Plasticity of microvascular oxygenation control in rat fast-twitch muscle: Effects of experimental creatine depletion

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Abstract

Aging, heart failure and diabetes each compromise the matching of O₂ delivery (QO₂)-to-metabolic requirements (O₂ uptake, VO₂) in skeletal muscle such that the O₂ pressure driving blood-myocyte O₂ flux (microvascular PO₂, PmvO₂) is reduced and contractile function impaired. In contrast, β-guanidinopropionic acid (β-GPA) treatment improves muscle contractile function, primarily in fast-twitch muscle (Moerland and Kushmerick, 1994). We tested the hypothesis that β-GPA (2% wt/BW in rat chow, 8 wk; n=14) would improve QO₂-to-VO₂ matching (elevated PmvO₂) during contractions (4.5 V @ 1 Hz) in mixed (MG) and white (WG) portions of the gastrocnemius, both predominantly fast-twitch). Compared with control (CON), during contractions PmvO₂ fell less following β-GPA (MG -54%, WG -26%, p<0.05), elevating steady-state PmvO₂ (CON, MG: 10±2, WG: 9±1; β-GPA, MG 16±2, WG 18±2 mmHg, P<0.05). This reflected an increased QO₂/VO₂ ratio due primarily to a reduced VO₂ in β-GPA muscles. It is likely that this adaptation helps facilitate the β-GPA-induced enhancement of contractile function in fast-twitch muscles.

Keywords

β-guanidinopropionic acid; microvascular O₂ exchange; muscle fiber type; O₂ extraction; phosphorescence quenching

1. Introduction

Skeletal muscle fibre types are highly stratified with respect to contraction speed (Armstrong and Phelps, 1984; Baldwin et al., 1972; Barclay et al., 1993; Bottinelli et al., 1996; Delp and Duan, 1996), oxidative capacity (Delp and Duan, 1996; Gollnick et al., 1972), capillarity (Folkow and Halicka, 1968), and O₂ uptake (VO₂) kinetics (Barstow et al.,...
1996), as well as blood flow (Q) and O\(_2\) delivery (QO\(_2\)) (Ferreira et al., 2006b).

Investigation of the mechanistic bases for the heterogeneity of Q and hence QO\(_2\) among muscles of disparate fiber types supports that inter fibre-type differences are attributable, in part, to a differential arteriolar vasomotor control (Behnke et al., 2011; Behnke et al., 2002a; Laughlin et al., 1997; McDonough et al., 2005).

We have previously demonstrated that, compared with its fast-twitch counterparts (medial and white gastrocnemius, peroneal), the contracting, slow-twitch soleus muscle achieved a higher QO\(_2\) per unit VO\(_2\) (Behnke et al., 2002a; Behnke et al., 2004). The QO\(_2\):VO\(_2\) ratio is of crucial importance because it determines the microvascular O\(_2\) partial pressure (PmvO\(_2\)) which drives blood-myocyte O\(_2\) flux and also helps “set” intracellular PO\(_2\) and thus muscle energetics (Behnke et al., 2002a; Haseler et al., 2004; Hogan and Welch, 1986; McDonough et al., 2005). The regulation of PmvO\(_2\) demonstrates considerable plasticity, for example, in aging (Behnke et al., 2005) and chronic diseases such as heart failure (Diederich et al., 2002) and diabetes (Behnke et al., 2002c; Padilla et al., 2007). Specifically, PmvO\(_2\) in the spinotrapezius muscle falls more rapidly and to far lower levels during contractions in aging and these disease states than observed in young healthy controls. Whereas these conditions are associated with slowed pulmonary VO\(_2\) kinetics and compromised muscle oxidative function (Behnke et al., 2004; Behnke et al., 2002c; Belardinelli et al., 1997; Brandenburg et al., 1999; Chilibeck et al., 1997; McDonough et al., 2004a; Pfeifer et al., 2001; Regenstein et al., 1998; Sietsema et al., 1994) (though see (Wilkerson et al., 2011) for an exception in long-term diabetic patients) the lowered PmvO\(_2\)indicates a reduced QO\(_2\)-to- VO\(_2\) ratio in skeletal muscle.

