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<td>• Combined training improves heart rate variability in postmenopausal women, • Isoflavone supplementation did not promote additional effects on heart rate variability in postmenopausal women</td>
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Isoflavone did not promote additional effects on heart rate variability of post-menopausal women submitted to combined exercise training: A clinical, controlled, randomized, double-blind study

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Abstract

The aim of the study is to investigate the effects of ingesting isoflavones associated with combined aerobic and resistance exercise training on heart rate variability (HRV) indices in postmenopausal women. Twenty-eight healthy postmenopausal women performed 10 weeks of combined exercise training associated with isoflavone ($n = 16$) or placebo ($n = 12$) supplementation. The RR intervals were collected for 20 min using a heart rate monitor. Analysis of HRV was performed in time (RMSSD, SDNN, and pNN50), frequency (LF%, HF%, and LF/HF), and nonlinear domains (SD1, SD2, and SD2/SD1). Student’s $t$-test did not show differences between groups in any general baseline characteristic variables. The results of the generalized estimating equation tests did not demonstrate interaction or group effects for any HRV indices. However, the results reported time effects for mean RR ($p < 0.001$), RMSSD ($p = 0.044$), and SD1 ($p = 0.044$), with increases in these indices in response to exercise training. There were no time effects for LF%, HF%, LF/HF, SDNN, pNN50, SD2, or SD2/SD1. In conclusion, isoflavone supplementation did not promote additional effects on HRV indices of postmenopausal women subjected to 10 weeks of combined exercise training.

Novelty bullets:

- Combined training improves heart rate variability in postmenopausal women
- Isoflavone supplementation did not promote additional effects on heart rate variability in postmenopausal women

Key words: Exercise; Autonomic; Supplementation; Climacteric; Isoflavones; Aerobic; Resistance; Combined; Menopause; Heart rate variability.
Introduction

The inclusion of aerobic and resistance exercises in training programs has been recommended to maintain and improve the health and function of the cardiovascular system and skeletal muscles of young and older adults (American College of Sports Medicine 2009; Garber et al. 2011). In postmenopausal women, combined exercise training (CET – aerobic and resistance exercises in the same session) may promote additional effects, attenuating climacteric symptoms, systemic inflammation markers, and oxidative stress, and improving bone health (Mendoza et al. 2016; Giolo et al. 2018). Furthermore, training programs that contain aerobic exercises may improve the heart rate variability (HRV) – i.e., a validated measure for evaluating cardiac autonomic modulation (Task Force 1996; Sandercock et al. 2005) – in postmenopausal women (Jurca et al. 2004; Sandercock et al. 2005). This improvement in HRV is an important effect as the reduced level of estrogen reported post menopause may reduce cardiac modulation by the autonomic nervous system (Brockbank et al. 2000; Mercuro et al. 2000; Neves et al. 2007), which is associated with an increased risk of arrhythmia and sudden cardiac death (Task Force 1996; Mercuro et al. 2000).

Although the effect of therapy with female sex hormones on HRV remains controversial (Fernandes et al. 2005, Kiselev et al. 2018), there is evidence reporting the role of estrogen in the modulation of the autonomic nervous system (Mercuro et al. 1999, 2000; Saleh and Connell 2007). Indirect and direct mechanisms may be involved in this modulation (Mercuro et al. 2000; Saleh and Connell 2007; Lee et al. 2011). Postmenopausal symptoms such as hot flashes and sleep problems, for example, are associated with altered autonomic control of the heart rate (Lee et al. 2011). Previous studies (Thurston et al. 2010; de Zambotti et al. 2013) showed significant decreases in cardiac vagal control during hot flashes in late perimenopausal and postmenopausal
women. Furthermore, postmenopausal women exhibited higher basal levels of noradrenaline than premenopausal women (Mercuro et al. 1999). As a direct mechanism, estrogen may act within central nuclei to modulate autonomic function (Saleh and Connell 2007), showing a central mediated action of estrogen. In this way, isoflavone has been used as an alternative treatment aiming to reduce postmenopausal symptoms (Glazier and Bowman 2001; Carbonel et al. 2018). Isoflavone is a phytoestrogen that exhibits a similar chemical structure to estrogen, presenting high affinity to estrogen receptors (Carbonel et al. 2018). This leads us to suggest that isoflavone consumption could provide additional beneficial effects on HRV indices increased by exercise practice. However, understanding of the effects of isoflavone on HRV is limited and it is important to investigate whether isoflavone provides additive effects on HRV in postmenopausal women submitted to CET.

