The Journal of Physiology

Intrathecal fentanyl abolishes the exaggerated blood pressure response to cycling in hypertensive men

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Key points

- The increase in blood pressure observed during physical activities is exaggerated in patients with hypertension, exposing them to a higher cardiovascular risk.
- Neural signals from the skeletal muscles appear to be overactive, resulting in this abnormal response in hypertensive patients.
- In the present study, we tested whether the attenuation of these neural signals in hypertensive patients could normalize their abnormal increase in blood pressure during physical activity.
- Attenuation of the neural signals from the leg muscles with intrathecal fentanyl injection reduced the blood pressure of hypertensive men during cycling exercise to a level comparable to that of normotensive men.
- Skeletal muscle afferent overactivity causes the abnormal cardiovascular response to exercise and was reverted in this experimental model, appearing as potential target for treatment.

Abstract Hypertensive patients present an exaggerated increase in blood pressure and an elevated cardiovascular risk during exercise. Although controversial, human studies suggest that group III and IV skeletal muscle afferents might contribute to this abnormal response. In the present study, we investigated whether attenuation of the group III and IV muscle afferent signal of hypertensive men eliminates the exaggerated increase in blood pressure occurring during exercise. Eight hypertensive men performed two sessions of 5 min of cycling exercise at 40 W. Between sessions, the subjects were provided with a lumbar intrathecal injection of fentanyl, a μ-opioid receptor agonist, aiming to attenuate the central projection of opioid-sensitive group III and IV muscle afferent nerves. The cardiovascular response to exercise of these subjects was compared with that of six normotensive men. During cycling, the hypertensive group demonstrated an exaggerated increase in blood pressure compared to the normotensive group (mean $\pm$ SEM: $+17 \pm 3$ *vs.* $+8 \pm 1$ mmHg, respectively; $P < 0.05$), whereas the increase in heart rate, stroke volume, cardiac output and vascular conductance was similar ($P > 0.05$). Fentanyl inhibited the blood pressure response to exercise in the hypertensive group ($+11 \pm 2$ mmHg) to a level comparable to that of the normotensive group ($P > 0.05$). Moreover, fentanyl increased the responses of vascular conductance and stroke volume to exercise ($P < 0.05$), whereas the heart rate response was attenuated ($P < 0.05$) and the cardiac output response was maintained ($P > 0.05$). The results of the present study show that attenuation of the exercise pressor reflex normalizes the blood pressure response to cycling exercise in hypertensive individuals.

 DOI: 10.1113/JP271335

(Received 23 July 2015; accepted after revision 7 December 2015; first published online 12 December 2015)
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Abbreviations a.u., arbitrary units; APBM, ambulatory BP monitoring; BL, baseline resting measurements; BP, blood pressure; CO, cardiac output; CTRL, control exercise session before fentanyl injection; FENT, exercise session after fentanyl injection; HR, heart rate; HT, hypertensive; MBP, mean blood pressure; MVC, maximal voluntary contraction; NO, nitric oxide; NT, normotensive; SV, stroke volume; TVC, total vascular conductance.

Introduction

Regular exercise is recommended as a coping resource to lower blood pressure (BP) in hypertensive (HT) patients (Pescatello *et al.* 2015). However, in these patients, exercise is often accompanied by exaggerated increases in BP (Papademetriou *et al.* 1989; Kokkinos *et al.* 2002), leading to greater myocardial demand and risk of cardiovascular events, particularly when associated with asymptomatic cardiovascular diseases (Thompson *et al.* 2007). In addition, an exaggerated BP response to low-intensity exercise is also a strong predictor of cardiovascular mortality (Mundal *et al.* 1996; Kurl *et al.* 2001). Hence, identification of the signals that drive the exaggerated pressor response to exercise in hypertension may help the development of interventions aiming to revert this abnormal response, reducing the potential risks of physical activity in this disease.

Cardiovascular responses to exercise are greatly influenced by adjustments of the autonomic nervous system, which are modulated by neural signals interacting within the brainstem. These signals originate from the brain ('central command') (Goodwin *et al.* 1972) and from group III and IV skeletal muscle afferent nerve endings ('exercise pressor reflex') in response to mechanical (mechanoreflex) and metabolic (metaboreflex) stimuli during muscular contractions (Coote *et al.* 1971; McCloskey & Mitchell, 1972; Fisher *et al.* 2015), as well as from the arterial and cardiopulmonary baroreflex, a negative feedback mechanism that adjusts cardiac and peripheral vascular autonomic neural outflow in a beat-to-beat basis, in response to abrupt changes in BP (Fadel & Raven, 2012). Evidence from animals suggests that both components of the exercise pressor reflex are overactive in hypertension (Leal *et al.* 2008). In HT humans, however, the BP response to handgrip exercise was either augmented (Delaney *et al.* 2010; Vongpatanasin *et al.* 2011; Greaney *et al.* 2015) or preserved (Rondon *et al.* 2006; Sausen *et al.* 2009), and responses to metaboreflex stimulation by muscle ischaemia after handgrip exercise were also controversial (Rondon *et al.* 2006; Sausen *et al.* 2009; Delaney *et al.* 2010). Furthermore, these findings are limited to upper limb exercise, whereas experiments involving leg exercise are lacking, and would have relevance for daily-life activities. Thus, in HT humans, the involvement of the exercise pressor reflex in mediating the exaggerated pressor response to physical activity remains unclear.

