

Influence of group III/IV muscle afferents on small muscle mass exercise performance: a bioenergetics perspective

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Key points

- This investigation assessed the influence of group III/IV muscle afferents on small muscle mass exercise performance from a skeletal muscle bioenergetics perspective.
- Group III/IV muscle afferent feedback was attenuated with lumbar intrathecal fentanyl during intermittent isometric single-leg knee-extensor all-out exercise, while ³¹P-MRS was used to assess skeletal muscle bioenergetics.
- Attenuation of group III/IV muscle afferent feedback improved exercise performance during the first minute of exercise, due to an increase in total ATP production with no change in the ATP cost of contraction. However, exercise performance was not altered during the remainder of the protocol, despite a sustained increase in total ATP production, due to an exacerbated ATP cost of contraction.
- These findings reveal that group III/IV muscle afferents directly limit exercise performance during small muscle mass exercise, but, due to their critical role in maintaining skeletal muscle contractile efficiency, with time, the benefit of attenuating the muscle afferents is negated.

Abstract The direct influence of group III/IV muscle afferents on exercise performance remains equivocal. Therefore, all-out intermittent isometric single-leg knee-extensor exercise and phosphorous magnetic resonance spectroscopy (³¹P-MRS) were utilized to provide a high time resolution assessment of exercise performance and skeletal muscle bioenergetics in control conditions (CTRL) and with the attenuation of group III/IV muscle afferent feedback via lumbar intrathecal fentanyl (FENT). In both conditions, seven recreationally active men performed 60 maximal voluntary quadriceps contractions (MVC; 3 s contraction, 2 s relaxation), while knee-extensor force and ³¹P-MRS were assessed during each MVC. The cumulative integrated

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force was significantly greater ($8 \pm 6\%$) in FENT than CTRL for the first minute of the all-out protocol, but was not significantly different for the second to fifth minutes. Total ATP production was significantly greater ($16 \pm 21\%$) in FENT than CTRL throughout the all-out exercise protocol, due to a significantly greater anaerobic ATP production ($11 \pm 13\%$) in FENT than CTRL with no significant difference in oxidative ATP production. The ATP cost of contraction was not significantly different between FENT and CTRL for the first minute of the all-out protocol, but was significantly greater ($29 \pm 34\%$) in FENT than in CTRL for the second to fifth minutes. These findings reveal that group III/IV muscle afferents directly limit exercise performance during small muscle mass exercise, but, due to their critical role in maintaining skeletal muscle contractile efficiency, with time, the benefit from muscle afferent attenuation is negated.

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Introduction

Despite extensive investigation, a clear understanding of the influence of group III/IV muscle afferents on exercise performance has remained elusive. However, it is evident that these afferents constrain motoneuronal output during exercise, as evinced by the marked increase in motoneuronal output when feedback from group III/IV muscle afferents is attenuated (Amann *et al.* 2009, 2011*a*; Sidhu *et al.* 2014; Blain *et al.* 2016). These muscle afferents also constrain the intramuscular metabolic perturbation and the development of peripheral fatigue during exercise, which are both exacerbated by the attenuation of group III/IV muscle afferent feedback (Amann *et al.* 2009, 2011*a*; Sidhu *et al.* 2014; Blain *et al.* 2016). Thus, it may be anticipated that these muscle afferents influence exercise performance, but, remarkably, exercise performance has either been unaltered or diminished with the attenuation of group III/IV muscle afferent feedback (Amann *et al.* 2009, 2011*a*; Sidhu *et al.* 2014, 2017; Blain *et al.* 2016). Importantly, it must be acknowledged that the impact of the group III/IV muscle afferents on exercise performance in these previous studies using whole-body exercise was likely influenced by a diminished oxygen delivery to the working skeletal muscle, secondary to the attenuated muscle afferent feedback (Amann *et al.* 2011*b*).

Recently, an intermittent isometric small muscle mass exercise protocol to task failure was utilized specifically to circumvent the reduction in oxygen delivery to the working skeletal muscle due to a fentanyl-induced attenuation of group III/IV muscle afferent feedback (Broxterman *et al.* 2017*b*). In doing so, it was revealed that the ATP cost of muscle contraction was exacerbated during exercise with attenuated muscle afferent feedback. This finding indicates that group III/IV muscle afferent feedback is critically involved in maintaining efficient muscle contraction during submaximal exercise. Of importance, this increased ATP cost of contraction

appeared to offset an increase in total ATP production, rendering exercise performance unaltered and, therefore, confounding conclusions regarding the influence of group III/IV muscle afferents on exercise performance. Unfortunately, due to large variations in time to task failure, an assessment of performance and skeletal muscle bioenergetics, throughout the exercise protocol, was precluded in this previous study (Broxterman *et al.* 2017*b*).

The intermittent isometric all-out exercise protocol presents a unique opportunity for the high time resolution assessment of both exercise performance and skeletal muscle bioenergetics during maximal exercise (Burnley, 2009; Burnley *et al.* 2010; Broxterman *et al.* 2017*a*). This all-out protocol is distinct from both time to task-failure and time-trial protocols, in that maximal exercise performance is assessed throughout the entire protocol, rather than only at task-failure, without the confounding influence of pacing strategies. Additionally, this all-out protocol recruits all motor units from the onset of exercise, and fatigue-prone motor units (i.e. Type II) progressively contribute less to force generation (Sargeant *et al.* 1981; McCartney *et al.* 1983; Burnley, 2009; Vanhatalo *et al.* 2011), which is coupled with the progressive intramuscular metabolic perturbation and development of neuromuscular fatigue (Burnley, 2009; Burnley *et al.* 2010; Broxterman *et al.* 2017*a*). As such, the relative influence of group III/IV muscle afferent feedback on motor unit recruitment patterns and strategies, and therefore exercise performance (Bigland-Ritchie *et al.* 1986; Garland & McComas, 1990; Hayward *et al.* 1991; Rossi *et al.* 2003; Martin *et al.* 2008; Kennedy *et al.* 2013), is likely progressively increased throughout the all-out protocol. It may, therefore, be anticipated that the attenuation of group III/IV muscle afferent feedback will exacerbate the ATP cost of contraction only during the latter portion of the all-out protocol, when activation of these muscle afferents is high and motor unit

recruitment strategies are highly influential on exercise performance. These characteristics of all-out exercise also provide a novel opportunity to further assess the influence of group III/IV muscle afferents on the perception of exertion during exercise. Thus, this all-out exercise protocol provides a unique opportunity to assess the influence of the group III/IV muscle afferents on exercise performance across time when the role of these muscle afferents on skeletal muscle bioenergetics is likely changing substantially.

