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

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Running title: Microvascular oxygenation, fiber type and β-guanidinopropionic acid

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Abstract

Aging, heart failure and diabetes each compromise the matching of O_2 delivery ($\dot{Q}O_2$)-to-metabolic requirements (O_2 uptake, $\dot{V}O_2$) in skeletal muscle such that the O_2 pressure driving blood-myocyte O_2 flux (microvascular PO_2, $P_{mv}O_2$) 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 $\dot{Q}O_2$-to-$\dot{V}O_2$ matching (elevated $P_{mv}O_2$) 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 $P_{mv}O_2$ fell less following β-GPA (MG -54%, WG -26%, $p<0.05$), elevating steady-state $P_{mv}O_2$ (CON, MG: 10±2, WG: 9±1; β-GPA, MG 16±2, WG 18±2 mmHg, $P<0.05$). This reflected an increased $\dot{Q}O_2/\dot{V}O_2$ ratio due primarily to a reduced $\dot{V}O_2$ in β-GPA muscles. It is likely that this adaptation helps facilitate the β-GPA-induced enhancement of contractile function in fast-twitch muscles.

Key words: β-guanidinopropionic acid; microvascular O_2 exchange; muscle fiber type; O_2 extraction; phosphorescence quenching

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 O_2 uptake ($\dot{V}O_2$) kinetics (Barstow *et al.*, 1996), as well as blood flow ($\dot{Q}$) and O_2 delivery ($\dot{Q}O_2$) (Ferreira *et al.*, 2006b). Investigation of the mechanistic bases for the heterogeneity of $\dot{Q}$ and hence $\dot{Q}O_2$ 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 $\dot{Q}O_2$ per unit $\dot{V}O_2$ (Behnke et al., 2002a; Behnke et al., 2004). The $\dot{Q}O_2$:$\dot{V}O_2$ ratio is of crucial importance because it determines the microvascular O_2 partial pressure ($P_{mv}O_2$) 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 $P_{mv}O_2$ 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, $P_{mv}O_2$ 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 $\dot{V}O_2$ 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; Regensteiner et al., 1998; Sietsema et al., 1994) (though see (Wilkerson et al., 2011) for an exception in long-term diabetic patients) the lowered P*mv*O_2 indicates a reduced $\dot{Q}O_2$-to-$\dot{V}O_2$ 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 P*mv*O_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 $\dot{Q}O_2$ versus $\dot{V}O_2$ 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 $\dot{Q}O_2$-to-$\dot{V}O_2$ 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 P*mv*O_2 fall (presumably due to faster blood flow

kinetics) during muscular contractions resulting in an elevated steady-state contracting P*mv*O_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 P*mv*O_2, which would then help facilitate transcapillary O_2 flux and act to increase intramyocyte PO_2.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University. Rats were housed individually at 23^{o}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*.

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 P*mv*O_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.

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.

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 P$mv$$O_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.

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 P*mv*O_2 from a measured phosphorescence lifetime using the following equation :

$$P\mathit{mv}O_2 = [(t^o/t)-1] / (k_Q * t^o)$$

where k_Q is the quenching constant (mmHg/s) and t^o and t are the phosphorescence lifetimes in the absence of O_2 and at the ambient O_2 concentration, respectively. For R2, in *in vitro* conditions similar to those found in the blood, k_Q is 409 $mmHg^{-1}.s^{-1}$ and t^o 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 PO_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.

Muscle blood flow and oxygen uptake. Muscle blood flow ($\dot{Q}$) 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

($ml.min^{-1}.100g^{-1}$). Microspheres (15 μm-diameter; ^{46}Sc, ^{85}Sr or ^{141}Ce; New England Nuclear, Boston, MA) were agitated via sonication and ~5 $x10^5$ 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 ($\dot{V}O_2$) was calculated as previously described (Behnke *et al.*, 2002b). Arterial O_2 content (C_aO_2) was measured directly (carotid arterial blood) and mixed venous O_2 content (C_vO_2) was calculated from $PmvO_2$ (assuming $PmvO_2$ is a valid approximation of mixed venous PO_2; (McDonough *et al.*, 2001)) using the rat O_2 dissociation curve (constructed using an "n" of 2.6, the measured [Hb], P_{50} of 38 mmHg and an O_2 carrying capacity of 1.39 $mlO_2.g\ Hb^{-1}$). $\dot{V}O_2$ was then calculated via the Fick equation, i.e. $\dot{V}O_2 = \dot{Q} * (C_aO_2 - C_vO_2)$.

