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Acute blockade of ATP-sensitive K$^+$ channels impairs skeletal muscle vascular control in rats during treadmill exercise

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Key points

- The ATP-sensitive K^+ (K_{ATP}) channel is activated, in part, by reductions in the ratio of ATP-to-ADP and may therefore contribute to the integration of cellular metabolism with vasomotor tone.

- The impact of K_{ATP} channels on exercising skeletal muscle blood flow (BF) may be dependent upon whether the metabolic demand elicits a sufficiently low O_2 tension to drive ADP accumulation and open K_{ATP} channels.

- Rats performed treadmill exercise (20 m min^{-1}, 5% incline) for the determination of BF and vascular conductance (VC) before and after K_{ATP} channel blockade via glibenclamide.

- K_{ATP} channel blockade reduced exercising skeletal muscle BF and VC in a fibre type-selective manner and increased arterial blood [lactate].

- These data suggest that although the VC response to exercise relies on potentially redundant mechanisms K_{ATP} channel function is requisite for healthy, large muscle mass exercise hyperaemia in rats.

Abstract

The ATP-sensitive K^+ (K_{ATP}) channel is a class of inward rectifier K^+ channels that can link cellular metabolic status to vasomotor tone across the metabolic transients seen with exercise. This investigation tested the hypothesis that if K_{ATP} channels are crucial to exercise hyperaemia then blockade via glibenclamide (GLI) would lower hindlimb skeletal muscle blood flow (BF) and vascular conductance (VC) during treadmill exercise. In 14 adult male Sprague Dawley rats mean arterial pressure (MAP), blood [lactate], and hindlimb muscle BF (radiolabelled microspheres) were determined at rest ($n = 6$) or during exercise ($n = 8$; 20 m min^{-1}, 5% incline) under control (CON) and GLI conditions (5 mg kg^{-1}, i.a). At rest and during exercise, MAP was higher (Rest, CON: 130 ± 6, GLI: 152 ± 8; Exercise, CON: 140 ± 4, GLI: 147 ± 4 mmHg, $P < 0.05$) and heart rate (HR) was lower (Rest, CON: 440 ± 16, GLI: 410 ± 18; Exercise, CON: 560 ± 4, GLI: 540 ± 10 beats min^{-1}, $P < 0.05$) with GLI. Hindlimb muscle BF (CON: 144 ± 10, GLI: 120 ± 9 ml min^{-1} (100 g)$^{-1}$, $P < 0.05$) and VC were lower with GLI during exercise but not at rest. Specifically, GLI decreased BF in 12, and VC in 16, of the 28 individual hindlimb muscles and muscle parts sampled during exercise with a greater fractional reduction present in muscles comprised predominantly of type I and type IIa fibres ($P < 0.05$). Additionally, blood [lactate] (CON: 2.0 ± 0.3; GLI: 4.1 ± 0.9 mmol L^{-1}, $P < 0.05$) was higher during exercise with GLI. That K_{ATP} channel blockade reduces hindlimb muscle BF during exercise in rats supports the obligatory contribution of K_{ATP} channels in large muscle mass exercise-induced hyperaemia.

Abbreviations

BF, blood flow; CON, control; GLI, glibenclamide; HR, heart rate; K_{ATP}, ATP-sensitive potassium channel; Kir, inward rectifier potassium channels; K_{NDP}, nucleotide-dependent potassium channel; MAP, mean arterial pressure; SMC, smooth muscle cell; SNA, sympathetic nerve activity; VC, vascular conductance

Introduction

Fundamental to cardiovascular control during exercise is an elevated sympathetic nerve activity (SNA) which increases heart rate (HR), stroke volume and therefore cardiac output. Appropriate redistribution of the increased cardiac output at exercise onset is achieved by vascular smooth muscle cell (SMC) relaxation in arterioles supplying active skeletal muscle and SMC contraction in arterioles supplying quiescent tissue. Accordingly, the elevation in SNA which drives cardiac output also precipitates increased vascular resistance in skeletal muscle via activation of α_2-adrenergic receptors (Thomas and Segal 2004). Within the active skeletal muscle vascular bed, local metabolic byproducts of muscle contraction (e.g. ATP, H^+, K^+, adenosine, etc.) as well as endothelium derived factors (e.g. NO, PGI_2) are important for promoting vasodilation and matching O_2 supply with O_2 demand during exercise (Dinenno and Joyner 2004).

The SMC resting membrane potential plays a prominent role in setting vascular tone as well as determining vasomotor sensitivity to depolarizing stimuli (i.e. SNA). Modulation of membrane potential may therefore represent an important mechanism by which sympathetic vasoconstriction is attenuated in active skeletal muscle (Thomas et al. 1997). It is well recognized that the inward rectifier K^+ channels (Kir) exert this control and are capable of hyperpolarizing the SMC membrane resulting in an inhibitory effect on excitability and thus relaxation (Nelson and Quayle 1995). Specifically, the ATP-sensitive K^+ (K_{ATP}) channel is activated, in part, by reductions in the ratio of subsarcolemmal ATP-to-ADP and may therefore contribute to the integration of cellular metabolism with vasomotor tone across the greater than 2 orders of magnitude increase in muscle metabolism seen with exercise. Importantly, K_{ATP} channel activation has been shown to result in SMC hyperpolarization, relaxation of vascular smooth muscle and attenuated α-adrenergic vasoconstriction (Quast et al. 1994; Nakai and Ichihara 1994; Tateishi and Faber 1995; Mori et al. 1995). In healthy human forearm muscle the activation of K_{ATP} channels increases vasodilation in response to ischemia indicating the potential for K_{ATP} channels to contribute substantially to skeletal muscle O_2 delivery during exercise (Biljstra et al. 1996b). Despite early studies demonstrating that blockade of K_{ATP} channels significantly

attenuates reactive and functional hyperaemia in humans and animals (Bank et al. 2000, Biljstra et al. 1996a, Banitt et al. 1996) more recent work has failed to confirm these results (Duncker et al. 2001, Farouque et al. 2003, Shrage et al. 2006). Thus, it remains unclear whether the K_{ATP} channel represents an obligatory mechanism supporting exercise-induced skeletal muscle hyperaemia. Additionally, given that K_{ATP} channels are sensitive to metabolic status and that there exists considerable heterogeneity of metabolic characteristics among and within skeletal muscles it is plausible that K_{ATP} channel-mediated vasodilation is dependent upon skeletal muscle fibre type distribution; a concept that cannot be addressed in humans with current technology.

