Swimming’s Prevention of Ovariectomy-Induced Obesity Through Activation of Skeletal-Muscle PPARα

Sunhyo Jeong and Michung Yoon

Ovariectomy leads to weight gain primarily in the form of adipose tissue in rodents. The authors investigated whether swimming improves ovariectomy-induced obesity through activation of peroxisome proliferator-activated receptor α (PPARα) in the skeletal muscle of female ovariectomized (OVX) mice, an animal model of postmenopausal women. Female mice were randomly divided into 3 groups (n = 8/group): a sedentary sham-operated group, a sedentary OVX group, and a swim-trained OVX group. After mice were subjected to swim training or kept sedentary for 6 wk, the authors studied the effects of swimming on not only body-weight gain, white adipose tissue (WAT) mass, adipocyte size, and skeletal-muscle lipid accumulation but also the expression of skeletal-muscle PPARα target genes. Sedentary OVX mice had significantly higher body weight and WAT than sedentary sham mice. However, swim training reduced body-weight gain, WAT mass, and adipocyte size of OVX mice. Swim-trained OVX mice had significantly lower levels of serum triglycerides and total cholesterol than sedentary OVX mice. Lipid accumulation in skeletal muscle was also markedly decreased by swimming. Concomitantly, swim training significantly increased mRNA levels of skeletal-muscle PPARα and its target enzymes, as well as uncoupling protein 3 (UCP3) responsible for fatty-acid oxidation. These results suggest that swimming can effectively prevent weight gain, adiposity, adipocyte hypertrophy, and lipid disorders caused by ovariectomy, in part through the activation of PPARα and UCP3, in the skeletal muscle of female mice and may contribute to the alleviation of metabolic syndrome, including obesity, hyperlipidemia, and Type 2 diabetes in postmenopausal women.

Keywords: swim training, UCP3, fatty-acid oxidation, female, lipid accumulation

Excess energy intake, combined with low levels of physical activity in modern societies, has led to increases in obesity and the related disorders of Type 2 diabetes, dyslipidemia, hypertension, and atherosclerosis (Jensen, 2006; Kissebah, 1997). In particular, being postmenopausal tends to be a risk factor for metabolic disease, including obesity, because visceral fat has an inverse relationship with estrogen levels (Bouchard, Despres, & Mauriege, 1993; Pilote et al., 2007; Tan, Gast, & van der Schouw, 2010). For example, ovariectomized (OVX) animals and many postmenopausal women not only show increased body weight and white adipose tissue (WAT) but also develop insulin resistance and cardiovascular disease, perhaps because of the development of obesity, whereas estrogen treatment antagonizes these effects in a positive way (Heine, Taylor, Iwamoto, Lubahn, & Cooke, 2000; Tan et al., 2010; Tchernof, Calles-Escandon, Sites, & Poehlman, 1998; Wade & Gray, 1979).

Although the primary treatment for obesity historically has been caloric restriction, a more promising treatment involves physical activity with appropriately increased frequency and duration in addition to controlling energy intake. Physical exercise rapidly increases energy expenditure and has been associated with improved weight control (King et al., 2001; Wier et al., 2001). Low-intensity, long-duration exercise also causes a preferential utilization of fat, resulting in less visceral fat in active individuals than sedentary controls (Morifuiji, Sanbongi, & Sugira, 2006; Redinger, 2009; Thompson, Townsend, Boughhey, Patterson, & Bassett, 1998). Whereas food restriction alone tends to cause loss of lean tissue, as well as fat tissue, physical activity appears to have a protein-sparing effect (Walberg, Mole, & Stern, 1982). Physical activity elicits physiological responses in skeletal muscle, so improved understanding of the molecular mechanisms of skeletal-muscle adaptation will help guide the proper use of regular exercise and physical activity in daily life, resulting in a reduced incidence of lifestyle-related disease in modern society (Rogge, 2009; Saltin & Pilegaard, 2002). In particular, swimming is effective for anyone, including overweight people, pregnant women, and seniors. It is regarded as one of the safest exercises, with very little risk of injury to joints, bones, and muscles (Iinattinem, Jokelainen, & Luukinen, 2008; Juhl, Kogevinas, Andersen, Andersen, & Olsen, 2010; Williams, 1999).