In addition to depleting intramuscular creatine and phosphocreatine stores (Moerland and Kushmerick, 1994; Moerland et al., 1989), chronic dietary β-guanidinopropionic acid (β-GPA) treatment up-regulates adenosine monophosphate -activated protein kinase (AMPK) activity (Bergeron et al., 2001; Williams et al., 2009). AMPK is a serine/threonine kinase that is expressed in several tissues including endothelial and smooth muscle cells and contributes to the regulation of endothelial nitric oxide synthase (eNOS) activation and NO synthesis (Morrow et al., 2003). Further, Bradley et al. (Bradley et al., 2010) have recently demonstrated that activation of AMPK has a direct vasodilatory action on skeletal muscle resistance arteries through increased NO activity. In muscle-specific AMPK dominant negative transgenic mice there is a faster PmvO\(_2\) decline (i.e., reduced time-constant) during the rest-to-contractions transition versus that observed from mice demonstrating a normal AMPK phenotype (Kano et al., 2011). Thus, the absence of AMPK induces a disproportionate slowing of QO\(_2\) versus VO\(_2\) kinetics across the rest-to-contractions transition (Kano et al., 2011). Therefore, long-term β-GPA treatment would likely have an indirect influence on vasomotor regulation (e.g., faster vasodilatory dynamics) through enhanced nitric oxide signaling elicited by increased AMPK activity. Based upon this reasoning, we investigated whether β-GPA treatment would elevate the QO\(_2\)-to-VO\(_2\) ratio during contractions in fast-twitch muscle (which is affected more than slow twitch muscle with β-GPA treatment at least with respect to mitochondrial adaptations (Bruton et al., 2003)). Specifically, we tested the hypotheses that chronic β-GPA supplementation would reduce the magnitude and slow the rate of the PmvO\(_2\) fall (presumably due to faster blood flow kinetics) during muscular contractions resulting in an elevated steady-state contracting PmvO\(_2\). As β-GPA treatment improves muscle contractile function primarily in fast-twitch muscle (Moerland and Kushmerick, 1994), we reasoned that these contractile improvements in fast-twitch muscle would result, in part, from an enhanced PmvO\(_2\), which would then help facilitate transcapillary O\(_2\) flux and act to increase intramyocyte PO\(_2\).
2. Methods

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University. Rats were housed individually at 23°C and were maintained on a 12:12-h light-dark cycle. All rats were fed rat chow (control or containing 2% β-GPA for 8 wk, see below) and water *ad libitum*.

2.1 Surgical Preparation

All rats were anesthetized prior to experimentation with pentobarbital sodium (40 mg/kg ip to effect) and supplemented (5–10 mg/kg) as needed. The carotid and tail (caudal) arteries were catheterized with polyethylene tubing (PE-10 connected to PE-50). This allowed for the infusion of the phosphorescent probe [palladium meso-tetra (4-carboxyphenyl) porphine dendrimer (R2)], measurement of arterial blood pressure (Digi-Med BPA model 200, Louisville, KY) and withdrawal of arterial blood for blood gas measurement (Nova Stat Profile M, Waltham, MA).

The muscles used in the current study (i.e., mixed gastrocnemius, (MG) and white gastrocnemius, (WG); (McDonough *et al.*, 2005)) were chosen as previous research demonstrates that the effects of β-GPA are manifest primarily in fast-twitch musculature (Freyssenet *et al.*, 1995; Moerland, 1995). Both the MG (3% type I, 6% type IIA, 34% type IID/x and 57% type IIB) and the WG (8% type IID/x, 92% type IIB; (Delp and Duan, 1996)) are comprised primarily of fast twitch fiber phenotypes. Whereas the soleus (predominantly slow-twitch) may have provided an interesting ‘control’ comparison this would have required more animals for what was expected to be a negative result.

Each muscle was exposed for PmvO$_2$ measurements as previously detailed (McDonough *et al.*, 2005). The tibial nerve was isolated and a stimulating electrode was attached. The ground electrode was attached distally, near the Achilles tendon. Care was taken to minimize the extent of the surgery in all cases. The exposed tissue was superfused with a Krebs-Henseleit bicarbonate-buffered solution (38°C, equilibrated with 5% CO$_2$-N$_2$ balance) and body temperature was maintained at ~38°C via a heating pad.

