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# Exercise training in ovariectomized rats stimulates estrogenic-like effects on expression of genes involved in lipid accumulation and subclinical inflammation in liver

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**Abstract**

We hypothesized that the reduction in liver fat accumulation known to occur with exercise training in ovariectomized (Ovx) rats is associated with reduced expression of genes involved in lipogenesis while favoring the expression of transcription factors regulating lipid oxidation. We also tested the hypothesis that liver fat accumulation in Ovx rats is associated with an increased gene expression of several inflammatory biomarkers and that exercise training would attenuate this response. Sprague-Dawley female rats (14 weeks of age) were randomly divided into 4 groups of sedentary sham-operated (Sham), Ovx, Ovx with 17$\beta$-estradiol (E2) supplementation using a pellet (0.72 mg; 0.012 mg/d) with a biodegradable carrier binder, and Ovx trained with endurance exercise. Endurance exercise training consisted of continuous running on a motor-driven rodent treadmill 5 times per week for 5 weeks. Fat accumulation in liver as well as in adipose fat depots was higher ($P < .01$) in Ovx than in Sham rats. This response was prevented in Ovx animals with 17$\beta$-estradiol supplementation and with endurance exercise training. Liver gene expressions of sterol regulatory element-binding protein 1-c, stearoyl coenzyme A desaturase 1 (and its protein content), carbohydrate response element binding protein, and acetyl–coenzyme A carboxylase were increased with estrogen withdrawal ($P < .01$). These responses were corrected with E2 supplementation alone as well as with training alone. Conversely, hepatic peroxisome proliferator–activated receptor $\alpha$ messenger RNA levels were lower ($P < .01$) after estrogen removal compared with Sham rats. The lower hepatic peroxisome proliferator–activated receptor $\alpha$ messenger RNA levels in Ovx rats were reincreased by E2 replacement or by exercise training. Gene expression of proinflammatory cytokines including inhibitor-$\kappa$B kinase $\beta$ and interleukin-6, as well as protein content of nuclear factor-$\kappa$B, was higher ($P < .01$) in Ovx than in Sham animals. E2 supplementation or exercise training prevented the expression of the proinflammatory markers. It is concluded that exercise training reduces fat accumulation in liver of Ovx rats possibly through regulation of key molecules involved in lipogenesis and lipid oxidation. Exercise training also acts as estrogens in properly regulating the expression of inflammatory biomarkers in liver of Ovx rats.


## 1. Introduction

Menopause is associated with the development of a state of hepatic steatosis [1,2]. The importance of this phenomenon is enlightened by the fact that excessive fat accumulation in liver plays an important role in the development of insulin resistance [3]. Recent findings even indicate that ectopic fat in liver may be more important than visceral fat in characterization of metabolically benign obesity in humans [4,5]. Hepatic and adipocytes fat accumulation is also well documented in animal models of menopause [6-9]. Ovariectomized (Ovx) rats as well as aromatase receptor knockout mice exhibit hepatic fat accumulation that seems to be triggered by changes in expression of genes that increase lipid synthesis and reduce lipid oxidation in liver [10,11]. There is also recent physiologic evidence that fatty acid

The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care after institutional approval.

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oxidation is reduced in liver of Ovx rats [12]. An alternative to counteract liver fat accumulation with estrogen withdrawal might be exercise training. It was reported that exercise training prevents fat accumulation in liver of high-fat–fed rats [13,14]. Specifically in Ovx rats, there is some evidence that resistance training in conjunction with food restriction reduces liver fat accumulation [15,16]. Recently, we reported that endurance exercise training when conducted concurrently with estrogen withdrawal prevented liver fat accumulation in Ovx rats [17]. These studies, however, did not provide any mechanistic information on the action of exercise training in preventing liver fat accumulation in Ovx animals. The first aim of the present study was to test the hypothesis that exercise training reduces the expression of key molecules involved in lipid synthesis while favoring the expression of molecules involved in fat oxidation.

In recent years, it has become clear that metabolic disturbances related to fat accumulation in adipocytes and ectopic tissues, such as liver, are associated with subclinical inflammation [18,19]. For instance, Cai et al [20] showed that inflammatory gene expression increases in liver of both transgenic and high-fat–fed mice with increasing adiposity. Regardless of the causes, the nuclear factor (NF)–$\kappa$B is activated in hepatocytes and proinflammatory cytokines including tumor necrosis factor (TNF)–$\alpha$ and interleukin (IL)-6 are overproduced in fatty liver [21]. As such, it is relevant to investigate if liver lipid accumulation resulting from estrogens deficit in Ovx rats leads to subacute hepatic inflammation and, if it is so, whether exercise training attenuates this response as it does for fat accumulation. Consequently, the second objective of the present study was to investigate the effects of ovariectomy and exercise training on gene expression of inflammatory biomarkers in the liver.

## 2. Methods

### 2.1. Animal care

Female Sprague-Dawley strain rats (Charles River, St-Constant, Quebec, Canada), weighing 180 to 200 g upon their arrival, were housed individually and had ad libitum access to food and tap water. All rats received usual pellet rat chow, referred to as the *standard diet* (12.5% fat, 63.2% carbohydrate, 24.3% protein, kilocalories; Agribrands Purina Canada, Woodstock, Ontario, Canada). Their environment was controlled in terms of light (12:12-hour light-dark cycle starting at 6:00 AM), humidity, and room temperature (20°C-23°C). The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care after institutional approval.

### 2.2. Groups

Six weeks after their arrival to our laboratory, rats were randomly divided into sedentary sham-operated (Sham), sedentary Ovx, sedentary Ovx with 17$\beta$-estradiol supplementation (OvxE2), and Ovx rats that underwent endurance exercise training (OvxTr) groups (n = 6-8 rats per group). Body weight and food intake were monitored 3 times per week. All animals were killed 6 weeks after the surgical manipulations.

### 2.3. Surgery

Ovariectomy surgery was conducted according to the technique described by Robertson et al [22]. Animals were injected with antibiotics (Tribrissen 24%, CDMV, St-Hyacinthe, QC, Canada; 0.125 mL/kg, subcutaneously) for 3 days, beginning the day before surgery. For surgery, rats were anesthetized with a mixture of ketamine-xylazine (61.5-7.6 mg/kg, intraperitoneally). In OvxE2 rats, a small 17$\beta$-estradiol pellet (0.72 mg; 0.012 mg/d) with a biodegradable carrier binder efficient for 60 days (catalog no. SE-121; Innovative Research of America, Sarasota, FL) was placed subcutaneously between the shoulder blades. El-Mas and Abdel-Rahman [23] previously showed that this estrogen regimen produces physiologic levels of the hormone. A placebo 60-day pellet containing the binding carrier only was used in all other rats (catalog no. SC-111).