The purpose of the present investigation was to test the hypothesis that blockade of K_{ATP} channels via glibenclamide (GLI) would reduce hindlimb skeletal muscle blood flow (BF) and vascular conductance (VC) and increase arterial blood [lactate] during submaximal treadmill exercise in healthy rats. Furthermore, given the purported coupling of cellular metabolism with vasomotor tone by K_{ATP} channels, it was anticipated that the reductions in VC with GLI would associate directly with the percentage of type I and type IIa fibres across muscles.

Methods

Ethical approval

All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University under the guidelines established by the National Institutes of Health and conducted according to the animal use guidelines mandated by *The Journal of Physiology* (Drummond, 2009). 14 adult male Sprague-Dawley rats (~4 months old, body mass = 366 ± 7 g) were maintained in accredited animal facilities (Association for the Assessment and Accreditation of Laboratory Animal Care) at Kansas State University on a 12-h light/12-h dark cycle with food and water provided *ad libitum*. Rats were separated into either rest ($n = 6$) or exercise ($n = 8$) groups and used for within-animal comparisons under control (CON) and K_{ATP} channel blockade (GLI) conditions. Rats were acclimatized to running during a familiarization period comprised of 5-7 sessions on a custom-built motor-driven treadmill set at an incline of 5%. Each session consisted of running at ~20 m min^{-1} over a total duration of no more than 5 min.

The pharmacological sulphonylurea derivative GLI (494 g mol^{-1}; 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-methoxybenzamide; Sigma-Aldrich, St Louis, MO, USA) was used to achieve blockade of vascular K_{ATP} channels. Briefly, 50 mg of GLI was dissolved in 4 ml of NaOH (0.1 M) to produce a 12.5 mg ml^{-1} stock solution. A 5 mg kg^{-1} dose was drawn from the stock solution and diluted to ~1 ml with heparinized saline. It has been reported previously that GLI is a selective K_{ATP} channel blocker at concentrations below 5 µmol L^{-1} (Beech et al. 1993, Sadraei and Beech 1995a). The current dose of 5 mg kg^{-1} for rats of a mean body mass of 366 g equates to a blood concentration of ~140 µmol L^{-1}. Given that 98-99% of GLI is bound to plasma protein, the effective blood concentration of GLI is ~2-3 µmol L^{-1} (George et al. 1990) which is in the range for GLI to be selective for K_{ATP} channels.

Surgical instrumentation

On the day of the final protocol the rats were anesthetized initially with a 5% isoflurane-O_2 mixture and maintained on a 3% isoflurane-O_2 mixture for the duration of the surgical instrumentation. Cannulation of both the carotid and caudal arteries was performed with PE-10 connected to PE-50 (Intra-Medic polyethylene tubing, BD, Franklin Lakes, NJ, USA). The catheters were then tunneled subcutaneously to the dorsal aspect of the cervical region where they were exteriorized through a puncture wound in the skin. Following closure of incisions the rat was removed from anesthesia and given a minimum recovery period of 2 h.

Experimental protocol

After the recovery period the exercise BF protocol ($n = 8$) was performed with the treadmill set at an incline of 5%. The rat was placed on the treadmill and the carotid catheter was attached to a pressure transducer (P23ID, Gould Statham Instruments, Hato Rey, Puerto Rico, USA) for the measurement of mean arterial pressure (MAP) and HR while the caudal catheter was connected to a 1-ml syringe attached to a Harvard pump (model 907, Holliston, MA, USA). Exercise was initiated at a speed of ~20 m min^{-1} and remained steady for ~3 min at which time pre-microspheres HR and pressures were recorded. At ~3.5 min of total exercise time blood withdrawal was initiated from the caudal catheter at a rate of 0.25 ml min^{-1}. The carotid catheter was then disconnected from the pressure transducer and ~0.5-0.6 10^6, 15 μm diameter microspheres (^{57}Co or ^{85}Sr in random order, Perkin Elmer Life and Analytical Sciences, Waltham, MA, USA) were rapidly infused into the aortic arch of the running animal for the determination of tissue BF. Upon reconnection of the carotid catheter to the pressure transducer a second pressure reading was immediately recorded post-microspheres. An arterial blood sample (0.2 ml) was then drawn from the carotid artery catheter for the determination of blood gases, hematocrit, pH, [lactate] and [glucose]. Exercise was terminated and the rat was continuously monitored during a minimum 30 min rest period before the second bout began.

A post-recovery pressure was recorded to establish resting pressure and HR values. The K_{ATP} channel inhibitor GLI (5 mg kg^{-1}) was infused via the caudal artery catheter. Pressure was monitored continuously until GLI elicited a persistent rise in MAP at which time the second exercise bout was initiated. The second bout and administration of microspheres were performed identically to the protocol described above. Upon exercise termination the rat was euthanized with an overdose of pentobarbital (>50 mg kg^{-1} body mass) via the carotid artery catheter. For a second group of rats ($n = 6$) administration of microspheres, blood sampling and pressure recordings were performed at rest under CON and GLI conditions as described above.

Determination of BF and VC

Correct placement of the carotid catheter in the aortic arch was verified by anatomical dissection. Hindlimb muscles and muscle portions as well as the lungs, kidneys, and representative organs of the splanchnic region were removed, weighed and placed in counting vials for the determination of radioactivity.

Radioactivity was measured for each tissue as well as the reference sample using a gamma scintillation counter (model 5230, Packard Auto Gamma Spectrometer, Downers Grove, IL, USA). Taking into account the cross-talk fraction between isotopes enabled radioactivity to be determined for separate microsphere injections (^{57}Co or ^{85}Sr). Based on this radioactivity BF to each tissue was determined for the individual conditions, CON or GLI, by comparison to the reference sample of known flow rate and measured radioactivity (Ishise et al. 1980, Musch and Terrell 1992). Tissue BFs were expressed as ml min^{-1} (100 g)$^{-1}$ of tissue and the results were also normalized to MAP and expressed as VC (ml min^{-1} (100 g)$^{-1}$ mmHg^{-1}). Adequate mixing of the microspheres for each BF determination was verified by a <15% difference in BF between the right and left kidneys or right and left hindlimbs.

