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The role of pH on the thermodynamics and kinetics of muscle biochemistry: An *in vivo* study by ^{31}P-MRS in patients with myo-phosphorylase deficiency

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ABSTRACT

In this study we assessed $\Delta G'_{ATP}$ hydrolysis, cytosolic [ADP], and the rate of phosphocreatine recovery using Phosphorus Magnetic Resonance Spectroscopy in the calf muscle of a group of patients affected by glycogen myo-phosphorylase deficiency (McArdle disease). The goal was to ascertain whether and to what extent the deficit of the glycogenolytic pathway would affect the muscle energy balance. A typical feature of this pathology is the lack of intracellular acidosis. Therefore we posed the question of whether, in the absence of pH decrease, the rate of phosphocreatine recovery depends on the amount of phosphocreatine consumed during exercise. Results showed that at the end of exercise both [ADP] and $\Delta G'_{ATP}$ of patients were significantly higher than those of matched control groups reaching comparable levels of phosphocreatine concentration. Furthermore, in these patients we found that the rate of phosphocreatine recovery is not influenced by the amount of phosphocreatine consumed during exercise. These outcomes provide experimental evidence that: i) the intracellular acidification occurring in exercising skeletal muscle is a protective factor for the energy consumption; and ii) the influence of pH on the phosphocreatine recovery rate is at least in part related to the kinetic mechanisms of mitochondrial creatine kinase enzyme.

1. Introduction

Phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS) affords the possibility of assessing the energy status of living tissues and has been used since the 1980's to study the human skeletal muscle energy metabolism *in vivo*. In fact, skeletal muscle is a very convenient tissue to study as it can be examined in different metabolic conditions such as rest, exercise and recovery [1]. Important metabolic parameters relevant for the knowledge of the bioenergetics of living tissues include ΔG of ATP hydrolysis, cytosolic [ADP] [1–5] and phosphocreatine (PCr) recovery [6–8]. Two main issues are connected with the *in vivo* measurement of ΔG of ATP hydrolysis, and cytosolic [ADP]. i) The first is that [ADP] cannot be directly measured *in vivo* by ^{31}P-MRS due to its low concentration in living tissues. As a consequence, [ADP] can only be calculated from the apparent equilibrium constant of the creatine kinase reaction (K'_{CK}) [9–14]. ii) The second is the choice of the ΔG of ATP hydrolysis: *i.e.* $\Delta G_{MgATP}{}^{2-}$ or $\Delta G'_{ATP}$. Since, $MgATP^{2-}$ is the active species in enzyme binding [15], the energy producing form in active transport [16,17] and muscular contraction [18,19] it can be argued that only $\Delta G_{MgATP}{}^{2-}$ should be relevant in describing the intracellular energetic status of a tissue [20–25]. Nevertheless, the equivalence between $\Delta G_{MgATP}{}^{2-}$ and $\Delta G'_{ATP}$ as a consequence of the demonstration of the general rule that $\Delta G = \Delta G'$ [26] has been recently shown. Therefore the long dispute on which between $\Delta G_{MgATP}{}^{2-}$ and $\Delta G'_{ATP}$ is more appropriate is now irrelevant [26].

Both [ADP] and $\Delta G'_{ATP}$, depend on $[H^+]$ and $[Mg^{2+}]$ [2]. Skeletal muscle displays marked changes in $[H^+]$ and $[Mg^{2+}]$ during exercise and recovery [27], therefore the assessment of the thermodynamic status of human skeletal muscle cannot forgo an equally accurate measurement of pH and pMg [2,27]. In this study we used a quantitative mathematical approach to assess *in vivo* by ^{31}P-MRS the cytosolic [ADP] and $\Delta G'_{ATP}$ in human skeletal muscle, taking into account pH and pMg changes [2].

PCr is mainly located in the mammalian brain and skeletal muscle and represents a storage of readily available energy able to buffer rapidly changing energy requirements. Typically, PCr is consumed during muscle contractions and is resynthesized to buffer ATP consumption. PCr resynthesis reaction has an unfavorable thermodynamics (backward reaction) and needs to be compartmentalized to take place. This goal is accomplished by the mitochondrial creatine kinase enzyme (MCK) localized in the mitochondrial inter-membrane

Abbreviations: ^{31}P-MRS, Phosphorus Magnetic Resonance Spectroscopy; PCr, phosphocreatine; MCK, mitochondrial creatine kinase enzyme; Cr, creatine; TR, repetition time; FWMH, Full Widths at Middle Height; $[PCr]_{cons}$, PCr consumed during exercise; TC_{PCr}, PCr recovery time constant; Vi, initial rate of PCr recovery; L, light exercise protocol; M, moderate exercise protocol; LBM, lean body mass

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space, where it produces PCr from mitochondrially-generated ATP and imported creatine (Cr) from the cytosol [28].

The rate of PCr recovery from exercise assessed *in vivo* by ^{31}P-MRS, which is taken as reliable index of muscle mitochondrial ATP production [6–8], is linked to the extent of cytosolic acidosis [4], being correlated with the minimum pH value reached during recovery [5]. The relationship between pH and the rate of PCr recovery, operationally useful, has been object of several studies [6,7,12,29–31], trying to understand its biochemical significance. The main question raised by these studies is whether in this context pH is an independent variable, as it is known that end-exercise pH covaries with the PCr consumed during exercise (PCr_{cons}). Recently, a study based on the kinetic analysis of the PCr re-synthesis reaction proposed a non-linear mathematical model to explain oscillatory PCr recovery pattern showing that the relationship between pH and PCr recovery rates can be explained on the basis of enzyme kinetic mechanisms [31].

