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Combinatory effects of phytoestrogens and exercise on body fat mass and lipid metabolism in ovariectomized female rats

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ABSTRACT

The purpose of this study was to investigate the combinatory effects of an isoflavone (ISO)-rich diet and exercise on fat mass and lipid metabolism in ovariectomized (OVX) rats. Therefore the female Wistar rats were sedentary, performed an intense treadmill uphill running, received ISOs, or a combination of ISOs and running after ovariectomy. The exercise reduced visceral fat mass, adipocyte size and serum leptin in Sham animals and antagonized the increases of these parameters induced by OVX. ISOs reduced OVX induced increase of serum leptin. The combination of training and ISOs was most effective in reducing serum triglyceride levels. In OVX rats the training stimulated the expression of genes associated with fatty acid synthesis (SREBP-1c and FAS) in adipose tissue, soleus muscle, liver and genes associated with fatty acid oxidation (PPARδ and PGC-1α) in adipose tissue. ISOs stimulated the expression of SREBP-1c and FAS in soleus muscle and PGC-1α in adipose tissue, whereas suppressed hepatic SREBP-1c and FAS expression. Strong additive effects of ISOs combined with the training were observed for PPARδ and PGC-1α expressions in soleus muscle. In conclusion our results demonstrate that both the training and ISOs affect fat mass and fatty acid metabolism in OVX rats. The training seems to have a higher impact than ISO exposure in regulating gene expression in adipose tissue. However, the strongest effects for several of the addressed parameters could be observed in the combination group especially in the soleus muscle. Therefore a combination of training and an ISO-rich diet may have beneficial effects on fatty acid metabolism and could be a concept for the prevention of obesity in postmenopausal females.

1. Introduction

Obesity is abnormal or excessive fat accumulation. BMI greater than 30 kg/m^2 is generally considered as obese. In our time it causes great public health concerns such as cardiovascular diseases, type 2 diabetes, dyslipidemia and insulin resistance [1–3]. More than 1.9 billion adults were overweight (BMI greater than 25 kg/m^2) and 600 million were obese (WHO, 2014) [4]. Postmenopausal women tend to have a higher risk of developing overweight or obesity because of changes in metabolic and hormonal parameters. Estrogen deficiency exerts a huge impact on mobilization of fatty acids, body fat distribution and bone mass [5–7]. According to the most recent data of the European Statistical Office (Eurostat, 2014), obese women aged 45–64 constitute 18.6% of women in the states of the European Union in 2014. The percentage of older obese women was about two-fold higher than in the younger group of women aged 18–44 [8]. Several exogenous factors such as diet and lifestyle could affect body composition and thereby also be associated with the risk of developing obesity or its co-morbidities [5].

As estrogen directly influences energy homeostasis through a modulation of lipid metabolism [9], hormone replacement therapy (HRT) was regarded as an effective treatment for preventing postmenopausal women from obesity [10]. However, it is still controversial whether HRT may be associated with increased risk of breast and endometrial cancer. Several findings did not support use of HRT for

Abbreviations: E_2, 17β-estradiol; FAS, fatty acid synthase; HDL, high-density lipoproteins; HRT, hormone replacement therapy; ISOs, isoflavones; LDL, low-density lipoproteins; OVX, ovariectomized; PGC, peroxisome proliferator-activated receptor-γ coactivator; PND, postnatal day; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; Tb.BMC, trabecular bone mineral content; Tb.BMD, trabecular bone mineral density

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chronic disease prevention [11,12], but the Women's Health Initiative and new studies reported that HRT has a complex pattern of risks and benefits which could be appropriate for symptom management in some women [13,14]. Therefore, there is a need for alternative treatment strategies such as administration of isoflavones (ISOs) which is controversially debated [15]. ISOs are the most well known phytoestrogens. They are structurally similar to 17β-estradiol (E_2) and display weak estrogenic activity in mammals [16]. ISOs derived from soybeans and their products are widely discussed regarding disease prevention [16]. Published data showed that soy ISOs have beneficial effects on preventing obesity and related metabolic diseases in both in vitro and in vivo studies [17].

Apart from nutrition exercise has also been used as a non-pharmacological intervention to overcome negative health impacts caused by menopause. It is well known that training has a potency to prevent the development of many diseases, such as obesity and osteoporosis [18,19]. Training experiments in animals have shown to decrease fat deposition and increase insulin sensitivity [20].

Previously studies of our group have used ovariectomized (OVX) rat models demonstrating that a combination of exercise and E_2 administration is an effective strategy to increase insulin sensitivity and prevent the development of obesity [21,22]. However, the combinatory effects of dietary ISO intake and training on obese-related risk prevention yet remain to be solved. Therefore, the major aim of this study was to investigate combinatory effects of dietary ISO intake and exercise on parameters related with lipid metabolism in an OVX Wistar rat animal model. Visceral fat mass, adipocyte size, serum leptin levels and lipid profile were determined. Bone mineral density was also measured. Furthermore, the expressions of genes involved in the regulation of lipid metabolism (sterol regulatory element-binding protein (SREBP)-1c, fatty acid synthase (FAS), peroxisome proliferator-activated receptor (PPAR) δ and peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α) were investigated in adipose tissue, liver and soleus muscle.

