ion transport (including SR Ca^{2+} reuptake). Therefore, any depletion in intramuscular ATP concentration during exercise could theoretically alter these ATP-dependent processes and result in the decline in force [9, 13, 22, 23, 81, 82].

ATP turnover in exercising muscle

It is striking to observe that the intramuscular concentration of ATP is relatively low at rest (averaging 6–8 mM, [83]) compared with the rate of ATP turnover for a high-intensity exercise (for example 200 mM ATP/min in [84]). In other words, such an ATP concentration would be enough for 2 s of sustained muscle activity. In fact, skeletal muscle has a high potential to generate ATP during exercise from various metabolic pathways, being able to adjust the rate of ATP production with a high precision to meet the rate of ATP utilization [13, 85]. To illustrate this capacity, it has been widely demonstrated that ATP concentration remains unchanged at the transition from rest to exercise, whereas ATP demand can increase by more than 100-fold [13, 85, 86]. At this stage of exercise, ATP regeneration is indeed insured whatever the intensity of muscle activity by the rapid breakdown of PCr and of glucose into lactate throughout anaerobic glycolysis. With the continuation of exercise, the contribution of these anaerobic pathways to ATP production decreases with a concomitant increase in the contribution of the mitochondrial oxidative phosphorylation of glucose and fatty acids. During low- and moderate-intensity exercise, ATP production is mainly oxidative with low anaerobic contribution. On the contrary, during high-intensity exercise, both oxidative phosphorylation and anaerobic processes contribute to energy supply [7]. Given that intramuscular stores of PCr and glycogen are limited, prolonged high-intensity exercise leads to the depletion of these metabolites, and ATP regeneration can therefore be-

come compromised. This imbalance between production and demand of ATP translates into a net decrease in intramuscular ATP concentration. Thus, whereas intramuscular concentration of ATP remains generally unchanged during low-intensity exercise when compared with its basal value, it can decrease up to 60–70% when exercise intensity is higher [7, 13, 87].

Glycogen supply and fatigue

Reduction in intramuscular stores of glycogen could play an important role in muscle fatigue [88, 89]. This hypothesis has been put forward from the observation that reduction of muscle performance coincides with the reduction in intramuscular glycogen pool during prolonged moderate or high-intensity exercise [90, 91]. The deleterious effect of intramuscular glycogen reduction on force may be explained by this concept. On the basis that glycogen and glycolytic enzymes are tightly associated with SR membrane [92, 93], the concept of microenvironment postulates that SR ionic pumps would preferentially use ATP from glycolysis, whereas ATP from mitochondrial oxidative phosphorylation would be mainly used by myofilaments [9, 51, 88, 89]. Because cellular ATP diffusion is limited [94], a large reduction in glycogen stores would lead to a local deficiency in ATP regeneration at the SR membrane level, which in turn would affect SR function by decreasing Ca^{2+} release [9, 88, 89]. This mechanism has been elegantly evidenced in isolated fibers from mouse flexor brevis muscle using photolytic activation of caged ATP (P^3-1(2-nitrophenyl)ethyl ester of ATP) into ATP by brief intense ultraviolet (UV) illumination [82]. Intramuscular injection of caged ATP at rest causes both a reduction in myoplasmic Ca^{2+} concentration and a decline in a force in response to tetanic stimulations. Photolytic activation of caged ATP in previously fatigued fibers leads to the increase in myoplasmic Ca^{2+} concentration and to partial force recovery. Consequently, these data clearly show a direct causal relationship between the sudden supply in intramuscular ATP and the improvement in force production in fatigued muscle. This phenomenon would be mediated by an improvement in SR function.