2.2 β-GPA supplementation

Experimental animals were fed β-GPA for 8 weeks which has been demonstrated to reduce phosphocreatine (PCr) (Moerland and Kushmerick, 1994) and up-regulate AMPK activity (Bergeron *et al.*, 2001; Chaturvedi *et al.*, 2009). The β-GPA was incorporated into the rat chow at 2% wt/body wt.

2.3 Contractions Protocol

The rat was positioned on a custom-built ergometer and secured as detailed previously (McDonough *et al.*, 2005). Fifteen minutes later the MG or WG was stimulated at 1-Hz for 3 minutes (twitch 4.5 V, 2 ms pulse duration) using a Grass S88 stimulator. This contraction intensity was chosen as it corresponds to approximately 65% of the voltage which produces a minimal PmvO$_2$ for these two muscles (McDonough, Behnke, Musch & Poole; Unpublished observations). All animals were euthanized with an overdose of pentobarbital sodium (>80 mg/kg i.a.) following the conclusion of the experimental protocol.

2.4 Phosphorescence Quenching

Fifteen minutes prior to the beginning of the contraction protocol the R2 probe was infused (15 mg/kg via the arterial catheter) and the probe of a PMOD 1000 Frequency Domain Phosphorimeter (Oxygen Enterprises Ltd., Philadelphia, PA) was positioned ~2 mm above the exposed muscle. A light guide contained within the probe focuses excitation light (524...
nm) on the medial region of the exposed muscle (~2.0 mm diameter, to ~500 μm deep). The PMOD 1000 uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and repeated every 2 seconds (for review see Vinogradov and Wilson, 1994). The phosphorescence lifetime was obtained computationally based on the decomposition of data vectors to a linearly independent set of exponentials (Moerland et al., 1989).

The Stern-Volmer relationship allows the calculation of PmvO\textsubscript{2} from a measured phosphorescence lifetime using the following equation:

\[
PmvO_2 = \frac{(t^0/t) - 1}{(k_Q * t^0)}
\]

where \(k_Q\) is the quenching constant (mmHg/s) and \(t^0\) and \(t\) are the phosphorescence lifetimes in the absence of O\textsubscript{2} and at the ambient O\textsubscript{2} concentration, respectively. For R2, in \textit{in vitro} conditions similar to those found in the blood, \(k_Q\) is 409 mmHg\textsuperscript{-1}.s\textsuperscript{-1} and \(t^0\) is 601 μs. Since the R2 is tightly bound to albumin in the plasma and is negatively charged, in combination with the extremely high albumin reflection coefficients in skeletal muscle, the O\textsubscript{2} measurements are ensured to result from signals within the microvasculature, rather than the surrounding muscle tissue (Poole et al., 2004). The phosphorescence lifetime is insensitive to probe concentration, excitation light intensity, and absorbance by other chromophores in the tissue. The effects of pH and temperature are negligible within the normal physiological range which was maintained herein.

### 2.5 Muscle blood flow and oxygen uptake

Muscle blood flow (Q) was measured using the radiolabelled microsphere technique (Musch and Terrell, 1992) at rest and at the end of the 3 minute contraction protocol and expressed as milliliters of blood per minute per 100g tissue (ml.min\textsuperscript{-1}.100g\textsuperscript{-1}). Microspheres (15 μm-diameter; \textsuperscript{46}Sc, \textsuperscript{85}Sr or \textsuperscript{141}Ce; New England Nuclear, Boston, MA) were agitated via sonication and ~5 × 10\textsuperscript{5} microspheres were injected into the ascending aorta at the specified time point. Tissue radiation counts were performed using a gamma scintillation counter (Packard Auto Gamma Spectrometer, Cobra model 5003). Adequate distribution of the microspheres was verified with a difference of ≤15% in blood flow between right and left kidneys.

Muscle oxygen uptake (VO\textsubscript{2}) was calculated as previously described (Behnke et al., 2002b). Arterial O\textsubscript{2} content (CaO\textsubscript{2}) was measured directly (carotid arterial blood) and mixed venous O\textsubscript{2} content (CvO\textsubscript{2}) was calculated from PmvO\textsubscript{2} (assuming PmvO\textsubscript{2} is a valid approximation of mixed venous PO\textsubscript{2}; (McDonough et al., 2001)) using the rat O\textsubscript{2} dissociation curve (constructed using an “n” of 2.6, the measured [Hb], P\textsubscript{50} of 38 mmHg and an O\textsubscript{2} carrying capacity of 1.39 mlO\textsubscript{2}.g Hb\textsuperscript{-1}). VO\textsubscript{2} was then calculated via the Fick equation, i.e. VO\textsubscript{2} = Q * (CaO\textsubscript{2} - CvO\textsubscript{2}).

