Impact of flaxseed and soy nuts as dietary supplements on lipid profile, insulin sensitivity and GLUT4 expression in ovariectomized rats

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Impact of flaxseed and soy nuts as dietary supplements on lipid profile, insulin sensitivity and GLUT4 expression in ovariectomized rats

Phytoestrogens, lipid profile and GLUT4

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ABSTRACT

We assessed the effects of a diet with flaxseed or soy nuts *versus* estradiol on lipid profile, insulin sensitivity, and GLUT4 expression in ovariectomized female rats. Forty-four Wistar female rats (90-days old) underwent ovariectomy were divided: C (standard diet); E (standard diet+subcutaneous 17 β -estradiol pellets); L (standard diet+flaxseed+subcutaneous placebo pellets); and S (standard diet+soy nuts+subcutaneous placebo pellets). Customized diets and the insertion of pellets were started 21 days after ovariectomy, and maintained for another 21 days. We measured body weight, insulin tolerance, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and GLUT4 (cardiac and adipose tissues). We found lower body weight and Lee index in group E; a trend for improved insulin sensitivity in S ($p=0.066$). Groups L and S showed lower lipid profile *vs.* C. Microsomal GLUT4 increased in L (cardiac and adipose tissues); plasma membrane GLUT4 increased in E, L and S (both tissues). We conclude that flaxseed and soy nuts as dietary supplements improve lipid profile and they increased GLUT4 expression.

Key-words: flaxseed, soy nuts, insulin resistance, GLUT4, ovariectomy.

1. INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality in menopausal women (Yusuf et al. 2001). As women age, their risk of cardiovascular disease and stroke rises, a risk that is even higher in women who have undergone early menopause or surgical menopause, especially if other risk factors are present. Mechanisms of cardiovascular disease in menopausal women are similar to men, and involve effects of estrogen's deficiency, such as endothelial dysfunction, increased body mass index, increased risk for the development of metabolic syndrome (Janssen et al. 2008), increased insulin resistance (Whitcroft and Herriot 2011), and cardiac changes related to oxidative stress (Al-Nakkash et al. 2010).

For many years, the prevailing belief has been that hormone therapy protects the heart, but cardioprotective effects of this therapy have been challenged by well-designed clinical trials. Thromboembolic events, coronary artery disease, stroke, and breast cancer have been reported in clinical trials evidencing that hormone therapy can be more harmful than previously expected (Herrington et al. 2000, Rossouw et al. 2002). Given its potential drawbacks, alternatives to hormone replacement therapy such as phytoestrogen supplements have been investigated to treat menopausal symptoms (Ricci et al. 2010, Lethaby et al. 2013).

Phytoestrogens are a diverse group of compounds that structurally and functionally mimic mammalian estrogens (Turner et al. 2007). The most widely researched phytoestrogens are soy isoflavones (genistein, daidzein and glycitein) and flaxseed lignans (secoisolariciresinol). Potential benefits were previously shown for human health, such as anticarcinogenic effect, cardioprotection, and reversal of some menopausal symptoms or deleterious effects of genotoxicity (Di Virgilio et al. 2004). Insulin resistance reduction by phytoestrogens is still controversial. A systematic review with meta-analysis of randomized clinical trials that examined the effect of phytoestrogens on glucose metabolism in perimenopausal and postmenopausal women did not find any changes in blood glucose and insulin levels despite the wide variety and doses of isoflavones investigated

across studies. However, when compared to placebo, isolated genistein had an effect of reducing fasting blood glucose (Ricci et al. 2010).

