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Swim Training Improves Fitness in High Fat Diet-fed Female Mice

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The peroxisome proliferator-activated receptor α (PPARα) is a nuclear transcription factor that plays a central role in lipid metabolism and obesity. Exercise also is a powerful modifier of the manifestations of the lipid metabolism and obesity in animal models and humans with obesity and metabolic syndrome. However, effects of exercise on lipid metabolism and obesity in normal-weight younger female subjects, having functional ovaries and not metabolic disease, remain unexplained. To explore the effects of exercise on the development of obesity and its molecular mechanism in high fat diet-fed female C57BL/6J mice, we experimented the effects of swim training on body weight, adipose tissue mass, serum lipid levels, morphological changes of adipocytes and the expression of PPARα target genes involved in fat oxidation in skeletal muscle tissue of female C57BL/6J mice. Swim-trained mice had significantly decreased body weight, adipose tissue mass, serum triglycerides compared with female control mice. Histological studies showed that swim training significantly decreased the average size of adipoctyes in parametrial adipose tissue. Swim training did not affect the expression of PPARα mRNA in skeletal muscle. Concomitantly, swim training did not increase mRNA levels of PPARα target genes responsible for fatty acid β-oxidation, such as carnitine palmitoyltransferase 1, medium chain acyl-CoA dehydrogenase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, and thiolase in skeletal muscle. In conclusion, these results indicate that swim training regulates lipid metabolism and obesity in high fat diet fed-female mice although swim training did not increase mRNA levels of PPARα target genes involved in fatty acid β-oxidation in skeletal muscle, suggesting that swim training may prevent obesity and improve fitness through other mechanisms in female with functioning ovaries, not through the activation of skeletal muscle PPARα.

Key Words: PPARα, Swim training, Muscle, Female

INTRODUCTION

Obesity arises from the imbalance between energy intake and energy expenditure, leading to a pathological accumulation of lipids in adipocytes. Obesity has been the subject of intense investigation due to its causative links to a number of metabolic diseases such as type 2 diabetes, atherosclerosis, hyperlipidemia and hypertension (Bray, 2003).

The family of peroxisome proliferator-activated receptors (PPARs) is thought to be involved in the control of energy homeostasis and obesity. Among the three PPAR isoforms, PPARα seems to be important in obesity and fat catabolism (Staels et al., 1998; Schoonjans et al., 2000). The activation of PPARα heterodimerizes with retinoid X receptor (RXR) and binds to PPAR response elements (PPREs) in the promoter region of target genes (Sander et al., 2000). PPARα target genes include those involved in the hydrolysis of plasma triglycerides, fatty acid uptake and binding, and fatty acid β-oxidation (Zhang et al., 1992; Hertz et al., 1995; Auwerx et al., 1996; Osumi et al., 1996; Schoonjans et al., 1996; Martin et al., 1997; Nicolas-Frances et al., 2000). Therefore, the activation of PPARα target genes promotes increased fatty acids oxidation and the increased breakdown, reduced synthesis, and secretion of triglycerides.

Many programs and strategies to prevent and treat obesity have been developed, such as a diet cure, exercise and pharmacological therapy (Muller et al., 2001; Swimburn

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and Egger, 2002; Halpern and Mancini, 2003). Exercise is a powerful modifier of the manifestations of the metabolic syndrome in the direction of health enhancement (Pedersen and Saltin, 2006). Understanding exercise physiology is important for the maintenance of our health. Indeed, physical exercise is a powerful modifier of the manifestations of the lipid metabolism and obesity in overweight men and animal models (Howley et al., 1995; Crampes et al., 2003; Morifuji et al., 2006; Pillard et al., 2010). This is achieved especially through alterations in metabolism caused by modifications in the activity and quantity of specific proteins. However, the effect of exercise training on the lipid metabolism and obesity in lean female is controversial, yet. It is reported that exercise trained female Wistar rats have no effects on body weight and visceral body fat mass as well as serum triglycerides, but exercise training in women increase the availability and oxidation of plasma fatty acids derived from adipose tissue triglycerides and levels of enzymes involved in fatty acid oxidation in skeletal muscle to regulate lipid metabolism (Friedlander et al., 1998; Horowitz et al., 2000). As data regarding the effects of exercise training on key proteins involved in lipogenesis and lipolysis are limited and controversial, the mechanisms by which exercise regulates lipid metabolism and protects development of obesity on lean female are unclear.