PCr synthesis and fatigue

Although a sudden increase in intramuscular ATP has never been reported in vivo on the contrary to what expected from caged ATP experiments [95], a net paradoxical PCr resynthesis has been reported a few times using ^{31}P MRS in electrically stimulated rat gastrocnemius muscle [27, 63, 68]. However, this phenomenon of energy recovery was not accompanied by any improvement in force production. In addition, this paradoxical energy recovery in exercising muscle remains very poorly studied under physiological conditions. In skeletal muscle, PCr resynthesis is carried out by the CK-catalyzed reaction, which transfers a high-energy phosphate group from ATP to Cr through the following reaction:

$$Cr^{2-} + ATP^{3-} \rightarrow PCr + ADP^{4-} + H^+$$

^{31}P MRS studies in human muscle have demonstrated that this resynthesis relies exclusively on oxidative phosphorylation during post-exercise recovery, i.e. as soon as muscle activity ceases [25, 96]. Net PCr resynthesis in contracting muscle indicates that some ATP would be paradoxically available to promote net PCr resynthesis instead of being used for contraction. This situation, which occurs during high-intensity stimulation, i.e. when ATP demand is important, is then in opposition with the common view that the rate of ATP production is adjusted with high precision to meet the rate of ATP utilization. In addition, the fact that the increase in ATP availability due to the net PCr resynthesis is not accompanied by any improvement in force production [27, 63, 68] challenges the concept of microenvironment.

Limitation of in vivo results

These challenging results must, however, be considered with caution by taking into account the limitation of in vivo ^{31}P MRS in relation to the mixed composition of mammalian skeletal muscle. Mammalian skeletal muscles are composed of a mixture of fast-twitch oxidative, fast-twitch glycolytic and slow oxidative fibers. Given that fast-twitch fibers are more fatigue resistant than slow-twitch fibers [55], it is possible that in the previous ^{31}P MRS studies exploring the net PCr resynthesis in stimulated muscle using high-intensity protocols, some fatigable fast fibers would be totally exhausted and produce no more force, whereas fatigue-resistant fibers would continue to be mechanically active. The net PCr resynthesis during muscle stimulation would then occur in inactivated fibers similarly to what occurs during post-exercise recovery [63]. This assumption is supported by the observation that in stimulated rat gastrocnemius muscle, the net PCr resynthesis relies exclusively on oxidative processes as evidenced from a comparative analysis of PCr time-dependent changes during aerobic and ischemic stimulation protocols [27].

Changes in energy demand associated with fatigue

It is clear that a decrease in ATP availability could lead to fatigue. It is therefore of interest to wonder whether muscle is able to use ATP sparingly in order to avoid or at least attenuate fatigue. Energy demand during contraction varies considerably between the various forms of exercise [85], depending on the type of activity, i.e. dynamic, concentric or isometric [97], on intensity and duration of exercise [51, 53] and on the fiber composition of the working muscle [57, 98]. Calculation of the energy cost of contraction, defined as the ratio between energy utiliza-

tion and force output, provides a good tool for comparing the bioenergetics of these various forms of exercise.

Energy cost of contraction and fatigue

Interestingly, it has been shown that the energy cost of contraction decreases during fatiguing exercise. This phenomenon, which might represent a way of optimizing ATP use to force production, is paradoxical because it means that fatigued muscle would need less energy to produce the same amount of isometric force compared with fresh muscle. Reduction in energy cost of contraction was first shown in vitro in animal skeletal muscle [53, 54]. For example, combination of both chemical and oxygen consumption measurements in isolated extensor digitorum longus muscle from mouse has shown that the energy cost of contraction was reduced by more than 30% at the end of a 15-s tetanic contraction inducing fatigue [53]. ^{31}P MRS studies have been useful in confirming these results under physiological conditions. A quantitative analysis of energy production showed that ATP cost of contraction decreased throughout fatiguing protocols consisting of maximal voluntary contraction (MVC) in human muscle [39, 66, 99] and during repeated isometric contractions in rat gastrocnemius muscle stimulated in situ [27].

Shift of the fiber type recruitment pattern could contribute to this energy cost reduction during fatiguing exercise. It is well known that slow-twitch fibers contract more economically compared with fast-twitch fibers [53, 100, 101]. In mixed muscle, the increase in the relative proportion of activated slow-twitch fibers during fatiguing activity could lead to more economical use of energy, resulting in a reduction in ATP cost of contraction [27].