Recent literature has given rising evidence, that during recovery from exercise starting at low levels of PCr, the rate of oxidative phosphorylation could be influenced by the rate of mitochondrial oxygen supply [32]. However, a study performing simultaneous *in vivo* measurements of oxygen saturation and PCr kinetics following calf muscle exercise in healthy humans has shown that recovery rates of oxyhemoglobin, is not affected by low cytosolic pH. This result suggests that in the exercising calf muscle the oxygen supply is not affected by the cytosolic proton load [33].

McArdle patients represent an ideal experimental model for investigation of the relationship between PCr recovery and PCr_{cons} in the absence of pH change since a lack of intracellular acidosis during muscle exercise is a typical feature of this disorder due to glycogen myo-phosphorylase deficiency [34,35]. In this study we assessed the cytosolic [ADP], $\Delta G'_{ATP}$ and the rate of PCr recovery of McArdle patients who were asked to perform muscular exercises at different intensities: i) to ascertain to what extent a deficit in the glycogenolytic pathway affects the muscle energy balance and 2) to understand whether, in the absence of cytosolic acidosis, the rate of PCr recovery depends on the amount of PCr depleted during exercise.

2. Methods

2.1. Controls and patients

Eight adults (2 F; 6 M, mean age ± s.d. 35 ± 10 years) with biochemically and molecularly proven McArdle's disease were enrolled for this study. Exclusion criteria were: hypertension; heart, kidney, or pulmonary disease; diabetes; use of any ACE inhibitor; ongoing long-term therapy with any drug; and pregnancy or lactation. Fourteen healthy volunteers were recruited (30 ± 9 years, 7 women and 7 men), as control subjects. The protocol was approved by our institutional ethical boards, and all subjects gave their informed consent to participate.

2.2. ^{31}P MRS acquisition

MR spectra were acquired on a General Electric 1.5 T Signa System whole-body scanner. Radiofrequency pulses at 25.866 MHz with a pulse width of 400 μs. A data table of 2048 complex points was collected for each FID. The band width was 2.5 kHz. The stimulation–response sequence was repeated every 5 s (TR = 5000 ms). Magnetic field homogeneity was optimized by shimming the ^{1}H water spectrum (FWMH 0.25–0.35 ppm). Subjects lay supine with a 7.5 cm diameter transmitter/receiver ^{31}P surface coil centered on the maximal circumference of the right calf muscle. One hundred-twenty-eight FIDs were averaged while the subject lay at rest. Then for each of two levels of work (see below), 12 FIDs were averaged during exercise while during recovery 2-FID data blocks (10 s) were recorded for 640 s.

2.3. Exercise protocols

Each subject performed isokinetic exercise in the scanner using two different protocols on separate occasions: Light (L) and Moderate (M), in order to reach different levels of PCr depletion. Exercise consisted in rhythmical plantar flexion against a pedal connected to a pneumatic ergometer. During the first 2 min. the force level of each contraction was the 10% of lean body mass (LBM), then the pressure in the ergometer was increased every minute by 5% of LBM. In protocol L subjects performed two contractions every 5 s. In protocol M subjects performed three contractions every 5 s. The time length of the exercise and the ergometer pressure were the same in both protocols for each subject, but varied between subjects according to their individual LBM.

2.4. Data analysis

Spectra were post-processed by a time-domain routine AMARES/JMRUI (http://www.mrui.uab.es/mrui/). The resonances of: ATP (α, β and γ), Pi, PCr, phosphomonoester (PME) and phosphodiester (PDE) resonance were used as the basis set. Prior knowledges were used for the quantification of the three resonances of ATP. In particular equal amplitude line-width and fixed relative frequency of both α-ATP and γ-ATP signals were imposed. Similarly we applied prior knowledges imposing the amplitude ratio of the triplet = 1:2:1 in fitting the three β-ATP peaks. Each metabolite signal was fitted to a Lorentzian line shape. For each metabolite signal, spectral fitting quality was estimated by using the Cramér–Rao bound [36], setting a maximum threshold of 20%. The PCr and Pi concentrations were calculated by assuming a ATP concentration of 8 mM [37] for the cohort of our control subjects. Three out of 8 patients showed an abnormally high value of both [PCr] and [Pi] (> control-mean + 2SD). We assumed that the simultaneous increase of [PCr] and [Pi] was due to a reduction of [ATP]. Therefore, we estimate the [ATP] of these three patients by using the following equation:

$$[\mathrm{ATP}] = \frac{1}{2}\sum_{j=1}^{2}\frac{1}{2}S\left(\beta - \mathrm{ATP}_j\right)\left(\frac{K_1}{S\left(\mathrm{PCr}_j\right)} + \frac{K_2}{S\left(\mathrm{Pi}_j\right)}\right) \quad (1)$$