The aim of this study was to determine whether swim training has an effect on mRNA expression of enzymes involved in fatty acid oxidation and prevents obesity through PPARα actions in female C57BL/6J mice with functional ovaries. This study indicate that swim training did not increase mRNA expression of PPARα target genes involved in fatty acid oxidation in skeletal muscle, but reduced accumulation of body and fat weight and serum triglycerides in high fat diet-fed female mice.

MATERIALS AND METHODS

Animal and swim training

For all experiments, eight-week-old female mice (C57BL/6J) were housed and bred at the Korea Research Institute of Bioscience and Biotechnology under pathogen-free conditions with a standard 12-h light/dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. Female mice each randomly divided into two groups (a non-swim control group and a swim group, n=8/group). Mice in swim group swam for 2 h daily for 6 weeks in a 35±1℃ water bath (1 m×1 m, Jeiotech, Seoul, Korea); during the first two weeks, the duration of daily training was increased from 10 min to 2 h. All the animals received high fat diet (45% kcal fat, Research Diets, New Brunswick, NJ) for 6 weeks and were sacrificed by cervical dislocation. Tissues were harvested, weighed, snap frozen in liquid nitrogen and stored at -80℃ until use. An additional section of parametrial adipose tissue was fixed in phosphate-buffered formalin for histological analysis.

Histological analysis

Adipose tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed in a routine manner for paraffin section. Sections (5 μm) were stained with hematoxylin and eosin for microscopic examination.

Serum assays

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

Analysis of target gene expression

Total RNA was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY) and relative levels of specific mRNA were assessed by reverse transcription-polymerase chain reaction (RT-PCR). Complementary DNA was synthesized from RNA samples by mixing 2 μg of total RNA and 0.5 μl of the reverse primer in a total volume of 14 μl in water, heating the mixture at 75℃ for 15 min, cooling the mixture immediately on ice for 5 min, and adding 5X M-MLV reaction buffer, 10 mM dNTP mixture (Promega) and 200 units M-MLV RT (Promega) in total volume of 25 μl. Samples were incubated at 42℃ for 60 min. A five μl aliquot of the RT reaction was then used for subsequent PCR amplification with specific primers.

Twenty five μl PCR sample contained 5 μl of the RT reaction, 10X buffer with $MgCl_2$, 10 mM dNTP, 5 units of

Table 1. Sequences of oligonucleotide primers and PCR conditions

Genes	Size (bp)	Primer sequences	Annealing (℃)	Cycle
PPARα	202	F : 5'-gcagctcgtacaggtcatca-3' R : 5'-ctcttcatccccaagcgtag-3'	56	37
HD	355	F : 5'-caaaaagatcggaaagattg-3' R : 5'-ctgataccaccgtttacctg-3'	58	45
Thiolase	294	F : 5'-ggataacctcggagaatgtggc-3' R : 5'-cactcacctgactggagttt-3'	52	45
CPT-1	587	F : 5'-tatgtgaggatgctgcttcc-3' R : 5'-ctcggagagctaagcttgtc-3'	58	43
MCAD	321	F : 5'-gacatttggaaagctgctagtg-3' R : 5'-tcacgagctatgatcagcctctg-3'	58	43
β-actin	350	F : 5'-tggaatcctgtggcatccatgaaac-3' R : 5'-taaaacgcagctcagtaacagtccg-3'	58	28

