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HEALTH SCIENCES

Fasted condition in multicomponent training does not affect health parameters in physically active post-menopausal women

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Abstract: Diet and exercise are the main modifiable factors for cardiovascular disease and may be particularly important in older adults. We investigated the effects of fasting during 12 weeks of multicomponent training in the context of the aging process in physically active post-menopausal women. Method: 25 women (60.6 ± 8.9 years) were randomized into two groups: fed (FED, n=12) or fasted (FASTED, n=13) and submitted to multicomponent training. The participants underwent anthropometric, body composition, blood pressure, biochemical blood and physical fitness assessments. Results: There was a reduction in both groups for waist circumference [FED: 100.4±6.8 and 99.1±7.1 cm before and after the intervention, respectively; F = 4.214, p = 0.048; FASTED: 93.1±10.2 and 92.2±8.4 cm before and after the intervention, respectively; p = 0.039]. No significant changes were observed for the other outcomes. Discussion: The current research results, the first in the context of aging, agree with previous studies that analyzed chronic effects of fasting, showing that fasted exercise training did not improve anthropometric measurements, body composition, or blood markers compared to the fed condition after long-term exercise training. Together, these findings suggest that fasting during multicomponent training does not affect health parameters in physically active post-menopausal women.

Key words*:* Aging, exercise, metabolism, nutrition.

INTRODUCTION

Diet and exercise are the main modifiable factors for cardiovascular disease and may be particularly important in older adults, who are at a higher risk of chronic disease (Atkins et al. 2016). The aging process is associated with increased fat and a reduction in muscle mass, strength, and mobility (Witard et al. 2016, Chernoff 2005). In this sense, current physical activity guidelines for older adults indicate exercise training including aerobic capacity, muscular strength, flexibility, coordination, agility, and balance (AMERICAN COLLEGE OF SPORTS MEDICINE 2009, Bouaziz et al. 2017). In this context, multicomponent training has been demonstrated as more effective for promoting significant improvements in physical fitness than other investigated exercise protocols in women over 50 years of age (Trapé et al. 2017).

Changes in dietary pattern have led to higher saturated fat intake, thereby raising cholesterol levels and the chances of developing chronic diseases including obesity, metabolic syndrome, and, consequently, cardiovascular diseases in older people (Nabuco et al. 2018, Trepanowski et al. 2017). Indeed, Nabuco et al. (2018) demonstrated an increased risk of developing metabolic syndrome in older women with lower levels of protein intake and/or higher consumption of carbohydrates. Considering the pivotal role of nutrition in training and performance, it is probably important to study training effects in a nutritional context, which is relevant to the aging process.

Although previous studies have investigated alternate-day fasting regimens (Trepanowski et al. 2017), nutritional profiles for overweight people (Magno et al. 2014), the effects on health markers during Ramadan fasting (Norouzy et al. 2017), and the effects of aerobic training in a fasted state (De Bock et al. 2005, Van Proeyen et al. 2010), the influence of multicomponent training in a fasted condition in the context of the aging process is unknown. Furthermore, Kersten et al. (2009) reported that physiological stressors, such as fasting and exercise, are required to elicit significantly raised plasma angiopoietin-like protein 4 (ANGPTL4) by an average of 80% in humans, mediated by elevated free fatty acids (FFAs) through activation of ANGPTL4 gene transcription via PPARs and ANGPTL4, which may raise plasma FFAs via stimulation of adipose tissue lipolysis (Kersten et al. 2009). In addition, Vendelbo et al. (2015) demonstrated that exercise induced stimulation of GH signaling activity in vivo is amplified by fasting and exercise at the level of SOCS and CISH target gene expression in skeletal muscle and adipose tissue in human subjects, thus GH acts as a critical conservator of protein during catabolic stress. However, these manuscripts are based on the acute effects of exercise. As aforementioned, no study has analyzed the chronic effects of fasting in humans in the context of aging, while in professional practice, there is huge speculation about the potential benefits of exercise training in the fasting condition. Moreover, it was demonstrated that a short period of fasting training (30 days) reduced triglycerides in older people (Oliveira 2013). As a primary outcome, this study aimed to analyze the influence of 12 weeks of multicomponent training in a fasted condition on body composition, weight loss, and markers of metabolic health in physically active women over 50 years of age. In addition, we compared the intake of micro and macronutrients on different days of the week (a training day, a day without training, and a weekend day) before and after the intervention to determine the effects of fasted *versus* fed training on food intake, energy, and macronutrient and micronutrient intake.

