CYTOSOLIC FREE $[Mg^{2+}]$ IN THE HUMAN CALF MUSCLE IN DIFFERENT METABOLIC CONDITIONS: *IN VIVO* ^{31}P MRS AND COMPUTER SIMULATION

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INTRODUCTION Skeletal muscles contain approximately 35% of total human body magnesium, a divalent cation acting as an essential cofactor in a number of cell processes. Magnesium ions influence the equilibria of many reactions involved in cellular bioenergetics by interacting with phosphorylated molecules (1) and interfere with the kinetics of ion transport across plasma membranes. In particular, Mg^{2+} is known to regulate Ca^{2+} traffic in smooth (2) and skeletal (3) muscle cells by acting as a blocker of Ca^{2+} channels. There is also considerable evidence that Mg^{2+} is actively transported and regulated, although the mechanisms are largely unknown (4). All this implies that accurate knowledge of intracellular magnesium concentration ($[Mg^{2+}]$) is crucial for a deeper understanding of both cellular bioenergetics and reaction kinetics *in vivo*, and that any changes in cellular $[Mg^{2+}]$ may alter critical regulatory mechanisms causing abnormal metabolism.

In the skeletal muscle variations of cytosolic pH, phosphocreatine (PCr) and inorganic phosphate (Pi) concentrations influence the complex multi-equilibrium system of the molecular species which bind magnesium ions. As a consequence free cytosolic $[Mg^{2+}]$ can change considerably in different metabolic conditions such as rest, exercise and recovery.

In this work we both assessed by ^{31}P MRS and calculated by computer simulation the cytosolic free $[Mg^{2+}]$ in the human calf muscle in different metabolic conditions, and compared the patterns.

METHODS Forty-two healthy controls (18 women and 24 men) aged 14-67 years (30 ± 15; mean ± SD) volunteered for this study. Informed consent was obtained in all cases.

^{31}P-MRS. MR spectra were acquired by a G.E. 1.5 T Signa System and a surface coil supplied by G.E. The repetition time (TR) of the sequence was 5 sec.

All studies were performed on calf muscle by placing the surface coil directly on the skin. Sixty transients were accumulated during rest (5 min). During exercise, data were collected for one min (12 FIDs) for each level of work. During recovery 2-FID data blocks (10 s) were recorded for 60 s, while longer time blocks were collected during the following 4 min. The accumulated spectra were processed using a 4 Hz line broadening and manual phasing.

Free cytosolic $[Mg^{2+}]$ was assessed from the chemical shift of β-ATP from PCr. We used an equation specifically developed for the human muscle (5) relying on a chemical model of a multi-equilibrium system of 25 species (6).

Computer simulation was performed, by HYSS (HYperquad Simulation and Speciation) a recently developed software package which supersedes HYPHEN in the HYPERQUAD suite of programs (7).

RESULTS AND DISCUSSION In 42 healthy subjects we found by ^{31}P-MRS a mean resting cytosolic free $[Mg^{2+}]$ of 0.32 ± 0.030 (mM). This value was used in the computer simulation to calculate the *in vivo* total amount of magnesium in the muscle cells, which resulted 7 mM.

The *In vivo* assessment showed that during exercise and recovery cytosolic free $[Mg^{2+}]$ remarkably changed, being influenced by the variations of [Pi], [PCr] and pH. The changes in cytosolic free $[Mg^{2+}]$ during exercise and recovery were mainly the result of the predominant effect of pH. The plot of all values of cytosolic free $[Mg^{2+}]$, measured during exercise and recovery in all subjects, as a function of pH showed an exponential pattern with a sharp increase of $[Mg^{2+}]$ below pH 6.5 (fig. 1A). Simulation by HYSS of the same changes of [PCr], [Pi] and pH found *in vivo* during exercise and recovery, with the assumption of 7mM total $[Mg^{2+}]$, showed a much smaller increase of free $[Mg^{2+}]$ in the pH region below 6.4 (fig. 1B).

Our results show:

i) our model well represents the *in vivo* biochemical conditions of muscle cells under different metabolic activation in the pH region 6.5-7.0;

ii) the existence of more Mg-binding sites releasing Mg at low pH, than those taken into account by our chemical model.

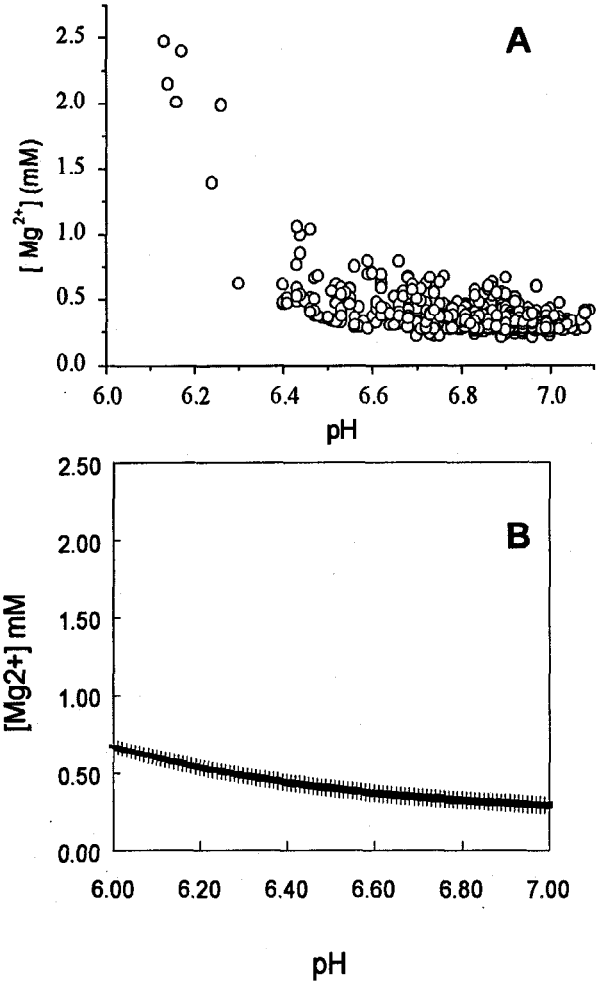

Figure 1. Patterns of free cytosolic $[Mg^{2+}]$ in human calf muscle during exercise and recovery reported as a function of cytosolic pH; A: assessed *in vivo* by ^{31}P MRS; B: calculated by computer simulation.

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