Experiments with anaesthetized cats found that the administration of a μ-opioid receptor agonist into the dorsal horn of the spinal cord attenuated the cardiovascular responses to electrically-induced muscle contraction and passive stretch, denoting inhibition of the exercise pressor reflex (Meintjes *et al.* 1995). Recently, this model was adapted to humans, investigating cardiovascular responses to leg exercise after injection of the μ-opioid receptor agonist fentanyl into the lumbar intrathecal (subarachnoid) space. In healthy young men, fentanyl reduced the BP response to cycling (Amann *et al.* 2010; Barbosa *et al.* 2015), without affecting muscle force production and central command activation (Amann *et al.* 2009). Using a similar approach, in the present study, we investigated the role of the exercise pressor reflex for the BP response of HT patients during cycling. We hypothesized that HT patients would present an exaggerated BP response, but this response would be normalized, i.e., reduced to the same level as that of normotensive (NT) subjects, following attenuation of the exercise pressor reflex with intrathecal fentanyl.

Methods

Ethical approval

All procedures were approved by the Ethical Committee for Research at Fluminense Federal University (CAAE: 09282812.3.0000.5243) and conformed to the *Declaration of Helsinki*. All individuals provided their written informed consent prior to participation.

Study population

Recreationally active and non-smoking men were divided into two groups: non-treated HT and NT (Table 1). They were screened for hypertension (HEM-742INT; Omron Healthcare, Kyoto, Japan) on two separate days in a seated position, with three measures per day using cuffs of an adequate size with respect to their arm circumference. To confirm the diagnosis of hypertension, we used ambulatory BP monitoring (ABPM)

Table 1. Subject characteristics

	HT	NT
Number of subjects	8	6
Number of systolic hypertension	7	–
Number of diastolic hypertension	6	–
Number of systolic and diastolic hypertension	5	–
Age (years)	49 ± 2	47 ± 5
Weight (kg)	86.7 ± 3.0	81.5 ± 6.8
Height (cm)	174 ± 2	176 ± 2
Body mass index (kg m^{-2})	28.7 ± 0.6	26.2 ± 2.3
24-h systolic BP (mmHg)	132 (130–177)*	122 (113–127)
24-h diastolic BP (mmHg)	84 (81–114)*	76 (68–80)
Office systolic BP (mmHg)	143 (142–145)*	111
Office diastolic BP (mmHg)	84 (80–103)	71

Twenty-four hour BP measurements included 5 HT and 5 NT. Office BP measurements included 3 HT and 1 NT.
Data are the mean ± SEM or median (minimum – maximum). *$P < 0.05$ *vs.* NT.

(Dyna-MAPA; Cardios, São Paulo, Brazil) because of its greater accuracy for describing the BP profile in the daily routine, avoiding 'white-coat' hypertension (O'Brien *et al.* 2013). The average BP values obtained in 24 h ABPM are usually lower than those obtained by office BP measurements as a result of nocturnal dipping. The consensus values for the diagnosis of hypertension using 24 h ABPM are systolic BP >130 mmHg and/or diastolic BP >80 mmHg (Mancia *et al.* 2013; O'Brien *et al.* 2013). These values yielded 10 year cardiovascular risks similar to those associated with high BP on office measurement (i.e. 140/90 mmHg) (Kikuya *et al.* 2007). During BP measurements in the awake period, subjects should relax their arms beside the body or over a support, either in a standing or seated position. Measures were obtained every 20 min during the 24 h monitoring. Four subjects were unable to participate in the 24 h ambulatory BP monitoring (three HT and one NT) and were diagnosed by office BP measurements only, when presenting average systolic BP >140 mmHg and/or diastolic BP >90 mmHg (Mancia *et al.* 2013). None of the HT patients was under pharmacological treatment of hypertension. All subjects were free of diseases other than hypertension.

Exercise protocol

Subjects were asked to avoid caffeine, alcohol and intense exercise for 24 h and to fast for 3 h prior to the experiment, which was conducted in a room with an ambient temperature of 22–24°C.

Figure 1 displays the timeline of experimental procedures. After a 30 min rest and instrumentation, subjects were positioned on a cycling ergometer (AB–RB 300 Magnetic Recumbent Bike; AIBI, Singapore) with support for the arms. The experiment comprised two exercise sessions. Initially, to assess a possible and undesired cephalad migration or vascular absorption of fentanyl that could attenuate cardiovascular responses to exercise by a direct effect on opioid receptors within the brainstem (Caringi *et al.* 1998), the HT group performed a static handgrip contraction for 2 min at 40% of the maximal voluntary contraction (MVC), with visual feedback of the force level. MVC of the hand was assessed in the first exercise session by squeezing a digital force transducer (Grip Force Transducer; ADInstruments, Sydney, Australia) with maximal effort for 2–3 s at least three times, with a 1 min interval. The greatest force achieved among trials ranging within 5% of each other was taken as the MVC.