MATERIALS AND METHODS

Participants

In total, 25 physically active post-menopausal women who participated in the Physical Education for the Elderly Program of the Escola de Educação Física e Esporte de Ribeirão Preto - Universidade de São Paulo (USP) (EEFERP-USP), with at least six months in the program (180 min/week) and a minimum attendance of 75%, signed a written informed consent form and were enrolled in the present study. This study was approved by the Ethics Committee of the Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo (CAAE 24579513.4.0000.5407), in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and according to the norms of Resolution 466/2012 of the National Health Council. The participants were randomized into two groups: FED (n=12) or FASTED (n=13) after two days of baseline evaluations, using Random Allocation Software (Saghaei 2004). Moreover, the participants in the fasted group were instructed to begin the fasting period 12 hours before each training session. The adherence to the fast condition was adequate for all participants. The inclusion and exclusion criteria have been previously described (Trapé et al. 2017). Briefly, the inclusion criteria were being post-menopausal women. Exclusion criteria were the presence of any medical, mental, or musculoskeletal conditions that could prevent performance of the motor tests and physical training program; body mass index > 35 kg/m2, systolic blood pressure >160 mmHg, maximal diastolic blood pressure >100 mmHg; participation in any other physical exercise program in the six months prior to or during the intervention proposed by this study; and presence <75% in the activities proposed by the intervention (Trapé et al. 2017).

Multicomponent training

The duration of the intervention was 12 weeks. Before starting each session, all the participants were asked if they were in a fasting condition or not. All sessions were conducted in the morning (two times per week from 7:30 to 9:00 am) by a physical education professional (blinded to group allocation) and the intensity of the training was controlled by the Subjective Effort Perception Scale (Borg & Noble 1974), maintaining an intensity between 13 (moderate) and 15 (intense). The characteristics of the multicomponent training have been demonstrated previously (Trapé et al. 2017). Briefly, the sessions (90 minutes each) were divided into four parts: (1) warm-up, including dynamic stretching exercises, coordination, and/or balance (about 20 to 30 minutes), (2) strength exercises performed in the form of a circuit using elastics, free weights, and body weight (about 30 to 40 minutes), (3) aerobic and ludic activities (dances or games) (about 20 to 30 minutes), and (4) “back to calm,” relaxation, massage, and stretching exercises (about 10 minutes). The assessment was completed in two visits: the first visit for anthropometric measurements, questionnaires, physical fitness tests, blood pressure measurements, and the second visit for blood collection. After 12 weeks of training, the participants were invited again for the same procedure. The flow of participants and study design are illustrated in Figure 1.

Dietary intake, sleep pattern, quality of life, and physical activity level

The participants were asked to complete a 3-day dietary record before and after the intervention on three different days of the week (a training day, a day without training, and a weekend day). Daily energy and macronutrient intakes were analyzed using *Dietpro* software version 4.0 (Laval University, Quebec). It is noteworthy that the usual diet did not alter for any groups during the study. However, subjects in the fasted group were asked to ingest only water during the physical training sessions and in the twelve hours preceding them.

In addition, sleep quality was evaluated by the Epworth Daytime Sleepiness Scale (Johns 1991), level of psychological stress by the Inventory of Stress Symptoms (ISS) (Lipp & Guevara 1994), quality of life by the SF-36 questionnaire (Ciconelli et al. 1999), and level of physical activity by the International Physical Activity Questionnaire (IPAQ) (Matsudo et al. 2001).