### 2.6 Citrate synthase measurement

Following the experimental protocol, contralateral (i.e., non-stimulated) muscles were excised rapidly, frozen in liquid N\textsubscript{2} and stored in sealed containers at ~80°C until analysis. Citrate synthase activity was measured in duplicate using spectrophotometric analysis from homogenates prepared from the MG and WG muscles according to the methods of Srere (Srere, 1969). Activity levels were expressed as μmol per minute per gram wet weight.
2.7 Curve Fitting and Statistical Analysis

For the PmvO\textsubscript{2} data, curve fitting was accomplished using KaleidaGraph software (version 3.5; Synergy Software, Reading, PA) and was performed on each data set using a one-component:

\[ \text{PmvO}_2(t) = \text{PmvO}_2(\text{BL}) + \Delta \text{PmvO}_2 \times (1 - e^{-(t-TD)/\tau}) \]

or a two-component model:

\[ \text{PmvO}_2(t) = \text{PmvO}_2(\text{BL}) + \Delta_1 \times (1 - e^{-(t-TD_1)/\tau_1}) + \Delta_2 \times (1 - e^{-(t-TD_2)/\tau_2}) \]

Where, PmvO\textsubscript{2} (t) is the PmvO\textsubscript{2} at any time t, PmvO\textsubscript{2} (BL) is the baseline pre-contracting PmvO\textsubscript{2}, \Delta_1 and \Delta_2 are the amplitudes of the PmvO\textsubscript{2} components, TD\textsubscript{1} and TD\textsubscript{2} are the independent time delays and \( \tau_1 \) and \( \tau_2 \) are the time constants for each component. Goodness of fit was determined by three criteria: 1. the coefficient of determination (i.e., r\textsuperscript{2}), 2. the sum of the squared residuals, and 3. visual inspection and analysis of the residual fit to a linear model.

The relative rate of change in PmvO\textsubscript{2} (dPO\textsubscript{2}/dt) was defined as the initial \( \Delta \text{PmvO}_2/\tau \) for the on-transient to contractions (McDonough et al., 2004b). In addition, area under the curve was calculated using the following formula for calculating the area of a trapezoid, \( \text{AUC} = ba + ((b*(c-a))/2) \), where \( a \) = the nadir of PmvO\textsubscript{2}, \( b \) = time and \( c \) = baseline PmvO\textsubscript{2}. AUC was calculated every 2 s and then summed to obtain a total AUC for the entire contractions protocol.

PmvO\textsubscript{2} values (e.g., baseline, steady-state contracting and delta), modeling dependent (e.g., TD, \( \tau \), MRT) results, VO\textsubscript{2} and Q data were analyzed using standard analysis of variance techniques between muscles (MG and WG). When a significant F value was demonstrated by the ANOVA, a Student-Newman-Keuls (SNK) post-hoc test was performed to determine differences among mean values. Pearson product-moment correlations were performed upon select variables. Statistical significance was accepted at P \( \leq 0.05 \).

3. Results

3.1 Body weight and citrate synthase activity

Body weight was significantly reduced in \( \beta \)-GPA vs. CON (275±4 vs. 317±10 g; P<0.05). Citrate synthase was not altered in either MG (MG: 25.9±0.8 vs. 25.7±3.7 \( \mu \)mol.min\textsuperscript{-1}.g\textsuperscript{-1} \( \beta \)-GPA vs. CON), or WG (WG: 11.3±0.6 vs. 11.0±0.7 \( \mu \)mol.min\textsuperscript{-1}.g\textsuperscript{-1}, both P>0.05 \( \beta \)-GPA vs. CON).

