Journal of Steroid Biochemistry & Molecular Biology 122 (2010) 100–105

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Physical activity and estrogen treatment reduce visceral body fat and serum levels of leptin in an additive manner in a diet induced animal model of obesity☆

Nora Zoth, Carmen Weigt, Ute Laudenbach-Leschowski, Patrick Diel*

Department of Cellular and Molecular Sports Medicine, Institute of Cardiovascular Research and Sports Medicine, German Sports University, Cologne, Germany

ARTICLE INFO

Article history:
Received 4 January 2010
Received in revised form 7 March 2010
Accepted 8 March 2010

Keywords:
Obesity
Metabolic syndrome
Leptin
Estrogens
Physical activity

ABSTRACT

Estrogen replacement and physical activity have been demonstrated to reduce the risk to develop a metabolic syndrome in postmenopausal women. In this study we investigate the combined effects of endurance training and estrogen substitution in a rat animal model of diet induced obesity. Effects on lipid and glucose metabolism were evaluated. Ovariectomized (OVX) or sham-operated (SHAM) female Wistar rats were fed with a high fat diet (HF) for 9 weeks. After 3 weeks of overnutrition the OVX rats either remained sedentary, performed treadmill training, received 17β-Estradiol (E_2), or combined treatment. The OVX rats had a greater increase in body weight and serum levels of cholesterol, triglyceride and low-density lipoprotein cholesterol (LDL). These parameters could be reduced by E_2 and more effectively E_2 in combination with exercise. Also the increase of visceral body fat and leptin could be improved by E_2 and exercise. This combination showed synergistic effects. Serum levels of insulin could be reduced by exercise training, E_2 substitution revealed no significant changes.

Our results indicate that ovariectomy increases the susceptibility to develop obesity. In addition they show that the combination of hormone replacement therapy (HRT) and physical activity may influence parameters related to lipid metabolism positively in an additive manner. The results of this study provide evidence that the combination of HRT with physical activity could be a very effective strategy to prevent the development of a metabolic syndrome induced by overnutrition.

1. Introduction

Metabolic syndrome, usually caused by overnutrition and a lack of physical activity, represents a heterogeneous cluster of obesity-related diseases. The combination of hypertension, dyslipidemia and insulin resistance is clinically interpreted as an indicator for increased risk of diabetes mellitus type 2 and cardiovascular diseases [1,2]. During the first half of life mainly men are affected, the female patients dominate from the age of 60 [3]. Especially the postmenopausal period tends to be a risk factor for cardiovascular diseases because hormonal alterations are associated with changes in metabolic situation [2,4]. In general it is well known that steroid hormones have a huge impact on the development of the metabolic syndrome [5]. There are many reports about the effects of androgens [6,7]. In the meantime it is well known that estrogen deficiency plays a role in the control of energy homeostasis in females [8]. It seems to be involved in changes of body fat distribution, mobilization of fatty acids and glucose-absorbing capacity of different tissues [9,10].

Ovariectomy in rats led to increases in energy intake, body weight and fat mass, whereas estrogen substitution antagonized these effects in a positive way [8,11,12]. Some studies have also observed an overall insulin resistance in animals after ovariectomy, and these effects could be prevented by hormone replacement therapy (HRT) too [13].

Still, older women trust HRT for treating estrogen deficiency symptoms. Although, Beral et al. [14] have opened up the discussion about the negative overall risk-benefit ratio of HRT and alternative strategies for the treatment of menopausal and postmenopausal disorders. It is still discussed if targeted exercise training might be a suitable alternative for HRT.

Exercise training in animals decreases fat deposition and enhances insulin sensitivity [15,16]. According to Latour et al. [17] physical training improved glucose stimulated insulin response and increased glucose transporter concentration in skeletal muscle in ovariectomized (OVX) rats as well. But the only existing study on fat deposition and exercise training has shown an activity-induced reduction in body fat content regardless of estrogen status [11]. Moreover, the interaction of estrogen substitution and exercise training to modulate metabolic syndrome in OVX rats has only been investigated in a limited number of studies [16].

☆ Article from special issue on "Steroids in Diabetes and Obesity".

* Corresponding author at: German Sports University Cologne, Institute of Cardiovascular Research and Sports Medicine, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany. Tel.: +49 221 49825420.
E-mail addresses: n.zoth@dshs-koeln.de (N. Zoth), diel@dshs-koeln.de (P. Diel).

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2010.03.029

So, in this context it is of interest to determine whether the effect of HRT on the classical cluster of metabolic syndrome can be supported by exercise training.