The aim of the present study was to investigate the effects of ingesting isoflavone in addition to CET on HRV indices in non-obese postmenopausal women. The hypothesis raised was that isoflavone would promote additional improvement in HRV indices compared to isolated CET.

**Methods**

**Participants**

A total of 260 postmenopausal women (amenorrhea for at least 12 months) aged 50–70 years were recruited through advertisements in traditional (newspapers, radio, and TV) and electronic media (social media), with the provision of a telephone contact for those who were interested. After contact, interviews were scheduled to verify compliance with the following inclusion criteria: being able to engage in treadmill and resistance
training; having no history of cardiovascular disease, diabetes, renal pathologies, or hypertension; being a non-smoker; not using hormone therapy or isoflavones for at least three years; and signing a consent form. The exclusion criteria were: not taking all capsules, not performing the initial or final evaluations, or initiating another exercise protocol concomitant to the study. All volunteers were instructed to maintain their diet and sleep habits throughout the study. The follow-up flowchart is presented in Figure 1. In total, 36 women who met the inclusion criteria were recruited and allocated (17 on placebo and exercise and 19 on isoflavone supplementation and exercise); of these, 32 completed the protocol and 4 were excluded from the HRV analyses due to bad signal quality, totaling 28 volunteers (12 on placebo and exercise and 16 on isoflavone supplementation and exercise). The sample and interventions used in the present study were the same as those used in a previous study aimed at verifying the effects of CET and isoflavone supplementation on climacteric symptoms in postmenopausal women (Costa et al. 2017). This study was approved by the local ethics committee (Federal University of Uberlândia; CAAE: 40622414.9.0000.5152) and recorded in the international registration of clinical trials at “ClinicalTrials.gov” (identifier: NCT03008785).

Study design

This study is a parallel randomized, double-blinded, placebo-controlled clinical trial. Initially, 38 possible samples (in accordance with the sample size calculation and estimated sample loss) were randomly assigned (by electronic software) to the PLA group \( (n = 19) \) who received placebo and to the ISO group \( (n = 19) \) who received isoflavone supplementation. However, after recruitment, only 36 women met the inclusion criteria and were allocated to the PLA group \( (n = 17) \) and the ISO group \( (n = 19) \). In
association with placebo or isoflavone consumption, participants performed 30 sessions of CET for 10 weeks. Before the first day of training, participants were characterized by anthropometric evaluation and a questionnaire on physical activity level. Furthermore, they performed a treadmill incremental test and a maximal strength test (one maximal repetition test – 1RM), with an interval of at least 48 hours, to determine the intensity of training. HRV was evaluated pre and post training, after at least 48 hours without exercise. Volunteers were instructed to abstain from alcohol and caffeine. All procedures were performed in the Cardiorespiratory and Metabolic Physiology Laboratory of the Faculty of Physical Education at the Federal University of Uberlândia from February to December 2015.

**Anthropometric measurements and physical activity level**

The anthropometric evaluations were performed in an isolated environment in the morning after 8 hours of fasting. The following variables were measured: body mass, on an electronic scale (Filizola®, São Paulo, SP, Brazil); height, using a fixed stadiometer (Sanny®, São Bernardo do Campo, SP, Brazil); abdominal, waist, and hip circumferences, with an inelastic tape 0.5 cm wide (Filizola®, São Paulo, SP, Brazil); and fat mass, using tetrapolar bioimpedance (Biodynamics Model 450c: Biodynamics, Shoreline, WA, USA). Physical activity level was assessed using the International Physical Activity Questionnaire (IPAQ, Short Version), validated for the Brazilian population (Matsudo et al. 2001).

