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Previous exercise attenuates muscle sympathetic activity and increases blood flow during acute euglycemic hyperinsulinemia

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¹Exercise Hemodynamic Laboratory, School of Physical Education and Sport, ²Hypertension Unit, General Hospital, ³Endocrinology Division, General Hospital, ⁴Unit of Cardiovascular Rehabilitation and Exercise Physiology, Heart Institute (InCor), Medical School, and ⁵Experimental Physiopathology Division, Medical School, University of São Paulo, Brazil

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Bisquolo, V. A. F., C. G. Cardoso, Jr., K. C. Ortega, J. L. Gusmão, T. Tinucci, C. E. Negrão, B. L. Wajchenberg, D. Mion Jr., and C. L. M. Forjaz. Previous exercise attenuates muscle sympathetic activity and increases blood flow during acute euglycemic hyperinsulinemia. *J Appl Physiol* 98: 866–871, 2005. First published November 12, 2004; doi:10.1152/jappphysiol.00251.2004.— Insulin infusion causes muscle vasodilation, despite the increase in sympathetic activity. In contrast, a single bout of exercise decreases sympathetic activity and increases muscle blood flow during the postexercise period. We tested the hypothesis that muscle sympathetic activity would be lower and muscle vasodilation would be higher during hyperinsulinemia performed after a single bout of dynamic exercise. Twenty-one healthy young men randomly underwent two hyperinsulinemic euglycemic clamps performed after 45 min of seated rest (control) or bicycle exercise (50% of peak oxygen uptake). Muscle sympathetic nerve activity (MSNA, microneurography), forearm blood flow (FBF, plethysmography), blood pressure (BP, oscillometric method), and heart rate (HR, ECG) were measured at baseline (90 min after exercise or seated rest) and during hyperinsulinemic euglycemic clamps. Baseline glucose and insulin concentrations were similar in the exercise and control sessions. Insulin sensitivity was unchanged by previous exercise. During the clamp, insulin levels increased similarly in both sessions. As expected, insulin infusion increased MSNA, FBF, BP, and HR in both sessions (23 ± 1 vs. 36 ± 2 bursts/min, 1.8 ± 0.1 vs. 2.2 ± 0.2 ml·min⁻¹·100 ml⁻¹, 89 ± 2 vs. 92 ± 2 mmHg, and 58 ± 1 vs. 62 ± 1 beats/min, respectively, $P < 0.05$). BP and HR were similar between sessions. However, MSNA was significantly lower (27 ± 2 vs. 31 ± 2 bursts/min), and FBF was significantly higher (2.2 ± 0.2 vs. 1.8 ± 0.1 ml·min⁻¹·100 ml⁻¹, $P < 0.05$) in the exercise session compared with the control session. In conclusion, in healthy men, a prolonged bout of dynamic exercise decreases MSNA and increases FBF. These effects persist during acute hyperinsulinemia performed after exercise.

insulin infusion; physical exercise; blood pressure; muscle sympathetic nerve activity; muscle blood flow

ALTHOUGH INSULIN-MEDIATED sympathetic activation is a well-described phenomenon in humans, its hemodynamic consequences are still controversial. The sympathoexcitatory effect of insulin may have clinical importance in some physiopathological states, such as hypertension and obesity, which commonly present hyperinsulinemia (10, 19, 25, 28, 38), sympathetic activation (2, 22, 27, 38), and cardiovascular disturbances (2, 22, 25, 27, 38).

It has been extensively demonstrated that insulin infusion increases muscle sympathetic nerve activity (MSNA) in

healthy subjects (6, 12, 16, 24, 30, 33, 37–39). However, it is interesting that acute insulin infusion does not always result in blood pressure (BP) increase (5, 38, 39). This apparent paradox can be explained by the insulin-induced muscle vasodilation (12, 20, 32, 33, 35, 37–39) that counteracts the pressor effect of sympathetic activation.

On the other hand, previous studies have demonstrated that acute dynamic exercise also provokes autonomic and cardiovascular adaptations during the postexercise period. In fact, a single bout of exercise decreases MSNA (12, 14) and increases muscle vascular conductance (12, 14, 15, 17, 31) during the postexercise period. The hemodynamic consequence of these neurovascular changes is a remarkable decrease in blood pressure during the recovery (11, 12, 14, 15, 17, 31).