This energy cost reduction could also be linked to an alteration of muscular relaxation processes. During muscular activity, ATP is used by both contractile (myosin ATPase) and noncontractile (mainly muscle relaxation due to Ca^{2+} pumping into the SR) processes. It has been shown that the amount of ATP hydrolyzed for Ca^{2+} pumping into the SR might account for 20–50% of the total amount of ATP utilized for contraction [44, 51, 56]. Any change in relaxation process during fatiguing protocol, due for example to local deficiency in ATP near the SR, could result in an overall reduction in the energy cost of contraction. This assumption is supported by studies using analytical methods on muscle extracts [55, 56, 102], VO_2 measurements [51] or in vivo ^{31}P MRS studies [57] demonstrating that for a given muscle, the energy cost of contraction is lower for a single sustained contraction compared with repeated contractions, all things (intensity and duration of exercise) being equal. Indeed, during a single sustained contraction ATP is mainly used to maintain force output, whereas additional ATP is needed for Ca^{2+} pumping during repeated contractions.

It is noteworthy that in previous studies looking at the reduction of the ATP cost of contraction, explorations were generally performed for a given fatiguing protocol. The relationships between the fatigue level and the extent of ATP cost reduction have been investigated in stimulated rat gastrocnemius muscle in situ for a variety of exercise conditions [27]. One nonfatiguing protocol and five protocols inducing increasing levels of fatigue were used, and the rates of ATP production from various metabolic pathways were quantified using ^{31}P MRS. It has thus been found that the reduction in ATP cost of contraction was larger for fatiguing protocols compared with nonfatiguing protocols. Moreover, the ATP cost of contraction was reduced to the same extent whatever the level of fatigue. The authors have therefore proposed that ATP utilization in contracting muscle would be systematically optimized whatever the intensity of contraction, up to a threefold, which could be associated with fatigue development. These results provide a novel view of the relationship between fatigue and bioenergetic metabolism, but further studies must be carried out to determine the exact causal relationship between these phenomena.

Combined investigations of muscle fatigue using MRS and surface EMG

As previously mentioned, the loss of maximum force-generating capacity, defined as fatigue, may occur at the various sites along the pathway from the central nervous system through to the intramuscular contractile machinery. Besides central factors [103], peripheral factors, which could interfere with force production, include metabolic inhibition of the contractile process and E-C coupling failure [69, 73, 104, 105]. Exclusive metabolic investigations thus offer a limited window towards the investigation of peripheral factors, and a few groups have reported combined investigations of electrical and metabolic changes associated with the failure of muscle force production in order to widen the experimental field of research related to fatigue.

Electromyographic components of fatigue

During the transition from rest to exercise, typical electromyographic changes, such as a rise in the integrated EMG (iEMG) and a shift of the power spectrum towards low frequencies, have been reported [106–109]. While the iEMG rise may indicate an increase in the firing rate of motor unit discharge and/or recruitment of additional muscle fibers, the reasons for the shift of the mean frequency of the EMG power spectrum remain a matter of debate. Alterations in the frequency components of the EMG power spectrum have been reported during static and dynamic contractions [107, 110] and variously related to accumulation of muscle lactate [108, 109] but not systematically to intracellular acidosis [108], muscle con-

duction velocity [111], force output [112], muscle thickness [113] and mechanical changes [114]. Overall, metabolic causes have sometimes been put forward as accounting for surface EMG (sEMG) alterations, and combined ^{31}P MRS and sEMG investigations have been performed to characterize the link, if any.

Methodological issues

This type of combined experiment is actually challenging mainly from a methodological point of view. On the one hand, surface electrodes have to be positioned beneath the muscles investigated, and such electrodes could introduce additional noise in the MR spectra. Similarly, radiofrequency field interferes with sEMG signals. On the other hand, the magnitudes of sEMG changes are relatively small, and the signal must be amplified very close to the source. In summary, technically challenging adaptations are necessary to perform such combined experiments. Several types of solutions have been proposed by a few research groups, and convincing combined measurements have been reported.

