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 used as indicators of mitochondrial respiration after brief (10–12 s) and exhaustive (1–4 min) dynamic knee extension exercise performed in placebo and creatine-supplemented conditions. The results show that the initial PCr resynthesis rate has a strong relationship with end-exercise PCr, Cr, and PCr/Cr (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 an indicator of mitochondrial respiration and that the initial PCr resynthesis rate is a more reliable indicator of mitochondrial respiration compared with the 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) recovery in creatine (Cr)-loaded muscle reveal direct effects of PCr and Cr, in addition to ADP, on mitochondrial respiration, as described in vitro (8). However:

If they are correct to assume that resting [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. Δ[PCr] (3) and invasive physiology is interesting (Fig. 1D). Furthermore, the regression fits Smith et al. (7) give for V vs. [ADP], Δ[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 Kₘ 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 Kₘ for ADP (8) cannot explain this, being independent of total Cr concentration. Effects of [PCr] on Kₘ (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.

---

**Fig. 1.** Mitochondrial ATP synthesis in vivo. A: phosphocreatine (PCr) resynthesis rate (V) vs. [ADP] (brackets denote concentration) in mean data (7) from rest and brief and exhaustive exercise in control and creatine (Cr) loading assuming that resting [ADP] does or does not increase. Sigmoid fits (5) assume 80 mM/min maximum from invasive measurements (4). B: least squares simulation assuming that Kₘ for ADP has a hyperbolic relationship to PCr-to-Cr ratio as in vitro (8). C: simulations assuming hyperbolic dependence of Kₘ on [PCr] (8). D: V vs. [PCr] fall. Lines from origin (= rest) have slope equal to PCr rate constant: extrapolation to zero PCr yields close to literature maximum in brief exercise but for acidifying exercise is unsound (1). Curved lines show V-[ADP] fits from A at exhaustive exercise pH; at brief exercise pH these are near linear (not shown). Also shown are the fits of Fig. 2, B and D (close to that of 2A) in Ref. 7. Smith et al. (7) report control [PCr] of 38.6 mmol/kg ~ 60 mM, very high for 31P-magnetic resonance spectroscopy, so to avoid argument V here is relative to control resting [PCr].

---

8750-7587/06 $8.00 Copyright © 2006 the American Physiological Society http://www.jap.org
CONTROL OF RESPIRATION IN CREATINE-LOADED MUSCLE

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 data. 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 epiphenomena, 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.

Graham Kemp
Division of Metabolic and Cellular Medicine
Faculty of Medicine
University of Liverpool
Liverpool, United Kingdom
e-mail: gkemp@liv.ac.uk

REFERENCES
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 31P-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.

REFERENCES


Sinclair A. Smith
Health Sciences Programs
College of Nursing and Health Professions
Drexel University
Philadelphia, Pennsylvania
e-mail: sas86@drexel.edu

Scott J. Montain
US Army Research Institute of Environmental Medicine
Natick, Massachusetts
e-mail: scott.montain@us.army.mil