3.2 Microvascular PO\textsubscript{2}

Representative PmvO\textsubscript{2} responses for both muscles from \( \beta \)-GPA and CON animals are illustrated in Figures 1A & B, respectively. Mean PmvO\textsubscript{2} profiles between groups for each muscle are illustrated in Figure 2 and kinetic parameters described in Table 1. Mixed Gastrocnemius: No difference was noted for baseline PmvO\textsubscript{2} prior to contractions (Table 1). However, the steady-state contracting PmvO\textsubscript{2} was significantly elevated in the \( \beta \)-GPA group. The delta PmvO\textsubscript{2} (i.e., pre-contracting baseline minus steady-state contracting value) was significantly reduced in \( \beta \)-GPA vs. CON (Table 1). Furthermore, delta PmvO\textsubscript{2} for the secondary component was significantly and directionally different for MG between CON and \( \beta \)-GPA (Table 1). In addition, the overall change in PmvO\textsubscript{2} per unit of time (dPO\textsubscript{2}/dt)
was significantly slowed for β-GPA (Table 1), indicative of a slower fall to an elevated steady state baseline. In addition, the area under the curve (a model independent representation of the time taken to achieve a particular steady state) was substantially greater for β-GPA (Table 1). White Gastrocnemius: Similar to the MG there were no differences in the pre-contracting baseline PmvO₂ in the WG between groups, but the contracting steady-state value was elevated in the β-GPA group. Similar to the results noted for the MG noted above, the overall (dPO₂/dt) was reduced and the AUC was greater for β-GPA (Table 1).

3.3 Muscle blood flow (Q) and oxygen uptake (VO₂)

Q was not different between conditions at rest or during contractions in either muscle (Figure 3). There was a slight reduction in the calculated resting muscle VO₂ in the WG for β-GPA vs. CON (Figure 4), and no change in resting VO₂ in the MG. However, contracting VO₂ was significantly reduced in both muscles for β-GPA vs. CON (Figure 4).

4. Discussion

The principal original finding of this investigation is that β-GPA supplementation induces marked changes in the PmvO₂ response to contractions (Figure 1, Table 1) within the fast-twitch MG and WG muscles. Specifically, in both the mixed and white portions of the gastrocnemius muscle β-GPA treatment reduced the fall in PmvO₂ with contractions which led to an elevated contracting steady-state PmvO₂. In addition, PmvO₂ dynamics were slowed in the MG (but not WG) after β-GPA demonstrating an enhanced QO₂-to-VO₂ ratio across the rest-to-contractions transition. In both muscles these effects enhanced blood-myocyte O₂ driving pressure (PmvO₂) throughout the majority of the on-transient (i.e. AUC was significantly greater in β-GPA). Further, the change in PmvO₂ with β-GPA occurred in the absence of an altered hyperemia; at least during the steady-state of contractions (Figure 3). Finally, there was a reduction in the steady-state contracting VO₂ (Figure 4) in both muscles, possibly indicating an enhanced efficiency of muscular contractions, which has been demonstrated previously following experimental creatine depletion (Moerland and Kushmerick, 1994). Thus, in many respects β-GPA transformed the PmvO₂ profile in these fast-twitch muscles such that the qualitative responses were similar to that of slow-twitch muscle (see McDonough et al., 2005). Interestingly, the slowed PmvO₂ response and elevated contracting steady-state PmvO₂ occurred in the absence of an elevated oxidative capacity (i.e., no change in citrate synthase activity for either muscle). These results indicate that, following experimental creatine depletion via β-GPA, the ratio of QO₂/VO₂ is raised during contractions, indicative of an overall enhancement of muscle O₂ availability which may contribute to the improved oxidative and contractile function demonstrated for this condition (Moerland and Kushmerick, 1994).

4.1 β-GPA and PmvO₂ dynamics

In β-GPA PmvO₂ dynamics were slowed (dPO₂/dt) and the AUC was greater indicating that QO₂ responded with faster dynamics than VO₂, across the rest-to-contractions transition (Table 1). Whereas we did not measure Q or VO₂ dynamics in separatum, based upon the modeling of Diederich et al. (Haseler et al., 2004) with β-GPA compared to control, the slower PmvO₂ dynamics could arise from either: 1) a faster increase in Q (and hence QO₂) relative to VO₂, 2) slower QO₂ and VO₂ dynamics but with a greater proportional slowing of the VO₂ response, or 3) a similar QO₂ response but slower VO₂ kinetics. According to Meyer’s electrical analog model of mitochondrial respiratory control (Meyer, 1988; as recently validated in vitro by Glancy et al. 2008):