### 2.4. Exercise protocol

One week (seventh week) was provided for surgery and recovery in all rats. During this time, all animals were submitted to a habituation running protocol (10-15 min/d) in the last 3 days of the week. Exercise training (Tr) consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA) 5 times per week for the duration of the experiment (5 weeks after recovery). The OvxTr rats progressively ran from 15 min/d at 15 m/min, 0% slope, up to 60 min/d at 26 m/min, 10% slope, for the last 3 weeks. All Tr animals were restrained from training 48 hours before sacrifice.

### 2.5. Blood and tissue sampling

Rats were killed between 9:00 AM and 12:00 PM. Food was removed from the animal's cage at least 3 hours before they were killed. Immediately after complete anesthesia (pentobarbital sodium; 50 mg/kg, intraperitoneally), the abdominal cavity was opened following the median line of the abdomen; and approximately 4 mL of blood was collected from the abdominal vena cava (<45 seconds) into syringes pretreated with ethylenediaminetetraacetic acid (15%). Blood was centrifuged (3000 rpm; 4°C; 12 minutes; Beckman GPR Centrifuge, Montreal, Canada), and the plasma was kept for further analysis. The liver was excised, and the median lobe was immediately snap-frozen and was used for triacylglycerol (TAG) determination, messenger RNA (mRNA) extraction and quantification, and Western blotting. The uterus and the mesenteric and retroperitoneal fat pads along with 4 skeletal muscles of the right hind limb (soleus, plantaris, medial gastrocnemius, and lateral gastrocnemius) were, thereafter,

rapidly excised and weighed. All tissue and plasma samples were stored at −78°C until analyses were performed. Finally, the right femur wet weight was obtained following a short boiling period in a 10% KOH solution to remove the surrounding tissue.

### 2.6. Biochemical analyses

Plasma insulin and leptin concentrations were determined with radioimmunoassay test kit distributed by LINCO Research (St Charles, MO). Plasma glucose concentrations were determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Plasma glucose and insulin values were used to calculate a homeostasis model assessment of insulin resistance (HOMA-IR) as follows: glucose (in millimoles per liter) × insulin (in micro–international units per liter)/22.5 [24]. Plasma C-reactive protein (CRP) concentrations were measured with Synchron LX Systems (Beckman Coulter) using Alpco Diagnostics kit (Salem, NH, catalog no. 41-CRPRT-E01). Liver TAG concentrations were estimated from glycerol released after ethanolic KOH hydrolysis by using commercial kit from Sigma (St Louis, MO).

### 2.7. Isolation of RNA and quantitative real-time polymerase chain reaction

#### 2.7.1. RNA extraction and complementary DNA preparation

Quick-frozen tissue samples of the liver were powdered with cold mortar and pestle, and approximately 100 mg was used for the isolation of RNA. Total RNA was extracted by the guanidine thiocyanate method, and mRNA was purified using PureLink RNA Mini Kit (Invitrogen, Burlington, ON, Canada) according to the manufacturer's instruction. Total RNA was reverse transcribed in a final volume of 100 μL using the High-Capacity cDNA (complementary DNA) Reverse Transcription Kit with random primers (Applied Biosystems, Foster City, CA) as described by the manufacturer. Reverse transcribed samples were stored at −20°C. A reference RNA (Human reference total RNA, Stratagene, La Jolla, CA) was also transcribed in cDNA.

#### 2.7.2. Quantitative polymerase chain reactions: ABI gene expression assay–endogenous controls

Gene expression level was determined using primer and probe sets from Applied Biosystems (ABI Gene Expression Assays, http://www.appliedbiosystems.com). Polymerase chain reactions (PCRs) for 384-well plate formats were performed using 2 μL of cDNA samples (20-50 ng), 5 μL of the Express qPCR SuperMix (Invitrogen), 0.5 μL of the TaqMan Gene Expression Assays (20×), and 2.5 μL of water in a total volume of 10 μL. The following predeveloped TaqMan assays were used as endogenous control: glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

#### 2.7.3. TaqMan reactions using Universal Probe Library

Gene expression level was also determined using primer and probe sets from Universal Probe Library from Roche (Laval, QC, Canada), a fast, specific and flexible format for quantitative real-time (RT) PCR (https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp). Polymerase chain reactions for 384-well plate formats were performed using 2 μL of cDNA samples (50 ng), 5 μL of the Express qPCR SuperMix (Invitrogen), 2 μmol/L of each primer, and 1 μmol/L of the Universal TaqMan probe in a total volume of 10 μL. The primer sets served to generate amplicons are presented in Table 1.

#### 2.7.4. Detection and analysis

The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) was used to detect the amplification level and was programmed FAST with an initial step of 3 minutes at 95°C, followed by 45 cycles of 5 seconds at 95°C and 30 seconds at 60°C. All reactions were run in triplicate, and the average values were used for quantification. Glyceraldehyde-3-phosphate dehydrogenase was used as endogenous control. The relative quantification of target genes was determined using the ΔΔCT method. Briefly, the threshold cycle (Ct) values of target genes were normalized to an endogenous control gene (GAPDH) ($\Delta CT = Ct_{target} - Ct_{GAPDH}$) and compared with a calibrator: $\Delta\Delta CT = \Delta Ct_{sample} - \Delta Ct_{calibrator}$. Relative expression was calculated using the Sequence Detection System 2.2.2 software (Applied Biosystems): relative expression $= 2^{-\Delta\Delta CT}$.

