Original Article

INFLUENCE OF SOY PROTEIN INTAKE AND WEIGHT TRAINING ON THE RESTING ENERGY EXPENDITURE OF POSTMENOPAUSAL WOMEN

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ABSTRACT

OBJECTIVE. The main objective of this study was to assess the influence of soy protein intake and weight training on resting energy expenditure (REE) in postmenopausal women.

METHODS. This 160-week clinical trial enrolled 60 women, with a mean age of 59 (7) years, allocated into four groups: G1 (soy protein plus exercise), G2 (placebo plus exercise), G3 (soy protein and no exercise) and G4 (placebo and no exercise). Soy protein and placebo (maltodextrin) were randomly distributed in powder form in servings of 25 grams/day. Ten weight exercises were performed, three times a week, with three sets of 8-12 repetitions per exercise per session, at a load of 60-80% of the one-repetition maximum (1RM). The REE was calculated from the O2 and CO2 values obtained by indirect calorimetry (Quinton QMC®), performed for 30 minutes under controlled temperature and humidity. For statistical analysis, ANOVA, Student t test and multiple regression were performed with the Stata 9.2 software package. Significance was set at $p<0.05$.

RESULTS. The study sample was homogeneous with regard to all variables. Significant increases in REE ($p<0.05$) were detected in G1 (158 kcal/day, 17%) and G2 (110 kcal/day, 9%), whereas a 4% decrease was detected in G4 ($p<0.05$).

CONCLUSION. Weight training is a determining factor for increased resting energy expenditure in post-menopausal women. This effect can be boosted by dietary consumption of soy protein.

KEY WORDS: Soy protein. Exercise. Energy metabolism. Postmenopause.

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INTRODUCTION

Life expectancy is on the rise worldwide. This phenomenon is particularly pronounced in Brazil, with the average lifespan for Brazilian women now at 76 years[1]. This entails an increase in the number of women who will be going through the post-menopausal period, which currently corresponds to one-third of the female lifespan[2] and constitutes an important milestone in the cycle of life.[3].

Estrogen concentrations decline slowly and steadily from the fourth decade of life onwards, leading to changes in body composition,[4], with decreases in muscle mass and muscle strength,[5], the latter occurring even when muscle mass remains constant.[6]. Several changes in energy metabolism occur in association with declining muscle mass, including a reduction in resting energy expenditure (REE), which, added to low levels of physical activity[7] sem controle na ingestão alimentar, resulta em maior quantidade de gordura corporal, proporcionalmente[8].

Skeletal muscle is an adaptable tissue that can undergo atrophy when individuals are bedridden or afflicted with consumptive illnesses or hypertrophy when stimulated by weight training.[9,10].

Weight training induces acute and chronic physiological responses, such as hormonal changes, which play a major role in muscle anabolism[11], and also increase resting energy expenditure.[12].

Several authors[13,14] have also ascribed beneficial effects

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to consumption of soy and/or its constituents, including soy proteins and phytoestrogens (isoflavones), by women. Proteins are capable of increasing thermogenesis and, consequently, resting energy expenditure.[15]. Isoflavones—structurally and functionally estrogen-like phenol-derived compounds—are an alternative to hormone replacement therapy,[16], but their biological effect varies depending on the stage of menopause. During the postmenopausal period, when body estrogen levels decline 60% on average, estrogen receptor availability is increased; this contributes to the effect of isoflavones, which eventually makes up for the endogenous estrogen deficiency of postmenopause.[17].

The objective of this study was to assess the influence of dietary consumption of isoflavone-enriched soy protein and weight training on the resting energy expenditure (REE) of postmenopausal women.

Methods

This was a prospective, longitudinal, double-blind randomized controlled trial conducted in three stages: pre-, during, and post-intervention (soy protein consumption and weight training).