Citrate synthase measurement. Following the experimental protocol, contralateral (i.e., non-stimulated) muscles were excised rapidly, frozen in liquid N_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.

Curve Fitting and Statistical Analysis. For the P*mv*O_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:

$$P\textit{mv}O_2\,(t) = P\textit{mv}O_{2\,(BL)} + \Delta\, P\textit{mv}O_2 * (1\text{-}e^{-(t\text{-}TD)/\tau})$$

or a two-component model:

$$P\textit{mv}O_2\,(t) = P\textit{mv}O_{2\,(BL)} + \Delta_1 * (1\text{-}e^{-(t\text{-}TD_1)/\tau_1}) + \Delta_2 * (1\text{-}e^{-(t\text{-}TD_2)/\tau_2})$$

Where, P*mv*O_2 (t) is the P*mv*O_2 at any time t, P*mv*$O_{2\,(BL)}$ is the baseline pre-contracting P*mv*O_2, $\Delta_1$and Δ_2 are the amplitudes of the P*mv*O_2 components, TD_1 and TD_2 are the independent time delays and τ_1 and τ_2 are the time constants for each component. Goodness of fit was determined by three criteria: 1. the coefficient of determination (i.e., r^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 P*mv*O_2 (dPo_2/dt) was defined as the initial Δ P*mv*O_2/τ 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, AUC = ba + ((b*(c-a))/2, where a = the nadir of P*mv*O_2, b = time and c = baseline P*mv*O_2. AUC was calculated every 2 s and then summed to obtain a total AUC for the entire contractions protocol.

P*mv*O_2 values (e.g., baseline, steady-state contracting and delta), modeling dependent (e.g., TD, τ, MRT) results, $\dot{V}O_2$ and $\dot{Q}$ 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 $P \leq 0.05$.

Results

Body weight and citrate synthase activity

Body weight was significantly reduced in β-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 $\mu mol.min^{-1}.g^{-1}$ β-GPA vs. CON), or WG (WG: 11.3±0.6 vs. 11.0±0.7 $\mu mol.min^{-1}.g^{-1}$, both $P>0.05$ β-GPA vs. CON).

Microvascular PO_2

Representative $P_{mv}O_2$ responses for both muscles from β-GPA and CON animals are illustrated in Figures 1A & B, respectively. Mean $P_{mv}O_2$ 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 $P_{mv}O_2$ prior to contractions (Table 1). However, the steady-state contracting $P_{mv}O_2$ was significantly elevated in the β-GPA group. The delta $P_{mv}O_2$ (i.e., pre-contracting baseline minus steady-state contracting value) was significantly reduced in β-GPA vs. CON (Table 1). Furthermore, delta $P_{mv}O_2$ for the secondary component was significantly and directionally different for MG between CON and β-GPA (Table 1). In addition, the overall change in $P_{mv}O_2$ per unit of time (dPO_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 $P_{mv}O_2$ 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 (dPO_2/dt) was reduced and the AUC was greater for β-GPA (Table 1).

Muscle blood flow ($\dot{Q}$) and oxygen uptake ($\dot{V}O_2$)

$\dot{Q}$ 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 $\dot{V}O_2$ in the WG for β-GPA vs. CON (Figure 4), and no change in resting $\dot{V}O_2$ in the MG. However, contracting $\dot{V}O_2$ was significantly reduced in both muscles for β-GPA vs. CON (Figure 4).

