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Saturated Fat Intake Is Related to Heart Rate Variability in Women with Polycystic Ovary Syndrome

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Keywords

Polycystic ovary syndrome · Heart rate variability · Autonomic modulation · Diet and foods · Saturated fatty acids · Testosterone

Abstract

Background/Aims: There is a heightened risk for cardiovascular diseases in women with polycystic ovary syndrome (PCOS). Alterations in heart rate variability (HRV) may reflect subclinical cardiovascular disease, with a putative association between HRV and dietary fat. This study evaluated HRV in PCOS and control women based on the dietary intake of saturated fatty acid (SFA). ***Methods:*** Biochemical/hormonal profile, resting metabolic rate, physical activity, HRV in response to the Stroop test, and dietary intake were assessed in 84 PCOS and 54 control women stratified by median SFA intake in the PCOS group (8.5% of daily energy intake). ***Results:*** Body mass index ($p = 0.041$), blood pressure ($p < 0.01$), and HOMA-IR ($p = 0.003$) were higher in PCOS vs. controls. PCOS women had higher testosterone ($p = 0.001$), dehydroepiandrosterone sulfate ($p = 0.012$), and free androgen index ($p = 0.001$), and lower sex hormone-binding globulin levels than controls ($p = 0.001$). In both groups, the clinical profile and calorie intake were similar between SFA categories. In PCOS, testosterone was lower when SFA intake <8.5%. PCOS women with SFA <8.5% consumed more beans, fruits, and vegetables and had better frequency and time domain HRV indices. No differences in HRV were detected between SFA categories in controls. In PCOS, age and SFA intake were independent predictors of HRV. ***Conclusions:*** Lower SFA intake is related to improved cardiovascular autonomic function in PCOS.

Introduction

Polycystic ovary syndrome (PCOS), a common endocrine disorder in women of reproductive age [1], is characterized by hyperandrogenism and chronic anovulation and is often associated with insulin resistance (IR), obesity and dyslipidemia, and consequently increased cardiovascular risk [2]. Preclinical cardiovascular alterations, such as decreased cardiac output, diastolic dysfunction, endothelial dysfunction, and elevation of inflammatory cytokines [3, 4], are more frequent in PCOS patients than in non-PCOS women of the same age. These preclinical changes occur in both lean and obese PCOS women but are probably more severe in the presence of higher adiposity [3, 4].

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In patients without established cardiovascular disease, previous studies have shown an association of IR, metabolic syndrome, and proinflammatory state [5] with heart rate variability (HRV) – oscillations in the interval between consecutive heart beats (R-R interval). HRV encompasses healthy autonomic mechanisms of adaptation [6]. Evidence indicates that decreased HRV (decreased parasympathetic modulation and/or increased sympathetic modulation) is associated with higher cardiovascular risk [7, 8] and may be a predictor of all-cause mortality [9]. In addition, studies have reported that alterations in HRV during standardized laboratory stress tests can predict increases in cardiovascular risk beyond traditional risk factors [10, 11], suggesting that HRV assessment could be a simple, non-invasive tool for cardiovascular risk screening in susceptible populations [12]. Therefore, changes in autonomic function in patients with PCOS could be regarded as an early, sub-clinical cardiovascular risk factor. In PCOS, studies have shown lower HRV when compared to controls [13–15]. Our group has also reported stress-induced alterations in heart autonomic function in women with classic PCOS phenotype in comparison to age-matched healthy controls [16].

Dietary pattern plays an important role in the primary and secondary prevention of cardiovascular disease [17], with a reduction in the intake of saturated fatty acids (SFA) being recommended [17]. An association between HRV and dietary fat has been investigated in healthy subjects [18] and overweight adults with risk for coronary disease [19], showing that consumption of fish and marine omega-3 fatty acids [18], and also of docosahexaenoic acid-rich fish oil [19], improves HRV. However, no studies have analyzed this relationship in PCOS.

Therefore, the aim of the present study was to evaluate cardiovascular autonomic function assessed by HRV in PCOS women and with lower vs. higher SFA dietary intake, and to compare these results with those obtained in a sample of non-hirsute, ovulatory control women.

Materials and Methods

Participants

The present study included participants with hirsutism and/or disturbed menstrual cycles recruited by advertisement in the media between 2009 and 2015. Eighty-four women met the inclusion criterion of the presence of PCOS according to the Rotterdam criteria [20] and body mass index (BMI) <40 kg/m^2. Fifty-nine patients were classified as classic PCOS (biochemical and/or clinical hyperandrogenism, oligo/amenorrheic or anovulatory cycles, with or without polycystic ovary [PCO] appearance at ultrasound) and 25 as ovulatory PCOS (biochemical and/or clinical hyperandrogenism, regular/ovulatory cycles [luteal-phase progesterone >3.8 ng/mL], and PCO) [20].

Fifty-four women were also recruited for the control group through public advertisement. These women did not have PCO or evidence of clinical/biochemical hyperandrogenism; they presented regular and ovulatory menstrual cycles (luteal-phase progesterone >3.8 ng/mL).