### 2.8. Western blot analysis

One hundred milligrams of liver was homogenized in TPER containing protease inhibitors (10 μL/mL pepstatin, and 1 mmol/L phenylmethanesulfonyl fluoride, and 100 units Trasylol; Sigma, Oakville, ON, Canada) using a polytron and centrifuged at 12 000*g*, 4°C, for 10 minutes. The infranatant was collected with a blunt-tipped Pasteur pipette, and the protein concentration was determined by modified Bradford assay and stored at −80°C until protein determination. Stearoyl coenzyme A desaturase 1 (SCD-1) and NF-κB contents in the liver were determined by Western blotting. All samples (10 μg of proteins) were separated on a sodium dodecyl sulfate polyacrylamide gel (12% for SCD-1 and 10% for NF-κB) and electrotransferred onto Hybond-Cextra nitrocellulose membranes (Amersham, Baie D'Urfe, QC, Canada). Membranes were blocked overnight in Tris-buffered saline containing 0.05% Tween 20 (TBST 0.05%) and 5% nonfat dry milk at 4°C. The blot was then incubated with specific primary antibodies SCD-1 (1:2000; kindly provided by Dr J. Ozols, University of Connecticut Health Center, Storrs, CT) and NF-κB p65 (1:2000; sc-109, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After 2 washes in TBST (0.05%) and 2 washes in TBST (0.05%) containing 0.5% nonfat dry milk, the membrane was incubated for 30 minutes with a horseradish peroxidase–conjugated anti-rabbit/anti-mouse immunoglobulin G (1:10 000; BM Chemiluminescence Western Blotting Kit, catalog no. 11520709001; Roche Diagnostics) at room

Table 1
Oligonucleotide primers used for quantitative RT-PCR

| Genes | Accession no. | Sense primer (5′-3′) | Antisense primer (5′-3′) |
|---|---|---|---|
| *SREBP-1c* | XM_213329 | TACAGCGTGGCTGGGAAC | GGCTGAGCGATACAGTTCAA |
| *SCD-1* | NM_139192 | GCCCTGTACGGGATCACA | CCCAGGGCACTGATAAGGTA |
| *ChREBP* | NM_133552 | AATCCCAGCCCCTACACC | CTGGGAGGAGCCAATGTG |
| *ACC* | NM_022193 | ACAGAGATGGTGGCTGATGTC | GATCCCCATGGCAATCTG |
| *PPAR-α* | NM_013196 | TCGGAGGGCTCTGTCATC | CATCTGTACTGGTGGGGACA |
| *PGC-1α* | NM_031347 | GAAGCGGGAGTCTGAAAGG | GTAAATCACACGGCGCTCTT |
| *CRP* | NM_017096 | CTTCTCTCAGGCTTTTGGTCA | GCTTCCAGTGGCTTCTTTGA |
| *IKKB* | NM_053355 | GAGAGCGTCAGCTGTGTCC | CCCCACACTTTCCTCATCTG |
| *IL-6* | NM_012589 | CCCTTCAGGAACAGCTATGAA | ACAACATCAGTCCCAAGAAGG |
| *TNF-α* | NM_012675 | GCCTCTTCTCATTCCTGCTC | GAGCCCATTTGGGAACTTCT |
| *IL-10* | NM_012854 | GCTCAGCACTGCTATGTTGC | AATGGCCTTTGCTGGTCTT |
| *GAPDH* | NM_017008 | CCCTCTGGAAAGCTGTGG | AGTGGATGCAGGGATGATG |

SREBP-1c, sterol regulatory element-binding protein-1c; SCD-1, stearoyl CoA desaturase-1; ChREBP, carbohydrate response element-binding protein; ACC, acetyl-CoA carboxylase; PPAR-α, peroxisome proliferator activated receptor-α; PGC-1α, peroxisome proliferatoractivated receptor-γ coactivator 1α; CRP, C-reactive protein; IKKB, inhibitor of kappa B kinase beta; IL-6, interleukin 6; TNF-α: tumor necrosis factor-α; IL-10, interleukin 10; GADPH, glyceraldehyde-3-phosphate dehydrogenase.

temperature. Afterward, the membrane was washed 4 times for 20 minutes each time in TBST (0.05%) before a chemiluminescence substrate (catalog no. 11520709001, Roche Diagnostics) was applied to the membrane. The resulting signal was detected on scientific imaging films (Amersham), and the image was acquired with the use of Alpha Imager software (Alpha Innotech, Santa Clara, CA). Densitometric measurement of the bands was performed using Image J software and expressed as arbitrary units. Equal protein loading was determined using mouse anti–*β*-actin primary antibodies as an internal control (1:2000; Sigma). For *β*-actin, the concentration of the second antibody was 1:5000.

### 2.9. Statistical analysis

Values are expressed as mean ± SE. Statistical analyses were performed using 1-way analysis of variance for nonrepeated measures. The Fisher protected least significant difference post hoc test was used in the event of a significant ($P < .05$) F ratio.

## 3. Results

Ovariectomy in rats resulted in higher body weight ($P < .05$), daily energy intake, and mesenteric and retroperitoneal fat depots weights ($P < .01$) as well as higher plasma concentrations of insulin ($P = .059$), glucose, leptin, and HOMA-IR index ($P < .01$) as compared with Sham rats (Table 2). Furthermore, Ovx resulted in lower femur (14%; $P < .05$) and uterus weight (80%; $P < .01$), indicating physiologic effects of ovariectomy. As a rule, almost all changes induced by ovariectomy were prevented either by 17*β*-estradiol supplementation or endurance exercise training (Table 2). The exceptions were uterus weight and an increase in plasma glucose concentrations in OvxTr rats. On the other hand, the higher uterus weight (29%; $P < .01$) in OvxE2 compared with Sham rats suggests that 17*β*-estradiol supplementation was slightly supraphysiologic. There was no significant difference between Sham and Ovx rats in relative muscle weight (Table 2). However, exercise training and 17*β*-estradiol supplementation in Ovx rats increased leg muscles weight ($P < .01$).

Table 2
Effects of ovariectomy, estrogen replacement, and exercise training on anthropometric and physiologic parameters

| | Sham | Ovx | OvxE2 | OvxTr |
|---|---|---|---|---|
| Body weight (g) | 345 ± 13 | 388 ± 12$^{abb}$ | 314 ± 5 | 357 ± 13$^{b}$ |
| Energy intake (kJ/d) | 333 ± 11 | 378 ± 11$^{aabbc}$ | 331 ± 9 | 341 ± 9 |
| Mesenteric fat weight (g) | 9.52 ± 0.9 | 13.6 ± 1.2$^{aabbcc}$ | 8.72 ± 0.6 | 7.83 ± 0.5 |
| Retroperitoneal fat weight (g) | 5.69 ± 0.8 | 8.69 ± 1$^{aabbcc}$ | 5.67 ± 0.6 | 5.0 ± 0.4 |
| Uterus weight (mg) | 602 ± 51 | 123 ± 9$^{aabb}$ | 774 ± 64$^{aa}$ | 123 ± 8$^{aabb}$ |
| Leg muscles weight (g/100 g BW) | 0.62 ± 0.02 | 0.6 ± 0.02$^{bbcc}$ | 0.67 ± 0.02 | 0.71 ± 0.02$^{aa}$ |
| Femur weight (g/100 g BW) | 0.21 ± 0.007 | 0.18 ± 0.01$^{abbcc}$ | 0.24 ± 0.009$^{aa}$ | 0.22 ± 0.007 |
| Plasma insulin (pmol/L) | 263 ± 22 | 387 ± 52 $^{(abc)*}$ | 231 ± 34 | 250 ± 50 |
| Plasma glucose (mmol/L) | 8.62 ± 0.3 | 10.5 ± 0.4$^{aabb}$ | 6.83 ± 0.3$^{aacc}$ | 9.59 ± 0.3$^{a}$ |
| HOMA-IR (mIU/L) | 16.8 ± 1.5 | 29.8 ± 4.5$^{aabbc}$ | 11.9 ± 1.8 | 17.7 ± 3.7 |
| Plasma Leptin (ng/mL) | 8.85 ± 1.1 | 13.6 ± 1.3$^{aabbcc}$ | 8.81 ± 0.9 | 6.63 ± 0.8 |