The study sample comprised 120 female volunteers who sought care at the Gynecology clinic of the Universidade Estadual Paulista Botucatu School of Medicine (FM-UNESP) or took part in the Mexa-se Pró-Saúde (roughly, "Get Moving for Health") project conducted by the FM-UNESP Center for Exercise Metabolism and Nutrition (CeMENutri). The inclusion criteria were: postmenopause (≥ one year of amenorrhea and a serum follicle-stimulating hormone [FSH] level >40 mUI/mL) [18] confirmed by a gynecologist; no physical exercise over a 3-month period prior to study enrollment; no use of hormone replacement therapy and no bone or muscle disease (for the exercise groups). Subjects were excluded if they did not complete at least 48 weight training sessions during the 16-week study period (groups 1 and 2); if bioimpedance, indirect calorimetry, blood test, or dietary recall data were missing for the pre- or post-intervention period (or pre- or post-cessation of soy protein consumption in groups 1 and 3). After sample selection, subjects were allocated into one of four groups; G1, weight training + soy protein; G2, weight training + placebo; G3, no weight training + soy protein; and G4, no weight training + placebo. Allocation to exercise or non-exercise groups was voluntary, whereas allocation to soy or non-soy ingestion groups was randomized. Of the initial sample, 60 subjects concluded all stages of the study (15 in each group), in accordance with the statistically estimated sample size[19], of 64 subjects. The study was approved by the FM-UNESP Research Ethics Committee on June 4, 2007, with ruling no. 183/2007-CEP.

The study variables were defined as resting energy expenditure, soy protein consumption, weight training, and control variables (age, FSH level, dietary protein, total calorie intake, body mass index [BMI] and muscle mass).

Soy protein and placebo (maltodextrin) were supplied as a chocolate- or vanilla-flavored powder and distributed among groups G1, G2, G3, and G4 as appropriate. Randomization was performed by a biomedical scientist using two numerical codes printed onto stickers placed on opaque plastic bags, each containing a 25-gram fraction of powder measured out by the CeMENutri nutrition team. Each 25-gram serving was enriched with 50 mg isoflavones (32 mg genistein, 15 mg daidzein and 3 mg glycitein). Each week, the investigator gave participants a packet containing daily servings for the whole week, instructed them to ingest the product throughout the day, adding it to milk, or oatmeal or blending it into smoothies, and addressed any questions or doubts.

Weight training began by determination of the maximum load for three major muscle groups (chest, back, and thighs). The subjective load for warm-up exercises, which consisted of one set of five to 10 repetitions, was set at approximately 40% to 60% of the one rep maximum (1RM); after a one-minute break, further load was added to roughly 60-80% of 1RM. Subjects performed three to five repetitions with this load, always taking care to avoid apnea. For the next load increase (presumed maximum load), subjects attempted 1RM no more than five times with a three- to five-minute recovery break between each attempt, to avoid any risk of injury [20]. After 1RM values had been determined, individual loads were set and a 16-week course of weight training was prescribed and supervised by exercise professionals. The first four weeks of the protocol were meant to equalize the physical aptitude level of all subjects. Training consisted of three weekly sessions, held every other day Monday through Friday, in the morning and afternoon, so as to meet the availability of all subjects. Each session lasted 90 minutes and included a warm-up before each exercise (10 repetitions of the exercise itself with no load) and, at the end of the session, stretching of each trained muscle group for up to 15 seconds. Ten weight exercises were selected to make up the training program: seven for the major muscle groups (three for the thighs, two for the chest and two for the back); and three for the remaining muscle groups (one for the biceps, one for the triceps and one for the abdomen). Exercises were performed in the following order, using appropriate exercise machines as needed: thighs (leg press), thighs (leg extension), thighs (leg curl), chest (bench press), chest (pec-deck fly), back (seated row), back (lat pull-down), triceps (pulley), biceps (barbell curls) and abdomen (crunches--unlike the other exercises, this part of the program comprised three sets of 30 reps each). Training progressed gradually until three sets of 8-12 repetitions at 60-80% of 1RM were achieved, with periodic adjustment to produce progressive overload and disrupt homeostasis. Subjects were instructed to take one- to two-minute breaks between sets and wait one to two seconds between repetitions.