The literature data are still not consistent with regard to the association between estrogen deprivation and changes in the glucose transporter type 4 (GLUT4). Insulin resistance may result from changes in the content and translocation of GLUT4 (Thorens 1996, White 2003). Results from ovariectomized female rats showed no changes in total GLUT4 content in muscle and adipose tissues. Moreover, no change in GLUT4 was observed in rats treated with both physiological and supraphysiological doses of estrogen, but not with progesterone (Campbell and Febbraio 2002). Oral phytoestrogens (red clover based) were indeed associated with reduced insulin sensitivity in postmenopausal women in one study (Lee et al. 2012), but other report showed improvement in insulin sensitivity by phytoestrogens (daidzein), as shown by GLUT4 overexpression *in vitro* and in obese-diabetic mice (Cheong et al. 2014). However, women with polycystic ovary syndrome have insulin resistance and a reduced content of GLUT4 in adipose tissue, regardless of obesity (Rosenbaum et al. 1993).

Although dietary flaxseed protein and soy protein may also have effects on some cardiovascular risk factors (Bhathena et al. 2002, Bhathena and Velasquez 2002), taking into consideration that insulin resistance can occur during menopause, adverse effects of traditional hormone replacement therapy and the potential of phytoestrogens as an alternative, this study aimed to examine the effects of a diet with flaxseed or soy nuts *versus* estradiol on lipid profile, blood pressure, insulin sensitivity, and GLUT4 expression in ovariectomized female rats.

2.MATERIAL AND METHODS

2.1 Ethical Standards

Our study complied with the ethical principles of the Use of Laboratory Animals and Canadian Council. Furthermore, the study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre, protocol #09-510. All animals were bred and kept under standard laboratory animal house conditions at the Animal Production and Research Unit of the Center for Scientific and Technological Development of *Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul*, Brazil. They were kept in special cages (4 rats/plastic cage) exposed to a 12-hour light and 12-hour dark cycle (6 a.m./6 p.m.) in a 20-25°C temperature-controlled room, and provided water *ad libitum*.

2.2 Animals and Groups

Forty-four Wistar female rats (90-days old) were ovariectomized at the beginning of the experiments: the animals were anesthetized by an intraperitoneal injection of 0.2 ml/100g body weight with ketamine (80 mg/kg body weight) + xylazine (10 mg/kg body weight), and a small abdominal incision was made; the ovaries were identified and excised connected. After that, a suture was performed, followed by analgesia (butorphanol 2 mg/kg, subcutaneous, 4 times for 24h). Customized diets (fully described in Table 1) and the insertion of pellets were started 21 days after ovariectomy, and maintained for another 21 days, when metabolic and cardiovascular evaluations were done. The pellets (0.03 mg/day; Innovative Research of America, Sarasota, Florida, USA) were inserted with the aid of a trocar, under anesthesia, as above described. Four groups were considered, according to interventions: C (n=12) received standard diet + placebo pellet; E (n=10) received standard diet + 1,5mg 17β-estradiol pellet; L (n=12) received flaxseed diet + placebo pellet; S (n=10) received soy nuts diet + placebo pellet.

2.3 Procedures

At the end of treatments, a Lee Index was calculated according to the formula: (body weight^{1/3}/naso anal length) (Machado et al. 1993). Also, an insulin tolerance test was performed as

proposed by Mori et al. (Mori et al. 2008) using commercially available regular insulin (Humulin, Eli Lilly, São Paulo, Brazil). Animals were anesthetized and insulin (0.75 U/kg) was administered into the tail vein. This test comprises six blood glucose measures using test strips (Accucheck Advantage, Roche, Mannheim, Germany) at baseline (before insulin administration), 4, 8, 12, 16, and 20 min post-insulin application. The rate constant for blood glucose disappearance (kITT) per min ($\% \cdot \text{min}^{-1}$) was then calculated.

After the insulin tolerance test, direct measurement of blood pressure (BP) was performed. A catheter filled with saline was implanted into the femoral artery (PE-10), under anesthesia. One day after catheter placement, the arterial cannula was connected to a strain-gauge transducer (P23Db, Gould-Statham, Oxnard, CA) and BP and heart rate (HR) signals were recorded (rats were conscious) during a 30-min period with a microcomputer equipped with an analog-to-digital converter board (CODAS, 2-kHz sampling frequency, Dataq instruments, Inc., Akron, OH).

