



ELSEVIER

Available online at www.sciencedirect.com

Maturitas xxx (2007) xxx–xxx

MATURITASTHE EUROPEAN
MENOPAUSE
JOURNALwww.elsevier.com/locate/maturitas

Neurovascular and hemodynamic responses to hyperinsulinemia in healthy postmenopausal women

Crivaldo Gomes Cardoso Jr.^a, Daniela Sakai^a, Luiz Gustavo Pinto^a, Eliana Labes^c,
Josiane Lima de Gusmão^b, Sandra Balieiro Abrahão^b, Taís Tinucci^a, Décio Mion Jr.^b,
Angela Maggio da Fonseca^c, Cláudia Lúcia de Moraes Forjaz^{a,*}

^a *Exercise Hemodynamic Laboratory, School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil*

^b *Hypertension Unit, General Hospital, University of São Paulo, Brazil*

^c *Gynecology and Climacteric Service, General Hospital, University of São Paulo, Brazil*

Received 21 July 2006; received in revised form 11 April 2007; accepted 7 May 2007

Abstract

Acute hyperinsulinemia produces sympathetic activation, vasodilation, and cardiovascular changes in healthy young men. Postmenopausal period is accompanied by sympathetic, vascular and cardiovascular changes. Nevertheless, the effects of acute insulin infusion were not known in postmenopausal women. To study this aspect, 26 postmenopausal healthy women were submitted to an euglycemic hyperinsulinemic clamp performed during 120 min. Heart rate (HR: ECG), blood pressure (BP: oscillometric method), forearm blood flow (FBF: plethysmography), plasma norepinephrine (NE), plasma epinephrine (EP), and cardiovascular autonomic modulation (spectral analysis of R–R interval and BP variabilities) were measured before and during the clamp. Glycemia was kept similar to baseline during the clamp (84.6 ± 1.2 mg/dl versus 87.1 ± 1.6 mg/dl), while plasma insulin increased significantly to a level of 89.3 ± 5.6 μ U/ml. Insulin infusion significantly increased plasma NE ($+45 \pm 17$ pg/ml), EP ($+20 \pm 9$ pg/ml), and low to high frequency ratio of R–R interval variability (LH/HF: 1.2 ± 0.4), but did not change low frequency component of BP variability. FBF ($+0.7 \pm 0.2$ ml min^{-1} 100 ml $^{-1}$) was also significantly enhanced by hyperinsulinemia. HR and systolic BP increased with insulin infusion ($+4 \pm 1$ bat/min and $+6 \pm 2$ mmHg, respectively, $P < 0.05$), while diastolic BP did not change. In conclusion, in healthy postmenopausal women, acute hyperinsulinemia produces sympathetic activation, and vasodilation, which results in HR and systolic BP enhancements, with no change in diastolic BP. This pattern of response is similar to the one usually observed in healthy young men.

© 2007 Elsevier Ireland Ltd. All rights reserved.

Keywords: Menopause; Hyperinsulinemia; Muscle blood flow; Sympathetic activity; Blood pressure; Spectral analysis

1. Introduction

Rich carbohydrate meals are usually accompanied by neurovascular adjustments, such as sympathetic activation and vasodilation [1]. Previous studies [2,3]

* Corresponding author at: Av. Prof. Mello Moraes, 65, Butantã, São Paulo, SP 05508-900, Brazil. Tel.: +55 11 3091 2149; fax: +55 11 3813 5921.

E-mail address: cforjaz@usp.br (C.L.M. Forjaz).

had demonstrated that these effects are due to hyperinsulinemia and not to hyperglycemia induced by such meals.

In fact, we [4,5] and others [3,6–11] had demonstrated that acute hyperinsulinemia, produced during an euglycemic hyperinsulinemia clamp, causes an expressive increase in muscle sympathetic nerve activity. Moreover, it also increases plasma norepinephrine levels [3,6,8,11–16], and sympathetic markers evaluated by spectral analysis of heart rate variability [15–17]. On the other hand, acute insulin infusion has a direct effect on muscle vasculature producing vasodilation [3,6–8,10,12,16,18–22]. These ambiguous actions of insulin result in blood pressure increase [4–6,10,11,14,15], decrease [8,12,15] or maintenance [3,7–13,15–17,20,23] during hyperinsulinemia.

It has been shown that neurovascular actions of insulin differ between subjects. Sympathetic activation produced during hyperinsulinemia is lower in obese than non-obese subjects [6,15,16], and in elderly [14]. Moreover, insulin induced vasodilation is also lower in obese than in non-obese subjects [6,16]. Nevertheless, all previous studies were conducted, predominantly, in young and middle aged men. Only one study [20] involved a group of young women, and observed that insulin-induced vasodilation was increased in young females compared with males.