Discussion

The principal original finding of this investigation is that β-GPA supplementation induces marked changes in the P*mv*O_2 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 P*mv*O_2 with contractions which led to an elevated contracting steady-state P*mv*O_2. In addition, P*mv*O_2 dynamics were slowed in the MG (but not WG) after β-GPA demonstrating an enhanced $\dot{Q}O_2$-to-$\dot{V}O_2$ ratio across the rest-to-contractions transition. In both muscles these effects enhanced blood-myocyte O_2 driving pressure (P*mv*O_2) throughout the majority of the on-transient (i.e. AUC was significantly greater in β-GPA). Further, the change in P*mv*O_2 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 $\dot{V}O_2$ (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 P*mv*O_2 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 P*mv*O_2 response and elevated contracting steady-state P*mv*O_2 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 $\dot{Q}O_2$/ $\dot{V}O_2$ is raised during contractions, indicative of an overall enhancement of muscle O_2 availability which may contribute to the improved oxidative and contractile function demonstrated for this condition (Moerland and Kushmerick, 1994).

β -GPA and PmvO_2 *dynamics*

In β-GPA P*mv*O_2 dynamics were slowed (↓dPO_2/dt) and the AUC was greater indicating that $\dot{Q}O_2$ responded with faster dynamics than $\dot{V}O_2$, across the rest-to-contractions transition (Table 1). Whereas we did not measure $\dot{Q}$ or $\dot{V}O_2$ dynamics *in separatum*, based upon the modeling of Diederich et. al. (Haseler et al., 2004) with β-GPA compared to control, the slower P*mv*O_2 dynamics could arise from either: 1) a faster increase in $\dot{Q}$ (and hence $\dot{Q}O_2$) relative to $\dot{V}O_2$, 2) slower $\dot{Q}O_2$ and $\dot{V}O_2$ dynamics but with a greater proportional slowing of the $\dot{V}O_2$ response, or 3) a similar $\dot{Q}O_2$ response but slower $\dot{V}O_2$ kinetics. According to Meyer's electrical analog model of mitochondrial respiratory control (Meyer, 1988; as recently validated in vitro by Glancy et al. 2008):

$$\tau\dot{V}O_2 = Rm \bullet C$$

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 $\dot{V}O_2$ kinetics (reduced $\tau\dot{V}O_2$) 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 ADP_{free} and $\dot{V}O_2$ 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 $\dot{V}O_2$ (i.e., finite $\dot{V}O_2$ kinetics) following the onset of contractions.

In accord with option 1 above, several lines of evidence support faster $\dot{Q}O_2$ 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 $\dot{Q}O_2$ response during contractions. In the current study, the fall in $P\bar{m}vO_2$ was slowed and the contracting steady-state elevated in the β-GPA group with no difference $P\bar{m}vO_2$ between groups at rest. Accordingly, the altered $P\bar{m}vO_2$ 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 $P\bar{m}vO_2$, but does slow the $P\bar{m}vO_2$ response across the rest-exercise transition (Ferreira *et al.*, 2006a; Ferreira *et al.*, 2006c), suggesting an enhanced $\dot{Q}O_2$ relative to $\dot{V}O_2$ during the exercise on-transient and the steady-state (Figures 2-4).

Effects of β-GPA on the O_2 Delivery ($\dot{Q}O_2$) Oxygen Uptake ($\dot{V}O_2$) Relationship

In healthy control muscles, there exists a strong linear relationship between $\dot{V}O_2$ and $\dot{Q}O_2$ such that increased $\dot{Q}O_2$ is driven by an elevated Q-to-Vo_2 ratio (typically 5-6 L $\dot{Q}$ /L $\dot{V}O_2$ (Poole et al., 2011). The unchanged steady-state $\dot{Q}$ (Figure 3, and therefore $\dot{Q}O_2$) in the face of decreased $\dot{V}O_2$ (Figure 4) indicates that β-GPA changed the fundamental relationship between $\dot{V}O_2$ and $\dot{Q}$.

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 P*mv*O_2 which would act to increase intramyocyte PO_2 and thereby reduce disturbance of the intracellular milieu (e.g., decrease [Pi]; (Wilson *et al.*, 1977)). As [Pi] has numerous negative effects on contractile function (e.g., reduces the number of force-generating cross bridges (Allen et al., 2008) and may decrease free Ca_2^+ available for release (Fryer et al., 1997), an enhanced intramyocyte PO_2 may mitigate increases in [Pi] and improve contractile function. Furthermore, this effect would be in addition to faster $\dot{V}O_2$ kinetics which in itself would allow a given $\dot{V}O_2$ to be achieved with less change in phosphate-linked controllers of mitochondrial function (i.e., Δ[PCr], [ADP_f],[Pi]). 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 O_2 transport (Saltin and Gollnick, 1983; Poole et al. 1989; Poole and Mathieu-Costello, 1990). Thus, while β-GPA results in faster $\dot{V}O_2$ kinetics, the mechanism is different than that achieved through exercise training

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 $\dot{V}O_2$ per unit work/tension (i.e., efficiency), 3) the heterogeneity of $\dot{Q}O_2$ 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.