\[ \tau V \dot O_2 = Rm \cdot C \]
where Rm is the mitochondrial resistance to energy transfer and C is the metabolic capacitance of the total creatine pool (i.e., PCr + Cr). In the present investigation, Rm as reflected by oxidative enzyme capacity, would be unchanged (as also found for superficial and mixed gastrocnemius by Shoubridge et al. (1985)) but β-GPA would act to reduce C, thereby speeding VO₂ kinetics (reduced τVO₂) and refuting options 2 and 3 above. In contrast, there is a wealth of experimental evidence supporting that impediments to the CK reaction evoked either by MM creatine kinase knockout (Roman et al. 2002) or by stripping CK of useable substrate (β-GPA, Moerland and Kushmerick, 1994; iodoacetamide, Kindig et al. 2005) lead to a faster increase of ADPfree and VO₂ during contractions (Freyssenet et al., 1995; Moerland and Kushmerick, 1994). Thus the CK reaction (and its ability to temporally buffer ATP levels) may be a large component of the “lag” in VO₂ (i.e., finite VO₂ kinetics) following the onset of contractions. In accord with option 1 above, several lines of evidence support faster QO₂ dynamics with β-GPA. Specifically, β-GPA supplementation up-regulates AMPK activity (Bergeron et al., 2001) even in the absence of increased oxidative capacity/mitochondrial volume (Williams et al., 2009)). The pleiotropic effects of AMPK include increased endothelial NO synthase (eNOS) activation (Chen et al., 2009; Chen et al., 1999) and inhibition of NADPH oxidase activity (Schulz et al., 2008), both of which may increase the bioavailability of NO. In addition, activation of AMPK enhances resistance artery vasodilation via activation of eNOS (Bradley et al., 2010).

Therefore, during conditions of increased vascular shear-stress (e.g., onset of contractions), a greater production (and bioavailability) of endothelium-derived NO would be expected in the β-GPA group, which may act to accelerate the QO₂ response during contractions. In the current study, the fall in PmvO₂ was slowed and the contracting steady-state elevated in the β-GPA group with no difference PmvO₂ between groups at rest. Accordingly, the altered PmvO₂ response with contractions indicates that any augmentation of NO bioavailability in the β-GPA group would have greater effects during contractions versus rest. Indeed, increasing NO bioavailability in healthy skeletal muscle does not alter resting PmvO₂, but does slow the PmvO₂ response across the rest-exercise transition (Ferreira et al., 2006a; Ferreira et al., 2006c), suggesting an enhanced QO₂ relative to VO₂ during the exercise on-transient and the steady-state (Figures 2–4).

4.2 Effects of β-GPA on the O₂ Delivery (QO₂) Oxygen Uptake (VO₂) Relationship

In healthy control muscles, there exists a strong linear relationship between VO₂ and QO₂ such that increased QO₂ is driven by an elevated Q-to-VO₂ ratio (typically 5–6 L Q/L VO₂ (Poole et al., 2011). The unchanged steady-state Q (Figure 3, and therefore QO₂) in the face of decreased VO₂ (Figure 4) indicates that β-GPA changed the fundamental relationship between VO₂ and Q.

A β-GPA-induced reduction in the steady-state energy cost (i.e., increased efficiency) of contractions is a consistent finding (Moerland and Kushmerick, 1994), (Shoubridge and Radda, 1984). In the absence of increased mitochondrial oxidative capacity one putative mechanism for the elevated contractile efficiency herein is the elevated PmvO₂ which would act to increase intramyocyte PO₂ and thereby reduce disturbance of the intracellular milieu (Wilson et al., 1977). Furthermore, this effect would be in addition to faster VO₂ kinetics which in itself would allow a given VO₂ to be achieved with less change in phosphate-linked controllers of mitochondrial function (i.e., Δ[PCr], [ADP], [Pi]). Within contracting skeletal muscle, conditions expected to elevate PmvO₂ (and by extension intracellular PO₂) at a given power output (e.g., hyperoxia, (Macdonald et al., 1997; Wilkerson et al., 2006); respiratory muscle unloading, (Cross et al., 2010)) reduce the VO₂ cost by lowering the so-called VO₂ slow component (rev. (Poole et al., 2008)). The VO₂ slow component represents a high phosphate (ATP) cost of force production and hence there is a tight relationship between PCr, Pi and VO₂ as the VO₂ slow component increases VO₂ (Dimenna et al., 2010;
Rossiter et al., 2002; Rossiter et al., 2003). Therefore, potential mechanisms by which βGPA might reduce VO₂ relate to reduced ΔPCr and ΔPi either consequent to the direct effects of βGPA or, alternatively, by increasing the QO₂/VO₂ ratio and elevating PmvO₂ and thus intracellular PO₂.