Because exercise has sympatholytic, vasodilatory, and hypotensive posteffects, it is possible to suppose that, after an acute bout of dynamic exercise, MSNA would be lower and muscle vasodilation would be higher even during acute hyperinsulinemia. Moreover, this hypothesis could have important implications for obesity and hypertension. However, to avoid possible influences from other disabilities present in these pathological states, it is important first to test the hypothesis on healthy subjects. Thus the aim of the present study was to investigate the effect of previous exercise on MSNA, forearm blood flow (FBF), BP, and heart rate (HR) measured before and during acute insulin infusion in healthy men.

METHODS

Subjects. Twenty-one healthy subjects gave written consent and were included in this study, which was approved by the Ethical Committee of the Heart Institute (InCor) and of the General Hospital, University of São Paulo. Subjects who had cardiovascular diseases, diabetes mellitus, hypertension, obesity, or high levels of cholesterol or triglycerides were excluded. No subject was engaged in any regular physical activity program. The physical and cardiovascular characteristics of the subjects are shown in Table 1.

Peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$) was measured during a maximal cardiopulmonary exercise test, performed on a cycle ergometer with a protocol of 30-W increment every 3 min, until the subjects were unable to continue. Oxygen uptake was directly measured by a metabolic cart (MGC, CAD/NET 2001).

Measurements. BP (mmHg) and HR (beats/min) were measured by an oscillometric automatic device (Dixtal, 2710), which was regularly calibrated by comparison against a mercury column.

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Table 1. *Physical and functional characteristics of the subjects*

Age, yr	31.6 ± 1.6
Weight, kg	75.1 ± 2.6
Height, cm	174 ± 2
BMI, kg/m ²	24.7 ± 0.6
SBP, mmHg	122 ± 3
MBP, mmHg	91 ± 2
DBP, mmHg	75 ± 3
HR, beats/min	56 ± 2
Peak power, W	195 ± 7
$\dot{V}O_{2\text{ peak}}$, ml·kg ⁻¹ ·min ⁻¹	36.9 ± 1.9

Values are means ± SE. BMI, body mass index; SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; HR, heart rate; $\dot{V}O_{2\text{ peak}}$, peak oxygen uptake.

FBF (ml·min⁻¹·100 ml tissue⁻¹) was measured by venous occlusion plethysmography (34). An air-filled latex plethysmographic cuff was applied to the forearm and connected to a differential pressure transducer (Gold, Validyne, RS3800). This arm was positioned above the right atrium. During measurements, circulation to the hand was interrupted by a wrist cuff inflated to 200 mmHg, while a venous occlusion cuff was placed around the upper arm and was inflated to 40–60 mmHg for 7 s of every 15 s. Forearm vascular resistance (FVR, in U) was calculated by the ratio of mean BP and FBF.

MSNA was directly recorded by microneurography (1). Briefly, a tungsten microelectrode (200 μm wide, tapering to an uninsulated tip of 1–5 μm) was inserted into a muscle fascicle of the common peroneal nerve, posterior to the fibular head, and it was adjusted until a spontaneous MSNA record was obtained. The recorded signal was fed to a preamplifier (gain: 1,000), an amplifier (variable gain: 40–60), and a band-pass filter (700–2,000 Hz). Then, signal was integrated to obtain a mean voltage display of MSNA neurogram (time constant: 0.1 s). MSNA was counted in terms of burst frequency (bursts/min) and bursts per 100 heart beats (burst/100 beats).

Euglycemic hyperinsulinemic clamp was performed by following DeFronzo's protocol (9). Regular human insulin (Novo-Nordisk, Novolin R), diluted in saline with 1 ml of subject's blood, was infused by a digital pump (Harvard, 55-2222) at a rate of 50.7 μU·m⁻²·min⁻¹ for 120 min to achieve a plasma insulin concentration of 100 μU/ml, as previously described by some of us (12). Blood glucose concentration was maintained at baseline level by adjusting the infusion rate of a 50% glucose solution. To make these adjustments, blood glucose was measured every 5 min with an automated device (Roche, Accutrend Advanced), and glucose infusion was initiated 4 min after insulin infusion. Moreover, blood samples were collected at baseline and every 5 min during the insulin infusion for plasma glucose and insulin analysis. These results were used to establish the steady-state period during the euglycemic hyperinsulinemic clamp.