Postmenopause is usually accompanied by metabolic and neurovascular changes. Insulin resistance and hyperinsulinemia were observed in long-term postmenopausal women [24,25]. Moreover, postmenopausal women usually presented greater sympathetic activity [26–29], lower blood flow [29], and high blood pressure levels [29] than premenopausal ones. In regard to neurovascular responses to stimuli, the only study [30] that investigated the vascular effects of hyperinsulinemia in postmenopausal women did not observe any change in blood flow. However, this study included only seven women without hormone therapy, and did not evaluate sympathetic activation.

Thus, the objective of the present study was to investigate, in healthy postmenopausal women without hormone therapy, the neurovascular and hemodynamic responses to acute hyperinsulinemia produced by a euglycemic-hyperinsulinemic clamp. We hypothesized that insulin infusion would produce sympathetic activation and vasodilation, resulting in a increase in heart

rate, and systolic blood pressure, but no change in diastolic blood pressure.

2. Methods

2.1. Subjects

Twenty-six healthy postmenopausal women were included in this study. Volunteers were recruited by advisements on newspapers, university bus stops, and hospital murals. All subjects were hysterectomized with or without ovariectomy. Natural menopause was observed in 16 women, and surgical menopause in 5. None of the subjects was receiving any medication during the study period. Thirteen women had never received hormone therapy before entering the study. In the others, previous treatment (eight oral, three injected, and two transdermal hormone) was withdrawn at least 3 months before the experiments. All volunteers were free of chronic diseases, as assessed by medical history, physical examination, standard hematological evaluation, and resting and exercise ECG. No subject was engaged in any regular physical activity program. Subjects who smoked or presented cardiovascular diseases (abnormal ECG), diabetes mellitus fast glycemia greater than 100 (mg/dl), hypertension (resting systolic/diastolic blood pressures equal or greater than 140/90 mmHg), obesity (body mass index equal or greater than 30 kg/m²), or high levels of total cholesterol (equal or greater than 240 mg/dl) were excluded. All subjects gave written informed consent to participate in the study, which was performed according with the Declaration of Helsinki, and approved by the Ethical Committee of the General Hospital, University of São Paulo, São Paulo, Brazil.

2.2. Procedures

Blood pressure (BP) and heart rate (HR) were measured by an oscillometric automatic device (Dixtal, 2710), which was regularly calibrated by comparison against a mercury column.

Forearm blood flow (FBF) was measured by venous occlusion plethysmography [31,32]. 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 a subdiastolic pressure of 40–60 mmHg for 7 s of every 15 s. This procedure interrupted venous return but not arterial inflow, resulting in an increase in forearm volume, which increased pressure inside the plethysmographic cuff. The slope of increase in this pressure determined FBF. Four measurements were taken each minute, and FBF was measured for 3 min in each time point. A mean value was calculated. The coefficient of variation of FBF was $14 \pm 2\%$. Forearm vascular conductance (FVC) was calculated by the quotient between FBF and mean BP.

Sympathetic activity was assessed by plasma catecholamine measurements, and by spectral analysis of R–R interval and BP variabilities. For epinephrine (EP) and norepinephrine (NE) assessments, blood samples were collected in iced tubes containing sodium citrate. For spectral analysis of R–R interval and BP variabilities, ECG was obtained in MC5, respiratory activity was assessed by a piezo-electric respiration transducer (UFI, 1132), and BP was recorded beat-by-beat by finger plethysmography (Ohmeda, Finapres). These signals were acquired in a computer with a sample frequency of 500 Hz/channel (WINDAC, DI-720). An autoregressive spectral analysis of R–R interval and BP variabilities was performed, and its theoretical and analytical procedures have been described before [33]. Briefly, on stationary segments of the time series, autoregressive parameters were estimated by the Levinson–Durbin recursion, and the order of the model was chosen according to Akaike's criteria [34]. An autoregressive spectral decomposition was then performed. The components were assigned based on their central frequency as low (LF: 0.04–0.15 Hz) and high (HF: 0.15–0.5 Hz) frequency components. HF power in R–R variability was considered in dependence to a coherence with respiratory signal. Normalized values of LF_{R-R} and HF_{R-R} were calculated by the quotient of each power component and the total power minus very low frequency component (VLF: 0–0.04 Hz).

Hyperinsulinemic-euglycemic clamp was performed during 2 h according with the method of DeFronzo et al. [35]. Briefly, regular human mono-component 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 $50.7 \mu\text{U m}^{-2} \text{min}^{-1}$ during 120 min to achieve a plasma insulin concentration of approximately $100 \mu\text{U/ml}$, as previously described by some of us [4,5]. Euglycemia was maintained by adjusting the infusion rate of a 50% glucose solution. Plasma glucose was monitored every 5 min, and dextrose infusion rate was adjusted to maintain euglycemia. The average of glucose infusion rate ($\text{mg kg}^{-1} \text{min}^{-1}$) was used to calculate glucose metabolized (M) based on DeFronzo's formula [35]. Insulin sensitivity index (M/I) was calculated by the ratio between M and plasma insulin concentration during this period.

