HEART RATE VARIABILITY IN METABOLICALLY HEALTHY AND METABOLICALLY UNHEALTHY OBESE PREMENOPAUSAL WOMEN

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Abstract

Content. Metabolically healthy obese (MHO) individuals are characterized by absence of metabolic syndrome. The role of autonomic nervous system in metabolic profile of obese subjects has not been sufficiently investigated.

Objective. We analyzed heart rate variability (HRV) in MHO and metabolically unhealthy obese (MUO) premenopausal women.

Design. In 42 women metabolic profile was defined as MHO and MUO.

Subjects and Methods. For metabolic profile Wildman, IDF and HOMA-IR criteria were used. Autonomic nervous system activity was assessed by analysis of heart rate variability.

Results. There was no significant difference in HRV between MHO and MUO premenopausal women. In Wildman division, after adjustment for systolic blood pressure, RRNN and LF/HF were statistically different between groups ($p=0.0001$; $p=0.029$). In IDF division, adjusting for waist circumference, LF was significantly different between groups ($p=0.004$). In HOMA division, adjusting for HOMA, groups were different in SDNN ($p=0.009$), RMSSD ($p=0.002$), pNN50 ($p=0.003$), HF ($p=0.002$) and TP ($p=0.005$).

Conclusions. Autonomic nervous system does not share the leading role in premenopausal women metabolic profile. The differences in HRV between MHO and MUO women depend on the metabolic health criteria. Systolic blood pressure, HOMA and waist circumference have significant effect on HRV differences between MHO and MUO premenopausal women.

Key words: heart rate variability, metabolically healthy obesity, metabolically unhealthy obesity.

INTRODUCTION

Obesity is a known risk factor for cardiometabolic disorders. Some obese people, however, present favorable cardiometabolic profiles.

Metabolically healthy obesity (MHO) is usually defined as the absence of metabolic syndrome and/or insulin resistance and inflammation, in conjunction with obesity (1). The nature of compensatory mechanisms which preserve insulin sensitivity and prevent complications in MHO people has not been recognized yet (2). It is suggested that genetic, biochemical, hormonal and neurohumoral factors could play a significant role (3). Regardless of the cardiometabolic profile, obesity can be associated with autonomic nervous system dysfunction, in terms of elevated sympathetic nervous system activity (4). An analysis of heart rate variability (HRV) presents the best non-invasive quantitative marker of autonomic nervous system activity in different conditions. Investigations showed that decreased HRV is associated with obesity, insulin resistance and metabolic syndrome (5, 6). To our knowledge, only one study compared HRV parameters between MHO and metabolically unhealthy obese (MUO) women who were postmenopausal, and found that MHO postmenopausal women had higher HRV indices (2). In order to exclude the influences of potential changes in body fat distribution, metabolic status and sympathetic and parasympathetic reactivity during menopausal transition (7), we analyzed the differences in HRV between MHO and MUO premenopausal women. We hypothesized that MHO women compared to MUO present favorable HRV profile.

MATERIALS AND METHODS

Study population

We examined 44 obese women (body mass index, BMI ≥ 30 kg/m²) aged between 19 and 51 years (average age: 35.6 ± 8.5 years), who were patients of general medical practice in Novi Sad and in Health centre in Novi Kneževac, both in north part of Serbia. The inclusion criteria were: premenopausal

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status (presence of menstrual cycles in the last three months), non-pregnancy, non-smokers, no alcohol intake, no use of hormone replacement therapy, no use of beta blockers, free of known infections, thyroid, adrenal and inflammatory disease. Subjects gave their signed voluntary informed consent to be part of study. The research has been approved by the responsible authorities at the Faculty of Medicine in Novi Sad, Clinical Centre of Vojvodina in Novi Sad and Health Centre in Novi Kneževac, in Serbia. The study was conducted in accordance with the Declaration of Helsinki.