One day after BP recording, rats were fed normally and then anesthetized with a high dose of pentobarbital sodium (100mg/kg), and a thoracotomy and cardiac puncture to draw and collect blood was made. After, epididymal white adipose tissue, uterus and heart were removed, washed with saline solution, to then be stored in a freezer -80. The heart and epididymal white adipose tissue were homogenized to GLUT4 analyses as previously described (Machado et al. 1993).

The protein concentration was determined using the Bradford method and GLUT4 expression was analyzed by Western blot, according to the standard procedure in our laboratory (Machado et al. 1994, Lehen et al. 2011). Briefly, 100 μg of each sample were solubilized in NuPAGE buffer (Invitrogen, Carlsbad, USA) and undergoes electrophoresis separation (SDS-PAGE, 10%). Proteins were then transferred to a Hybond ECL nitrocellulose membrane (Ge Healthcare, New York, USA) and incubated with anti-GLUT4 antibody (Millipore, Billerica, USA) titrated to 1:2000 overnight at 4° C followed by an additional 3-h incubation at 37° C. After, incubation for 1h with a secondary antibody, 1:12000 goat anti-rabbit IgG (Millipore, Billerica, USA) was performed. Blot intensity was quantified by optic densitometry using a public domain

software, Scion Image. For tissue final analysis, the membranes were reprobated with anti- β -actin antibody (Monoclonal anti- β -actin antibody AC-74, A2228, Sigma-Aldrich), GLUT4 values were normalized by the respective β -actin value, and results were expressed as arbitrary units (AU/ μ g protein).

Total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides concentrations were analyzed using commercial kits (LabtestW, Lagoa Santa, MG, Brazil); reading was performed through spectrophotometry (Spectramax, Molecular Devices Corporation, Sunnyvale, CA, USA) and compared to a standard curve provided by kits.

2.4 Statistical Analysis

Our sample size was determined by the feasibility of recruitment (animal experimental). Thus, taking into consideration the lipid profile as main outcome, 44 animals and $\alpha=5\%$, the power calculation was 91% and Cohen's test was $d=1.680$ and effect-size was $r=0.643$.

The Shapiro-Wilk test was performed to assess the distribution of numerical data and, therefore, they are described as means and standard deviations. Results were compared using analysis of variance (one-way for analysis) followed by Tukey's post-hoc test. A 5% level of significance was set for all tests performed.

3. RESULTS

Table 2 shows the metabolic and hemodynamic changes observed in rats during the experiments. The different diets produced a variation in body weight at the end of the protocols ($p=0.040$). Compared to group C, group E had lower body weight ($p=0.002$) and lower Lee index ($p<0.001$) (Table 2). Groups L and S showed similar Lee indexes compared to group C and lower Lee index than group E ($p<0.001$ and $p=0.020$, respectively). Group S showed a trend for improved insulin sensitivity when compared to other groups, though not statistically significant ($p=0.066$). Uterine weight was ~2.8-fold greater ($p<0.001$) in group E compared to other groups.

Table 2 also present lipid profiles of the rats. Diets containing phytoestrogens resulted in lower total cholesterol ($p=0.001$). There was ~28% reduction in group L ($p=0.037$) compared to group C, and ~35% reduction in group S ($p=0.011$) compared to group C. LDL-cholesterol (LDL-C) levels were also reduced ($p=0.001$): ~40% reduction in group L ($p=0.031$) while group S showed a greater reduction of ~56% ($p=0.003$), both compared to group C. No change was noted in HDL-cholesterol (HDL-C) levels ($p=0.052$). Triglyceride levels were reduced at the end of the experiment ($p=0.007$) by ~60% in group L ($p=0.036$) and ~71% in group S ($p=0.014$) compared to group C. We found no difference in the lipid profile between groups E and C.