**Incremental treadmill test**

The submaximal incremental treadmill test was performed with a fixed velocity of 5.5 km/h and intensity imposed by the incline (%) to identify exercise intensity between
ventilatory thresholds 1 and 2 for exercise prescription. After a 5-min warm-up with a 0% incline, the test began with a 1% incline. The protocol consisted of 2-min stages with 1% increments in incline per stage until the volunteers reached 85% of their predicted maximum heart rate or 18 for the rate of perceived exertion (Borg 1982). Oxygen uptake and carbon dioxide output were recorded during the tests using a gas analyzer (Cosmed Quark CPET, Rome, Italy) to identify the ventilatory thresholds based on ventilatory equivalents (Wasserman 1984).

*One maximal repetition test*

For the 1RM test, participants performed a specific warm-up consisting of the same exercise as the test, with two sets at intensities of around 40–50% and 60–80% of the subjective estimate of 1RM and with 8–10 and 3–5 repetitions, respectively. After this warm-up, a maximum of five attempts were allowed per exercise to find the highest workload at which the participant could only perform one complete movement with the correct technique (Maud and Foster 2006). If the 1RM score was not found in the first session, a new session was scheduled after an interval of at least 48 hours. The order of exercises tested was: leg press, bench press, lateral pulldown, pec deck, and seated cable row.

*Combined exercise training program*

The training program consisted of combined aerobic and resistance exercises performed three times a week in 45-min sessions for 10 weeks. The sessions began with a 5-min warm-up on a treadmill at 5.5 km/h without inclination, followed by 20 min of aerobic exercises and 20 min of resistance exercises. The aerobic training was performed at a velocity of 5.5 km/h with the treadmill inclination corresponding to between ventilatory
thresholds 1 and 2 determined in an incremental treadmill test. Intensity increments of 20% were performed in the fifth week of training. Data on volunteers who were absent for more than 15% of training were excluded from the analysis.

The resistance exercises were performed in two sets of 15 repetitions, with 30 seconds between exercises and sets. Seven resistance exercises were performed: leg press 45° (hip and knee extension); chest press in vertical machine (shoulder horizontal abduction and elbow extension); anterior latissimus dorsi pulldown (shoulder abduction and elbow flexion); seated cable row (shoulder extension and elbow flexion); pec deck (shoulder horizontal adduction with flexed elbows); squat with lumbar Swiss ball support (hip and knee extension); and classic abdominal crunch (spine flexion with fixed hip and flexed knee on a flat surface). The resistance exercise intensity corresponded to 60% of 1RM. A new 1RM test was carried out in the fifth week of training for load readjustment.

Heart rate analysis

RR intervals (RRI) were collected for 20 min in a seated position, with spontaneous breathing, in a well-lit room using a heart rate monitor (Polar® RS800cx: Polar Electro Oy, Finland; sampling frequency, 1000 Hz) and without the influence of sensorial stimuli. Heart rate data were transferred to a computer using Polar Pro trainer5® software (Polar Electro, Kempele, Finland), after which the RRI were visually inspected and artifacts were replaced by the mean of the adjacent values. Samples were selected from the range of 300 seconds with the fewest artifacts closest to the time series end, and signals with more than 2% of artifacts were discarded (Task Force 1996). HRV analyses were performed in time, frequency, and nonlinear domains (Task Force 1996)
using validated (Tarvainen et al. 2014) software (Kubios® HRV 3.0.0: University of Kuopio, Kuopio, Finland).

The analyzed time-domain indices included the square root of the mean squared difference of successive R Ri (RMSSD), the standard deviation of all normal R Ri (SDNN), and the percentage of adjacent R Ri differing by more than 50 milliseconds (pNN50). For frequency-domain analysis, time series were interpolated at 4 Hz and the linear trend component signal was removed using the smooth prior technique. Next, the signal was multiplied by the Hanning window and a fast Fourier transform of the product was calculated. Thus, spectral bands were calculated through the integral of the power spectral density curve and specified in low (LF: 0.04–0.15 Hz) and high frequencies (HF: 0.15–0.4 Hz), as well as the ratio (LF/HF). Both LF and HF were normalized (LF% and HF%, respectively), representing the relative contribution of each component to the total power minus the very-low-frequency component. For nonlinear indices the Poincaré plot was analyzed, and also the standard deviation of the instantaneous variability of the beat-to-beat interval (SD1) and the long-term variability of the continuous R Ri (SD2) were analyzed, along with the ratio (SD2/SD1).

