The heterogeneity of intramuscular metabolism using simultaneous ^{31}P 2D-CSI and pulmonary oxygen uptake (VO_2) during incremental knee-extensor exercise in humans

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Introduction

The mechanisms limiting tolerance in high-intensity exercise are poorly understood. Hypotheses range from O_2 delivery/diffusion limitation, depletion of local energy stores (e.g. intramuscular PCr, glycogen), build-up of fatigue-inducing metabolites (e.g. Pi, H^+) and neural limitation of the recruited muscle mass. During ramp-incremental exercise pulmonary O_2 uptake (VO_2) responds as a linear function of work rate (WR), following a short delay, to attain its peak value at the limit of tolerance. This response is often considered to be consistent with a system of uniform metabolic characteristics. However, as muscle fibers of different types (e.g. type I or II) express different metabolic responses during exercise (Crow & Kushmerick, 1982; Meyer et al., 1982), we were interested in how the discrete responses of the recruited muscles are integrated into a linear metabolic response during ramp-incremental exercise.

Methods

We used 2D chemical shift imaging (2D CSI; similar to Jeneson et al., 1992 and Nelson et al., 1991) and quadriceps knee-extensor exercise to determine the dynamics of [PCr], [Pi], [ADP], pHi and VO_2 in subjects (n=6) lying prone in the bore of a 1.5T whole-body MR system (*Signa Advantage*, G.E., USA). Each subject performed repeated ramp-incremental exercise to the limit of tolerance using a computer-controlled knee-extensor ergometer (Lode, NL). Protocol: 4 min at rest, 4 min at 5W, incremental-WR at 1W/20s or 1W/30s (designed to limit the subject in ~12 min). ^{31}P spectra were obtained from an 8" transmit–5" receive surface coil under the right quadriceps, synchronous with the recovery phase of each contraction. FID's were acquired from an 8cm axial slice every 2s and averaged to estimate ^{31}P metabolites every 16s. VO_2 was measured breath-by-breath using a custom-designed non-magnetic turbine and a remote mass spectrometer (Whipp et al., 1999). On a separate day the protocol was repeated for spatial determination of ^{31}P changes using 2D CSI of an 8cm thick slice, with 16x16 phase encode steps over a 48cm field of view, 1 average and TR of 1s to obtain ^{31}P metabolite maps every 4 min. Post-processing of the CSI data using SAGEIDL version dev2000.3 allowed voxel shifting to determine localized ^{31}P spectra (Fig.1). Analysis was in jMRUI (Naressi et al., 2001) assuming [ATP] of 8.2 mM. Metabolite changes in each voxel were referenced to their initial resting values. Significance was at $P<0.05$ using ANOVA.

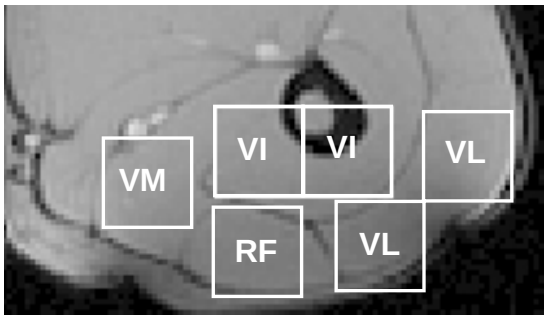

Fig.1 – Example of 2D CSI voxel-shifts for the *m.quadriceps femoris* (RF: *rectus femoris*; VL: *vastus lateralis*; VI: *vastus intermedius*; VM *vastus medialis*). The maximum number of unique voxels were obtained from each muscle.

Results

Peak VO_2 averaged 1.98 (0.3, ±SD) l/min at a WR of 42 (9) W (reproducible to ~3W). ^{31}P measures at rest were similar ($P>0.05$) between the pulse-acquire and CSI tests (CSI mean: [PCr] 29.1 (3.2) mM; [Pi] 3.8 (1.1) mM; [ADP] 13.2 (5.7) μM; pHi: 7.06 (0.01)). At end-exercise, [PCr] and pHi fell to 19.9 (4.1) mM and 6.91 (0.08) while [Pi] and [ADP] rose to 19.3 (5.0) mM and 26.4 (8.5) μM, respectively. Both the summed CSI voxels and pulse-acquire data demonstrated similar linear responses of ^{31}P metabolites. However, this was achieved with a surprisingly wide variability of metabolic responses among the muscles (Table 1; $P<0.05$). In all subjects, the greatest contribution to the energetics was from the RF muscle (end-exercise: [PCr] 14.4 (7.8) mM; range 7.75-20.30 mM), while the other muscles of the *vastus* contributed to a variable degree, both within and among subjects: the range within quadriceps muscles averaging ~±40% of the mean for all subjects (Table 1). Similarly, [Pi] and pHi varied widely, extreme values at peak exercise averaging 22.2 (9.4) mM and 6.53 (0.15) respectively.

Table 1 - Heterogeneity of resting and end-exercise quadriceps [PCr] (mM) and pHi

	RF	VM	VI	VL
[PCr] rest	30.4 (5)	30.9 (4)	27.8 (4)	27.1 (4)
[PCr] end exercise	14.4 (8)	25.7 (5)	17.7 (3)	23.8 (4)
pHi rest	7.06 (0.02)	7.06 (0.02)	7.06 (0.04)	7.08 (0.04)
pHi end exercise	6.80 (0.22)	7.03 (0.07)	7.01 (0.04)	6.99 (0.11)

Discussion

While the VO_2, [PCr], [Pi] and [ADP] responses to ramp-incremental exercise were essentially linear, following a short delay, the contributions to the total from the involved quadriceps muscles were highly variable. Heterogeneities were evident both among subjects and within muscles (evidenced from intra-voxel Pi-peak splitting). At end-exercise, neither the quadriceps as a whole nor any of the individual muscles showed complete [PCr] depletion, suggesting that peak VO_2 was achieved with a muscle energy store still remaining. Furthermore, during knee-extensor exercise, the limit of tolerance is likely to be achieved with both a muscle-recruitment and cardiac output (i.e. O_2 delivery) reserve, suggesting that aerobic exercise intolerance results from the regional build-up of fatigue-inducing metabolites. Intramuscular [Pi], a putative fatigue-inducing metabolite, reached levels as high as ~37 mM with pHi as low as ~6.3, but only in a small proportion of the exercising muscle. The net linear metabolic (pHi, [PCr], [Pi] and [ADP]) and VO_2 responses are thus an aggregate of a marked heterogeneity of regional muscle metabolic responses (ranging from ~-50% to 70% of the global average). However, the processes eliciting peak VO_2 and limiting tolerance in this form of exercise are likely to be determined within only some 15% or less of the recruited muscle mass.

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References: Crow & Kushmerick, J. Gen Physiol. 79:147, 1982; Meyer et al., AJP 242:C1, 1982; Nelson et al., NMR Biomed, 4:268, 1991; Jeneson et al., AJP 263:C357, 1992; Whipp et al., JAP 86:742, 1999; Naressi et al., MAGMA 12:141, 2001.