Blood analysis

All tests were performed in the morning (7:00-8:30 am) after 12 h overnight fasting. Before the tests, all volunteers remained seated for approximately five minutes, after which a venous blood sample was performed to verify fasting lipid profile (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol) and serum glucose level. The analyzes were performed in the clinical laboratory of the Faculty of Pharmaceutical Sciences of Ribeirão Preto of the University of São Paulo, by means of an enzymatic analysis kit (Wiener Lab, Argentina) on an automatic device (Konelab 600i, Wiener Lab, Argentina).

Anthropometric measurements and body composition

Height (cm) was measured using a fixed stadiometer (Welmy W200ALCD) with an accuracy of 1.0 mm. Body mass (kg) was evaluated using a

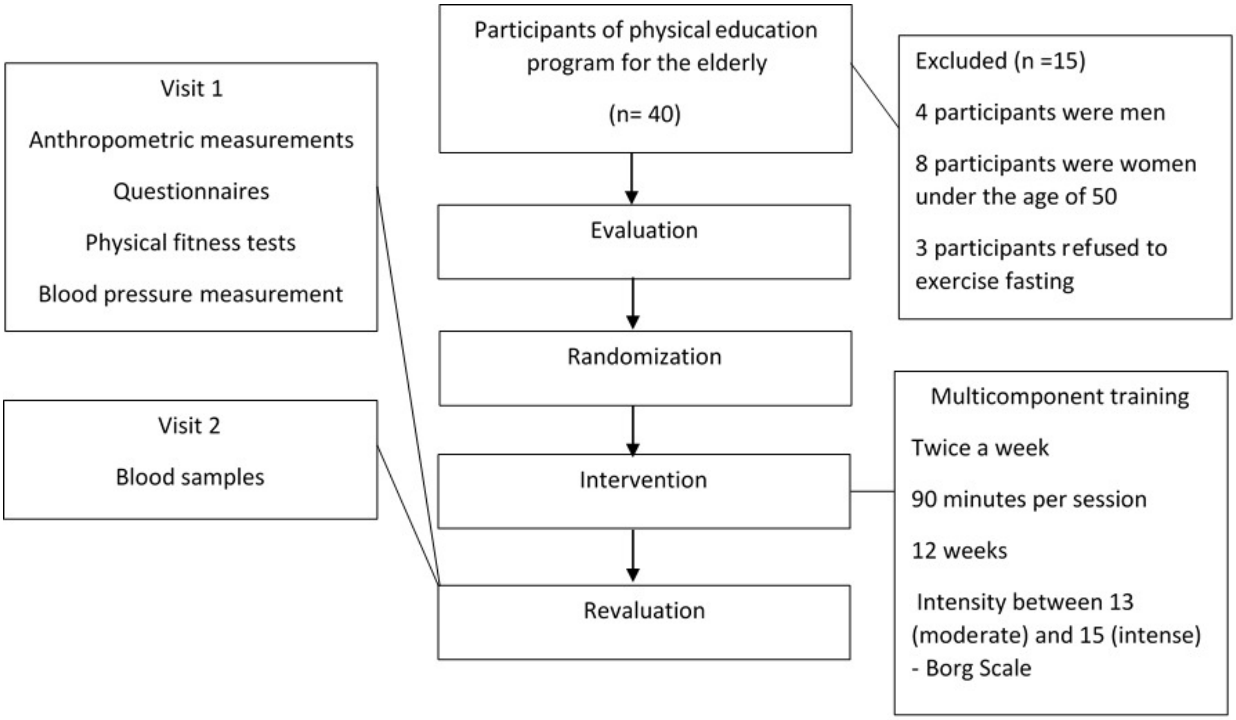

Figure 1. Flow chart and sample design.

digital scale (Filizola PL 31, Filizzola Ltda., Brazil), with an accuracy of 0.1 kg. The body mass index (BMI) was calculated using the formula weight/height^2 (kg/cm^2). The waist circumference measurement was performed at the midpoint between the last costal arch and the iliac crest. Body composition was measured with a tetrapolar bioimpedance analyzer (model HBF-510W, Brazil) (Hasnan et al. 2014).