Glucose metabolized (M) was calculated by DeFronzo's formula (9) on the basis of glucose infusion rate and plasma glucose concentration at the beginning and at the end of the steady-state period, which was defined as a 20-min period during which glycemia was constant and similar to baseline (±10%) and plasma insulin concentration was ~100 μU/ml. Moreover, this period was established at least 20 min after the beginning of insulin infusion and, in each subject, it was set at similar times of insulin infusion in the control and exercise sessions. The insulin sensitivity index (M/I) was calculated by the ratio between M and plasma insulin concentration during the steady-state period.

Experimental protocol. All subjects underwent two experimental sessions (control and exercise), which were randomly performed with at least a 30-day interval. Both sessions were conducted in the morning after an overnight fast. In the control session, subjects rested for 45 min in the sitting position before the beginning of the experi-

mental protocol, whereas in the exercise session they exercised for 45 min on a cycle ergometer (Funbec, Cycle 2) at 50% of $\dot{V}O_{2\text{ peak}}$. After seated rest or exercise, subjects were prepared for the experimental protocol (catheter insertion and nerve location).

Baseline measurements were initiated ~90 min after completion of exercise or seated rest and lasted for 10 min. Afterward, the euglycemic hyperinsulinemic clamp procedure was initiated and lasted for 120 min. FBF was measured for 3 min during baseline period and every 15 min during insulin infusion. MSNA was continuously recorded, and BP and HR were measured every minute during FBF determination.

Biochemical analysis. Plasma glucose concentration was measured by a colorimetric enzymatic method (GOD/POD; Merck, Darmstadt, Germany). Plasma insulin was analyzed in duplicate by radioimmunoassay (CIS-Bio International kit, ICN Biomedicals, Costa Mesa, CA).

Statistical analysis. M and M/I in both sessions were compared by Wilcoxon's test. The effects of previous exercise on plasma insulin, plasma glucose, HR, BP, MSNA, FBF, and FVR responses to insulin infusion were compared by a two-way ANOVA for repeated measures (Statistica for Windows 4.3, Statsoft, 1993), establishing session (control and exercise) and period (baseline and steady state) as the main factors. Post hoc comparisons were done by Newman-Keuls test. $P < 0.05$ was accepted as statistically significant. Data are presented as means ± SE.

RESULTS

Ten subjects were randomly assigned to initiate the protocol with the control session, whereas eleven started it with the exercise session. The exercise bout was performed with 91.2 ± 3.7 W. In 14 of the 21 subjects, oxygen uptake was measured during exercise, and it was 20.5 ± 1.4 ml·kg⁻¹·min⁻¹, which corresponded to 55 ± 2% of $\dot{V}O_{2\text{ peak}}$. The mean interval period between sessions was 74 ± 7 days. Baseline measurements and insulin infusion started at similar times in the control and exercise sessions (baseline = 94 ± 7 vs. 88 ± 6 min; insulin infusion = 107 ± 8 vs. 100 ± 6 min, respectively). Moreover, the steady-state period was initiated at a similar time of insulin infusion in the control and exercise sessions (66 ± 6 vs. 65 ± 6 min, respectively), and, in the exercise session, the steady-state period was initiated 164 ± 7 min after the end of the exercise bout.

Plasma glucose and insulin levels measured at the beginning and end of the steady-state period are shown in Fig. 1. Moreover, plasma glucose, plasma insulin, M, and M/I values measured in both sessions are shown in Table 2. Plasma glucose concentration did not change throughout the experimental protocol. Similarly, there was no difference in plasma insulin concentration between the control and the exercise sessions, and insulin levels increased significantly and similarly with insulin infusion in both sessions. M values and M/I index did not differ between the experimental sessions.

BP and HR responses to exercise and insulin infusion are shown in Table 2. BP and HR were similar in the control and exercise sessions. Regardless of the session, BP and HR increased significantly with insulin infusion.

A typical recording of MSNA and FBF measured in both experimental sessions at baseline and during insulin infusion is shown in Fig. 2.

