Postexercise responses of muscle sympathetic nerve activity and blood flow to hyperinsulinemia in humans

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Forjaz, Cláudia L. M., Paulo R. Ramires, Taís Tinnuci, Kátia C. Ortega, Heloísa E. H. Salomão, Edna C. Ignés, Bernardo L. Wajchenberg, Carlos E. Negrao, and Décio Mion, Jr. Postexercise responses of muscle sympathetic nerve activity and blood flow to hyperinsulinemia in humans. J. Appl. Physiol. 87(2): 824–829, 1999.—Although insulin and exercise cause dramatic changes in physiological parameters, the impact of exercise on neural and hemodynamic responses to insulin administration has not been described. In a study of the effects of a single bout of exercise on blood pressure (BP), muscle sympathetic nerve activity (MSNA), and forearm blood flow (FBF) responses to insulin infusion during the postexercise period, 11 healthy men underwent, in a random order, two hyperinsulinemic euglycemic clamps performed after 45 min of

exercise; insulin sensitivity; blood pressure

THE PREVALENCE of diabetes mellitus and hypertension has increased in Western societies. The fact that these disorders usually develop together suggests a common pathogenic factor, which has been proposed to be the insulin resistance and its consequent hyperinsulinemia (8). Because exercise reduces insulin resistance and hyperinsulinemia (4, 6, 11, 21, 29) and alters cardiovascular parameters (5, 7, 12, 13, 15–17, 23), it has been recommended as a nonpharmacological treatment for hypertensive and diabetic patients. Anderson et al. (2) demonstrated, in healthy subjects, that insulin increased muscle sympathetic nerve activity (MSNA) and decreased forearm vascular resistance (FVR) without changing arterial blood pressure (BP). In contrast, other investigators (22, 25) observed that acute insulin infusion increased mean BP levels. Insulin-induced enhancement of sympathetic nerve activity seems to be dose dependent (2, 22, 25) and greater in insulin-resistant subjects (20). Similarly, insulin-induced vasodilation is also dose dependent (2, 19, 28) and directly related to insulin sensitivity (3, 19, 20).

Physical exercise causes metabolic, neural, and cardiovascular effects. Wasserman et al. (29) found that a single bout of exercise increased insulin sensitivity and reduced plasma insulin levels during the postexercise period. Halliwill et al. (15) observed that, after an acute bout of exercise, muscle blood flow was higher, and MSNA and BP were lower than in a nonexercise control session. Because exercise induces changes in MSNA, muscle blood flow, insulin sensitivity, and BP, it could be expected that physiological responses to hyperinsulinemia may be modified after a single bout of exercise.

To test this hypothesis, we studied the aftereffects of a single bout of exercise on MSNA, forearm blood flow (FBF), and arterial BP responses to a euglycemic and hyperinsulinemic clamp in healthy men.

METHODS

Subjects

This study was approved by the Ethical Committee of the Heart Institute, Medical School, University of São Paulo. After giving their written consent, 11 healthy normotensive men participated in this study. Subjects who presented with obesity, cardiovascular diseases, diabetes mellitus, or high levels of total cholesterol or triglycerides were excluded. No subjects were excessively sedentary or participated in any regular physical activity program. Their diet was calorically suited to their energetic needs. Subjects’ physical and functional characteristics are shown in Table 1.

Peak oxygen uptake (V̇O₂peak) was measured directly by a metabolic cart (CAD/NET 2001, Medical Graphics) during a graded maximal exercise test performed on a cycle ergometer, with 30-W increments every 3 min until the subjects were unable to continue.

Measurements

BP and heart rate (HR). BP and HR were measured by an oscillometric automatic sphygmomanometer (Dixtal, 2710) that was calibrated regularly by comparison with a mercury column. Muscle blood flow, FBF (ml/min per 100 ml of tissue) was measured by venous occlusion plethysmography (26). An air-filled latex plethysmography cuff was applied to the forearm and connected to a differential pressure transducer (Gould, Validyne). The arm was elevated above the right atrium. During measurements, circulation to the hand was interrupted by a wrist cuff inflated to 180–200 mmHg, while a
of the volunteers

| Age, yr | 30 ± 2 |
| Weight, kg | 73.7 ± 2.9 |
| Height, m | 1.73 ± 0.02 |
| Body mass index, kg/m² | 24.6 ± 0.6 |
| Supine systolic blood pressure, mmHg | 125 ± 3 |
| Supine diastolic blood pressure, mmHg | 74 ± 2 |
| Supine heart rate, beats/min | 59 ± 2 |
| Peak oxygen uptake, ml·kg⁻¹·min⁻¹ | 33.2 ± 1.7 |