Therefore, the purpose of this investigation was to elucidate the influence of group III/IV muscle afferent feedback on exercise tolerance from a skeletal muscle bioenergetics perspective using lumbar intrathecal fentanyl and ^{31}P -MRS during all-out intermittent isometric knee-extensor exercise. We hypothesized that, compared to control conditions (CTRL), the attenuation of group III/IV muscle afferent feedback (FENT) during all-out exercise would (1) increase total ATP production throughout the entire exercise protocol, (2) increase the ATP cost of contraction during the later portion of the exercise protocol, and therefore (3) exercise performance would be improved during the initial, but not the latter, portion of the protocol.

Methods

Ethical approval

The experimental protocol was approved by the Institutional Review Boards of the University of Utah and the Salt Lake City Veterans Affairs Medical Center (reference no. 00062889), and conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. Written informed consent was attained after subjects were informed of the experimental protocol and the potential risks of participation.

Subjects

Eight subjects completed the testing after providing written informed consent. Unique analyses of the control data, independent of the current study, were previously published (Broxterman *et al.* 2017a). The results of the current study are from seven healthy men (age, 27 ± 5 years; stature, 178 ± 4 cm; and body mass, 78 ± 9 kg), as data from one subject were excluded due to technical difficulties during data collection. Subjects were instructed to abstain from vigorous activity during the 24 h preceding each visit to the laboratory and to arrive at the laboratory having abstained from food and caffeine during the preceding 3 h. Subjects reported to the laboratory a minimum of three times with at least 72 h between each visit.

Experimental protocol

A single-leg intermittent isometric knee-extensor exercise protocol (60 maximal voluntary contractions; MVCs) was performed during a familiarization visit and two subsequent experimental visits. The 60 MVCs were conducted over 5 min using a 3 s contraction and 2 s relaxation duty cycle, maintained by audio cues. Subjects performed the exercise in a semi-recumbent position ($\sim 15^\circ$ elevation of the trunk) with the knee supported ($\sim 45^\circ$ knee joint angle) and the ankle fixed to an immovable strain gauge (SSM-AJ-250, Interface Inc., Scottsdale, AZ, USA). Non-elastic straps were positioned over the hips and thigh to minimize extraneous movements. For the experimental visits, the 60 MVC protocol with 5 min of recovery was conducted inside a whole-body magnetic resonance imaging (MRI) system under both CTRL and FENT conditions, with at least 72 h between visits. Testing order and leg strength (i.e. MVC peak force) were balanced across subjects. The integrated force, mean force, and peak force were determined for each of the 60 MVCs and end-test force was defined as the mean force of the final six MVCs (Burnley, 2009). At the 60th MVC, a rating of perceived exertion (RPE) was obtained from the subjects using the Borg modified CR10 scale (Borg, 1998).

Intrathecal fentanyl administration

For the fentanyl administration, subjects adopted an upright seated posture with the torso slightly flexed. After the skin and subcutaneous tissue at the L_3 – L_4 vertebral interspace were anaesthetized using 1% lidocaine (10 mg ml^{-1}), a 25-gauge Whitacre needle was advanced into the intrathecal space through a 20-gauge spinal introducer needle. Correct placement was confirmed by free-flowing cerebrospinal fluid, which was aspirated and injected with 0.025 mg of fentanyl (0.050 mg ml^{-1}) in order to attenuate feedback arising from μ -opioid receptor-sensitive group III and IV leg muscle afferents. The upright seated position was maintained for at least 5 min prior to positioning for the exercise protocol. Previous studies have documented that this intrathecal fentanyl administration protocol does not result in fentanyl migration to the brain (Amann *et al.* 2009, 2011a; Hilty *et al.* 2011; Sidhu *et al.* 2014, 2015; Blain *et al.* 2016) and does not affect motor nerve activity or maximal force output (Grant *et al.* 1996; Wilson & Hand, 1997; Standl *et al.* 2001; Amann *et al.* 2009).

^{31}P -MRS

A clinical 2.9 Tesla MRI system (Tim-Trio, Siemens Medical Systems, Malvern, PA, USA) operating at 49.9 MHz for ^{31}P resonance was utilized for MRS. ^{31}P -MRS data acquisition was obtained using a dual

^{31}P - ^1H surface coil (110 mm ^1H coil loop surrounded by the ^{31}P single-loop coil with a diameter of 125 mm) with linear polarization (Rapid Biomedical GmbH, Rimpf, Germany). The surface coil was secured with elastic straps at mid-thigh level and advanced localized volume shimming was performed after a three-plane scout proton image was acquired in order to ensure sampling of all major quadriceps muscles. Two fully relaxed spectra were acquired at rest (3 averages per spectrum and a repetition time of 30 s) prior to the commencement of the 60 MVC protocol. MRS data acquisition throughout exercise and recovery were performed using a free-induction decay pulse sequence with a 2.56 ms adiabatic-half-passage excitation RF pulse, a repetition time of 2.5 s, a receiver bandwidth of 5 kHz, 1024 data points, and two averages per spectrum. Saturation factors were quantified by the comparison between fully relaxed (repetition time = 30 s) and partially relaxed (repetition time = 2.5 s) spectra.

Relative concentrations of intramuscular PCr, P_i and ATP were determined by a time-domain fitting routine using the AMARES algorithm (Vanhamme *et al.* 1997) incorporated into CSAIPO software (Layec *et al.* 2008; Le Fur *et al.* 2010), while absolute concentrations were estimated assuming a resting [ATP] of 8.2 mM (Harris *et al.* 1974). Intracellular pH was calculated from the chemical shift difference between the P_i and PCr signals. The free cytosolic [ADP] was calculated from [PCr] and pH using the creatine kinase (CK) equilibrium constant ($K_{\text{CK}} = 1.66 \times 10^9 \text{ m}^{-1}$), with the assumption that PCr represents 85% of the total creatine content (Jeneson *et al.* 1995). Resting concentrations were calculated from the average peak areas of the two fully relaxed spectra. When P_i splitting was evident, the pH corresponding to each P_i pool was calculated separately as pH_1 and pH_2 on the basis of the chemical shift of each peak relative to PCr, such that the overall pH was then calculated as:

$$\text{pH} = \text{pH}_1(\text{area } \text{P}_{i,1}/\text{total } \text{P}_i \text{ area}) + \text{pH}_2(\text{area } \text{P}_{i,2}/\text{total } \text{P}_i \text{ area}).$$

The concentration of the H_2PO_4^- was calculated as (Lanza *et al.* 2006):

$$\text{H}_2\text{PO}_4^- = [\text{P}_i]/(1 + 10^{\text{pH}-6.75})$$

Free cytosolic adenosine monophosphate (AMP) was calculated based on the equilibrium of the adenylate kinase reaction corrected for the effects of pH, assuming a free magnesium concentration of 1 mM (Golding *et al.* 1995). ATP free energy (ΔG_{ATP}) was calculated using the following equation and constants (Kemp *et al.* 2001):

$$\Delta G_{\text{ATP}} = \Delta G_{\text{ATP}}^0 + RT \ln \frac{[\text{ADP}] \times [\text{P}_i]}{[\text{ATP}]}$$

$$\Delta G_{\text{ATP}}^0 = RT \left[\ln (K_{\text{ATP}}^{\text{true}}) + \ln \left(\frac{f_{\text{ATP}}}{f_{\text{ADP}} \times f_{\text{P}_i}} \times \frac{1}{[\text{H}^+]} \right) \right]$$

With $K_{\text{ATP}}^{\text{true}} = 0.722$ (Golding *et al.* 1995), $R = 8.3145 \text{ K}^{-1} \text{ m}^{-1}$ and $T = 310 \text{ K}$. In all cases, relative amplitudes were corrected for partial saturation due to the repetition time relative to T_1 via the fully relaxed spectra acquired at rest.

