The following is the abstract of the article discussed in the subsequent letter:

Smith SA, Montain SJ, Zientara GP, and Fielding RA. Use of phosphocreatine kinetics to determine the influence of creatine on muscle mitochondrial respiration: an in vivo 31P-MRS study of oral creatine ingestion. J Appl Physiol 96: 2288–2292, 2004.—Recent human isolated muscle fiber studies suggest that phosphocreatine (PCr) and creatine (Cr) concentrations play a role in the regulation of mitochondrial respiration rate. To determine whether similar regulatory mechanisms are present in vivo, this study examined the relationship between skeletal muscle mitochondrial respiration rate and end-exercise PCr, Cr, PCr-to-Cr ratio (PCr/Cr), ADP, and pH by using 31P-magnetic resonance spectroscopy in 16 men and women (36.9 ± 4.6 yr). The initial PCr resynthesis rate and time constant (Tc) were not strongly related to PCr, Cr, PCr/Cr, and ADP (r < 0.80, P < 0.001), a moderate relationship with end-exercise ADP (r = 0.77, P < 0.001), and no relationship with end-exercise pH (r = -0.14, P = 0.34). The PCr Tc was not as strongly related to PCr, Cr, PCr/Cr, and ADP (r < 0.77, P < 0.001–0.18) and was significantly influenced by end-exercise pH (r = -0.43, P < 0.01). These findings suggest that end-exercise PCr and Cr should be taken into consideration when PCr recovery kinetics are used as indicators of mitochondrial respiration and that the initial PCr resynthesis rate is a more reliable indicator of mitochondrial respiration compared with PCr Tc.

Mitochondrial respiration in creatine-loaded muscle: is there 31P-MRS evidence of direct effects of phosphocreatine and creatine in vivo?

To the Editor: Smith et al. (7) argue that 31P-magnetic resonance spectroscopy (MRS) studies of phosphocreatine (PCr) resynthesis rate (V) vs. [ADP] (brackets denote concentration) is unaffected by Cr loading (7), their data are compatible with a single initial PCr resynthesis rate (V)-vs.-[ADP] relationship (solid line, Fig. 1A), arguably the dominant mechanism matching mitochondrial ATP production to demand in exercising muscle (5).

Relationships of V vs. other concentrations can be seen as epiphenomena, although the concordance between maximum rates inferred from V vs. ∆[PCr] (3) and invasive physiology is interesting (Fig. 1D). Furthermore, the regression fits Smith et al. (7) give for V vs. [ADP], ∆[PCr] (Fig. 1, A and D), PCr/Cr, and [Cr] (not shown) cover a limited range, excluding rest, and have no generalizable significance.

Simulation (Fig. 1B) suggests that direct effects of PCr/Cr on mitochondrial K_m for ADP (8) might explain the sigmoidicity of V vs. [ADP] in vivo (5) across the full dynamic range. However, Cr loading reduces resting PCr-to-total Cr ratio by 7% (mean of 7 biopsy studies of which Ref. 2 is typical), implying increased [ADP] and thus a different V-[ADP] relationship (dashed line, Fig. 1A). Effects of PCr/Cr on K_m for ADP (8) cannot explain this, being independent of total Cr concentration. Effects of [PCr] on K_m (8) might contribute, although the simulated fit (Fig. 1C) is imperfect.

