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Short communication

Chronic L-citrulline supplementation improves cardiac sympathovagal balance in obese postmenopausal women: A preliminary report☆

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ABSTRACT

The present study evaluated the impact of chronic L-citrulline (L-cit) supplementation on resting heart rate variability (HRV) and blood pressure (BP) in obese postmenopausal women. Participants were randomly assign to either an L-cit group (n = 12) or a control group (n = 11). HRV and BP were measured before and after 8 weeks. There were significant decreases ($P < 0.05$) in nLF (sympathetic activity), LnLF/LnHF (sympathovagal balance), and BP as well as a significant increase ($P < 0.05$) in nHF (vagal tone) following L-cit compared with no changes after control. Our findings indicate that L-cit supplementation improves sympathovagal balance in obese postmenopausal women.

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1. Introduction

Heart rate variability (HRV) is a non-invasive tool for the evaluation of cardiac autonomic function by quantifying the beat-to-beat fluctuations in R-R intervals (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). HRV has been proven to be negatively influenced by menopause (Brockbank et al., 2000) and obesity in women (Zahorska-Markiewicz et al., 1993), which has been associated with adverse cardiac events and mortality (Schwartz et al., 1992). Therefore, HRV is a worthwhile target intervention in obese postmenopausal women.

L-arginine (L-arg), the nitric oxide (NO) precursor, has been shown to have significant physiological effects related to improved NO synthesis including an improved cardiac vagal activity (Nomura et al., 1998)(Chowdhary et al., 2002). For instance, acute intravenous infusion of L-arg has been shown to acutely increase HRV and HRV parameters of vagal activity in healthy British (Chowdhary et al., 2002) and Japanese (Nomura et al., 1998) populations. Although L-arg is obtained naturally in the human diet, it has been suggested that an increased arginase activity in the intestines and the liver makes chronic oral L-arg consumption somewhat ineffective (Romero et al., 2006). Conversely, oral L-citrulline (L-cit) provides greater plasma L-arg levels due to an efficient conversion of L-cit to L-arg, augmenting NO and NO dependent-signaling (cGMP) (Schwedhelm et al., 2008). However, evidence on the effects of oral L-cit supplementation on cardiac autonomic function is limited to one study. Kameda et al. showed decreases in QT interval (a marker of cardiac death in myocardial infarction) in healthy subjects after acute L-cit ingestion (Kameda et al., 2005). However, the effect of chronic L-cit supplementation on cardiac autonomic activity remains unclear. Since obese postmenopausal have a high prevalence of cardiac autonomic dysfunction (Brockbank et al., 2000)(Zahorska-Markiewicz et al., 1993), a condition associated with increased mortality in individuals with high cardiovascular risk (Schwartz et al., 1992), it would be of clinical importance to examine the effects of L-cit supplementation on HRV in this population.

The purpose of this study was to evaluate the effectiveness of an 8-week L-cit supplementation on HRV in obese postmenopausal women. We hypothesized that L-cit supplementation would improve HRV in obese postmenopausal women.

2. Methods

2.1. Participants

Twenty-three (age, 50–65 years) obese (body mass index [BMI] > 30 and <40 kg/m^2) postmenopausal women participated in the present study. Menopause was defined as the absence of menstruation for at least 1 year. Exclusion criteria included smoking, cardiovascular diseases, diabetes, and the use of medications or hormone replacement therapy during the 6 months before the study. All women were sedentary, defined as having <1 h of regular exercise per week in the previous year. Participants were recruited from Tallahassee, FL, and surrounding areas through flyers and word of mouth. All participants gave written informed consent prior to their inclusion in the study. The study

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protocol was approved by The Florida State University Human Subject committee and registered in Clinicaltrial.gov (NCT02143817).

2.2. Study design

Before baseline measurements, participants were familiarized with the study tests and procedures and were randomly assigned to a L-cit supplementation group (n = 12) or a control group (n = 11) for 8 weeks. Allocation was stratified for BMI (>30.0, <35 or ≥35, <40 kg/m^2), and the sequence was generated by a computer-based number. R-R intervals and blood pressure (BP) measurements were performed in the supine position after at least 10 min of rest in a quiet temperature controlled room (23 ± 1 °C) following an overnight fast and abstinence from caffeinated drinks and alcohol for at least 24 h. Measurements were repeated at the end of the study in the morning at the same time of day (±1 h) and under similar conditions. Participants were instructed not to alter their regular lifestyle habits during the study.

2.3. Supplementation

Participants in the L-cit group were supplemented with 6 g/day of L-cit. The L-cit was ingested in the form of 750 mg capsules corresponding to 4 capsules (3 g) before breakfast and 4 capsules before sleeping. Likewise, the control group consumed 4 capsules of maltodextrin before breakfast and 4 capsules before sleeping to match the number of capsules ingested daily by the L-cit group. The selected dose and times of ingestion were based on previous studies with L-cit supplementation that showed increases in NO levels and decreases in BP in several populations (Ochiai et al., 2012; Schwedhelm et al., 2008), including postmenopausal women (Wong et al., 2016). The last dose of L-cit and placebo was ingested 48 h prior to the last visit in order to avoid any possible acute cardiac/vascular effects of L-cit (Wong et al., 2016). Participants self-recorded supplementation logs on a weekly basis. Participants were required to return the logs and unused capsules after 4 and 8 weeks to verify their adherence to the supplementation. Compliance was calculated by dividing the consumed capsules by the expected number of capsules.