No PCOS or control participants had received any drugs known to interfere with hormonal levels for ≥3 months before the study. Exclusion criteria were diabetes mellitus, pregnancy, smoking, BMI >40 kg/m^2, blood pressure >160/100 mm Hg, and use of antihypertensive medications. The study protocol was approved by the Institutional Review Board. Written informed consent was obtained from all subjects.

Study Protocol

Anthropometric measurements included body weight, height, and waist circumference. BMI was calculated by dividing weight in kilograms by square of height in meters.

Hirsutism was defined as a modified Ferriman-Gallwey score ≥8 [20]. All participants underwent transvaginal or transabdominal pelvic ultrasound. Hormonal and metabolic assessments were made between the 2nd and 10th days of the menstrual cycle or on any day if the patient was amenorrheic. Samples were obtained between 8 and 10 a.m. Blood samples were drawn after an overnight 12-hour fast for determination of plasma cholesterol (TC), high-density lipoprotein cholesterol (HDLc), and triglyceride (TG) levels. Glucose was measured before and 2 h after the ingestion of a 75-g oral glucose load.

Blood samples were also drawn for measurements of insulin, sex hormone-binding globulin (SHBG), androstenedione, dehydroepiandrosterone sulfate (DHEAS), and total testosterone (TT). IR was estimated by homeostasis model assessment (HOMA-IR). HOMA-IR index was calculated by multiplying insulin (mIU/mL) by glucose (mmol/L) and dividing the product by 22.5. Free androgen index was estimated by dividing TT (nmol/L) by SHBG (nmol/L) and multiplying by 100.

Biochemical and Hormonal Assays

TC, HDLc, TG, and glucose levels were determined by colorimetric-enzymatic methods (Advia 1800 Siemens). LDLc was determined indirectly with the formula LDLc = TC-HDLc-TG/5. Plasma insulin levels were measured by electrochemiluminescence (Centaur XP Siemens); TT levels were measured by chemiluminescence (Centaur XP Siemens). SHBG, androstenedione and DHEAS were measured by chemiluminescence (Immulite 2000 Siemens).

Assessment of Habitual Physical Activity and Resting Metabolic Rate

Habitual physical activity was estimated with the use of a digital pedometer (BP148 Techline), as previously reported [21]. The participants were instructed not to modify their usual physical activity during the study.

Resting metabolic rate was obtained by indirect calorimetry (Fitmate®, Cosmed, Rome, Italy). Patients were evaluated in the morning, after a fast of ≥5 h, in a quiet, low-light, temperature-controlled environment. Patients were instructed to avoid exercise and consumption of caffeine and alcohol the day before and on the day of the test [21].

Assessment of Food Consumption

Dietary intake was assessed in order to quantify the consumption of SFA, macronutrients, and energy, as well as to evaluate whether high/low SFA intake was related to an overall healthy dietary pattern, according to the intake of macronutrients and food groups.

A food frequency questionnaire consisting of 121 items was used to assess dietary intake during the preceding month. This questionnaire has been validated in the adult population of the city of Porto Alegre, Rio Grande do Sul [22], where the present study was performed. Questionnaires were applied by 2 experienced dieticians (S.G. and F.M.), and there were no missing entries. Nutritional composition was calculated using the Brazilian Table of Food Composition [23]. There were no implausible energy intake assessments for any of the participants.

Food group portions were calculated according to the Feeding Guide for the Brazilian Population [24], including fruits, vegetables, beans, whole grain, refined grain, processed meats, meat and eggs, sweets and desserts, and dairy foods, as previously reported [25]. Glycemic index and glycemic load were calculated as previously described [21].

Heart Rate Variability

Participants were submitted to a 30-min electrocardiogram recording (ECG) with a SEER Light digital recorder (GE Medical Systems Information Technologie, Milwaukee, WI, USA) for HRV analysis. ECG data were analyzed with a MARS 8000 analyzer (GE Medical Systems Information Technologies) by an experienced investigator (R.M.).

ECG recording was performed in the morning, after a minimum 2-hour fast. Participants were instructed not to consume alcoholic beverages, caffeine, or other products containing stimulants, and not to perform heavy exercise for 24 h before the test.

The participants rested quietly in the supine position, in a silent, semi-dark room for 20 min. After resting, they underwent a Stroop color-word conflict test during 10 min. HRV was evaluated during the last 5 min of the rest and Stroop test periods. In the color-word test, subjects are shown the printed names of colors on screens of a different color (e.g., the word "blue" on a red screen), and are asked to name the color of the screen rather than the word [16].

The following time and frequency domain HRV indices were calculated using 5-min segments [12]: mean of all normal R-R intervals (mean R-R), root mean square of successive differences of normal adjacent R-R intervals (rMSSD), percentage of successive differences between normal adjacent R-R intervals exceeding 50 ms (pNN50), low frequency (LF) component (0.04–0.15 Hz), high frequency (HF) component (0.15–0.5 Hz), and LF-to-HF ratio. Spectral components were expressed in normalized units. The difference between HRV results obtained during stress and rest (delta) was calculated for each variable.