Therefore, the mayor aim of this study was to investigate the combined effects of physical activity and estrogen replacement on parameters related with insulin sensitivity, glucose and fat metabolism in the animal model of the OVX Wistar rat. OVX female Wistar rats were fed with a high fat diet (HF) for 9 weeks. Meanwhile the animals either remained sedentary, performed treadmill training, received 17β-Estradiol (E_2), or underwent both exercise training and estrogen treatment. Sham-operated (SHAM) animals served as controls. Insulin sensitivity, HOMA-index, body weight, subcutaneous and visceral body fat, leptin, serum levels of insulin and lipids (cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL)) were investigated.

2. Materials and methods

2.1. Animals

Female Wistar rats (Janvier, Le Genest St Isle, France), weighing 200–220 g upon their arrival were housed in groups of five and had a libitum access to food (SSniff GmbH, Soest, Germany) and water. They were maintained under controlled conditions of illumination (12/12-h light/dark cycle) and room temperature (20 ± 1 °C, relative humidity 50–80%). All animal procedures were approved by the Committee on Animal Care and complied with accepted veterinary medical practice.

2.2. Animal treatment and tissue preparation

Previous studies have demonstrated that HF diet alone does not induce obesity in SHAM animals compared to animals on low fat diet (data not shown). Therefore in this study on arrival all animals were fed with a HF diet (SSniff GmbH, Soest, Germany). It consisted of pellet rat chow with a gross energy of 22.6 MJ/kg and metabolizable energy of 19.2 MJ/kg. The details of the diet, especially the fatty acids, are presented in Table 1. After estrous synchronization rats on the same cycle were sham-operated ($n = 13$), the remaining were ovariectomized ($n = 27$). After 14 days of endogenous hormonal decline, all animals were randomly assigned to one of the experimental groups for 6 weeks. A subset of OVX rats ($n = 12$) were treated with E_2 (4 μg kg^{-1} day^{-1}). The 17β-Estradiol (Estra-1,3,5(10)-trien-3,16α,17β-diol) provided by Sigma–Aldrich (Deisenhofen, Germany) was administered via ALZET® osmotic mini pumps. Beyond that all treatment groups were subdivided into a sedentary and an exercise training group (Fig. 1).

At the end of the experiment all animals were decapitated after light anesthesia with CO_2 inhalation. Blood samples were collected (complete blood after decapitation), uteri prepared free of fat and visceral body fat deposits were excised. Altogether visceral body fat content consisted of periovarian, perirenal, mesenteric and omental fat pads. Afterwards all wet weights were determined.

Table 1
Composition of the high fat diet (HF).

Ingredients	AmountHF diet
Dry matter (%)	96.2
Crude fat (%)	23.1
Fatty acids (%)	
C12:0	0.02
C14:0	0.29
C16:0	5.15
C16:1	0.62
C18:0	2.83
C18:2	8.99
C18:3	3.19
C20:0	0.37
C20:4	0.01
Cholesterol (mg/kg)	175
Crude protein (%)	22.5
Crude fibre (%)	4
Crude ash (%)	5.9
Nitrogen-freeextracts (%)	40.7
Starch (%)	6.7
Sugar/dextrines (%)	31.7

2.3. Exercise training protocol

Exercise training, in terms of endurance training, took place on a motor-driven rodent treadmill (Columbus Instruments, Ohio, USA) 5 days/week over 6 weeks. The intensity was progressively increased from 10 min once a day at 22 m/min, 5% upgrade, up to 15 min twice a day at 25 m/min, 5% upgrade, after the first week of practicing. All animals were urged to run. The exercise intensity was chosen based on previous experiments.

2.4. Determination of serum lipids

Blood samples were collected at the day of decapitation, centrifuged and serum was stored at −20 °C. Serum levels of cholesterol, high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were determined via photometric systems using DIALAB (Wiener Neudorf, Austria). Serum levels of triglyceride were analyzed by colorimetry using ABX Pentra (ABX Diagnostics Montpellier, France).