ATP synthesis rates and ATP cost of contraction

The rate of ATP production from the breakdown of PCr through the creatine kinase reaction (ATP_{CK} , mM min^{-1}) was calculated from the change in [PCr] for each time point of the exercise period (Kemp & Radda, 1994):

$$\text{ATP}_{\text{CK}} = \Delta \text{PCr} / \Delta t$$

Based on the sigmoid relationship between the oxidative ATP production rate (ATP_{OX} , mM min^{-1}) and free cytosolic [ADP], the rate of mitochondrial ATP production was calculated as:

$$\text{ATP}_{\text{OX}} = V_{\text{max}} / (1 + (K_m / [\text{ADP}])^{2.2})$$

in which K_m (the [ADP] at half-maximal oxidation rate) is $\sim 30 \mu\text{M}$ in skeletal muscle (Kemp & Radda, 1994), 2.2 is the Hill coefficient for a sigmoid function (Jeneson *et al.* 1996) and V_{max} is the peak rate of *in vivo* oxidative ATP synthesis.

During exercise, changes in intramuscular pH result from glycogen breakdown to pyruvate and lactate, proton efflux, buffering capacity, protons produced by oxidative phosphorylation, and the consumption of protons by the CK reaction (Kemp & Radda, 1994). Assuming that the glycogenolytic production of 1 mol of H^+ , when coupled to ATP hydrolysis, yields 1.5 mol of ATP, the ATP production from anaerobic glycolysis (ATP_{GLY} , mM min^{-1}) can be deduced from the total number of protons ($\text{H}_{\text{total}}^+$) produced throughout exercise (Hochachka & Mommsen, 1983; Kemp *et al.* 1993; Kemp & Radda, 1994):

$$\text{ATP}_{\text{GLY}} = 1.5 \times \text{H}_{\text{total}}^+$$

where

$$\text{H}_{\text{total}}^+ = \text{H}_{\text{CK}}^+ + \text{H}_{\beta}^+ - \text{H}_{\text{OX}}^+ + \text{H}_{\text{efflux}}^+$$

H_{CK}^+ (in mM min^{-1}) was calculated from the time-dependent changes in [PCr] and from the stoichiometric coefficient (γ):

$$\text{H}_{\text{CK}}^+ = -\gamma \times \text{ATP}_{\text{CK}}$$

where γ is the proton stoichiometric coefficient of the coupled Lohmann reaction as previously described (Kushmerick, 1997). H_{β}^+ (in mM min^{-1}) was calculated from the apparent buffering capacity β_{total} (in Slykes,

millimoles acid added/unit change in pH) and from the rate of pH changes:

$$H_{\beta}^{+} = -\beta_{\text{total}} \times \Delta\text{pH}/\Delta t$$

where

$$\beta_{\text{total}} = \beta_{\text{non-bicarbonate-non-P}_i} + \beta_{\text{P}_i} + \beta_{\text{bicarbonate}}$$

where

$$\beta_{\text{non-bicarbonate-non-P}_i} = \beta_a - \beta_{\text{P}_i}$$

in which β_a was determined from the initial change in PCr (ΔPCr_i) and alkalization of pH (ΔpH) (Conley *et al.* 1997):

$$\beta_a = \gamma \times (\Delta\text{PCr}_i \times \Delta\text{pH})$$

β_{P_i} was determined based on the dissociation constant of the buffer (K) according to the standard formula (Conley *et al.* 1998):

$$\beta_{\text{P}_i} = (2.303 \times H^{+} \times K \times [P_i]) / (K + H^{+})^2$$

where $K = 1.77 \times 10^{-7}$. In agreement with previous studies and assuming that muscle is a closed system during exercise (Kemp *et al.* 1993; Conley *et al.* 1998), $\beta_{\text{bicarbonate}}$ was set to zero. H_{OX}^{+} (in mM min^{-1}) was calculated from the factor $m = 0.16/[1 + 10^{(6.1-\text{pH})}]$, which accounts for the amount of protons produced through oxidative ATP production (Kemp *et al.* 1993, 1997):

$$H_{\text{OX}}^{+} = m \times \text{ATP}_{\text{OX}}$$

H_{efflux}^{+} (in mM min^{-1}) was calculated for each time point of exercise using the proportionality constant λ relating proton efflux rate to ΔpH (Kemp *et al.* 1993, 1997):

$$H_{\text{efflux}}^{+} = -\lambda \Delta\text{pH}$$

This proportionality constant λ (in $\text{mM min}^{-1} \text{pH unit}^{-1}$) was calculated during the recovery period:

$$\lambda = -V_{\text{efflux}}/\Delta\text{pH}$$

During the recovery period, PCr is regenerated through the CK reaction as the consequence of oxidative ATP production in mitochondria. Thus, H_{efflux}^{+} can be calculated from the rates of proton production from the CK reaction (H_{CK}^{+} , in mM min^{-1}) and mitochondrial ATP production (H_{OX}^{+} , in mM min^{-1}) on one side and the rate of pH changes on the other side. At this time, ATP production is exclusively aerobic and lactate production is considered negligible:

$$V_{\text{efflux}} = \beta_{\text{total}} \times \Delta\text{pH}/\Delta t + \gamma \times V_{\text{iPCr}} + m \times \text{ATP}_{\text{OX}}$$

To improve precision, a modified version of this calculation was used (Kemp *et al.* 1993, 1997), in which the total proton disappearance (i.e. $\int \text{Edt}$) is estimated cumulatively from the start of recovery and then fitted

to an exponential function to obtain the initial recovery rate (V_{efflux}).

