

Effect of 6 months of exercise and isoflavone supplementation on clinical cardiovascular risk factors in obese postmenopausal women: a randomized, double-blind study

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Abstract

Objective: To investigate whether 6 months of exercise combined with isoflavone supplementation could improve clinical risk factors that predispose to cardiovascular disease in obese postmenopausal women.

Design: This was a randomized, double-blind, controlled trial in which 50 healthy obese postmenopausal women were divided into two groups and assigned to isoflavone supplementation (n = 25) or a placebo (n = 25) for 1 year. For the last 6 months, both groups participated in an exercise program (three times per week), at the end of which cardiovascular disease risk factors were compared between groups. Body composition (using dual-energy x-ray absorptiometry), metabolic profile (blood lipids, fasting insulin, fasting glucose, sex hormone-binding globulin, C-reactive protein) were determined at baseline and at 6 and 12 months.

Results: We observed a significant effect of exercise and isoflavone supplementation on body weight, total and abdominal fat mass (kilograms and percentage), body mass index, appendicular fat-free mass, fat-free mass/fat mass ratio, and sex hormone-binding globulin, but not with exercise alone. No difference was observed for other biochemical characteristics, although the quantitative insulin sensitivity check index increased equally in both groups. Conversely, although not significant, we observed a tendency for a treatment effect on body mass index ($P = 0.07$) and on absolute (kilograms) ($P = 0.07$) and percentage of ($P = 0.053$) abdominal fat mass, whereas no effect of treatment was found for other variables using the Mann-Whitney test.

Conclusions: Compared to an aerobic exercise program alone, 70 mg/day of isoflavones combined with exercise may promote significant improvements in body composition parameters that are known to influence cardiovascular disease risk in postmenopausal women.

Key Words: Isoflavones – Cardiovascular disease – Obese – Menopause – Body composition – Biochemical parameters.

Menopause is associated with alterations in body composition, such as a gain of fat mass (FM), essentially at the abdominal level, which increases cardiovascular disease (CVD) risks,^{1,2} and a loss of fat-free mass (FFM), which leads to sarcopenia.³ Moreover, it is known that these menopause-associated changes lead to an increase in lipid profile^{4,5} and type 2 diabetes.^{6,7}

It has been shown that hormone therapy (HT) can prevent the gain of FM,³ the alterations in lipid profile⁸ and insulin

sensitivity,⁹ and the loss of FFM.^{10,11} Conversely, the role of physical activity to counteract the changes in body composition has been well established.¹² Moreover, it has been demonstrated in observational and interventional studies that HT combined with an exercise program can produce a synergistic effect on FM, FFM, and insulin sensitivity.^{6,13,14} Nevertheless, these results are controversial.¹⁵ Furthermore, the Women's Health Initiative study showed that the combination of estrogen and progestin does not confer cardiac protection and may increase the risk of CVD among generally healthy postmenopausal women.¹⁶ Obviously, the use of HT has thus become controversial.

Phytoestrogens are plant-derived substances that are structurally comparable to 17 β -estradiol and that may have estrogenic properties because of their high affinity for the β estrogen receptors and, to a lesser extent, for the α estrogen receptors. In this sense, soybean is a rich source of isoflavones, such as genistein and daidzein, which show fewer estrogenic effects on several organs.¹⁷ In some metabolic studies,¹⁸⁻²⁰ but not all,²¹⁻²⁴ soy that naturally contains isoflavones has been found to exert lipid-lowering effects. Other favorable cardiovascular effects of soy or

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isoflavone supplementation have also been observed, such as beneficial effects on vasodilatation and arterial compliance²⁵ and a favorable effect on fasting glucose and insulin levels in humans.²⁶ Furthermore, some studies,^{27,28} but not all,^{24,26,29,30} have demonstrated a beneficial effect on body composition of ingesting isoflavones for 6 months in postmenopausal women with a normal body mass index (BMI). However, some were observational studies of the effect of a diet rich in isoflavones, and therefore isoflavones were not used as a supplement.^{28,31} Moreover, the discrepancies between the results may be due to the variation in factors such as the duration of intervention, whether isoflavones were part of a diet or added as a supplement, and the fact that in some studies the diet was strictly vegetarian (high protein).