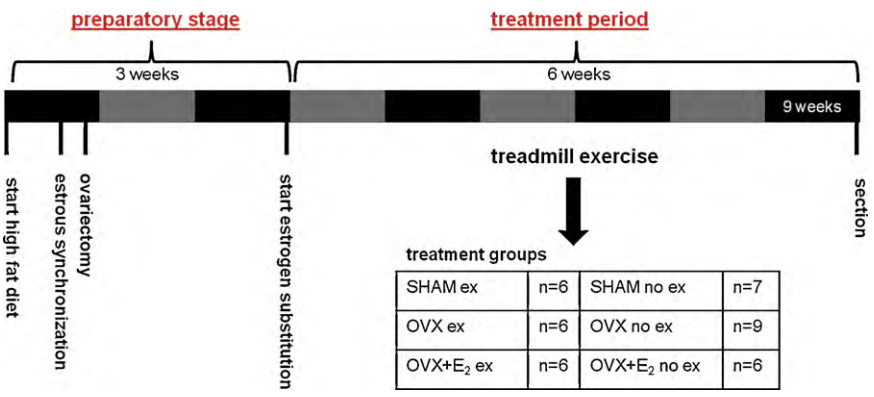

Fig. 1. Experimental design. Experimental design of the study; SHAM = sham-operated, OVX = ovariectomized, E_2 = treated with 17β-Estradiol, ex = exercise, no ex = rest

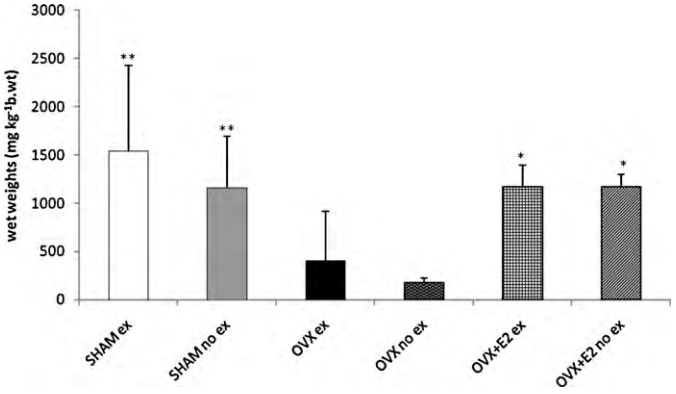

Fig. 2. Effects of estradiol treatment and physical activity on the uterine wet weights. SHAM = sham-operated, OVX = ovariectomized, E_2 = treated with 17β-Estradiol, ex = exercise, no ex = rest, data shown are means ± S.E.M. Mean values were significantly different for the following comparisons: * vs OVX no ex ($p \leq 0.05$), ** vs OVX no ex ($p \leq 0.01$)

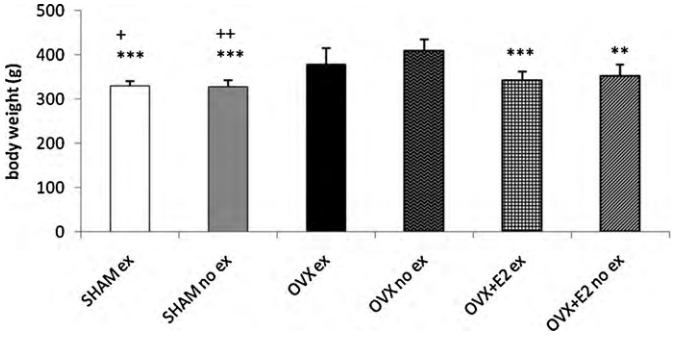

Fig. 3. Effects of estradiol treatment and physical activity on the body weight. SHAM = sham-operated, OVX = ovariectomized, E_2 = treated with 17β-Estradiol, ex = exercise, no ex = rest, data shown are means ± S.E.M. Mean values were significantly different for the following comparisons: ** vs OVX no ex ($p \leq 0.01$), *** vs OVX no ex ($p \leq 0.001$), + vs OVX ex ($p \leq 0.05$), ++ vs OVX ex ($p \leq 0.01$)

2.5. Determination of leptin, insulin and HOMA-index

Leptin levels were measured by an Enzyme-Immunoassay (Alpco diagnostics, Salem NH) which utilizes two specific polyclonal antibodies for mouse and rat leptin. The practical sensitivity of the assay was 10 pg/ml and the intra-assay variation coefficient was 4.4%. Serum levels of insulin were assessed with an Enzyme-Immunoassay (Alpco Diagnostics, Salem, NH) in which mouse monoclonal antibodies for insulin were used. The practical sensitivity of the assay was 0.06 ng/ml and the intra-assay variation coefficient was 3.7%. The evidence of insulin resistance was provided by the Homeostasis model assessment (HOMA-index). Therefore the mathematical model of Matthews et al. [18] was used.