Main results

sEMG recordings can be analyzed in the time domain (root mean square) and in the frequency domain (mean power frequency), and exercise-induced changes associated with both variables provide information related to the amount and chronological activation of different motor units. When muscle contractions are not performed voluntarily, electrical stimulations may be used to acquire information regarding peripheral activation, i.e. the excitability of the neuromuscular junction and muscle membrane. In that case, supramaximal stimulations are used, and corresponding compound muscle action potentials (CMAP or M-wave) are recorded. These variables or, more exactly, alterations of these variables have been reported during fatiguing exercise and analyzed in the light of simultaneous metabolic changes such as depletion of high-energy phosphates and accumulation of metabolic products, e.g. ADP, P_i and H^+. Since the first study reported by Miller et al., a few combined analyses have been devoted to the study of both metabolic and electrical changes linked to muscle fatigue. As highlighted below, the results are highly controversial and a unified position cannot be proposed at the moment. Miller's results were mainly based on analysis of the recovery phase following exercise and not on the exercise period per se. They proposed at least three components of fatigue following the completion of exercise with one of them, i.e. the recovery rate of MVC being associated with time-dependent changes in both PCr and intracellular pH [105]. They also reported altered M-wave (reduced in amplitude and prolonged in duration), reflecting impaired muscle membrane excitation and a decrease in neuromuscular efficiency (NME) that persists for a longer time as compared with both M-wave and metabolic changes. However, both variables were not closely associated with metabolic changes. In addition, the delayed recovery of NME indicates a component of impaired muscular function that is independent of high-energy phosphates and intracellular pH. Interestingly, in both adductor pollicis and tibialis anterior muscles, low-intensity exercise produced a marked depression of twitch tension with only minimal changes in MVC, M-wave, PCr and pH [115]. These observations clearly indicate that for a low intensity-exercise, fatigue is largely due to changes in E-C coupling with no contribution of either central factors, impairment of the contractile mechanism, altered membrane properties or impaired neuromuscular transmission [115]. However, the exact mechanism is poorly understood. Similar conclusions have been put forth by Bendahan et al. from combined measurements performed during isometric contractions of the forearm flexor muscles [116]. On the basis of their measurements, the existence of causal relationships between the extent of acidosis and EMG signs of fatigue could not be firmly established. A shift in the median frequency of the EMG power spectrum was indeed observed as a sign of fatigue, but its time-dependent evolution was not at all linked to the pH of PCr time-dependent changes, given that EMG changes characterizing fatigue were only noted beyond critical metabolic values [116]. Also of interest was their observation of a large scattering of data in a group of untrained subjects despite standardization of the exercise protocol using MVC [116]. The effect of oxygen availability on a potential relationship between electrical signs of fatigue and alterations in muscle energetics has also been investigated [26]. Hypoxemia did not affect the magnitude of metabolic changes and the duration of contraction. However, the rate of changes in integrated sEMG was significantly modified, changing the correlated evolution of metabolic and electrical changes [26]. The downward shift of the relationships between myoelectrical and metabolic changes under hypoxemia points to the existence of better E-C coupling and could indicate an adaptive mechanism [26].

On the other hand, a relationship between the loss of MVC and accumulation of P_i or, more precisely, its diprotonated form has been reported [76], although the exact causative nature of this relationship is unclear. Wilson et al. [76] and Miller et al. [105] have reported such a relationship in a single group of subjects and suggested that accumulation of $H_2PO_4^-$ could account for the failure of muscle force production, in agreement with experiments conducted in skinned fibers [117]. In contrast, Kent-Braun et al. have recently reported that this relationship is modulated by age and gender, indicating that the causative links and the underlying mechanisms could differ among various groups of subjects [118]. Accumulation of $H_2PO_4^-$ has been advocated as accounting for the loss of MVC but also for the shift of the mean power fre-

quency (MPF) of the EMG spectrum [119, 120]. A quasilinear decrease in MPF was found during an exhausting calf muscle exercise test, and it was significantly correlated with $H_2PO_4^-$ concentration, which can be considered as resulting from both Pi accumulation and intramuscular acidosis. A similar inverse relationship has been reported between muscle lactate accumulation and the MPF shift in the vastus lateralis muscle, but surprisingly the expected link with intracellular pH was not observed [108]. Bouissou et al. also reported that systemic alkalosis was associated with a greater spectrum shift toward lower frequencies at exhaustion despite a level of muscle acidosis similar to that under placebo conditions, although the muscle lactate concentration was higher in alkalosis [108]. It is noteworthy that the shift in EMG power spectrum toward lower frequencies can also account for the increased root mean square (RMS). When the low frequency component of the EMG signal is increased, more myoelectrical signals will be recorded since muscle tissue and skin act as low-pass filters [121]. In addition, for submaximal (>25% of MVC) exercise, the drop in MPF is independent of force output [112].