Statistical analysis

BF and VC were compared between and within groups using mixed two-way ANOVA's and Student-Newman-Keuls *post hoc* tests where appropriate. Muscle fibre-type composition was based on the percentage of type I, type IIa, type IIb, and type IId/x fibres in the individual muscles and muscle parts of the rat hindlimb as reported by Delp and Duan (1996). Pearson Product Correlations were used to test the relationship between changes in VC and muscle fibre type. Significance was set at $P < 0.05$ and values are expressed as mean $\pm$ SEM.

Results

There was no significant difference in body mass between groups (Rest: 365 ± 5, Exercise: 367 ± 13 g, $P > 0.05$).

Effects of GLI on HR, MAP, blood gases, pH, hematocrit, [lactate] and [glucose]

At rest and during exercise MAP was higher and HR was lower with GLI compared to CON (Fig. 1). During exercise hematocrit was higher with GLI (CON: 31 ± 1, GLI: 33 ± 1 %, $P < 0.05$), but pH, PO_2, PCO_2 and O_2 saturation were not different between conditions (data not shown, $P > 0.05$ for all). Blood [lactate] was higher with GLI during exercise but not at rest (Fig. 4). Blood [glucose] was not significantly different with GLI during exercise (CON: 99.1 ± 4.0, GLI: 98.7 ± 5.8 mg dL^{-1}, $P > 0.05$).

Effects of GLI on skeletal muscle BF and VC

At rest there were no differences between groups in total hindlimb skeletal muscle BF or VC (Fig. 2, $P = 0.23$) or BF and VC to 26 of 28 individual muscles or muscle portions (exception: VC was lower with GLI in the red gastrocnemius and vastus intermedius (Tables 2 and 3)). During exercise both total hindlimb skeletal muscle BF and VC were lower with GLI compared to CON (Fig. 2). Specifically, GLI resulted in a lower BF in 12, and lower VC in 16, of the 28 individual hindlimb muscles or muscle portions during exercise (Tables 2 and 3). Furthermore, there was a significant negative correlation between the percentage of type I and type IIa fibres and the percent change in VC for the 28 muscles or muscle portions of the hindlimb (Fig. 3; r = -0.53, $P < 0.05$).

Effects of GLI on renal and splanchnic BF and VC at rest and during exercise

Renal BF and VC were reduced by GLI at rest but not during exercise (Table 3). Among the representative organs of the splanchnic region BF was reduced at rest to the stomach, adrenals and large intestine with GLI. VC was reduced at rest to the stomach, adrenals, spleen, small intestine and large intestine with GLI. BF during exercise with GLI

was reduced to the pancreas and small intestine, and VC was reduced to the stomach, pancreas and small intestine (Table 3).

Discussion

The primary original findings of this investigation were that the blockade of K_{ATP} channels in the rat via GLI increased MAP and decreased HR at rest and during treadmill exercise, reduced exercising hindlimb skeletal muscle BF and VC and increased exercising blood [lactate]. Furthermore, the decreases in VC with GLI reflected a fibre type-selective effect such that VC was reduced primarily to the muscles comprised of type I and type IIa fibres. These data suggest that K_{ATP} channels contribute significantly to exercise-induced skeletal muscle hyperaemia during treadmill exercise.

The finding that GLI increased MAP with a consequent decrease in HR is not surprising as this is consistent with previous reports in rats (Moreau et al. 1994, Gardiner et al. 1996), swine (Duncker et al. 2001) and humans (Farouque et al. 2003). Specifically, the higher MAP and lower HR at rest with K_{ATP} channel blockade were coincident with large reductions in renal and splanchnic VC. This is in agreement with studies demonstrating a significant contribution of K_{ATP} channels to basal vasomotor tone in the systemic circulation of conscious rats (Gardiner et al. 1996, Moreau et al. 1994, Parekh and Zou 1996), hamsters (Jackson et al. 1993, Saito et al. 1996) and dogs (Vanelli and Hussain 1994, Comtois et al. 1994).

The similar hindlimb skeletal muscle BF and VC at rest with GLI is most likely due to the inhibition of K_{ATP} channels at physiological ATP concentrations or a compensatory vasodilation by redundant pathways (e.g. NO, PGI_2, baroreflex) to maintain appropriate basal vasomotor tone (Hellsten et al. 2012). Similar observations have been made across species (Duncker et al. 2001, Farouque et al. 2003) although there is some evidence for modest skeletal muscle vascular K_{ATP} channel activity at rest (Neilsen et al. 2003). In the present study, for example, the vastus intermedius was one of few hindlimb muscles to evince a GLI-induced reduction in BF and VC at rest. Given that the rat vastus intermedius is a postural muscle it is plausible that it would possess a relatively low subsarcolemmal ATP-to-ADP ratio resulting in a greater K_{ATP} channel open probability at resting metabolic

rates compared to the vast majority of the hindlimb muscle vasculature evidencing little or no basal K_{ATP} channel activation.

Previously, K_{ATP} channel blockade in rats and hamsters has been shown to reduce the skeletal muscle hyperaemic response to electrically-induced contractions (Thomas et al. 1997, Saito et al. 1996). In this regard, our findings indicate that, in rats, K_{ATP} channel blockade also reduces the *in vivo* skeletal muscle hyperaemic response to treadmill running. This corroborates the notion that K_{ATP} channels represent an important mechanistic link between metabolism and vasomotor tone during exercise. The 20% decrease in exercising hindlimb skeletal muscle VC with GLI suggests that the rat locomotor muscle vascular bed contains a substantial pool of K_{ATP} channels which are activated even at moderate exercise intensities. Importantly, the 16% decrease in exercising hindlimb skeletal muscle BF occurred simultaneous with a doubling of arterial blood [lactate] (2.0 to 4.1 mmol L^{-1}) with GLI. Thus, not only is vasodilation attenuated in the hindlimb vasculature, but this decrement represents an impaired vasomotor control failing to meet the BF demands of the active skeletal muscle. These intriguing findings support the hypothesis that K_{ATP} channels are obligatory for exercise-induced hyperaemia with respect to exercise tolerance and work capacity; at least within the rat.