Physical fitness

The tests used in the current study are specific for older and elderly adults, validated and with normative reference values (Trapé et al. 2017). Aerobic capacity was evaluated by the six-minute walk test, the strength of upper limbs was evaluated by the elbow extension-flexion and handgrip test, and the strength of lower limbs was evaluated by the sit and stand up from a chair test.

Statistical analysis

Normally distributed variables were tested and confirmed by the Kolmogorov-Smirnov test, enabling the description of data as mean ± standard deviation. Non-normally distributed variables are presented as median (interquartile range). In order to assess differences before and after the intervention, as well as between groups (FED and FASTED), a repeated measure analysis of variance (ANOVA) with fixed factors (i.e., ‘condition’ and ‘time of response’) or the Kruskal-Wallis test was performed. Bonferroni’s post hoc test was applied to determine the differences between conditions and time points. The statistical analysis was performed using Sigma Stat software version 3.1 and the level of significance was set at *p-value* <0.05.

RESULTS

The mean (SD) age of the participants was 60.6±8.9 years. Table I presents the anthropometric characteristics, body composition, and blood analysis between the groups before and after the multicomponent training. Both groups presented a reduction in waist circumference [F = 4.214, p = 0.048 (FED) and p = 0.039 (FASTED)].

Table II shows the physical tests, level of physical activity, level of sleepiness during the day, level of stress, and quality of life. There was no effect of time, group, or interaction between the groups before and after the training period for these variables.

The changes in energy intake are shown in Figure 2a. Increased energy intake was observed on the weekend day only in the fasted group (F = 4.536, p = 0.026). Macronutrient intake, comparing the fed and fasted conditions, before and after the multicomponent training, on different days of the week, is also shown in Figure 2. Increased intake of carbohydrates was observed after the training period on the weekend day only in the fasted group (F = 4.340, p = 0.027) (Figure 2b). In addition, there was increased intake of protein in the fasted group on the training day (F = 11.397, p = 0.011). In the baseline condition, the intake of protein was higher in the fed group compared to the fasted group on the weekend day (F = 3.480, p = 0.049) (Figure 2c). Finally, there was increased intake of lipids in the fed group on the training day (F = 5.869, p = 0.011) (Figure 2d).

Table III presents the intake of micronutrients between the fed and fasted conditions before and after the multicomponent training intervention on different days of the week. Increased intake of zinc was observed in both groups on the training day [F = 28.616, p = 0.014 (FED) and p < 0.001 (FASTED)]. Iron intake reduced only in the fed group on the weekend day (F = 5.081, p = 0.006). In counterpart, an increase in folic acid intake was observed only in the fasted group on the weekend day (F = 9.327, p = 0.010). The intake of sodium was different between the groups after the multicomponent training, being higher in the

Table I. Anthropometric, body composition, and blood analysis characteristics between the fed and fasted conditions before and after the multicomponent training intervention.

	Fed		Fasted	
	Pre	***Post***	***Pre***	***Post***
Body mass (kg)	80.6±15.0	80.1±15.6	70.9±11.0	70.7±10.8
Height (m)	1.63±0.1		1.59±0.1	
BMI (kg/m^2)	29.6±2.2	30.1±2.1	28.3±4.3	27.9±4.1
WC (cm)	100.4±6.8	99.1±7.1*	93.1±10.2	92.2±8.4*
FAT (%)	38.1±7.3	37.9±4.0	37.2±8.2	37.4±7.6
BMR (kcal)	1327.6±244.8	1324.9±212.6	1233.3±109.4	1226.8±96.4
Blood glucose (mg/dL)	109.8±35.2	107.2±44.9	106.5±42.4	92.5±12.8
HDL-c (mg/dL)	49.7±13.4	49.8±12.2	52.2±14.4	50.3±13.8
LDL-c (mg/dL)	114.8±20.0	115.1±22.3	116.9±30.0	109.8±33.9
Triglycerides (mg/dL)	143.6±76.8	142.0±70.5	135.2±87.5	144.9±90.2
Cholesterol total (mg/dL)	196.6±20.4	193.5±20.9	196.5±37.1	188.9±30.9