Because V is never maximal in MRS experiments, extrapolation is required to estimate “mitochondrial capacity” (3, 6). The physiology is debated, but these results (7) do not prove that PCr and Cr must be allowed for as well as ADP.
CONTROL OF RESPIRATION IN CREATINE-LOADED MUSCLE

REFERENCES


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REPLY

To the Editor: We appreciate the comments regarding our 2004 publication (2) and agree, in part, with Dr. Kemp’s statement that ADP accumulation is the primary mechanism responsible for increasing mitochondrial ATP production, particularly at the onset of skeletal muscle exercise. However, during exhaustive exercise the ADP activation of mitochondrial respiration approaches maximum and the relationship between [ADP] (brackets denote concentration) and mitochondrial respiration appears to decline. This is clearly observed in the dispersion of our ADP results (Ref. 2, Fig. 2D) where [ADP] and mitochondrial respiration are significantly related during brief exercise (r = 0.61, P = 0.001) and not during exhaustive exercise (r = 0.36, P = 0.07). Our results (Ref. 2, Fig. 2, A–C) show that phosphocreatine (PCr)-to-creatine (Cr) ratio, [PCr], and [Cr] are significantly related to mitochondrial respiration during exhaustive exercise, suggesting that PCr and Cr may modulate ADP activated mitochondrial respiration via direct or indirect mechanisms as discussed.

Dr. Kemp suggests that [ADP] may rise with Cr ingestion and that this may account for the increase in mitochondrial respiration rendering our [PCr] and [Cr]-vs.-mitochondrial respiration relationships epiphenomenal. Muscle biopsy results indicate that creatine ingestion does not change [ADP] during rest, exercise, or recovery (3). Because creatine ingestion may increase the Cr-to-PCr ratio and the calculated ADP, it could be argued that the blunted resynthesis rate we observed with increasing ADP is actually artifact caused by overestimation of ADP. However, if our ADP data are adjusted for possible influences of Cr ingestion, the same relationship persists. Furthermore, if the Cr-supplemented data are removed from the regression analyses, thereby eliminating the ADP issue, the same relationships exist between mitochondrial respiration and our metabolic variables.

Dr. Kemp used our mean values from rest, brief exercise, and exhaustive exercise to support his argument that ADP alone is the primary mechanism controlling mitochondrial respiration (Fig. 1A in Letter to the Editor). It is concerning that mean data and only three actual data points were used to determine a curve fit. Additionally, the data in Dr. Kemp’s Fig. 1A do not appear to agree with our published figure. Figure 1A herein presents the actual association between our mean [ADP] results and the fit we originally presented as well as the [ADP] curve fit from previous work by Dr. Kemp (Ref. 1, Fig. 2A). The remarkably similar curvilinear fit from these independent experiments suggests that our metabolic relationships are, in fact, generalizable. Although we cannot totally discount that our PCr and Cr relationships may in part be epiphenomenal, our PCr-to-Cr ratio vs. respiration rate relationship derived from in vivo magnetic resonance spectroscopy (MRS) agrees with the results from human isolated muscle fiber experiments in

Fig. 1. A: exponential fit for initial phosphocreatine (PCr) resynthesis rate (PCr rate) and [ADP] (brackets denote concentration) from Smith et al. (2) on left axis and Kemp et al. (1) on right axis and the [ADP] mean and SE for placebo (solid symbols) and creatine (open symbols) conditions and brief (triangles) and exhaustive exercise (circles). B: exponential fit for PCr rate, and PCr-to-creatine ratio (PCr/Cr) from Smith et al. (2) on left axis and the PCr/Cr mean and SE from Walsh et al. (4) on right axis.

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which [ADP] is tightly controlled (4), as shown by Fig. 1B herein.

The practical importance of our study is that it clearly shows that experiments estimating mitochondrial respiratory rate using $^{31}$P-MRS need to consider end-exercise [PCr] and [Cr]. Whether the associations between [PCr] or [Cr] with mitochondrial respiration rate are epiphenomenal or a result of direct interaction is practically irrelevant, because end-exercise PCr resynthesis is the parameter used to estimate mitochondrial respiration rate in MRS experiments. Our data clearly show that PCr resynthesis rate is significantly affected by the end-exercise [PCr].

We thank Dr. Kemp for his active interest in our work and the Journal of Applied Physiology for the opportunity to address Dr. Kemp’s intriguing questions. We hope our answers clarify any confusion regarding the outcomes and interpretation of our paper.

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