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 $\dot{Q}O_2$-to-$\dot{V}O_2$ during the rest-to-contractions transition, including a slower $dP\textit{mv}O_2/dt$ (MG) and an elevated contracting steady-state $P\textit{mv}O_2$ (MG and WG). Further, despite an unaltered steady-state contracting $\dot{Q}$ between groups, β-GPA supplementation resulted in a lower contracting $\dot{V}O_2$ in both muscles versus their controls. Interestingly, the altered $P\textit{mv}O_2$ profile and reduced $\dot{V}O_2$ occurred in the absence of any changes in citrate synthase activity (marker of oxidative capacity) but, with respect to the dynamic matching of $\dot{Q}O_2$ and $\dot{V}O_2$, make these fast-twitch muscles resemble slow twitch muscles (e.g., soleus, Behnke et al. 2003; McDonough et al. 2005).

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Figure Legends

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_2 partial pressure ($P_{mv}O_2$) 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 ($\dot{Q}$) 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 $\dot{Q}$ compared to MG ($p<0.05$).

Figure 4. Calculated muscle oxygen uptake ($\dot{V}O_2$) 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 PO_2 following the onset of contractions in mixed (MG) and white (WG) gastrocnemius muscles from Control and β-GPA rats.

	MG	WG
Control		
Primary component		
Pre-contractions P*mv*O_2 (mmHg)	25.1 ± 1.1	20.2 ± 2.0
Delta P*mv*O_2 (mmHg)	-16.3 ± 1.7	-12.3 ± 1.8
Time delay (s)	5.3 ± 1.0	5.1 ± 1.1
Time constant (s)	7.0 ± 1.3	12.6 ± 3.0
dPO_2/dt (mmHg/s)	3.2 ± 0.4	1.4 ± 0.2
Secondary component		
Delta P*mv*O_2 (mmHg)	2.1 ± 1.4	1.3 ± 0.5
Time delay (s)	34.5 ± 8.1	63.0 ± 2.7
Time constant (s)	50.4 ± 22.0	72.0 ± 27.7
Overall		
SS P*mv*O_2 (mmHg)	10.3 ± 1.6	8.7 ± 1.0
dPO_2/dt (mmHg/s)	1.0 ± 0.3	0.6 ± 0.2
AUC (mmHg/180s)	3932 ± 195	3535 ± 228
β-GPA		
Primary component		
Pre-contractions P*mv*O_2 (mmHg)	25.9 ± 1.4	25.0 ± 2.1
Delta P*mv*O_2 (mmHg)	-7.5 ± 1.5#	-9.1 ± 1.8#
Time delay (s)	7.1 ± 1.6	5.6 ± 1.1
Time constant (s)	9.6 ± 6.0	10.8 ± 2.8
dPO_2/dt (mmHg/s)	2.1 ± 0.2	1.3 ± 0.2
Secondary component		
Delta P*mv*O_2 (mmHg)	-4.0 ± 2.5	2.1 ± 2.6
Time delay (s)	30.3 ± 8.4	39.7 ± 8.9
Time constant (s)	36.8 ± 10.9	52.9 ± 15.4
Overall		
SS P*mv*O_2 (mmHg)	15.5 ± 2.1#	17.4 ± 1.9#
dPO_2/dt (mmHg/s)	0.4 ± 0.1#	0.3 ± 0.1#
AUC (mmHg/180s)	4642 ± 394#	4423 ± 308#

Values presented as mean ± SE. # denotes significant difference from Control. SS P*mv*O_2, the steady-state contracting P*mv*O_2. AUC (area under the curve), the sum P*mv*O_2 from the onset of contractions through the end of the contracting paradigm (180 s).