Supplementation
Volunteers took one capsule of isoflavone or placebo every day of the week (including weekends) from the first day to the last day of training, totaling 70 capsules per volunteer during the 10 weeks of training. Every Monday, each volunteer received a plastic refill containing the substances (isoflavone or placebo) with markings for the days. In the initial and final evaluations, volunteers did not receive supplementation. At every training session, participants were reminded and encouraged to maintain supplementation. The ISO capsules contained 100 mg of isoflavone (composition: 3.3%
genistein, 93.5% daidzein, and 3.2% glycitein) derived from soybean, corresponding to approximately 37.58 g of soy (Wang and Murphy 1994), whereas the PLA capsules contained 100 mg of cornstarch. All capsules were identical in appearance, taste, and smell.

**Statistical analysis**

The sample calculation was performed using G. Power software (version 3.1.9.2) and considering RMSSD as the main variable. An “*a priori*” family test for within–between interaction repeated-measures ANOVA was performed, with a possible effect size (*f*) of 0.3, a probability of error *α* of 0.05, power (1−β) of 0.8, correlation between repeated measures of 0.5, and a non-sphericity correction of 1. Thus, a total sample size (summed of over all groups) of 24 individuals was determined.

The pre and post HRV results are presented as mean ± standard deviation, variation (Δ), and lower and upper limits of the 95% confidence interval. Normality of data was tested using the Shapiro-Wilk test. Student’s *t*-test was used to compare HRV and the general characteristics of participants at the pre-intervention phase, and data are presented as mean ± standard deviation. The Mann-Whitney test was performed for variables without normal distribution, and these data are presented as median and interquartile range (25–75%). Pearson’s chi-square test was used to compare the physical activity level (by IPAQ) between groups, followed by the Monte Carlo test when the expected frequency was less than 5. A two-factor (time and group) generalized estimating equation technique was performed for between, within, and interaction comparisons. All analyses were performed using IBM® SPSS® Statistics 21. The significance level adopted was *p* < 0.05.
Results

The IPAQ analyses (data not shown) demonstrated that although no participants practiced regular exercises, none of the women were sedentary. The levels of physical activity were not different between groups ($\chi^2 = 0.609; p = 0.772$). No differences were found between groups at any pre-intervention HRV index (values can be checked in Table 2; statistical data not shown). However, there was a significant difference in mean RR values ($p = 0.026$). The general baseline characteristics are presented in Table 1. There were no differences between groups in any general baseline characteristic variables.

Table 2 presents the HRV data. The results of the generalized estimating equation tests did not show interaction or group effects for any HRV indices. However, the results reported time effects for mean RR, RMSSD, and SD1, with an increase in these indices in response to CET. There were no differences between moments for LF%, HF%, LF/HF, SDNN, pNN50, SD2, or SD2/SD1.

Discussion

The present study aimed to investigate if isoflavone promoted additional benefits to HRV indices over those provided by CET in postmenopausal women. Our hypothesis was based on similarity of chemical structure between isoflavone and estrogen and its high affinity to estrogen receptors (Carbonel et al. 2018). When stimulated, estrogen receptors may directly (i.e., acting within central nuclei) or indirectly (i.e., regulation of hot flashes and sleep problems; change in basal level of noradrenaline) modulate autonomic function (Mercuro et al. 1999, 2000; Saleh and Connell 2007; Lee et al.
2011). However, the results refuted the hypothesis raised, as only time effects were found, in accordance with studies that did not find any benefits of female sex hormonal therapy on cardiac autonomic modulation (Fernandes et al. 2005, Kiselev et al. 2018).