Calculation of resting energy expenditure was based on pre- and post-intervention oxygen (O_2) and carbon dioxide (CO_2), measurements, obtained by open-circuit respiration calorimetry using the mixing-chamber method in a Quinton QMC® (Quinton Instruments, Bothell, WA). Subjects were fitted with a silicone face mask and remained in supine position, in a climate-controlled environment (temperature, 23-24 °C; relative humidity, 40-60%) for 30 minutes while calorimetry was performed. Only the last 20 minutes of the assessment were taken into account for calculation of resting energy expenditure (REE) using the equation proposed by Weir[21], with results expressed in food calories (kcal). Indirect calorimetry was performed at the Physical Aptitude Assessment Laboratory (Laboratório de Avaliação da Aptidão Física, LADAF) by qualified lab personnel and by the chief investigator. Subjects were instructed to maintain a 12-hour fast and abstain from physical exercise, consumption of alcoholic or caffeinated beverages (mate, black tea, coffee, colas or energy drinks) for 24 hours before the test, and were also instructed to remain silent, still, and awake during calorimetry.

For measurement of FSH levels, subjects underwent a 12-hour fast, after which 12-mL samples of blood were collected, by a technician wearing disposable gloves, directly into serum-separating Vacutainer™, vacuum blood collection tubes (BD Vacutainer Systems, Plymouth, UK). After collection, samples were centrifuged for 10 minutes at 3000 rpm and serum was stored at -80°C until the time of analysis. All samples were analyzed simultaneously. FSH levels were determined by electrochemiluminescence immunoassay (ECLIA) with an Elecsys® 1010/2010 automatic system (Roche Diagnostics, Mannheim, Germany). Both blood collection and FSH measurement were performed at CeMENutri by its team of laboratory technicians and biomedical scientists, before the intervention portion of the study.

Data on dietary protein intake and DCI were obtained from three-day dietary recalls, which included Sunday and two nonconsecutive days between Monday and Friday[22]; recorded before and after intervention. Protein intake and DCI were calculated by trained Dietetics students conducting undergraduate research at CeMENutri, under the supervision of the chief investigator, using the NutWin software package [23] Protein intake values were expressed as grams (g), grams per kilogram of body mass (g/kg) and percentage (%) of DCI. DCI was expressed in absolute (kcal) and relative (kcal/kg body mass) values. Mean protein intake was calculated as the arithmetic mean of pre- and post-intervention protein intake, and DCI was calculated in a similar fashion. In groups 1 and 3, 25 grams and 100 kcal were added to each subject's protein intake and DCI respectively, to account for soy protein consumption. Subjects were instructed to maintain their usual dietary habits, but exclude any other soy product from their diet.

BMI (kg/m^2) was determined from weight measurements (kg) obtained with a 150-kg capacity, 0.1-kg resolution digital platform scale (Filizola, São Paulo, Brazil) and height measurements (m) obtained with a portable stadiometer (SECA®) to 0.1 cm precision. Subjects were weighed and measured while barefoot, standing, and wearing bikinis. Muscle mass (MM) was estimated by electrical bioimpedance testing with a Maltron® BF-900 device, measuring the resistance (W) to passage of a 0.7 mA, 50 kHz current through the body by four electrodes, two placed on the right foot and two on the right hand. For bioimpedance testing, subjects were told to follow all instructions as for indirect calorimetry, and to remove any metal jewelry or accessories and void their bladders before the test as well. All measurements were performed by undergraduate Dietetics students. Resistance (R), height and age values were used in the calculation of absolute muscle mass (kg), using the equation proposed by Janssen[24], and muscle mass as a percentage of total body mass (%MM).

Histograms were used to roughly assess the normality of data. The subject profile was characterized by descriptive statistics. Between-group differences before and after interaction were assessed by the Student t test, whereas between-group comparisons, assessment of pre-intervention homogeneity and assessment of the percentage influence of the study intervention on REE were accomplished by analysis of variance (ANOVA) followed by Bonferroni correction. Association between control variables and percentage variation in REE was assessed by multiple linear regression. The Stata 9.2 statistical software package[25] was used for analysis. The significance level was set at $p<0.05$.

RESULTS

The final sample comprised 60 women, between the ages of 36 and 71, who were allocated across four intervention groups. A profile of the sample is provided in Table 1. Comparative analysis of baseline variables confirmed sample homogeneity. All subjects were overweight (BMI≥25 kg/m2), had borderline low muscle mass (≤28% MM), underreported daily calorie intake and tended to consume excessive amounts of dietary protein.

Between-group comparison of pre- and post-intervention REE (Figure 1) showed a statistically significant difference ($p<0.05$) between G1 (17% increase post-intervention) and G4 (4% reduction). Subjects in G2 experienced a 9% increase in REE, although it did not reach statistical significance.