Until now, only one study has examined the effect of isoflavones combined with exercise. Wu et al³² studied the effect of 1 year of isoflavone supplementation (75 mg/d) and exercise (walking three times per week) in healthy postmenopausal Japanese women and observed an effect of the combined interventions on trunk FM and high-density lipoprotein cholesterol. Nevertheless, it is important to note that Japanese women have a diet that includes regular ingestion of isoflavones.

Hence, to our knowledge, no study in Western women has examined whether isoflavone supplementation combined with an aerobic exercise program could have an effect on body composition (more specifically on abdominal and total FM), lipid profile, and insulin sensitivity, which are important clinical factors that predispose to CVD risks, specifically in obese postmenopausal women. Thus, the purpose of this randomized, double-blind, controlled trial was to investigate

whether 6 months of exercise combined with isoflavone supplementation could improve clinical risk factors that predispose obese postmenopausal women to CVD.

METHODS

Participants

Fifty-six postmenopausal women ages 50 to 70 years (58 ± 5 y) were recruited through advertisements in a local newspaper to participate in this double-blind, controlled trial. Of these, 50 women were randomly assigned to one of two groups, isoflavones (ISO) or placebo (PLA). To be included in the study, women had to meet the following criteria: obese (total FM >35%), healthy, no major physical disability, not taking HT (at the time of the study, women had never taken HT or had stopped HT for at least 1 y), sedentary, weight stable (± 2 kg) for the past 6 months, nonsmoker, moderate drinker (maximum of 15 g of alcohol, the equivalent of one alcoholic beverage, per day), no medication that could influence glucose or lipid metabolism, no medical contraindication to participate in an exercise program, and absence of menses for the past 12 months.

Overview of experimental protocol

A telephone interview was conducted to screen for the aforementioned inclusion criteria. After the nature and goals of the study were thoroughly explained to the women, they provided written informed consent. After screening, the women underwent metabolic testing (baseline) at the Research Centre on Aging (Geriatric Institute of the University of Sherbrooke). Upon arrival, a 12-hour fasting blood