Fifteen minutes later, the subjects performed cycling exercise for 5 min at 40 W and 60 rpm, with visual feedback of the cadence. NT subjects also performed this cycling exercise protocol. Approximately 30 min after the end of the first session (CTRL; i.e. control exercise session before fentanyl injection), the HT group was provided with an intrathecal administration of fentanyl and then repeated the exercise protocol in a second exercise session. Therefore, HT subjects performed the CTRL session before the fentanyl session (FENT; i.e. exercise session after fentanyl injection). Static handgrip and leg cycling exercises were repeated within 29–37 min and 51–58 min, respectively, of fentanyl administration.

Subjects reported the rate of perceived exertion according to the modified Borg rating scale (Noble *et al.* 1983) at the end of each exercise trial, with scores ranging from 0 (minimal perception of effort) to 10 (maximal perception of effort).

Lumbar intrathecal fentanyl

After finishing CTRL cycling exercise, HT patients rested supine for 30 min and then underwent lumbar puncture.

When seated with the trunk flexed, a 27 G, 9 cm Whitacre spinal needle was inserted into the L2–L3 or L3–L4 intervertebral intrathecal space. With presentation of spinal fluid, 1 ml of a solution containing 50 μg of fentanyl was injected through a filter. After fentanyl injection, the needle was removed. Then, subjects returned to the cycle ergometer and performed FENT exercise session. They remained upright until the end of the experiment and were instructed to report any sensations after fentanyl administration, including pruritus, nausea and dyspnoea (Chaney, 1995). To prevent hypotension and headache after lumbar puncture, subjects received 500 ml of saline solution at the end of the experiment and were followed for 1 week.

Cardiovascular measurements

Mean BP (MBP) was obtained from a catheter into the brachial or radial artery of the non-exercising arm, linked to a transducer kept at the heart level (TruWave Disposable Pressure Transducer; Edwards Life Sciences, Irvine, CA, USA) and a monitor (Dialogue 2000; IBC-Danica, Copenhagen, Denmark). Heart rate (HR) was recorded using an electrocardiogram (Dual Bio Amp and 5 Lead Shielded Cable; ADInstruments). These signals were recorded at a 1000 Hz sampling rate using an analogue-to-digital data acquisition system (PowerLab 16/35, software LabChart 7 for Mac OS; ADInstruments). Stroke volume (SV) was derived from the BP waveform using the Modelflow method (Wesseling *et al.* 1993) (Beatscope 1.1a; Finapres Medical Systems BV, Amsterdam, The Netherlands). Cardiac output (CO) was the product of HR and SV. Total vascular conductance (TVC) was the ratio of CO to MBP.

Statistical analysis

Resting baseline values (average from 2–3 min immediately pre-exercise) and the responses to exercise [differences between values during exercise (average of the entire period of static handgrip or 1 min averages during cycling) and resting baseline (Δ)] of the HT group in CTRL and FENT sessions were compared by Student's paired t test and two-way repeated measures ANOVA (factors: Time of exercise $\times$ Fentanyl), whereas Student's t test for independent samples and two-way repeated measures ANOVA (factors: Time of exercise $\times$ Group) were used for comparisons between groups (HT CTRL *vs.* NT, HT FENT *vs.* NT). When a significant interaction was found by ANOVA, Fisher's least significant difference *post hoc* tests were conducted for pairwise comparisons. Statistical analysis was performed using Statistica, version 10 (StatSoft Inc., Tulsa, OK, USA).

Results

Fentanyl-related effects and responses to static handgrip

All experiments were completed within 80 min of fentanyl administration. None of the HT subjects reported nausea or felt dyspnoeic, although seven subjects reported pruritus over the legs, pelvis and abdomen. This symptom disappeared within less than 1 h after the end of the experiments.

HT subjects sustained the target force level during static handgrip in both trials (HT CTRL: 39.5 $\pm$ 0.3% MVC, HT FENT: 39.6 $\pm$ 0.3% MVC; $P = 0.74$). Also, the rate of perceived exertion was similar in both conditions (HT CTRL: 6 $\pm$ 0 a.u., HT FENT: 5 $\pm$ 1 a.u.; $P = 0.25$).