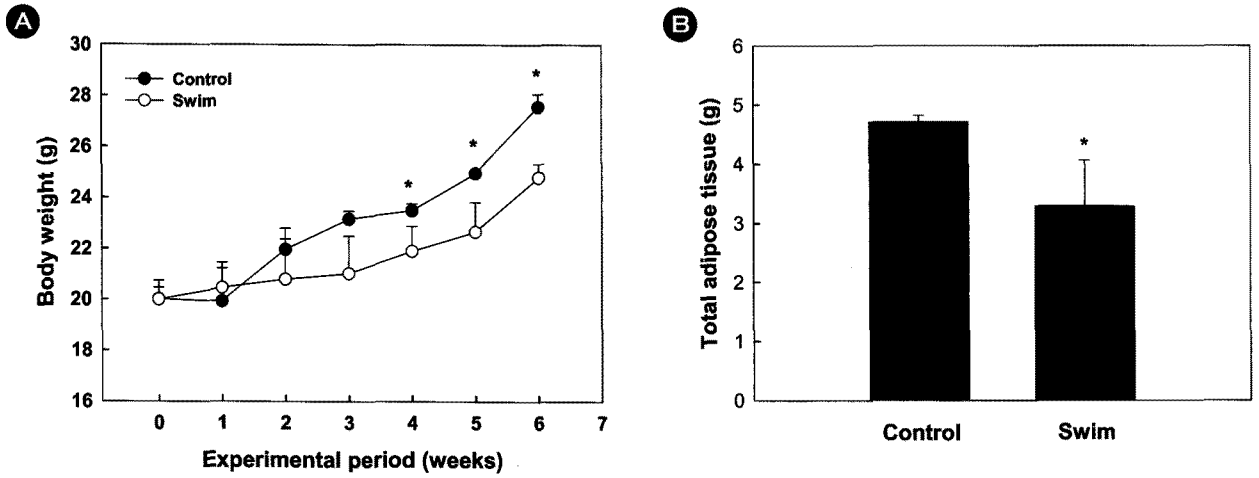

Fig. 1. Effects of swim training on body weight (A) and total adipose tissue mass (B) in female C57BL/6J mice. Female C57BL/6J mice (n=8/group) were subjected to swim training for 2 h daily in a 35±1℃ water bath (1 m×1 m, Jeiotech, Seoul, Korea) for 6 weeks; during the first two weeks, the duration of daily training was increased from 10 min to 2 h. Control mice of similar initial body weights were kept sedentary for 6 weeks. All values are expressed as mean ± SD. *P<0.05 Significantly different from control mice.

Tag polymerase (Solgent, Taejon, Korea) and 10 μM of each primer. Primer sequences and PCR conditions are shown in Table 1. PCR was performed in a PTC-100™ Programmable Thermal Controller (MJ Research, Watertown, MA, USA). PCR products were electophoresed on a 1% agarose gel.

Statistics

Unless otherwise noted, all values are expressed as mean ± standard deviation (SD). All data were analyzed by ANOVA for statistically significant differences between each group.

RESULTS

To determine whether swim training regulates obesity in female C57BL/6J mice, we measured body weight and total adipose tissue mass (Fig. 1). At week 6, swim trained-mice had significantly decreased body weight and total adipose tissue mass compared with control mice (P<0.05). Since total adipose tissue mass is related to serum lipid levels, serum total cholesterol and triglycerides were examined (Fig. 2). In comparison with control mice, swim-trained mice had significantly decreased circulating triglycerides (P<0.05). However, swim training did not affect serum

levels of total cholesterol in female mice. Our data show a strong correlation between decreased serum triglycerides, body weight and total adipose tissue mass following swim training in female mice.

To determine whether swim training regulates morphological changes of adipocytes in female mice, the size and number of parametrial adipocytes were measured by histological analysis and image analysis system (Fig. 3). In