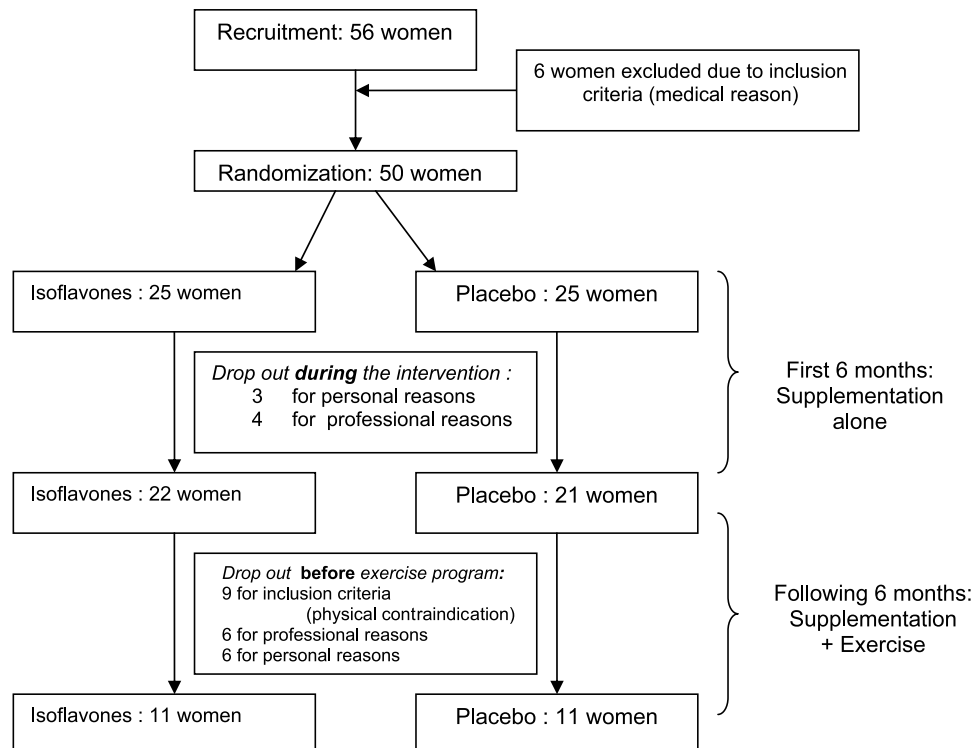


FIG. 1. Study design.

TABLE 1. Physical characteristics before and after interventions (mean \pm SD)

Variables	ISO (n = 11)			PLA (n = 11)		
	Baseline	6 mo	12 mo	Baseline	6 mo	12 mo
Weight (kg)	79 \pm 16	78 \pm 17	75 \pm 16 ^a	77 \pm 7	76 \pm 5	76 \pm 5
BMI (kg/m ²)	30 \pm 5	30 \pm 6	28 \pm 4 ^a	30 \pm 2	30 \pm 3	30 \pm 2
Total FM (kg)	34.5 \pm 9.5	33.8 \pm 10.2	31.6 \pm 10.7 ^a	33.2 \pm 5.9	33.0 \pm 5.3	31.9 \pm 4.8
Abdominal FM (kg)	16.7 \pm 5.5	16.6 \pm 6.2	14.8 \pm 7 ^a	15.93 \pm 3.5	16.3 \pm 3.3	15.54 \pm 3.2
Total FM (%)	45.8 \pm 3.5	44.9 \pm 4.2	42.8 \pm 4.4 ^b	44.7 \pm 4.8	44.9 \pm 4.8	43.5 \pm 4.3
Abdominal FM (%)	46 \pm 5	45.3 \pm 6.1	42.4 \pm 5 ^a	44.7 \pm 5	45.4 \pm 6.1	44.4 \pm 5
Appendicular FFM (kg)	18 \pm 2.2	18.1 \pm 2.4	18.9 \pm 1.8 ^a	18.4 \pm 1.8	17.8 \pm 1.6	19.0 \pm 1.9 ^a
FFM/FM ratio (kg)	1.2 \pm 0.18	1.2 \pm 0.2	1.3 \pm 0.2 ^b	1.3 \pm 0.27	1.2 \pm 0.2	1.3 \pm 0.2

ISO, isoflavone; PLA, placebo; BMI, body mass index; FM, fat mass; FFM, fat-free mass. Appendicular fat-free mass = sum of leg and arm fat-free mass. Mann-Whitney test/Wilcoxon test.

^a*P* < 0.05, significantly different from baseline values.

^b*P* < 0.01, significantly different from baseline values; clinically significant.

sample was obtained, and measurements of body composition were performed. After this first visit, women were randomized to the PLA or ISO group. The double-blind randomization was done by Arkopharma Ltd. For the next 6 months, women received isoflavone or placebo supplementation and returned each month to receive a new container of capsules. At 6 months, women underwent a second metabolic evaluation. During the next 6 months, half of the women continued the study and participated in a weight loss exercise program, after which they came back for a final metabolic evaluation (Fig. 1). Among the women who were excluded from the study, 9 were excluded because they had a physical contraindication to exercise, and 12 dropped out for professional or personal reasons (in total, there were 11 in the ISO group and 10 in the PLA group who dropped out prior to the exercise phase of the study). All procedures were approved by the Ethics Committee of the Geriatric Institute of the University of Sherbrooke.

Isoflavone supplementation

Participants received either four isoflavone or four placebo capsules daily for 12 months. Each capsule contained 17.5 mg of isoflavones extracted from natural soy. The total dose of isoflavones was thus 70 mg/day, which corresponded to 44 mg of diadzein, 16 mg of glycitein, and 10 mg of genistein. This supplementation cannot be considered protein supplementation but only isoflavone supplementation. Identical active and PLA capsules were supplied by Arkopharma Ltd. (Carros, France).

