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J A Timmons, … , I A Macdonald, P L Greenhaff

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Research Article

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Increased Acetyl Group Availability Enhances Contractile Function of Canine Skeletal Muscle during Ischemia

James A. Timmons,* Simon M. Poucher,‡ Dumitru Constantin-Teodosiu,* Viv Worrall,‡ Ian A. Macdonald,* and Paul L. Greenhaff*

**Department of Physiology and Pharmacology, University Medical School, Queens Medical Center, Nottingham NG7 2UH; and ‡Cardiovascular and Metabolism Research Department, Zeneca Pharmaceuticals, Macclesfield SK10 4TG, United Kingdom*

Abstract

Skeletal muscle contractile function is impaired during acute ischemia such as that experienced by peripheral vascular disease patients. We therefore, examined the effects of dichloroacetate, which can alter resting metabolism, on canine gracilis muscle contractile function during constant flow ischemia. Pretreatment with dichloroacetate increased resting pyruvate dehydrogenase complex activity and resting acetylcarnitine concentration by ~ 4- and ~ 10-fold, respectively. After 20-min contraction the control group had demonstrated an ~ 40% reduction in isometric tension whereas the dichloroacetate group had fatigued by ~ 25% ($P < 0.05$). Dichloroacetate resulted in less lactate accumulation (10.3±3.0 vs 58.9±10.5 mmol·kg^{-1} dry muscle [dm], $P < 0.05$) and phosphocreatine hydrolysis (15.6±6.3 vs 33.8±9.0 mmol·kg^{-1} dm, $P < 0.05$) during contraction. Acetylcarnitine concentration fell during contraction by 5.4±1.8 mmol·kg^{-1} dm in the dichloroacetate group but increased by 10.0±1.9 mmol·kg^{-1} dm in the control group. In conclusion, dichloroacetate enhanced contractile function during ischemia, independently of blood flow, such that it appears oxidative ATP regeneration is limited by pyruvate dehydrogenase complex activity and acetyl group availability. (*J. Clin. Invest.* 1996. 97:879–883.) Key words: dichloroacetate • pyruvate dehydrogenase complex • peripheral vascular disease • phosphocreatine • lactic acid

Introduction

Skeletal muscle contractile function is impaired during acute ischemia (1) and this reduction in force production is analogous to that experienced by peripheral vascular disease (PVD)[1] patients while walking (2). The accumulation of the metabolites of anaerobic metabolism have been associated with an inhibition of contractile function (3, 4). To maintain both contractile function and ATP replenishment it would be advantageous to decrease anaerobic ATP regeneration and increase the contribution made by oxidative phosphorylation. Furthermore, oxidation of carbohydrate allows the greatest amount of ATP to be regenerated during conditions of limited oxygen availability (5), such that it would be beneficial to contractile function if it were the sole oxidative fuel.

Pyruvate dehydrogenase complex (PDC) is responsible for the regulation of pyruvate decarboxylation and hence carbohydrate oxidation. Increased flux through PDC, and therefore carbohydrate oxidation, can be achieved by increasing the degree of activation of the enzyme complex (6–8) or by reducing the amount of acetyl-CoA derived from noncarbohydrate sources (9, 10), thus reducing substrate competition for entry into the tricarboxylic acid cycle. This latter point is distinct from the classical regulation of substrate oxidation as described by the glucose-fatty acid cycle, since operation of the glucose-fatty acid cycle describes a direct effect of acetyl groups derived from noncarbohydrate sources on the amount of PDC in its active form (PDCa, 11). In contracting skeletal muscle, however, there is little evidence that increasing acetyl group availability from noncarbohydrate sources leads to a reduction in PDCa (9, 10).