More recently, combined analyses of central and peripheral contributions to muscle fatigue were reported by Kent-Braun et al. [11, 118]. In order to quantify the respective contributions of central and peripheral factors to fatigue development, they performed simultaneous noninvasive measurements of central activation (using electromyography), neuromuscular junction and muscle membrane excitability (using electrical stimulation) and muscle energetics (using ^{31}P MRS). They mainly concluded that during a 4-min maximum isometric exercise involving the ankle dorsiflexor muscles, central factors contributed modestly (16%) to fatigue development. The remaining 80% was apparently due to intramuscular sources, primarily increased proton concentration, given that intracellular acidosis was significantly linked to both the fall in MVC and the integrated EMG decrease [11]. The role of H^+ accumulation in muscle fatigue has often been considered as minor, but Kent-Braun's [11, 118] and other studies [73, 119] have reported that the decline in force was tightly linked to it. Likewise, Miller et al. observed such a relationship [77]. This strong association between fatigue and pH would be consistent with the role of pH in feedback to the central nervous system and a subsequent alteration in central motor drive during the development of fatigue. Similar measurements have been performed during an incremental isometric exercise with the purpose of comparing the magnitude and mechanisms of ankle dorsiflexor muscle fatigue in young and older subjects [118]. From this study, the authors mainly reported that young subjects fatigued more than older subjects regarding MVC measurements. On the other hand, at the end of exercise there was a significant gender effect in that men had a higher P_i and/or PCr (i.e. greater energy consumption) compared with women. Meanwhile, intracellular pH fell more, and accumulation of P_i and/or $H_2PO_4^-$ was larger in young compared with older subjects and in men compared with women. Whatever the group, a significant linear relationship was found between $H_2PO_4^-$ and the fall in MVC regardless of the magnitude of fatigue or degree of metabolite accumulation. Although men had a nearly twofold greater increase in $H_2PO_4^-$ during exercise, they developed no more fatigue than women. As a result, the slope of the relationship between fatigue and $[H_2PO_4^-]$ appears to be steeper for women. Similar observations were reported for pH and the overall P_i concentration. Kent-Braun et al. suggested that alterations in contractile function did not explain the age-related difference in fatigue. During their moderately fatiguing exercise, whatever the group, CMAP amplitude did not change, suggesting that peripheral excitability was not affected, whereas its duration was shorter as a sign of an increased conduction velocity across the neuromuscular junction or along the muscle membrane. Overall, neither central nor peripheral (compound muscle action potential) played a significant role in fatigue in any group. The varied metabolic responses to exercise suggest that the mechanisms of fatigue change with age and gender [118].

Functional investigation of exercising muscle using MRI

As previously mentioned, the acute effects of exercise on muscle MRI contrast were first reported in 1988 by Fleckenstein et al. [5], and from that time, it was recognized that MRI could be used to distingish between active and nonactive muscle groups, providing some functional assessment of exercising muscle.

^1H MRI

Contrast differences in MRI come from differences in T1, T2 values and nuclear density. Bone, fat, muscle, connective tissue and blood have different proton nuclear densities, which, together with T1 and T2 relaxation processes, have a significant impact on the final contrast obtained on the image produced. In muscle MRI, signals arise from the protons of water, lipids and bone marrow (fig. 2). It has been theorized that there are three basic proton spins groups within cells: organic protons on macromolecules (which would not contribute to the MRI signal), protons of water within the hydration shell of macromolecules (bound) and the protons of bulk water (free) [122]. These fractions also exist in the extracellular compartments of muscle. The MR behaviour of intracellular water is believed to result from interactions between the surface of macromolecules and a bound water layer and exchange between this layer and the relatively free cellular water. Proton motion within the free cellular water is it-