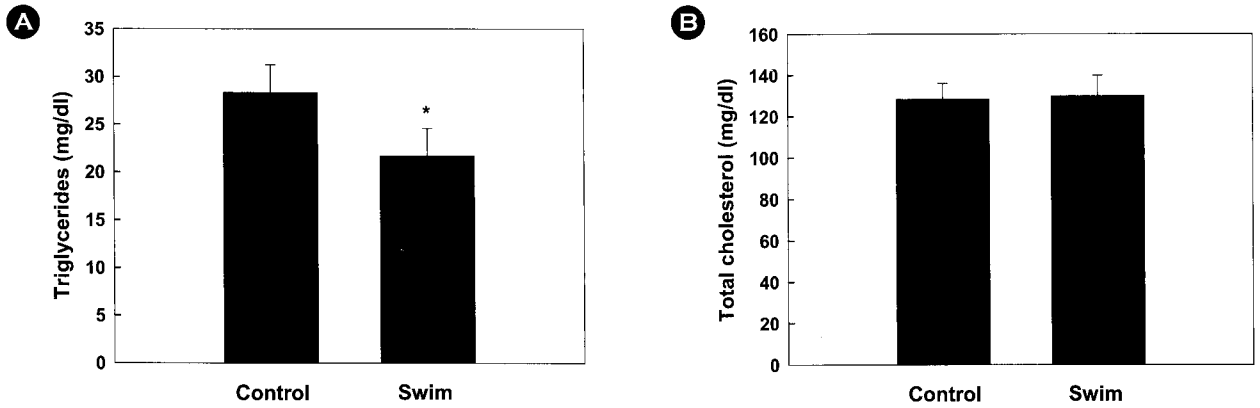

Fig. 2. Effects of swim training on circulating triglycerides (A) and total cholesterol (B) in female C57BL/6J mice. Female C57BL/6J mice (n=8/group) were subjected to swim training as described in the legend of Fig. 1, with control mice of similar initial body weights kept sedentary, for 6 weeks. All values are expressed as mean ± SD. *P<0.05 Significantly different from control mice.