Values are means ± SE; n = 11 men.

venous occlusion cuff around the upper arm was inflated to 60 mmHg for 10 min. The calibration procedure was performed at the beginning and end of each experimental procedure by adding known volumes of air to the plethysmographic cuff, which was still placed on the subject's forearm. FVR (in U) was calculated by the mean BP and FBF ratio. No systemic hemodynamic measurements were performed in the present study.

MSNA. Multiunit, postganglionic MSNA was recorded by microneurography, as described previously (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 peroneal nerve, posterior to the fibular head, and an uninsulated reference electrode was positioned 1-3 cm away. The recorded signal was fed to a preamplifier (gain: 1,000), an amplifier (variable gain: 40-60), a bandpass filter (700-2,000 Hz), and a resistance capacity integrating network (time constant: 0.1 s) to obtain a mean voltage neurogram. MSNA was distinguished from other sources of nerve activity by the following criteria: 1) electrical stimulation elicited muscle contraction but not paresthesias; 2) tapping or stretching innervated muscle elicited afferent mechanoreceptor discharges, whereas stroking the skin did not; and 3) neurogram revealed spontaneous, intermittent, pulse-synchronous bursts that increased during held expiration and Valsalva maneuvers. MSNA was quantified in terms of burst frequency (bursts/min).

Euglycemic and hyperinsulinemic clamp. Regular human insulin (Novolin R, Novo-Norvisk), diluted in saline with 1 ml of subject's blood, was infused by digital pump (model 55-2222, Harvard Apparatus). To achieve a plasma insulin concentration of 100 µU/ml, an initial 10-min priming infusion of insulin was started according to the procedure described by DeFronzo et al. (9) and was followed by a 110-min period of insulin was started according to the procedure described by DeFronzo et al. (9) and was followed by a 110-min period of insulin clamping. The exercise bout was conducted at 91 ± 4 W. During exercise, VO₂ was measured in eight subjects, and the
mean value found was 18.0 ± 1.1 ml O$_2$.kg$^{-1}$.min$^{-1}$, which corresponded to 54 ± 2% of V˙O$_2$peak.

**Metabolic Responses**

Plasma glucose levels were kept close to baseline during the steady-state period in both sessions (Control: 87 ± 2 vs. 86 ± 3 mg/dl; Exercise: 82 ± 2 vs. 83 ± 2 mg/dl, baseline vs. steady state, respectively). Glucose concentration was significantly lower in the Exercise than in the Control session. The coefficients of variation of plasma glucose during the steady-state period were 4.7 ± 0.4% in the Control session and 5.2 ± 1.3% in the Exercise session.

Insulin levels were similar between the two experimental sessions. Baseline plasma insulin concentration averaged 11.0 ± 2.1 µU/ml in the Control session and 9.7 ± 1.8 µU/ml in Exercise session. During euglycemic-hyperinsulinemic clamp, the insulin concentration increased to a similar level in both experimental sessions (102.5 ± 7.3 vs. 95.3 ± 4.7 µU/ml, Control vs. Exercise, respectively).

M values (8.16 ± 1.39 vs. 8.01 ± 1.11 mg·kg$^{-1}$.min$^{-1}$, Control vs. Exercise, respectively) and MCR values (0.100 ± 0.022 vs. 0.097 ± 0.013 ml·kg$^{-1}$.min$^{-1}$, Control vs. Exercise, respectively) did not change significantly with exercise. Similarly, the M/I index (0.085 ± 0.017 vs. 0.079 ± 0.012 mg·kg$^{-1}$.min$^{-1}$/µU·ml$^{-1}$, Control vs. Exercise, respectively) was not different between Control and Exercise sessions.