2. Materials and methods

2.1. Animals

Forty-four female Wistar rats (~250 g, Janvier, Le Genest St Isle, France) were kept under controlled conditions of illumination (12/12 h day/night cycle) and constant room temperature (20 ± 1 °C, relative humidity 50–70%). The rats had free access to food (SSniff GmbH, Soest, Germany) and water. Body weight and food intake were monitored twice/week. All animal handling and experiments were approved by the Committee of Animal Care.

2.2. Animal treatment and diets

The study design is shown in Fig. 1 and performed as previously described [23]. Treatment groups were assigned as follows: (1) Sham; (2) Sham-operated with training (Sham + T); (3) OVX; (4) OVX + T; (5) OVX with an ISO-rich diet (OVX + ISO); (6) OVX + ISO + T.

One week before the training started, rats either remained on the standard ISO-depleted diet or received an ISO-rich diet, which were 4 mg or 479 mg ISO aglycone equivalent ((sum of genistein, daidzein and glycitein)/kg diet; Ssniff Sm R/M-H, 10 mm, phytoestrogen-free, Ssniff, Soest, Germany). The ISO-rich diet is based on the ISO-depleted diet with an added ISO extract (NovaSoy650, ADM, Decatur, Illinois, USA). Animals were sacrificed two days after the last training session, whereby Sham rats were sacrificed in the proestrous or estrous phase and blood was collected. Visceral fat (periovarian, perirenal, omental) was removed, pooled and weighed. Perirenal fat pads were immediately fixed in 4% formalin solution for morphological analysis. Adipose tissue, liver and soleus muscle for gene analysis were snap frozen immediately after removal.

2.3. Training protocol

An uphill intense training protocol (Fig. 1) was designed for the exercise groups [23]. The training was conducted on a motor-driven treadmill with an incline of 25° and lasted for 61 days. Rats were trained for 10 min, twice/day, three training days followed by a rest day. The velocity was gradually increased from 12 to 20 m/min and kept at 20 m/min until the end of the training. The 10 min training was divided into a 5 min training, a 5 min break and a 5 min training when the velocity reached 20 m/min. The break was necessary, as a continuous running performance of 10 min was too exhausting for the rats.

2.4. HE staining and determination of adipocyte size

HE staining and determination of adipocyte size were performed as previously described [24].

2.5. Determination of leptin in serum

The serum concentration of leptin was measured in triplicates using ELISA kits for rats according to the manufacturer's instruction (mouse/rat leptin ELISA E06, Mediagnost, Reutlingen, Germany).

2.6. Determination of serum lipids

Serum triglyceride levels were analyzed by colorimetry using ABX Pentra reagent (ABX Diagnostics Montpellier, France). Total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) were determined by photometry using reagents from DIALAB (Wiener, Neudorf, Austria). To measure serum lipids, a chemistry analyzer (Roche Hitachi Cobas Mitra Plus) was used.

2.7. Peripheral quantitative computed tomography measurements of bone

Trabecular bone mineral content (Tb.BMC) and trabecular bone mineral density (Tb.BMD) were measured by peripheral quantitative computed tomography (pQCT, XCT Research SA+, StraTec Medizintechnik, Pforzheim, Germany) as previously described [25].

2.8. Real-time PCR experiments

mRNA analysis and PCRs were performed as previously described [22,26]. Total RNA was extracted from frozen adipose tissue, soleus muscle and liver of individual rats using the standard TRIzol method (Invitrogen, Germany). cDNA synthesis of each group was performed with equal amount of pooled RNA samples which was a mixture of same amount of RNA from each rat (QuantiTect Reverse Transcription Kit, Qiagen, Germany). Real-time PCR was performed with Taq DNA polymerase (Invitrogen, Germany) and a fluorescent dye (SYBR Green, BioRad, USA) on an Mx3005P™ qPCR System (Stratagene, USA). The final results of real-time PCR consisted of a minimal of three different cDNA synthesis. Relative mRNA amounts of target genes were calculated after normalization to an endogenous housekeeping gene (Hypoxanthine-guanine phosphoribosyltransferase-HPRT was used with adipose tissue, cytochrom c oxidase subunit-1A was used with hepatic tissue, cyclophillin was used with soleus muscle). Specific primer pairs were designed as previously described [26] and depicted in the Supplementary Table 1.

2.9. Western blotting

Protein extraction and concentration measurement were performed as previously described [25,26]. After electrophoretic protein separation by size, proteins were transferred onto a nitrocellulose membrane (PROTRANR Nitrocellulose Transfer Membrane, WhatmanR, Little Chalfont, UK) and blocked with 5% non-fat milk in a Tris buffered