where: j represents the number of the exams for each patient; S(β − ATP) is the amplitude of the triplet of the β-ATP at rest; S(PCr) and S(Pi) are the amplitude respectively of the PCr and Pi peaks at rest; K_1 and K_2 are the values of mean [PCr] and [Pi] plus 2 SD at rest of our control subjects expressed in mM. Eq. (1) is designed to take simultaneously into account the increase of [PCr] and [Pi] averaging the values of these metabolites when both exceeded the control mean + 2SD. It must be underlined that the values of [ATP] obtained by Eq. (1) gives a conservative estimation of the [ATP] reduction, since the reference values K1 and K2 are taken at the upper edge of control range (mean + 2SD). The [ATP] obtained in these three patients was used to assess the [PCr] in different metabolic conditions (*i.e.* rest and end-exercise). The rate of PCr recovery was assessed by the mono-exponential equation best-fitting the PCr recovery pattern (Eq. (2)) and reported as time constant (TC_{PCr}) expressed in seconds:

$$\mathrm{PCr(a.u.)} = C_1 e^{-kt} + C_2 \quad (2)$$

where C_1 is the amount of PCr consumed during the exercise, C_2 is the amount of PCr at rest and k is the rate constant of PCr recovery in s^{-1}. The time constant of PCr recovery rate is defined as: TC_{PCr} =

$-1/k$. The initial rate of PCr recovery (Vi) was calculated according [38] to the following:

$$Vi(mM/s) = \frac{[PC]_{cons}}{TC} \quad (3)$$

where $[PCr]_{cons}$ is the PCr consumed during the exercise in mM calculated from the difference between rest and end-exercise PCr concentration.

The pMg and pH were assessed from the chemical shift of β-ATP and Pi from PCr respectively according to [27] the software package MAGIC-MC available at http://www.cermiv.unibo.it. Both $\Delta G'_{ATP}$ and cytosolic [ADP] were calculated taking into account pH and pMg changes according the approach developed by Iotti et al. [2] by the software package BMMG2 available at http://www.cermiv.unibo.it.

In the group of MD patients studied we did not find any systematic decrease in the beta-ATP signal during exercise. The oscillation in beta-ATP signal during exercise and recovery was always less than 15% compared to rest as in control subjects.

An unpaired 2-sided Student t-test was used to compare average patient and control metabolite concentrations at rest and end of exercise, and metabolic constants. For the McArdle patients only, end-of-exercise PCr and recovery constants for the two exercise intensities were compared using a paired t-test. Values of $p<0.05$ were considered to be significant.

3. Results

Fig. 1 shows ^{31}P-MRS spectra acquired at rest and at the end of exercise in the calf muscle of a McArdle patient compared to that of a control subject reaching a similar level of PCr depletion.

Table 1 reports the values of the metabolic parameters measured at rest in McArdle patients compared to controls. At rest patients showed a higher pH and [PCr] and lower pMg, accompanied by a more negative $\Delta G'_{ATP}$.

Table 1
Values (mean ± SD) of metabolic parameters measured at rest in McArdle patient and control groups. P-value shows result of group comparison using Student t-test.

	pMg	pH	PCr (mM)	$\Delta G'_{ATP}$ (kJ/mol)	ADP (μM)
McArdle (n = 8)	3.46 ± 0.03	6.96 ± 0.01	32.8 ± 1.7	−62.10 ± 1.34	47.35 ± 11.76
Control (n = 14)	3.52 ± 0.05	6.92 ± 0.01	29.4 ± 2.6	−59.51 ± 1.75	62.45 ± 19.84
P	<0.01	<0.001	<0.01	<0.001	N.S.

All patients performed calf muscle exercise at two different intensities in order to reach different amount of PCr depletion. The resulting 16 exercises were divided into three groups: Low [PCr] (<14 mM), Medium [PCr] and High [PCr] (>19 mM) according to the level of PCr concentration reached at the end of exercise ($[PCr]_{end}$).

We did not find any decrease in [ATP] of patients during exercise as described by Zange [39], possibly because of a worse signal-to-noise ratio due to the lower magnetic field strength of the MR system used in this study.

Table 2 reports the values of metabolic parameters measured at the end of exercise in the three groups compared to matched controls. Results showed that for a comparable level of $[PCr]_{end}$ the pMg_{end} of patients did not differ significantly from that of matched controls, whereas pH_{end} was always higher as a consequence of the lack of intracellular acidosis during muscle exercise which is a typical feature of the pathology [33,34]. Among the patients in the Low [PCr] and Medium [PCr] groups, $\Delta G'_{ATPend}$ was considerably less negative than in matched controls at the end of exercise, while [ADP] end was considerably higher. In the High [PCr] group $[ADP]_{end}$ was slightly higher than for matched controls while $\Delta G'_{ATPend}$ did not differ significantly from control values. These outcomes are better illustrated