The hemodynamic results (Table 2) showed no changes in systolic BP, diastolic BP and HR. However, there was a slight reduction in mean BP in group S compared to group L, though not statistically significant ($p=0.065$).

Figure 1 presents GLUT4 expression in cardiac tissue. Flaxseed diet increased by ~78% the expression of microsomal GLUT4 in cardiac tissue ($p<0.001$) when compared to the C group (Panel A). The same was not observed with soy nuts and estradiol. The analysis of plasma membrane GLUT4 (Panel B) showed that estradiol, flaxseed and soy nuts increased GLUT4 content when compared to the control group ($p=0.026$, $p=0.001$; $p=0.040$ respectively).

Figure 2 shows a quantitative analysis of GLUT4 in white adipose tissue. The expression of microsomal GLUT4 (Panel A) was higher in groups treated with estradiol and flaxseed when

184 compared to the control group ($p=0.030$ and $p=0.019$, respectively). Interestingly, there was no
185 difference in the expression of GLUT4 in group S compared to other groups. However, when
186 plasma membrane GLUT4 was analyzed (Panel B), all dietary interventions (estradiol, flaxseed,
187 and soy nuts) increased GLUT4 levels compared to the C group (group E: 102%, $p<0.001$; group L:
188 92%, $p=0.011$, group S: 81%; $p=0.020$).

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4. DISCUSSION

This study showed in an animal model of hypoestrogenism that soy nuts and flaxseed phytoestrogens may have health benefits by reducing cholesterol and triglycerides with no effects on body weight, BP and insulin sensitivity. These effects were accompanied by increased GLUT4 expression at cardiac and adipose tissue level mostly in the plasma membrane.

The ovariectomized rat model has previously been used (Kalu 1991) and it was satisfactorily reproduced in our laboratory. The findings of reduced uterine weight in the control group and increased uterine weight in female rats treated with physiological doses of estrogen (Hernandez et al. 2000) suggest that the procedure was successful and the desired hormone replacement achieved. We also observed no effects of phytoestrogens on uterine weight, which corroborates the findings of other studies investigating soy in a similar model (Yoneda et al. 2011) and genistein in human subjects (D'Anna et al. 2009). It may be a valuable finding to support the safety of phytoestrogens whether these results translate into long-term benefits (Tempfer et al. 2009).

According to the literature, our study showed that ovarian hormone deprivation caused an increase in body weight and Lee index in C rats, which corroborates the findings of some studies that evaluated the effects of ovariectomy on body weight (Saruhan and Ozdemir 2005). Estrogen may be related to higher energy expenditure (Guyard B 1991), which could explain lower weight gain in the group treated with this hormone. We found similar weight gain in female rats treated with phytoestrogens and controls, which may suggest that this treatment does not have an effect on energy balance. Similar results were reported in ovariectomized rats treated with genistein and daidzein injections (Banz et al. 2004) and postmenopausal women treated with soy (Charles et al. 2009). On the other hand, postmenopausal women treated with dietary flaxseed showed lower body weight (Morisset et al. 2009). However, these findings are from observational studies and high flaxseed consumption may be associated with healthier lifestyles and thus lower body weight.

Female hormones act on both lipogenesis by reducing lipoprotein lipase activity and lipolysis by increasing the activity of hormone-sensitive lipase (Cooke and Naaz 2004). Although

not directly related to lipogenesis, in our study, ovariectomized rats treated with estradiol did not show any changes in plasma lipid profile. However, total cholesterol, LDL-C and triglycerides were reduced in the groups treated with soy nuts and flaxseed. In contrast, no changes in lipid levels have been found in response to dietary flaxseed reported in humans (Simbalista et al. 2010). These contrasting findings could be due to the use of different amounts of phytoestrogens relative to both subjects' body weight (normal weight vs. overweight/obesity) and other dietary macronutrients. The potential benefits of soy nuts and flaxseed in menopausal women should be taken into account as they may help treating common conditions such as dyslipidemia.