Weight loss exercise program

All women participated in a weight loss exercise program that consisted of three 1-hour sessions per week. Each session consisted of aerobic exercise, such as a step workout, circuit training, or a fast walk. All sessions were supervised by a kinesiologist. To be included in the final analyses, women had to have participated in 85% of the sessions. We attempted to maintain exercise intensity between 65% and 75% of maximal heart rate. The maximal heart rate was calculated from the carotid pulse using the following equation: 220 - age. The maximal heart rate was then multiplied by 65% and 75% to obtain the target heart rate range.

Body composition measurements

Body weight (in kilograms; \pm 0.2 kg) was determined using an electronic scale (SECA707, Hamburg, Germany). Height was measured using a tape measure fixed to the wall with the participant in stocking feet. FM and FFM was determined with the woman in a supine position using dual-energy x-ray absorptiometry (GE Prodigy Lunar, Madison, WI). In our laboratory, the coefficients of variation for repeated measures of FM and FFM in 10 adults (measured 1 wk apart) are 4.7% and 1.1%, respectively.³³ FFM is defined here as the mass of tissue representing soft tissue exclusively (mineral body mass excluded).

Blood collection and biochemical analyses

Blood samples were obtained in the morning after a 12-hour fast. Venipuncture was done with the participants in a sitting position. Venous blood was drawn and placed in Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ).

Plasma lipid profile (high-density lipoprotein, low-density lipoprotein, total cholesterol, total cholesterol/high-density lipoprotein, triglycerides), plasma glucose, and C-reactive protein were analyzed immediately in the clinical laboratory of the Geriatric Institute. Sex hormone-binding globulin (SHBG) and plasma insulin were analyzed at the Sherbrooke University Hospital Center.

Insulin sensitivity

Insulin sensitivity (IS) was assessed indirectly with the quantitative IS check index (QUICKI) using 12-hour fasting glucose and insulin values with the following formula: $1/\{\log [\text{fasting insulin (mU/mL)}] + \log [\text{fasting glucose (mg/dL)}]\}$.³⁴ Not only does QUICKI correlate with the IS derived from the hyperinsulinemic-euglycemic clamp technique, but it has also been shown to be a good predictor of IS.³⁵⁻³⁷ As such, QUICKI provides an estimate of IS with a variability and discriminant power comparable to that of the hyperinsulinemic-euglycemic clamp technique.³⁸

Statistical analyses

Results are presented as mean \pm SD. At baseline, the two groups were compared by nonparametric Mann-Whitney tests for all body composition and metabolic profile

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variables. Nonparametric Wilcoxon tests were used to verify the effect of treatment after 6 months of isoflavone supplementation for all variables. Thereafter, a nonparametric Mann-Whitney test was used to examine the effect of the intervention for each variable in each group. Nonparametric Wilcoxon tests were used to verify the effect of the exercise program combined with isoflavone supplementation for all variables. *P* values below 0.05 were considered statistically significant. Analyses were performed using the SPSS 11.0 program (Chicago, IL).

RESULTS

At baseline, the ISO and PLA groups were similar in age (57 ± 5 vs 58 ± 5 y), years of menopause (8 ± 6 vs 9 ± 7 y), history of obesity (9 women in each group were nonobese before menopause), and all other physical characteristics (Table 1). Moreover, no differences were found between groups for metabolic characteristics except for fasting plasma insulin ($P = 0.04$). In fact, the ISO group had a lower level of fasting plasma insulin (53.29 vs 74.10 pmol/L) than the PLA group at baseline (Table 2).

We first examined data after 6 months of isoflavone supplementation or placebo and found no effect of treatment for any variables. After the exercise program, however, we found significant changes in body weight, BMI, total and abdominal FM (kg and %), FFM/FM ratio, and SHBG for the ISO group but not for PLA group (Table 1). A significant increase was observed for both groups for QUICKI, whereas no difference was observed in either group for all other metabolic parameters (Table 2).