Postmenopausal symptoms (such as hot flashes and sleep problems) associated with reduced levels of estrogen are related to decreased autonomic control of the heart rate (Lee et al. 2011). A systematic review and meta-analysis of randomized controlled trials concluded that soy isoflavone supplements are significantly more effective than placebo in reducing the frequency and severity of hot flashes (Taku et al. 2012). Therefore, it was speculated that isoflavone supplementation could promote an additive reduction in postmenopausal symptoms occasioned by exercise practices (Ivarsson et al. 1998; Costa et al. 2017), and consequently promote an indirect additional effect on HRV. Although hot flashes and sleep disturbance symptoms were not analyzed in the present study, a previous study showed that isoflavone supplementation did not promote additive effects in improving these climacteric symptoms when ingested concomitantly with 10 weeks of CET (Costa et al. 2017). Therefore, the speculation made in the present study was not confirmed.

Another hypothesis was that isoflavone could interact with estrogen receptors in central nuclei to modulate autonomic function (Saleh and Connell 2007), promoting additive improvement in HRV promoted by CET. Modulation in central areas in response to exercise (Michelini and Stern 2009; Martins-Pinge 2011), which reduces the response efficiency of isoflavone, may explain the lack of additive effect found in the present study. Furthermore, β-endorphin released during exercise can stabilize thermoregulation and prevent hot flashes (Ivarsson et al. 1998). Up to now, no additive effect of isoflavone combined with CET on HRV has been found (Costa et al. 2017).
The time effects reported in mean RR, RMSSD, and SD1 suggest that CET increased the resting cardiac autonomic modulation of postmenopausal women. Mean RR is suggested as a global parameter of cardiac autonomic control (Task Force 1996). On the other hand, RMSSD and SD1 are most affected by high frequency variations in the heart rate, and are used as a marker of cardiac vagal control (Task Force 1996). Improvement in global or vagal indices of autonomic control of the heart rate in postmenopausal women is an important result due to the elevated risk of cardiovascular disease in this population (Kuo et al. 1999; Brockbank et al. 2000; Neves et al. 2007; Pathak et al. 2017). These results suggest that CET promoted intrinsic and/or central cardiovascular adaptations (Michelini and Stern 2009; Martins-Pinge 2011), which is in accordance with the supposition made in previous paragraphs.

The lack of a group with only isoflavone supplementation, a group without CET, and evaluation of the amount of isoflavone that appears in the blood could be some limitations of this study. However, as the aim of the current study was to investigate if isoflavone supplementation could have additive effects on the exercise-derived responses in HRV, we believe that our study could help to answer this question. Further studies are needed to investigate other doses of isoflavone and the association of this supplementation with other kinds of exercises.

The class of isoflavone used in the present study may be another limitation. The three primary isoflavones found in soy are genistein, daidzein, and glycitein (Murphy et al. 1999). Apparently, studies that show effects of isoflavone on climacteric symptoms use compounds containing at least 15 mg of genistein (Scambia et al. 2000; Williamson-Hughes et al. 2006), which is a larger quantity than that used in the present study (3.3 mg). A previous study that used a similar quantity of isoflavone compounds also did not show additive effects on a reduction in climacteric symptoms promoted by
CET (Costa et al. 2017). However, to date, no studies have investigated the effects of different classes of isoflavone on HRV modulation.

In summary, isoflavone did not promote additional effects on HRV indices of postmenopausal women submitted to 10 weeks of CET. The study was conducted in generally healthy, non-obese women, therefore the results might not be applicable to other groups receiving treatment with higher potency medication or for longer than 10 weeks. It is also important to note that this result is applicable only for isoflavone supplementation and may not be extrapolated to isoflavone consumption from natural and regular foods.

Acknowledgments and Authorships

The study was designed by IMM, JGC and GMP; data were collected and analyzed by IMM, JGC, JPB, TCFS, ALA, MLR and VHVC; data interpretation and manuscript preparation were undertaken by IMM, VHF and GMP. All authors approved the final version of the paper.

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Disclosure of Potential Conflicts of Interest

All authors declare no conflicts of interest.
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severity: systematic review and meta-analysis of randomized controlled trials.