In the ischemic contracting heart, the amount of PDCa is low and under these conditions limits pyruvate oxidation (12). However, by increasing PDCa and therefore carbohydrate oxidation, an improvement in myocardial function has been achieved (6, 12). In contrast, during short lasting contraction in human skeletal muscle with total occlusion of blood flow, complete activation of PDC occurs (13). However, the extent of PDC activation in contracting skeletal muscle during prolonged partial ischemia is not known, and it is possible that attenuation of the normal PDC response under these conditions may contribute to the development of skeletal muscle fatigue. This question is of particular importance in PVD, where the normal hyperemic response to muscle contraction is reduced and where it is generally accepted that exercise training or pharmacological interventions have little impact on total limb blood flow during exercise (14). Therefore, it was the aim of the present study to maximally activate PDC, using dichlo-

Address correspondence to James A. Timmons, Department of Physiology and Pharmacology, University Medical School, Queens Medical Center, Nottingham NG7 2UH, United Kingdom. Phone: 44-115-970-9465; FAX: 44-115-970-9259; E-mail: mqxjat@mqn1.phpharm.nottingham.ac.uk

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1. *Abbreviations used in this paper:* DCA, dichloroacetate; dm, dry muscle; PCr, phosphocreatine; PDC, pyruvate dehydrogenase complex; PDCa, active form of PDC; PVD, peripheral vascular disease.

roacetate (DCA), to evaluate the possibility of improving skeletal muscle contractile function during ischemia. Our hypotheses were that DCA would, firstly, reduce lactic acid accumulation and hence attenuate the possible deleterious effects of anaerobic metabolism, and secondly, would increase the relative contribution made from carbohydrate oxidation, thus increasing the aerobic ATP yield and consequently improving contractile function.

Methods

Animals. 12 female Beagle dogs (Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Cheshire, UK) weighing 14.7±1.5 kg and aged 11.7±1.9 mo were used in the present experiment.

Surgical procedures. After an overnight fast, each animal was premedicated with morphine (10 mg intramuscularly) 30 min before the induction of anesthesia with sodium pentobarbitone (46±1 $mg \cdot kg^{-1}$ body mass, i.v., followed by continuous infusion at 0.11±0.01 $mg \cdot kg^{-1} \cdot min^{-1}$, Sagatal, Rhône Merieux, Harlow, UK). The trachea was intubated, and the dogs were artificially ventilated (24 $cycles \cdot min^{-1}$, 13–15 $ml \cdot kg^{-1}$, model 16/20; Palmer Bioscience, London, UK). Muscle and rectal temperatures were maintained at 37°C and between 36–38°C, respectively. The right brachial artery was then cannulated, and systemic blood pressure was recorded using a pressure transducer (PDCR 75; Druck Ltd., Barendeecht, The Netherlands) and an eight channel chart recorder (Mk 8 WR3500; Graphtec Linearcorder, Nantwich, UK). The left brachial artery and left brachial vein were cannulated for collection of arterial blood samples and infusion of heparin, saline, or DCA.

The gracilis muscle was vascularly isolated, leaving only the arterial and venous blood flows intact. The distal tendon of the muscle was attached to an isometric force transducer (Grass FTC 10; Quincy, Medfield, MA). The popliteal artery was catheterized for recording gracilis muscle perfusion pressure. Heparin was infused (Multiparin, 1 $U \cdot kg^{-1} \cdot min^{-1}$) for the duration of the experiment. The femoral artery supplying the gracilis was cannulated proximally and distally and was attached to a perfusion pump (Minipuls 3; Gilson, Villiers le bel, France). The resting muscle perfusion pressures and venous oxygen saturations were set by altering the blood flow such that they were similar to those values observed before insertion of the pump. This flow rate was maintained for the duration of the experiment such that during contraction we observed no functional hyperemia and a fall in muscle perfusion pressure, with blood flow being ~ 25% of the normal flow observed when this muscle was stimulated to contract with the blood supply intact. After a 20-min equilibration period, the animals were infused either with 45 ml saline ($n = 6$) or 300 $mg \cdot kg^{-1}$ DCA (in 45 ml saline, $n = 6$) over a period of 45 min. The dose of DCA used in the present study has been shown to completely transform PDC to PDCa, in vivo (6, 15).

Muscle stimulation parameters. The resting length of the muscle was altered to obtain a standard 300 g resting tension. 60 min after the onset of DCA or saline infusion, muscle contraction was induced via electrical stimulation of the obturator nerve (Grass S88 stimulator; Quincy). Square-wave impulses of 0.1 ms duration, 3 Hz frequency, and 10 V intensity were applied for 20 min. It was determined, in a pilot study, that the stimulus intensity of 10 V resulted in maximal twitch force production from this muscle indicating that complete muscle fiber recruitment was achieved (unpublished observation).