2.3. Experimental protocol

All volunteers arrived at the laboratory between 7 and 8 am after an overnight fast, preceded by 3 days of unrestricted diet with at least 250 g of carbohydrate. They abstained of any physical exercise for at least 48 h and from alcohol ingestion for at least 24 h before the experiments.

Two catheters were introduced in both antecubital veins, and were maintained patent by a saline infusion. One catheter was used to infuse insulin and glucose, and the other to collect blood samples. Before and every 15 min during the insulin infusion, FBF was measured during 3 min, while BP and HR were measured every minute. Moreover, data for spectral analysis were collected for 10 min before and during insulin infusion. Blood samples for glycemia and plasma insulin analyses were collected before and every 5 min during the clamp, while samples for EP and NE analyses were collected immediately before and at the end of the insulin infusion.

2.4. Biochemical analysis

Plasma glucose concentration was measured by hexoquinase enzymatic method (GLUL 0-991, Roche). Plasma insulin was analyzed in duplicate by immunofluorimetric method (Auto-Delfia, PerkinElmer Life and Analytical Sciences, Wallac Oy, Mustionkatu 6). The coefficients of variation were 2.0 ± 0.4 and $6.1 \pm 1.3\%$ for plasma glucose and insulin, respectively. Plasma NE and EP levels were quantified by high-performance liquid chromatography with electrochemical detection (HPLC–ED).

3. Statistical analysis

Considering a power of 80%, an alpha error of 5%, and a standard deviation of $0.6 \text{ ml min}^{-1} 100 \text{ ml}^{-1}$ for FBF, the minimal sample size necessary to detect a difference of $0.5 \text{ ml min}^{-1} 100 \text{ ml}^{-1}$ in FBF during insulin infusion was calculated to be 14 subjects.

As all metabolic and hemodynamic data were stable between 60 and 105 min of insulin infusion, a mean value was calculated for this period. This value was compared with baseline by a paired *T*-student test. $P < 0.05$ was accepted as statistically significant. Data are presented as means \pm S.E.

4. Results

The characteristics of the subjects are shown in Table 1. All subjects were healthy, non-obese, without cardiovascular risk factors, and with hormonal status coherent with postmenopause.

Plasma glucose and insulin measured before and during insulin infusion are shown in Fig. 1. Plasma glucose was maintained similar to baseline during infusion ($84.6 \pm 1.2 \text{ mg/dl}$ versus $87.1 \pm 1.6 \text{ mg/dl}$), while plasma insulin increased significantly from a baseline value of $4.4 \pm 0.5 \mu\text{U/ml}$ to a steady-state value of $89.3 \pm 5.6 \mu\text{U/ml}$. *M* and *M/II*

Table 1

Anthropometric, metabolic, cardiovascular and menopausal characteristics of the subjects

<i>n</i>	26
Age, years	51 ± 1
Weight, kg	61.3 ± 1.6
BMI, kg/m^2	24.8 ± 0.5
Fasting blood glucose, mg/dl	85 ± 1
Fasting insulin, $\mu\text{U/ml}$	4.4 ± 0.5
Total cholesterol, mg/dl	208 ± 5
LDL cholesterol, mg/dl	126 ± 5
HDL cholesterol, mg/dl	61 ± 3
Tryglicerides, mg/dl	105 ± 9
SBP, mmHg	119 ± 2
DBP, mmHg	76 ± 2
E ₂ , pg/ml	17 ± 1
FSH, U	86 ± 6
LH, U	41 ± 3

Data are the mean \pm S.E. BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; E₂, estradiol; FSH, follicle stimulating hormone; LH, luteizing hormone.

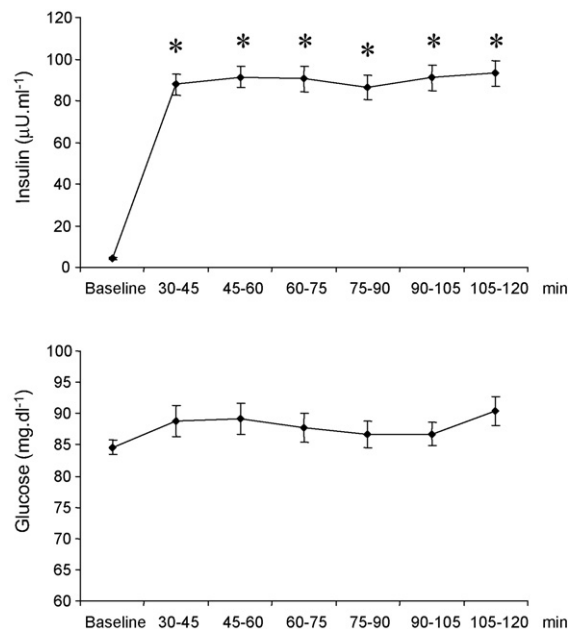


Fig. 1. Plasma glucose and insulin measured before and during the euglycemic hyperinsulinemic clamp. *significantly different from baseline ($P < 0.05$).