The total ATPase rate ($\text{ATP}_{\text{TOTAL}}$, in mM min^{-1}) was calculated for each time point as:

$$\text{ATP}_{\text{TOTAL}} = \text{ATP}_{\text{OX}} + \text{ATP}_{\text{CK}} + \text{ATP}_{\text{GLY}}$$

and the anaerobic ATPase rate (ATP_{ANA} , mM min^{-1}) was calculated for each time point as:

$$\text{ATP}_{\text{ANA}} = \text{ATP}_{\text{CK}} + \text{ATP}_{\text{GLY}}$$

The ATP cost of contraction (in mM N^{-1}) was calculated as the ratio between $\text{ATP}_{\text{TOTAL}}$ and the force integral. The rates of ATP synthesis and the ATP cost of contraction were calculated for each for each minute of the 60 MVC protocol and were normalized to the first minute of CTRL exercise.

PCr recovery kinetics

The [PCr] kinetics during the recovery period were described by a mono-exponential curve:

$$[\text{PCr}](t) = [\text{PCr}]_{\text{end}} + \Delta[\text{PCr}](1 - e^{-(t/\tau)})$$

where $[\text{PCr}](t)$ is the [PCr] at a given time (t), $[\text{PCr}]_{\text{end}}$ is the [PCr] at task failure, $\Delta[\text{PCr}]$ is the amount of PCr resynthesized during the recovery period, and τ represents the time constant of the PCr offset kinetics. The initial rate of PCr resynthesis (V_{iPCr}) was calculated from the derivative of the mono-exponential equation at the onset of recovery:

$$V_{\text{iPCr}} = k\Delta[\text{PCr}]$$

where $\Delta[\text{PCr}]$ is the amount of PCr resynthesized during the recovery period and the rate constant $k = 1/\tau$ (Kemp & Radda, 1994). V_{max} was calculated using V_{iPCr} and the [ADP] at task failure (Trenell *et al.* 2006):

$$V_{\text{max}} = V_{\text{iPCr}} [1 + (K_m/[ADP]_{\text{end}})^{2.2}]$$

where K_m (the [ADP] at half the highest oxidative rate) is $\sim 30 \mu\text{M}$ in skeletal muscle (Kemp & Radda, 1994).

Model variables were determined with an iterative process by minimizing the sum of squared residuals (RSS) between the fitted function and the observed values. Goodness of fit was assessed by visual inspection of the residual plot and the frequency plot distribution of the residuals, with the chi square values and the coefficient of determination (r^2) calculated as follows:

$$r^2 = 1 - (\text{SS}_{\text{reg}}/\text{SS}_{\text{tot}})$$

Statistical analysis

The peak force per contraction, the integral force per contraction and the ^{31}P -MRS data were analysed using

two-way ANOVAs (condition \times time) with repeated measures. Student's paired t test was performed for each minute when a significant interaction effect was detected. The peak force of the exercise protocol, cumulative integrated force for each minute, RPE and peak rate of mitochondrial ATP synthesis were compared between conditions using Student's paired t test. Within each condition, ATP_{OX} for each minute was compared with the 95% confidence interval around V_{\max} to determine whether maximal oxidative ATP production was attained. Significance for the statistical analysis was accepted at $P \leq 0.05$. Results are presented as means \pm SD, except for in figures where SEM is used for clarity.

Results

Effect of afferent blockade on exercise performance and RPE

FENT had no effect on peak force production (FENT: 592 ± 121 vs. CTRL: 572 ± 135 N, $P = 0.22$). The integrated force per contraction significantly decreased over time with a significant condition \times time interaction (Fig. 1). For the first minute of exercise, the integrated force was significantly greater for FENT than CTRL ($17,373 \pm 2731$ vs. $16,197 \pm 3049$ N; Fig. 1), while there was no difference for the second to fifth minutes ($40,068 \pm 5803$ vs. $40,772 \pm 6365$ N, $P = 0.63$). In both conditions, force did not change over the final six MVCs ($P > 0.05$) and the resulting end-test force values were not different between conditions (FENT: 220 ± 52 vs. CTRL: 210 ± 41 N, $P = 0.60$). RPE at the 60th MVC was significantly lower for FENT than CTRL (4.3 ± 2.6 vs. 9.9 ± 0.4 ; Fig. 2).

Effect of afferent blockade on intramuscular metabolic perturbation

Recognizing the assumption that resting [ATP] was not different between FENT and CTRL, data required for the calculation of other metabolite concentrations at rest, baseline metabolic indices were not different between FENT and CTRL for [PCr] (FENT: 25.4 ± 4.1 vs. CTRL: 24.4 ± 1.6 mM, $P = 0.32$), [P_i] (FENT: 1.3 ± 0.2 vs. CTRL: 1.0 ± 0.2 mM, $P = 0.92$), [$H_2PO_4^-$] (FENT: 0.5 ± 0.1 vs. CTRL: 0.4 ± 0.1 mM, $P = 0.95$), pH (FENT: 7.00 ± 0.02 vs. CTRL: 6.99 ± 0.02 , $P = 0.48$) and ΔG_{ATP} (FENT: -65.5 ± 1.4 vs. CTRL: -66.5 ± 1.0 kJ mol⁻¹,

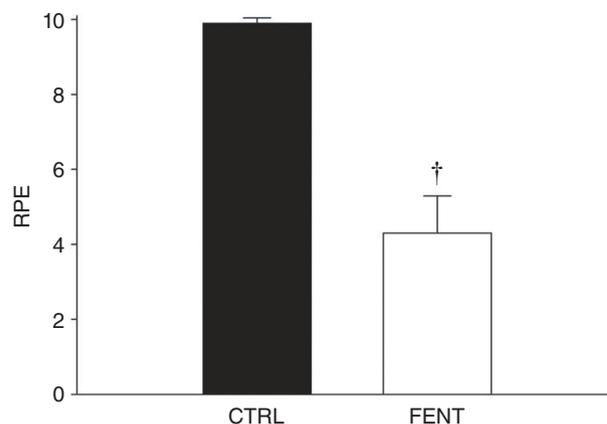


Figure 2. Rating of perceived exertion at the completion of the all-out intermittent isometric single-leg knee-extensor exercise protocol

Ratings of perceived exertion (RPE) were obtained, using the Borg modified CR10 scale, from the subjects immediately upon completion of the exercise protocol in both control (CTRL) and lumbar intrathecal fentanyl (FENT) conditions. Data are presented as means \pm SEM. †Significantly different from CTRL