Note: BMI: body mass index; WC: waist circumference; BMR: body metabolic ratio; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol. Two-way ANOVA for repeated measurements. p <0.05. Data presented as mean ± standard deviation. *against pre in the same group.

fed group compared to the fasted group during the day without training (F = 4.681, *p* = 0.042). Intake of vitamin D increased in the fed group on the training day (F = 10.503, *p* = 0.013), while the intake of vitamin A reduced in the fed group on the day without training (F = 9.882, *p* = 0.024).

DISCUSSION

The results of this study demonstrate that the body composition, weight loss, and health markers of physically active post-menopausal women were similar regardless of whether an individual was fasted or fed during 12 weeks of a multicomponent training intervention. Despite the growing popularity of exercise in the fasted state, to our knowledge, no randomized clinical trials have evaluated its efficacy or compared this regimen before and after 12 weeks of multicomponent exercise in the context of aging. In addition, one innovation of the present study was to analyze the consumption of micro and

Table II. Characteristics of physical abilities and participant questionnaires between the fed and fasted conditions before and after the multicomponent training intervention.

	Fed		Fasted	
	Pre	***Post***	***Pre***	***Post***
6 minute walk (m)	544.6±58.4	564.2±69.8	574.9±59.4	578.6±55.7
Sit and stand up (reps)	18.0±5.4	18.5±5.3	16.2±7.0	18.4±5.8
RHGS (KGS)	30.2±5.7	29.8±5.7	28.5±6.7	29.2±6.5
LHGS (KGS)	28.8±9.1	29.3±7.7	26.2±8.2	26.8±7.0
EFE (reps)	22.1±5.1	22.8±4.1	20.7±3.8	21.5±3.3
IPAQ Walk (minute/week)	190.1 (20-790)	170.3 (30-940)	180.3 (30-780)	270.1 (40-970)
Moderate (minute/week)	215.1 (40-840)	305.1 (60-1202)	182.4 (90-1300)	240.1 (30-1420)
Vigorous (minute/week)	40.3 (10-220)	30.2 (10-260)	45.3 (30-185)	33.1 (30-170)
ESS (points)	8.3±3.9	7.7±3.9	6.4±4.8	7.2±4.2
ISS Alert phase (points)	1.6 ±2.5	1.5±1.7	1.2±1.2	1.2±1.5
Phase of resistance and near-exhaustion (points)	1.4±2.2	1.6±2.3	1.2±1.7	0.8±1.3
Exhaustion phase (points)	1.8±2.4	1.8±2.8	1.3±2.1	0.8±1.3
SF36 Physical score (points)	65.7±8.2	66.1±9.5	68.3±3.6	63.9±9.1
Mental score (points)	59.8±11.0	63.0±13.2	60.8±4.3	56.2±7.1

Note: RHGS: right-hand grip strength; LHGS: left-hand grip strength; EFE: elbow flexion and extension; ESS: Epworth sleepiness scale; ISS: Inventory of stress symptoms; SF36: Quality of life questionnaire. Normally distributed variables are presented as mean ± standard deviation. Non-normally distributed variables are presented as median (interquartile range). *p*<0.05.