Body composition

Body mass was measured using a balanced beam scale to the nearest 0.1 kg. Body height was measured using Harpenden anthropometer (Holtain Ltd, Croswell, UK), to the nearest 0.1 cm. Body fat mass (FAT%) was assessed using bioimpedance analyzer Omron BF-511 (Omron Matsusaka Co, Ltd, Matsusaka, Japan). Subjects did not drink liquid 4 hours before the test and urinated 30 minutes before the test. BMI was calculated as a quotient of weight in kilograms divided by the square of body height in meters. Waist circumference was measured halfway between the lowest rib and the iliac crest using a flexible tape to the nearest 0.1 cm.

Blood pressure

Blood pressure was measured early in the morning after 5 minutes of resting, on the left arm, using Riva - Roccy sphygmomanometer.

Measurement of metabolic parameters

Total cholesterol and triglyceride levels were determined by the standard enzyme-based method, HDL-cholesterol levels were determined by the precipitation method with sodium phospho-wolframate, and LDL-cholesterol levels were calculated using the Friedwald and all formula (8). Fasting glucose levels were determined by the Dialab glucose GOD-PAP method, and serum insulin levels were determined by ELISA. The high-sensitivity C-reactive protein was measured by latex enhanced nephelometry. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: fasting glucose (mmol/L) x fasting insulin level (mIU/L)/ 22.5 (6).

Identification of MHO individuals

Metabolic profile was defined in three ways: using the International Diabetes Federation (IDF)

criteria for metabolic syndrome, using the Wildman criteria, and using HOMA alone. IDF criteria for MHO include central obesity (waist circumference ≥ 80 cm) plus less than any two of the following factors: raised triglycerides level (≥ 1.7 mmol/L), reduced HDL-cholesterol (< 1.29 mmol/L), or antilipidemic medication usage, raised blood pressure (systolic blood pressure ≥ 130 mmHg, or diastolic blood pressure ≥ 85 mmHg), or antihypertensive medication usage, raised fasting glucose level (≥ 5.6 mmol/L), or previously diagnosed diabetes mellitus (9). The Wildman's criteria for MHO were BMI ≥ 30 kg/m² and less than two cardiometabolic abnormalities among followed: blood pressure level $\geq 130/85$ mmHg or antihypertensive medication usage, elevated fasting triglyceride level (≥ 1.7 mmol/L), decreased HDL-cholesterol level (< 1.3 mmol/L), elevated fasting glucose level (≥ 5.5 mmol/L), or antidiabetic medication usage, insulin resistance (HOMA-IR > 3.62 , the 90th percentile) and systemic inflammation (CRP ≥ 9.76 mg/L, the 90th percentile) (6). By the third criterion, subjects with HOMA values in the lower tertile were categorized as MHO (2, 10).

Heart rate variability

Five-minute digital electrocardiography was recorded in a comfortable supine position (VNS-Spekt, Neurosoft, Ivanovo, Russia). All undesirable beats were excluded. Time and frequency-domain analysis was obtained (11). RRNN (ms) is calculated as a mean duration of all normal R-R intervals, SDNN is the standard deviation of R-R intervals, RMSSD is calculated as a root mean square of the successive differences of all R-R intervals, pNN50 is the number of adjacent intervals differing more than 50 ms expressed as a percentage of all the intervals in the collecting period. LF (low frequency), HF (high frequency), LF/HF and TP (total power) are determined when HRV is plotted as a frequency at which the length of the R-R intervals changes. LF norm and HF norm are normalized low and high frequency (11). SDNN, RRNN and Tp demonstrate overall HRV activity, both vagal and sympathetic influences, RMSSD, pNN50 and HF correspond to parasympathetic activity, LF presents sympatovagal interaction and LF/HF indicates sympatovagal balance (4).

Statistical analysis

Data were analyzed using the SPSS 11.5 for Windows® and reported using mean values and standard deviations. The statistical differences were evaluated using independent-samples T-test. The Pearson

correlation analyses were used to analyze the correlation between parameters. Partial correlations were adjusted for age. In order to evaluate differences between MHO and MUO women after adjustment for metabolic and anthropometric factors that were different between groups we conducted multivariate analysis. We also used variance of component estimation whose basic goal was to estimate the premenopausal obese women covariation between random factors and the dependent variable. Depending on the method used to estimate variance components, the population variances of the random factors can also be estimated, and significance tests can be performed to test whether the population covariations between the random factors and the dependent variable are nonzero. We also analyzed variables with fixed effect and random effect.