Considering the previous findings, we looked for treatment effects. Although not significant, we observed a tendency for a treatment effect on BMI ($P = 0.07$) and absolute ($P = 0.07$) and relative abdominal FM ($P = 0.053$), whereas no effect of treatment was observed for other variables. The lack of statistical significance may have been due to the small sample size at the end of the 12 months.

The aim of this study was to investigate whether the combination of exercise and isoflavone supplementation could improve clinical risk factors that predispose obese postmenopausal women to CVD. The major findings of this study were the significant effects on body weight, total and abdominal FM (kg and %), BMI, appendicular FFM, FFM/FM ratio, and SHBG in the ISO group (but not in the PLA group). No difference was observed for other biochemical characteristics, although QUICKI increased equally in both groups. We observed a tendency for a treatment effect on BMI ($P = 0.07$) and abdominal FM (kg, $P = 0.07$); %, $P = 0.053$) with the combination of exercise and isoflavone supplementation. The lack of effect may have been due to the small sample size at the end of the study, which could have prevented statistical significance from being reached.

Important changes in body composition occur during the menopausal period. These changes include a decrease of FFM and an increase of FM that may be modulated by HT, exercise, or a combination of both.³⁹ Conversely, two studies have reported that soy isoflavone supplementation and moderate exercise can prevent FM gain in ovariectomized mice⁴⁰ and, recently, in Asian women.³² In fact, Wu et al³² found that 1 year of isoflavone supplementation combined with walking decreased the percentage of trunk FM in normal weight women, which is in accordance with our observations in Western obese postmenopausal women. These results may be explained by the fact that estrogens modulate central body fat deposition in women.⁴¹

Conversely, it is important to note that Japanese women, as compared to Western women, have a diet that includes large amounts of isoflavones. In fact, Asians consume a diet that is higher in isoflavones (average: 44.4 to 49.4 mg/d) than the diet of Western women.²³ Thus, without any treatment, the amount of isoflavones ingested by Asian women is almost as high as the amount of isoflavones ingested by Western women during supplementation. However, to our knowledge, ours is the first study to observe an

TABLE 2. Metabolic profile before and after interventions (mean \pm SD)

Variables	ISO (n = 10) ^a			PLA (n = 10) ^a		
	Baseline	6 mo	12 mo	Baseline	6 mo	12 mo
Glucose (mmol/L)	4.8 \pm 0.4	4.7 \pm 0.4	4.9 \pm 0.2	5.1 \pm 0.5	5.0 \pm 0.4	5.0 \pm 0.4
Insulin (pmol/L)	46 \pm 11 ^b	38 \pm 11	41 \pm 15	81 \pm 42	62 \pm 54	58 \pm 22
Insulin sensitivity (QUICKI)	0.15 \pm 0.07	0.29 \pm 0.01	0.37 \pm 0.02 ^c	0.14 \pm 0.01	0.27 \pm 0.02	0.35 \pm 0.02 ^c
LDL-C (mmol/L)	3.31 \pm 0.79	3.29 \pm 0.59	3.38 \pm 0.79	3.41 \pm 0.80	3.39 \pm 0.53	3.59 \pm 0.92
TG-C (mmol/L)	1.31 \pm 0.77	1.31 \pm 0.64	1.20 \pm 0.47	1.43 \pm 0.68	1.55 \pm 1.07	1.31 \pm 0.45
TC (mmol/L)	5.40 \pm 0.82	5.26 \pm 0.54	5.45 \pm 0.83	5.63 \pm 0.77	5.54 \pm 0.83	5.78 \pm 0.93
HDL-C/TC (mmol/L)	3.82 \pm 1.18	4.01 \pm 1.13	3.71 \pm 1.00	3.81 \pm 1.14	4.11 \pm 1.39	3.84 \pm 1.28
HDL-C (mmol/L)	1.48 \pm 0.31	1.37 \pm 0.25	1.52 \pm 0.25	1.55 \pm 0.32	1.44 \pm 0.38	1.59 \pm 0.35
SHBG (nmol/L)	29.62 \pm 12.17	31.36 \pm 15.58	44.22 \pm 21.38 ^c	30.09 \pm 16.04	33.13 \pm 23.26	35.57 \pm 23.58
CRP (mg/L)	4.5 \pm 3.9	6.3 \pm 2.9	4.2 \pm 5.4	2.5 \pm 3.5	3.8 \pm 2.1	3.8 \pm 4.2