Table 1 – General baseline characteristics

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<th>PLA (n=12)</th>
<th>ISO (n=16)</th>
<th>p</th>
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</thead>
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<tr>
<td>Age (years)</td>
<td>52.6 ± 5.3</td>
<td>56.1 ± 5.5</td>
<td>0.100</td>
</tr>
<tr>
<td>Time after menopause (years)</td>
<td>3.0 (1.4-5.8)</td>
<td>4.5 (2.0-12.0)</td>
<td>0.217</td>
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<tr>
<td>Body mass (kg)</td>
<td>63.2 ± 7.5</td>
<td>65.9 ± 8.8</td>
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</tr>
<tr>
<td>Height (m)</td>
<td>1.55 ± 0.05</td>
<td>1.58 ± 0.05</td>
<td>0.830</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>27.1 ± 2.6</td>
<td>26.4 ± 3.4</td>
<td>0.555</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>90.3 (87.3-96.8)</td>
<td>100.5 (84.5-104.3)</td>
<td>0.763</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>81.0 (76.0-86.3)</td>
<td>82.3 (74.7-91.5)</td>
<td>0.561</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>102.3 ± 6.8</td>
<td>103.7 ± 7.3</td>
<td>0.614</td>
</tr>
<tr>
<td>Waist-Hip ratio</td>
<td>0.79 ± 0.06</td>
<td>0.78 ± 0.06</td>
<td>0.648</td>
</tr>
<tr>
<td>Leg press 1RM (kg)</td>
<td>169.6 ± 32.4</td>
<td>158.2 ± 41.6</td>
<td>0.439</td>
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<tr>
<td>Bench press 1RM (kg)</td>
<td>27.3 ± 4.2</td>
<td>25.0 ± 5.2</td>
<td>0.230</td>
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<tr>
<td>Lat pull down 1RM (kg)</td>
<td>30.0 (25.0-35.0)</td>
<td>30.0 (30.0-33.8)</td>
<td>0.807</td>
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<tr>
<td>Pec deck 1RM (kg)</td>
<td>19.2 ± 5.1</td>
<td>19.5 ± 4.4</td>
<td>0.855</td>
</tr>
<tr>
<td>Seated cable row 1RM (kg)</td>
<td>57.1 ± 8.4</td>
<td>56.6 ± 12.1</td>
<td>0.899</td>
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</table>

ISO: Isoflavone group; PLA: Placebo group; 1RM: One repetition maximum test. Data are presented as mean ± standard deviation in variables with normal distribution (p from Student t test) and median with interquartile range (25-75%) in variables without normal distribution (p from Mann-Whitney test).
Table 2 – Heart Rate Variability