The doubling of arterial blood [lactate] is not surprising given that GLI-induced decreases in BF were correlated with the percent type I and IIa fibres such that the predominantly oxidative muscles and muscle portions experienced the most consistent decrease in BF during exercise. However, muscle fibre type composition only explains a modest portion (~28%) of the VC decrease with GLI. The preferential recruitment of type I and type IIa fibres during the low speed exercise protocol (~55-65% $\dot{V}O_{2max}$) likely explains a significant portion of the fibre type-selective effect. In addition, NO, which has a proportionally greater role in the control of oxidative fibre type vascular beds (Hirai et al. 1994), has been shown to operate through the activation of K_{ATP} channels (Tare et al. 1990, Murphy and Braden 1995). Thus, blockade of K_{ATP} channels in the vasculature supplying type I and IIa fibres would result in a greater decrement to VC during exercise as it may block a portion of this NO-mediated vasodilation.

With respect to the interpretation of the effects of GLI on altered BF and VC as purely local muscle effects, the possible impacts of K_{ATP} channel blockade on sympathetic nerve discharge must be considered. The loss of K_{ATP} channel-mediated hyperpolarization could conceivably result in increased sympathetic nerve discharge for a given stimulus which would account, in part, for the decrements in BF and VC seen herein. However, we consider this unlikely because it has been demonstrated, in male Sprague Dawley rats, that direct injection of GLI into the rostral ventrolateral medulla has no effect on blood pressure, HR or renal SNA (Guo et al. 2011). Furthermore, preliminary studies from our lab demonstrate that arterial infusion of GLI in the rat actually reduced renal and lumbar SNA consistent with an appropriate baroreflex response to the reduction in VC elicited by GLI (CT Holdsworth, TI Musch, DC Poole, unpublished observations). Taken together, these findings indicate that GLI did not directly impact sympathetic nerve discharge in the current study and that the reductions in BF and VC are the result, specifically, of muscle vascular K_{ATP} channel blockade.

It is important to note that the BF and VC results seen herein run contrary to data from exercising swine (Duncker et al. 2001). This may be the result of cross-species differences in vascular K_{ATP} channel expression and/or function. Indeed, while the octameric structure of vascular K_{ATP} channels is presumed to be the Kir6.1 pore-forming units with associated SUR2B subunits, considerable heterogeneity of functional expression has been demonstrated through readily occurring heteromultimerization of the pore forming units (Kir6.x) as well as the sulphonylurea receptor subunits (SURx) which confer the channel's ATP sensitivity. Accordingly, there is substantial variation in unitary conductance which may impact the relative importance of the K_{ATP} channel in vasomotor control across species, tissue beds, and metabolic rates (Teramoto et al. 2006). This is particularly true for vascular K_{ATP} channels as the range of unitary conductance appears to be much greater (~20 to >200 pS) than for pancreatic β cells (~50 to 90 pS) and some regions of the heart and brain (~40 to 80 pS) (Cole and Clément-Chomienne 2003). Further exploration of the discrepancies in channel molecular structure across tissues may lead to

better understanding of the functional consequences resulting from global K_{ATP} channel blockade.

The role of K_{ATP} channels in the control of human skeletal muscle BF has been elucidated primarily by the use of K_{ATP} channel agonists, namely nicorandil and diazoxide, as well as the K_{ATP} channel inhibitor GLI. Seminal results include K_{ATP} channel agonists eliciting forearm vasodilation (Biljstra et al. 1996b) and K_{ATP} channel blockade reducing the BF response to both reactive and functional hyperaemia in some (Bank et al. 2000, Biljstra et al. 1996a, Banitt et al. 1996) but not all studies (Farouque et al. 2003, Schrage et al. 2006). The reason for the differential conclusions drawn from use of K_{ATP} channel agonists versus blockers is unclear. In healthy humans, K_{ATP} channels may not be obligatory for achieving adequate skeletal muscle BF during exercise given the great redundancy of vasodilatory mediators (Hellsten et al. 2012). The potential for parallel pathways such as NO- and PGI_2-mediated vasodilation to adequately meet BF requirements is present at relatively low workloads. This notion is consistent with K_{ATP} blockade studies in humans involving the forearm musculature which represents a small, non-locomotory portion of the total body muscle mass. Furthermore, the putative variability of K_{ATP} channel expression among skeletal muscle vascular beds with vastly different metabolic characteristics may confound interpretation of the available human data. It appears that the vascular K_{ATP} channel subtype uniquely requires nucleotide diphosphates (i.e. ADP) for activation and thus is designated as a nucleotide-dependent K^+ channel (K_{NDP}) (Beech et al. 1993). As such, the channel would only demonstrate obligatory function where sufficiently low blood-smooth muscle cell O_2 driving pressures result in pronounced subsarcolemmal ADP accumulation. Under these conditions even a small impact of K_{ATP} channel activation on SMC membrane potential could drive large increases in vasodilation. This is due to the remarkably steep relationship between membrane potential and Ca^{2+} influx in smooth muscle; changes of only a couple millivolts can reduce $[Ca^{2+}]_i$ ~30% (Nelson et al. 1990). For this reason we are in agreement with Shrage and colleagues (2006) who have recognized that the determination of K_{ATP} channel function across more diverse muscle

vascular beds and a greater range of exercise intensities/paradigms are necessary to resolve the circumstances under which these channels may impact O_2 delivery.

The results seen herein appear to suggest that the role of K_{ATP} channels may be potentiated under conditions of low O_2 tension (i.e. when muscle fibres are recruited) which can increase glycolytic flux and drive greater intracellular ADP accumulation. This environment is exacerbated in muscle fibres with low oxidative potential when exposed to high metabolic demand as well as conditions characterized by skeletal muscle hypoxia such as heart failure (Poole et al. 2012) type II diabetes (Padilla et al. 2007) and ageing (Poole and Ferreira 2007). Therefore, the potential for K_{ATP} channels to contribute significantly to exercise-induced skeletal muscle hyperaemia has important implications for characterizing the etiology of blood-muscle O_2 transport decrements in these conditions. For example, in diabetics, increased blood glucose levels cause overexposure to the carboxyl radical methylglycoxal which has been shown to decrease membrane expression of vascular K_{ATP} channels (Yang et al. 2012). Accordingly, K_{ATP} channel-mediated vasodilation may be attenuated in diabetes and would contribute to the reduction in skeletal muscle microvascular O_2 driving pressures described for this condition (Padilla et al. 2007). Additionally, this would explain why GLI administration for the management of insulin release in diabetics does not impact exercise tolerance as the K_{ATP} channel-mediated vasodilation may already demonstrate reduced function prior to blockade (Cunha et al. 2008). The development of novel therapeutics targeting K_{ATP} channel membrane expression and activation may provide an exciting and powerful tool for improving O_2 transport and subsequent exercise performance in populations exhibiting vascular dysfunction (Poole et al. 2012).