Values are mean ± SE; n = 6 to 8 rats per group. BW indicates body weight.
Significantly different from Sham: $^{a}P < .05$ and $^{aa}P < .01$.
Significantly different from OvxE2: $^{b}P < .05$ and $^{bb}P < .01$.
Significantly different from OvxTr: $^{c}P < .05$ and $^{cc}P < .01$.
$^{(abc)*}P = .059$.

Liver TAG levels were 71% higher (25.1 ± 2.7 vs 14.6 ± 1 mg/g; $P < .01$) in Ovx than in Sham rats (Fig. 1A). The Ovx-induced hepatic fat accumulation was prevented either by 17 $\beta$-estradiol replacement or by exercise training. Quantitative RT-PCR analysis showed higher ($P < .01$) gene expression of hepatic lipogenic transcription factors sterol regulatory element-binding protein 1-c (SREBP-1c) (57%; Fig. 1B), SCD-1 (87%; Fig. 2A), and carbohydrate response element binding protein (ChREBP) (63%; Fig. 2C), as well as acetyl–coenzyme A carboxylase (ACC) (68%; Fig. 2D) mRNA levels, in Ovx than in Sham rats. Protein quantification by Western blot analysis confirmed results obtained by RT-PCR for SCD-1. We found greater SCD-1 (40%; $P <$ .01; Fig. 2B) protein abundance in the liver of Ovx rats. These Ovx-induced higher lipogenic gene expressions and protein content were totally prevented either by E2 replacement or by endurance training. Conversely, the hepatic oxidative transcription factor peroxisome proliferators–activated receptor α (PPAR-α) mRNA was lower (31%; $P < .01$) in Ovx than in Sham rats, whereas the hepatic peroxisome proliferators–activated receptor-γ coactivator 1α mRNA showed the same trend, although the differences did not reach significant statistical levels (Fig. 3). Again, lower PPAR-α mRNA levels were reestablished in OvxE2 animals and OvxTr rats.

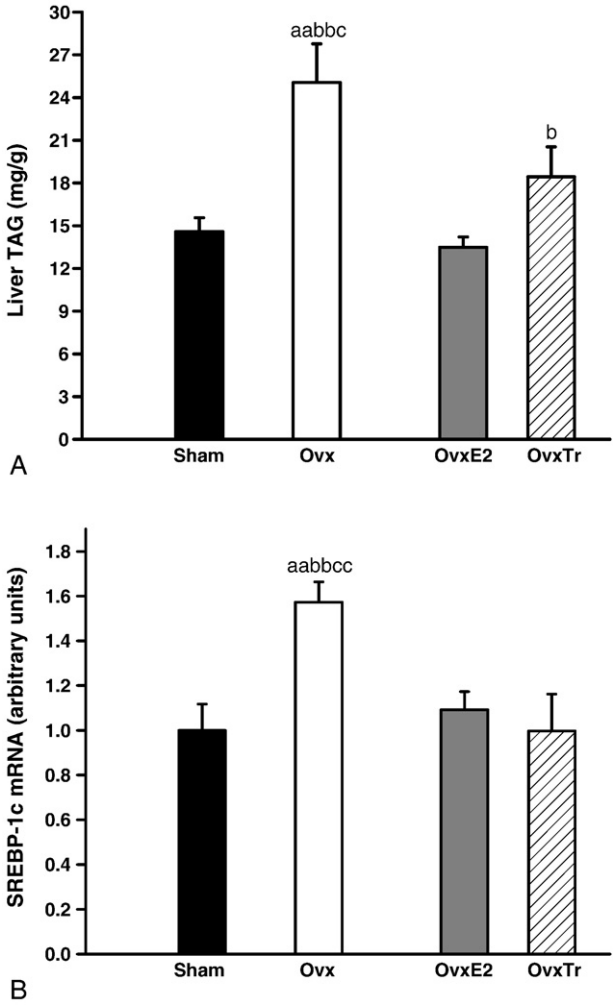


Fig. 1. Liver TAG (A) and SREBP-1c mRNA (B) in Sham, Ovx, OvxE2, and OvxTr rats. Significantly different from Sham: $^{aa}P < .01$. Significantly different from OvxE2: $^{b}P < .05$ and $^{bb}P < .01$. Significantly different from OvxTr: $^{c}P < .05$ and $^{cc}P < .01$. Values are mean ± SE; n = 6 to 8 rats per group.

To get an insight into how estrogen withdrawal and endurance exercise training affect the hepatic inflammatory response, we measured gene expression of several inflammatory markers in liver. The Ovx as compared with Sham rats had higher ($P < .01$) hepatic proinflammatory IL-6 (37%) and inhibitor of κB kinase β (IKKB) (35%) mRNA expressions (Fig. 4). Moreover, we found higher NF-κB (51%; $P < .01$; Fig. 4C) protein level in liver of Ovx rats than in Sham control. Like the metabolic markers, Ovx-induced changes of inflammatory markers were totally prevented with estrogen replacement as well as with endurance training. Tumor necrosis factor–α and IL-10 mRNA was not significantly changed by the ovariectomy (Fig. 5). Nevertheless, TNF-α gene expression was significantly ($P <$ .05) lower in OvxE2 and OvxTr rats compared with Ovx animals. Interestingly, the IL-10/TNF-α ratio was higher ($P < .05$) in OvxE2 and OvxTr animals compared with Ovx group, indicating better inflammatory status under these physiologic conditions (E2 supplementation and exercise training) (Fig. 5).