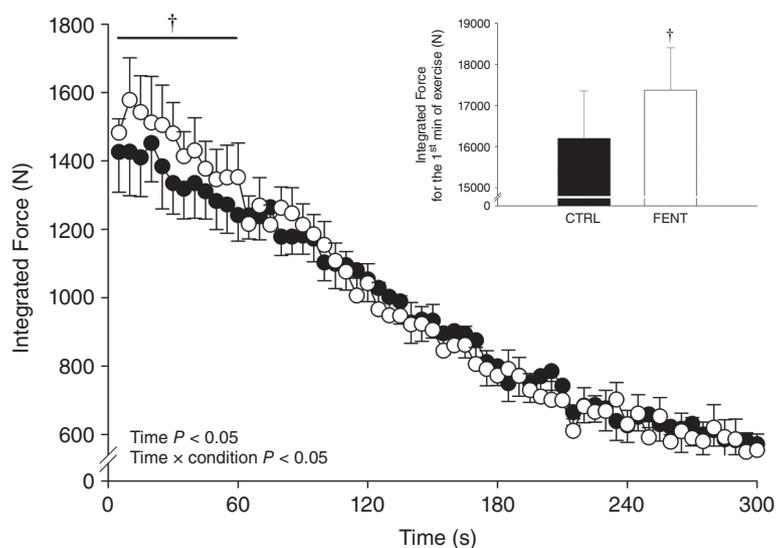


Figure 1. Force production during the all-out intermittent isometric single-leg knee-extensor exercise protocol

Seven subjects performed a series of 60 intermittent maximal voluntary contractions (3 s contraction, 2 s relaxation) over 5 min in both control (CTRL) and lumbar intrathecal fentanyl (FENT) conditions. The integrated force was determined per maximal voluntary contraction. Inset, the cumulative integrated force production for the first minute of exercise. Data are presented as means \pm SEM. Significant time and interaction effects are indicated on the graph. †Significantly different from CTRL.

$P = 0.15$). All measured intramuscular variables changed significantly over time during the 60 MVCs and there were significant interaction effects only for $[P_i]$, $[H_2PO_4^-]$ and $[ATP]$ (Fig. 3). There were no changes in intramuscular variables over the final six MVCs ($P > 0.05$) (Fig. 3). Intramuscular metabolite concentrations at the 60th MVC were significantly different between conditions for $[P_i]$ (FENT: 37.4 ± 17.8 vs. CTRL: 27.6 ± 11.9 mM) and $[H_2PO_4^-]$ (FENT: 25.0 ± 12.7 vs. 18.0 ± 8.5 mM), but not for $[PCr]$ (FENT: 5.4 ± 2.4 vs. 5.0 ± 1.9 mM, $P = 0.69$), pH (FENT: 6.46 ± 0.07 vs. CTRL: 6.49 ± 0.04 , $P = 0.20$) and $[ATP]$ (FENT: 70.9 ± 28.4 vs. $78.8 \pm 21.4\%$, $P = 0.29$; Fig. 3).

Effect of afferent blockade on ATP synthesis rates, ATP cost of contraction and PCr recovery kinetics

For the first minute of exercise, CTRL values were as follows: ATP_{CK} , 19.6 ± 1.3 mM min^{-1} ; ATP_{GLY} , 8.8 ± 3.0 mM min^{-1} ; ATP_{ANA} , 28.4 ± 4.0 mM min^{-1} ; ATP_{OX} , 45.8 ± 7.0 mM min^{-1} ; ATP_{TOTAL} , 52.6 ± 5.2 mM min^{-1} ; and ATP cost of contraction, 0.04 ± 0.01 mM $\text{min}^{-1} \text{N}^{-1}$. All ATP synthesis rates and the ATP cost of contraction changed significantly over time during the 60 MVCs. There were significant condition effects between FENT and CTRL for ATP_{CK} , ATP_{GLY} , ATP_{ANA} , ATP_{TOTAL} and ATP cost of contraction, with significant interaction effects for ATP_{CK} and ATP cost of contraction (Fig. 4). For the 60 MVCs, ATP_{ANA} was significantly greater ($\sim 11\%$) for FENT than CTRL, arising from significantly greater ATP_{CK} ($\sim 9\%$) for the first minute and significantly greater ATP_{GLY} ($\sim 35\%$) throughout the 60 MVCs. ATP_{OX} was not significantly different between FENT and CTRL ($P = 0.14$), and for both conditions ATP_{OX} for each minute was within the 95% confidence interval of V_{max} . There were no differences in the percentage contribution of ATP_{ANA} and ATP_{OX} to ATP_{TOTAL} between FENT and CTRL ($P > 0.05$; Fig. 4). For the PCr recovery kinetics, there were no differences between FENT and CTRL: amplitude, 23.9 ± 4.3 vs. 22.2 ± 4.2 mM, $P = 0.15$; V_{max} , 34.4 ± 12.3 vs. 28.3 ± 5.6 mM min^{-1} , $P = 0.24$; τ , 54.2 ± 13.4 vs. 54.9 ± 11.3 s, $P = 0.89$; V_{IPCr} , 27.7 ± 7.6 vs. 24.6 ± 3.8 mM min^{-1} , $P = 0.391$; and H_{efflux}^+ , 6.6 ± 2.8 vs. 4.3 ± 1.4 mM min^{-1} , $P = 0.07$).

Discussion

This study sought to elucidate the influence of group III/IV muscle afferents on exercise performance during maximal small-muscle mass exercise from a skeletal muscle bioenergetics perspective. Consistent with our first hypothesis, a greater total ATP production was sustained throughout the all-out exercise protocol when group III/IV muscle afferent feedback was attenuated.

This metabolic reserve was provided by the anaerobic energetic pathways of the active skeletal muscle. In support of our second hypothesis, when group III/IV muscle afferent feedback was attenuated, the ATP cost of contraction was exacerbated during the latter, but not the initial, portion of the exercise protocol. This finding reinforces the important role of group III/IV muscle afferents in maintaining efficient skeletal muscle contractile function during high-intensity exercise. Supporting our third hypothesis, the attenuation of group III/IV muscle afferent feedback improved exercise performance ($\sim 8\%$) during the initial, but not the latter, portion of the all-out protocol. The time-dependent alterations in exercise performance appear to have been the result of the ensemble product of the changes in total ATP production and the ATP cost of contraction. These findings reveal that group III/IV muscle afferents directly limit exercise performance during small muscle mass exercise, but, due to their critical role in maintaining skeletal muscle contractile efficiency, with time, the benefit of attenuating muscle afferents is negated.