were $6.8 \pm 0.6 \text{ mg kg}^{-1} \text{ min}^{-1}$ and $0.0697 \pm 0.0053 \text{ mg kg}^{-1} \text{ min}^{-1}/(\mu\text{U ml}^{-1})$, respectively.

Neurovascular and hemodynamic responses to acute hyperinsulinemia are shown in Table 2. The main responses are also shown in Fig. 2. Plasma NE and EP increased significantly with insulin infusion. Moreover, R–R interval and normalized $\text{HF}_{\text{R-R}}$ decreased, while normalized $\text{LF}_{\text{R-R}}$ and $\text{LF}/\text{HF}_{\text{R-R}}$ increased significantly with hyperinsulinemia. Absolute $\text{LF}_{\text{R-R}}$, $\text{HF}_{\text{R-R}}$, and total power did not change significantly, but absolute $\text{HF}_{\text{R-R}}$ tended to decrease. Furthermore, LF component of systolic and diastolic BP variabilities did not change with hyperinsulinemia. FBF and FVC increased significantly during insulin infusion. In regard to cardiovascular variables, HR, systolic and mean BPs increased significantly, while diastolic BP did not change with hyperinsulinemia.

5. Discussion

The main findings of the present study are that, in postmenopausal healthy women, acute hyperinsulinemia produces sympathetic activation and vasodilation,

Table 2

Neurovascular and hemodynamic variables measured before (baseline) and during insulin infusion in the euglycemic hyperinsulinemic clamp

	Baseline	Infusion	P-value
Plasma catecholamines			
NE, pg/ml	87 ± 11	131 ± 20	0.02
AD, pg/ml	29 ± 3	49 ± 9	0.03
R–R variability			
R–R interval, ms	0.910 ± 0.037	0.872 ± 0.030	0.03
TV _{R–R} , ms ²	1542 ± 287	1221 ± 284	0.06
LF _{R–R} , ms ²	282 ± 50	258 ± 50	0.51
HF _{R–R} , ms ²	378 ± 114	158 ± 39	0.06
LF _{R–R} , nu	41 ± 4	54 ± 5	0.01
HF _{R–R} , nu	47 ± 4	36 ± 4	0.01
LF/HF _{R–R}	1.1 ± 0.2	2.3 ± 0.5	0.01
Blood pressure variability			
TV _{SBP} , mmHg ²	33 ± 7	31 ± 8	0.89
LF _{SBP} , mmHg ²	4 ± 1	8 ± 2	0.10
TV _{DBP} , mmHg ²	9 ± 2	9 ± 2	0.70
LF _{DBP} , mmHg ²	2 ± 1	2 ± 0	0.61
Hemodynamic variables			
SBP, mmHg	143 ± 3	148 ± 4	0.02
MBP, mmHg	92 ± 2	97 ± 3	0.02
DBP, mmHg	77 ± 2	78 ± 2	0.27
HR, bat/min	64 ± 2	68 ± 2	0.00
FBF, ml min ⁻¹ 100 ml ⁻¹	2.1 ± 0.2	2.8 ± 0.3	0.00
FVC, ml min ⁻¹ 100 ml ⁻¹ /mmHg	0.022 ± 0.002	0.029 ± 0.02	0.00

Data are the mean ± S.E. NE, norepinephrine; EP, epinephrine, TV, total variance; LF low-frequency; HF, high frequency; nu, normalized units; SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; HR, heart rate; FBF, forearm blood flow; FVC, forearm vascular conductance.

which results in HR and systolic BP enhancements without any change in diastolic BP.

In the present study, acute insulin infusion, achieving levels similar to those observed after a rich carbohydrate meal (85% of carbohydrate) [1] produced an expressive increase in sympathetic activity. Global sympathetic activation was observed by the increase in plasma NE levels. Moreover, the decrease in normalized HF_{R–R}, observed simultaneously with an increase in normalized LF_{R–R} and LF/HF, clearly indicated a shift of cardiac autonomic control towards a sympathetic predominance, showing that acute insulin infusion also produced cardiac sympathetic activation.