Statistical Analysis

Recommended SFA intake is below 10% of energy intake and less than 7% of total energy for high-risk groups [26–28]. However, because in a previous study [21], we found that most PCOS and control women from the same region had SFA intake within the range of 7–10% of the total energy value/day, and only a few of them were in extreme ranges (SFA intake <7 and >10%), we chose to stratify the groups according to the median SFA intake of PCOS women, which was 8.5%.

Estimation of sample size was based on the study by Yildirir et al. [14], considering a power of 80 and alpha of 5%. To detect a difference of 0.6 in LF/HF between the 2 categories of median SFA dietary intake in PCOS, 28 women would be required in each category.

Results are presented as mean ± SD or median and interquartile range. Comparisons between 2 means were analyzed by Student *t* test; comparisons between median values were analyzed with the Mann-Whitney U test. A multiple linear regression analysis was performed to detect independent effects of age, BMI, SFA intake, and testosterone on HRV. For this analysis, delta LF-to-HF ratio, considered the most robust parameter in the frequency domain, was used as the dependent variable. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 18). Data from FFQ were entered in duplicate in Epidata software, version 3.1 (Epidata Association) and subsequently transported to SPSS for analysis. Data were considered to be significant at $p < 0.05$.

Results

Table 1 presents clinical, hormonal, and metabolic characteristics of PCOS and control women. Women with PCOS were younger and presented higher BMI, blood pressure, and HOMA-IR, and lower HDLc than the control group. The PCOS group also had higher testosterone, DHEAS, and free androgen index, and lower SHBG levels than control women. Among all participants, 91.3% were white. The remaining subjects were of mixed African and European ancestry. The prevalence of metabolic syndrome was higher in the PCOS group in comparison with the control group (25.6 vs. 4.4%, $p = 0.003$).

Median SFA intake among PCOS women was 8.5% of daily energy intake. Table 2 summarizes clinical, anthropometric, hormonal, and metabolic features of patients with PCOS and controls, stratified by median SFA intake (< or ≥8.5%). In the PCOS group, only TT was significantly higher in the subgroup with SFA ≥8.5%. The prevalence of classic PCOS (71.4 *vs.* 69.0%; $p = 0.811$) and metabolic syndrome (25.0 vs. 24.4%; $p = 0.790$) was similar in participants with SFA < or ≥8.5%. In the control group, no differences in anthropometric, metabolic, and hormonal variables were observed between participants with SFA < or ≥8.5%.

Table 3 shows dietary intake stratified by SFA categories in both PCOS and control groups. Protein intake was similar between SFA categories in PCOS and slightly higher in control women with SFA ≥8.5%. Calorie intake was similar between the SFA categories both groups. Also, in both groups, the percentage of total fat was slightly higher in women with SFA ≥8.5%; consequently, the percentage of carbohydrates was lower in this SFA category. However, consumption (servings per day) of whole and refined grains, sweets, and sweet beverages was similar in women with SFA < or ≥8.5% from both PCOS and control groups. Glycemic index and glycemic load were also similar between SFA categories in PCOS and control groups. Women with PCOS and controls with SFA <8.5% consumed less red meat, and PCOS women with SFA <8.5% consumed more beans, fruits, and vegetables. The groups had similar intake of other food groups.

A multiple linear regression analysis of the independent effect of age, BMI, SFA intake, and testosterone on HRV in PCOS patients and control women is presented in Table 4. Age and SFA intake were independent predictors of HRV in PCOS patients only, explaining 22% of variance in HRV.

Table 1. Clinical, anthropometric, hormonal, and metabolic features of PCOS and control women

Variables	PCOS (n = 84)	Controls (n = 54)	p value
Age, years	23.5±6.3	26.2±6.5	0.014
BMI, kg/m^2	29.4±6.4	27.2±5.8	0.041
Waist circumference, cm	86.6±14.1	83.6±12.3	0.201
Resting metabolic rate, kcal/day	1,463±248	1,468±253	0.810
Steps/day	5,821 (3,821–7,664)	6,002 (4,375–7,427)	0.957
Glucose 0', mg/dL	87.4±8.4	86.8±7.9	0.673
Glucose 120', mg/dL	103.6±31.5	97±20.9	0.201
Total cholesterol, mg/dL	173.7±34.3	174.0±30.4	0.961
HDL-cholesterol, mg/dL	45.8±12.2	50.6±11.0	0.024
Triglycerides, mg/dL	88.0 (61.0–135.5)	77.5 (53.5–102.8)	0.059
LDL-cholesterol, mg/dL	106.5±27.4	105.4±24.3	0.814
HOMA-IR	3.4 (1.8–4.7)	2.1 (1.5–2.8)	0.003
Total testosterone, ng/mL	0.67±0.25	0.51±0.18	0.001
SHBG, nmol/L	28.1 (19.2–40.1)	39.1 (31.1–61.6)	0.001
Free androgen index	7.9 (5.3–15.0)	4.1 (2.7–6.5)	0.001
Androstenedione, ng/mL	2.5 (2.0–3.5)	2.1 (1.6–3.3)	0.168
DHEAS, μg/dL	219.0 (126.5–308.8)	170.5 (117.0–210.0)	0.012
Classic PCOS diagnosis, % (n)	70.2 (59)	–	–
Metabolic syndrome diagnosis, % (n)	25.6 (20)	4.4 (2)	0.003
White, % (n)	92.9 (78)	88.9 (48)	0.538
SBP, mm Hg	118.2±13.0	112.4±11.1	0.008
DBP, mm Hg	77.4±9.9	72.8±10.0	0.010

Values are expressed as mean ± SD (Student t test), median (interquartile range) (Mann-Whitney test), or percentage (n) (chi-square test).