Peroxisome proliferator-activated receptor α (PPARα) appears to be important in both fat catabolism

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and obesity (Jeong et al., 2004; Yoon et al., 2003; Yoon et al., 2002). Because the PPARα ligand fenofibrate increases hepatic fatty-acid (FA) oxidation and thus decreases the levels of plasma triglycerides (TGs) responsible for adipose cell hypertrophy and hyperplasia (Jeong et al., 2004; Jeong & Yoon, 2009; Yoon et al., 2003), it may also inhibit an increase in body weight. This hypothesis is supported by a report that PPARα-deficient mice showed abnormalities in TG and cholesterol metabolism and became obese with age (Costet et al., 1998). FA-oxidative PPARα target genes and PPARα expression levels are suppressed in obese mice (Kamei et al., 2005). Furthermore, studies have shown that fenofibrate can modulate body weight in animal models of diabetes, obesity, and insulin resistance, such as fatty Zucker rats, high-fat-fed C57BL/6 mice, and high-fat-fed obese rats (Chaput, Saladin, Silvestre, & Edgar, 2000; Guerre-Millo et al., 2000; Mancini et al., 2001). PPARα has a potential role in the metabolic response to exercise training (Horowitz, Leone, Feng, Kelly, & Klein, 2000; Iemitsu et al., 2002; Morifuji et al., 2006). In addition to PPARα, uncoupling proteins (UCPs) are thought to play roles in regulating energy expenditure, body weight, body temperature, and FA metabolism. Chemical uncoupling of the mitochondrial membrane reduces weight gain and adiposity, so UCPs could potentially be used to treat human obesity (Clapham et al., 2000; Li et al., 2000). Transgenic mice overexpressing UCP3 in their skeletal muscles exhibit increased FA oxidation and are resistant to diet-induced obesity (Clapham et al., 2000). Because menopause is associated with a rise in obesity and swimming is a good choice to treat obesity in postmenopausal women, we thus hypothesized that swimming would likely improve ovariectomy-induced obesity through activation of skeletal-muscle PPARα and UCP3.

In the current study, we investigated whether swim training reduces weight gain, fat mass, and adipocyte size, as well as skeletal-muscle lipid accumulation, in female OVX mice, an animal model of postmenopausal women, and examined whether activation of PPARα and UCP3 in skeletal muscle is involved in this regulation.

Materials and Methods

Animals and Swim Training

For all experiments, 8-week-old female mice (C57BL/6J) were housed and bred at Mokwon University under a standard 12-hr light/dark cycle. Mice were randomly divided into three groups (n = 8/group): a sedentary sham-operated group, a sedentary OVX group, and a swim-trained OVX group. Mice in the swim-trained OVX group swam for 2 hr daily for 6 weeks in a 35 ± 1 °C water bath (1 × 1 m, Jeiotech, Seoul, Korea); during the first 2 weeks, the duration of daily training was gradually increased from 10 min to 2 hr. All animals received a high-fat diet with 45% kcal fat (#D12451, Research Diets, New Brunswick, NJ) for 6 weeks and then were sacrificed by cervical dislocation. Tissues were harvested, weighed, snap frozen in liquid nitrogen, and stored at –80 °C until use. Additional sections of parametrial adipose tissue and gastrocnemius muscle were prepared for histological analyses. All animal experiments were approved by the Institutional Animal Care and Use Committees of Mokwon University and followed National Research Council Guidelines.

Histological Analysis

Parametrial adipose tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed in a routine manner for paraffin sections. Sections (5 μm) were stained with hematoxylin and eosin for microscopic examination. To quantitate adipocyte size, hematoxylin- and eosin-stained sections were analyzed using an image-analysis system (Image Pro-Plus, Silver Spring, MD).

Gastrocnemius muscle tissues were embedded in a frozen-section compound (Leica, Bannockburn, IL) and frozen in liquid nitrogen. Transverse and longitudinal sections (7 μm) were cut using a cryostat at –20 °C and thawed on slides. Lipid accumulation in muscle tissue was assessed by Oil red O staining. Briefly, the sections were incubated with 1,2-propanediol for 1 min at room temperature and rinsed with deionized water. The sections were stained with 0.5% Oil red O solution for 2 hr at room temperature, incubated with 85% 1,2-propanediol for 1 min, and rinsed with deionized water. Morphological measurements of lipid accumulation were made under light-microscopic examination.

Serum Analysis

Serum concentrations of total cholesterol and TGs were measured using an automatic blood chemical analyzer (CIBA, Corning, OH).