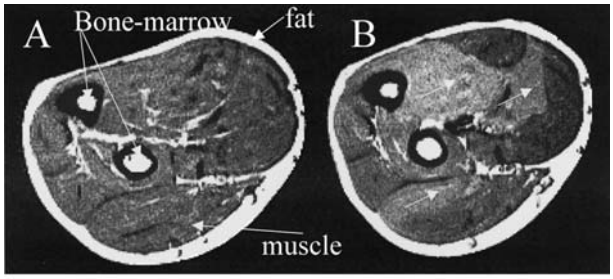

Figure 2. Typical T2-weighted ^{1}H MR images representing transverse sections of the forearm recorded at rest (*A*) and after a finger-flexion exercise (*B*). Areas with a higher contrast on image *B* indicate activated muscles during exercise.

self fast enough to average out molecular interactions; thus, this large fraction of cellular water may not contribute to the MR relaxation process. The smaller slowly exchanging bound water fraction may determine much of the MR relaxation character of muscle cells. Muscle cell contraction involves conformational changes in the large contractile proteins as well as mechanical alterations in intracellular surfaces [123]. Such surface alterations may affect the bound water layer, which in turn may determine MR relaxation [122]. In addition to alteration in the size, shape and charge of surfaces in muscle cells, contraction involves the production and translocation of ions and metabolites. These processes would have osmotic effects that alter the concentration and MR relaxation of water.

T2 changes in exercising muscle

Changes in proton signal intensity cause exercised muscles to appear 'light-up' in T2-weighted images, as shown in figure 2. The mechanism of the T2 increase underlying muscle functional MRI is still poorly understood but is definitely different from the well-known blood oxygenation level-dependent (BOLD) effect underlying brain functional MRI investigations. T2 measurements in muscle have been performed in order to elucidate the signal shift in muscle that is related to exercise. More particularly, MRI measurements focuse on the intrinsic property of water and its exchange between compartments, as well as the binding capacity of the water molecule to subcellular structures. Changes in T2 accounting for the discrimination between active and inactive muscles result from the dependence of relaxation on the local molecular environment of the nuclei under study [122]. Given that T2 changes affect exclusively muscle and neither fat nor bone marrow, it is reasonable to conclude that the signal changes arise from one or more of the water compartments in muscle, i.e. from intra- and extracellular spaces.

T2 and volume changes

For instance, different T2 values in resting muscle have been reported as a sign of different size in extracellular spaces [124]. Increased perfusion would therefore seem an obvious factor, which could account for exercise-induced T2 changes. The volume of exercising muscle is known to increase because of redistribution of body water [125, 126]. This redistribution could be attributed to increased perfusion and the production and translocation of ionic species, which could alter the osmotic behaviour of muscle cells [127, 128]. Low-intensity exercise is believed to be associated with an increase in extracellular water, whereas high-intensity exercise is primarily associated with change in intracellular water [126, 129]. Changes in either intra- or extracellular water would be expected to alter the relaxation characteristics of the excited nuclei in a muscle sample [122]. However, changes in muscle volume can be induced by venous occlusion to the same extent as exercise, and similar T2 changes could be expected. Fisher et al. [130] demonstrated that venous occlusion had little effect on T2 despite significant changes in muscle volume, thereby clearly indicating that exercise-induced enhanced MRI contrast does not result from the simple increase in fluid volume linked to increased perfusion. More complex, probably intracellular events would be responsible for exercise-induced contrast enhancement.

Exercise-induced changes in MRI T2 contrast result from some complex combination of several processes occurring during muscle contraction, and several studies have been devoted to determining of the mechanisms involved in such changes.