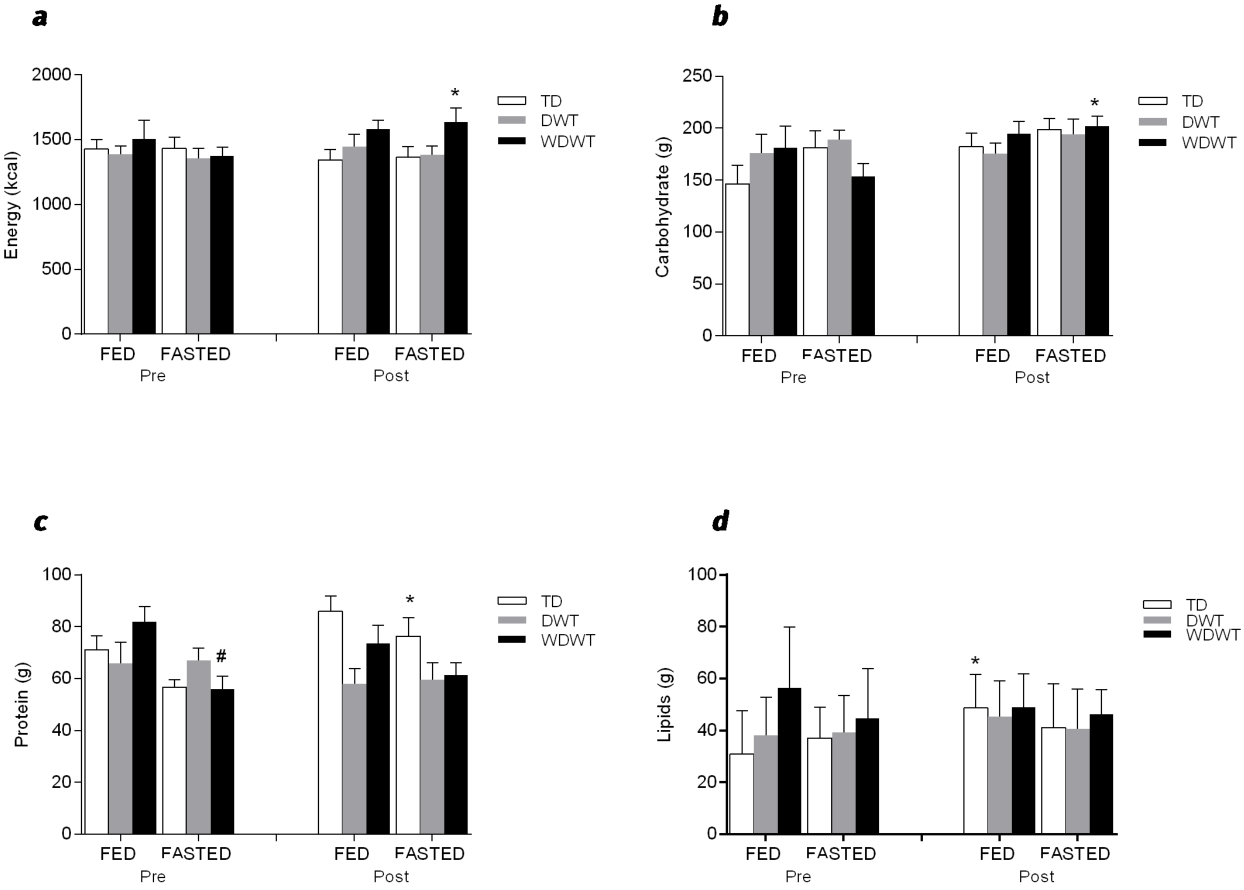

Figure 2. TD: training day; DWT: day without training; WDWT: weekend day without training. Two-way ANOVA for repeated measurements. Data presented as mean ± standard deviation. *p* <0.05. *against pre in the same group. $^{\#}$against fed at the same time.

macronutrients on different days of the week (a training day, a day without training, and a weekend day) before and after the intervention.