Intramuscular metabolic perturbation

In the current study, a greater accumulation of P_i and $H_2PO_4^-$, and a greater depletion of ATP occurred during the all-out exercise when feedback from the group III/IV muscle afferents was attenuated (Fig. 3). Of note, this greater accumulation of P_i and $H_2PO_4^-$ was incurred despite no difference in PCr depletion between FENT and CTRL, which was likely due to the greater depletion of ATP (Fig. 3) and the substantial increase in glycolytic energy production (Fig. 4) during FENT compared to CTRL (Marsh *et al.* 1991; Chilibeck *et al.* 1998). These findings are consistent with two previous reports during both whole-body cycling exercise (Blain *et al.* 2016) and submaximal small muscle mass exercise (Broxterman *et al.* 2017b). Collectively, these data demonstrate that group III/IV muscle afferents constrain the maximal level of intramuscular metabolic perturbation during severe intensity exercise, which appears to be consistent during both large and small muscle mass exercise (Hogan *et al.* 1999; Burnley *et al.* 2010; Vanhatalo *et al.* 2010, 2016; Chidnok *et al.* 2013). The intramuscular metabolic data from the current study have important implications for the influence of group III/IV muscle afferents on the development of neuromuscular fatigue. Specifically, the greater accumulation of P_i and $H_2PO_4^-$ suggests a greater development of peripheral fatigue (Blain *et al.* 2016; Broxterman *et al.* 2017a), which, coupled with the similar fall in MVC between conditions, implies that the attenuation of group III/IV muscle afferent feedback decreased the development of central fatigue. These findings are consistent with previous demonstrations

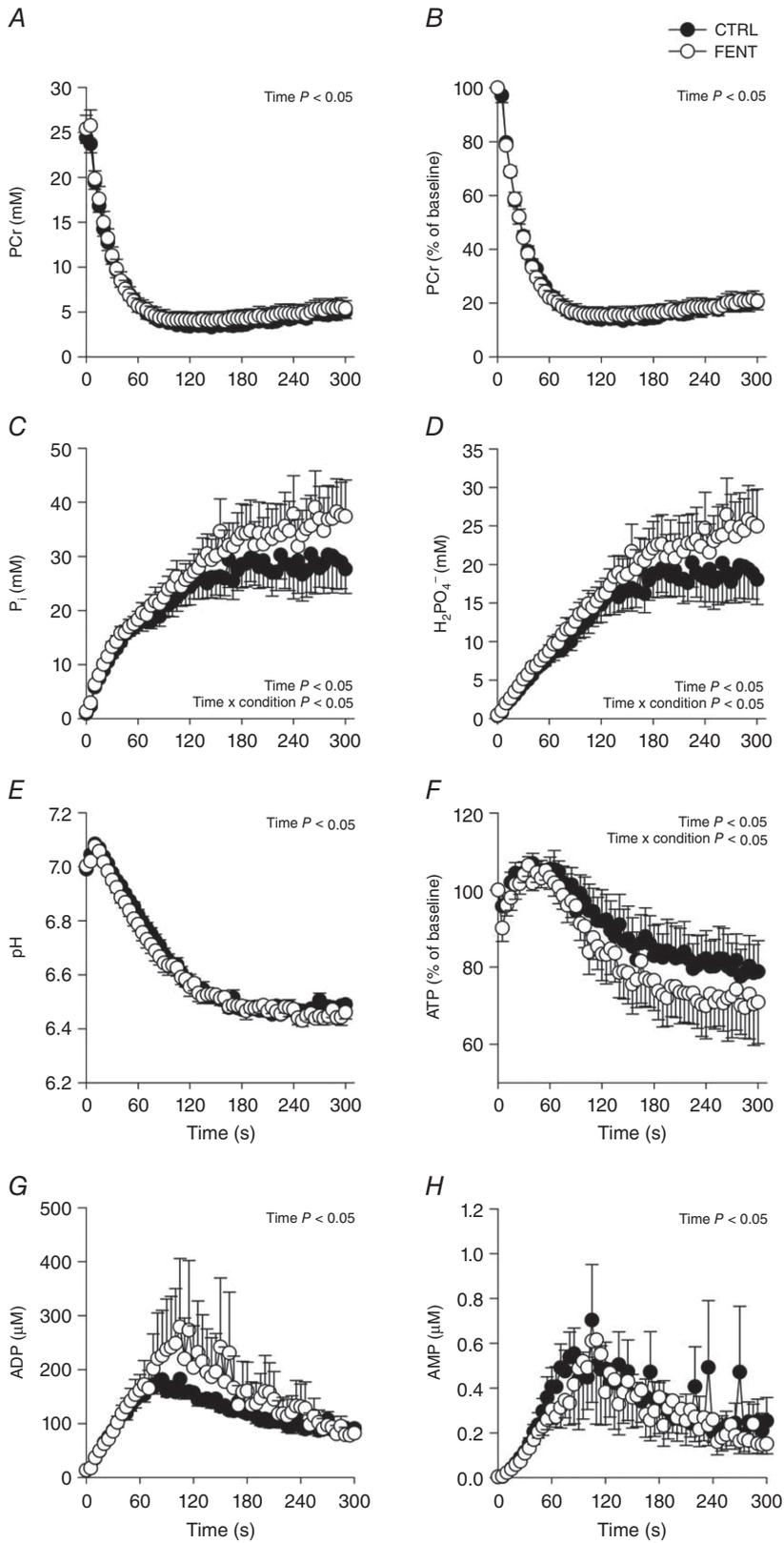


Figure 3. Intramuscular metabolites during the all-out intermittent isometric single-leg knee-extensor exercise protocol

Intramuscular phosphocreatine (PCr, A and B), inorganic phosphate (P_i , C), diprotonated phosphate ($H_2PO_4^-$, D), pH (E), ATP (F), ADP (G) and AMP (H) were determined with phosphorous magnetic resonance spectroscopy (^{31}P -MRS) in both control (CTRL) and lumbar intrathecal fentanyl (FENT) conditions. Data are presented as means \pm SEM. Significant time and interaction effects are indicated on the graphs.