RESULTS

Based on the Wildman and IDF criteria most subjects were MHO (59.1% and 63.64%, respectively),

while using HOMA criterion 27.3% were MHO. Groups were not different in age, under any criteria of division (Table 1). According to Wildman criteria, MHO women had significantly lower BMI, waist circumference, systolic and diastolic blood pressure, level of triglycerides and significantly higher HDL cholesterol (Tables 1, 2). According to IDF criteria, MHO women had significantly lower waist circumference, systolic and diastolic blood pressure, triglycerides and HOMA index, and significantly higher HDL cholesterol level (Tables 1, 2). Under HOMA criterion, MHO women had significantly lower body weight, HOMA index and insulin level (Tables 1, 2).

There were no significant differences observed in HRV analysis indices in MHO and MUO (Table 3). Although MHO women had higher mean values of the HRV parameters (SDNN, RMSSD, pNN50, LF, LF/HF, LF norm), and lower HF parameter, in all criteria, there were no statistically significant differences comparing to MUO women.

Since women differed in anthropometric and

Table 1. Anthropometric characteristics of examined patients

	WILDMAN				IDF				HOMA			
	MHO		MUO		MHO		MUO		MHO		MUO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	35.6	8.5	35.3	9.5	36.0	8.3	34.7	9.9	32.2	8.8	36.8	8.6
Body weight (kg)	91.5	15.0	97.6	13.9	92.1	15.2	97.4	13.6	86.3*	12.64	96.9	14.5
Body height (cm)	164.7	7.15	162.5	7.8	163.9	7.53	163.6	7.48	160.4	6.67	165.1	7.38
BMI (kg/m ²)	33.5**	3.5	37.0	4.2	34.0	3.83	36.5	4.42	33.5	3.9	35.5	4.20
Waist circumference (cm)	94.4**	8.38	103.6	7.98	94.9**	8.54	103.7	8.11	93.88	9.59	99.8	8.9
Body fat (%)	45.78	3.75	46.86	4.22	45.78	3.73	46.98	4.29	46.13	3.9	46.3	4.0

*Significantly different comparing to MUO group (at the level $p < 0.05$); **Significantly different comparing to MUO group (at the level $p < 0.01$); BMI- body mass index; IDF- International Diabetes Federation; HOMA- homeostasis model assessment of insulin resistance; MHO – metabolically healthy obese; MUO – metabolically unhealthy obese; WC-waist circumference.

Table 2. Metabolic characteristics of examined patients

	WILDMAN				IDF				HOMA			
	MHO		MUO		MHO		MUO		MHO		MUO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Systolic blood pressure (mmHg)	114.9**	15.3	137.3	19.1	116.4**	15.95	137.6	19.9	120.3	10.25	125.5	22.7
Diastolic blood pressure	73.85**	11.07	89.44	14.54	75.89**	12.91	87.8	14.83	77.1	13.39	81.4	15.1
Total cholesterol (mmol/L)	5.24	1.21	5.49	1.09	5.21	1.20	5.5	1.08	5.2	1.19	5.4	1.1
Triglycerides (mmol/L)	1.18**	0.43	2.12	1.14	1.18**	0.41	2.2	1.15	1.1	0.41	1.7	1.0
HDL-cholesterol (mmol/L)	1.55*	0.33	1.35	0.28	1.57**	0.32	1.3	0.24	1.6	0.22	1.4	0.35
LDL-cholesterol (mmol/L)	3.15	1.08	3.19	0.83	3.11	1.1	3.3	0.81	3.1	1.11	3.2	0.94
Fasting plasma glucose (mmol/L)	5.08	0.55	5.29	0.73	5.10	0.54	5.29	0.767	5.01	0.57	5.23	0.65
Insulinemia (mIU/L)	9.34	3.85	11.45	4.96	9.44	4.29	11.74	4.34	5.23**	1.57	12.17	3.53
CRP (mg/L)	4.80	3.23	7.62	12.72	4.48	2.98	8.99	14.17	5.63	2.67	6.03	10.21
HOMA	2.10	0.87	2.68	1.19	2.09*	0.95	2.76	1.09	1.15**	0.34	2.78	0.84