**HR and BP Responses**

HR and systolic, mean, and diastolic BP responses to insulin infusion in both experimental sessions are shown in Fig. 1. Systolic BP was similar in Control and Exercise sessions, and it increased significantly with insulin infusion (127 ± 2 vs. 131 ± 2 mmHg, baseline vs. steady state, respectively; P < 0.05). Mean BP was significantly lower in the Exercise than in the Control session (89 ± 2 vs. 92 ± 2 mmHg, respectively; P < 0.05). Mean BP did not change significantly with insulin infusion. Exercise and insulin infusion had no significant effects on diastolic BP or HR levels.

**MSNA Responses**

Baseline and steady-state levels of MSNA in both Exercise and Control sessions are shown in Figs. 2 and 3. Baseline MSNA was significantly lower in the Exercise than in the Control session (19.5 ± 2.4 vs. 22.4 ± 2.5 bursts/min; P < 0.05). MSNA was significantly increased by insulin infusion in both sessions, and this enhancement was significantly greater in the Exercise than in the Control session (absolute change = 5 ± 1.4 vs. 7.9 ± 1.9 bursts/min, respectively; P < 0.05). Thus, during the steady-state period, MSNA was significantly greater in the Exercise than in the Control session (34.0 ± 3.3 vs. 30.3 ± 3.1 bursts/min, respectively; P < 0.05).

**FBF Responses**

Baseline and steady-state levels of FBF and FVR in both Exercise and Control sessions are shown in Figs. 2 and 3. FBF was significantly higher (2.10 ± 0.14 vs. 1.65 ± 0.11 ml·min$^{-1}$.100 ml$^{-1}$, P < 0.05) and FVR was significantly lower (47 ± 3 vs. 63 ± 5 U, P < 0.05) in the Exercise than in the Control session. FBF and FVR responses to insulin infusion were not significantly different between the Control and the Exercise ses-
sions. Insulin infusion did not significantly change FBF and FVR; however, FVR tended to be lower in the steady-state condition than at baseline ($P = 0.0782$).

Saline Session

Time and vehicle did not modify physiological responses significantly. Changes from 10 to 120 min during saline administration were -2.2 and +1.4 bursts/min for MSNA, 3.6 and 5.0 mmHg for mean BP, 2.0 and 1.3 beats/min for HR, +0.63 and -0.45 ml/(min·100 ml) for FBF, and -8.1 and +14.8 U for FVR in Control and Exercise sessions, respectively.

DISCUSSION

The main findings of the present investigation are that, in healthy men, a 45-min period of bicycle exercise at 50% of $V_o_{2\text{peak}}$ 1) decreases MSNA, increases FBF, and decreases mean BP during postexercise period; 2) does not change $M$; and 3) exacerbates insulin-induced enhancement in MSNA without changing FBF, FVR, and BP responses to acute postexercise hyperinsulinemia.

Aftereffects of Exercise on Neural and Hemodynamic Responses

In agreement with other studies (7, 15), in the present investigation, exercise simultaneously reduced MSNA and mean BP during the postexercise period. This suggests an alteration in the baroreflex control. In fact, Halliwill et al. (15) reported that, after exercise, the baroreflex set point of sympathetic nerve activity control was shifted to lower levels without changing the
reflex gain. Moreover, the alteration on the reflex control was better explained by a modification on central pathway rather than on baroreceptors (15). Exercise-induced increase in endogenous opioid secretion (27) and cardiopulmonary sensitivity (5) might be involved in such inhibition of the central sympathetic nerve activity control after acute exercise. The increase in muscle blood flow after exercise has also been extensively reported (7, 13, 15–17). Reduction in sympathetic nerve activity (7, 15), decrease in α-adrenergic responsiveness (15, 17), need of heat dissipation (13), release of local metabolites (18), and increased production of nitric oxide (23) and opioids (16) have been suggested as the most important mechanisms to explain this vasodilatory response to exercise.

Effects of Insulin on Neural and Hemodynamic Responses

As previously reported (2, 20, 22, 25), insulin infusion significantly increased MSNA, possibly mediated by a direct effect of insulin on the central nervous system. Lombo et al. (20) observed that systemic, but not local, insulin infusion increased norepinephrine release in skeletal muscle. Pereda et al. (24) found that insulin infusion in dogs’ carotid artery, at doses that have no systemic effects, produced cardiovascular responses compatible with sympathetic activation.