MSNA recorded at baseline and during insulin infusion in the control and exercise sessions is shown in Fig. 3. In both sessions, MSNA increased significantly and similarly with insulin infusion (baseline = 22.9 ± 1.8 vs. steady state =

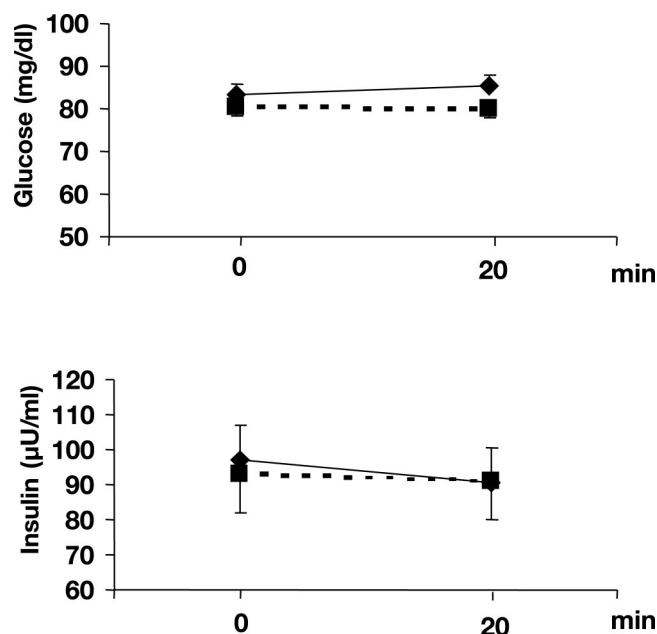


Fig. 1. Plasma glucose and insulin concentrations measured at the beginning (0) and the end (20 min) of the steady-state period in the control (◆, solid line) and exercise sessions (■, dashed line).

35.9 ± 2.7 bursts/min, $P < 0.05$). Moreover, MSNA recorded throughout the exercise session was significantly lower than in the control session (27.4 ± 2.5 vs. 31.4 ± 3.0 bursts/min, $P < 0.05$). Similar findings were observed when MSNA was corrected by heart rate (bursts/100 beats).

FBF measured at baseline and during insulin infusion in both experimental sessions is shown in Fig. 4. FBF increased significantly and similarly with insulin infusion in both sessions (baseline = 1.78 ± 0.15 vs. steady state = 2.24 ± 0.21 ml·min⁻¹·100 ml⁻¹, $P < 0.05$). FBF measured throughout the exercise session was significantly greater than in the control session (2.21 ± 0.17 vs. 1.82 ± 0.16 ml·min⁻¹·100 ml⁻¹, $P < 0.05$).

FVR measured at baseline and during insulin infusion in both experimental sessions is also shown in Fig. 4. FVR decreased significantly and similarly with insulin infusion in both sessions (baseline = 58.0 ± 5.2 vs. steady state = 48.1 ± 4.6 U, $P < 0.05$). FBF measured throughout the exercise session was significantly lower than in the control session (47.9 ± 4.3 vs. 58.2 ± 5.3 U, $P < 0.05$).

DISCUSSION

The new finding of the present study is that a previous bout of dynamic exercise decreases MSNA, and increases FBF, even during acute hyperinsulinemia.

In regard to MSNA, the reduction was observed at baseline, and although sympathetic activity increased with insulin infusion, it was still lower after exercise than after rest during the hyperinsulinemic state. The mechanisms underlying the decrease in MSNA after exercise are out of the scope of the present study. However, postexercise decrease in MSNA has already been reported (12, 14) and seems to be mediated by many factors such as opioid secretion, baroreflex resetting, and insulin sensitivity enhancement (13). In the present investigation, MSNA decreased after exercise without any change in

insulin sensitivity, suggesting that other mechanisms, besides insulin sensitivity enhancement, may have been responsible for MSNA fall during the postexercise period. As expected, insulin infusion increased MSNA (6, 12, 16, 24, 30, 33, 37–39), and this response has been attributed to the central effect of insulin stimulating the vasomotor center by nitric oxide secretion (24). However, it is interesting to notice that insulin-induced MSNA increase was similar in the control and exercise sessions, showing that previous exercise did not change insulin action on sympathetic nerve system. Thus, as the increase was similar, MSNA was still lower during hyperinsulinemia performed after exercise than after rest, showing that previous exercise can decrease sympathetic activity even during a condition of sympathetic excitation. The combined effect of exercise and insulin may be explained by the fact that these interventions influence MSNA through different mechanisms, because insulin stimulates MSNA by nitric oxide release (24) and exercise decreases MSNA by other mechanisms, such as opioid secretion and baroreflex resetting (13, 15).