Complementary data for Figure 1 on pre- and post-intervention REE changes in each group showed statistically significant changes ($p<0.05$) only in G1 (1,212 to 1,370 kcal, a 158 kcal increase) and G2 (1,302 to 1,412 kcal, a 110 kcal increase).

No association between control variables and percentage change in REE was detected during the intervention period (Table 2).

The study intervention was associated with statistically significant increases in muscle mass in G1 (7%), G2 (10%) and G3 (2%), but only G1 and G2 were statistically different from G4, for which no change was detected.

DISCUSSION

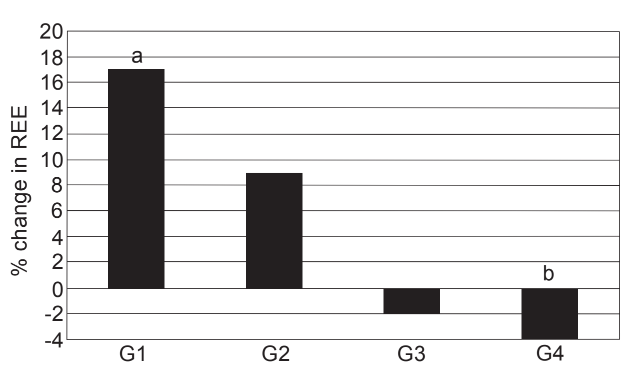
The study intervention was associated with significant increased in REE and muscle mass percentage in groups 1 and 2 (the weight training groups). Campbell et al.[26] reported a 6.8% REE increase in a sample of 12 subjects (eight male, four female) between the ages of 56 and 80 after 12 weeks of weight training. Although a 1.4-kg increase in fat-free mass

Table 1 - Study variables (mean +/- SD) at baseline (pre-intervention)

VARIABLE	G1	G2	G3	G4	p
Age (years)	58 (7)	57 (9)	60 (5)	60 (7)	0.7
Body mass (kg)	68 (12)	69 (13)	72 (17)	66 (10)	0.6
Height (cm)	156 (7)	158 (4)	157 (5)	155 (6)	0.7
BMI (kg/m2)	28 (4)	28 (5)	29 (6)	27 (4)	0.7
FSH (mIU/mL)	82 (43)	80 (32)	105 (36)	104 (44)	0.1
MM (%)	28 (3)	28 (4)	27 (4)	27 (3)	0.9
DCI (kcal)	1302 (499)	1308 (278)	1518 (672)	1554 (602)	0.4
DCI (kcal/kgBM)	20 (8)	19 (4)	23 (14)	24 (10)	0.4
Protein (g)	60 (21)	59 (39)	73 (31)	66 (26)	0.5
Protein (g/kgBM)	0.9 (0.3)	0.9 (0.5)	1.1 (0.6)	1.4 (0.5)	0.5
Protein (%)	19 (5)	17 (7)	19 (6)	17 (5)	0.5
REE (kcal)	1212 (322)	1302 (153)	1432 (290)	1435 (212)	0.05

BM, body mass; BMI, body mass index; DCI, daily calorie intake; FSH, follicle-stimulating hormone; MM, muscle mass; REE, resting energy expenditure.

Figure 1 - Influence of soy protein intake or placebo and weight training on percentage change in resting energy expenditure (REE) of postmenopausal women



a, b – between-group difference (p<0.05)

was detected (as measured by dual-energy X ray absorptiometry, DEXA), it was due to water retention and was unrelated to increased REE. Likewise, Treuth et al.[27] found a 9% increase in REE in a sample of 13 women (mean age, 67 years) after 16 weeks of weight training, but no changes in lean body mass. Although the sample profiles were similar to those of the present study, the authors did not measure muscle mass, but lean body mass and fat-free mass, which includes other body constituents (such as bone tissue and water); furthermore, one of the studies had a very short intervention period (12 weeks). In a study by Silva et al.[28], comparing the effect of 12 weeks of weight training on muscle strength and body composition in 30 women (mean age, 61.1 ± 7.3 years), found no changes in bioimpedance-estimated lean body mass.