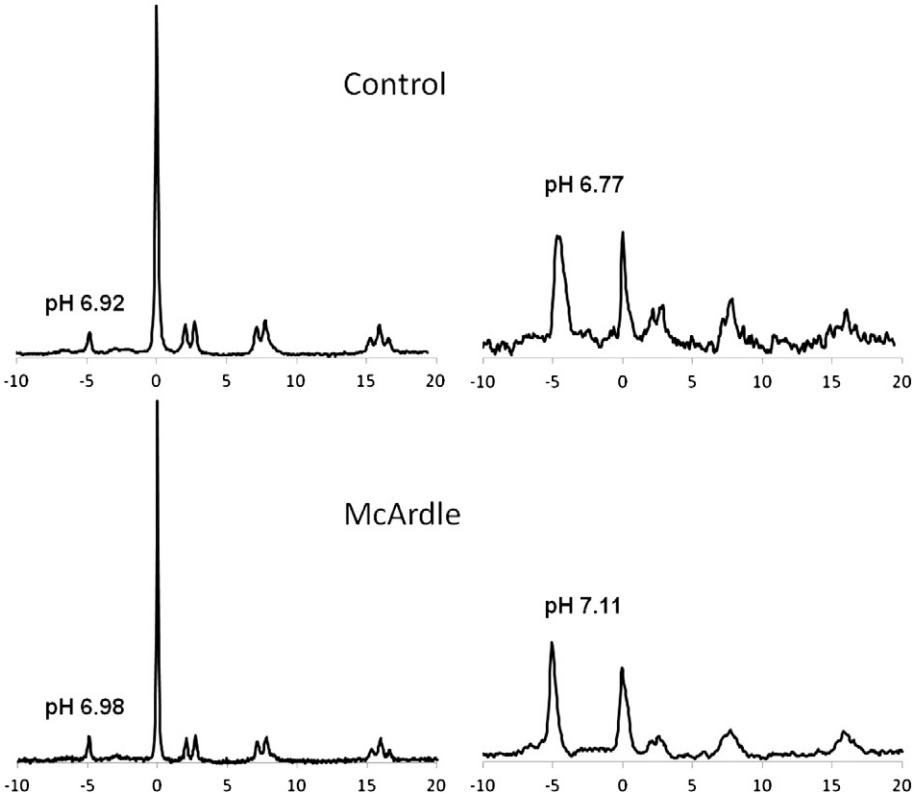

Fig. 1. Calf muscle spectra. Fig. 1 shows rest and end-exercise ^{31}P-MRS spectra of calf muscle acquired in a control subject and McArdle patient reaching a similar PCr depletion. ^{31}P-MRS spectra of human skeletal muscle typically show the peaks corresponding to inorganic phosphate (Pi), phosphocreatine (PCr) and the three phosphate groups α, β, γ of ATP.

Table 2
End exercise values of McArdle patient compared to controls. Subjects were divided into three groups: Low [PCr], Medium [PCr] and High [PCr] according to the PCr concentration reached at the end of exercise. P-value shows result of group comparison using Student t-test.

		pMg_{end}	pH_{end}	PCr_{end} (mM)	$\Delta G'_{ATPend}$ (KJ/mol)	$[ADP]_{end}$ (μM)
Low [PCr]	McArdle (n = 6)	3.58 ± 0.11	7.13 ± 0.07	13.0 ± 0.6	−52.04 ± 0.25	573 ± 128
	Control (n = 12)	3.48 ± 0.10	6.77 ± 0.08	13.8 ± 0.7	−52.61 ± 0.33	258 ± 57
	P	N. S.	<0.001	N. S.	<0.001	<0.001
Medium [PCr]	McArdle (n = 5)	3.54 ± 0.08	7.08 ± 0.01	16.7 ± 0.9	−53.42 ± 0.34	335 ± 72
	Control (n = 8)	3.50 ± 0.09	6.81 ± 0.09	16.9 ± 1.0	−53.97 ± 0.31	187 ± 30
	P	N. S.	<0.001	N. S.	<0.01	<0.01
High [PCr]	McArdle (n = 5)	3.56 ± 0.07	7.07 ± 0.03	20.8 ± 2.1	−54.87 ± 0.81	231 ± 44
	Control (n = 8)	3.47 ± 0.05	6.89 ± 0.05	20.5 ± 1.9	−55.05 ± 0.72	151 ± 31
	P	N. S.	<0.001	N. S.	N. S.	<0.05

in Fig. 2 which shows that the lower $[PCr]_{end}$ the higher the difference of $\Delta G'_{ATPend}$ and $[ADP]_{end}$ between patients and matched controls.

We calculated the amount of PCr depleted during exercise ($[PCr]_{cons}$) both in L and M exercise protocols of each patient. Table 3 reports the comparison of $[PCr]_{cons}$, TC_{PCr} and Vi of PCr recovery obtained in L and M exercise protocols for each patient. In each patient, more PCr was consumed and the initial rate of recovery (Vi) was slower in the more intense exercise protocol, yielding a highly significant ($P<0.001$) group comparison. Notwithstanding the larger $[PCr]_{cons}$, TC_{PCr} recovery values were not significantly different. A typical example of this phenomenon is shown in Fig. 3. The two exponential recovery curves show almost overlapping TC_{PCr} values despite the different workload performed by the patient in the two exercise protocols as shown by the different amount of $[PCr]_{cons}$ at the end of the exercise (9.4 and 17.8 for L and M protocols respectively). We posed the question whether, in the absence of cytosolic acidosis, the rate of PCr recovery depends on the amount of PCr depleted during exercise. Therefore for all 16 patient data sets we correlated the time constants of PCr recovery with the respective $[PCr]_{cons}$ obtained in exercise protocols L and M. TC_{PCr} of PCr recovery did not show any statistically significant correlation with the $[PCr]_{cons}$ ($r = 0.36$; $p>0.05$).

4. Discussion

In this study we assessed the cytosolic [ADP] and $\Delta G'_{ATP}$ in different metabolic conditions in the calf muscle of a group of patients affected by glycogen myo-phosphorylase deficiency (McArdle disease) to ascertain how and to what extent the muscle energy balance is affected by the deficit of the glycogenolytic pathway. The lack of intracellular acidosis of McArdle patients also allowed us to investigate the relationship between the amount of PCr consumed (PCr_{cons}) during exercise and the rate of PCr recovery in the absence of the effect of pH [7].