The reduction in sympathetic activity after exercise resulted, as expected, in an increase in FBF during the recovery period (12, 14, 15, 17, 31). Moreover, as it was also previously shown (12, 20, 32, 33, 35, 37–39), insulin infusion caused vasodilation, probably by its local effects on nitric oxide release (32, 35, 36), resulting in muscle blood flow increase, which is mainly due to an increase in nutritive capillaries blood flow (8). The increase in blood flow was similar in the exercise and control sessions. This result is similar to the one observed by Rhéaume et al. (28), who showed that previous exercise did not change FBF response to glucose infusion. Thus FBF during hyperinsulinemia was even higher after exercise than after rest. In fact, because insulin infusion (12, 20, 32, 33, 35, 37–39) and exercise (12, 14, 15, 17, 31) promote vasodilation, in part by a common pathway (nitric oxide release) (13, 15, 18, 32, 35, 36), but also because exercise has other vasodilatory mechanisms, such as sympathetic withdrawal, thermoregulation, and local

Table 2. Metabolic and cardiovascular data measured at baseline and steady-state period of euglycemic hyperinsulinemic clamp procedure in both control and exercise sessions

		Control Session	Exercise Session
<i>Metabolic measurements</i>			
Glucose, mg/dl	Baseline	85±2	81±2
	Steady state	84±2	80±2
Insulin, µU/ml	Baseline	11±1	11±1
	Steady state	94±9*	92±9*
M, mg·kg ⁻¹ ·min ⁻¹	Steady state	9.2±1.2	9.4±1.0
M/I, mg·kg ⁻¹ ·min ⁻¹ /µU·ml ⁻¹	Steady state	0.12±0.02	0.11±0.01
<i>Cardiovascular measurements</i>			
SBP, mmHg	Baseline	126±2	125±2
	Steady state	131±2*	131±2*
MBP, mmHg	Baseline	90±2	88±2
	Steady state	92±2*	91±2*
DBP, mmHg	Baseline	73±2	71±2
	Steady state	75±2*	73±2*
HR, beats/min	Baseline	55±1	60±2
	Steady state	61±1*	63±2*

Values are means ± SE. M, glucose metabolized; M/I, insulin sensitivity index. *Significantly different from baseline ($P < 0.05$).