PCOS, polycystic ovary syndrome; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Heart rate variability indices in response to the Stroop test, stratified by SFA intake, are shown in Figures 1 and 2 for PCOS and control groups respectively. Stroop stress promoted a significant change in time and frequency domain indices in both groups and SFA categories, indicating the induction of vagal withdrawal and sympathetic stimulation as expected (data not shown).

Concerning frequency-domain HRV indices, PCOS participants with lower SFA intake had higher delta LF-to-HF ratio (Fig. 1a), delta LFnu (Fig. 1b), and lower delta HFnu (Fig. 1c) than participants with higher SFA intake. The two SFA categories had similar LF/HF ratio, LFnu, and HFnu at rest, whereas during Stroop test, the LF/HF ratio and LFnu values were significantly higher (3.94 [2.60–6.21] vs. 2.66 [1.52–3.56], $p = 0.012$; 0.75 ± 0.12 vs. 0.67 ± 0.14, $p = 0.011$) and HFnu values were significantly lower (0.21 ± 0.11 vs. 0.29 ± 0.12, $p = 0.016$) in the group with SFA <8.5%.

PCOS participants with lower SFA intake had lower mean R-R intervals (Fig. 1d), pNN50 (Fig. 1e), and rMSSD (Fig. 1f) values during the stress test when compared with the group with higher SFA intake. The groups had similar R-R intervals, pNN50, and rMSSD at rest.

No changes were found in any of the heart rate variability indices in response to the Stroop test stratified by SFA intake in the control group (Fig. 2).

Discussion

In this study, while control women in both SFA categories had similar performance in HRV assessment, PCOS women with lower SFA intake achieved better results in HRV testing. In addition, SFA consumption was an independent predictor of HRV only in the PCOS group. To the best of our knowledge, this is the first study evaluating HRV indices and dietary pattern, specifically macronutrients and food group intake, in PCOS women compared to control women.

Indeed, while time and frequency domain HRV indices reflect mainly vagal activity during rest [29], under controlled sympathetic stimulation, a pronounced reduction in time domain indices and HF component is related to vagal withdrawal, and increases in LF component and LF-to-HF ratio are associated with sympathetic modulation [30]. Thus, the evaluation of HRV derived from ECG recordings during rest and after the Stroop stress test enabled us to assess both parasympathetic and sympathetic

Table 2. Clinical, anthropometric, hormonal, and metabolic features of PCOS and control women, stratified by saturated fatty acids intake

Variables	PCOS		*p*	Controls		*p*
	SFA <8.5% (*n* = 42)	SFA ≥8.5% (*n* = 42)		SFA <8.5% (*n* = 33)	SFA ≥8.5% (*n* = 21)	
BMI, kg/m^2	29.8±6.1	29.1±6.8	0.615	27.8±6.4	26.3±4.6	0.370
Waist circumference, cm	87.6±14.2	85.7±14.1	0.537	84.6±13.6	82.1±10.1	0.488
Resting metabolic rate, kcal/day	1,483 (1,283–1,675)	1,424 (1,250–1,536)	0.134	1,473 (1,368–1,672)	1,401 (1,229–1,557)	0.183
Steps/day	5,959 (3,982–7,964)	5,579 (3,699–7,548)	0.571	5,634 (3,398–7,428)	6,497 (5,091–8,133)	0.137
Glucose 0', mg/dL	89.1±9.8	85.8±6.5	0.081	87.3±9.1	85.9±5.6	0.547
Glucose 120', mg/dL	109.0±36.9	98.4±24.4	0.134	96.3±22.5	98.0±18.9	0.792
Total cholesterol, mg/dL	177.3±36.3	170.2±32.3	0.353	170.6±32.6	179.3±26.7	0.326
HDL-cholesterol, mg/dL	44.0±10.1	47.5±13.8	0.193	51.0±11.1	50.0±11.1	0.743
Triglycerides, mg/dL	104 (62–140)	84 (60–133)	0.183	67.5 (51.8–94.5)	94.0 (70.3–115.5)	0.050
LDL-cholesterol, mg/dL	109.8±29.6	103.4±25.0	0.297	102.8±24.7	109.3±23.8	0.360
HOMA-IR	3.6 (2.0–4.6)	2.8 (1.4–5.0)	0.453	2.1 (1.4–2.6)	2.4 (1.5–3.0)	0.418
Total testosterone, ng/mL	0.61±0.22	0.73±0.27	0.031	0.54±0.19	0.49±0.17	0.339
SHBG, nmol/L	28.4 (18.2–41.7)	28.1 (19.5–38.1)	0.899	42.3 (30.0–63.8)	35.1 (31.9–43.1)	0.251
Free androgen index	7.1 (5.1–11.0)	7.9 (5.8–15.9)	0.395	4.2 (2.5–7.0)	4.1 (3.5–6.3)	0.924
Androstenedione, ng/mL	2.5 (1.9–3.2)	2.7 (2.0–3.9)	0.228	2.6 (1.6–3.5)	2.0 (1.6–3.1)	0.502
DHEAS, µg/dL	215 (117–306)	225 (162–317)	0.620	171.0 (109.5–221.5)	164.0 (117.0–210.0)	0.992
Classic PCOS diagnosis, % (*n*)	71.4 (30)	69.0 (29)	0.811	–	–	–
Metabolic syndrome diagnosis, % (*n*)	25.0 (10)	24.4 (10)	0.790	6.9 (2)	0 (0)	0.531
White, % (*n*)	92.9 (39)	92.9 (39)	1.000	84.8 (28)	95.2 (20)	0.386