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre (mean ± SD)</th>
<th>Post (mean ± SD)</th>
<th>Δ (95% CI)</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RR (ms)</td>
<td>ISO 844.8±84.8</td>
<td>885.4±139.7</td>
<td>40.6 (-7.2 to 88.5)</td>
<td>0.125</td>
<td>&lt;0.001</td>
<td>0.113</td>
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<td></td>
<td>PLA 760.1±104.5</td>
<td>855.3±114.2</td>
<td>95.2 (47.6 to 142.8)</td>
<td></td>
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<tr>
<td>SDNN (ms)</td>
<td>ISO 25.3±11.0</td>
<td>25.9±10.4</td>
<td>0.6 (-8.1 to 9.4)</td>
<td>0.934</td>
<td>0.172</td>
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<tr>
<td></td>
<td>PLA 21.0±14.3</td>
<td>29.7±17.2</td>
<td>8.7 (-1.4 to 18.8)</td>
<td>0.883</td>
<td>0.044</td>
<td>0.338</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>ISO 19.5±10.9</td>
<td>23.1±15.2</td>
<td>3.6 (-5.2 to 12.5)</td>
<td>0.779</td>
<td>0.094</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td>PLA 15.6±11.4</td>
<td>25.9±14.9</td>
<td>10.2 (0.1 to 20.4)</td>
<td>0.574</td>
<td>0.339</td>
<td>0.672</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>ISO 4.1±7.4</td>
<td>8.0±15.7</td>
<td>3.9 (-3.4 to 11.3)</td>
<td>0.578</td>
<td>0.342</td>
<td>0.674</td>
</tr>
<tr>
<td></td>
<td>PLA 2.4±6.7</td>
<td>8.1±10.5</td>
<td>5.6 (-2.8 to 14.0)</td>
<td>0.156</td>
<td>0.760</td>
<td>0.522</td>
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<tr>
<td>LF% (n.u.)</td>
<td>ISO 74.3±13.6</td>
<td>71.9±22.2</td>
<td>-2.3 (-13.6 to 8.9)</td>
<td>0.883</td>
<td>0.044</td>
<td>0.337</td>
</tr>
<tr>
<td></td>
<td>PLA 73.7±9.5</td>
<td>67.6±18.2</td>
<td>-6.0 (-19.1 to 6.9)</td>
<td>0.322</td>
<td>0.311</td>
<td>0.843</td>
</tr>
<tr>
<td>HF% (n.u.)</td>
<td>ISO 25.7±13.6</td>
<td>28.0±22.2</td>
<td>2.3 (-8.9 to 13.6)</td>
<td>0.994</td>
<td>0.295</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>PLA 26.3±9.5</td>
<td>32.3±18.1</td>
<td>6.0 (-7.0 to 19.0)</td>
<td>0.322</td>
<td>0.311</td>
<td>0.843</td>
</tr>
<tr>
<td>LF/HF</td>
<td>ISO 4.2±2.9</td>
<td>5.1±5.7</td>
<td>0.9 (-1.5 to 3.4)</td>
<td>0.779</td>
<td>0.094</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td>PLA 3.4±1.9</td>
<td>3.1±2.1</td>
<td>-0.3 (-3.2 to 2.5)</td>
<td>0.574</td>
<td>0.339</td>
<td>0.672</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>ISO 13.8±7.7</td>
<td>16.4±10.8</td>
<td>2.6 (-3.7 to 8.8)</td>
<td>0.883</td>
<td>0.044</td>
<td>0.337</td>
</tr>
<tr>
<td></td>
<td>PLA 11.1±8.1</td>
<td>18.4±10.5</td>
<td>7.3 (0.1 to 14.5)</td>
<td>0.574</td>
<td>0.339</td>
<td>0.672</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>ISO 32.8±14.0</td>
<td>32.0±12.1</td>
<td>-0.7 (-11.9 to 10.5)</td>
<td>0.779</td>
<td>0.094</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td>PLA 27.4±18.8</td>
<td>37.3±22.7</td>
<td>9.9 (-3.1 to 22.8)</td>
<td>0.574</td>
<td>0.339</td>
<td>0.672</td>
</tr>
<tr>
<td>SD2/SD1</td>
<td>ISO 2.6±0.8</td>
<td>2.4±0.9</td>
<td>-0.2 (-0.7 to 0.3)</td>
<td>0.994</td>
<td>0.295</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>PLA 2.4±0.6</td>
<td>2.2±0.8</td>
<td>-0.2 (-0.7 to 0.4)</td>
<td>0.322</td>
<td>0.311</td>
<td>0.843</td>
</tr>
</tbody>
</table>

CI: Confidence interval; ISO: Isoflavone group; PLA: Placebo group; SDNN: Standard deviation of normal RR intervals; RMSSD: Root Mean Square of the Successive Differences of RR intervals; pNN50: percentage of pairs of adjacent RR intervals differing by more than 50 milliseconds; LF%: Low frequency; HF%: High frequency; SD1: Standard deviations of the distances from points to diagonal Y = X of the scattergram.; SD2: Standard deviations of the distances from points to straigtn Y = -X+RRmean of the scattergram. n.u.: normalized units.
Figure caption

Figure 1 – Follow-up flowchart. HR: Heart rate.
Assessed for eligibility (n = 260)

Excluded (n=224)
- Did not meet the inclusion criteria (n=220)
- Refused to participate (n=4)

Allocated (n=36)

Follow-up

Placebo and Exercise (n=17)
Loss to follow-up (n=5)
- Personal problems (n=1)
- Health problems not related to the study (n=1)
- Poor HR signal quality (n=3)

Isoflavone supplementation and exercise (n=19)
Loss to follow-up (n=3)
- Personal problems (n=1)
- Health problems not related to the study (n=1)
- Poor HR signal quality (n=1)

Analyzed (n=12)

Analyzed (n=16)