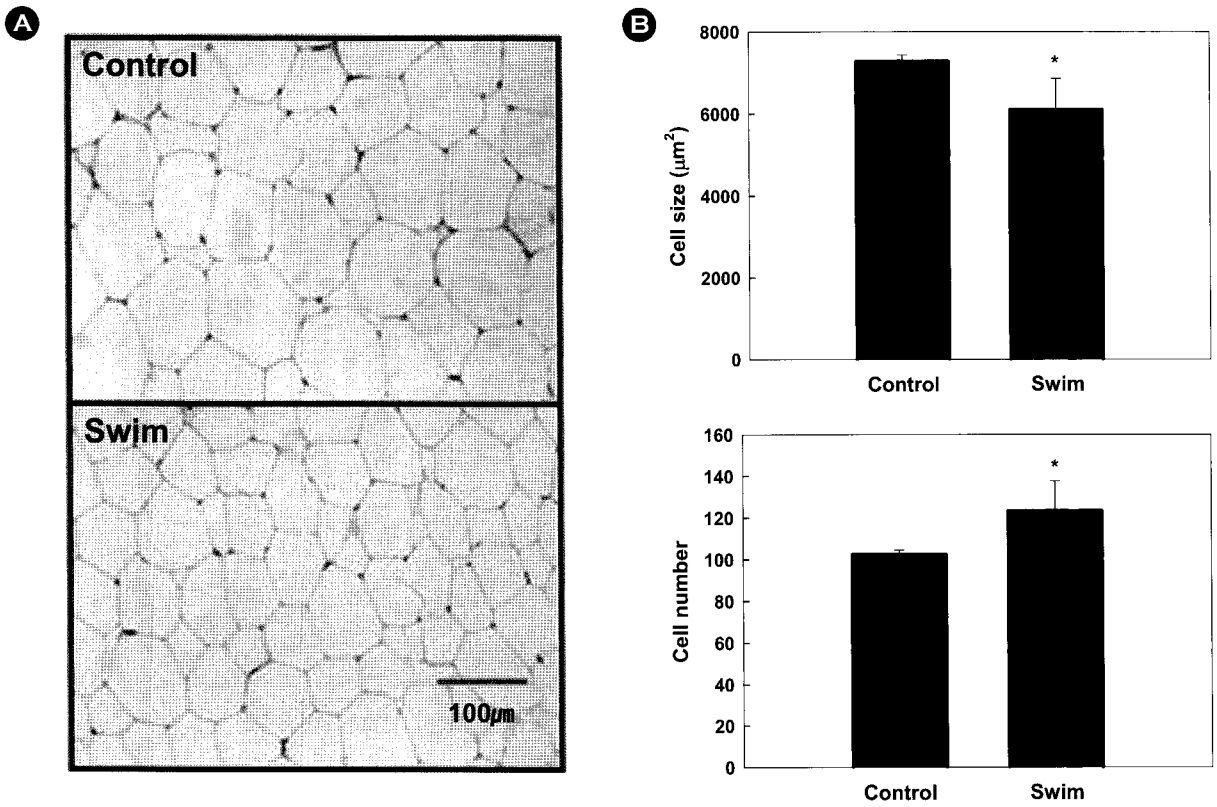

Fig. 3. Light microscopy of parametrial adipose tissue stained with hematoxylin and eosin (original magnification × 200). (A) Female C57BL/6J mice (n=8/group) were subjected to swim training as described in the legend of Fig. 1, with control mice of similar initial body weights kept sedentary, for 6 weeks. Shown are representative hematoxylin- and eosin-stained sections (5 μm thick) of female parametrial adipose tissue. (B) Hematoxylin and eosin-stained sections were analyzed with an image analysis system, and the size and number of adipocytes in a fixed area (1,000,000 μm^2) were quantified. All values are expressed as mean ± SD. *P<0.05 Significantly different from control mice.

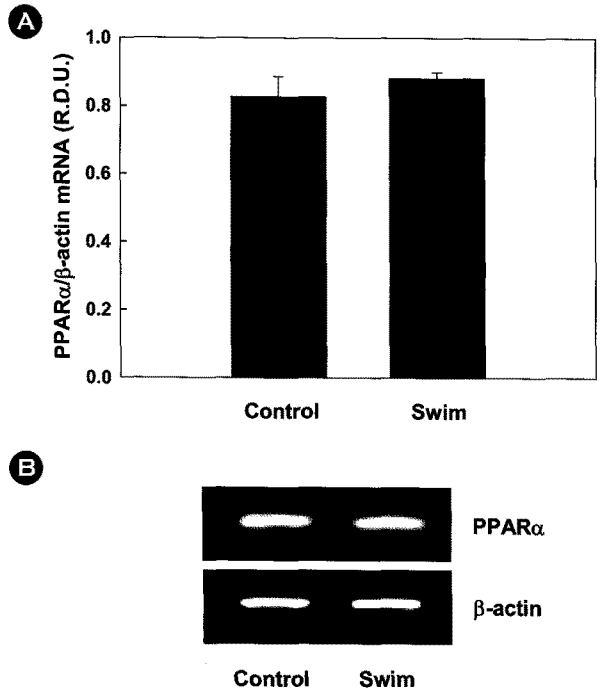

Fig. 4. Modulation of PPARα gene expression by swim training in skeletal muscle of female C57BL/6J mice. (A) Female C57BL/6J mice (n=8/group) were subjected to swim training as described in the legend of Fig. 1, with control mice of similar initial body weights kept sedentary, for 6 weeks. RNA was extracted from the skeletal muscle, and all values are expressed as mean ± SD of R.D.U. (relative density units) using β-actin. (B) Representative RT-PCR photographs from one of three independent experiments are shown. RT-PCR; reverse transcription-polymerase chain reaction

comparison with control mice, swim-trained mice significantly decreased in the average size of adipocytes and increased the number of adipoctyes in parametrial adipose tissue of female mice ($P<0.05$). These results are consistent with reductions in circulating triglyceride levels, from which lipids accumulated in the adipose tissue largely derive.

To evaluate whether the effects of swim training on lipid profiles were caused by PPARα actions in skeletal muscle, we determined the expression of PPARα and PPARα target genes involved in the lipid metabolism, such as enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (HD), thiolase, carnitine palmitoyltransferase 1 (CPT-1) and medium chain acyl-CoA dehydrogenase (MCAD); these have crucial roles in the control of mitochondrial and peroxisomal fatty acid β-oxidation system (Fig. 4 and 5). Compared with control mice, swim training did not increase the expression levels of PPARα as well as PPARα target genes involved in the lipid metabolism, such as HD, thiolase, CPT-1 and MCAD, suggesting that swim training may not stimulate PPARα actions in skeletal muscle of female mice.