Values are expressed as mean ± SD (Student *t* test) or median (interquartile range) (Mann-Whitney test) or percentage (*n*) (Chi-square test).

PCOS, polycystic ovary syndrome; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DHEAS, dehydroepiandrosterone sulfate; HOMA-IR, homeostasis model assessment of insulin resistance; SHBG, sex hormone-binding globulin; SFA, saturated fatty acids intake. 8.5% is the median SFA value of PCOS sample.

modulation in young women with PCOS and in control non-hirsute, ovulatory women.

While no changes were found in HRV indices in control women with SFA intake < or ≥8.5%, we observed that PCOS women with higher SFA consumption had a worse sympathetic response to the Stroop test, as shown by a significantly lower increase in LFnu and LF-to-HF ratio when compared with the group with lower SFA intake. Furthermore, in response to the Stroop test, the group with higher consumption of SFA showed less reduction in predominantly vagal HRV indices, such as HFnu, mean R-R, pNN50, and rMSSD. Differences in HRV between the SFA categories were detected in response to the Stroop test but not during rest – perhaps because the PCOS women in our sample were young, without alterations in autonomic modulation during rest conditions. Meanwhile, during the stress test, which demanded fast and effective autonomic modulation, an impaired response was produced by the PCOS group – but not in controls – with higher SFA intake. This autonomic imbalance in PCOS women denotes a failure to adapt to stress that may predispose to the development of a sustained perturbation of sympathovagal balance over time, possibly with higher risk of hypertension and IR.

In this study, although PCOS women in both SFA categories were similar in relation to hormonal and metabolic profile, women with higher SFA intake had higher testosterone levels, as described previously [31–33]. In fact, while weight loss by hypocaloric diets has been linked to decrease in androgen levels in PCOS women [34–36], no such associations have been established with SFA content.

Interestingly, increasing evidence suggests a regulation of androgens by dietary fat [37]. A controlled, randomized, crossover trial has shown an increase in adrenal androgen precursors DHEA, DHEAS, androstenedione, and in androgens testosterone and 5α-dihydrotestosterone during lipid infusion in healthy young women [37]; this effect appears to be independent of subsequent changes in insulin sensitivity.

Conversely, it is possible that higher testosterone levels influence the relationship between higher saturated fat intake and lower HRV. Some authors have indicated an association between testosterone and HRV [16, 38]. A

Table 3. Dietary intake of PCOS and control women, stratified by saturated fatty acids intake