It is pertinent to note that exercise training also facilitates this ‘tighter’ metabolic control. While β-GPA achieves this through a decrease in the total usable creatine pool (“C” in Meyer’s model; see above), exercise training will typically result in an increased mitochondrial enzyme activity (or decreased Rm in Meyer’s model) (Phillips et al., 1996) in addition to improvements in capillarity that will enhance blood-myocyte O₂ transport (Saltin and Gollnick, 1983; Poole et al. 1989; Poole and Mathieu-Costello, 1990). Thus, while β-GPA results in faster VO₂ kinetics, the mechanism is different than that achieved through exercise training.

4.3 Directions for future research

This study raises several intriguing possibilities that could usefully be addressed in future investigations using the β-GPA intervention. These include determining whether: 1. β-GPA supplementation alters the dynamics of resistance artery vasodilation, 2) despite an unaltered CS activity, biochemical adaptations in mitochondrial phenotype (i.e., intermyofibrillar versus subsarcolemmal) elicited by creatine depletion (Roussel et al., 2000) alter the “metabolic work” of the muscle due to the differential bioenergetics behaviors of distinct mitochondrial populations (Cogswell et al., 1993) and impact the VO₂ per unit work/tension (i.e., efficiency), 3) the heterogeneity of QO₂ is reduced and/or its speed increased with β-GPA treatment (likely through an AMPK mediated pathway (Poole et al., 2011)) during exercise. Addressing these possibilities may provide important insight into how alterations in the creatine kinase and/or AMPK pathways might contribute to oxidative and contractile function in skeletal muscle.

5. Conclusions

In the two fast-twitch skeletal muscles utilized in the current study (i.e., the mixed and superficial white portion of the gastrocnemius muscle) β-GPA supplementation induced significant changes in matching of QO₂-to-VO₂ during the rest-to-contractions transition, including a slower dPmvO₂/dt (MG) and an elevated contracting steady-state PmvO₂ (MG and WG). Further, despite an unaltered steady-state contracting Q between groups, β-GPA supplementation resulted in a lower contracting VO₂ in both muscles versus their controls. Interestingly, the altered PmvO₂ profile and reduced VO₂ occurred in the absence of any changes in citrate synthase activity (marker of oxidative capacity) but, with respect to the dynamic matching of QO₂ and VO₂, make these fast-twitch muscles resemble slow twitch muscles (e.g., soleus, Behnke et al. 2003; McDonough et al. 2005).

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References


Folkow B, Halicka HD. A comparison between “red” and “white” muscle with respect to blood supply, capillary surface area and oxygen uptake during rest and exercise. Microvascular Research. 1968; 1.


Kano Y, Poole DC, Sudo M, Hirachi T, Miura S, Ezaki O. Control of microvascular PO2 kinetics following onset of muscle contractions: Role for AMPK. Am J Physiol Regul Integr Comp Physiol. 2011