DISCUSSION

Metabolic syndrome, usually caused by overnutrition and a lack of physical activity, represents a heterogeneous cluster of obesity-related diseases. It is well known that physical activity has beneficial effects on the metabolic syndrome (Haram et al., 2009). In this study we wanted to investigate that swim training may activate PPARα in skeletal muscle, leading to reductions in body weight, total adipose tissue mass and circulating trilgycerides in female mice.

Our results demonstrated that swim-trained female mice for 6 weeks decreased body weight and total adipose tissue mass. We previously showed that reductions in body weight gain could be correlated with reductions in fat mass, indicating that reduced fat may lead to reduced body weight (Yoon et al., 2002; Yoon et al., 2003; Jeong et al., 2004a). Similarly, swim-trained mice were significantly reduced total adipose tissue mass.

It was reported that the increase in adipose tissue mass was due to the enlargement of the preexisting adipocytes with increased lipid accumulation (Ogawa et al, 2004; Villena et al., 2004; Yagi et al., 2004). In accordance with the effects of swim training on adipose tissue mass, we found that the size of adipocytes was considerably smaller in swim-trained mice than in control mice. Morphometric analysis of adipose tissue histology showed that swim training decreased the number of large adiocytes and increased the number of small adipoctyes. Adipocyte size seems to be influenced by circulating triglycerides, since lipids that accumulate in adipose tissue are largely derived from circulating triglycerides (Bourgeois et al., 1983; Costet et al., 1998; Chaput et al., 2000). Expectedly, swim-trained female mice had decreased circulating triglycerides compared with controls. It was reported that swim training decreased in serum triglycerides, indicating that swim training improves circulating lipid metabolism (Miyasaka et al., 2003; Savage et al., 2003).