Variables	PCOS		*p* value	Controls		*p* value
	SFA <8.5% (*n* = 42)	SFA ≥8.5% (*n* = 42)		SFA <8.5% (*n* = 33)	SFA ≥8.5% (*n* = 21)	
Macronutrients and energy						
Carbohydrate, %	58.4±6.1	52.0±7.8	0.000	59.0±6.1	49.9±5.4	<0.001
Protein, %	16.2±3.0	17.2±5.0	0.270	15.8±3.5	18.3±3.8	0.017
Fat, %	24.4±4.0	29.9±4.7	0.000	23.7±4.0	31.2±4.3	<0.001
Energy intake, kcal/day	2,295 (1,522–2,981)	2,232 (1,674–3,492)	0.610	2,056 (1,582–2,489)	1,951 (1,624–2,773)	0.756
Polyunsaturated fatty acids, %	3.3±1.1	3.8±1.3	0.066	3.5±1.1	3.7±0.9	0.579
Saturated fatty acids, %	7.0±1.2	10.2±2.2	0.000	6.7±1.4	11.2±2.5	<0.001
Fiber, g/day	26.7 (17.8–39.6)	24.7 (13.9–28.4)	0.071	22.6 (18.1–37.5)	20.9 (18.0–25.6)	0.192
Glycemic index	57.0±5.0	58.5±5.7	0.209	57.3±6.1	56.4±3.9	0.557
Glycemic load	197.5 (121.1–253.8)	191.3 (117.4–250.5)	0.886	160.2 (135.3–202.1)	134.5 (99.0–174.9)	0.090
Food groups						
Fruits and vegetables, servings/day	6.0 (2.6–9.3)	4.4 (1.7–6.7)	0.041	5.0 (3.2–9.7)	3.9 (2.3–6.7)	0.108
Beans, servings/day	1.4 (0.6–2.8)	0.6 (0.3–1.7)	0.017	1.4 (0.6–2.1)	0.9 (0.6–1.4)	0.099
Whole grains, servings/day	0.7 (0.2–1.3)	0.5 (0.2–0.9)	0.295	0.5 (0.3–1.1)	0.6 (0.3–1.2)	0.619
Refined grains, servings/day	3.4 (2.0–5.0)	3.4 (1.5–5.0)	0.635	2.6 (1.9–4.6)	2.1 (1.6–3.5)	0.231
Fried and fast food, servings/day	1.0 (0.6–1.9)	1.2 (0.5–2.6)	0.700	0.9 (0.6–1.5)	1.2 (0.6–1.6)	0.729
Processed meat, servings/day	0.2 (0.0–0.4)	0.3 (0.1–0.5)	0.074	0.2 (0.1–0.3)	0.3 (0.2–0.6)	0.031
Sweets, servings/day	1.8 (1.1–3.2)	2.8 (1.4–5.1)	0.054	1.9 (1.3–2.8)	2.1 (1.5–5.4)	0.112
Sweet beverages, servings/day	0.8 (0.3–2.3)	1.0 (0.3–2.9)	0.687	0.8 (0.1–1.6)	0.6 (0.3–1.2)	0.852
Total meat and eggs, servings/day	1.3 (0.9–2.0)	1.9 (0.9–2.8)	0.081	1.1 (0.7–1.6)	1.6 (1.4–2.4)	0.001
Red meat, servings/day	0.9 (0.5–1.5)	1.4 (0.6–2.2)	0.047	0.7 (0.5–1.2)	1.2 (0.9–1.8)	<0.001
White meat, servings/day	0.4 (0.1–0.6)	0.3 (0.1–0.6)	0.604	0.2 (0.1–0.4)	0.3 (0.1–0.5)	0.315
Dairy/dairy products, servings/day	1.1 (0.6–2.3)	1.1 (0.5–2.1)	0.658	1.3 (0.8–2.2)	1.2 (0.6–1.8)	0.601
Alcoholic beverage, drinks/day	0.0 (0.0–0.2)	0.0 (0.0–0.2)	0.764	0.5 (0.0–4.4)	0.2 (0.0–2.0)	0.598

Values are expressed as mean ± SD (Student *t* test) or median (interquartile range) (Mann-Whitney test).
PCOS, polycystic ovary syndrome; SFA, saturated fatty acids intake; 8.5% is the median SFA value of PCOS sample.

Table 4. Multiple linear regression analysis for independent effect of age, BMI, saturated fatty acid intake, and testosterone on HRV in PCOS patients and control women

	PCOS		Controls	
Delta LF/HF vs.	coefficient (B) ± SE	*p* value	coefficient (B) ± SE	*p* value
Age, years	0.019±0.009	0.040	0.009±0.016	0.577
Body mass index, kg/m²	0.001±0.010	0.895	0.008±0.025	0.766
Saturated fatty acids intake, %	–0.062±0.022	0.008	0.032±0.033	0.346
Testosterone, ng/mL	0.160±0.227	0.483	–0.812±0.985	0.419

In PCOS patients: *p* value of regression = 0.009 and model $r^2 = 0.220$. In control patients: *p* value of regression = 0.850 and model $r^2 = 0.060$.

previous study by our group [16] had already demonstrated a negative correlation between testosterone levels and frequency domain HRV indices during stress in PCOS women. Similarly, Neufeld et al. [38] demonstrated that, in premenopausal women, testosterone was correlated with HRV (SDNN, PNN50, RMSSD, and power of HF band). In fact, in the present study, PCOS women with higher SFA intake also had higher testosterone levels, and only SFA intake was an independent predictor of HRV in the multiple linear regression analysis. Therefore,