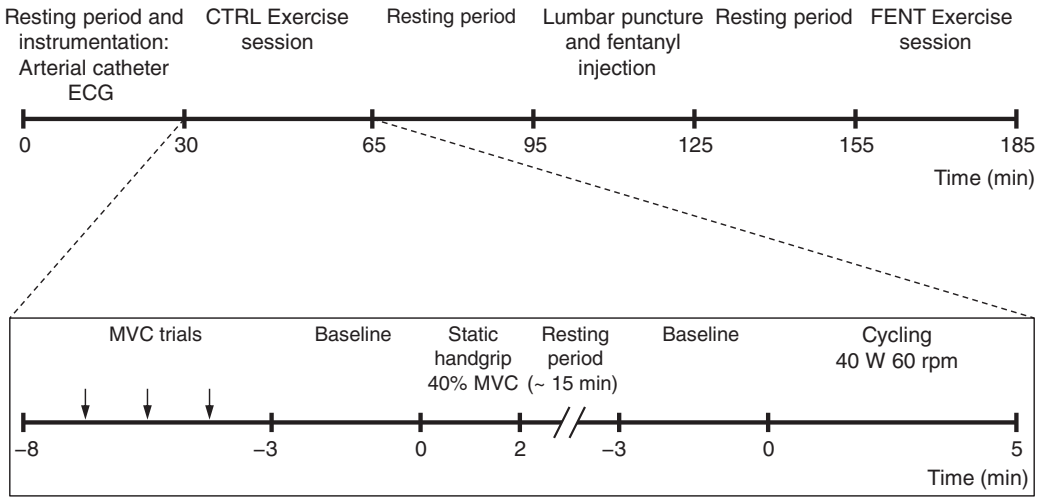

Figure 1. Timeline of experimental procedures
The FENT exercise session was identical to the CTRL exercise session, except for the MVC trials. Normotensive subjects performed the CTRL cycling only.

Table 2. Cardiovascular measures at resting baseline and during 2 min static handgrip

	Baseline	Static handgrip	Difference
Mean BP (mmHg)			
HT CTRL	117 ± 3*	150 ± 4	+37 ± 5
HT FENT	103 ± 6	134 ± 7	+33 ± 3
Heart rate (beats min^{-1})			
HT CTRL	67 ± 4	85 ± 5	+19 ± 2
HT FENT	68 ± 3	84 ± 5	+15 ± 2
Stroke volume (mL)			
HT CTRL	77 ± 4*	73 ± 4	−8 ± 3
HT FENT	72 ± 4	69 ± 6	−5 ± 2
Cardiac output (L min^{-1})			
HT CTRL	5.2 ± 0.4	6.2 ± 0.6	+0.8 ± 0.2
HT FENT	4.9 ± 0.4	5.8 ± 0.6	+0.6 ± 0.2
Total vascular conductance (mL min^{-1} mmHg^{-1})			
HT CTRL	45 ± 4*	42 ± 4	−6 ± 2
HT FENT	50 ± 6	45 ± 6	−7 ± 1

Values are average from 2–3 min of rest before, and during the entire period of 2 min static handgrip at 40% MVC. Resting values and the differences between exercising and resting values were used for comparisons. HT CTRL, hypertensive subjects before fentanyl administration; HT FENT, hypertensive subjects after fentanyl administration. Data are the mean ± SEM. *$P < 0.05$ *vs.* HT FENT.

Table 2 presents resting cardiovascular measurements and their responses to static handgrip. Following fentanyl administration, HT patients presented lower resting MBP ($P = 0.02$ *vs.* HT CTRL), accompanied by higher TVC ($P = 0.05$ *vs.* HT CTRL) and lower SV ($P = 0.01$ *vs.* HT CTRL), although HR ($P = 0.36$ *vs.* HT CTRL) and CO ($P = 0.07$ *vs.* HT CTRL) did not change. Cardiovascular responses to static handgrip were maintained after fentanyl administration ($P > 0.05$ for all comparisons). Further analysis considering only the first 30 s of static handgrip had equivalent results.

Responses to cycling

Subjects reported similar rates of perceived exertion (HT CTRL: 3 ± 0 a.u., HT FENT: 3 ± 1 a.u., NT: 4 ± 1 a.u.; $P > 0.05$ for all comparisons). Table 3 presents the resting cardiovascular measurements before each cycling trial. MBP was greater ($P = 0.03$) and TVC was smaller ($P = 0.03$) for HT CTRL compared to the NT group. HR, SV and CO were similar between groups ($P > 0.05$). Following fentanyl administration, the HT group presented lower MBP ($P < 0.01$ *vs.* HT CTRL) and higher TVC ($P = 0.02$ *vs.* HT CTRL), whereas resting HR, SV and CO remained similar ($P > 0.05$ *vs.* HT CTRL). An original BP recording of one HT patient during cycling is presented in Fig. 2. Changes in MBP during cycling are presented in Fig. 3, whereas HR, SV, CO and TVC are presented in Fig. 4. Cycling elicited an exaggerated increase of MBP in HT CTRL compared to NT (Interaction $P = 0.02$). HR, SV, CO and TVC also increased, although with the same magnitude in HT CTRL and NT (Interaction $P > 0.05$). Fentanyl decreased the MBP response of the HT subjects (Interaction $P < 0.01$ *vs.* HT CTRL), accompanied by a larger increase in TVC (Interaction $P = 0.02$ *vs.* HT CTRL). The MBP response of the HT subjects after fentanyl injection was similar to that of NT subjects (Interaction $P = 0.75$). Moreover, fentanyl not only attenuated the HR response of the HT subjects (Interaction $P < 0.01$ *vs.* HT CTRL), but also increased SV (Interaction $P = 0.01$ *vs.* HT CTRL), resulting in similar CO (Interaction $P = 0.36$ *vs.* HT CTRL). The *post hoc* tests indicated that the differences between-groups or within-group were apparent from the first to the fifth minute of cycling.