2.6. Statistical analysis

All available data were sampled in a SPSS-Database and statistically evaluated by SPSS 16.0 (SPSS, Chicago). The values are presented as arithmetic means ± S.E.M. Significant differences among all groups were calculated using one-way ANOVA with a following Tukey's HSD test.

3. Results

3.1. Body and tissue weights

The effectiveness of ovariectomy was proved by uterine wet weights as OVX rats showed a 6 to 7 fold lower uterine wet weight compared to SHAM rats. Treatment with E_2 led to a strong stimulation of uterine wet weights (each $p \leq 0.05$). Interestingly, each exercise training group tended to show higher uterine wet weights than the ones who stayed sedentary (Fig. 2).

After the experiment OVX rats without exercise training revealed the highest body weights. Their values were about 20% higher than those of SHAM rats. This OVX induced body weight gain could be reversed by exercise training (7%, ns) or by E_2 substitution (14%, $p \leq 0.01$). The strongest reduction was observed in OVX rats with combined intervention (16%, $p \leq 0.001$) (Fig. 3).

3.2. Serum levels of lipids

Fig. 4 demonstrates serum levels of cholesterol, triglyceride, LDL and HDL. Compared to SHAM rats, estrogen deprivation by ovariectomy resulted in a significant increase in serum levels of cholesterol, triglyceride and LDL (each $p \leq 0.05$). This negative course could be hindered by exercise training or estrogen replacement. In addition, combined exercise training and substitution of E_2 brought about the greatest reduction in serum levels of cholesterol and LDL (ns.).

Regarding serum levels of HDL no significant changes were observed (Fig. 4).

3.3. Body fat

After 9 weeks of HF diet the OVX rats revealed the highest visceral body fat content and according to this also the highest serum levels of leptin. Both parameters could be lowered with exercise training or estrogen replacement (Fig. 5). But the most effectual reduction could be reached with the combined intervention of hormone supplement and exercise training (each $p \leq 0.001$). Particularly, serum levels of leptin decreased by a factor 5–5.5 compared to the sedentary OVX rats. Interestingly, the serum levels of leptin were more responsive to the intervention than the visceral body fat contents (Fig. 5).

3.4. Glucose metabolism

Six weeks of intervention did not have any influence on the glucose metabolism. Neither serum levels of insulin nor HOMA-index showed significant differences compared to the SHAM groups. Thus, OVX rats without exercise training tended to have the highest levels of insulin which could be suppressed by exercise training. Interestingly, the treatment of E_2 was ineffective. Concerning the HOMA-index no significant changes were assessed (Fig. 6).

4. Discussion

The role of estrogen in the regulation of energy homeostasis in females is well established. They seem to have an influence on both carbohydrate and lipid metabolism [19]. On the other had it is also well known that physical activity has beneficial effects on the metabolic syndrome [20]. In this study we wanted to investigate the combined effects of physical activity and estrogen treatment in a dietary induced model of obesity in OVX Wistar rats. As expected ovariectomy resulted in lowered uterine wet weights compared to those of SHAM rats whereas estrogen substitution raised them again (Fig. 2). This demonstrates that an adequate delivery of E_2 is achieved by the use of osmotic mini pumps, as an agreement to earlier results [21]. After 9 weeks of HF diet OVX rats of the present study exhibited some symptoms of the metabolic syndrome, namely, adiposity through increased visceral body fat content (Fig. 4). In addition there was a nonsignificant increase of serum levels of insulin in untreated and untrained animals (Fig. 5).