Experimental considerations

Since GLI is not selective for skeletal muscle K_{ATP} channels it might be argued that GLI-induced K_{ATP} channel blockade in the coronary vasculature (or some other vascular compartment) could account for the reduced BF during exercise seen herein. Measurements of coronary BF and VC were not available in the present investigation. However,

decrements in cardiac output would not be expected to support a selective decrease in BF for 12 of the 28 muscles or muscle portions as seen herein. This observation suggests that GLI would not impact coronary BF to the extent that decrements in cardiac function could impact hindlimb skeletal muscle BF during submaximal treadmill exercise.

Conclusions

Our principal novel findings show that blockade of K_{ATP} channels via GLI (5 mg kg^{-1}) attenuates hindlimb skeletal muscle BF and VC and elevates blood [lactate] during submaximal treadmill exercise. These results provide evidence that K_{ATP} channel-induced hyperpolarization constitutes an important mechanism of vasomotor control in exercising skeletal muscle and suggest that K_{ATP} channels represent an obligatory pathway for skeletal muscle vascular control during large muscle mass exercise in healthy rats.

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Additional information

Competing interests

None.

Author contributions

Conception and design of the experiments: C.T.H., T.I.M. and D.C.P. Data collection and analysis: C.T.H., S.W.C., S.K.F., G.E.S., T.I.M. and D.C.P. Manuscript preparation and revision: S.W.C., C.T.H., S.K.F., G.E.S., T.I.M. and D.C.P.

Table 1. Effects of GLI on total hindlimb skeletal muscle BF for rest and exercise groups
(ml min^{-1} (100 g)$^{-1}$)

	Rest		Exercise	
	CON	GLI	CON	GLI
Ankle extensors				
Soleus (91%)	115 ± 20	91 ± 28	382 ± 19	277 ± 21*
Plantaris (20%)	18 ± 4	16 ± 4	253 ± 25	209 ± 21
Gastrocnemius, red (86%)	57 ± 18	46 ± 22	419 ± 40	337 ± 33
Gastrocnemius, white (0%)	11 ± 3	16 ± 5	53 ± 13	69 ± 9
Gastrocnemius, mixed (9%)	20 ± 5	20 ± 9	161 ± 9	152 ± 16
Tibialis posterior (27%)	26 ± 10	11 ± 4	192 ± 23	161 ± 16
Flexor digitorum longus (32%)	57 ± 31	65 ± 48	118 ± 17	105 ± 20
Flexor halicus longus (29%)	16 ± 4	9 ± 2	107 ± 14	87 ± 12
Ankle flexors				
Tibialis anterior, red (37%)	59 ± 17	33 ± 12	383 ± 24	337 ± 39
Tibialis anterior, white (20%)	24 ± 8	17 ± 4	136 ± 12	139 ± 18
Extensor digitorum longus (24%)	22 ± 5	20 ± 5	70 ± 5	72 ± 5
Peroneals (33%)	27 ± 7	18 ± 7	169 ± 13	144 ± 19
Knee extensors				
Vastus intermedius (96%)	134 ± 34	101 ± 44	419 ± 25	299 ± 18*
Vastus medialis (18%)	38 ± 14	30 ± 12	249 ± 15	186 ± 11*
Vastus lateralis, red (65%)	88 ± 37	87 ± 45	464 ± 27	306 ± 42*
Vastus lateralis, white (0%)	10 ± 2	14 ± 4	56 ± 20†	57 ± 14
Vastus lateralis, mixed (11%)	24 ± 8	24 ± 8	200 ± 12†	139 ± 16*
Rectus femoris, red (34%)	38 ± 11	28 ± 13	324 ± 29	233 ± 25*
Rectus femoris, white (0%)	21 ± 4	16 ± 5	146 ± 15	113 ± 10*
Knee flexors				
Biceps femoris anterior (0%)	12 ± 3	12 ± 2	34 ± 4	41 ± 6
Biceps femoris posterior (8%)	20 ± 7	16 ± 5	106 ± 11	93 ± 10
Semitendinosus (17%)	23 ± 7	15 ± 4	77 ± 7	77 ± 15
Semimembranosus, red (28%)	25 ± 12	17 ± 4	137 ± 19	123 ± 14
Semimembranosus, white (0%)	13 ± 2	10 ± 1	40 ± 6	47 ± 7
Thigh adductors				
Adductor longus (95%)	97 ± 14	74 ± 20	396 ± 18	251 ± 32*
Adductor magnus & brevis (11%)	31 ± 14	22 ± 7	142 ± 10	99 ± 6*
Gracilis (23%)	23 ± 9	14 ± 3	81 ± 13	58 ± 7
Pectineus (31%)	29 ± 10	20 ± 6	104 ± 19	58 ± 8*

Data are mean ± SEM. Values in parentheses indicate % type I + IIa according to Delp & Duan (1996). Rest; $n = 6$, Exercise; $n = 8$. *$P < 0.05$ *versus* control.

Table 2. Effects of GLI on total hindlimb skeletal muscle VC for rest and exercise groups
(ml min^{-1} (100 g)$^{-1}$ mmHg^{-1})