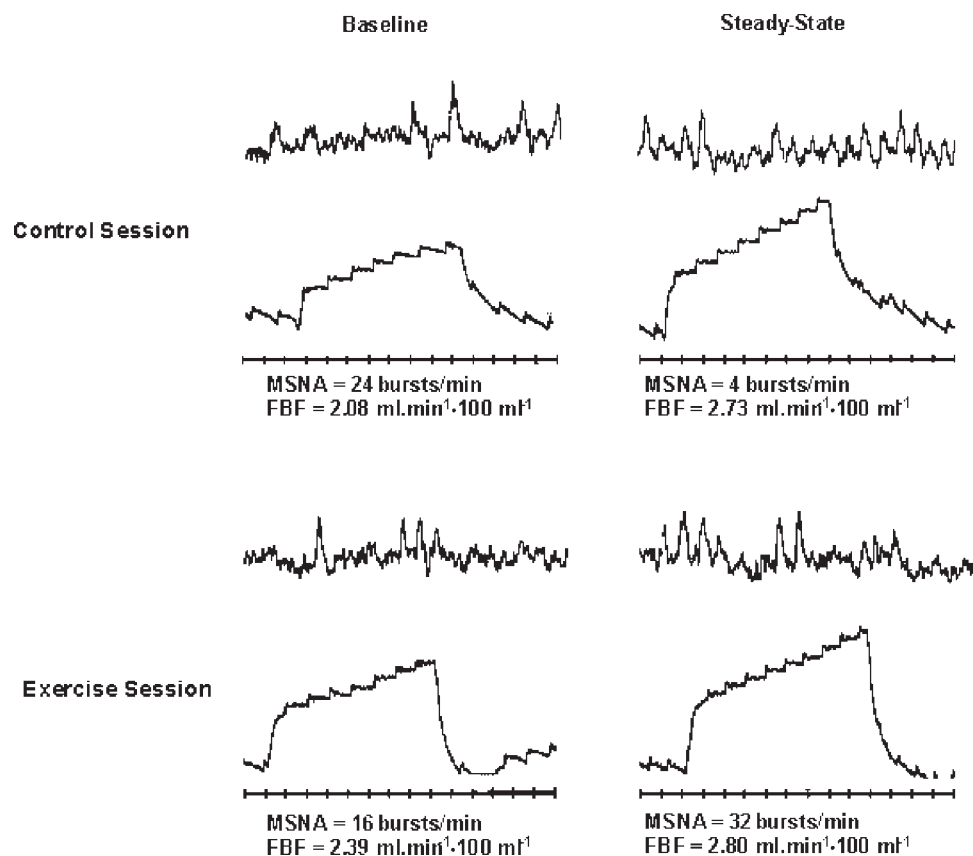


Fig. 2. Muscle sympathetic nerve activity (MSNA) and forearm blood flow (FBF) recorded in 1 subject at baseline and during acute hyperinsulinemia in the control and exercise sessions.

metabolites (13), the sum of these mechanisms may explain the additive effect of exercise and insulin and the higher levels of muscle blood flow observed during acute hyperinsulinemia conducted after exercise.

The lower MSNA and higher FBF achieved during hyperinsulinemia conducted after exercise in healthy subjects suggest that a single bout of exercise could have important clinical implications, especially in some hyperinsulinemic populations, such as obese (10, 25, 38) and hypertensive (19, 28) patients,

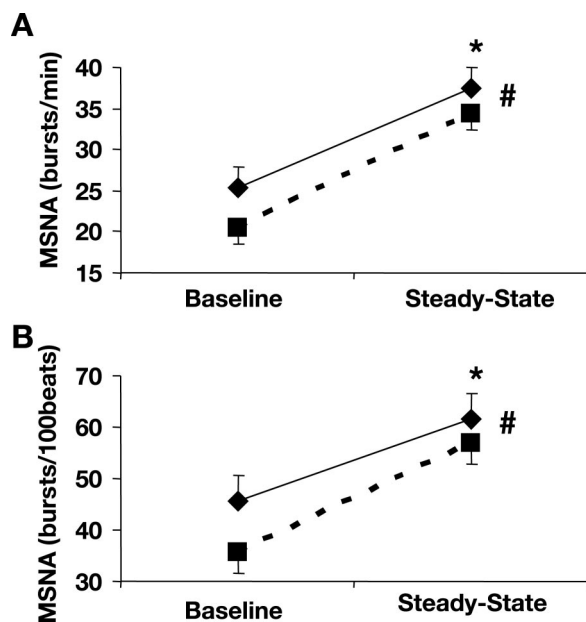


Fig. 3. MSNA recorded in burst frequency (A) and bursts per 100 heart beats (B) at baseline and during insulin infusion (steady state) in the control (◆, solid line) and exercise (■, dashed line) sessions. *Significantly different from baseline ($P < 0.05$). #Significant difference between sessions ($P < 0.05$).

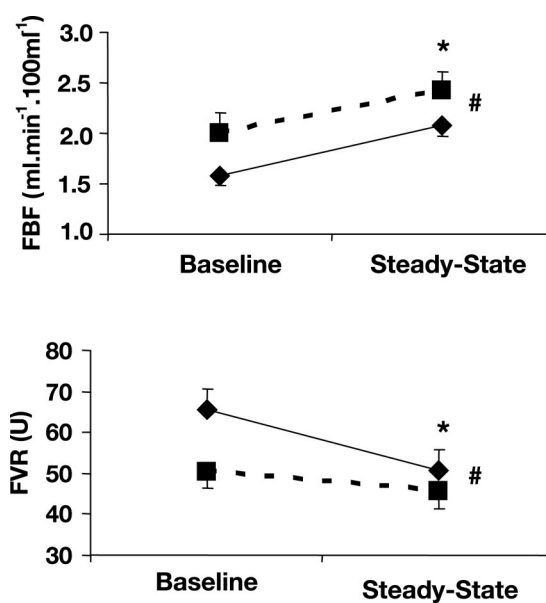


Fig. 4. FBF and forearm vascular resistance (FVR) measured at baseline and during insulin infusion (steady state) in the control (◆, solid line) and exercise (■, dashed line) sessions. *Significantly different from baseline ($P < 0.05$). #Significant difference between sessions ($P < 0.05$).

