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

**McMillen, J., C. M. Donovan, J. I. Messer, and W. T. Willis.** Energetic driving forces are maintained in resting rat skeletal muscle after dietary creatine supplementation. *J Appl Physiol* 90: 62–66, 2001.—The total creatine (TCr) pool of skeletal muscle is composed of creatine (Cr) and phosphocreatine (PCr). In resting skeletal muscle, the ratio of PCr to TCr (PCr/TCr; PCr energy charge) is ~0.6–0.8, depending on the fiber type. PCr/TCr is linked to the cellular free energy of ATP hydrolysis by the Cr kinase equilibrium. Dietary Cr supplementation increases TCr in skeletal muscle. However, many previous studies have reported data indicating that PCr/TCr falls after supplementation, which would suggest that Cr supplementation alters the resting energetic state of myocytes. This study investigated the effect of Cr supplementation on the energy phosphates of resting skeletal muscle. Male rats were fed either rodent chow (control) or chow supplemented with 2% (wt/wt) Cr. After 2 wk on the diet, the gastrocnemius and soleus muscles were freeze clamped and removed from anesthetized animals. Cr supplementation increased TCr, PCr, and Cr levels in the gastrocnemius by 20, 22, and 17%, respectively (P < 0.05). A numerical 6% higher mean soleus TCr in Cr-supplemented rats was not statistically significant. All other energy phosphate concentrations, free energy of ATP hydrolysis, and PCr/TCr were not different between the two groups in either muscle. We conclude that Cr supplementation simply increased TCr in fast-twitch rat skeletal muscle but did not otherwise alter resting cellular energetic state.

**Look Before You Leap**

To the Editor: In the article by McMillen et al. (5), the authors set out “to evaluate the effect of dietary creatine supplementation on the energy phosphate status of resting soleus and gastrocnemius muscles in the rat.” They hypothesized “that dietary creatine supplementation would not alter the phosphocreatine/total creatine ratio in resting type I or type II skeletal muscle,” having cited, but subsequently dismissed, a whole series of independent studies involving healthy human volunteers showing the contrary [i.e., the ratio declined because most of the increase in total creatine (TCr) being in the form of free creatine (Cr)].

The authors reported that feeding rats chow containing 2% (wt/wt) Cr for 2 wk had, first, no effect on soleus muscle free Cr, phosphocreatine (PCr), and TCr contents. Second, it increased free Cr, PCr, and TCr contents in the gastrocnemius muscle but did not change the PCr-to-TCr ratio. The authors concluded, “Cr supplementation increased TCr in rat fast-twitch skeletal muscle. Because no change in the PCr/TCr occurred, Cr supplementation simply provided an increased TCr content and metabolic capacitance in skeletal muscle.” It is the opinion of this reader that the authors have neglected to compare their own muscle metabolite data with analogous published work; as a result, they have arrived at a spurious conclusion. In particular, comparison of the data presented in Fig. 1 (Ref. 5) with the literature shows that the free Cr concentration of the gastrocnemius (~7.5 μmol/g wet wt) was ~50% lower than that routinely reported for white gastrocnemius and ~30% lower than that reported for red gastrocnemius (e.g., Refs. 3 and 7; the authors do not report whether they analyzed red or white gastrocnemius). Furthermore, the gastrocnemius free Cr concentration shown in Fig. 1 is depicted to be the same as that measured in soleus, which is also contrary to what has been widely published previously (it is ~40–75% higher in red and white gastrocnemius, respectively; Ref. 3).

With this information in mind, and given that ATP and PCr contents of the gastrocnemius depicted in Table 1 and Fig. 1 were comparable with that shown in published literature for red gastrocnemius (3, 7), it seems that the authors found the PCr-to-TCr ratio to be unchanged by Cr supplementation simply because they failed to measure muscle free Cr concentration correctly. This could be at least partly attributable to the authors failing to use independent methods for PCr and Cr determination.

The authors state in their discussion that they were not surprised by their findings because they can think of no compelling reasons to suspect that Cr supplementation would compromise the forces or flows generated by mitochondria. This statement reflects a bias on the part of the authors because they failed to refer to published evidence in support of the existence of the “creatine-phosphocreatine circuit” in the manuscript. Indeed, contrary to the view of McMillen et al. (5), there is substantial evidence to support the view that a change in the cellular Cr concentration is an important regulator of cellular energetics in vivo (1, 6, 8, 9).

It is also important to appreciate that the lack of an effect of Cr supplementation on Cr and PCr concentrations in rat soleus muscle reported by McMillen et al. (5) illustrates that it is unwise for investigators to use an animal model that will not allow the hypothesis under scrutiny to be tested.