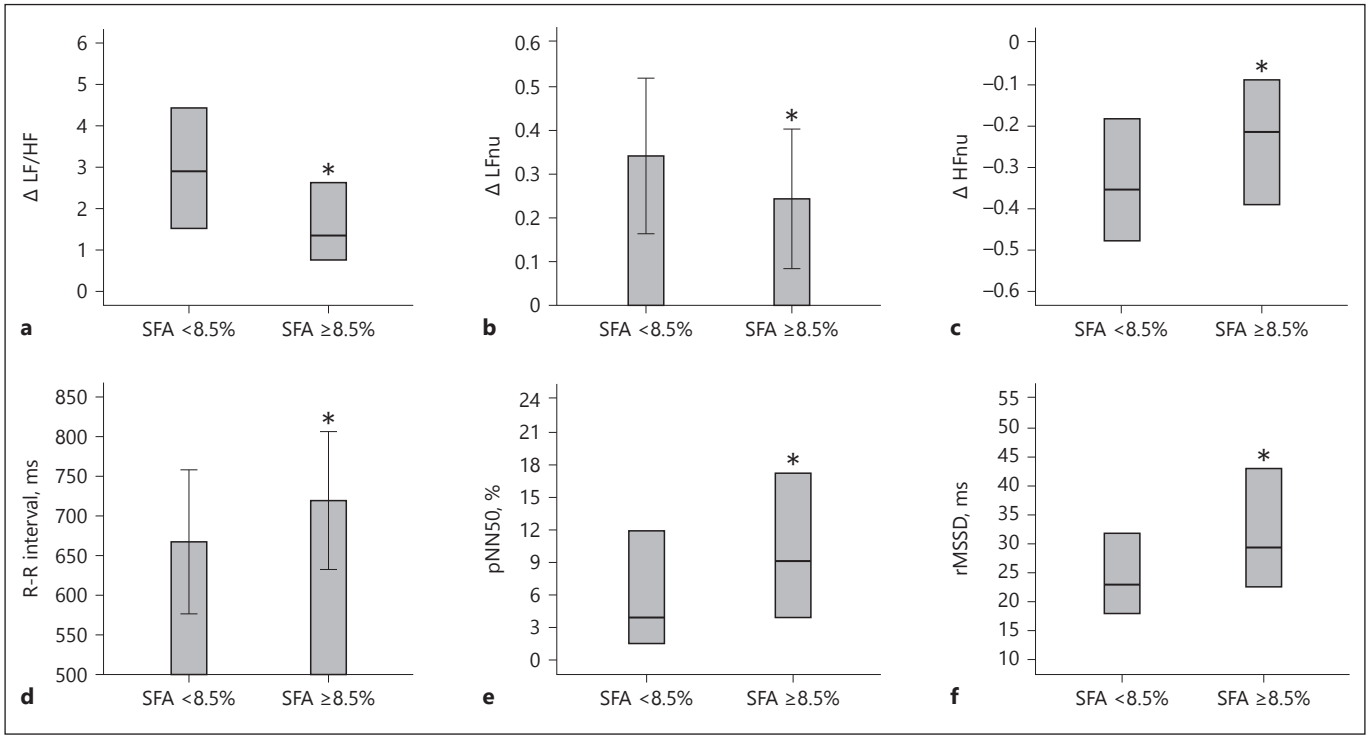

Fig. 1. Heart rate variability indices in response to Stroop test in PCOS, stratified by median saturated fatty acid intake. **a** Delta (Δ) of low-frequency to high-frequency (LF/HF) ratio. **b** Delta of low-frequency component (LF) in normalized units (nu). **c** Delta of high-frequency component (HF) in normalized units. **d** Normal R-R intervals during the stress test. **e** Percent differences between normal adjacent R-R intervals exceeding 50 ms (pNN50) during the stress test. **f** Root mean square of successive differences of normal adjacent R-R intervals (rMSSD) during the stress test. Delta is the difference between the results obtained in heart rate variability for each variable during Stroop test – value obtained at rest. * $p < 0.05$ by Mann-Whitney test. Values are expressed as median and 25–75% interquartile range.

further randomized dietary trials are needed in order to explore the effect of dietary SFA content on androgen levels and autonomic function markers in PCOS.

Because a perfect comparison between the SFA categories regarding all macro and micronutrients in the usual diet was not possible, the pattern of food group intake was considered. The PCOS group with lower SFA intake consumed less red meat and more beans, fruits, and vegetables. In addition, this group had a slightly lower intake of total fat and slightly higher intake of carbohydrates. Women from the control group consumed almost the same diet pattern, with mild differences only in fruit and vegetable intake.

The percent intake of total dietary fat and carbohydrates in both groups and SFA categories met the Dietary Reference Intakes – 20–35 and 45–65% of total energy intake respectively [39]. Regarding the consumption of beans, fruits, and vegetables, only the lower SFA intake category of PCOS and control groups met the Brazilian recommendations (one serving of beans, thee servings of fruits, and 3 servings of vegetables per day) [24]. While the link between dietary components and risk of cardiovascular diseases is still not fully understood, vegetables, fruits, and beans are sources of key nutrients, such as fiber, vitamins, minerals, and antioxidants, which have been associated with lower risk of cardiovascular disease [40]. In this sense, the present results raise the question of whether the association between dietary SFA intake and HRV in PCOS might be linked to a putative direct mechanism or else related to an overall healthier pattern of macronutrient and food group intake.

In the present study, the total meat and egg intake in the category of higher SFA intake was higher than the daily recommended allowance in both PCOS and control groups (one serving of meat, preferably chicken and low-fat meats, fish, and eggs) [24]. De Oliveira Otto et al. [41] suggested that associations of saturated fat with health might depend on food-specific fatty acids or other nutrient constituents in foods that contain this fat. In their study with a large multiethnic cohort, the only in-