Opposite to the other inflammatory markers, we found that plasma CRP level and hepatic CRP mRNA expression were significantly lower ($P < .01$) in Ovx rats than in Sham animals (Fig. 6). These responses were reincreased when E2 replacement was provided. On the other hand, plasma CRP remained decreased in OvxTr; and hepatic CRP mRNA level in this group was midway between Ovx and OvxE2 groups at the Sham group's level.

## 4. Discussion

The present observation that liver TAG content was 71% higher in Ovx than in Sham rats is consistent with previous findings showing that estrogen withdrawal results in a state of hepatic steatosis [7,9,25]. We also confirmed previous reports that 17β-estradiol supplementation and endurance exercise training prevent the accretion of lipids in the liver of Ovx rats [10,12,17]. This indicates that endurance exercise training has an important estrogenic-like effect on the prevention of hepatic steatosis in Ovx animals. It is important to recall that pair-feeding in Ovx rats does not completely prevent fat accumulation in liver (Heine et al [26] and unpublished data from our laboratory). Consequently, Ovx-induced hepatic fat accumulation cannot be totally attributed to the increased food intake. To shed some light on possible mechanisms involved in the metabolic action of exercise training in liver of Ovx rats, we analyzed the expression of influential genes that regulate hepatic fat accumulation.

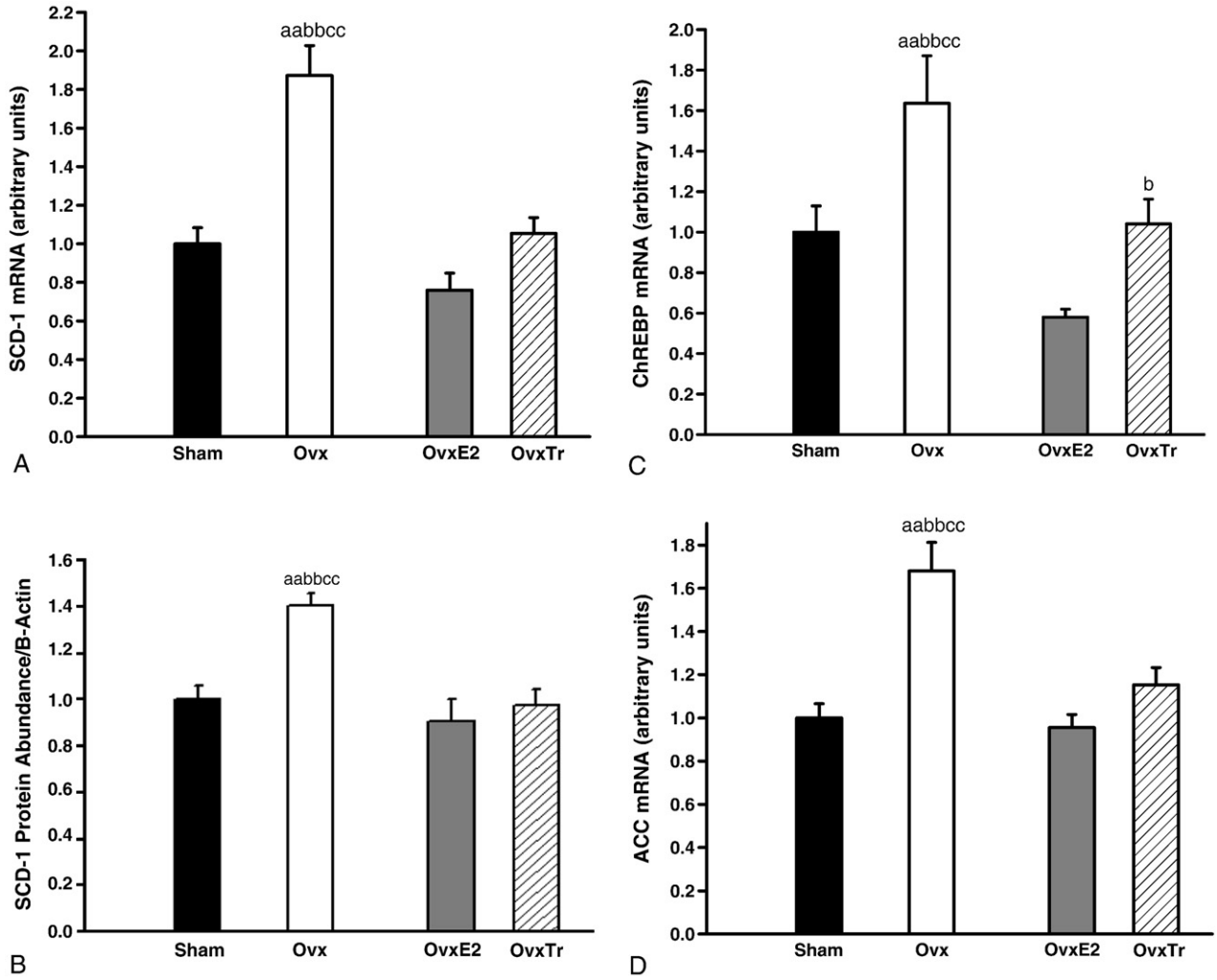


Fig. 2. Hepatic lipogenic mRNA and protein abundance in Sham, Ovx, OvxE2, and OvxTr rats. Significantly different from Sham: $^{aa}P < .01$. Significantly different from OvxE2: $^{b}P < .05$ and $^{bb}P < .01$. Significantly different from OvxTr: $^{cc}P < .01$. Values are mean ± SE; n = 6 to 8 rats per group.

Kinetic studies in human subjects indicate that approximately 26% of hepatic TAG accumulation can be accounted for by de novo lipogenesis [27]. Therefore, it has been assumed that the enhancement of de novo lipid synthesis is a primary disorder in hepatic steatosis that is tightly stimulated by lipogenic molecules such as the transcription factor SREBP-1c and the ACC downstream enzyme [28]. Similarly, SCD-1 is an enzyme that represents a pivotal control point in lipid homeostasis by catalyzing a rate-limiting step in the biosynthesis of monounsaturated fats, which are required for TAG synthesis [29,30]. On the other hand, the transcription factor ChREBP also plays an essential role in the regulation of gene expression of enzymes (ie, ACC and fatty acid synthase) involved in lipogenesis derived from glucose metabolism [31]. Recently, it was reported that ovariectomy increased SREBP-1c and SCD-1 gene expressions [10]. The present results complement these previous findings by showing that the gene expression of transcription factor ChREBP and the important downstream ACC enzyme is also elevated in Ovx rats. The main contribution of the present study, however, is the original finding that endurance exercise training, similarly to E2 supplementation, counteracted these hepatic molecular disturbances by canceling the increase in SREBP-1c, SCD-1, ChREBP, and ACC transcripts in Ovx rats. This effect was confirmed at the protein level for SCD-1. These molecular responses strongly suggest that endurance training depressed lipogenesis in liver of Ovx rats, thus constituting a possible mechanism that contributes to the decrease in liver fat accumulation.