McArdle patient group showed at rest a higher pH and a lower pMg compared to controls, corresponding to a lower cytosolic $[H^+]$ and a higher $[Mg^{2+}]$ as already found in a previous study [40]. We assessed [PCr] taking into account the estimated [ATP] reduction in 3 out of 8 patients of at least 12% as explained in the Methods section. Nevertheless, at rest [PCr] was still higher in patients group than controls. This result, which may be explained as an adaptive response to metabolic deficit, is the direct cause of a statistically significant lower $\Delta G'_{ATP}$ found at rest in patient group compared to controls. In fact, despite the different pH and pMg, using in the patients group a value of resting [PCr] equal to control group, we obtained $\Delta G'_{ATP}$ and [ADP] values of −59.27 kJ/mol and 69.58 μM respectively that are even higher than control values reported in Table 1. As a consequence the value of $\Delta G'_{ATP}$ of patients group did not show a statistically significant difference.

All patients displayed a lack of cytosolic acidosis at the end of exercise as a consequence of a complete glycogen phosphorylase deficiency, reaching a pH that was always slightly higher than rest, irrespective of the PCr consumed during exercise (Table 2).

As expected, for any given patient the degree of PCr depletion at the end of exercise depended on the degree of exercise intensity. A striking results was that both [ADP] and $\Delta G'_{ATP}$ of patients were significantly higher than those of matched control groups reaching comparable levels of PCr concentration at the end of exercise. These results show an imbalance of the thermodynamic status of patient muscle related to the amount of PCr consumed during exercise. At low and medium $[PCr]_{end}$ both $[ADP]_{end}$ and $\Delta G'_{ATPend}$ values were significantly higher than controls while at high $[PCr]_{end}$ only $[ADP]_{end}$ showed a value higher than controls. We interpret this finding as direct consequence of the lack of intracellular acidosis occurring in

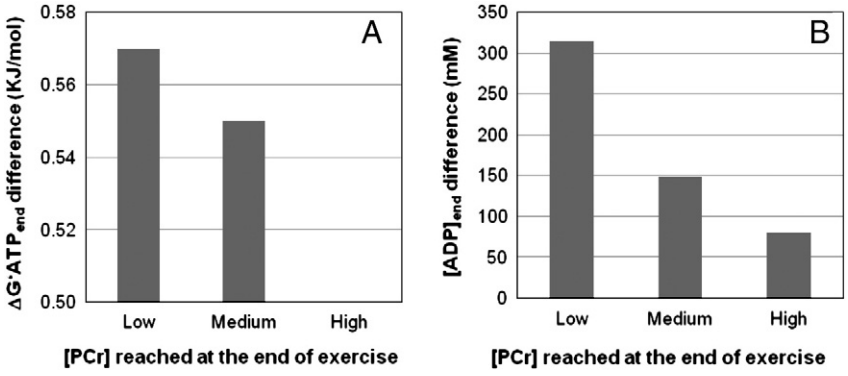

Fig. 2. Plot of $\Delta G'_{ATPend}$ (A) and $[ADP]_{end}$ (B) difference between patients and controls. Fig. 2 shows $\Delta G'_{ATPend}$ (A) and $[ADP]_{end}$ (B) difference between McArdle patients and control subjects assessed at the end of exercises. Patients and controls were divided in three different groups according to the level of PCr concentration reached at the end of exercise. In panel A the value for the High [PCr] group is assumed to be zero because the difference in $\Delta G'_{ATPend}$ between McArdle patients and matched controls was not statistically significant (see Table 2).

Table 3
Paired t-test of $[PCr]_{cons}$, TC_{PCr} and Vi of PCr recovery obtained in L and M exercise protocols for each McArdle patient. P-value shows result of comparisons across protocol using pairwise Student t-test.

Patient	L $[PCr]_{cons}$ (mM)	M $[PCr]_{cons}$ (mM)	L TC_{Pcr} (s)	M TC_{Pcr} (s)	L Vi (mM/s)	M Vi (mM/s)
1	16.7	19.3	91	76	0.184	0.250
2	9.4	17.8	48	53	0.196	0.336
3	15.8	21.9	88	79	0.179	0.278
4	11.0	21.9	72	74	0.153	0.296
5	14.4	22.0	57	68	0.253	0.324
6	16.0	20.5	64	63	0.250	0.325
7	18.7	26.1	74	75	0.253	0.348
8	15.2	21.8	73	80	0.209	0.273
P	<0.001		N.S.		<0.001	

these patients. The influence of pH on both [ADP] concentration and $\Delta G'_{ATP}$ has been postulated on the basis of theoretical calculation, indicating that a decreasing pH, occurring during muscle exercise, tends to counteract the [ADP] and $\Delta G'_{ATP}$ increase due to [PCr] depletion [2]. This study represents the *in vivo* confirmation that the intracellular acidification occurring in exercising skeletal muscle is *per se* a protective factor for the energy consumption. It is interesting to note that the effect of the lack of pH decrease is more evident at lower end exercise [PCr], showing that when the energy demand is higher the lack of protective effect of a low pH is more disruptive and the thermodynamic imbalance is further increased (Fig. 2). It must be underlined that the value of $\Delta G'_{ATP}$ also depends on $[Mg^{2+}]$ whose functional relationship with the energy metabolism is well known [14,24,27]. Therefore, we compared the $\Delta G'_{ATP}$ in groups of patients and controls reaching comparable level of PCr consumption also measuring muscle cytosolic $[Mg^{2+}]$. Different values of $\Delta G'_{ATP}$ mean different amount of work that can be extracted from the chemical reaction of ATP hydrolysis. This concept pertains to biochemical thermodynamics and not to muscle contractility or mechanical performance which are governed by the kinetic of ATP hydrolysis.