	Rest		Exercise	
	CON	GLI	CON	GLI
Ankle extensors				
Soleus (91%)	0.88 ± 0.15	0.62 ± 0.21	2.76 ± 0.20	1.92 ± 0.20*
Plantaris (20%)	0.14 ± 0.03	0.11 ± 0.03	1.83 ± 0.21	1.45 ± 0.18
Gastrocnemius, red (86%)	0.44 ± 0.15	0.30 ± 0.14*	3.02 ± 0.32	2.33 ± 0.27*
Gastrocnemius, white (0%)	0.09 ± 0.03	0.10 ± 0.03	0.37 ± 0.08	0.47 ± 0.07
Gastrocnemius, mixed (9%)	0.16 ± 0.04	0.13 ± 0.06	1.15 ± 0.06	1.04 ± 0.13
Tibialis posterior (27%)	0.21 ± 0.08	0.07 ± 0.02	1.36 ± 0.14	1.10 ± 0.10
Flexor digitorum longus (32%)	0.41 ± 0.21	0.38 ± 0.26	0.84 ± 0.12	0.71 ± 0.13
Flexor halicus longus (29%)	0.12 ± 0.03	0.06 ± 0.01	0.75 ± 0.09	0.59 ± 0.09
Ankle flexors				
Tibialis anterior, red (37%)	0.45 ± 0.13	0.20 ± 0.06	2.76 ± 0.20	2.32 ± 0.28
Tibialis anterior, white (20%)	0.19 ± 0.06	0.11 ± 0.03	0.98 ± 0.09	0.96 ± 0.12
Extensor digitorum longus (24%)	0.16 ± 0.04	0.13 ± 0.03	0.50 ± 0.04	0.49 ± 0.03
Peroneals (33%)	0.22 ± 0.06	0.12 ± 0.04	1.22 ± 0.10	0.99 ± 0.13
Knee extensors				
Vastus intermedius (96%)	1.04 ± 0.28	0.70 ± 0.30*	3.00 ± 0.18	2.06 ± 0.16*
Vastus medialis (18%)	0.30 ± 0.12	0.20 ± 0.08	1.79 ± 0.13	1.29 ± 0.11*
Vastus lateralis, red (65%)	0.71 ± 0.31	0.59 ± 0.29	3.34 ± 0.24	2.12 ± 0.34*
Vastus lateralis, white (0%)	0.08 ± 0.01	0.10 ± 0.03	0.40 ± 0.15	0.40 ± 0.10
Vastus lateralis, mixed (11%)	0.19 ± 0.07	0.16 ± 0.05	1.44 ± 0.11	0.96 ± 0.13*
Rectus femoris, red (34%)	0.29 ± 0.08	0.18 ± 0.09	2.35 ± 0.26	1.62 ± 0.21*
Rectus femoris, white (0%)	0.17 ± 0.04	0.11 ± 0.03	1.05 ± 0.13	0.78 ± 0.08*
Knee flexors				
Biceps femoris anterior (0%)	0.10 ± 0.03	0.08 ± 0.02	0.24 ± 0.03	0.28 ± 0.04
Biceps femoris posterior (8%)	0.16 ± 0.05	0.10 ± 0.03	0.76 ± 0.08	0.64 ± 0.07
Semitendinosus (17%)	0.19 ± 0.06	0.10 ± 0.02	0.55 ± 0.05	0.52 ± 0.10
Semimembranosus, red (28%)	0.20 ± 0.09	0.12 ± 0.03	0.98 ± 0.14	0.84 ± 0.09
Semimembranosus, white (0%)	0.10 ± 0.02	0.07 ± 0.01	0.28 ± 0.04	0.32 ± 0.04
Thigh adductors				
Adductor longus (95%)	0.76 ± 0.11	0.50 ± 0.15	2.85 ± 0.16	1.74 ± 0.25*
Adductor magnus & brevis (11%)	0.25 ± 0.11	0.15 ± 0.05	1.02 ± 0.08	0.68 ± 0.05*
Gracilis (23%)	0.18 ± 0.07	0.09 ± 0.02	0.58 ± 0.09	0.39 ± 0.04*
Pectineus (31%)	0.23 ± 0.08	0.13 ± 0.04	0.75 ± 0.14	0.40 ± 0.05*

Data are mean ± SEM. Values in parentheses indicate % type I + IIa according to Delp & Duan (1996). Rest; n = 6, Exercise; n = 8. *$P < 0.05$ *versus* control

Table 3. Effects of GLI on BF (ml min^{-1}(100 g)$^{-1}$) and VC (ml min^{-1}(100 g)$^{-1}$ mmHg^{-1}) of the kidneys and organs of the splanchnic region for rest and exercise groups.

	Rest		Exercise	
	CON	GLI	CON	GLI
BF				
Kidney	488 ± 50	380 ± 27*	455 ± 42	417 ± 47
Stomach	100 ± 21	48 ± 8*	101 ± 15	73 ± 27
Adrenals	718 ± 144	405 ± 45	448 ± 55	419 ± 69
Spleen	397 ± 82	307 ± 88	113 ± 28	115 ± 31
Pancreas	142 ± 26	108 ± 15	198 ± 31	92 ± 22*
Sm. Intestine	304 ± 41	257 ± 37	304 ± 40	229 ± 40*
Lg. Intestine	218 ± 48	98 ± 14*	153 ± 30	116 ± 28*
Liver**	31 ± 6	37 ± 9	20 ± 7	20 ± 6
VC				
Kidney	3.82 ± 0.50	2.53 ± 0.24*	3.30 ± 0.36	2.88 ± 0.36
Stomach	0.76 ± 0.15	0.32 ± 0.04*	0.64 ± 0.08	0.33 ± 0.05*
Adrenals	5.73 ± 1.32	2.69 ± 0.33	3.17 ± 0.35	2.92 ± 0.55
Spleen	3.08 ± 0.66	1.99 ± 0.54*	0.83 ± 0.23	0.82 ± 0.24
Pancreas	1.13 ± 0.22	0.72 ± 0.12	1.46 ± 0.26	0.65 ± 0.16*
Sm. Intestine	2.35 ± 0.31	1.71 ± 0.27	2.23 ± 0.35	1.62 ± 0.31*
Lg. Intestine	1.72 ± 0.38	0.64 ± 0.07*	1.11 ± 0.25	0.90 ± 0.21
Liver**	0.24 ± 0.05	0.24 ± 0.06	0.14 ± 0.04	0.14 ± 0.04