In regard to peripheral sympathetic control, muscle or skin sympathetic activities were not directly measured in the present study. Previous researches from us [4,5] and others [3,6–11] have reported that muscle sympathetic nerve activity, measured by microneurography, increases with hyperinsulinemia, while skin sympathetic activity does not change [11]. In the

present investigation, vasomotor sympathetic modulation was assessed by the spectral analysis of BP variability [33]. To our knowledge, this is the first study to do this kind of analysis during clamp. LF component of systolic and diastolic BP variabilities did not change with insulin infusion, which suggests that vasomotor sympathetic activity was not influenced by hyperinsulinemia. Controversy between previous studies and the actual one might be explained by differences in methodologies to assess peripheral sympathetic activity. Microneurography measured sympathetic traffic through a specific nerve [36], while spectral analysis of BP variability allows an evaluation of whole body peripheral sympathetic activation coupled with vascular response [37,38]. Thus, it is possible that muscle sympathetic activity was increased by insulin infusion, but as insulin acts directly on the vasculature producing vasodilation, the sympathetic activation was masked when analyzed by BP variability. Besides, sympathetic traffic for other vascular beds, such as skin [11], might

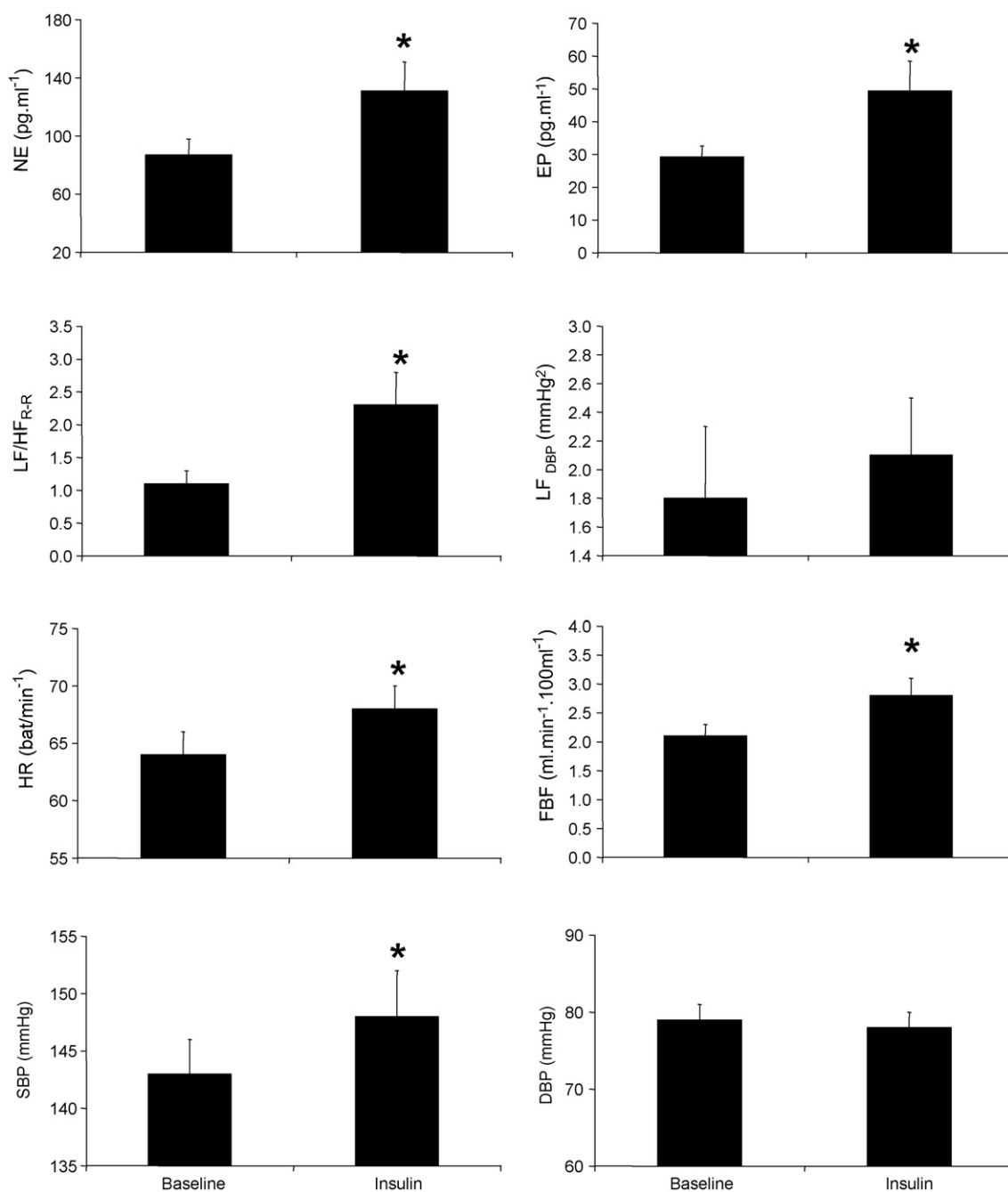


Fig. 2. Plasma norepinephrine (NE), plasma epinephrine (EP), R–R interval low to high frequency ratio (LF/HF_{R-R}), diastolic blood pressure low frequency component (LF_{DBP}), heart rate (HR), forearm blood flow (FBF), systolic blood pressure (SBP), and diastolic blood pressure (DBP) measured before (baseline) and during insulin infusion promoted by euglycemic hyperinsulinemic clamp. *significantly different from baseline ($P < 0.05$).

not have changed with insulin, resulting in no modification in BP variability. Mechanisms responsible for insulin-induced sympathetic activation were out of the scope of the present study, but previous researches [39] have suggested that they were related to a central action of insulin.

Insulin-induced sympathetic activation has been extensively reported in healthy young men, regardless it was studied by plasma NE levels [3,6,8,11–16], microneurographic measurement [3–11] or heart rate variability [15–17]. Nevertheless, this is the first time this activation was demonstrated in women, and especially in postmenopausal ones. This might have clinical relevance in this population, since postmenopausal period was usually accompanied by sympathetic activation [26–29], and the present results suggested that high carbohydrate meals, that could enhanced expressively insulin levels, might result in a greater increase in sympathetic activity.

The increase in HR observed during hyperinsulinemia is in accordance with the increase in cardiac sympathetic activity. Previous studies, performed with young men [12] and women [20], demonstrated an increase in cardiac output with acute insulin infusion, and this increase was, at least in part, explained by the increase in HR. Thus, in the present study, cardiac output might also be enhanced during hyperinsulinemia, which might explain the increase in systolic BP.