Since the discovery that the rate of PCr recovery is linked to the extent of cytosolic acidosis [6,7], the inverse correlation between pH and rate of PCr recovery has been used as an invariance criterion to assess the functionality of muscle mitochondrial respiration in health and disease (see Ref. [1] for a review). The relationship between the rate of PCr recovery and pH has been studied correlating the time constant of the monoexponential fit of PCr recovery (TC_{PCr}) and the minimum pH measured during recovery [7]. The TC_{PCr} represents the time when about 67% of the total PCr has been re-synthesized. In healthy subjects minimum pH is always reached between 30 and 90 s [7]. The TC_{PCr} values measured in the control subjects ranged between 20 and 60 s [7]. This means that at the time of the minimum pH the PCr has been almost completely re-synthesized.

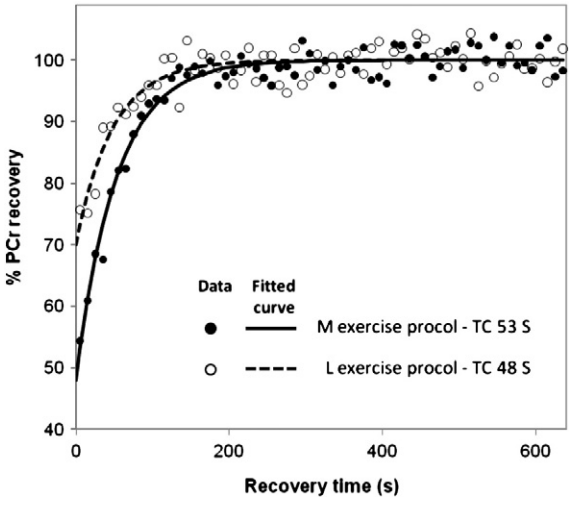

Fig. 3. Patterns of PCr recovery for different exercise intensities. Plot of PCr recovery, expressed as percentage of [PCr] rest values, for a single patient performing L and M exercise protocols. The patient showed a similar time constant of PCr recovery despite reaching a different level of PCr depletion: $[PCr]_{cons}$ of 9.4 and 17.8 mM in L and M protocols respectively.

Nevertheless, the biochemical significance of this relationship has not been completely clarified. Several studies posed question whether pH is a true independent variable in the assessment of the rate of PCr recovery [1,6,7,12,29,30], in that the pH change and the amount of PCr consumed during exercise (PCr_{cons}) appear to be mutually inter-dependent.

Our results showed that the rate of PCr recovery is not influenced by the amount of [PCr] reached at the end of exercise for a given pH value. Looking at the reaction of PCr resynthesis from a chemical kinetics perspective both [PCr] and $[H^+]$ could influence the rate of reaction being the products of the chemical reaction. The results give experimental evidence that $[H^+]$ rather than [PCr] influence the kinetic of PCr resynthesis (according to the theory of chemical kinetics it could be a sort of first order kinetic). A recently published non-linear mathematical model shows that the relationship between pH and PCr recovery can be explained on the basis of enzyme kinetic mechanisms [31].

In particular according to this model, since H^+ ions are produced inside the MCK, their activity inside MCK during PCr recovery is higher than at rest. For this reason, when the pH of the cytoplasmic medium is low, the release of H^+ and PCr is less favored than at neutral pH, where a larger difference in proton activity occurs. This provides the rationalization at molecular level of the known inverse correlation between pH and PCr recovery rate so that when pH decreases the TC_{PCr} increases [5] and is consistent with the result of the present study.

Another way to assess PCr recovery is to use the initial rate of recovery, Vi [29,38]. In our study we found that Vi is affected by the different amount of PCr consumed during exercise. However, this is not a surprising result, as by definition Vi is function of PCr_{cons}. Nevertheless, since we also showed that TC_{PCr} is independent from PCr_{cons}, we conclude that the use of Vi is appropriate to evaluate PCr recovery rate of a cohort of subjects when their end exercise pH values are similar, while in the presence of pH variation the use of TC_{PCr} is preferable.

5. Conclusions

Two main conclusions can be drawn from this study:

a) The intracellular acidification occurring in exercising skeletal muscle reduces energy consumption hence increasing the thermodynamic efficiency of ATP hydrolysis. This effect is more pronounced when energy demand is higher.
b) The time constant of the mono-exponential equation best-fitting PCr recovery (TC_{PCr}) depends on cytosolic pH and it is not influenced by the amount of PCr consumed during exercise. This support the hypothesis that the influence of pH on the PCr recovery rate is at least in part related to the kinetic mechanisms of mitochondrial creatine kinase enzyme.

Acknowledgments

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References

[1] B. Chance, J. Im, S. Nioka, M. Kushmerick, Skeletal muscle energetics with PNMR: personal views and historic perspectives, NMR Biomed. 19 (2006) 904–926 S.