*Significantly different comparing to MUO group (at the level $p < 0.05$); **Significantly different comparing to MUO group (at the level $p < 0.01$); CRP- C reactive protein; IDF- International Diabetes Federation; HOMA- homeostasis model assessment of insulin resistance; MHO – metabolically healthy obese; MUO – metabolically unhealthy obese.

metabolic parameters, we conducted a multivariate analysis to exclude all potential confounding factors that could lead to such results. After adjustment for BMI, waist circumference, systolic and diastolic blood pressure, levels of triglycerides in Wildman division, there was significant difference in RRNN and LF/HF between MHO and MUO (Table 4), when adjusted

for systolic blood pressure ($p < 0.001$ and $p < 0.05$, respectively).

In order to see the effect of systolic blood pressure against RRNN and LF/HF in MHO and MUO, we computed 3D surface plot diagrams (Figs 1, 2), where there are notable differences in dispersion of used parameters and more uniform connection of

Table 3. Heart rate variability characteristics of examined patients

	WILDMAN				IDF				HOMA			
	MHO		MUO		MHO		MUO		MHO		MUO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
RRNN (ms)	841.7	90.6	793.0	76.6	838.5	90.9	792.5	75.4	795.6	105.3	831.6	79.69
SDNN (ms)	48.38	18.5	45.8	26.17	49.6	19.2	43.43	25.6	49.83	16.39	46.4	23.55
RMSSD (ms)	40.62	21.6	39.1	36.4	41.7	21.6	37.0	37.7	40.67	19.07	39.7	31.24
pNN50 (%)	19.04	17.2	15.68	21.75	19.94	17.26	13.67	21.77	20.97	18.20	16.4	19.46
LF (ms ²)	808.5	676.1	736.8	609.4	848.7	682.4	657.5	568.8	917.50	443.1	727.3	703.0
HF (ms ²)	806.5	712.3	1111.9	2354.3	849.6	709.3	1074.8	2500.7	868.0	684.6	955.3	1820.0
LF/HF	1.37	0.93	1.19	0.93	1.34	0.90	1.23	0.987	1.65	1.19	1.16	0.78
TP (ms ²)	2843.5	2013.7	3010.3	3336.3	2998.3	2121.2	2760.3	3355.0	3086.3	1833.7	2846.3	2859.1
LF norm (nu)	52.8	14.5	53.7	17.3	52.3	14.04	54.7	18.23	56.14	16.1	52.1	15.4
HF norm (nu)	47.2	14.5	46.2	17.3	47.6	14.04	45.3	18.24	43.86	16.1	47.9	15.4

*Significantly different comparing to MUO group (at the level $p < 0.05$); **Significantly different comparing to MUO group (at the level $p < 0.01$); IDF- International Diabetes Federation; MHO – metabolically healthy obese; MUO – metabolically unhealthy obese; HOMA- homeostasis model assessment of insulin resistance; HRV- heart rate variability; HF- high frequencies; HF norm- normalized high frequencies; LF- low frequencies; LF norm- normalized low frequencies; LF/HF- ratio of low to high frequencies; pNN50- proportion of interval differences of successive normal-to-normal intervals greater than 50 ms; RMSSD- square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; RRNN- mean duration of all normal R-R intervals; SDNN-, the standard deviation of normal-to-normal intervals; TP-total power.

Table 4. Multivariate analysis of effects of waist circumference, body mass index, systolic and diastolic blood pressure, triglyceride level, HDL cholesterol level on differences in RRNN and LF/HF between MHO and MUO premenopausal women

	RRNN(ms)		LF/HF	
	F	p	F	p
WC (cm)	1.83	0.191	0.770	0.387
BMI (kg/m ²)	0.69	0.409	0.084	0.773
SBP (mmHg)	19.0	0.0001*	5.15	0.029*
DBP (mmHg)	0.10	0.744	2.08	0.157
TG (mmol/L)	1.57	0.216	3.34	0.075
HDL (mmol/L)	3.52	0.068	0.003	0.950

p- level of statistically significant difference in Multivariate analysis; F- comparison of the error variance /covariance matrix and the effect variance/covariance matrix; BMI- body mass index; DBP- diastolic blood pressure; HDL-high density level cholesterol; LF/HF- ratio of low to high frequencies; RRNN- mean duration of all normal R-R intervals;SBP- systolic blood pressure; TG- triglycerides; WC-waist circumference.