Ovariectomy was not associated with changes in BP, which is consistent to that reported in young normotensive female rats (Clark et al. 2004). Estrogen deficiency has been linked to increased BP levels in postmenopausal women, though other factors may also be involved (Boldo and White 2011). Time of estrogen deprivation was key to inducing increased BP levels in ovariectomized female rats, which has been reported as occurring later in another study (Clark et al. 2004). Treatment with estrogen did not change BP levels, possibly because it was administered subcutaneously rather than orally (Percegoni et al. 2009) or because our experiment included young normotensive rats at low risk of hypertension.

Similar to that observed with estrogen administration, in this study, phytoestrogen replacement also did not change BP levels. The possible mechanisms for reduced BP in ovariectomized rats treated with phytoestrogens include reduced aortic contractility (as showed *in vitro*) (Li et al. 2004), reduced arterial stiffness (Teede et al. 2003), increased expression of eNOS, and improved endothelial function (Mahn et al. 2005) (as showed *in vivo*). However, high soy nuts and flaxseed intake had no effect on BP possibly because the rats in our experiment were young and normotensive, or even because they were administered different amounts from those that have been associated with BP effects in rats (Mahn et al. 2005).

Hormonal fluctuations above or below normal physiological levels, as seen during menopause (Cagnacci et al. 1992), may cause insulin resistance and increased risk of diabetes and

cardiovascular diseases. In view of potential benefits of phytoestrogens on insulin sensitivity and glucose metabolism in women (Villa et al. 2009, Ricci et al. 2010), one can speculate that a longer treatment period with soy nuts or treatment with larger amounts of soy nuts in a larger sample would provide evidence on the metabolic benefits in these animals. Our study did not show any difference in insulin sensitivity among the groups studied, but there was a trend for increased insulin sensitivity in the group treated with soy nuts compared to the C group ($p=0.066$).

Soy nuts increased the expression of GLUT4 in cardiac and adipose tissues, but this effect was seen only in the plasma membrane fraction. A study with equol (a metabolite of daidzein) in 3T3-L1 cells showed increased expression of GLUT4 and IRS-1 through activation of *peroxisome proliferator-activated receptor gamma* (PPAR γ) transcription factor involved in fatty acid storage and glucose metabolism (Cho et al. 2010). Flaxseed also increased the expression of GLUT4 in cardiac and adipose tissues evidencing a potential estrogenic effect. Interestingly, treatment with genistein injections for 2 weeks did not promote any changes in the expression of GLUT4 in ovariectomized rats in another study (Al-Nakkash et al. 2010). In addition, several studies have showed increased insulin sensitivity with oral phytoestrogens (Villa et al. 2009, Ricci et al. 2010). In our study, estradiol increased the expression of microsomal and plasma membrane fractions of GLUT4 in both cardiac and adipose tissues.

This study has some limitations. Although the composition of the two experimental diets was fully presented, no specific measure to assess the content of major phytoestrogens was performed and this may be a limitation of our results. Also, the experimental design of our study cannot differentiate if the hypolipidemic effects of soy nuts and flaxseed observed were derived from other diet components such as proteins, as some previous data suggest that both dietary flaxseed protein and soy protein may also have beneficial effects in lipids (Bhathena et al. 2002). Which chemical constituent has the highest impact on health still needs to be assessed.

We conclude that flaxseed and soy nuts as dietary supplements may improve lipid profile. Also, flaxseed and soy nuts increased GLUT4 expression, particularly in adipose tissue, which may

268 be associated with a tendency to improve insulin resistance. Thus, these dietary supplements, used
269 in an animal model of menopause, may have health benefits with potential clinical implications by
270 improving lipid profile and increasing GLUT4 expression in insulin-sensitive tissues with no
271 harmful estrogen-like effects on both reproductive and cardiovascular systems.