[2] S. Iotti, C. Frassineti, A. Sabatini, A. Vacca, B. Barbiroli, Quantitative mathematical expressions for accurate in vivo assessment of cytosolic [ADP] and ΔG of ATP hydrolysis in the human brain and skeletal muscle, Biochim. Biophys. Acta-Bioenerg. 1708 (2005) 164–177.
[3] S.J. Harkema, R.A. Meyer, Effect of acidosis on control of respiration in skeletal muscle, Am. J. Physiol. 272 (1997) 491–500.
[4] D.L. Arnold, P.M. Matthews, G.K. Radda, Metabolic recovery after exercise and the assessment of mitochondrial function in vivo in human skeletal muscle by means of ^{31}P NMR, Magn. Reson. Med. 1 (1984) 307–315.
[5] B. Chance, J.S. Leigh Jr., J. Kent, K.K. McCully, S. Nioka, B.J. Clark, J.M. Maris, T. Graham, Multiple controls of oxidative metabolism in living tissues as studied by phosphorus magnetic resonance, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 9458–9462.
[6] D. Bendahan, S. Confort-Gouny, G. Kozak-Reiss, P.J. Cozzone, Heterogeneity of metabolic response to muscular exercise in humans, new criteria of invariance defined by in vivo phosphorus-31 NMR spectroscopy, FEBS Lett. 272 (1990) 155–158.
[7] S. Iotti, R. Lodi, C. Frassineti, P. Zaniol, B. Barbiroli, In vivo assessment of mitochondrial functionality in human gastrocnemius muscle by ^{31}P MRS. The role of pH in the evaluation of phosphocreatine and inorganic phosphate recoveries from exercise, NMR Biomed. 6 (1993) 248–253.
[8] B. Chance, S. Eleff, J.S. Leigh Jr., D. Sokolow, A. Sapega, Mitochondrial regulation of phosphocreatine/inorganic phosphate ratios in exercising human muscle: a gated ^{31}P NMR study, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 6714–6718.
[9] S.M. Eleff, P.B. Barker, S.J. Blackband, J.C. Chatham, N.W. Lutz, D.R. Johns, R.N. Bryan, O. Hurko, Phosphorus magnetic resonance spectroscopy of patients with mitochondrial cytopathies demonstrates decreased levels of brain phosphocreatine, Ann. Neurol. 27 (1990) 626–630.
[10] G.J. Kemp, G.K. Radda, Quantitative interpretation of bioenergetic data from 31P and 1H magnetic resonance spectroscopic studies of skeletal muscle: an analytical review, Magn. Reson. Q. 10 (1994) 43–63.
[11] G.J. Kemp, A.L. Sanderson, C.H. Thompson, G.K. Radda, Regulation of oxidative and glycogenolytic ATP synthesis in exercising rat skeletal muscle studied by ^{31}P magnetic resonance spectroscopy, NMR Biomed. 9 (1996) 261–270.
[12] R. Lodi, G.J. Kemp, S. Iotti, G.K. Radda, B. Barbiroli, Influence of cytosolic pH on *in vivo* assessment of human muscle mitochondrial respiration by phosphorus magnetic resonance spectroscopy, MAGMA 5 (1997) 165–171.
[13] S. Bluml, E. Zuckerman, J. Tan, B.D. Ross, Proton-decoupled 31P magnetic resonance spectroscopy reveals osmotic and metabolic disturbances in human hepatic encephalopathy, J. Neurochem. 71 (1998) 1564–1576.
[14] B. Barbiroli, S. Iotti, P. Cortelli, P. Martinelli, R. Lodi, V. Carelli, P. Montagna, Low brain intracellular free magnesium in mitochondrial cytopathies, J. Cereb. Blood Flow Metab. 19 (1999) 528–532.
[15] S.A. Kuby, E.A. Noltman, ATP-creatine transphosphorylase, in: P.D. Boyer (Ed.), The enzymes, 2nd ed., Academic Press, New York, 1959, pp. 515–603.
[16] J.C. Skou, The sodium–potassium ATPase: coupling of the reaction with ATP to the reaction with sodium ion and potassium ion, Ann. N. Y. Acad. Sci. 402 (1982) 169–184.
[17] K.R.H. Repke, On the mechanism of energy release, transfer, and utilization in sodium-potassium ATPase transport work: old ideas and new findings, Ann. N. Y. Acad. Sci. 402 (1982) 272–286.
[18] F. Ramirez, J.F. Marecek, Coordination of magnesium with adenosine 5′-diphosphate and triphosphate, Biochim. Biophys. Acta 589 (1980) 21–29.
[19] J.A. Wells, C. Knoeber, M.C. Sheldon, M.M. Werber, R.G. Yount, Cross-linking of myosin subfragment 1. Nucleotide-enhanced modification by a variety of bifunctional reagents, J. Biol. Chem. 255 (1980) 11135–11140.
[20] K. Roth, M.W. Weiner, Determination of cytosolic ADP and AMP concentrations and the free energy of ATP hydrolysis in human muscle and brain tissues with phosphorus-31 NMR spectroscopy, Magn. Reson. Med. 22 (1991) 505–511.
[21] R.A. Alberty, Recommendation for nomenclature and tables in biochemical thermodynamics, Pure Appl. Chem. 66 (1994) 1641–1666.