RT-PCR

Total cellular RNA from skeletal-muscle tissue was prepared using the Trizol reagent (Invitrogen, Carlsbad, CA). After 2 μg total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase (MMLV-RT) and an antisense primer, cDNA was generated. The RNA was denatured for 5 min at 72 °C and then immediately placed on ice for 5 min. Denatured RNA was mixed with MMLV-RT, MMLV-RT buffer, and a dNTP mixture and incubated for 1 hr at 42 °C. Synthesized cDNA fragments were amplified by PCR in an MJ Research Thermocycler (Waltham, MA). The PCR primers used for gene-expression analysis are shown in Table 1. The cDNA was mixed with PCR primers, Taq DNA polymerase (Nanohelix, Daejeon, Korea), and a dNTP mixture. The reaction consisted of 28–45 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 52–58 °C, and elongation for 1 min at 72 °C. The PCR products were analyzed by electrophoresis on a 1% agarose gel. Relative expression levels were presented as...
a ratio of target gene cDNA versus β-actin cDNA. PCR products were quantified from agarose gels using the GeneGenius (Syngene, Cambridge, UK).

**Statistics**

All values are expressed as M ± SD. Statistical analysis was performed by ANOVA followed by Tukey’s multiple-comparison test. Statistical significance was defined as a p < .05.

**Results**

To determine whether swim training regulates obesity in OVX mice, we measured body-weight gain, adipose tissue mass, and adipocyte size. Sedentary OVX mice had significantly higher body weight and WAT mass than sedentary sham mice. Body weight, body-weight gain, and WAT mass in sedentary OVX mice were 36.0%, 72.9%, and 69.9% higher, respectively, than values in sedentary sham mice. Body weight, body-weight gain, and WAT mass in sedentary OVX mice were 36.0%, 72.9%, and 69.9% higher, respectively, than values in sedentary sham mice. Swimmer training had significantly higher body weight and WAT mass than sedentary sham mice (0.6 g, respectively; p < .05), and WAT mass (6.97 ± 0.8 g vs. 2.1 ± 0.6 g, respectively; p < .05). These results indicate that swim training suppresses lipid disorders, leading to the prevention of hypertriglyceridemia and hypercholesterolemia in mice with ovariectomy-induced obesity.

To determine the TG accumulation in skeletal muscle, we examined the cryostat sections of skeletal muscle by light microscopy after staining with Oil red O. Figures 4(A) and (B) show the microscopic appearance of transverse sections of skeletal muscle; Figure 4(C) shows the microscopic appearance of longitudinal sections. The number of stained fibers and intensity of staining of the positive fibers were greatly increased in OVX controls compared with sham mice. In contrast, reductions in both the number of stained fibers and the intensity of staining of the positive fibers were observed in the swim-trained mice compared with OVX controls.

To evaluate whether the inhibitory effects of swim training on body-weight gain, adipose tissue mass, and serum TG levels in OVX mice are caused by PPARα activation in skeletal muscle, we measured the mRNA levels of PPARα and PPARα target genes in skeletal muscle (Figure 5). As expected, mRNA levels of PPARα and its target FA-oxidative enzymes were lower in OVX control mice than in sham mice, although carnitine palmitoyltransferase 1 mRNA was not reduced. However, swim training increased the mRNA levels of PPARα and its target genes. Compared with sedentary OVX controls, swim-trained OVX mice showed significantly elevated mRNA levels of PPARα and its targets including medium-chain acyl-CoA dehydrogenase, carnitine

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<tr>
<th>Table 1</th>
<th>Sequences of Oligonucleotide Primers and PCR Conditions</th>
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<tr>
<td>Gene</td>
<td>Size (bp)</td>
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<tr>
<td>Peroxisome proliferator-activated receptor α</td>
<td>202</td>
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<tr>
<td>Thiolase</td>
<td>294</td>
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<tr>
<td>Carnitine palmitoyltransferase 1</td>
<td>587</td>
</tr>
<tr>
<td>Medium-chain acyl-CoA dehydrogenase</td>
<td>321</td>
</tr>
<tr>
<td>Uncoupling protein 3</td>
<td>180</td>
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<td>β-actin</td>
<td>350</td>
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Figure 1 — Effects of swim training on (A) body weight, (B) body weight gain, and (C) white adipose tissue mass in female sham and ovariectomized (OVX) mice. Mice were randomly divided into three groups ($n = 8$/group): a sedentary sham group, a sedentary OVX group, and a swim-trained OVX group. All values are expressed as $M \pm SD$. *Significantly different from sham mice, $p < .05$. **Significantly different from OVX mice, $p < .05$.

Palmitoyltransferase 1, and thiolase of 13.6%, 8.1%, 15.7%, and 20.8%, respectively ($p < .05$).