2.4. Blood pressure and heart rate variability

Resting brachial BP was measured using an automatic device (VP-2000; Omron Healthcare, Vernon Hills, IL, USA). R-R intervals were collected during 6 min using a validated wireless monitor (Polar 800CX; Polar Electro OY, Kempele, Finland) via a chest strap interfaced with a PC. All R–R intervals were inspected for artifacts and premature beats. HRV was quantified from 5-min segments free of artifacts. As a time domain index of HRV, we calculated the root mean square of successive differences (RMSSD), which reflects parasympathetic modulation. The autoregressive model was used to estimate the power spectrum in the total power (TP, 0.00–0.40 Hz) and its main components: low-frequency (LF, 0.04–0.15 Hz) and high-frequency (HF, 0.15–0.40 Hz). TP of HRV is an estimation of the global activity of the autonomic nervous system. The HF power is a marker of cardiac parasympathetic activity (McCraty and Shaffer, 2015). The LF component of HRV in absolute units is mediated by both sympathetic and parasympathetic activities (McCraty and Shaffer, 2015). The HF and LF were normalized (nHF and nLF) and expressed as a percentage of the TP. The ratio of LF to HF (LF/HF) could be used to quantify the relationship between sympathetic and parasympathetic nerve activities (sympathovagal balance) (McCraty and Shaffer, 2015). Increased sympathovagal balance was considered to reflect predominant sympathetic activity. We followed the standards for the measurement and interpretation of the HRV recommended by the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Controlled breathing (12 breaths/min) was maintained following a metronome.

2.5. Statistical analysis

Data were examined for normality with the Shapiro–Wilk test. Since TP, RMSSD, LF, HF, LF/HF were not normally distributed, a logarithmic transformation (Ln) was performed for these variables. Student's *t*-test was used to detect possible difference in parameters between groups at baseline. A two-way analysis of variance with repeated measures (group [control and L-cit] × time [before and after 8 weeks]) was used to determine the effects of the intervention over time. If a significant interaction or main effect was noted, a paired *t*-test was used for post hoc comparisons. Data are shown as means ± SE. Statistical significance was defined a priori as $P < 0.05$. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL).

3. Results

Compliance to the supplementation was 98% for both the L-cit and control groups. Age, height and anthropometry values before and after 8 weeks for the control and L-cit groups are presented in Table 1. Baseline parameters in the two groups were not significantly different. There were no significant changes in body weight and BMI after L-cit or control. There was a significant group x time interaction ($P < 0.05$) for brachial systolic BP and diastolic BP, indicating that BP significantly decreased ($P < 0.05$) following L-cit compared to no changes after control.

Autonomic variables at baseline and after 8 weeks for the control and L-cit groups are presented in Table 2. There was a significant group × time interaction ($P < 0.05$) for LnLF/LnHF, which significantly decreased ($P < 0.01$) following L-cit compared to no changes after control. There was a significant ($P < 0.05$) decrease in nLF and increase in nHF in the L-cit group when compared to baseline and control group. LnRMSSD ($P < 0.05$) increased in the L-cit group in comparison to baseline, but the change was not different compared to the control. There were no significant changes in LnTP and R-R intervals after L-cit or control.

4. Discussion

Our major finding is that 8 weeks of L-cit improved the cardiac sympathovagal balance in obese postmenopausal women. To our knowledge, this is the first study that has shown beneficial effects of chronic L-cit supplementation on resting HRV.

Development of new avenues to efficiently improve cardiac autonomic activity remains an important task in the healthcare field. Autonomic dysfunction is associated with a variety of cardiovascular conditions, ranging from arterial hypertension to stroke and coronary heart disease (Schwartz et al., 1992). Evidence suggests that NO plays an important role in increasing vagal modulation (Chowdhary and Townend, 1999) and decreasing sympathetic tone (Choate and Paterson, 1999). Since L-cit supplementation is well known for

Table 1
Subjects characteristics before and after 8 weeks of L-cit or Control. Abbreviations: L-cit, L-citrulline; BMI, body mass index; BP, blood pressure. $^{\#}P < 0.05$ different than before. †$P <$ 0.05 different than control. Data are mean ± SEM.

Variable	CONTROL		L-CIT	
	Before	After	Before	After
Age (years)	58 ± 1	–	58 ± 1	–
Height (m)	1.61 ± 0.03	–	1.60 ± 0.02	–
Body weight (kg)	86.2 ± 4.4	85.9 ± 4.5	82.0 ± 2.2	82.0 ± 2.0
BMI (kg/m^2)	32.9 ± 1.1	32.8 ± 1.1	32.2 ± 0.7	32.2 ± 0.6
Systolic BP (mm Hg)	137 ± 4	138 ± 4	138 ± 4	131 ± 5$^{\#\dagger}$
Diastolic BP (mm Hg)	80 ± 3	80 ± 3	81 ± 4	75 ± 4$^{\#\dagger}$