Besides sympathetic activation, FVC increased with insulin infusion, which was associated to an increase in FBF that overcame the increase in mean BP. Insulin-induced vasodilation has already been extensively observed in healthy young men [8,12,19–21], and women [20]. Nevertheless, the only other study that involved postmenopausal women [30] did not observe any change in FBF during acute hyperinsulinemia in subjects without hormone therapy. Differences might be explained by the higher number of subjects involved in the present study (24 versus 7), and/or by the higher levels of insulin achieved in the present study. Anderson et al. [8] observed that insulin-induced vasodilation is dose-dependent, and Hausberg et al. [9] did not observed vasodilation with a low dose of insulin. Nevertheless, as stated before, plasma insulin levels achieved in the present study are similar to the ones achieved after a high carbohydrate meal (85% of carbohydrate) [1]. The mechanisms responsible for insulin-induced vasodilation have been suggested to be

related to a direct effect of insulin mediated by nitric oxide release [40,41], and capillary recruitment [21]. However, the increase in EP levels, observed in the present study, suggested that vascular beta-adrenergic stimulation, at least in postmenopausal women, might also be involved.

Although FBF and FVC increased during insulin infusion, diastolic BP did not change. In fact, insulin-induced muscle vasodilation might have been compensated by sympathetic activation, which might have constricted other vascular beds [22]. Moreover, the maintenance of diastolic BP during insulin infusion is in accordance with the maintenance of LF component of BP variability.

Neurovascular actions of insulin have been proposed to be related to insulin sensibility in carbohydrate metabolism. Subjects resistant to insulin, such as obese, presented lower insulin-induced sympathetic stimulation [6,15,16]. Moreover, in healthy men, only those who were more sensitive to insulin presented an increase in sympathetic activity during hyperinsulinemia [17]. Similarly, insulin-induced vasodilation was greater in non-obese than in obese subjects [6,16], and a direct relationship has been observed between insulin sensitivity and insulin-induced vasodilation [12].

The relationship between menopause and insulin sensitivity is complex. When compared with premenopausal women, postmenopausal ones are usually insulin resistant [24,25]. However, the transition from pre to postmenopause did not affect insulin sensitivity [25]. In the present study, M value was $6.8 \pm 0.6 \text{ mg kg}^{-1} \text{ min}^{-1}$, which was similar to values observed previously in pre and immediately postmenopausal women [25,30], and were higher than the ones observed in long term menopausal women [30]. Moreover, M value presented a large variation between subjects (from 3.5 to $17.8 \text{ mg kg}^{-1} \text{ min}^{-1}$), showing that even in healthy postmenopausal women, insulin sensitivity varies a lot. However, when these values were compared with the ones predicted for each subject based on their age and body mass index [17], only three subjects presented an index lower than expected, showing that the sample was mainly composed by subjects sensitive to insulin, which is in accordance to their healthy status. It is interesting to point out that M values did not correlated with FBF increase, neither to R–R LF or HF component variations. However, there was a positive correlation between M value and plasma NE

increase ($r = 0.460$, $P < 0.05$), showing that global sympathetic activation was greater in women more sensitive to insulin.