Table 5. Multivariate analysis of differences in LF between MHO and MUO, after adjustment for waist circumference, systolic and diastolic blood pressure, triglycerides, HDL cholesterol and HOMA index

	LF	
	F	p
WC (cm)	9.35	0.004*
SBP (mmHg)	0.067	0.796
DBP (mmHg)	1.18	0.282
TG (mmol/L)	0.952	0.335
HDL (mmol/L)	0.122	0.728
HOMA	0.083	0.774

p- level of statistically significant difference in Multivariate analysis; F- comparison of the error variance /covariance matrix and the effect variance/covariance matrix; DBP- diastolic blood pressure; HDL-high density level cholesterol; LF- low frequencies; SBP- systolic blood pressure; TG- triglycerides; WC-waist circumference; HOMA- index of insulin resistance.

Table 6. Multivariate analysis of statistical differences between SDNN, RMSSD, pNN50, HF, and TP between MHO and MUO after adjustment for body mass, insulin level and HOMA

	SDNN (ms)		RMSSD (ms)		pNN50(%)		HF (ms ²)		TP (ms ²)	
	F	p	F	p	F	p	F	p	F	p
BM(kg)	0.910	0.345	0.537	0.467	0.448	0.507	0.45	0.506	1.261	0.268
Insulin (mIU/L)	2.18	0.147	3.77	0.059	1.32	0.256	9.27	0.004*	5.94	0.019*
HOMA	7.46	0.009*	10.20	0.002*	9.54	0.003*	10.13	0.002*	8.73	0.005*

p-level of statistically significant difference; F- comparison of the error variance /covariance matrix and the effect variance/covariance matrix; BMI-body mass; HF- high frequencies; HOMA-index of insulin resistance; pNN50- proportion of interval differences of successive normal-to-normal intervals greater than 50 ms; RMSSD- square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; SDNN-, the standard deviation of normal-to-normal intervals; TP- total power.

TA syst-systolic blood pressure; MHO- metabolically healthy obese; RRNN- mean duration of all normal R-R intervals; LF/HF- ratio of low to high frequencies.

Figure 1. 3D surface plot diagram of systolic blood pressure against RRNN and LF/HF in metabolically healthy obese (MHO) premenopausal women.

TA syst-systolic blood pressure; MUO- metabolically unhealthy obese; RRNN- mean duration of all normal R-R intervals; LF/HF- ratio of low to high frequencies.

Figure 2. 3D surface plot diagram of systolic blood pressure against RRNN and LF/HF in metabolically unhealthy obese (MUO) premenopausal women.

p-level of statistically significant difference; IDF- International diabetes federation; MHO-metabolically healthy obese; MUO-metabolically unhealthy obese;

Figure 3. Mean plot diagrams of Waist circumference in MHO and MUO, by IDF criteria.

systolic blood pressure with RRNN and LF/HF in MHO, and elevated connection in MUO group.

For IDF division, after adjustment for waist circumference, systolic and diastolic blood pressure, triglycerides, HDL cholesterol and HOMA index, there was a significant difference in LF between MHO and MUO (Table 5), after adjustment for waist circumference ($p < 0.005$).

In mean plot diagrams are presented trends of waist circumference and LF between MHO and MUO (Figs 3, 4).

As for the HOMA division, after adjustment for

p-level of statistically significant difference; IDF- International diabetes federation; LF- low frequencies; MHO-metabolically healthy obese; MUO-metabolically unhealthy obese;

Figure 4. Mean plot diagrams of LF in MHO and MUO, by IDF criteria.

weight, insulin level and HOMA (Table 6), there were significant differences after adjustment for HOMA in SDNN, RMSSD, pNN50, HF, TP ($p < 0,01$) and after adjustment for insulin in HF and TP ($p < 0,01$; $p < 0,05$).