When subgroups of individuals with systolic HT, diastolic HT or concurrent systolic and diastolic HT were considered, statistical analyses to compare each of these HT subgroups with the NT group and to test whether fentanyl attenuated their cardiovascular responses led to the same conclusions.

Discussion

In clinical exercise tests using a treadmill, an abnormal increase of BP in HT patients was detected during the first 6 min of exercise, at intensities of ∼5–7 metabolic-equivalents (Papademetriou *et al.* 1989; Kokkinos *et al.* 2002). The present study reproduced this condition in an experimental protocol. In detail, using beat-to-beat measurement, we show that the BP abnormally rises since the first minute of leg cycling exercise in HT patients. More importantly, we assessed the role of the group III and IV leg muscle afferent signal in this exaggerated BP response to leg exercise, using a lumbar intrathecal injection of fentanyl to attenuate the muscle afferent signal. Intrathecal fentanyl

Table 3. Cardiovascular variables before cycling exercise

	HT CTRL	HT FENT	NT
Mean BP (mmHg)	121 ± 4*†	109 ± 5	106 ± 5
Heart rate (beats min^{-1})	66 ± 3	67 ± 3	70 ± 4
Stroke volume (mL)	76 ± 4	74 ± 5	84 ± 5
Cardiac output (L min^{-1})	5.0 ± 0.4	5.0 ± 0.4	5.8 ± 0.3
Total vascular conductance (mL min^{-1} $mmHg^{-1}$)	42 ± 4*†	47 ± 5	56 ± 3

Values are average from 2–3 min of rest before cycling. HT CTRL, hypertensive subjects before fentanyl administration; HT FENT, hypertensive subjects after fentanyl administration; NT, normotensive subjects. Data are the mean ± SEM. *$P < 0.05$ *vs.* NT. †$P < 0.05$ *vs.* HT FENT.

normalized the increase of BP during cycling in HT men, suggesting an overactivity of the exercise pressor reflex in this population. These findings demonstrate that a key mechanism driving an increased cardiovascular risk in HT patients can be corrected, with potential application for the development of treatments for this abnormal response to exercise.

Cardiovascular responses to cycling

During dynamic exercise, the integration of central command, the exercise pressor reflex and other neural signals within the brainstem modulates sympathetic and parasympathetic activity directed to the heart, evoking increases in CO, and also increasing sympathetic activity to peripheral blood vessels. In healthy conditions, the sympathetic vasoconstriction is attenuated in the exercising muscles because of a number of substances released from the active muscle, a physiological phenomenon termed functional sympatholysis (Remensnyder *et al.* 1962). The result is an increase in blood flow to the exercising muscles, with BP maintained in a narrow range. However, HT animals presented impaired functional sympatholysis, with significant decrease of vascular conductance in the exercising limb during superimposed sympathetic stimulation (Zhao *et al.* 2006; Mizuno *et al.* 2014; Thomas, 2015). Similarly, HT humans presented impaired functional sympatholysis in the exercising forearm, associated with an exaggerated increase of BP and sympathetic activity (Vongpatanasin *et al.* 2011). During one-leg exercise, although the functional sympatholysis was not significantly impaired in HT humans, they consistently had lower leg vascular conductance compared to NT subjects (Mortensen *et al.* 2014). In the present study, although TVC responses to cycling only tended to be different between HT and NT subjects, a clear indication of blunted increase of TVC driving the exaggerated BP in the HT group is the effect of fentanyl with respect to reducing the BP response, accompanied by a greater increase of TVC and similar CO. Possibly, the attenuation of the exercise pressor reflex resulted in less sympathetic vasoconstriction, counterbalancing the effects of the impaired functional sympatholysis with regard to TVC and BP.