5.1. Limitations

The present study did not involve a control experiment with saline infusion. However, many previous researches [5,9,11,16] have already stated that saline infusion does not alter neural, vascular and hemodynamic variables investigated in the present study.

5.2. Conclusion

In postmenopausal women, acute insulin infusion produces sympathetic activation, resulting in a shift of cardiac autonomic modulation towards a sympathetic predominance, which results in heart rate and systolic BP increases. On the other hand, it produces muscle vasodilation that counterbalance sympathetic activation, resulting in no change of diastolic blood pressure. This pattern of response is similar to the one usually observed in healthy young men and women.

Acknowledgements

We gratefully acknowledge the volunteers involved in this study. We also thank Alberto Porta for providing the software for spectral analysis, Dulce Casarini and Luciana Cristina Teixeira for catecholamine analysis, and Mariana Curi and Carlos Ugrinowitsch for statistical assistance. This study was supported by FAPESP (01/14989-7).

References

- [1] Scott EM, Greenwood JP, Vacca G, Stoker JB, Gilbey SG, Mary DA. Carbohydrate ingestion, with transient endogenous insulinaemia, produces both sympathetic activation and vasodilatation in normal humans. *Clin Sci (London)* 2002;5: 523–9.
- [2] van Gurp PJ, Rongen GA, Lenders JW, Al Nabawy AK, Timmers HJ, Tack CJ. Sustained hyperglycaemia increases muscle blood flow but does not affect sympathetic activity in resting humans. *Eur J Appl Physiol* 2005;5/6:648–54.
- [3] Vollenweider P, Tappy L, Randin D, et al. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J Clin Invest* 1993;1:147–54.
- [4] Bisquolo VA, Cardoso Jr CG, Ortega KC, et al. Previous exercise attenuates muscle sympathetic activity and increases blood flow during acute euglycemic hyperinsulinemia. *J Appl Physiol* 2005;3:866–71.
- [5] Forjaz CL, Ramires PR, Tinucci T, et al. Postexercise responses of muscle sympathetic nerve activity and blood flow to hyperinsulinemia in humans. *J Appl Physiol* 1999;2:824–9.
- [6] Vollenweider P, Randin D, Tappy L, Jequier E, Nicod P, Scherrer U. Impaired insulin-induced sympathetic neural activation and vasodilation in skeletal muscle in obese humans. *J Clin Invest* 1994;6:2365–71.
- [7] Vollenweider L, Tappy L, Owlya R, Jequier E, Nicod P, Scherrer U. Insulin-induced sympathetic activation and vasodilation in skeletal muscle. Effects of insulin resistance in lean subjects. *Diabetes* 1995;6:641–5.
- [8] Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 1991;6:2246–52.
- [9] Hausberg M, Mark AL, Hoffman RP, Sinkey CA, Anderson EA. Dissociation of sympathoexcitatory and vasodilator actions of modestly elevated plasma insulin levels. *J Hypertens* 1995;9:1015–21.
- [10] Hausberg M, Sinkey CA, Mark AL, Hoffman RP, Anderson EA. Sympathetic nerve activity and insulin sensitivity in normotensive offspring of hypertensive parents. *Am J Hypertens* 1998;11:1312–20. Pt 1.
- [11] Berne C, Fagius J, Pollare T, Hjemdahl P. The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia* 1992;9:873–9.
- [12] Baron AD, Brechtel G. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol* 1993;1(Pt 1):E61–7.
- [13] Arauz-Pacheco C, Lender D, Snell PG, et al. Relationship between insulin sensitivity, hyperinsulinemia, and insulin-mediated sympathetic activation in normotensive and hypertensive subjects. *Am J Hypertens* 1996;12(Pt 1):1172–8.
- [14] Minaker KL, Rowe JW, Young JB, Sparrow D, Pallotta JA, Landsberg L. Effect of age on insulin stimulation of sympathetic nervous system activity in man. *Metabolism* 1982;12: 1181–4.
- [15] Muscelli E, Emdin M, Natali A, et al. Autonomic and hemodynamic responses to insulin in lean and obese humans. *J Clin Endocrinol Metab* 1998;6:2084–90.
- [16] Paolisso G, Manzella D, Tagliamonte MR, Rizzo MR, Gambardella A, Varricchio M. Effects of different insulin infusion rates on heart rate variability in lean and obese subjects. *Metabolism* 1999;6:755–62.
- [17] Bergholm R, Westerbacka J, Vehkavaara S, Seppala-Lindroos A, Goto T, Yki-Jarvinen H. Insulin sensitivity regulates autonomic control of heart rate variation independent of body weight in normal subjects. *J Clin Endocrinol Metab* 2001;3:1403–9.
- [18] Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 1995;2:786–92.