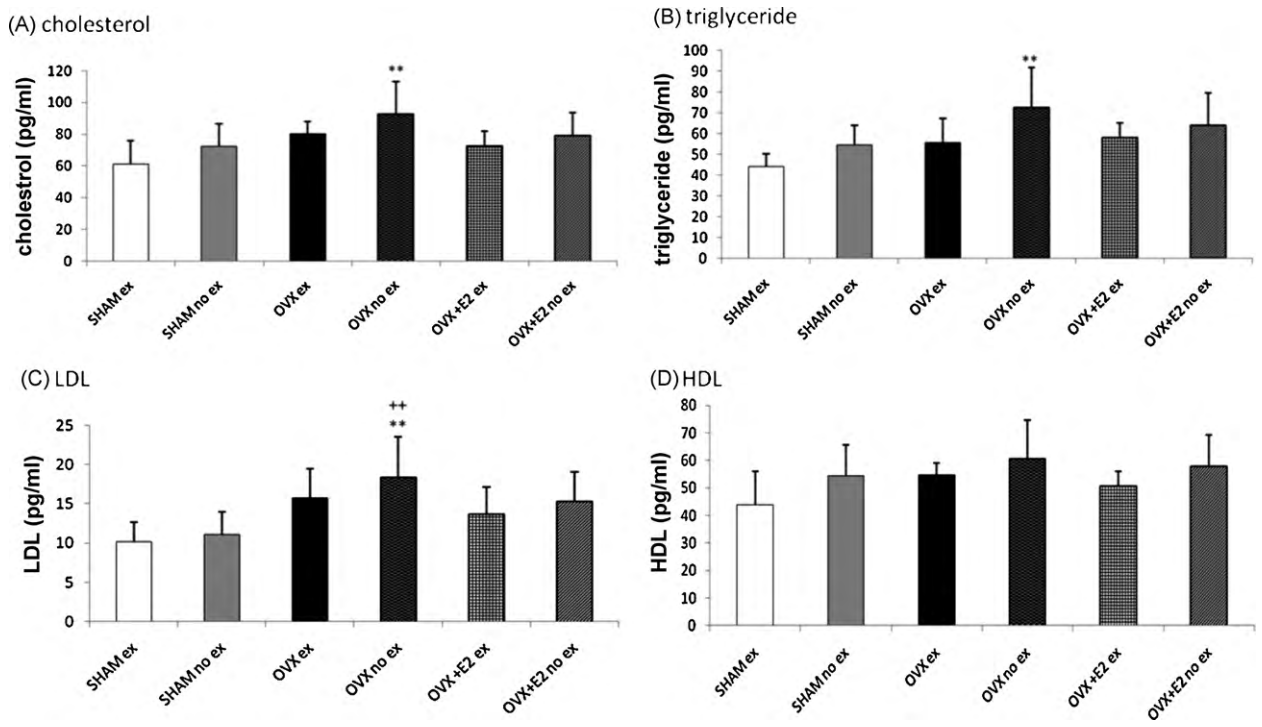

Fig. 4. Effects of estradiol treatment and physical activity on the serum levels of cholesterol, triglycerides, HDL and LDL. SHAM = sham-operated, OVX = ovariectomized, E_2 = treated with 17β-Estradiol, ex = exercise, no ex = rest, data shown are means ± S.E.M. Mean values were significantly different for the following comparisons: A** vs SHAM ex ($p \leq 0.01$); B ** vs SHAM ex ($p \leq 0.01$); C ** vs SHAM ex ($p \leq 0.01$), ++ vs SHAM no ex ($p \leq 0.01$); D no significance

In agreement to earlier studies of our group [22] OVX nontrained rats possessed a significant higher final body weight compared to SHAM and E_2 treated animals (Fig. 3). As demonstrated in a previous study the increase in body weight is not caused by hyperphagia [23]. This effect was prevented, in part, by exercise or E_2, but most effectively by combined treatment (Fig. 3). This observation indicates that the prevention of weight gain cannot only be the result

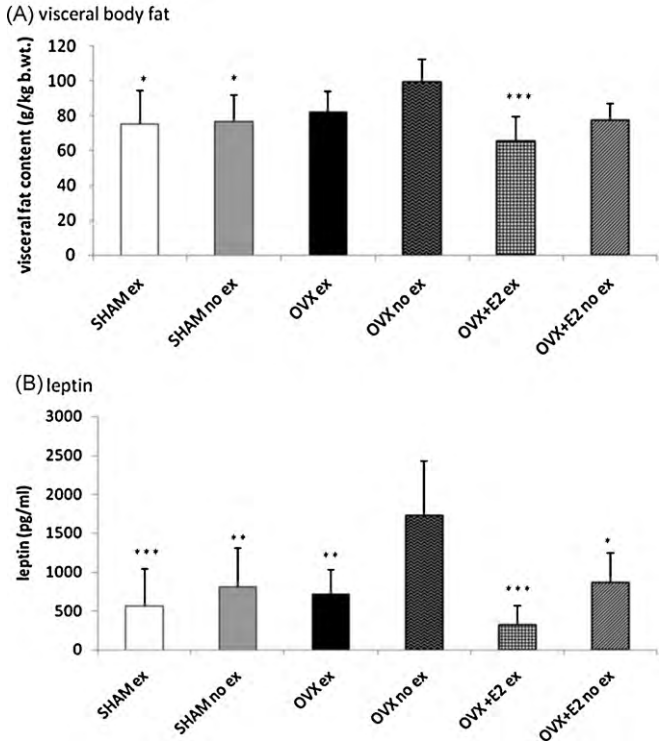

Fig. 5. Effects of estradiol treatment and physical activity on visceral body fat content and serum levels of leptin. SHAM = sham-operated, OVX = ovariectomized, E_2 = treated with 17β-Estradiol, ex = exercise, no ex = rest, data shown are means ± S.E.M. Mean values were significantly different for the following comparisons: A * vs OVX no ex ($p \leq 0.05$), *** vs OVX no ex ($p \leq 0.001$); B * vs OVX no ex ($p \leq 0.05$), ** vs OVX no ex ($p \leq 0.01$), *** vs OVX no ex ($p \leq 0.001$)