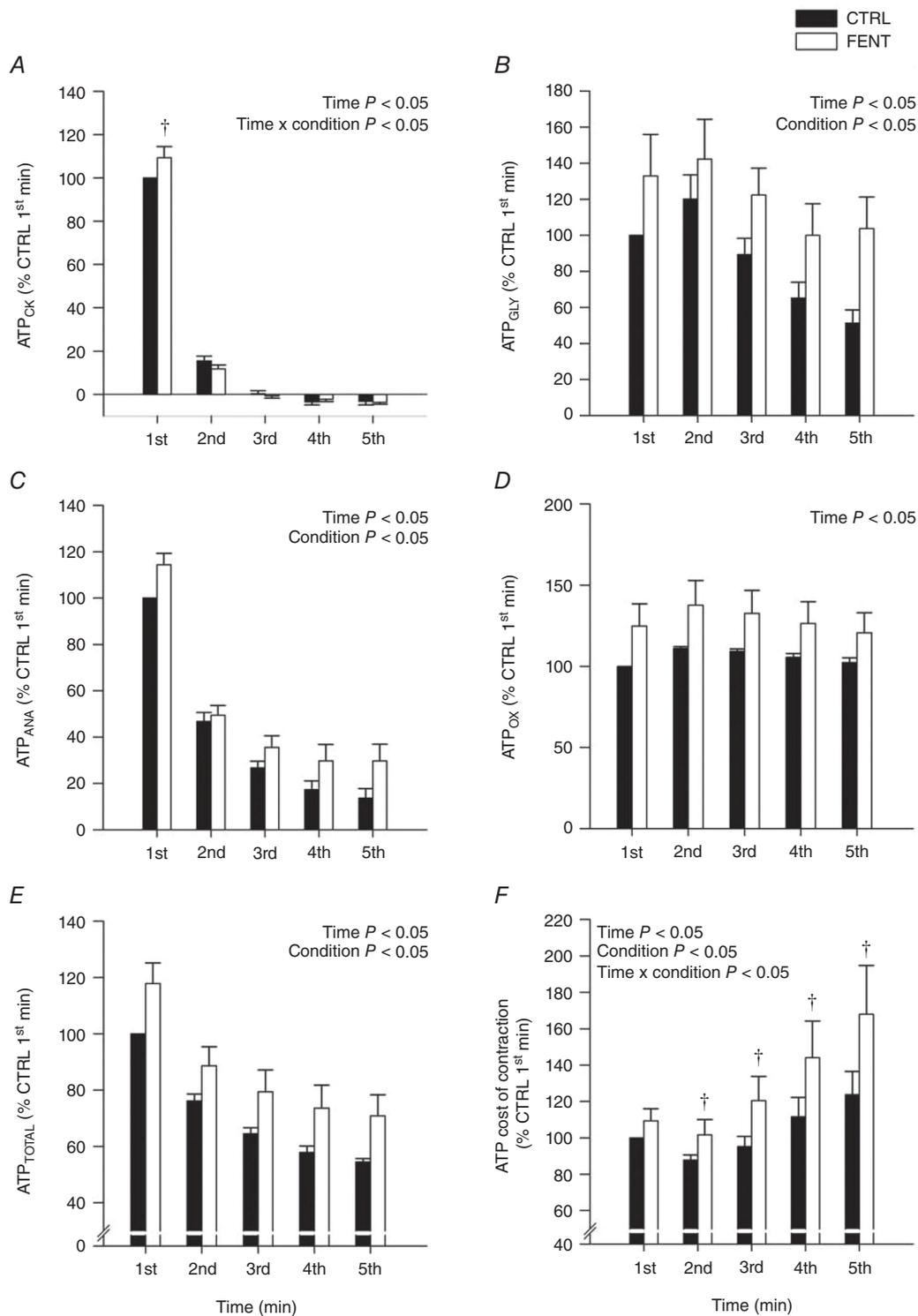


Figure 4. Adenosine triphosphate (ATP) synthesis rates and the ATP cost of contraction during the all-out intermittent isometric single-leg knee-extensor exercise protocol

Rates of ATP synthesis through the creatine kinase reaction (ATP_{CK}, A), anaerobic glycolysis (ATP_{GLY}, B), cumulative anaerobic metabolism (ATP_{ANA}, C), oxidative phosphorylation (ATP_{OX}, D), total ATPase rate (ATP_{TOTAL}, E) and the ATP cost of contraction (F) were determined in both control (CTRL) and lumbar intrathecal fentanyl (FENT) conditions. These variables were calculated for each minute of the 60 MVC protocol and were normalized to the first minute of CTRL exercise. Data are presented as means ± SEM for each minute of exercise. Significant time, condition and interaction effects are indicated on the graphs. †Significantly different from CTRL at specific time point.

of reduced central fatigue and exacerbated peripheral fatigue development during whole-body exercise with attenuated group III/IV muscle afferent feedback (Amann *et al.* 2009, 2011a; Sidhu *et al.* 2014, 2017; Blain *et al.* 2016). Importantly, the current study also reveals that an increased ATP cost of contraction is another factor, in addition to decreased oxygen delivery (Amann *et al.* 2011b), that may explain the exacerbated development of peripheral fatigue during exercise with attenuated group III/IV muscle afferent feedback.

Exercise performance and skeletal muscle biogenetics

Exercise performance has been unaltered or diminished with attenuation of group III/IV muscle afferent feedback, despite the lifting of constraints on motoneuronal output, intramuscular perturbation and neuromuscular fatigue (Amann *et al.* 2009, 2011a; Sidhu *et al.* 2014, 2017; Blain *et al.* 2016). However, it has, over time, been recognized that the exercise performance outcomes in these previous studies were likely the result of a diminished oxygen delivery to the working skeletal muscle (Amann *et al.* 2011b) leading to an exacerbated rate of intramuscular perturbation and neuromuscular fatigue development (Blain *et al.* 2016; Broxterman *et al.* 2017a). These effects offset any direct benefit of attenuating group III/IV muscle afferent feedback on exercise performance (Amann & Calbet, 2008). With this in mind, an important aspect of the current study is that oxygen delivery to the working skeletal muscle was likely similar between conditions, as indicated by the oxygen delivery-sensitive variables of oxidative ATP synthesis, H_{efflux}^+ , and the peak rate of mitochondrial ATP synthesis (Kemp *et al.* 1993, 1997; Haseler *et al.* 1999, 2007; Layec *et al.* 2013; Broxterman *et al.* 2017b), facilitated by the substantial hyperaemia that occurs between contractions during intermittent isometric exercise (Lind & Williams, 1979; Wigmore *et al.* 2004, 2006). Interestingly, however, despite circumventing the issue of diminished oxygen delivery, overall exercise performance was still not altered by the attenuation of group III/IV muscle afferent feedback (Fig. 1). This finding is consistent with our previous report during submaximal exercise to task failure (Broxterman *et al.* 2017b). In both of these studies, the attenuation of group III/IV muscle afferent feedback exacerbated the ATP cost of contraction, which, as with previous reductions in oxygen delivery, appears to have offset the greater total ATP production resulting from the attenuation of group III/IV muscle afferent feedback on exercise performance (Figs 1 and 4).

A novel aim of the present study was, however, to perform a high time resolution assessment of maximal exercise performance and skeletal muscle bioenergetics. Importantly, this revealed a substantial improvement (~8%) in exercise performance during the first minute of

exercise (Fig. 1). Thus, it appears that for the first minute of exercise, attenuating group III/IV muscle afferent feedback likely unbridled motoneuronal output, which resulted in an increase in total ATP production which was translated into improved force production. Unlike subsequent time periods, the initial minute of exercise without muscle afferent feedback did not result in an increase in the ATP cost of contraction, facilitating the successful translation of the greater total ATP production into improved force production (Figs 1 and 4). The higher rate ATP synthesis was attributable to the anaerobic energetic pathways (i.e. creatine kinase and glycolysis). This reserve in anaerobic ATP production demonstrates that exercise performance in control conditions is not limited by the maximal capacity of skeletal muscle metabolism, but, rather, the group III/IV muscle afferents constrain exercise performance, likely through their direct effect on motoneuronal output (Amann *et al.* 2009, 2011a; Sidhu *et al.* 2014; Blain *et al.* 2016).

