ΔG of ATP hydrolysis and cytosolic [ADP] assessed at the end of exercise by 31P-MRS in the calf muscle of patients with myophosphorylase deficiency

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Introduction.

Phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS) affords the possibility of assessing *in vivo* the energy status of living tissues. The main thermodynamic variables relevant for the knowledge of the health of living tissues are: ΔG of ATP hydrolysis, and cytosolic [ADP], the latter as calculated from the apparent equilibrium constant of the creatine kinase reaction (Kck) (1). We recently formulated novel quantitative mathematical expressions of ΔG of ATP hydrolysis, and of the Kck as a function of total [PCr], pH and pMg, all quantities directly measurable by *in vivo* ^{31}P MRS (2). The above mathematical expressions allow the *in vivo* assessment of cytosolic [ADP] and ΔG of ATP hydrolysis, calculated as ΔG $MgATP^{2-}$, which is the relevant form, in human brain and skeletal muscle, as taking into account pH and pMg changes occurring in physiological and pathological conditions. In this study we assessed the cytosolic [ADP] and ΔG $MgATP^{2-}$ in the calf muscle of a group of patients with glycogen myophosphorylase deficiency (McArdle disease) at the end of exercise to ascertain whether and to what extent the deficit of the glycogenolytic pathway would affect the muscle energy balance.

Methods.

We studied 6 male patients (age 38 ± 10), affected by glycogen McArdle disease, as detected by histochemical/biochemical analysis of muscle biopsy. Informed consent was obtained from each patient. Subjects were studied in a 1.5T GE Signa Horizon LX whole-body scanner. Subjects lay supine with a 6 cm surface coil centred on the maximum circumference of the right calf muscle. Muscle aerobic exercise consisted of repetition of plantar flexion at incremental intensity (10% lean body mass for 2 min, then increasing 5% LBM per minute). Spectra were acquired with a repetition time (TR) of 5 sec. One hundred-twenty-eight FIDs at rest, and 12 FIDs during exercise for each level of work were averaged. Spectra were post-processed by a time-domain fitting routine AMARES/MRUI (http://carbon.uab.es/mrui). The pMg and pH were assessed from the chemical shift of β-ATP and Pi from PCr respectively (3). The control values were from 9 sex and age matched healthy subjects (age 39 ± 12). The ΔG of ATP hydrolysis was calculated as ΔG $MgATP^{2-}$, as only the Gibbs free energy of hydrolysis of this ionic species of ATP is relevant in describing the intracellular energetic status of a tissue (2,4). Both ΔG $MgATP^{2-}$ and cytosolic [ADP] were calculated by the software package BMMG2 available at http://www.cermiv.unibo.it/ containing the equations developed by Iotti et al. (2).

Result and Discussion.

All six patients displayed a lack of cytosolic acidosis at the end of exercise as a consequence of a complete glycogen phosphorylase deficiency. At the end of exercise patients reached a PCr depletion above 60% of rest level achieving a PCr concentration comparable to that of control group, although exercise duration was longer in healthy subjects. Despite the same content of PCr at the end of exercise both the cytosolic [ADP] and ΔG $MgATP^{2-}$ of patients were significantly higher than those of control group. These results show an imbalance of the thermodynamic status of patients muscle at the end of exercise, irrespective of the same amount of PCr consumed during exercise. However, we ascribe this finding as direct consequence of the lack of intracellular acidosis occurring in these patients, and not the effect of the deficit of glycogen break down. The influence of pH on both [ADP] concentration and ΔG $MgATP^{2-}$ has been postulated on the basis of theoretical calculation (2), indicating that changes in both pH and free [Mg^{2+}] occurring during muscle exercise tend to counteract the [ADP] and ΔG $MgATP^{2-}$ increase due to [PCr] depletion. This study represents the *in vivo* confirmation that the intracellular acidification due to lactate production occurring in exercising skeletal muscle is *per se* a protective factor for the energy consumption

Table 1. Values of [PCr], pH, pMg, ΔG $MgATP^{2-}$ and [ADP] assessed at the end of exercise in 6 patients affected by glycogen myophosphorylase (Mc Ardle) compered to controls.

	$[PCr]_{end}$ (mM)	pH_{end}	pMg_{end}	ΔG_{end} (kJ/mol)	$[ADP]_{end}$ (μM)
Mc Ardle (*n* = 6)	9.6 ± 2.4	7.09 ± 0.04	3.54 ± 0.09	-50.59 ± 1.14	851 ± 273
Control (*n* = 9)	10.3 ± 1.6	6.59 ± 0.16	3.50 ± 0.09	-52.09 ± 0.69	256 ± 79
t-test	N.S.	< 0.001	N.S.	< 0.01	< 0.001

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