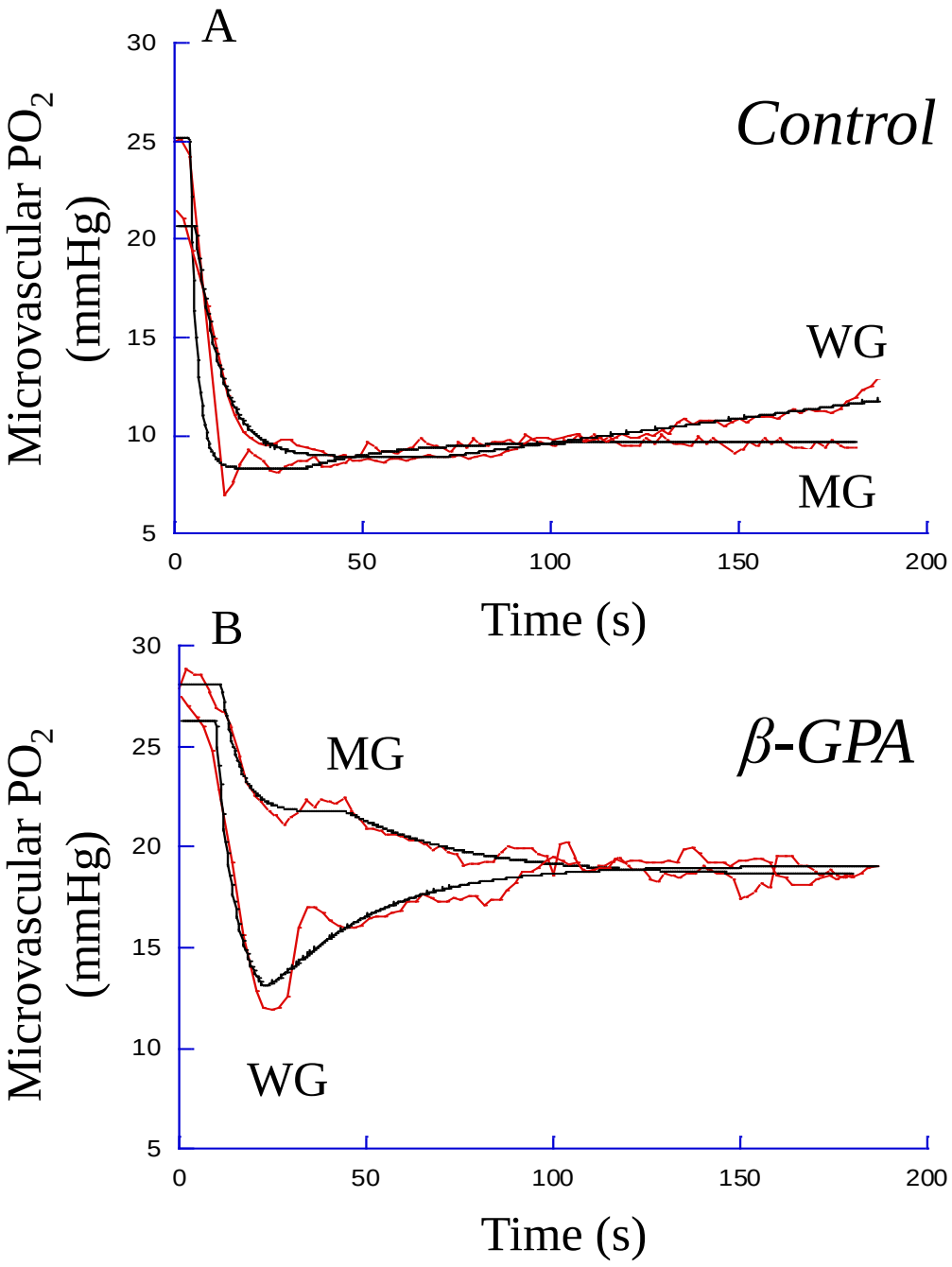

A
Control
WG
MG
Microvascular PO_2 (mmHg)
Time (s)
B
β-GPA
MG
WG
Microvascular PO_2 (mmHg)
Time (s)