During the second to fifth minutes of the all-out exercise, performance was not altered by the attenuation of group III/IV muscle afferent feedback (Fig. 1). The total ATP production during this portion of the protocol remained substantially elevated with the attenuated group III/IV muscle afferent feedback, likely as the result of the continued unbridling of motoneuronal output. However, this did not translate into an improved force production, as the ATP cost of contraction progressively increased due to the secondary effects of attenuating group III/IV muscle afferent feedback (Figs 1 and 4). Speculatively, these secondary effects may have included compromised afferent mediated reciprocal inhibition of antagonist muscle (Enoka & Duchateau, 2008; Martin *et al.* 2008; Kennedy *et al.* 2013), motor unit rotation during exercise (Bawa & Murnaghan, 2009), or alterations of motor unit firing rate during exercise (Bigland-Ritchie *et al.* 1986). The current findings may also indicate that group III/IV muscle afferent feedback is important for the appropriate interplay between substrate level and oxidative phosphorylation during exercise, preventing an untenable rise in mitochondrial respiration stimulants. Overall, the time course dependent nature of exercise performance with attenuated group III/IV muscle afferent feedback, was likely the ensemble product of alterations in total ATP production and the ATP cost of contraction, as illustrated in Fig. 5.

Implications for the force–duration relationship

The relationship between exercise intensity and exercise tolerance within the severe-intensity domain is well-described by a hyperbolic equation (Hill & Lupton, 1923; Hill, 1925; Monod & Scherrer, 1965; Moritani *et al.* 1981; Poole *et al.* 1988). The asymptote of this

relationship defines the highest attainable steady state for aerobic energy production without continually utilizing the finite capacity for work above this asymptote, defined by the curvature constant of this relationship (Monod & Scherrer, 1965; Moritani *et al.* 1981; Poole *et al.* 1988; Jones *et al.* 2008; Copp *et al.* 2010; Vanhatalo *et al.* 2010; Dekerle *et al.* 2012; Broxterman *et al.* 2014, 2015a). The asymptote of this relationship can be determined from the end-test force of the all-out exercise protocol utilized in the current study (Burnley, 2009). Interestingly, the end-test force was not affected by group III/IV muscle afferent attenuation (Fig. 1). Together with no effect on oxidative ATP production, this finding suggests that aerobic metabolism was not diminished by attenuating group III/IV muscle afferent feedback. Cumulating evidence supports the concept that group III/IV muscle afferents are critically involved in determining the curvature constant of the force–duration relationship (Broxterman *et al.* 2015b; Hureau *et al.* 2016). The current findings support this, as attenuation of group III/IV muscle afferent feedback increased exercise performance during the initial portion of the all-out

protocol, when the bioenergetic sources associated with the curvature constant are primarily utilized. However, as the curvature constant cannot be precisely determined with this specific all-out exercise protocol (Burnley, 2009), this remains to be confirmed. Collectively, the current data suggest that group III/IV muscle afferents are likely critically involved in determining the curvature constant of the force–duration relation, while the asymptote of this relationship does not appear to be influenced by these muscle afferents.

Perception of effort during all-out exercise

It is becoming increasingly apparent that, amongst other factors, group III/IV muscle afferent feedback directly influences the perception of effort during exercise (Amann *et al.* 2013). Recently, it was demonstrated that attenuation of group III/IV muscle afferent feedback reduced RPE by ~50% at task-failure during small muscle mass exercise (Broxterman *et al.* 2017b). In agreement with this previous study, RPE in the current study was reduced by ~60% with the attenuation of feedback from group III/IV muscle afferents during all-out small muscle mass exercise (Fig. 2). Thus, the current findings offer additional evidence that group III/IV muscle afferents influence the perception of effort during exercise.

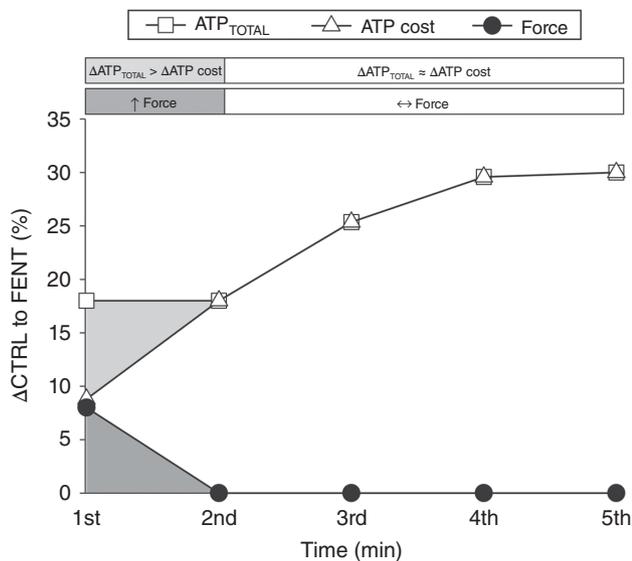


Figure 5. Graphical illustration of the skeletal muscle bioenergetic influence on exercise performance during the all-out intermittent isometric single-leg knee-extensor exercise protocol

The percentage difference between control (CTRL) and lumbar intrathecal fentanyl (FENT) conditions (Δ CTRL to FENT) for integrated force, total adenosine triphosphate production ($\text{ATP}_{\text{TOTAL}}$) and the ATP cost of contraction are plotted for each minute of the protocol. Integrated force was significantly greater in FENT than CTRL for the first minute of exercise (dark grey shading), when $\text{ATP}_{\text{TOTAL}}$ was significantly increased (light grey shading) with no significant change in the ATP cost of contraction. Integrated force was not significantly different between FENT and CTRL for the 2nd–5th min of exercise despite the sustained increase in $\text{ATP}_{\text{TOTAL}}$, due to the significantly increased ATP cost of contraction in FENT compared to CTRL.

Conclusions

This study revealed two novel findings regarding the influence of group III/IV muscle afferents on small muscle mass exercise performance. First, there is an anaerobic metabolic reserve within skeletal muscle that, when group III/IV muscle afferent feedback is attenuated, allows for a greater ATP production and integrated force generation compared to control conditions. Second, group III/IV muscle afferents are critically involved in maintaining efficient skeletal muscle contraction during the latter portion of an all-out exercise protocol. As such, the time course-dependent nature of all-out exercise performance with attenuated group III/IV muscle afferent feedback was the ensemble effect of alterations in total ATP production and the ATP cost of contraction. Therefore, group III/IV muscle afferents directly limit exercise performance during small muscle mass exercise, but, due to their critical role in maintaining skeletal muscle contractile efficiency, with time, the benefit of attenuating muscle afferents is negated.

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Additional information

Competing interests

The authors declare no competing interests.

Author contributions

The work was conducted at the Utah Vascular Research Laboratory (UVRL) housed in the Salt Lake City Veterans Affairs Hospital, Utah. All authors contributed to data collection/analysis and manuscript preparation. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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