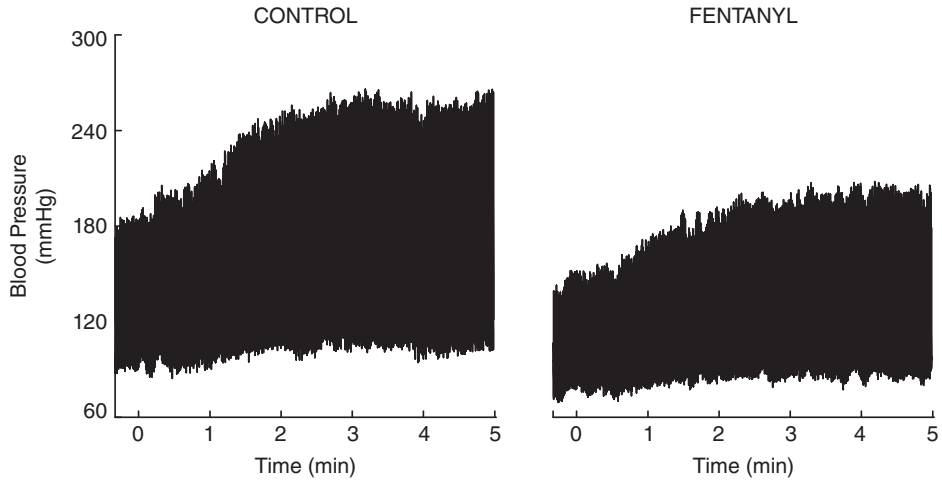

Figure 2. Representative original data
Blood pressure tracing from one hypertensive subject during the trials of cycling before and after intrathecal fentanyl administration.

In line with our findings, lumbar intrathecal fentanyl injection also resulted in a decrease of BP during leg exercise in heart-failure patients, accompanied by an increase in the exercising leg blood flow and vascular conductance (Amann *et al.* 2014).

Regulation of HR during low-intensity exercise is mostly attributed to the central command (Williamson *et al.* 1995), with the mechanoreflex having some influence (Williamson *et al.* 1995; Gladwell & Coote, 2002). We found that fentanyl decreased the HR response to cycling in the HT subjects, corroborating a possible overactivity of the mechanoreflex in HT patients (Greaney *et al.* 2015). However, the lower HR was compensated by a greater SV, and so the CO increase during cycling remained unchanged.

At the molecular level, two important factors associated with the overactive exercise pressor reflex in hypertension are the lower nitric oxide (NO) bioavailability and the greater oxidative stress mediated by angiotensin II (Smith *et al.* 2015; Thomas, 2015). NO modulates neural signalling in the nucleus tractus solitarius and rostral ventrolateral medulla, two areas related to autonomic and cardiovascular regulation. An increase of NO concentration in these areas significantly decreases BP and sympathetic activity, as well as their responses to exercise, in HT rats (Kishi *et al.* 2002; Leal *et al.* 2013*a*; Smith *et al.* 2015), suggesting that NO bioavailability in the central nervous system may be decreased in hypertension. In this sense, oxidative enzymes activated by angiotensin II increase reactive oxygen species, such as superoxide anions. These substances rapidly react with NO, generating peroxynitrite, thus reducing NO bioavailability (Kishi & Hirooka, 2012). Peripherally, oxidative stress also decreases NO bioavailability in the skeletal muscle vasculature, resulting in impaired functional sympatholysis (Zhao *et al.* 2006) and, with the accumulation of metabolites, further sensitization of the muscle afferents (Smith *et al.* 2015; Thomas, 2015). Moreover, alterations in the expression of proteins related to NO formation (Murphy *et al.* 2013), oxidative stress (Felix & Michelini, 2007) and muscle afferent receptors (Mizuno *et al.* 2011) have been demonstrated in HT rats, indicating that multiple alterations are required, at the central and peripheral level, to significantly impair the homeostasis and cause an exaggerated pressor response to exercise.

Fentanyl-related effects and cardiovascular responses to static handgrip

One major concern regarding the use of intrathecal fentanyl is the need to rule out the possibility of cephalad migration through the cerebrospinal fluid or its vascular absorption. A direct action of fentanyl on opioid receptors within the brainstem can decrease HR and BP responses to exercise (Caringi *et al.* 1998) and this would mislead interpretations concerning the role of muscle afferents during cycling. Therefore, we assessed cardiovascular responses to static handgrip, which are expected to be impaired only if fentanyl had migrated within the cerebrospinal fluid to a