T2 and metabolic changes

As indicated above, it has been known from invasive studies that the volume of exercising muscle increases as a result of redistribution of body water [125, 126]. While changes in muscle volume as a factor involved in post-exercise T2 changes have been refuted on the basis of venous occlusion experiments [131], it has been clearly demonstrated that T2 changes were graded with exercise intensity during dorsiflexion exercises [130], thereby indicating that T2 changes during exercise are dependent on the force generated. Besides these observations, a direct relationship between changes in osmotically active metabolites such as lactate and P_i has also been correlated with the extent of T2 changes [132, 133], indicating that they are likely related to osmotically driven fluid shift. This relationship between the extent of T2 and pH changes in exercising muscle has been confirmed from a functionnal analysis of McArdle patients. Such patients suffer from glycogenolysis deficiency and are unable to produce lactate [134]. As a result of this deficiency, intracellular pH cannot change upon exercise, as clearly shown by ^{31}P MR measurements [135]. In association with the absence of intracellular acidosis, a couple of studies have reported an absence of T2 changes in these patients, confirming the tight relationship between MRI T2 contrast changes and pH [136]. The cause-effect rela-

tionship between exercise intensity, pH and MRI contrast measurements have been further illustrated from a combined MRI and electromyography study [131]. This study has suggested that shifts in MRI contrast after exercise are an excellent measure of muscle use. Indeed, EMG and T2 changes were both different in eccentric and concentric actions. Interestingly, both measurements were linked [131]. To go further with the determination of mechanisms underlying T2 changes in activated muscles and in keeping with the hypothesis that these changes must result primarily from altered relaxation within the active muscle cells and not from changes in the extracellular fluid space [137, 138], a series of experiments in humans and animals have considered the metabolic dependence of T2 increase. These studies were based on the hypothesis that an increase in intracellular fluid during exercise is likely caused by the accumulation of osmolites such as P_i and lactate. Therefore, if the osmotic expansion of an intracellular fluid compartment is linked to post-exercise T2 changes, these intensity changes ought to vary with the extent of metabolic changes. In that respect, it has been reported that T2 changes are linked to exercise intensity [131] and aerobic capacity of a given muscle [139, 140]. However, experiments conducted in marine invertebrates have provided interesting information about the relationship between T2 and metabolic changes [141]. Contraction of tail muscle of crayfish (an osmoregulator species with osmolarity near 340 mosM) was linked to T2 changes, whereas contraction of lobster tail (a marine osmoconformer with osmolarity equal to that of seawater, i.e. 1 osM) was not linked to any. These results clearly point out that T2 changes are linked to redistribution of tissue fluid caused by accumulation of intracellular osmolites as previously indicated in rat muscle [140]. However, pH changes occurred only in lobster tail muscle upon contraction and not in crayfish tail muscle, suggesting that muscle acidification per se is not a necessary or dominant cause of T2 increase during stimulation [141]. Overall, one can conclude that T2 increase in active mamalian muscles is caused by osmotically driven fluid shifts between subcellular compartments. However, in order to exploit these changes as either a mapping tool or an index of exercise intensity, one has to address the issue of normalizing the T2 changes between different muscles and different individuals. Indeed, heterogeneous exercise-induced T_2 changes have been reported among subjects with different training status or among muscles within the same subject, with no clear interpretation regarding the underlying mechanisms [132, 142]. It has been clearly shown from comparative analysis between eccentric and concentric exercises that T2 increase after exercise is not dependent on the absolute work rate per se but is linearly linked to exercise intensity relative to maximum aerobic power [139, 143]. In addition, Prior et al. [140] have recently reported that the T2 increase after exercise varied inversely with known aerobic aptitudes. In keeping with the results published by Reid et al. [139], such standardization should allow reliable comparison.

Conclusion

For many years, there has been considerable interest in trying to determine whether or not biochemical changes occurring in exercising muscle actually contribute to the subjective manifestation of fatigue. The development of fatigue is attributable to both central and peripheral factors. The relative contribution of these factors may be estimated using a combination of voluntary and electrically stimulated force measurements, ^{31}P MRS and EMG recordings. This type of approach should improve and shed some light on our understanding of the mechanisms of human muscle fatigue by simultaneously assessing functions at various stages along the pathway of force production.

Regarding mapping of muscles activated during exercise, investigation of T2 changes using MRI provides reliable information as long as standardization procedures are properly used. Although it has been quite clearly established that the mechanisms underlying these T2 changes are linked to altered relaxation within the active muscle cells likely due to accumulation of osmotic metabolites, the exact nature of these metabolites still remains to be precisely determined.

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