In the present study, insulin infusion did not significantly change muscle blood flow. This response may be explained by the increase in sympathetic nerve activity, which might have counteracted the vasodilatory effect of insulin (2, 3, 19, 20, 28).

The increase of systolic BP, concomitant with the tendency of decrease in FVR during insulin infusion, suggests that the pressor effect of insulin may be caused by an increase in cardiac output. This increase might happen because of the stroke volume, once HR did not change with hyperinsulinemia.

Aftereffects of Exercise on Physiological Responses to Insulin

The new finding of the present investigation is that a single bout of exercise, which did not change insulin sensitivity, induces an exacerbation of the MSNA response to postexercise insulin infusion.

Although the mechanisms underlying this response were not investigated in the present study, we speculate that exercise-induced alterations to central pathways of the sympathetic nerve activity control might be involved, because insulin stimulates MSNA by central actions (24). Moreover, this response might be modulated by opioids released during exercise, because Hara and Floras (16) observed that the control of sympathetic activity outflow after exercise was altered by naloxone. Future studies may address this issue.

Despite the greater increase of MSNA during insulin infusion after exercise, FBF and FVR responses to hyperinsulinemia did not change. This suggests that exercise counteracts the increase in MSNA by a reduction of α-adrenergic responsiveness (15, 17) or even an increase in insulin vasodilatory effects.

It is also interesting to point out that, in four subjects, M increased significantly with exercise (7.28 ± 2.98 vs. 11.23 ± 4.23 mg·kg⁻¹·min⁻¹, Control vs. Exercise session, respectively; P < 0.05). Also, in this subset of subjects, exacerbated response of MSNA to hyperinsulinemia after exercise was not observed (9.5 ± 8.3 vs. 13.6 ± 5.2 bursts/min, Control vs. Exercise session, respectively; P = 0.115). Despite the small number of subjects, these results suggest a possible association between the increased MSNA response to insulin infusion after exercise and insulin resistance. We believe this is the first time in the literature that this behavior has been described. Therefore, it should be more deeply investigated by future studies.

Aftereffects of Exercise on Insulin Sensitivity

Some authors (4, 6, 11, 21, 29), but not others (14, 30), have observed that acute exercise increases insulin sensitivity. The results from the present study agree with those of investigators who found no change in insulin sensitivity after exercise. The combination of methodological approach, population studied, exercise protocol, and time between exercise and measurements may explain the disparate results among studies. First, most studies (21, 29) employed a dose-response curve, whereas we used a single dose of insulin. Second, exercise-induced increase in insulin sensitivity is greater in insulin-resistant subjects (11). In the present study, subjects presented a large range of insulin sensitivity indexes [M/I values varied from 0.262 to 0.186 mg·kg⁻¹·min⁻¹/(μU·ml⁻¹)]. Third, the aspects of an exercise protocol, such as exercise intensity (4), muscle mass involved (6), and time between exercise and measurements (14), are known to influence M; however, the ideal protocol for postexercise enhancement of M is not known.

Study Limitations

Although vehicle and time control sessions were not performed in all subjects, saline studies carried out in three subjects suggested that time and vehicle cannot explain the response to insulin infusion that was observed in both Control and Exercise sessions. Similar results have already been obtained by other authors (2) in control trials for a longer period of time.

Plasma glucose and insulin concentrations can be influenced by changes in plasma volume that are caused by exercise. However, these changes seem not to be the case in the present investigation, because hemato-crit and hemoglobin, as measured in our laboratory in eight subjects before and after a bout of exercise similar to the one employed in this study (cycle ergometer, 45 min, 50% V̇O₂peak), did not change significantly (hemato-crit, 46.1 ± 2.1 vs. 45.6 ± 2.7, P = 0.302; hemoglobin, 15.5 ± 0.7 vs. 15.3 ± 0.9 mg/dl, P = 0.318).

Although MSNA was measured on the lower limb and muscle blood flow was measured on the upper limb, it is unlikely that the difference in measurement sites has limited our interpretation. Delius et al. (10) demonstrated that MSNA recorded in different sites was, in fact, similar.
Conclusion

In conclusion, in healthy subjects, a single bout of prolonged exercise, which does not alter insulin sensitivity, exacerbates insulin-induced increase in MSNA without changing FBF, FVR, and BP responses to hyperinsulinemia after exercise.

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