- [19] Dela F, Stallknecht B, Biering-Sorensen F. An intact central nervous system is not necessary for insulin-mediated increases in leg blood flow in humans. *Pflugers Arch* 2000;2/3:241–50.
- [20] Lind L, Fugmann A, Millgard J, Berne C, Lithell H. Insulin-mediated vasodilatation, but not glucose uptake or endothelium-mediated vasodilatation, is enhanced in young females compared with males. *Clin Sci (London)* 2002;2:241–6.
- [21] Baron AD, Tarshoby M, Hook G, et al. Interaction between insulin sensitivity and muscle perfusion on glucose uptake in human skeletal muscle: evidence for capillary recruitment. *Diabetes* 2000;5:768–74.
- [22] Hoffman RP, Sinkey CA, Dopp JM, Phillips BG. Lack of effect of alpha- and beta-adrenergic inhibition on forearm glucose uptake despite differences in forearm blood flow in healthy humans. *Metabolism* 2002;11:1506–13.
- [23] Berne C, Fagius J, Niklasson F. Sympathetic response to oral carbohydrate administration. Evidence from microelectrode nerve recordings. *J Clin Invest* 1989;5:1403–9.
- [24] Walton C, Godsland IF, Proudler AJ, Wynn V, Stevenson JC. The effects of the menopause on insulin sensitivity, secretion and elimination in non-obese, healthy women. *Eur J Clin Invest* 1993;8:466–73.
- [25] Toth MJ, Sites CK, Eltabbakh GH, Poehlman ET. Effect of menopausal status on insulin-stimulated glucose disposal: comparison of middle-aged premenopausal and early postmenopausal women. *Diab Care* 2000;6:801–6.
- [26] Vongpatanasin W, Tuncel M, Mansour Y, Arbique D, Victor RG. Transdermal estrogen replacement therapy decreases sympathetic activity in postmenopausal women. *Circulation* 2001;24:2903–8.
- [27] Liu CC, Kuo TB, Yang CC. Effects of estrogen on gender-related autonomic differences in humans. *Am J Physiol Heart Circ Physiol* 2003;5:H2188–93.
- [28] Narkiewicz K, Phillips BG, Kato M, Hering D, Bieniaszewski L, Somers VK. Gender-selective interaction between aging, blood pressure, and sympathetic nerve activity. *Hypertension* 2005;4:522–5.
- [29] Moreau KL, Donato AJ, Tanaka H, Jones PP, Gates PE, Seals DR. Basal leg blood flow in healthy women is related to age and hormone replacement therapy status. *J Physiol* 2003;309–16. Pt 1.
- [30] Vehkavaara S, Westerbacka J, Hakala-Ala-Pietila T, Virkamaki A, Hovatta O, Yki-Jarvinen H. Effect of estrogen replacement therapy on insulin sensitivity of glucose metabolism and preresistance and resistance vessel function in healthy postmenopausal women. *J Clin Endocrinol Metab* 2000;12:4663–70.
- [31] Anderson EA. Measurement of blood flow and venous distensibility. In: Schneiderman N, Weiss SM, Kauffman PG, editors. *Handbook of research methods and cardiovascular behavioral medicine*. New York: Plenum Publishing Corporation; 1989.
- [32] Siggaard-Andersen J. Venous occlusion plethysmography on the calf. Evaluation of diagnosis and results in vascular surgery. *Dan Med Bull* 1970;1–68. Suppl I.
- [33] Task TFotESoCatNASoPaE. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; 5: 1043–65.
- [34] Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation* 1991;2:482–92.
- [35] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;3:E214–23.
- [36] Grassi G, Esler M. How to assess sympathetic activity in humans. *J Hypertens* 1999;6:719–34.
- [37] Pagani M, Montano N, Porta A, et al. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation* 1997;6:1441–8.
- [38] Malliani A, Pagani M, Lombardi F. Physiology and clinical implications of variability of cardiovascular parameters with focus on heart rate and blood pressure. *Am J Cardiol* 1994;10:3C–9C.
- [39] Muntzel MS, Malena H, Druke T. Inhibition of nitric oxide synthesis attenuates insulin-mediated sympathetic activation in rats. *J Hypertens* 2001;9:1625–31.
- [40] van Veen S, Chang PC. Prostaglandins and nitric oxide mediate insulin-induced vasodilation in the human forearm. *Cardiovasc Res* 1997;1:223–9.
- [41] Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab* 2003;1:E123–9.