## Highlights

- In this study we examined the effects of experimental creatine depletion on muscle oxygenation, blood flow and $O_2$ uptake.
- We contracted fast-twitch muscle in control and animals that consumed beta-GPA to deplete creatine stores.
- We found that beta-GPA enhanced muscle oxygenation across the rest-to-contractions transition.
- We also found that blood flow was not different between groups, but contracting $O_2$ uptake was reduced in the beta-GPA group.
- We conclude that experimental creatine depletion enhances muscle microvascular oxygenation which may lead to enhanced contractile performance in fast-twitch muscle.
Figure 1.
Microvascular O$_2$ partial pressure (P$_{mv}$O$_2$) responses for the mixed (MG) and white gastrocnemius (WG) from representative animals in A) control and B) β-GPA groups following the onset of 1 Hz contractions (time 0). Note that while baseline P$_{mv}$O$_2$ is not different between muscles or conditions, β-GPA supplementation significantly increased the steady state contracting P$_{mv}$O$_2$. The thin line is the measured P$_{mv}$O$_2$ value whereas the smoothed line is the model-fit of the response.
Figure 2.
Mean microvascular O\textsubscript{2} partial pressure (P\textsubscript{mvO\textsubscript{2}}) profiles from control and β-GPA treated animals across the rest-to-contractions transition in the A) mixed gastrocnemius and B) white gastrocnemius. Contractions were initiated at time zero. Average kinetic parameters are described in Table 1.
Figure 3.
Muscle blood flow (Q) in the A) mixed and B) white portions of the gastrocnemius muscle at rest (i.e., immediately prior to the onset of contractions) and during the steady-state of contractions (i.e., 3 min after the onset of contractions) in both control and β-GPA groups. * denotes significantly higher Q compared to MG (p<0.05).
Figure 4.
Calculated muscle oxygen uptake (VO₂) in the A) mixed and B) white portions of the gastrocnemius muscle at rest (i.e., immediately prior to the onset of contractions) and during the steady-state of contractions (i.e., 3 min after the onset of contractions) in both control and β-GPA groups. * denotes significantly different compared to MG for the same condition (p<0.05). # P<0.05 versus control group.
Table 1

Microvascular PO₂ following the onset of contractions in mixed (MG) and white (WG) gastrocnemius muscles from Control and β-GPA rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MG</th>
<th>WG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary component</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-contractions PmvO₂ (mmHg)</td>
<td>25.1 ± 1.1</td>
<td>20.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Delta PmvO₂ (mmHg)</td>
<td>−16.3 ± 1.7</td>
<td>−12.3 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Time delay (s)</td>
<td>5.3 ± 1.0</td>
<td>5.1 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Time constant (s)</td>
<td>7.0 ± 1.3</td>
<td>12.6 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>dPO₂/dt (mmHg/s)</td>
<td>3.2 ± 0.4</td>
<td>1.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Secondary component</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta PmvO₂ (mmHg)</td>
<td>2.1 ± 1.4</td>
<td>1.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Time delay (s)</td>
<td>34.5 ± 8.1</td>
<td>63.0 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Time constant (s)</td>
<td>50.4 ± 22.0</td>
<td>72.0 ± 27.7</td>
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</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS PmvO₂ (mmHg)</td>
<td>10.3 ± 1.6</td>
<td>8.7 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>dPO₂/dt (mmHg/s)</td>
<td>1.0 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>AUC (mmHg/180s)</td>
<td>3932 ± 195</td>
<td>3535 ± 228</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>β-GPA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary component</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-contractions PmvO₂ (mmHg)</td>
<td>25.9 ± 1.4</td>
<td>25.0 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Delta PmvO₂ (mmHg)</td>
<td>−7.5 ± 1.5#</td>
<td>−9.1 ± 1.8#</td>
<td></td>
</tr>
<tr>
<td>Time delay (s)</td>
<td>7.1 ± 1.6</td>
<td>5.6 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Time constant (s)</td>
<td>9.6 ± 6.0</td>
<td>10.8 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>dPO₂/dt (mmHg/s)</td>
<td>2.1 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Secondary component</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta PmvO₂ (mmHg)</td>
<td>−4.0 ± 2.5</td>
<td>2.1 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Time delay (s)</td>
<td>30.3 ± 8.4</td>
<td>39.7 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>Time constant (s)</td>
<td>36.8 ± 10.9</td>
<td>52.9 ± 15.4</td>
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</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SS PmvO₂ (mmHg)</td>
<td>15.5 ± 2.1#</td>
<td>17.4 ± 1.9#</td>
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</tr>
<tr>
<td>dPO₂/dt (mmHg/s)</td>
<td>0.4 ± 0.1#</td>
<td>0.3 ± 0.1#</td>
<td></td>
</tr>
<tr>
<td>AUC (mmHg/180s)</td>
<td>4642 ± 394#</td>
<td>4423 ± 308#</td>
<td></td>
</tr>
</tbody>
</table>

Values presented as mean ± SE.

# denotes significant difference from Control. SS PmvO₂: the steady-state contracting PmvO₂. AUC (area under the curve), the sum PmvO₂ from the onset of contractions through the end of the contracting paradigm (180 s).