Data are mean ± SEM. Rest; *n* = 6, Exercise; *n* = 8.* *P* < 0.05 *versus* control. **Indicates arterial, not portal, BF and VC
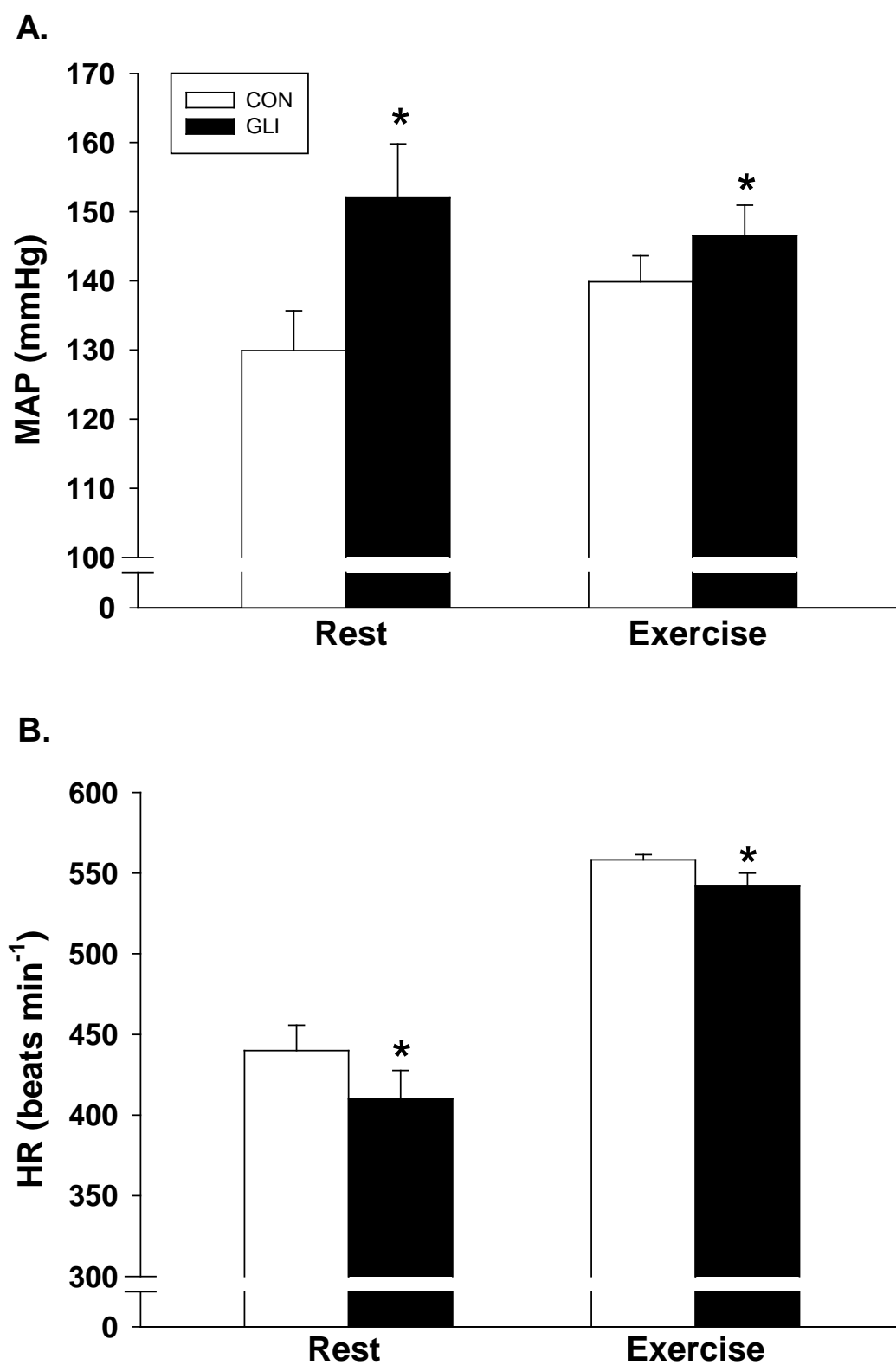
Figure 1

A.

B.

Figure 1. GLI increased MAP (A) and decreased HR (B) at rest
and during exercise compared to CON. *, P < 0.05 versus control.
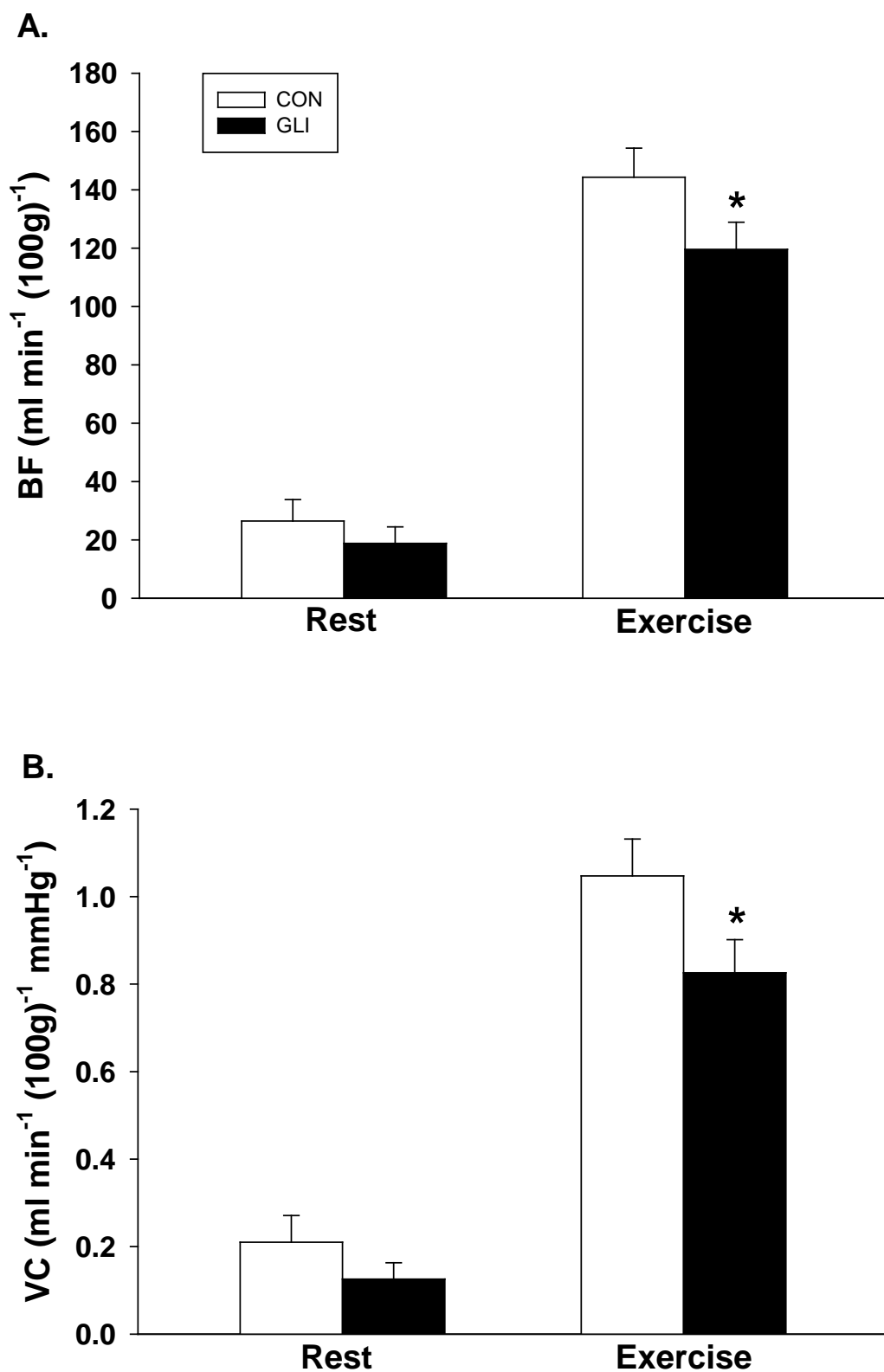
Figure 2

A.