ISO, isoflavone; PLA, placebo; QUICKI, quantitative insulin sensitivity check index; LDL-C, low-density lipoprotein cholesterol; TG-C, triglyceride cholesterol; TC, total cholesterol; HDL-C/TC, high-density lipoprotein/total cholesterol; SHBG, sex hormone-binding globulin; CRP, C-reactive protein. Mann-Whitney test/Wilcoxon test.

^aBlood was not drawn from one woman in the ISO group and one woman in the PLA group.

^bSignificantly different from PLA at baseline; $P < 0.05$.

^c $P < 0.05$ significantly different from baseline values.

effect on body composition in Western obese postmenopausal women when isoflavones are combined with an aerobic exercise program. Thus, our results are interesting because they support the idea that isoflavones combined with exercise can decrease cardiovascular risk in obese Western women.

We also found that the ISO group, after 6 months of exercise, lost a significant amount of body weight (5%). It is now known that a loss of 5% to 10% of initial body weight is effective at significantly and clinically improving the metabolic profile of older obese individuals.⁴²⁻⁴⁴ Furthermore, it is important to note that in women taking isoflavones, appendicular FFM increased significantly and FM decreased after 6 months of the exercise program. In this sense, it has been demonstrated that a diet-induced loss of body weight normally engenders a loss of FM as well as FFM.^{43,44} However, the intervention used in this study both decreased CVD risk and prevented the loss of FFM associated with weight loss.

It has been demonstrated that circulating estradiol increases the SHBG level in the liver.⁴⁵ We also observed a significant increase in SHBG in the ISO group but not in the PLA group ($P = 0.025$), likely due to the estrogenic properties of isoflavones. This may explain the loss of FM since the SHBG level increases with isoflavone-rich food (soy) consumption in postmenopausal women⁴⁶ and is inversely associated with body fat.³¹ Furthermore, it has been demonstrated that the level of SHBG is inversely related to the level of insulin and insulin resistance.^{47,48} Nevertheless, further studies are required to clarify the mechanisms leading to these effects.

Our study had several limitations. Because the isoflavone supplementation was started 6 months before the exercise program, it cannot be ruled out that the potential effect of isoflavones in combination with exercise is mainly or partly due to the longer duration of isoflavone supplementation. Hence, further research using randomized, controlled trials is needed to confirm our findings. Moreover, participants, even if obese, had normal lipid and insulin levels (although groups had significantly different IS indexes [QUICKI], they were all in the range of normal values), which could explain the absence of effect on these variables. Another hypothesis could be that a greater duration of treatment was needed to observe a significant effect on the metabolic profile, especially in healthy women. Finally, the high dropout rate, before the start of the exercise program, led to the study ending with a small sample. Thus, the numerous analyses conducted with the small sample may have increased the risk of type I error. However, our results are in accordance with the results of Wu et al³² who demonstrated that isoflavone supplementation combined with regular walking has more effect on FM than either one of these interventions alone. Unfortunately, the small sample size may have prevented us from detecting a treatment effect. Despite the risk of type I error, our results suggest a potential synergistic effect between isoflavone

supplementation and exercise that deserves to be further studied.

CONCLUSION

In conclusion, our results strongly suggest that 70 mg/day of isoflavones combined with an aerobic exercise program provides significant improvements in body composition parameters that are known to influence CVD risk in postmenopausal women. To our knowledge, this is the first study to examine the combination of these interventions in obese Western postmenopausal women. Nonetheless, further studies with more robust designs are necessary to confirm our findings.

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