In the current research, we observed that fasted exercise training did not improve anthropometric measurements, body composition, or blood markers compared to the fed condition after 12 weeks of multicomponent training (Table I). In addition, our results did not demonstrate any significant differences in physical abilities, physical activity level, stress, quality of life, or mental score between the groups. Only the waist circumference reduced in both groups after the training period (Table II). It is known that the aging process is characterized by several changes in the different components of body composition, especially a decline in muscle mass and subcutaneous fat, with a concomitant increase in visceral fat and intramuscular fat (Cruz-Jentoft et al. 2010). Therefore, improving health outcomes or even avoiding deterioration due to the aging process through exercise becomes an important strategy. In addition, it is essential to highlight that the participants in the current study were physically active, which could explain the absence of differences in the health parameters analysed - the trainability principle states that the more fully a person is trained with respect to a given fitness component, the less there remains of that component to be trained in the future (Foster 1998).

Fasting is characterized by the absence of food and/or energy beverage intake for a period, which may last from several hours to a few weeks (Moro et al. 2016). Some weight loss studies have used fasting on alternate days as a strategy to change body composition (Trepanowski et al. 2017). In theory, strategies involving training after an overnight fast might accelerate the loss of body fat, as fasting greatly enhances the aerobic metabolism, promoting fat oxidation during activity (Kang et al. 2013), although some studies have shown an absence of differences. Recently, Schoenfeld et al. (2014), for example, did not find differences in body composition changes in young women after four weeks of aerobic exercise performed in the fasted *versus* fed state, while subjects maintained a caloric deficit. The authors observed no differences between conditions in any outcome measured, regardless of pre-exercise feeding status (Schoenfeld et al. 2014).

De Bock et al. (2005) hypothesized that six weeks of endurance training while fasting would increase the relative contribution of fat oxidation to total energy production, for a given absolute workload and exercise duration, even in the presence of carbohydrate intake during exercise. However, the authors showed that neither intramyocellular triglyceride content breakdown nor rate of total fat oxidation was affected by the differential dietary context of the training sessions. Using *Diet Pro* software, the 3-day diet record was uploaded and provided information on an extensive battery of macro and micronutrient intake data from which 10 were selected (calcium, zinc, iron, acid folic, sodium, vitamin D, vitamin B6, vitamin A, vitamin B12, vitamin C), which may be associated with deficiencies in elderly women. According to the Dietary Guidelines for Americans (Trumbo et al. 2002), a healthier eating pattern includes habits based on fruit and vegetable intake and reduction in energy-dense foods. The mean intake of all micronutrients was similar between groups (*p*>0.05), except for sodium after the training period (Table III).

Van Proeyen et al. (2010) investigated whether exercise training in the fasted state is more potent than exercise in the fed state to rescue whole-body glucose tolerance and insulin sensitivity during a period of hyper-caloric fat-rich diet. The authors showed that a given amount of endurance training in a fasted state is more potent than training in a fed state to improve glucose tolerance during an episode of dietary lipid challenge (six weeks of high fat diet) (Van Proeyen et al. 2010). Our results did not demonstrate differences between the groups for glucose levels (Table I). In addition, we showed that the intake of protein was lower in the fasted condition before the intervention compared to the fed group at the same moment on the weekend day and the intake of carbohydrate increased in the fasted group after the intervention on the weekend day. Finally, the fasted group presented increased protein intake on the training day while the fed group increased lipid intake on the training day after the intervention (Figure 2c, d). It is important to highlight that although we did not impose a diet for the participants during the intervention, for all participants the intake of micro and macronutrients was balanced as recommended by the Dietary Reference Intake (Trumbo et al. 2002). Regarding the measurement instruments, it is difficult to compare research findings due to the different methodologies used to evaluate nutritional status.

Finally, it is important to state that no individual experienced any discomfort throughout the intervention period. Furthermore, the small sample size is a significant limitation. Besides that, the participants' blood was collected at least 48h after the last training session on random days. However, this fact does not entirely

Table III. **Micronutrient intake of participants between the fed and fasted conditions before and after the multicomponent training intervention on different days of the week.**