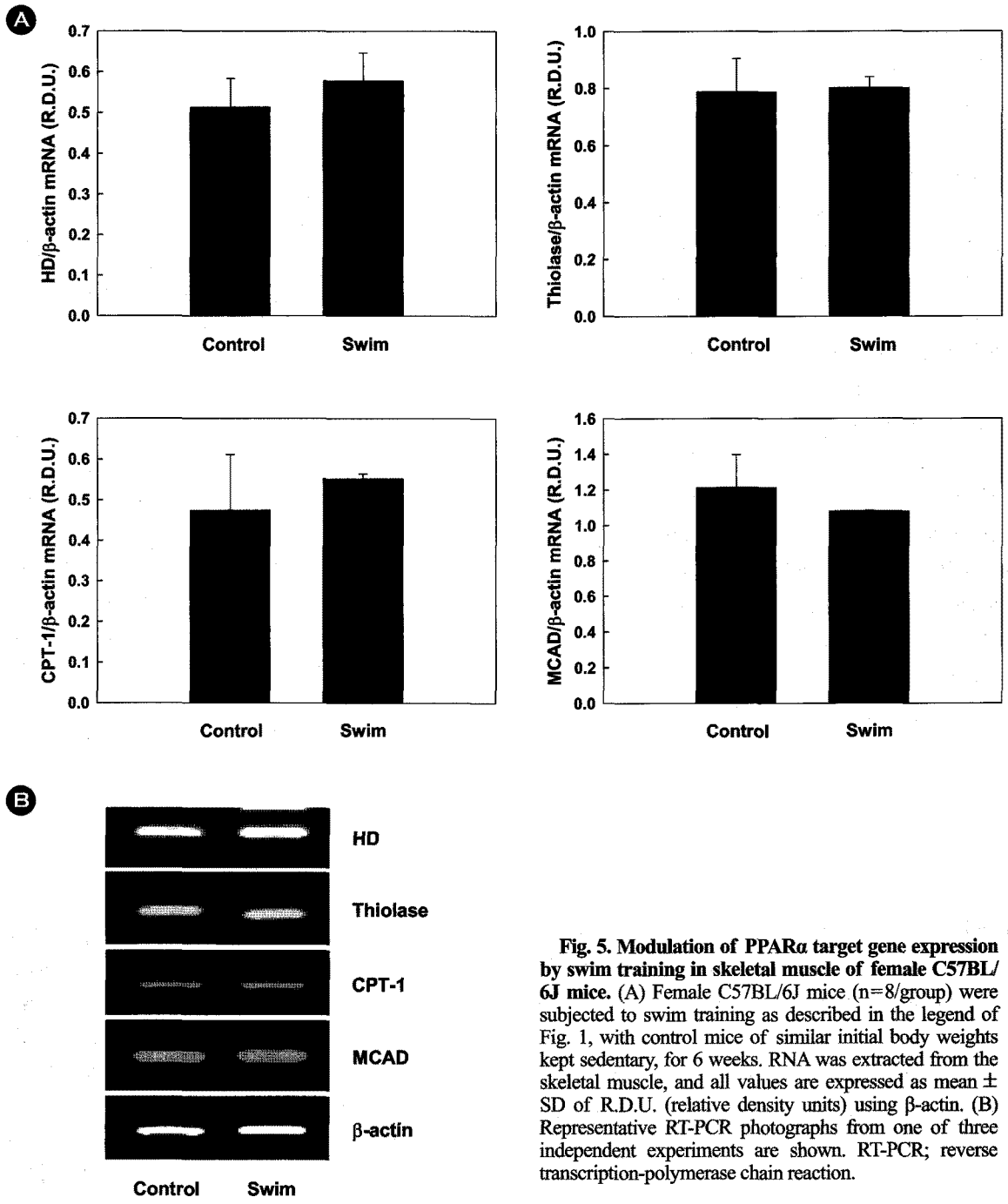

Fig. 5. Modulation of PPARα target gene expression by swim training in skeletal muscle of female C57BL/6J mice. (A) Female C57BL/6J mice (n=8/group) were subjected to swim training as described in the legend of Fig. 1, with control mice of similar initial body weights kept sedentary, for 6 weeks. RNA was extracted from the skeletal muscle, and all values are expressed as mean ± SD of R.D.U. (relative density units) using β-actin. (B) Representative RT-PCR photographs from one of three independent experiments are shown. RT-PCR; reverse transcription-polymerase chain reaction.

In this present study, to gain insight into the molecular mechanisms underlying the effects described above, we have examined, in female skeletal muscle, the effects of swim training on the expression of genes involved in fat catabolism, as well as their regulatory transcription factor PPARα. We found no differences between trained and untrained mice in the skeletal muscle PPARα. In addition, swim training did not significantly alter the expression of genes involved in fatty acid β-oxidation (HD, thiolase, CPT-1 and MCAD). The finding of no difference in muscle PPARα is in agreement with that of Tunstall et al (Tunstall et al., 2002) and Petridou A (Petridou et al., 2007) who found no effect of training on the PPARα mRNA content of skeletal muscle of human and rat. PPARα is known to be activated by fatty acids (Kliewer et al., 1997), whose concentrations in plasma increase immediately with exercise

(Mougios et al., 2003). It has been shown that the binding of fatty acids to PPARα increases the DNA binding activity of PPARα (Mochizuki et al., 2006). However, the action of PPARα on lipid metabolism and obesity may be influence by estrogens, ovarian factor in female (Yoon et al., 2003; Jeong et al., 2004b). There is evidence to show that a bidirectional signal cross-talk exists between PPARα and ERs (Wang and Kilgore, 2002; Jeong and Yoon, 2007). There are data indicating that estrogen receptor (ER) regulated PPARα transactivation and that expression of ERα or ERβ inhibited the basal expression of PPRE-mediated reporter gene activity. Altogether, estrogens may inhibit the actions of PPARα on lipid metabolism and obesity through its effects on PPARα-dependent regulation of target genes. Accordingly, the unchanged expression levels of PPARα target genes by swim training may be due to interference of estrogens of female with functional ovaries, although swim training increase serum fatty acids.

In conclusion, our results demonstrated that swim training for 6 weeks decreased body weight, total adipose tissue mass and circulating triglycerides in female mice although the expression of PPARα target genes involved in lipid catabolism was not decreased in skeletal muscle of female mice. Further studies will be necessary to determine the factors contributing obesity and lipid metabolism following swim training in female mice and need to investigate whether swim training stimulates the expression of PPARα target genes involved in lipid catabolism in female ovariectomized mice, an animal model of postmenopausal women, in order to better understand the molecular mechanisms that swim training regulates lipid metabolism and obesity.

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