Muscle collection and analysis. Resting muscle biopsies were obtained after the infusion period using the Bergström needle biopsy technique (16). After 20 min of stimulation, the muscle was freeze clamped during contraction and a thin piece of muscle was excised, divided into two portions, and stored under liquid nitrogen. Subsequently, one portion was freeze dried, dissected free from visible connective tissue and blood, powdered, and extracted in 0.5 M perchloric acid containing 1 mM EDTA. After centrifugation, the supernatant was neutralized with 2.2 M $KHCO_3$ and used for spectrophotometric determination of ATP, phosphocreatine (PCr), creatine, and lactate (17). The extract was also used for the determination of free carnitine and acetyl-carnitine by enzymatic assays using radioisotopic substrates, as described previously (18). Freeze-dried muscle powder was used for the determination of muscle glycogen (17). The remaining portion of frozen muscle was used to assess PDC activity, and PDCa was expressed as nmol $acetyl\text{-}CoA \cdot min^{-1} \cdot mg^{-1}$ protein at 37°C (19).

Calculations and statistics. All data are reported as mean±SE. Comparisons between treatments were made using ANOVA for repeated measurements (20). When a significant F value was found ($P < 0.05$) a Tukey post-hoc test was used to locate any differences. Significance was accepted at the 5% level. Anaerobic ATP production was estimated from: Δ PCr + (1.5 × Δ lactate) + (2 × Δ ATP), where Δ equals the difference between rest and contraction concentrations (21). An estimation of muscle glycogen oxidation was calculated from muscle glycogen degradation and lactate accumulation assuming that lactate accumulation was derived solely from muscle glycogen degradation. This assumption is based on the observations that the contribution made by blood glucose, to glycolytic flux, is minimal during intense fatiguing contraction (22).

Results

Peak tension did not differ between control and DCA treatments (4,461±181 vs 4,019±445 g $tension \cdot 100\ g^{-1}$ wet muscle, respectively). The DCA-treated muscle demonstrated a significant reduction in the decline from peak tension during contraction when compared to the control, such that the decline in force production was ~ 40% less (Fig. 1, $P < 0.05$). No differences in blood flow (control = 20.1±1.5 vs DCA = 21.3±1.3 $ml \cdot min^{-1} \cdot 100$ g muscle) and perfusion pressure (control = 62.9±3.2 vs DCA = 62.1±3.2 mmHg) were observed between treatments during contraction.

There was no significant difference in the ATP concentration between control and DCA treated muscles either at rest

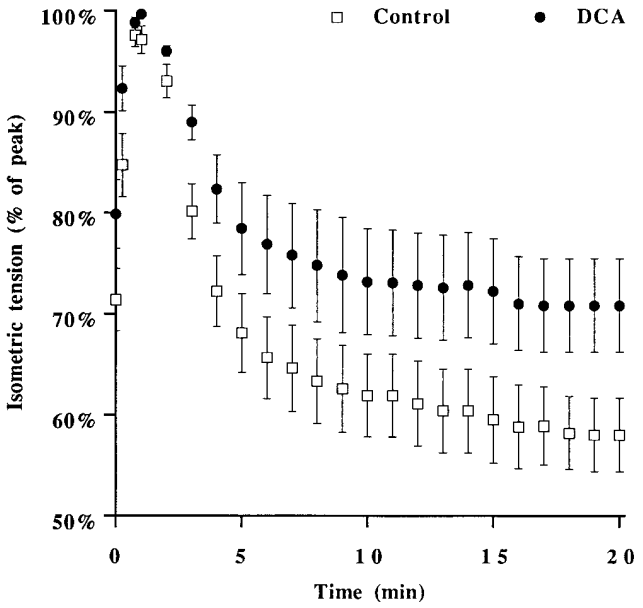

Figure 1. The decline in tension from peak, during 20 min of ischemic contraction with dichloroacetate (*filled circles*) and control (*open squares*). Data are expressed as a percentage of peak tension. The decline in tension in the control group was significantly greater than the corresponding DCA group ($P < 0.05$).