DISCUSSION

Our results, at first, did not confirm differences in autonomic nervous system activity between obese premenopausal women of different metabolic profiles. After adjustment for anthropometric and metabolic factors, differences in autonomic nervous system

between MHO and MUO premenopausal women appeared. Significant correlations between HRV parameters and age (not shown in tables) lead us to hypothesize that lower HRV is more closely related to the metabolic risk with advancing age.

Among obese people, based on the previous researches, 3.3-43% are actually MHO (2, 12-15). There are several definitions of MHO phenotypes that include presence of central obesity, hypertension, parameters of lipo - and glycoregulation and markers of systemic inflammation (12). The prevalence of MHO in Spanish population was 39.94% according to Consensus Societies for metabolic syndrome, 16.29% according to modified Wildman criteria, and 9.65% according to original Wildman criteria (12). The prevalence of MHO individuals in our study group was 59.1% (Wildman criteria), 63.64% (IDF criteria), respectively. The lowest percent of MHO subjects was registered when using HOMA index as a criterion (27.3%), which is similar to the results published by Robillard *et al.* (2) and Phillips *et al.* (16) who used the same diagnostic criterion (2).

Some research suggests that MHO people show specific phenotype differences compared with MUO people such as lower total fat mass and abdominal fat mass, and higher tendency to peripheral accumulation of adipose tissue (3, 13). Reduced overall HRV has been shown to be associated with higher body mass index (17) and total body fat (18). In our study, BMI was significantly lower in MHO, by Wildman criteria (Table 1). Waist circumference was significantly lower in metabolically healthy subjects, using Wildman and IDF criteria. Using HOMA, body weight was significantly lower in MHO women.

Our results reported no statistically significant difference in HRV indicators between MHO and MUO premenopausal women (Table 3), so we conducted the multivariate analysis to exclude influences of anthropometric and metabolic indices that were statistically significantly different between groups. We adjusted Wildman groups division for BMI, waist circumference, systolic and diastolic blood pressure, HDL cholesterol and triglycerides, which were different between MHO and MUO women. Adjustment of systolic blood pressure appeared to play an important role in differences in RRNN ($p < 0.0001$) and LF/HF ($p < 0.05$) between groups (Table 4). Lower HRV has already been found to be associated with hypertension (19). It is possible that reparatory mechanisms in obese people with normal blood pressure can still preserve better HRV profile but other metabolic factors

cause loss of differences in HRV between MHO and MUO. Prehypertensive people had lower SDNN, TP, LF, HF than normotensive ones (20). Metabolic signals that provoke prehypertensive obese people to develop hypertension in future, could lower HRV before hypertension itself manifests. In hypertensive people sympathetic influence has dominancy among parasympaticus. This could be attributed to the effects of obesity on autonomic nervous system (21). It is surely possible that hypertension and impairment of HRV profile have the same background and that they are provoked by the same factor. 3D plot diagrams of systolic blood pressure against RRNN and LF/HF (Figs 1, 2) show more uniform connections in MHO group, and obvious differences in dispersion of controlled parameters between groups.

In IDF division, waist circumference seems to be an important factor for HRV difference between MHO and MUO women. After adjustment for waist circumference, systolic and diastolic blood pressure, HDL cholesterol, triglycerides and HOMA (which were different between groups) there was a significant difference in LF ($p < 0.01$) between groups when adjusted for waist circumference (Table 4). This reveals waist circumference, and central fat mass this way, as essential factor for loss of differences in sympathetic impulses in MHO and MUO. The waist circumference was smaller in MHO, but still larger than in normal weight women. It is possible that waist circumference, among others IDF criteria of metabolic health has the strongest influence in sympathetic impulses, and that enlargement of waist circumference in MHO profile leads to impairment of HRV profile even if waist circumferences in MHO still did not reach high values as in MUO group. Mean plot (Figs 3 and 4) show that MHO phenotype has lower waist circumference but higher LF (LF still not statistically significant). Predominantly central fat distribution in MUO subjects is in line with earlier observations about the protective role of the peripheral accumulation of adiposetissue (22). Few studies indicated association between specific fat distribution and autonomic nervous system modulation (4). Questions arise about additional functional differences that could explain protective mechanisms in metabolically healthy obese individuals, including autonomic nervous system activity. Also remains unclear what are the first dysmetabolic and adiposity distribution signals that lead to impairment of MHO profile of obese women. Our results clearly confirmed decreasing of HRV parameters with aging (not shown in tables), which is in agreement with previous findings