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273 **CONFLICT OF INTEREST**

274 The authors have no conflicts of interest to report.

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Draft

Table 1: Composition of experimental diets

Diet	Component	Qty (%)	Qty (g) ^a	Balance
Standard diet (AIN 93G)				
	Corn starch	39.750	397.500	
	Casein	20.000	200.000	17.0% (protein)
	Dextrinized corn starch	13.200	132.000	
	Saccharose	10.000	100.000	
	Lard	7.000	70.000	7.0 % (lipids)
	Microcrystalline cellulose	5.000	50.000	5.0 % (fibers)
	L-cystine	0.300	3.000	
	B. choline (41.1%)	0.250	2.500	
	BHT (antioxidant)	0.0028	0.028	
	Mineral mix G	3.500	35.000	
	Vitamin mix	1.000	10.000	
Flaxseed				
	Corn starch	37.160	371.600	
	Casein	15.923	159.230	17.3 % (protein)
	Dextrinized corn starch	13.200	132.000	
	Saccharose	10.000	100.000	
	Flaxseed	17.500	175.000	7.1 % (lipids)
	Microcrystalline cellulose	1.168	11.680	4.9% (fibers)
	L-cystine	0.300	3.000	
	B. choline (41.1%)	0.250	2.500	
	BHT (antioxidant)	0.0028	0.028	
	Mineral mix G	3.500	35.000	
	Vitamin mix	1.000	10.000	
Soy nuts				
	Corn starch	28.203	282.030	
	Casein	3.740	37.4000	17.3% (protein)
	Dextrinized corn starch	13.200	132.000	
	Saccharose	10.00	100.000	
	Soy nuts	38.900	389.000	7.1 % (lipids)
	Microcrystalline cellulose	0.905	9.050	5.0% (fibers)
	L-cystine	0.300	3.000	
	B. choline (41.1%)	0.250	2.500	

BHT (antioxidant)	0.003	0.028
Mineral mix G	3.500	35.000
Vitamin mix	1.00	10.000

^a Amounts in grams for 1 kg of feed. Supplier: Pragsoluções Biociências (Jaú, SP, Brazil).
The amounts of corn starch and casein were adjusted to ensure the isocaloric and isoprotein characteristics in each diet.

Table 2: Characteristics of the study animals after ovariectomy and treatments

	C (n=12)	E (n=10)	L (n=12)	S (n=10)	p-value
Body weight (g)	363 ± 31	260 ± 21 ^a	333 ± 26 ^b	315 ± 30 ^b	0.040
Lee index	0.323 ± 0.009	0.304 ± 0.005 ^a	0.318 ± 0.009 ^b	0.315 ± 0.007 ^b	<0.001
kITT (%.min ⁻¹)	3.11 ± 0.63	3.93 ± 1.10	3.58 ± 0.69	4.21 ± 0.99	0.066
Uterine weight (g)	0.16 ± 0.05	0.65 ± 0.23 ^a	0.17 ± 0.05 ^b	0.16 ± 0.03 ^b	0.001
Total cholesterol (mg/dL)	114.5 ± 33	122.4 ± 39	81.9 ± 20 ^{ab}	75.5 ± 12 ^{ab}	0.001
LDL-cholesterol (mg/dL)	85.0 ± 37	80.7 ± 38	50.7 ± 17 ^a	38.0 ± 14 ^{ab}	0.001
HDL-cholesterol (mg/dL)	29.5 ± 11	41.6 ± 13	31.2 ± 7	37.2 ± 11	0.052
Triglycerides (mg/dL)	85.8 ± 9	71.4 ± 5	34.3 ± 3 ^a	24.8 ± 7 ^{ab}	0.007
SBP (mmHg)	122.3 ± 9.5	121.8 ± 7.2	126.9 ± 9.5	117.4 ± 9.7	0.744
DBP (mmHg)	88.1 ± 6.4	86.4 ± 7.7	91.0 ± 8.8	82.9 ± 7.9	0.819
MAP (mmHg)	104.9 ± 6.3	104.1 ± 6.6	108.9 ± 8.8	100.1 ± 8.1	0.065
HR (bpm)	370.2 ± 45.7	351.1 ± 32.9	370.5 ± 29.1	365.7 ± 27.9	0.120