In addition to increased lipogenesis, recent data indicate that fatty acid oxidation is reduced in the liver of estrogen-deficient animals [10-12]. The present decrease in gene expression of PPAR-α in liver of Ovx rats, which is reincreased in OvxE2 animals, is in agreement with this finding. Peroxisome proliferators–activated receptor α is the key transcriptional regulator of peroxisomal, mitochondrial, and microsomal fatty acid oxidation systems in the liver [32,33]. Although less pronounced, PPAR-α transcript was also higher in OvxTr than in Ovx rats. This suggests that, in addition to suppression of higher rates of liver lipogenesis, a

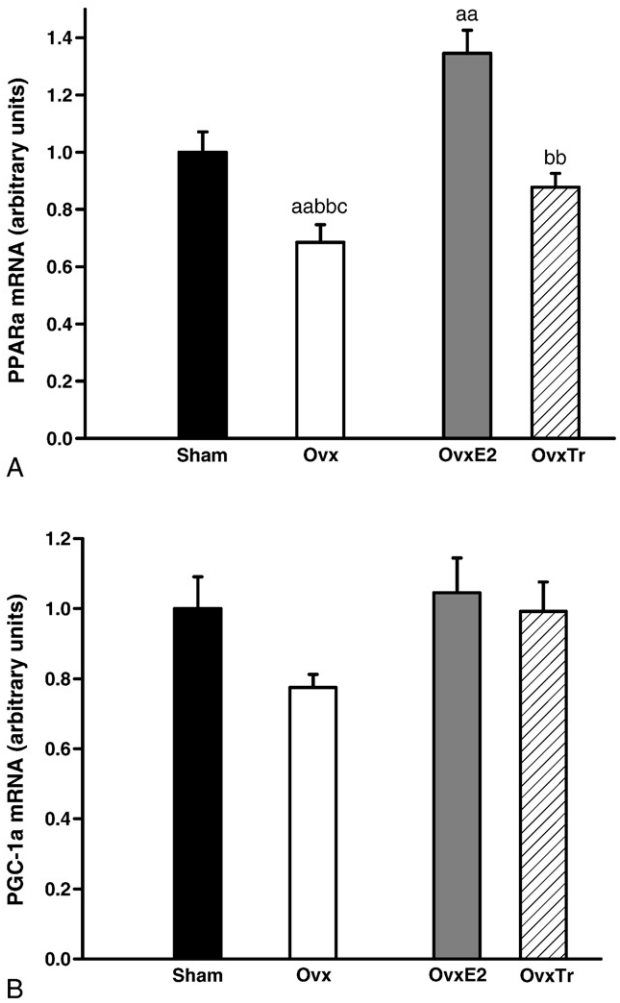


Fig. 3. Hepatic lipid oxidative mRNA abundance in Sham, Ovx, OvxE2, and OvxTr rats. Significantly different from Sham: $^{aa}P < .01$. Significantly different from OvxE2: $^{bb}P < .01$. Significantly different from OvxTr: $^{c}P < .05$. Values are mean ± SE; n = 6 to 8 rats per group.

reincrease in lipid oxidation in exercise-trained Ovx rats may also contribute to the prevention of liver lipid accumulation in Ovx rats.

The fact that exercise training acts similarly as estrogen supplementation in changing gene expression of key molecules involved in fat metabolism in the liver of Ovx rats raises the question of whether both of these actions take place through a similar pathway. The molecular and biological mechanisms underlying the metabolic actions of estrogen in liver are weakly understood. Estrogen is a steroid hormone whose actions are predominantly mediated by genomic mechanisms of E2 action through its nuclear receptors (ER) $\alpha$ or $\beta$ [34]. Estrogen has also been shown to have rapid nongenomic biological actions through membrane bound subpopulations of ER [35-37]. D`Eon et al [38] recently reported that E2 treatment decreases gene expression of SREBP-1c and its target genes fatty acid synthase and ACC in liver. It is possible that E2 directly regulates SREBP-1c, which contains an estrogen response element in its promoter region [39]. On the other hand, D`Eon et al [38] showed that E2 rapidly activates adenosine monophosphate–activated protein kinase (AMPK) in skeletal muscle. Because SREBP-1c expression is down-regulated by AMPK [40], they suggested that the decreased expression of SREBP-1c in