Table I. Skeletal Muscle Metabolites in Saline and Dichloroacetate Pretreated Groups at Rest and during Ischemic Contraction

	Resting		Ischemic contraction	
	Control	DCA	Control	DCA
ATP	27.1±0.9	26.2±0.6	21.9±1.2	23.1±1.0
PCr	58.1±2.5	55.1±1.7	28.5±6.9	38.9±2.9*
Lactate	10.5±0.9	5.6±0.9‡	53.7±12.6	18.1±3.5‡
Glycogen	248±18	247±17	158±24	157±15

Dog gracilis muscles were stimulated at 3 Hz via the obturator nerve for 20 min with restricted blood flow (to ~ 25% of normal contraction induced flow). Data are expressed as mmol·kg^{-1} dm (mean±SE). *$P <$ 0.05, ‡$P < 0.01$ indicates that the DCA pretreated muscle significantly differed from the corresponding control muscle.

or during contraction (Table I). However, when examining the change in muscle ATP concentration within each group the control muscles demonstrated a significant fall in ATP during contraction ($P < 0.05$), whereas the DCA group did not. PCr concentration at rest did not differ between groups. PCr degradation was, however, significantly greater in the control group when compared with DCA ($P < 0.05$). Muscle lactate concentration was lower at rest ($P < 0.05$) and during stimulation ($P < 0.01$) after DCA when compared with the control group. Resting muscle glycogen and glycogen degradation during contraction (control = 93.4±26.5 vs DCA = 93.4±26.0 mmol·kg^{-1} dry muscle [dm]) did not differ between groups (Table I). Similarly, glycogen oxidation over the 20-min period was not statistically different between groups (control = 64.0±20.7 vs DCA = 87.4±22.5 mmol·kg^{-1} dm) although the trend was for greater glycogen oxidation in the DCA group.

Resting PDCa was threefold greater in the DCA-treated group (Fig. 2) falling slightly but nonsignificantly during contraction. However, PDCa increased in the control group during contraction such that there was no difference in PDCa between groups at the end of contraction. Resting muscle acetylcarnitine concentration in the DCA group was ~ 10-fold greater than in the control group (Fig. 3, $P < 0.01$). During contraction acetylcarnitine concentration declined by 5.4±1.8 mmol·kg^{-1} dm in the DCA group (Fig. 3, $P < 0.05$). However, in the control group, acetylcarnitine concentration increased by 10.0±1.9 mmol·kg^{-1} dm (Fig. 3, $P < 0.05$), such that no statistical difference existed between the groups after 20-min stimulation. Free carnitine concentration at rest and during contraction mirrored stoichimetrically the differences in acetylcarnitine between the groups. Thus, at rest, free carnitine was approximately fourfold lower in the DCA group (Fig. 3, $P < 0.05$). During contraction, free carnitine declined in the control group but significantly increased in the DCA group (Fig. 3, both $P < 0.05$), such that no difference existed between the groups after 20-min stimulation. The sum of acetylcarnitine and free carnitine concentration remained constant throughout.

Anaerobic ATP regeneration, calculated from lactate accumulation and ATP and PCr degradation, was 120.7±26.2 mmol ATP·kg^{-1} dm in the control group and is likely to reflect the initial period of contraction. The corresponding figure in the DCA group was ~ 70% lower than the control (37.1±8.2 mmol ATP·kg^{-1} dm). However, the sum of the anaerobic ATP regeneration and ATP regeneration calculated from the net fall in acetyl groups in the DCA group was 119.8±29.9 mmol ATP·kg^{-1} dm.

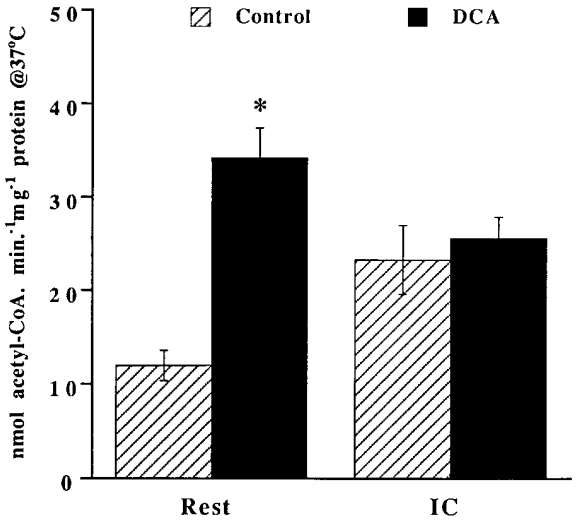

Figure 2. The active form of PDC, at rest and during ischemic contraction (*IC*), pretreated with saline (*control*) or DCA. PDCa is expressed as nmol acetyl-CoA·min^{-1}·mg^{-1} protein at 37°C (mean±SE). *$P < 0.05$, indicates DCA significantly differed from the control group.