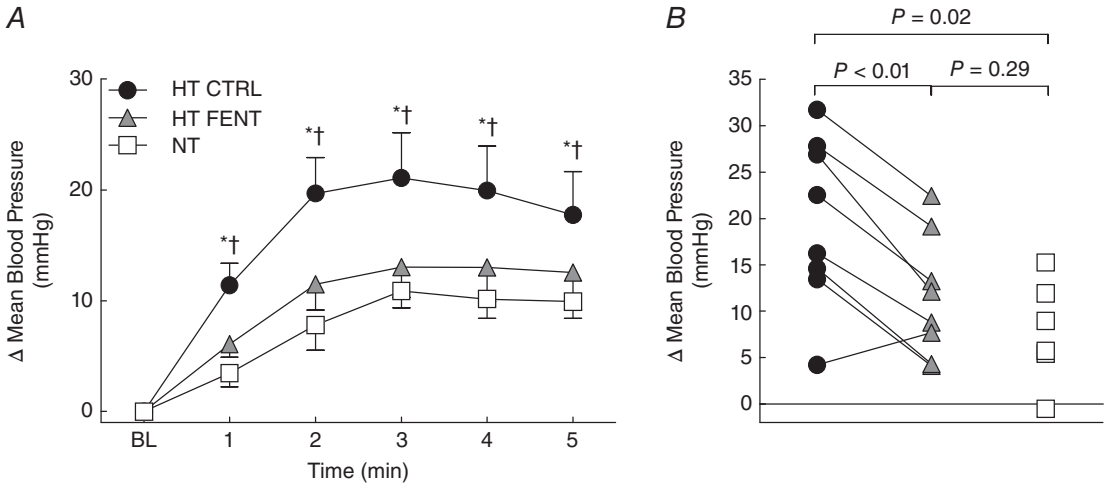

Figure 3. Blood pressure response to cycling in hypertensive and normotensive subjects
A, responses of mean BP during each minute of cycling. HT CTRL (black circles), hypertensive subjects before fentanyl administration; HT FENT (grey triangles), hypertensive subjects after fentanyl administration; NT (white squares), normotensive subjects; BL, resting baseline. Data are the mean ± SEM. *$P < 0.05$ HT CTRL *vs.* NT. †$P < 0.05$ HT CTRL *vs.* HT FENT. *B*, individual mean BP change from resting baseline at the second minute of cycling. Connecting lines indicate that, except for one individual, the BP response of every hypertensive subject to cycling exercise was lower after fentanyl. These statistical comparisons had equivalent results from the first to the fifth minutes. HT CTRL (black circles), hypertensive subjects before fentanyl administration; HT FENT (grey triangles), hypertensive subjects after fentanyl administration; NT (white squares), normotensive subjects.

higher level of the spinal cord and the brainstem. Because these responses were not affected, along with the absence of nausea, dyspnoea and pruritus above the abdominal level, a central effect of fentanyl can be considered most improbable in our experiments. Moreover, studies with similar doses of fentanyl excluded its central effect by observing the hyperventilatory response to CO_2 breathing and the preserved cardiovascular responses to arm exercise (Amann *et al.* 2010; Trinity *et al.* 2010).

Methodological aspects

The design of the present study presents some limitations worthy of consideration. We did not include sham injection or injection of saline and, considering the symptoms that could manifest after fentanyl injection, we did not blind the subjects about the pharmacological intervention. However, a previous study showed no difference between the absence of injection or saline injection (Amann *et al.* 2009), suggesting that the blinding of subjects is not crucial for the responses observed.

HT subjects presented a significant decrease of ∼12 mmHg in the resting MBP before the cycling session following fentanyl. Some level of decrease in resting MBP (∼3 mmHg) following fentanyl administration has been reported by our group and in other studies conducted in healthy young men (Amann *et al.* 2010; Trinity *et al.* 2010; Amann *et al.* 2011; Amann *et al.* 2014; Olson *et al.* 2014; Barbosa *et al.* 2015; Ives *et al.* 2015; Sidhu *et al.* 2015), raising the question of whether the stimulation of spinal opioid receptors could affect BP regulation at rest. Some possible mechanisms are a reduced activity of preganglionic sympathetic neurons as a result of activation of opioid receptors in the intermediolateral cell column of the spinal cord (Li & Han, 1984; D'Angelo *et al.* 1994), as well as a local anaesthetic effect (Gissen *et al.* 1987; Power *et al.* 1991) affecting sympathetic nerves. The psychological stress associated with the experimental procedures (Huang *et al.* 2013) and a hypotensive effect of cumulative exercise sessions (da Nobrega, 2005; Halliwill *et al.* 2013) might have affected resting BP. Nevertheless, a central effect of fentanyl is most improbable in our experiments because of