who present sympathetic activation (2, 22, 25, 27, 38) and blunted vasodilation (3, 26, 38) during acute hyperinsulinemia induced, for example, by a meal. Nevertheless, it is important to point out that the insulin level achieved after meals is lower than the one used in the present study and occurs concomitantly to hyperglycemia (33). However, it is known that neural and vascular alterations observed after meals are mainly due to plasma insulin and not to glycemia (33, 39). Moreover, insulin actions on MSNA and blood flow are dose dependent (6, 30). Thus it would be expected that insulin actions would be similar but of lower magnitude if insulin level was similar to the one achieved after meals. However, this hypothesis needs to be tested by future research in hypertensive and obese patients.

It is also important to point out that the lower sympathetic activity and the higher muscle blood flow, obtained during hyperinsulinemia performed after exercise, were observed in the absence of insulin sensitivity enhancement, showing that even an exercise bout that does not promote insulin sensitization may have clinical relevance. In fact, although some studies (4, 7, 10, 23, 28) observed an increase in insulin sensitivity after exercise, others (12, 28, 40) did not. Some factors, such as exercise protocol (4, 7), time after exercise (4, 7, 23, 28, 29), level of fitness (40), and degree of obesity (10), might explain this controversy. Moreover, it has been observed that the effect of exercise increasing insulin sensitivity is greater in more resistant subjects (10, 28). Also, some studies (25, 26, 28, 38) have reported that neural and hemodynamic responses to hyperinsulinemia depend on insulin sensitivity status. Thus the results observed in the present study should be even more pronounced in insulin-resistant subjects, in whom previous exercise may enhance insulin sensitivity, increasing its clinical relevance. This issue should be investigated by future studies.

Although MSNA and FVR decrease after exercise, BP and HR did not change. Some previous studies (4, 11, 13–15, 17, 31) have observed an important BP fall after exercise; however, this fall was especially observed 60 min after exercise (13). Thus it may have been missed in the present study because baseline and steady-state measurements were taken ~90 and 160 min after exercise. As expected, insulin infusion increases BP and HR, which may reflect the cardiac effects of sympathetic activation promoted by hyperinsulinemia. Some authors showed that insulin infusion can stimulate sympathetic drive to the heart (5, 25), increasing heart rate and cardiac contractility (21), which may result in a cardiac output increase (25) that may overcome vascular resistance fall, therefore increasing BP. Thus, because previous exercise did not change BP and HR levels at baseline, and because it did not change MSNA and FBF responses to insulin infusion, it is not surprising that HR and BP responses during hyperinsulinemia were not changed by previous exercise.

Study limitations. For technical reasons, a vehicle and time control session was not performed in the present study. However, previous studies (6, 16, 30) have already stated that vehicle infusion does not promote time-dependent changes in MSNA, FBF, HR, and BP.

Because the objective of the study was to compare insulin action without glucose influence, the steady-state period had to be established at different insulin infusion times in each subject to ensure euglycemia. This time difference might somehow interfere in the results; however, it was controlled in the

present study by setting the steady-state period at similar times of insulin infusion in the exercise and control sessions.

In the present investigation, sympathetic activity was recorded in the peroneal nerve, whereas blood flow was measured in the forearm. Thus it is possible that different sites of measurement may lead to different results. However, that does not seem to be true, because many studies (33, 37–39) measured blood flow and sympathetic activity in the same limb and reported results similar to ours.

Because of technical procedures in the present study, baseline and steady-state measurements were taken approximately after 90 and 160 min of exercise. Because postexercise effects may be time dependent, the results observed are limited to the periods analyzed and may be different at other moments after exercise.

In conclusion, in healthy men, a single bout of prolonged dynamic exercise decreases MSNA and increases muscle blood flow at baseline. These effects persist during sympathetic excitation induced by acute hyperinsulinemia conducted postexercise.

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