We also tested the effects of swim training on the mRNA level of UCP3, which is also involved in the regulation of FA oxidation, in mice with ovariectomy-induced obesity. Swim training significantly increased the levels of UCP3 mRNA by 22.2% compared with those of OVX mice ($p < .05$; Figure 6). These results suggest that swim training stimulates FA oxidation via activation of skeletal-muscle PPAR$\alpha$ and UCP3 and that the resulting fat catabolism may contribute to alleviation of obesity and lipid disorders in OVX animals.

Discussion

With a decrease in sex steroid hormones as a result of aging or gonadectomy, there is a tendency to increase adipose tissue mass (Björntorp 1996). Menopause accentuates obesity and the associated metabolic diseases such as insulin resistance, Type 2 diabetes, lipid disorders, and hypertension. The incidence of obesity is higher in postmenopausal women than in age-matched premenopausal women (Svendsen, Hassager, & Christiansen, 1995; Tchernof, Poehlman, & Després, 2000). Animal studies have shown that ovariectomy increases body weight and
Figure 2 — Effects of swim training on adipocyte size in female sham and ovariectomized (OVX) mice. Mice were randomly divided into three groups (n = 8/group): a sedentary sham group, a sedentary OVX group, and a swim-trained OVX group. (A) Histology of white adipose tissue (WAT). Shown are representative hematoxylin- and eosin-stained sections (5 μm thick) of parametrial WAT. Adipocyte size in the swim group was smaller than that in the sedentary group. (B) Adipocyte size in WAT. All values are expressed as M ± SD. *Significantly different from sham mice, p < .05. **Significantly different from OVX mice, p < .05.

Figure 3 — Effects of swim training on serum (A) triglycerides and (B) total cholesterol in female sham and ovariectomized (OVX) mice. Mice were randomly divided into three groups (n = 8/group): a sedentary sham group, a sedentary OVX group, and a swim-trained OVX group. All values are expressed as M ± SD. *Significantly different from sham mice, p < .05. **Significantly different from OVX mice, p < .05.
fat accumulation, and 17β-estradiol replacement reverses these effects (Geary & Asarian, 2001; Wade & Gray, 1979). Because PPARα and UCP3 are both involved in the regulation of energy homeostasis, we hypothesized that swim training regulates ovariectomy-induced obesity and lipid disorders via skeletal-muscle PPARα and UCP3 in female OVX mice, an animal model of postmenopausal women.

We observed that body-weight gain and WAT mass were significantly increased in OVX mice compared with sham mice with functioning ovaries. However, swim training substantially decreased ovariectomy-induced increases in body-weight gain and WAT mass by 72.9% and 69.9%, respectively, indicating that it acts as an efficient weight regulator in OVX mice. Similarly, a moderate-intensity training program of swimming prevented the weight gain after ovariectomy in older rats despite their excessive caloric intake of fat (Melton et al., 2000). In this respect, swim training demonstrates a potential for use in the treatment of postmenopausal obese women.

In parallel with WAT mass, OVX mice that received swim training were found to have significantly smaller adipocytes than sedentary OVX mice. Adipocyte size in parametrial WAT was 55.5% lower in swim-trained OVX mice than in sedentary OVX mice. Our results also showed that swim training increased the number of small adipocytes while decreasing the number of large adipocytes in a fixed area, suggesting that it induced the conversion of large adipocytes to smaller adipocytes (Oh et al., 2006). Because large adipocytes are associated with insulin resistance, whereas smaller adipocytes are associated with insulin sensitivity (Kadowaki, 2000; Kubota et al., 1999), swim training may improve insulin sensitivity in part because of its ability to reduce adipocyte size in OVX mice.

Loss of ovaries is associated with dyslipidemia, such as increased TG and low-density lipoprotein cholesterol levels and decreased levels of high-density lipoprotein cholesterol (Pilote et al., 2007; Tan et al., 2010). Our current results showed that swim training significantly decreased the serum levels of both TGs and
Figure 5 — Effects of swim training on the mRNA levels of peroxisome proliferator-activated receptor α (PPARα) and its target genes in the skeletal muscle of female sham and ovariectomized (OVX) obese mice. (A) Mice were randomly divided into three groups (n = 8/group): a sedentary sham group, a sedentary OVX group, and a swim-trained OVX group. RNA was extracted from the skeletal muscle, and all values are expressed as M ± SD of relative density units (R.D.U.) using β-actin. *Significantly different from sham mice, p < .05. **Significantly different from OVX mice, p < .05. (B) Representative RT-PCR photographs from one of three independent experiments. CPT-1 = carnitine palmitoyltransferase 1; MCAD = medium-chain acyl-CoA dehydrogenase.
total cholesterol caused by ovariectomy. This finding is consistent with the decreased WAT mass and adipocyte size because lipids that accumulate in adipose tissue are largely derived from circulating TGs. Thus, it appears that swim training can be used to treat postmenopausal women with hypertriglyceridemia and hypercholesterolemia. It may also be effective in treating elderly female patients with coronary heart disease because hypertriglyceridemia is a more significant risk factor of coronary heart disease for women than for men (Pilote et al., 2007; Tan et al., 2010).