[22] R.A. Alberty, IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (JCBN). Recommendations for nomenclature and tables in biochemical thermodynamics. Recommendations 1994, Eur. J. Biochem. 240 (1996) 1–14 k.
[23] R.A. Alberty, Thermodynamics of biochemical reactions, John Wiley and Sons, New York, 2003.
[24] R.W.J. Lawson, R.L. Veech, Effects of pH and free magnesium ion on the Keq of the creatine kinase reaction and other phosphate hydrolyses and phosphate transfer reactions, J. Biol. Chem. 254 (1979) 6528–6537.
[25] R.L. Veech, R.W.J. Lawson, N.W. Cornell, H.A. Krebs, Cytosolic phosphorilation potential, J. Biol. Chem. 254 (1979) 6538–6547.
[26] S. Iotti, A. Sabatini, A. Vacca, Chemical and biochemical thermodynamics: from ATP hydrolysis to a general reassessment, J. Phys. Chem. B 114 (2010) 1985–1993.
[27] S. Iotti, C. Frassineti, L. Alderighi, A. Sabatini, A. Vacca, B. Barbiroli, In vivo ^{31}P-MRS assessment of cytosolic [Mg^{2+}] in the human skeletal muscle in different metabolic condition, Mag Reson Imaging 18 (2000) 607–614.
[28] T. Wallimann, M. Wyss, D. Brdiczka, K. Nicolay, H.M. Eppenberger, Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis, Biochem. J. 281 (1992) 21–40 Review.
[29] J.P. Mattei, G. Kozak-Ribbens, M. Roussel, Y. Le Fur, P.J. Cozzone, D. Bendahan, New parameters reducing the interindividual variability of metabolic changes during muscle contraction in humans. A ^{31}P MRS study with physiological and clinical implications, Biochim. Biophys. Acta 1554 (2002) 129–136.
[30] S. Iotti, G. Gottardi, V. Clementi, B. Barbiroli, The monoexponential pattern of phosphocreatine recovery after muscle exercise is a particular case of a more complex behaviour, Biochim. Biophys. Acta-Bioenerg. 1608 (2004) 131–139.
[31] S. Iotti, M. Borsari, D. Bendahan, Oscillation in energy metabolism (Review), Biochim. Biophys. Acta-Bioenerg. 1797 (2010) 1353–1361.
[32] J. Zange, M. Beisteiner, K. Müller, V. Shushakov, N. Maassen, Energy metabolism in intensively exercising calf muscle under a simulated orthostasis, Pflugers Arch. 6 (2008) 1153–1163.
[33] K.K. McCully, S. Iotti, K. Kendrick, Z. Wang, J.D. Posner, J. Leigh Jr., B. Chance, Simultaneous in vivo measurements of HbO2 saturation and PCr kinetics after exercise in normal humans, J. Appl. Physiol. 1 (1994) 5–10.
[34] K. Sahlin, N.H. Areskog, R.G. Haller, K.G. Henriksson, L. Jorfeldt, S.F. Lewis, Impaired oxidative metabolism increases adenine nucleotide breakdown in McArdle's disease, J. Appl. Physiol. 69 (1990) 1231–1235.
[35] S.M. McConchie, J. Coakley, R.H.T. Edwards, R.J. Beynon, Molecular heterogeneity in McArdle's disease, Biochim. Biophys. Acta 1096 (1991) 26–32.
[36] H. Ratiney, M. Sdika, Y. Coenradie, S. Cavassila, D. van Ormondt, D. Graveron-Demilly, Time-domain semi-parametric estimation based on a metabolite basis set, NMR Biomed. 1 (2005) 1–13.
[37] R.C. Harris, E. Hultman, L.O. Nordesjö, Glycogen, glycolytic intermediates, and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values, Scand. J. Clin. Lab. Invest. 33 (1974) 109–120.
[38] D. Bendahan, G. Kozak-Ribbens, S. Confort-Gouny, B. Ghattas, D. Figarella-Branger, M. Aubert, P.J. Cozzone, A noninvasive investigation of muscle energetics supports similarities between exertional heat stroke and malignant hyperthermia, Anesth. Analg. 3 (2001) 683–689.
[39] J. Zange, T. Grehl, C. Disselhorst-Klug, G. Rau, K. Müller, R. Schröder, M. Tegenthoff, J.P. Malin, M. Vorgerd, Breakdown of adenine nucleotide pool in fatiguing skeletal muscle in McArdle's disease: a noninvasive ^{31}P-MRS and EMG study, Muscle Nerve 6 (2003) 728–736.
[40] E. Malucelli, R. Lodi, A. Martinuzzi, C. Tonon, B. Barbiroli, S. Iotti, Free Mg^{2+} concentration in the calf muscle of glycogen phosphorylase and phosphofructokinase deficiency patients assessed in different metabolic conditions by ^{31}P MRS, Dyn Med. 6 (2005) 4–7.

Glossary

^{31}P-MRS: Phosphorus Magnetic Resonance Spectroscopy
K'_{CK}: apparent equilibrium constant of the creatine kinase reaction
$\Delta G_{MgATP^{2-}}$: Gibbs energy change of $MgATP^{2-}$ hydrolysis
$\Delta G'_{ATP}$: transformed Gibbs energy change of ATP hydrolysis
pMg: $-\log [Mg^{2+}]$
MCK: mitochondrial creatine kinase enzyme
PCr: phosphocreatine
Cr: creatine
PCr_{cons}: PCr consumed during exercise
Pi: inorganic phosphate ($H_2PO_4^- + HPO_4^{2-}$)
TC_{PCr}: rate of PCr recovery
Vi: initial rate of PCr recovery