B.

Figure 2. GLI decreased total hindlimb skeletal muscle BF (A) and VC (B) during submaximal exercise but not at rest compared to CON. *, P < 0.05 versus control.
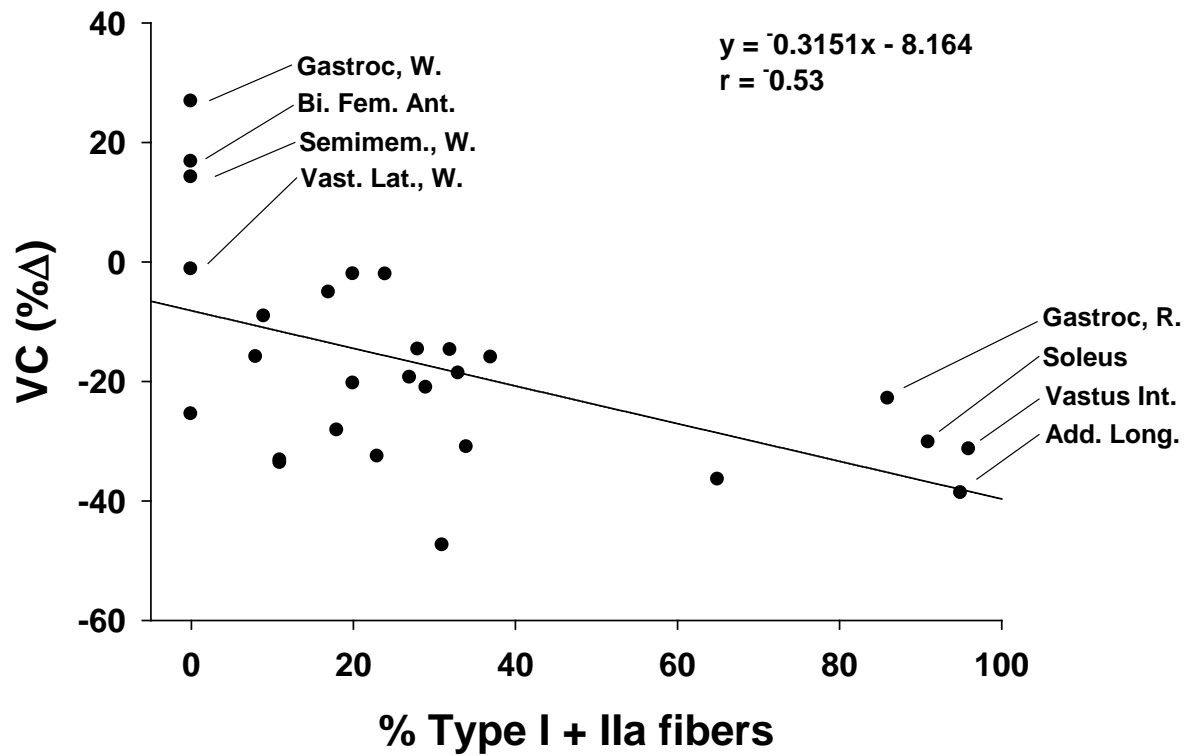
Figure 3

Figure 3. The percent decreases in hindlimb VC with GLI were positively correlated with the percent type I and type IIa fibres of the muscles or muscle portions. P < 0.05.
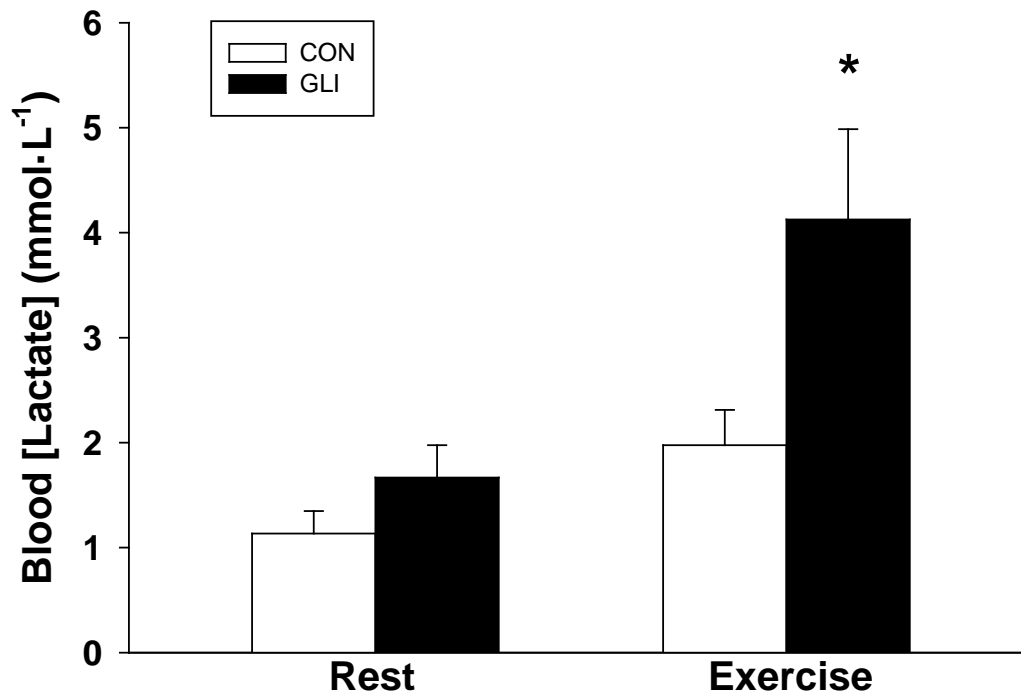
Figure 4

Figure 4. GLI increased blood [lactate] during submaximal exercise but not at rest compared to CON. *, P < 0.05 versus control.