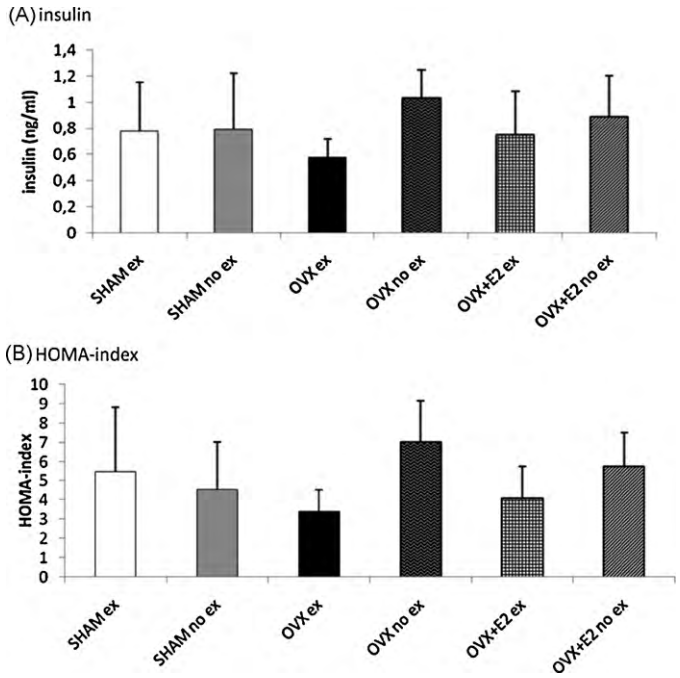

Fig. 6. Effects of estradiol treatment and physical activity on serum levels of insulin and HOMA-index. SHAM = sham-operated, OVX = ovariectomized, E_2 = treated with 17β-Estradiol, ex = exercise, no ex = rest, data shown are means ± S.E.M.

of exercise training but also of metabolic changes under control of estradiol. Observations about the combined effects of E_2 and physical activity are controversial. Some previous studies could not share the present observations [17,24,25]. In a similar study with OVX Sprague–Dawley rats only E_2 substitution reduced body weight gain in the OVX, exercise training did not have any influence [17]. In contrast our data presented in this paper are in line with earlier studies where similar effects were observed in Wistar rats. There the animals had been on a normal phytoestrogen free diet and were trained by volunteer wheel running [22]. A possible explanation for the controversial results could be that the other groups did not prove the rodents' body composition. According to Picard et al. [26] the OVX induced weight gain is ascribed to an increased visceral body fat content of OVX rats. The results of the present study are in line with this proposition. As shown in Fig. 5A the significant reduction of body weight by combined E_2 treatment and training is in line with the data regarding visceral body fat. Exercise training alone lowered visceral body fat content of OVX by 18% in combination with E_2 even by 34%. Moreover, trained OVX rats showed nearly the same visceral body fat content as sedentary intact rats. This indicates that exercise training has a strong influence on lowering body fat accumulation following a decrease in estrogen levels. A similar study with Sprague–Dawley rats provided additive effects of exercise training and E_2 treatment on the visceral body fat content [16]. These findings are in agreement with those of other studies [27,28].

The reduction in fat deposition with training in OVX rats raises the question of whether the level of leptin is improved in these rats. Leptin is characterized as an adipocytokine that is mainly expressed in adipose tissue [29]. Leptin plays an important role in regulating appetite and energy expenditure [30]. It is able to resist insulin secretion and features a positive correlation with body fat content [31]. In our study ovariectomy resulted in significantly elevated levels of leptin (Fig. 5B). Increase of leptin levels could be antagonized by exercise and estrogen replacement and most efficiently in combination together. The effect of estrogen treatment and training is in line with the visceral body fat content in these groups (Fig. 5A). It is well known that serum levels of leptin reflect adiposity levels [32].