Both weight loss and improved lipid metabolism resulting from swim training presented in this study may be attributed to the stimulatory effects of swim training on FA \( \beta \)-oxidation via skeletal-muscle PPAR\( \alpha \) activation. We found that swim training significantly increased the mRNA levels of PPAR\( \alpha \) and its target genes, such as carnitine palmitoyltransferase 1, medium-chain acyl-CoA dehydrogenase, and thiolase, all of which are responsible for FA \( \beta \)-oxidation, in the skeletal muscle of OVX mice. According to the results of Horowitz et al. (2000), endurance training increased the skeletal-muscle protein content of PPAR\( \alpha \) and its target mitochondrial FA-oxidative enzymes in lean premenopausal women, suggesting that this important adaptation to exercise training is intrinsic to skeletal muscle and that the increase in fat oxidation induced by exercise is primarily associated with an increase in skeletal-muscle FA-oxidative capacity. Morifuji et al. (2006) also found increases in PPAR\( \alpha \)mRNA expression levels in the skeletal muscle of swim-trained rats, although PPAR\( \alpha \) activity was not altered in the skeletal muscle of cycle-ergometer-trained humans and wheel-running-trained rats (Bruce et al., 2006; Petridou et al., 2007; Tunstall et al., 2002). Different exercise modes, exercise intensities, and experimental subjects may have contributed to the discrepancies in those previous reports. However, our current results and other data suggest that swim training regulates obesity and lipid disorders by activating PPAR\( \alpha \), which is a candidate gene activated during exercise training.

In addition to swim-training-induced expression of PPAR\( \alpha \) target enzymes for FA oxidation, we examined alterations in the skeletal-muscle expression of UCP3, which is known to be regulated by PPAR\( \alpha \), based on the role of UCP3 in the regulation of energy expenditure, body weight, and FA metabolism (Boss, Hagen, & Lowell, 2000). UCP3 mRNA and protein expression were elevated by endurance exercise in the skeletal muscle of rodents and humans (Schrauwen et al., 2002; Tsuboyama-Kasaoka et al., 1998); similarly, we observed that swim training increased the mRNA levels of skeletal-muscle UCP3 in obese OVX mice. These results suggest that skeletal-muscle UCP3 is involved in swim training’s inhibition of ovariectomy-induced weight gain and lipid disorders.
Lipid accumulation in skeletal muscle was examined after swim training. Swim training decreased skeletal-muscle TG levels, as shown by reductions in the number of Oil red O–stained myocytes and in the intensity of staining of the positive myocytes. These results are consistent with swim-training-induced activation of PPARα and UCP3 responsible for FA oxidation in skeletal muscle. In both human and rodent skeletal muscle, PPARα regulates lipid metabolism. Activation of PPARα by the potent agonist GW7647 in differentiated human myotubes stimulated lipid oxidation and decreased accumulation of TGs (Muoio et al., 2002). Moderate expression of UCP3 protein in L6 myotubes specifically increases FA oxidation (MacLellan et al., 2005).

These results suggest that swim training stimulates fat catabolism in part because of PPARα and UCP3 activation in skeletal muscle of female O VX mice. Although swimming induced mRNA expression of these genes, it is necessary to investigate whether swimming affects their protein levels and to examine the upstream events of PPARα and UCP3 activation.

In conclusion, these results suggest that swim training can effectively prevent weight gain, adiposity, adipocyte hypertrophy, and lipid disorders caused by ovariectomy, in part through the activation of PPARα and UCP3 in skeletal muscle of female mice. These benefits may further contribute to alleviating metabolic syndrome, including obesity, hyperlipidemia, and Type 2 diabetes, in postmenopausal women. Further studies will be necessary to show whether PPARα deficiency and UCP3 deficiency would prevent swimming effects to prove our current results.

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References