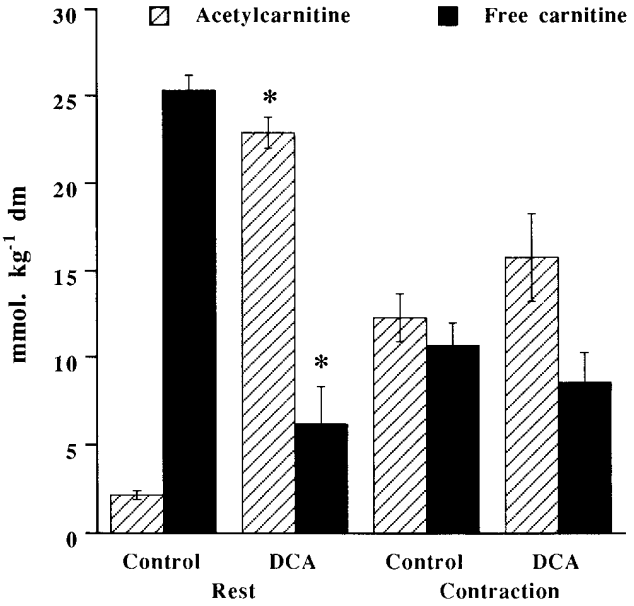

Figure 3. Acetylcarnitine and free carnitine concentrations at rest and during contraction in canine skeletal muscle pretreated with saline (*control*) or DCA. Data are expressed as mmol·kg^{-1} dm (mean±SE). *$P < 0.01$ indicates DCA significantly differed from the control condition.

Discussion

This study demonstrated that increasing acetyl group availability at rest resulted in a greater oxidative contribution to ATP regeneration, a substantial reduction in anaerobic metabolism,

and a marked improvement in skeletal muscle contractile function during ischemic conditions. We can, therefore, accept our first hypothesis that DCA's principal action would be to reduce lactic acid accumulation and the associated deleterious effects of anaerobic metabolism. However, because there was no significant increase in carbohydrate oxidation over the 20-min contraction period, after DCA, it would seem that an increased ATP yield from altering the oxidative substrate used was not achieved such that we are unable to accept our second hypothesis.

The mechanisms underlying the regulation of skeletal muscle substrate oxidation during contraction are not fully understood. It has been shown that accumulation of acetyl-CoA and NADH resulting from fat oxidation in heart and resting skeletal muscle reduces the amount of PDCa, leading to an inhibition of carbohydrate oxidation. This observation forms the basis of the glucose-fatty acid cycle (11), which describes a potential mechanism for the regulation of substrate oxidation. Several studies have failed, however, to demonstrate the operation of the glucose-fatty acid cycle in contracting skeletal muscle (9, 10, 13, 23). Indeed, there is no in vivo evidence to show that increasing acetyl group availability from noncarbohydrate sources affects the degree of PDC activation in healthy contracting skeletal muscle with blood flow intact (9, 10). However, it has been demonstrated that during ischemic contraction in PVD a proportionally greater contribution is made from fat than carbohydrate to oxidative metabolism (24). A consequence of increasing fat oxidation through a reduction in PDCa would be a lower ATP yield per molecule of oxygen used. We hypothesized, therefore, that the role of PDC may be of particular importance in regulating skeletal muscle substrate oxidation and contractile function during ischemia. This possibility is supported by studies showing that the amount of PDCa is reduced in ischemic contracting myocardium (12), and from observations in heart failure patients showing some evidence of an improvement in ischemic myocardial function after increasing the proportionate contribution from carbohydrate to oxidative ATP regeneration using DCA (25). Other in vivo and in vitro studies have also demonstrated a significant functional benefit from administration of DCA to ischemic myocardium (26). If the inhibition of PDC observed in ischemic myocardium (12) also occurs in ischemic skeletal muscle then a reduction in carbohydrate oxidation might be expected and therefore, by increasing PDC activity using DCA, an increase in carbohydrate oxidation and contractile function may occur. In the present study, however, DCA administration only demonstrated a trend towards increased muscle glycogen oxidation and, therefore, an increased ATP yield from altering the oxidative substrate used by the muscle is an unlikely explanation for the observed improvement in contractile function.