Finally, the authors cite their own animal-based study, and that of Brannon et al. (2), to indicate that type II muscle becomes better suited for burst activity following Cr supplementation. However, they failed to mention that this was clearly shown to be the case and moreover was shown in human type II fibers several years previously (4).
REFERENCES


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REPLY

To The Editor: Greenhaff and his collaborators have indeed published “a whole series of independent studies” reporting decreased phosphocreatine (PCr) to total creatine (TCr) ratios in resting human skeletal muscle in response to dietary creatine (Cr) supplementation (Cr-Sup). However, they have seemingly dismissed the rather profound implications this purported finding would bring on the resting metabolic rate of creatine-loaded skeletal muscle.

We are not biased against the “Cr-PCr energy circuit” as Greenhaff suggests. We studied resting skeletal muscle. There is general agreement that the creatine kinase reaction maintains thermodynamic equilibrium in resting muscle (14). Under this condition, the energy phosphate system possesses only one degree of freedom (5); thus all kinetic (4, 13, 18) and energetic (6, 16) models of respiratory control reduce to the same prediction: a fall in PCr/TCr should result in an elevated rate of mitochondrial oxidative phosphorylation. Thus the bias that Greenhaff seems to believe is present in our report would have no bearing on our conclusions in any case because the molecular signals of all models are linked by the equilibrium. Greenhaff and his co-authors must include themselves in this consensus view, as evidenced by their use of the creatine kinase equilibrium to calculate the free ADP concentration ([ADP]f) of resting skeletal muscle (11). With their calculation, they reported that the fall in PCr/TCr after Cr-Sup indicated a ~24% increase in [ADP]f in resting myocytes, to quote (11) “our findings point to Cr supplementation having a substantial impact on cellular energetics.” Substantial indeed, especially since the mitochondrial response to ADP has been shown to be at least second order (13). For example, using an S0.5 for ADP of 44 μM (13), the most conservative Hill coefficient of two, and the values for resting [ADP]f reported by Green et al. (11) would predict that Cr-Sup increases the resting ATP turnover of myocytes by >40%. We were curious as to what cellular energy-conserving or energy-dissipating reactions might accommodate such an impressive increase in mitochondrial energy delivery in resting Cr-Sup cells and why such profound implications of decreased PCr/TCr appeared to be of no concern. On the other hand, it was conceivable that Cr-Sup impaired mitochondrial sensitivity to the signal(s); thus resting metabolic rate would remain unchanged in the face of a depressed energy phosphate status. A final possibility was that Cr-Sup precipitated a phosphate (P_i) limitation. Because we are interested in the control of mitochondrial respiration, we carried out a complete assessment of the impact of Cr-Sup on muscle energy phosphates, including enzymatically assayed Pi. We found that a modest 20% rise in TCr resulted in neither any predicted rise in resting metabolic rate nor any impairment in the ability of mitochondria to maintain the resting cellular energy state, an outcome we sincerely consider unsurprising. Thus we agree that “creatine concentration is an important regulator of cellular energetics in vivo.” This is why we did the study and why a Cr term appears in three of the five equations of our paper. As we discussed (15), unchanged PCr/TCr in human skeletal muscle after Cr-Sup has been reported by Febbraio et al. (10).

In response to Greenhaff’s criticism of our assay, we would point out that the colorimetric assay for Cr and PCr we employed (8) has been widely used for decades (e.g., Refs. 7, 17, 19). The colorimetric assay correlates with the enzymatic assay demonstrating a slope of unity and r = 0.997 (1), achieves ~100% recovery of Cr added to tissue samples (9), and has no interfering molecules in skeletal muscle (9). Our own pilot data confirmed all of these published reports. In addition, the colorimetric assay provides an extinction coefficient about twice that of NAD⁺-linked assays, all Cr and PCr assays were run in triplicate, and the coefficient of variation of the assay in our hands is <3%. Our assay indicated that dietary Cr-Sup increased TCr in rat whole gastrocnemius muscle by 20%, and the unchanged PCr/TCr we measured before and after dietary Cr-Sup is very close to, but slightly below, published values (5). This directionality is opposite to the criticism advanced by Greenhaff.
In the past, Greenhaff et al. (3) ascribed little relevance to type I fiber creatine status, yet he now expresses concern that the rat soleus fails to exhibit increased TCr in response to Cr-Sup (2, 15). However, if we had defined “Cr-responders” as only those Cr-Sup solei demonstrating TCr above the mean value of the control group (a strategy inspired by the human study in Ref. 12), then Cr-Sup would have yielded 28% higher TCr in our soleus “responders” but still no change in PCr/TCr.

REFERENCES


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