Figure 1

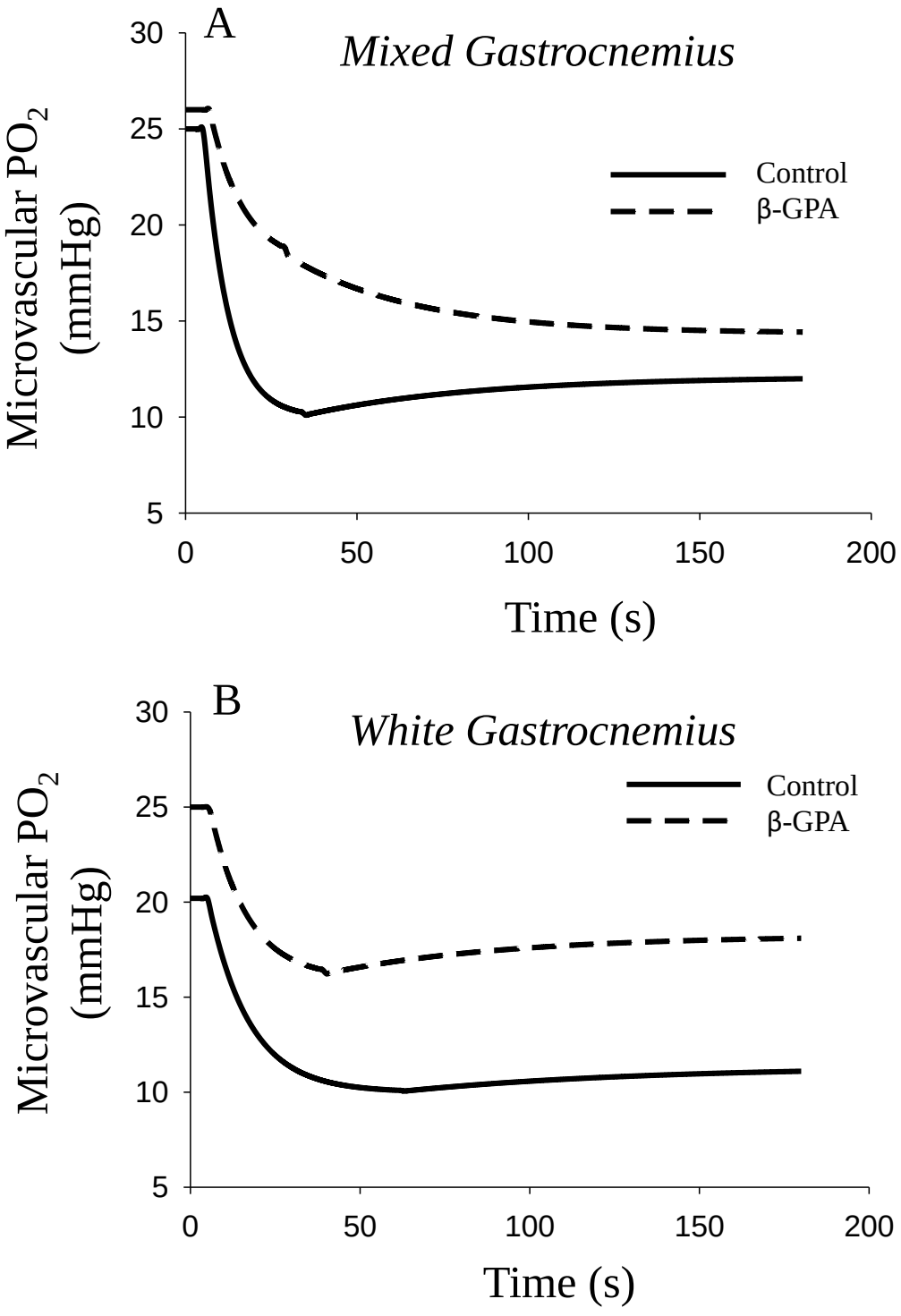

A
Mixed Gastrocnemius
Microvascular PO_2 (mmHg)
30
25
20
15
10
5
0
50
100
150
200
Time (s)
Control
β-GPA
B
White Gastrocnemius
Microvascular PO_2 (mmHg)
30
25
20
15
10
5
0
50
100
150
200
Time (s)
Control
β-GPA