Examination of the pattern of acetylcarnitine accumulation does, however, provide a potential explanation for the enhanced contractile function. During moderate to intense exercise, skeletal muscle acetylcarnitine accumulation occurs, principally during the initial period of contraction (23). This reflects a period during contraction where an imbalance exists between the rate of acetyl group formation and the rate of utilization by the tricarboxylic acid cycle. In the present study, pretreatment with DCA resulted in an $\sim$ 10-fold elevation in acetylcarnitine at rest. By the end of the contraction period, however, a decline in the acetylcarnitine concentration had occurred in the DCA group in contrast to the normal accumulation observed in the control group. This suggests, therefore, that during contraction in the DCA group, there was a period where acetyl group utilization exceeded flux through PDC and by elevating acetyl group availability before contraction, we provided a mechanism for increasing the regeneration of ATP oxidatively, thereby reducing the contribution from anaerobic metabolism. In support of this explanation was the finding that the lower contribution from anaerobic ATP regeneration in the DCA group ($\sim$ 84 mmol·kg^{-1} dm) was closely matched by the calculated increase in ATP regenerated from the net fall in acetylcarnitine ($\sim$ 83 mmol·kg^{-1} dm). It appears, therefore, that at some stage during contraction, acetyl group availability limits oxidative phosphorylation, consequently these alterations in resting muscle metabolism result in a significant improvement in contractile function during ischemia.

The mechanisms underlying the production of lactic acid during muscle contraction are still the subject of intense debate (27). It seems clear, however, that lactate and hydrogen ion accumulation may both, independently, influence skeletal muscle contractile function (3, 4, 28). In the present study, DCA substantially reduced muscle lactic acid accumulation during contraction. Clearly, this together with the better maintenance of ATP and PCr concentrations may have reduced other mediators of contractile failure (e.g., ADP, inorganic phosphate, and Mg^{2+}) thus favorably influencing contractile function (4, 28). However, DCA's primary action is to inhibit the kinase responsible for phosphorylating and therefore inactivating PDC (15), such that DCA's primary influence on lactic acid formation is mediated through changes in PDCa. Since a large difference existed in the amount of PDCa between treatments at rest and yet no difference in PDCa was evident at the end of contraction, it is likely that DCA's main effect on energy metabolism occurred during the initial period of contraction. In support of this interpretation are the wealth of data demonstrating that the highest rate of glycogenolysis occurs during the first few minutes of contraction and that this is accompanied by substantial lactate formation and PCr hydrolysis (27, 29). In short, the observed reduction in lactate accumulation, in the DCA group, may have reflected the immediate ability of PDC to oxidize pyruvate. Alternatively, the reduction in PCr hydrolysis seen with DCA may explain the reduction in lactate accumulation in this group. Presumably the reduction in PCr hydrolysis would have reduced ADP and inorganic phosphate accumulation in the DCA group, reducing the stimulus to glycogen phosphorylase, glycogenolysis, and hence glycolysis during the initial period of contraction (27). Both these explanations reflect a better matching of the initial glycolytic flux and flux through PDC suggesting that, even during ischemia, the majority of lactate accumulated is unrelated to oxygen availability per se but rather reflects the availability of oxidizable substrate and PDC activity. Interestingly, the reduction in lactate accumulation observed in the current study resulted in $\sim$ 40% attenuation of contractile fatigue, which is similar to that observed by Hogan et al. when the intramuscular lactate anion concentration was altered, independently of pH, over a similar concentration range observed in the present study (3).

In conclusion, pretreatment of skeletal muscle with DCA significantly maintained contractile function during ischemia. This may prove to be a useful strategy in the treatment of pa-

tients suffering from peripheral vascular disease, since an increase in exercise capacity could be achieved without additional demands being made on the cardiovascular system. The proposed mechanisms underlying this improvement in contractile function are the marked elevations in resting muscle PDCa and acetylcarnitine concentration leading to a reduction in the contribution made by PCr hydrolysis and lactate production to ATP regeneration, thus attenuating the decline in muscle contractile function originating from the reliance on anaerobic metabolism. These alterations in skeletal muscle metabolism suggest that PDC and acetyl group availability limit oxidative ATP regeneration.

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