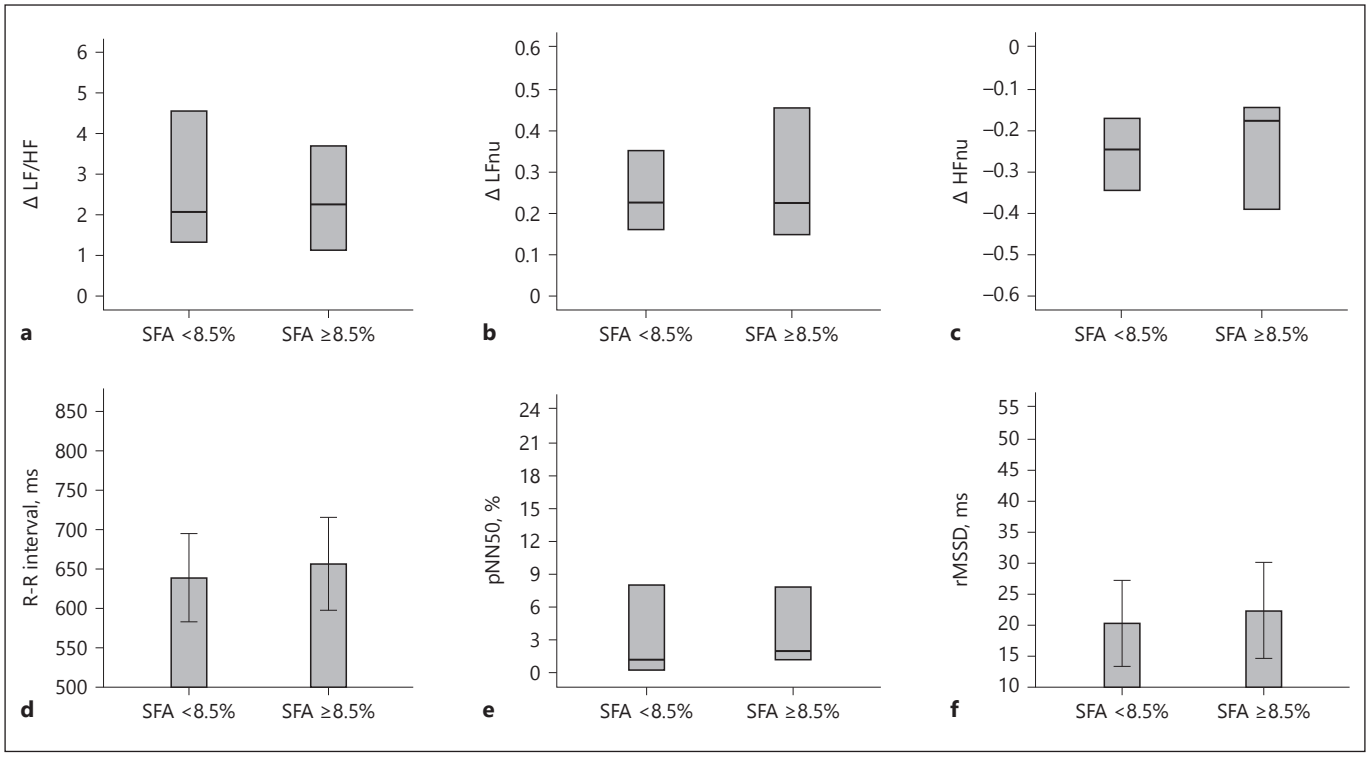

Fig. 2. Heart rate variability indices in response to Stroop test in the control group, stratified by median saturated fatty acid intake. **a** Delta (Δ) of low-frequency to high-frequency (LF/HF) ratio. **b** Delta of low-frequency component (LF) in normalized units (nu). **c** Delta of high-frequency component (HF) in normalized units. **d** Normal R-R intervals during the stress test. **e** Percent differences between normal adjacent R-R intervals exceeding 50 ms (pNN50) during the stress test. **f** Root mean square of successive differences of normal adjacent R-R intervals (rMSSD) during the stress test. Delta is the difference between the results obtained in heart rate variability for each variable during Stroop test – value obtained at rest. Values are expressed as median and 25–75% interquartile range.

take of saturated fat from meat was associated with higher CVD risk [41]. Siri-Tarino et al. [42] also suggest that the choice of macronutrient to replace fat is of crucial importance, and that saturated fat should ideally be replaced with polyunsaturated fat and minimally processed grains. Evidence from a meta-analysis suggests that high intake of red meat may be related to increased risk of cardiovascular mortality [43]. A possible mechanism for the negative effect of red meat consumption has been described as the partial degradation by intestinal bacteria of phosphatidylcholine [44] and carnitine [45], generating potentially atherogenic trimethylamine-N-oxide.

Strengths of the present study include the absence of earlier analyses of dietary intake and HRV in PCOS women, a comparison control group, and the use of a robust validated FFQ that assesses 121 items of food consumption during the preceding month. Limitations are the relatively small sample size, which could not be stratified according to PCOS phenotype, and the cross-sectional nature of the study, which does not allow causal inferences. Also, the sample size of the control group might not have been sufficient for the analysis of an eventual association between dietary SFA intake and HRV in this specific group, constituted by young and healthy women. Another limitation is that androgen levels were measured by chemiluminescence assay with quality control standards. However, while liquid chromatography mass spectrometry is regarded as the gold standard for quantifying serum testosterone levels in women, the accuracy and low cost of well-chosen automated assays still warrant their use to measure TT in hyperandrogenic women.

In conclusion, the present results indicate that lower consumption of saturated fat is related to a better autonomic response to the Stroop test in women with PCOS. Additionally, low dietary SFA content was linked to a healthier diet including lower intake of meat and higher intake of fruits, vegetables, and beans.

Further studies on the effects of dietary composition and SFA content on autonomic function in different PCOS phenotypes and distinct ethnicities are needed in order to enhance the understanding of these associations and confirm the generalizability of the present results.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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