As an essential component of the metabolic syndrome blood lipid profiles were also investigated in this study. Ovariectomy resulted in an increase in serum lipid levels. Cholesterol, triglyceride and LDL levels showed significant changes (Fig. 4). This is in line with reports in literature [16,28]. Exercise training and E_2 treatment resulted in a reduction of the OVX increased levels of these factors whereas serum levels of HDL remained unaffected. The beneficial effects of estrogens on blood lipid profile have been known for a long period of time [33]. Also physical activity is well known to effect blood lipid profiles in a positive manner [34]. Even we could not observe significant combinatory effects in our study; we believe that the combination of both may also result in additive effects with respect to the prevention of arteriosclerosis. To prove this hypothesis future investigations in the cardiovascular tissue of these animals are ongoing. Interestingly, our HF diet does not seem to influence glucose metabolism in the exposed animals. Neither serum levels of insulin nor HOMA-index showed significant changes in OVX rats compared to SHAM rats. However, OVX nontrained rats tended to have the highest levels of insulin (not significant), which could be taken as an indication that they were progressively developing an insulin-resistant condition (Fig. 6). Obviously the treatment period of the present study was too short to demonstrate insulin resistance. E_2 treatment has no effect on serum levels of insulin, but interestingly exercise training could lower them. The results of other studies do not conform to our observations. Latour et al. [17] for example found significant increased levels of insulin in OVX rats after 8 weeks of treatment which could not be influenced by exercise training.

In summary, the results of the present short experiment indicated that a combined treatment with estrogen and exercise training is able to influence parameters related to lipid metabolism in OVX rats positively. In contrast glucose metabolism remains unaffected by E2 treatment. Here, exercise training seems to have the strongest effect. As a conclusion we believe that our data provides some evidence that HRT combined with physical activity could be a very effective strategy to prevent the development of a metabolic syndrome induced by overnutrition. Also as a consequence we believe that the data indicates that the dose of estrogen used in HRT can be lowered if treatment is combined with training. This is in agreement with earlier studies of our group on animal bones [12].

Acknowledgement

This project was in part supported by the DFG grant DI 716/10-3.

References

[1] P.W.F. Wilson, W.B. Kannel, H. Silbershatz, R.B. D' Agostino, Clustering of metabolic factors and coronary heart disease, Arch. Intern. Med. 159 (1999) 1104–1109.
[2] Y.W. Park, S. Zhu, L. Palaniappan, S. Heshka, M.R. Carnethon, S.B. Heymsfield, The metabolic syndrome, Arch. Intern. Med. 163 (2003) 427–436.
[3] H.U. Janka, D. Michaelis, Epidemiology of diabetes mellitus: prevalence, incidence, pathogenesis and prognosis, Z. Arztl. Fortbild. Qualitätssich 96 (2002) 159–165.
[4] R. Rosi, A. Nuzzo, G. Origliani, M.G. Modena, Metabolic syndrome affects cardiovascular risk profile and response to treatment in hypertensive postmenopausal women, Hypertension 52 (2008) 865–872.
[5] R. Pasquali, V. Vicennati, Steroids and the metabolic syndrome, J. Steroid Biochem. Mol. Biol. 109 (3–5) (2008) 258–265.
[6] F. Saad, L. Gooren, The role of testosterone in the metabolic syndrome: A review, J. Steroid Biochem. Mol. Biol. 114 (1–2) (2009) 40–43.
[7] T. Yanase, W. Fan, K. Kyoya, L. Min, R. Takayanagi, S. Kato, H. Nawata, Androgens and metabolic syndrome: lessons from androgen receptor knock out (ARKO) mice, J. Steroid Biochem. Mol. Biol. 109 (3–5) (2008) 254–257.
[8] E. Simpson, M. Jones, M. Kylie, R. Hill, L. Maffei, C. Carani, W.C. Boon, Estrogen, a fundamental player in energy homeostasis, J. Steroid Biochem. Mol. Biol. 95 (1–5) (2005) 3–8.
[9] M. Toth, A. Tchernof, C. Sites, E. Poehlman, Menopause-related changes in body fat distribution, Ann. N. Y. Acad. Sci. 904 (2000) 502–506.
[10] M. Jones, K.J. Mc Innes, W.C. Boon, E. Simpson, Estrogen and adiposity–Utilizing models of aromatase deficiency to explore the relationship, J. Steroid Biochem. Mol. Biol. 106 (1–5) (2007) 3–7.
[11] D. Richard, L. Rochon, Y. Deshaies, Effects of exercise training on energy balance of ovariectomized rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 253 (1987) R740–R745.
[12] T. Hertrampf, M.J. Gruca, J. Seibel, U. Laudenbach, K.H. Fritzemeier, P. Diel, The bone-protective effect of the phytoestrogen genistein is mediated via ER alpha-dependent mechanisms and strogly enhanced by physical activity, Bone 40 (6) (2007) 1529–1535.
[13] C.J. Bailey, H. Ahmed-Sorour, Role of ovarian hormones in the long-term control of glucose homeostasis, Diabetologia 19 (1980) 475–481.
[14] V. Beral, Breast cancer and hormone-replacement therapy in the Million Women Study, Lancet 362 (2003) 419–427.
[15] D.E. James, E.W. Kraegen, D.J. Chisholm, Effect of exercise training on whole-body insulin sensitivity and responsiveness, J. Appl. Physiol. 56 (1984) 1217–1222.
[16] V. Saengsirisuwan, S. Pongseeda, M. Prasannarong, K. Vichsiwong, C. Toskulkao, Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement, Metabolism 58 (2008) 38–47.
[17] M.G. Latour, M. Shinoda, J.M. Lavoie, Metabolic effects of physical training in ovariectomized and hyperestrogenic rats, J. Appl. Physiol. 90 (2001) 235–241.
[18] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.
[19] E.S. Campbell, M.A. Febbraio, Effects of ovarian hormones on exercise metabolism, Curr. Opin. Clin. Nutr. Metab. Care 4 (2001) 515–520.
[20] P.M. Haram, O.J. Kemi, S.J. Lee, M.Ø. Bendheim, Q.Y. Al-Share, H.L. Waldum, L.J. Gilligan, L.G. Koch, S.L. Britton, S.M. Najiar, U. Wisløff, Aerobic interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected for low aerobic capacity, Cardiovasc. Res. 81 (2009) 723–732.