Figure 2

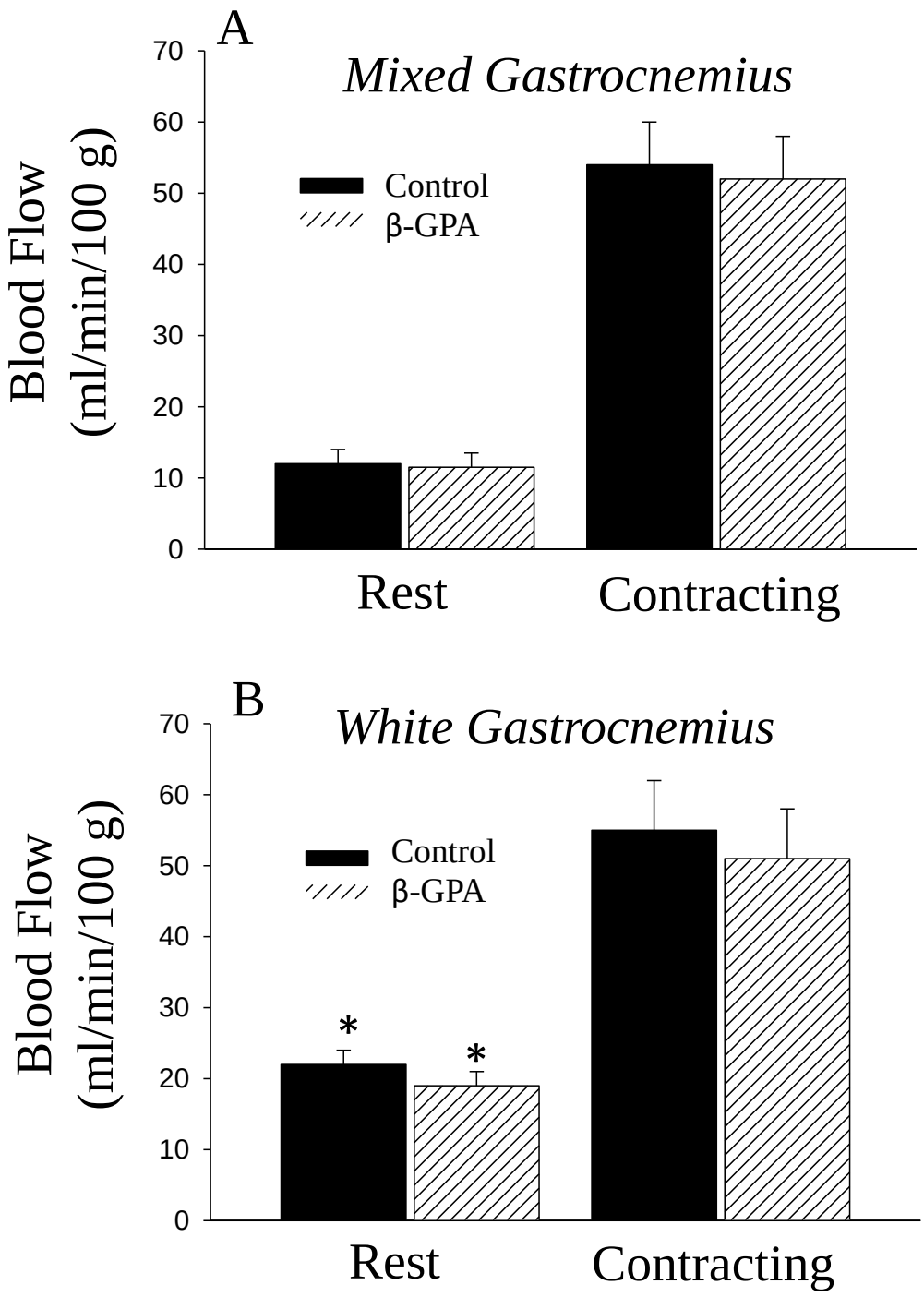
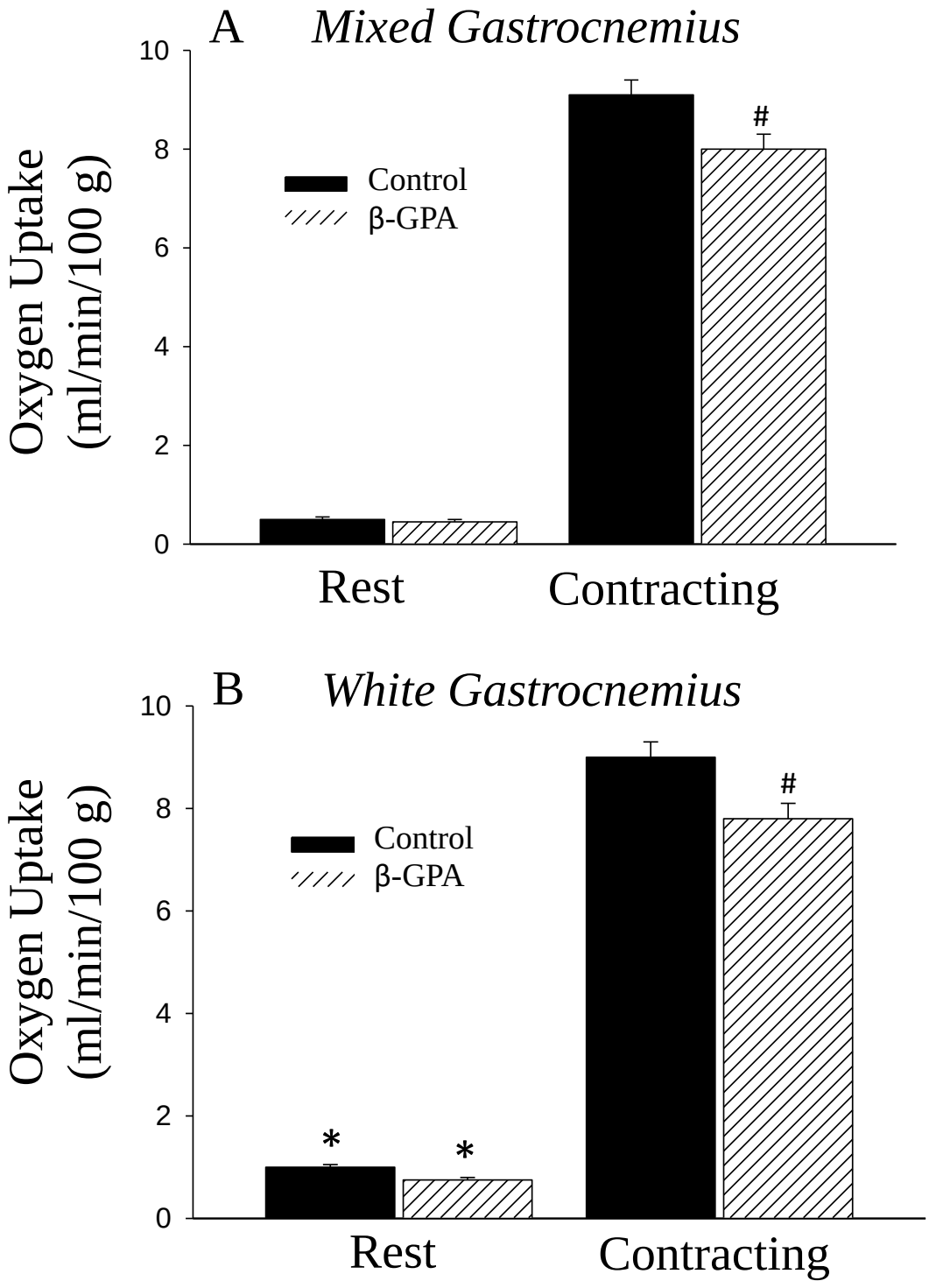

A
Mixed Gastrocnemius
Blood Flow
(ml/min/100 g)
70
60
50
40
30
20
10
0
Control
β-GPA
Rest
Contracting
B
White Gastrocnemius
Blood Flow
(ml/min/100 g)
70
60
50
40
30
20
10
0
Control
β-GPA
*
*
Rest
Contracting

Figure 3


A
Mixed Gastrocnemius
Oxygen Uptake
(ml/min/100 g)
10
8
6
4
2
0
Control
β-GPA
#
Rest
Contracting
B
White Gastrocnemius
Oxygen Uptake
(ml/min/100 g)
10
8
6
4
2
0
Control
β-GPA
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Rest
Contracting

Figure 4