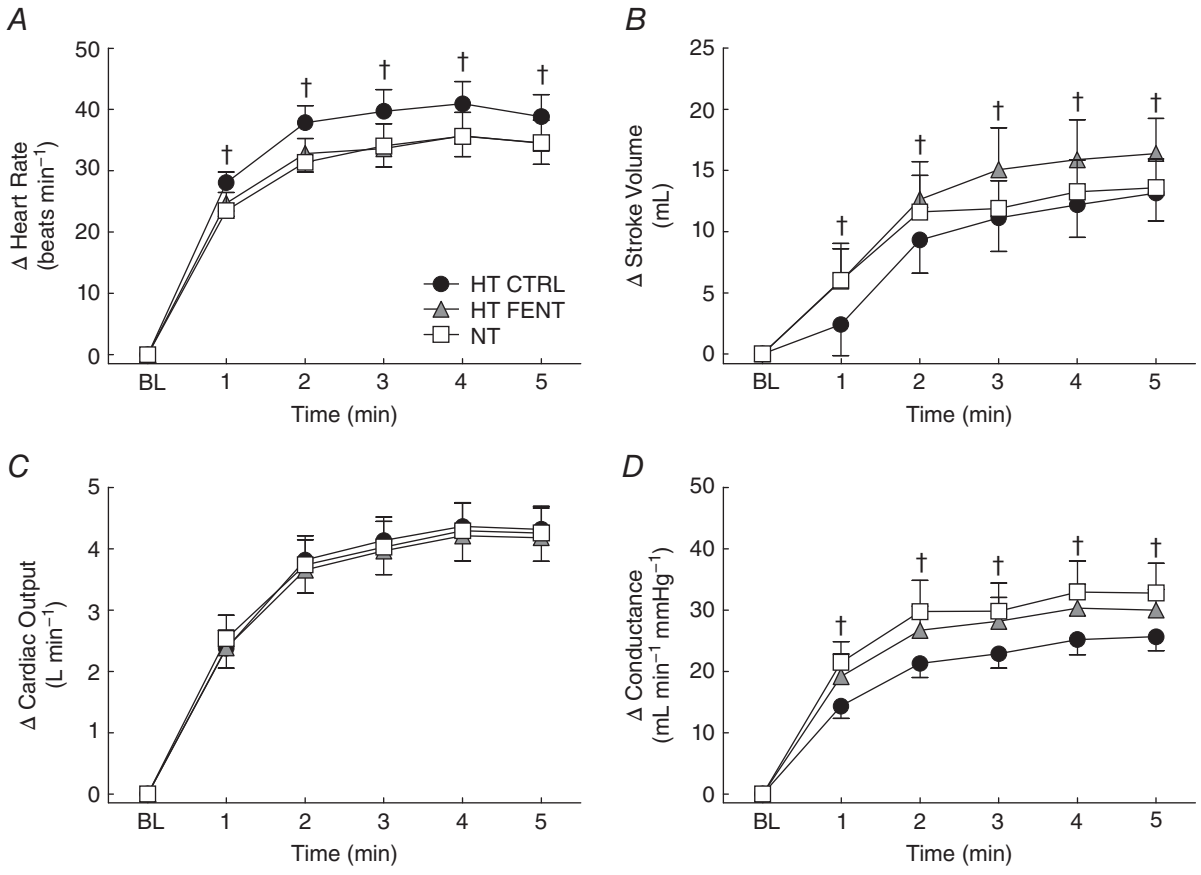

Figure 4. Cardiovascular responses to cycling in hypertensive and normotensive subjects
Responses of heart rate (*A*), stroke volume (*B*), cardiac output (*C*) and total vascular conductance (*D*) during each minute of cycling. HT CTRL (black circles), hypertensive subjects before fentanyl administration; HT FENT (grey triangles), hypertensive subjects after fentanyl administration; NT (white squares), normotensive subjects. BL, resting baseline. Data are the mean ± SEM. †$P < 0.05$ HT CTRL *vs.* HT FENT.

the preserved MBP response to handgrip and an absence of sensory symptoms.

The blockade of the muscle afferent signal by fentanyl was probably incomplete, as a result of limited delivery to μ-opioid receptor and afferent fibres expressing δ-opioid receptors, including some mechanosensitive group III fibres (Leal *et al.* 2013*b*).

Perspectives

The present study is the first to report that an overactive exercise pressor reflex can cause exaggerated increase of BP in HT individuals during low-intensity leg exercise. These potentially dangerous elevations in BP possibly increase the cardiovascular risk during many daily activities (e.g. playing with children, cleaning duties, walking upstairs, etc.) (Ainsworth *et al.* 2011), limit regular exercise training, and are associated with an increased risk of developing chronic cardiovascular diseases. Correction of the exercise pressor reflex overactivity in HT patients would potentially protect them from the acute increased risk of cardiovascular events and improve safety during regular physical activities, which is beneficial for the cardiovascular system over the long term. From a clinical perspective, more research is needed to create a new intervention aiming to restore the normal exercise pressor reflex, probably via action on the free nerve endings of the group III and IV skeletal muscle afferents.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

Experiments were performed at Antonio Pedro University Hospital and Laboratory of Exercise Sciences, Fluminense Federal University, Brazil. TCB was involved in the conception and design of the experiments; the collection, assembly, analysis and interpretation of data; and the drafting of the first version of the manuscript. LVC, NHS and ACLN were involved in the conception and design of the experiments; the interpretation of data; the drafting of the manuscript; and the revision of the article for important intellectual content. IAF, EP, HNMR, VPG and NGR were involved in the collection, analysis and interpretation of data and the revision of the article for important intellectual content. All authors revised and approved the final version and submission of the 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.

Funding

This study was supported by grants and scholarships from the Brazilian National Council of Scientific and Technological Development (CNPq – 87584/2013-9), the Foundation for Research Support of Rio de Janeiro (FAPERJ – E-26/110.097/2013), Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES – 99999.008878/2014-05) and the Brazilian Funding Agency for Studies and Projects (FINEP – MCT/FINEP/CT-INFRA-PROINFRA-01/2007). NHS was sponsored by the program Science without Borders (PVE 051/2012 – CNPq/CAPES).

Acknowledgements

The authors appreciate the time and effort expended by all volunteer subjects in this study. We would also like to thank Dr Lisbeth G. Jorgensen, Dr Alessandra C. Toledo-Arruda, Dr Gustavo Mataruna and Igor R. C. Cardoso for their support during the experiments.