	Fed		**Fasted**	
	Pre	***Post***	***Pre***	***Post***
Calcium TD (mg)	635.7±345.3	467.0±248.6	549.7±367.0	424.6±210.3
Calcium DWT (mg)	738.0±382.6	614.1±251.5	526.5±367.9	537.6±293.3
Calcium WDWT (mg)	573.1±302.4	522.0±166.8	337.8±198.3	496.7±277.9
Zinc TD (mg)	7.6±4.0	11.6±5.8*	5.7±1.9	12.8±5.3*
Zinc DWT (mg)	8.6±2.7	9.0±6.1	7.9±3.6	9.1±5.2
Zinc WDWT (mg)	10.3±4.6	9.0±5.1	8.8±4.8	7.4±4.2
Iron TD (mg)	11.1±4.8	11.9±3.3	10.1±4.6	11.4±3.4
Iron DWT (mg)	10.2±6.1	8.5±2.3	9.3±4.2	8.9±3.2
Iron WDWT (mg)	11.0±3.9	7.5±1.6*	8.7±4.0	8.5±2.9
Folic acid TD (µg)	103.2±73.5	139.9±75.9	106.8±80.1	131.6±64.7
Folic acid DWT (µg)	93.7±47.7	102.7±64.9	121.7±49.8	133.3±77.8
Folic acid WDWT (µg)	88.1±55.5	116.1±32.9	76.7±34.9	124.8±41.3*
Sodium TD (g)	2477.8±1103.2	2707.8±650.1	2475.3±821.8	2446.6±676.0
Sodium DWT (g)	2628.0±447.0	2715.1±820.8	2454.4±626.0	2143.7±644.9#
Sodium WDWT (g)	2411.6±2381.3	2520.8±1005.4	3248.0±3006.2	4569.9±5510.4
Vitamin D TD (µg)	1.5±1.3	5.0±4.6*	2.0±1.9	4.3±4.5
Vitamin D DWT (µg)	2.4±1.5	2.2±2.7	2.5±1.5	3.4±3.2
Vitamin D WDWT (µg)	1.6±1.4	2.0±1.3	7.0±22.7	2.1±1.4
Vitamin B6 TD (mg)	1.4±0.6	1.4±0.6	1.3±0.6	1.6±0.4
Vitamin B6 DWT (mg)	1.3±0.4	1.1±0.6	1.4±0.4	1.3±0.6
Vitamin B6 WDWT (mg)	1.2±0.6	1.2±0.4	1.3±0.6	1.4±0.4
Vitamin A TD (µg)	1055.5±2190.1	442.5±401.3	412.2±212.1	2975.6±9731.1
Vitamin A DWT (µg)	587.7±370.7	310.3±215.1*	500.4±310.3	277.6±128.8
Vitamin A WDWT (µg)	292.8±361.1	386.2±388.4	205.6±94.5	299.3±235.9
Vitamin B12 TD (µg)	13.7±40.5	3.0±2.0	1.5±0.8	3.2±2.5
Vitamin B12 DWT (µg)	2.8±2.3	2.7±2.4	2.0±1.2	2.5±2.2
Vitamin B12 WDWT (µg)	3.0±2.6	2.3±1.5	3.1±2.3	3.2±1.6
Vitamin C TD (mg)	106.8±111.9	105.6±91.6	90.5±82.1	111.2±105.9
Vitamin C DWT (mg)	83.0±67.5	114.7±72.9	89.5±99.7	102.9±63.9
Vitamin C WDWT (mg)	73.8±63.8	77.2±24.8	100.4±70.5	87.3±48.3

Note: TD: training day; DWT: day without training; WDWT: weekend day without training. Two-way ANOVA for repeated measurements. *p*<0.05. Data presented as mean ± standard deviation. *against pre in the same group; $^{\#}$against fed at the same time.

put away the effect on the blood outcomes of higher energy and carbohydrates intakes during the weekends in the fasted group.

CONCLUSION

In conclusion, the current study, for the first time, shows that multicomponent training in a fasted or fed condition does not affect health parameters in physically active post-menopausal women, except for waist circumference, which reduced in both groups. These results add important information regarding the chronic effects of exercise during fasting for physically active post-menopausal women.

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