[21] P. Diel, U. Laudenbach-Leschowski, A. Friedel, A. Voss, J. Roussel, Pulsed estradiol exposure has a limited ability to induce uterine proliferation in ovariectomized female Wistar rats, J. Mol. Cell Endocrinol. 230 (2005) 7–15.
[22] T. Hertrampf, G.H. Degen, A.A. Kaid, U. Laudenbach-Leschowski, J. Seibel, A.L. Di Virgilio, P. Diel, Combined effects of physical activity, dietary isoflavones and 17beta-estradiol on movement drive, body weight and bone mineral density in ovariectomized female rats, Planta Med. 72 (2006) 484–487.
[23] B Schleipen, M. Velders, U. Laudembach, K.H. Fritzemeier, P. Diel, Estrogen receptor subtype-specific effects on markers of bone homeostasis, Mol. Cell Endocrinol. 291 (2008) 104–108.
[24] G.L. Dohm, G.R. Beecher, The ovariectomized female rat as a model animal for the study of adaptation to endurance training, Lab Anim. Sci. 31 (1981) 146–148.
[25] R.S. Mazzeo, S.M. Horvath, Effects of training on weight, food intake, and body composition in aging rats, Am. J. Clin. Nutr. 44 (1986) 732–738.
[26] F Picard, Y. Deshaies, J. Lalonde, P. Samson, C. Labrie, A. Bélanger, F. Labrie, D. Richard, Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats, Int. J. Obes. 24 (2000) 830–840.
[27] J.M. Gray, G.N. Wade, Food intake, body weight, and adiposity in female rats: actions and interactions of progestins and antiestrogens, Am. J. Physiol. (Endocrinol. Metab.) 240 (1981) E474–E481.
[28] M. Shinoda, M.G. Latour, J.M. Lavoie, Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats, Int. J. Obes. 26 (2002) 335–343.
[29] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.
[30] J.M. Friedman, J.L. Halaas, Leptin and the regulation of body weight in mammals, Nature 395 (1998) 763–770.
[31] K.E.L. Hashimi, D.D. Pierroz, S.M. Hileman, C. Bjorbaek, J.S. Flier, Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesitiy, J. Clin. Invest. 105 (2000) 1827–1832.
[32] G.N. Wade, Regulation of body fat content? Am. J. Physiol. Regul. Integr. Comp. Physiol. 286 (2004) R14–R15.
[33] J.C. Stevenson, Cardiovascular effects of oestrogens, J. Steroid. Biochem. Mol. Biol. 74 (5) (2000) 387–393.
[34] M.L. Mestek, Physical activity, blood lipids and lipoproteins, Am. J. Lifestyle Med. 3 (2009) 279–283.