C: ovariectomized rats treated with standard diet and placebo pellets; E: ovariectomized rats treated with standard diet with 1.5 mg 17β-estradiol pellets; L: ovariectomized rats treated with flaxseed and placebo pellets; S: ovariectomized rats treated with soy nuts and placebo pellets; n: number of animals per group; kITT: rate constant for insulin tolerance test; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate. Data described as mean ± standard deviation. One-way ANOVA followed by post-hoc Tukey's test; ^a p<0.05 vs. C, ^b p<0.05 vs. E.

FIGURE LEGENDS

Figure 1 – Expression of GLUT4 in cardiac tissue. Overview of quantitative Western blot analysis. C: Ovariectomized female rats receiving standard diet and placebo pellets (control group), E: Ovariectomized female rats receiving standard diet and estrogen pellets (Estradiol group), L: Ovariectomized female rats receiving dietary supplementation with flaxseed and placebo pellets (Flaxseed group), S: Ovariectomized female rats receiving dietary supplementation with soy nuts and placebo pellets (soy nuts group). Panel A and B: overview of the expression of microsomal and plasma membrane GLUT4 tissue contents. One-way ANOVA (Panel A: $p=0.022$; Panel B: $p=0.039$) followed by post-hoc Tukey's test.

Figure 2 – Expression of GLUT4 in adipose tissue. Overview of quantitative Western blot analysis. C: Ovariectomized female rats receiving standard diet and placebo pellets (control group), E: Ovariectomized female rats receiving standard diet and estrogen pellets (Estradiol group), L: Ovariectomized female rats receiving dietary supplementation with flaxseed and placebo pellets (Flaxseed group), S: Ovariectomized female rats receiving dietary supplementation with soy nuts and placebo pellets (Soy protein group). Panel A and B: overview of the expression of microsomal and plasma membrane GLUT4 tissue contents. One-way ANOVA (Panel A: $p=0.040$; Panel B: $p=0.016$) followed by post-hoc Tukey's test.

Figure 1 – Expression of GLUT4 in cardiac tissue. Overview of quantitative Western blot analysis. C: Ovariectomized female rats receiving standard diet and placebo pellets (control group), E: Ovariectomized female rats receiving standard diet and estrogen pellets (Estradiol group), L: Ovariectomized female rats receiving dietary supplementation with flaxseed and placebo pellets (Flaxseed group), S: Ovariectomized female rats receiving dietary supplementation with soy nuts and placebo pellets (soy nuts group). Panel A and B: overview of the expression of microsomal and plasma membrane GLUT4 tissue contents. One-way ANOVA (Panel A: $p=0.022$; Panel B: $p=0.039$) followed by post-hoc Tukey's test.

119x71mm (300 x 300 DPI)

Adipose Tissue

Figure 2 – Expression of GLUT4 in adipose tissue. Overview of quantitative Western blot analysis. C: Ovariectomized female rats receiving standard diet and placebo pellets (control group), E: Ovariectomized female rats receiving standard diet and estrogen pellets (Estradiol group), L: Ovariectomized female rats receiving dietary supplementation with flaxseed and placebo pellets (Flaxseed group), S: Ovariectomized female rats receiving dietary supplementation with soy nuts and placebo pellets (Soy protein group). Panel A and B: overview of the expression of microsomal and plasma membrane GLUT4 tissue contents. One-way ANOVA (Panel A: $p=0.040$; Panel B: $p=0.016$) followed by post-hoc Tukey's test.

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