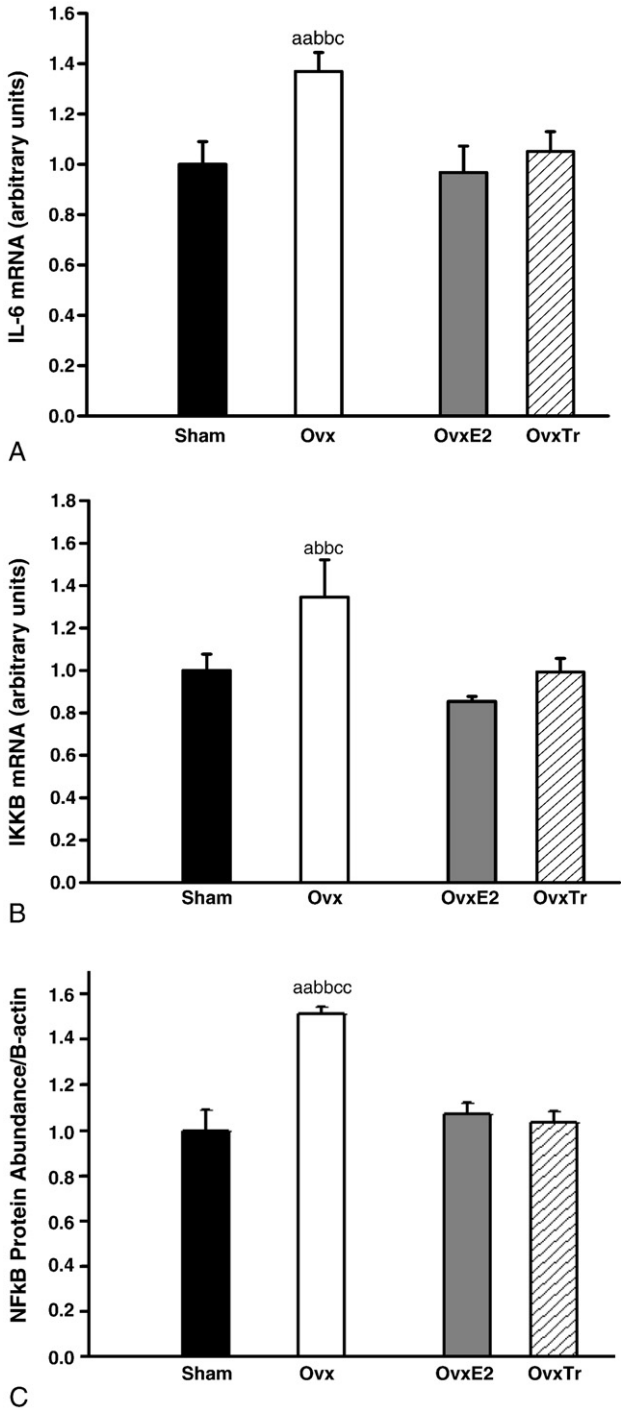


Fig. 4. Hepatic gene expression of IL-6 (A) and IKKB (B), and the protein abundance of NF-$\kappa$B (C) in Sham, Ovx, OvxE2, and OvxTr rats. Significantly different from Sham: $^{a}P < .05$ and $^{aa}P < .01$. Significantly different from OvxE2: $^{bb}P < .01$. Significantly different from OvxTr: $^{c}P < .05$ and $^{cc}P < .01$. Values are mean ± SE; n = 6 to 8 rats per group.

muscle reflects AMPK activation by nongenomic action of E2 [38]. Because physical exercise has been reported to activate AMPK in liver [41], it is thus possible that the decreased expression of SREBP-1c and its downstream

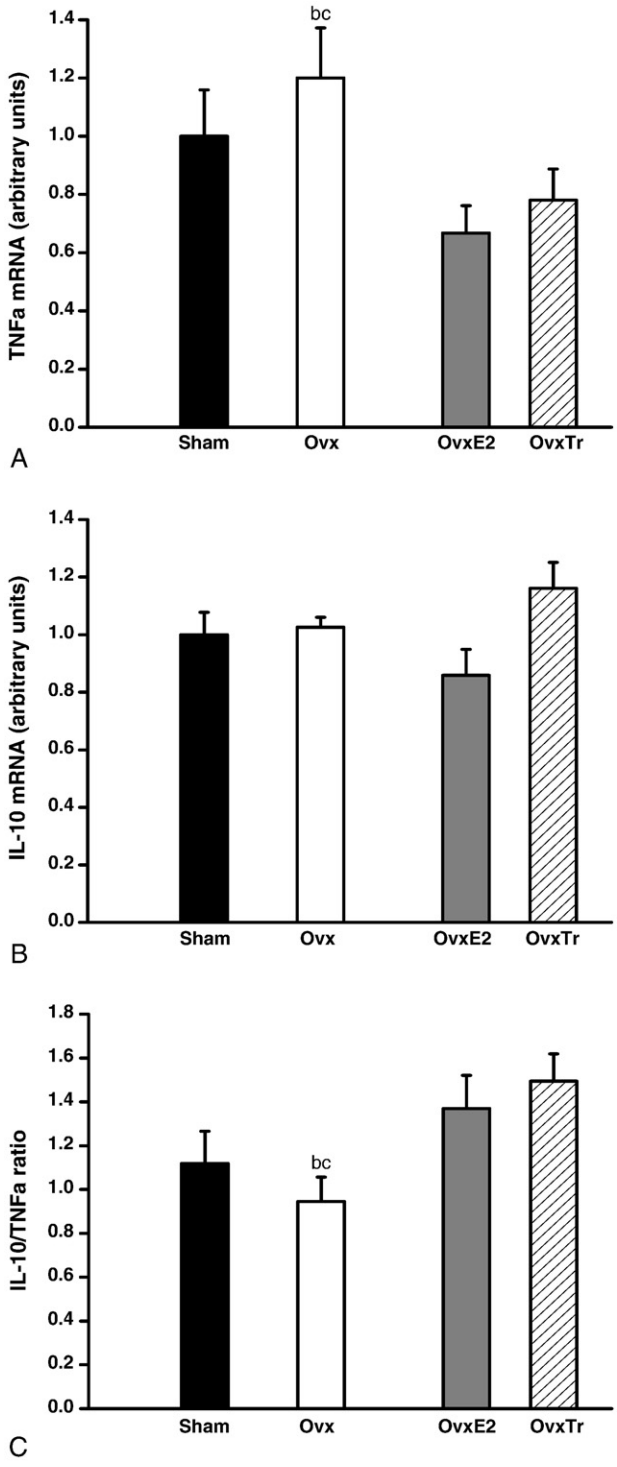


Fig. 5. Hepatic gene expression of TNF-α (A), IL-10 (B), and IL-10/TNF-α ratio (C) in Sham, Ovx, OvxE2, and OvxTr rats. Significantly different from OvxE2: $^{b}P < .05$. Significantly different from OvxTr: $^{c}P < .05$. Values are mean ± SE; n = 6 to 8 rats per group.