A recent study[29] using similar methods to those reported above but enrolling a sample of younger (mean age, 21 ± 0.5 years), sedentary men and women also found a 7% increase in REE after six months of weight training. Lemmer et al.[30] also found a 7% increase in REE after 24 weeks of weight training, regardless of gender or age. Several studies[31,32,12], assessing the effect of weight training on REE have reported increases in energy expenditure, lean body mass, fat free mass, and even muscle mass. However, changes in resting energy expenditure may be due to factors independent of lean body mass, the main such factor being muscle mass, which has led to increased research interest in other components of energy expenditure, including genetic factors[33], and dietary aspects,

Table 2 - Association between control variables and percentage change in resting energy expenditure (REE) by intervention (soy protein vs. placebo, with or without weight training)

	% CHANGE IN REE			
VARIABLE	Coefficient	p	95% confidence	interval
G1	23.4	<0.05	8.0	38.8
G2	15.6	<0.05	0.1	31.2
G3	1.7	0.8	-13.4	16.7
Age (years)	-0.3	0.5	-1.3	0.7
FSH (mIU/mL)	0.02	0.8	-0.1	0.2
DCI (kcal/kgBM)	0.5	0.5	-0.8	1.7
Protein (g/kgBM)	-6.1	0.6	-27.8	15.6
BMI (kg/m2)	-0.1	0.9	-2.1	1.9
MM (%BM)	-1.4	0.3	-4.1	1.3

BM, body mass; BMI, body mass index; DCI, daily calorie intake; FSH, follicle-stimulating hormone; G1-G3, groups 1 through 3; MM, muscle mass; REE, resting energy expenditure. p<0.05 = statistically significant.

such as protein intake[15,34].

In this study, muscle mass increased in the weight training groups; however, group 1, whose subjects received soy protein in addition to exercise, showed the greatest increase in REE (8% higher than G2) despite a lesser increase in muscle mass. Protein intake increases diet induced thermogenesis (DIT) and, consequently, energy expenditure[15]. This effect, which corresponds to 20-30% of DIT, is due to protein synthesis, consumption of adenosine triphosphate (ATP) for peptide bond formation, as well as other aspects of increased protein turnover associated with higher protein intake[35]. Low-calorie, high-protein diets reportedly allow preservation of lean body mass while contributing to fat loss, increasing insulin sensitivity, particularly in people with obesity or diabetes[36].

In a study by Mikkelsen et al.[37], replacement of 17-18% of dietary carbohydrate intake with pork or soy protein led to a 3% increase in 24-hour energy expenditure in healthy overweight subjects. In addition to protein, soy stands out among the legumes due to its high content of phytoestrogens, including isoflavones[16], which have been investigated (among other reasons) for their stimulating effect on production of thyroxine, a potent mediator of energy metabolism[38]. Isoflavones also appear to assist in reducing body fat by decreasing lipogenesis and increasing lipolysis[39], which contributes to a proportional increase in muscle mass.

Although the scientific literature has pinpointed the variables included in this study (age, menopause[8], decreased muscle mass[12] and protein intake[15]) as risk factors for REE, we found no association between these variables as measured at baseline and increases in REE due to the study intervention. That is, older women with a longer menopausal period, lower muscle mass and higher protein intake may not necessarily show a greater response in REE. Some factors must be taken into account when considering the findings of this study, however. No special dietary recommendations were prescribed; due to the length of the intervention period and to the sample size, subjects followed their usual diet. Furthermore, subjects were allocated to the exercise groups on a purely voluntary basis, as adherence to and maintenance of an exercise protocol requires motivation. Of the 30 women between the ages of 36 and 69 studied by Prado[40], 100% regarded physical exercise as essential to proper health; however, the obstacle to exercise mentioned most often was a lack of willpower (96.7%).

Both weight training and soy protein intake may thus aid maintenance of REE through advancing age and menopause, and may also provide an alternative to hormone replacement therapy in many women. In a study conducted by Valadares et al[41] with 378 women between the ages of 40 and 65, 16% reported that physicians feared to or lacked the confidence to prescribe hormone replacement therapy. On the other hand, men and women between the ages of 18 and 40 reportedly have a positive opinion of soy and soy products[42] and of the benefits of weight training[43,44], but, according to Behrens et al.[42], still consume little soy or soy-derived compounds.

CONCLUSION

Weight training is a determining factor of increased resting energy expenditure in postmenopausal women. This effect can be boosted by dietary consumption of soy protein.

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