(18, 23). This may explain inverse association between HRV and waist circumference. The decreasing trend of HRV parameters in women is pronounced by reduction of estrogen levels after menopause (24). Yang *et al.* (25) found that HRV profile in postmenopausal women becomes similar to that in men, while premenopausal women significantly differ from both postmenopausal women and men. To our knowledge, only one study compared HRV between MHO and MUO women who were postmenopausal, reporting favorable HRV profiles (significantly higher RR intervals, SDNN and LF) in MHO subjects (2).

Reduced HRV in obese individuals may be a result of chronic hyperinsulinemia that causes desensitization of sinus node to autonomic stimuli (26). In our results, groups divided by HOMA differed in body mass, HOMA and insulin (Tables 1, 2). After adjustment for these three factors, there was a statistically significant difference in SDNN ($p < 0.01$), RMSSD ($p < 0.05$), pNN50 ($p < 0.05$), HF ($p < 0.05$) and TP ($p < 0.01$) between groups, when adjusted for HOMA, and in HF ($p < 0.01$) and TP ($p < 0.05$) when adjusted for insulin level (Table 5). Changes in autonomic nervous system activity in state of hyperglycaemia and hyperinsulinaemia happen after longer exposition to hyperinsulinaemia. Insulin resistance is an etiological factor for metabolic disease. Circadian rhythm of ANS activity can rapidly change if metabolic changes happen in healthy people (27). In our findings, groups divided by HOMA did not differ in any other metabolic factor. This could be the reason for absence of differences in HRV, from the first point of view. The fact that differences appeared when controlling for HOMA, points out that HOMA should be taken into account when calculating the differences in HRV indices in the context of obese premenopausal women.

Our results of correlations between HRV and metabolic markers in obese premenopausal women (not shown in tables) are mostly in line with previous investigations in different subpopulations: RRNN, as a marker of overall HRV activity, positively correlated with HDL-cholesterol (28), and negatively with systolic and diastolic blood pressure (20), while markers of vagal activity (HF and RMSSD) negatively correlated with HOMA, which indicates an association between parasympathetic activity and insulin sensitivity (29). In contrast to the previous findings (30, 31) we did not find a significant correlation between HRV indices and inflammatory markers.

The main limitation of the present study is the small study sample. Also, we included only non-

diabetic patients. To evaluate the effects of menopausal transition, it is necessary to compare HRV differences between premenopausal and postmenopausal women considering their metabolic profiles including hormonal status. It would be interesting to analyze differences between MHO and MUO men of different ages. Until about sixth decade of life, HRV is considered to be greater among males vs. females (32).

Also there are differences in HRV between MHO and MUO. According to our results, it is difficult to determine the role of the autonomic nervous system in the determination of metabolic profile, and HRV does not necessarily need to be included in the assessment of metabolic risk among premenopausal women. The difference in metabolism and autonomic nervous system activity between MHO and MUO depends on the criterion of metabolic division. Differences in HRV are strongly influenced by metabolic and body phenotype differences, especially by systolic blood pressure, waist circumference and HOMA. Obviously, metabolic disturbances in obesity precede autonomic nervous system changes. More likely, aging contributes to both metabolic and autonomic nervous system changes. However, visceral fat, in conjunction with hypertension and insulin resistance seems to be a common denominator for autonomic and cardiometabolic perturbations that have different age-dependent dynamics. Prospective studies should confirm our assumptions and extend these findings.

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Conflict of interest

All authors disclose any commercial or similar associations that might create a conflict of interest in connection with submitted manuscript. All authors state that no competing financial interests exist.

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