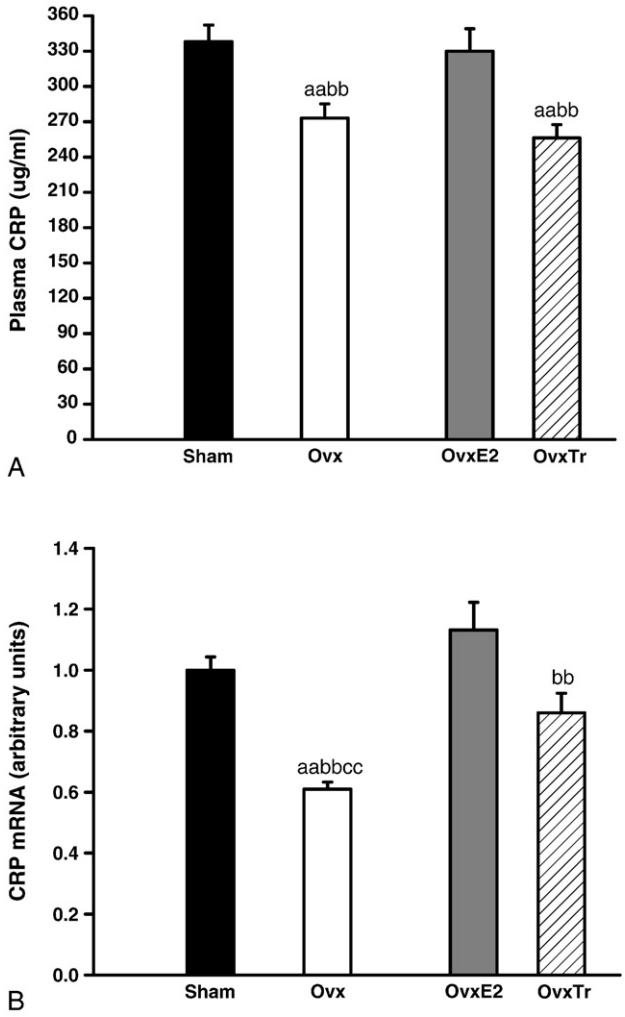


Fig. 6. Plasma CRP levels (A) and hepatic gene expression of CRP (B) in Sham, Ovx, OvxE2, and OvxTr rats. Significantly different from Sham: $^{aa}P < .01$. Significantly different from OvxE2: $^{bb}P < .01$. Significantly different from OvxTr: $^{cc}P < .01$. Values are mean ± SE; n = 6 to 8 rats per group.

targets (SCD-1 and ACC) by exercise training may be mediated through the AMPK pathway. Important increases in hepatic ERα mRNA levels have also been found in endurance-trained rats [42]. Although further work is needed to clarify the precise mechanism, the present data support the contention that both E2 and exercise training act on hepatic expression of the same target genes to reduce de novo lipogenesis while favoring fat oxidation.

An additional support to the interpretation that exercise training acts similarly as estrogen supplementation is the finding that exercise training had similar effects as E2 supplementation in reducing peripheral fat accumulation and plasma leptin levels, as well as peripheral insulin resistance (as measured from HOMA-IR index) in the present Ovx rats. These results are in line with the recent report of Saengsirisuwan et al [43] who showed that Ovx-induced features of the insulin resistance syndrome are largely attenuated by endurance exercise training alone and estrogen

replacement alone. On the whole, the present results suggest that exercise training in Ovx rats acts similarly as estrogen supplementation in regulating not only liver fat but also peripheral fat accumulation and its metabolic consequence, the insulin resistance.

The second objective of the present study was to investigate if gene expressions of some important inflammatory biomarkers were increased in liver of Ovx rats and the impact of exercise training on this response. Regardless if the cause is the liver fat accumulation or the proinflammatory substances in the portal circulation, proinflammatory cytokines such as IL-6 and TNF-α are overproduced in fatty liver [21]. Two main signaling pathways have been linked to inflammation associated with obesity: the NF-κB pathway, activated by IKKB, and the c-Jun NH2-terminal kinase pathway [44]. In recent years, it was found that the NF-κB and IKKB signaling pathway activated by proinflammatory cytokines is a key modulator of inflammation and insulin resistance [45]. Cai et al [20] demonstrated that lipid accumulation in liver (induced either by high-fat diet or by transgenic expression of IKKB in mice) leads to subacute hepatic inflammation and downstream cytokine production, with IL-6 showing the strongest evidence of pathologic involvement. In the present study, mRNA levels of IL-6 and IKKB as well as protein content of NF-κB increased in liver of Ovx rats. Estrogen replacement neutralized these elevated gene expressions, indicating that estrogens contribute to the maintenance of low expression level of these inflammatory biomarkers in the liver. This is in line with results of Kireev et al [46] and Hamilton et al [47] who reported an increase in IL-6 and TNF-α gene expression in liver and heart of Ovx rats, respectively, that was corrected by 17β-estradiol replacement. To our knowledge, the present study is the first to report that endurance exercise training acts like estrogens in neutralizing increased gene expression of IL-6 and IKKB as well as the protein expression of NF-κB in liver of Ovx rats. Although this effect of exercise training in liver might be solely linked to the reduction of fat accumulation, it is important to recall that physical activity mediates anti-inflammatory effects in skeletal muscle and fat tissue [18]. Regular exercise protects against diseases associated with chronic low-grade systemic inflammation, and the long-term effect of exercise training may be ascribed to the anti-inflammatory response elicited by an acute bout of exercise [48].

Tumor necrosis factor–α plays a central role in initiating and sustaining inflammation [49], whereas IL-10 demonstrates potent anti-inflammatory properties through inhibiting the production of various proinflammatory cytokines including IL-6 and TNF-α [50]. In fact, Kaur et al [51] showed that a proper balance between IL-10 and TNF-α rather than any of these individual cytokine responses is of physiologic importance. Hashem et al [52] reported that the IL-10/TNF-α ratio is a convenient predictive biomarker for investigation of fatty liver of different grades including steatohepatitis and nonalcoholic fatty liver disease. The present increase in gene expression ratio of IL-10/TNF-α with E2 replacement as well as with endurance exercise training in Ovx animals, therefore, indicates an improvement in the status of the liver in both of these conditions.

An intriguing response related to inflammatory biomarkers in the present study is the observation that plasma CRP as well as CRP mRNA levels in liver decreased in Ovx animals and reincreased with E2 replacement. A number of investigations have reported that hormone replacement therapy increases plasma CRP levels [53-55] especially in response to oral conjugated estrogens [56]. Although it has been argued that rats are not an appropriate model to investigate the relationship between estrogen and CRP, Yang et al [57] showed that ovariectomy in rat reduces plasma CRP and that estrogen replacement raises the plasma CRP levels. It has been suggested that E2-mediated increase in CRP may not represent an up-regulation of proinflammatory response mediated by upstream cytokines but rather is related to a secondary mechanism [56]. Nevertheless, in the present study, plasma and liver mRNA expression of CRP did not increase in endurance exercise–trained Ovx rats. This indicates that whatever the clinical significance of the action of E2 on increasing CRP levels, this is not carried out in exercise-trained rats.

In summary, results of the present study indicate that exercise training acts like estrogen supplementation in properly regulating gene expressions of molecular markers involved in liver fat accumulation and biomarkers of subclinical inflammation in Ovx rats. On a clinical point of view, the present results reiterate the importance of exercise training as a tool to alleviate some of the metabolic consequences of low estrogenic status in postmenopausal women.

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