The effect of exercise training on upregulation of molecular markers of bile acid metabolism in the liver of ovariectomized rats fed a cholesterol-rich diet

Zahra Farahnak[(1)], Luciane Magri Tomaz[(2)], Raynald Bergeron[(3)], Natalie Chapados[(4)], Jean-Marc Lavoie[(5)]

Original Article

Abstract

BACKGROUND: Small heterodimer partner (SHP) is an important transcriptional factor involved in the regulation of glucose, lipid, and bile acid metabolism in the liver. SHP has been reported to be down-regulated in ovariectomized (Ovx) mice and up-regulated by estrogens suggesting a link between estrogens and SHP. The aim of the present study was to determine the effects of exercise training on SHP and key molecular markers of cholesterol and bile acid homeostasis in Ovx rats under cholesterol feeding.

METHODS: Our main experimental group was composed of Ovx rats fed a high-cholesterol diet (Ovx-Chol) that was compared to a group of Ovx rats fed a standard diet (Ovx-SD) and a group of sham operated rats fed the cholesterol diet (Sham-Chol). These three groups of Ovx and sham rats were subdivided into either voluntary wheel running (Tr) or sedentary (Sed) groups for 5 weeks. The mRNA expression of all genes was measured by quantitative real-time polymerase chain reaction.

RESULTS: Liver total cholesterol levels were not affected by exercise training in any of the experimental conditions. Cholesterol feeding in both sham and Ovx rats resulted in significantly higher hepatic cholesterol accumulation than in Ovx-SD ($P < 0.001$). Hepatic low density lipoprotein receptor (LDL-R) involved in cholesterol uptake from circulation was not influenced by training. A main effect of training was, however, found for transcripts of SHP and cholesterol 7 alpha-hydroxylase (CYP7A1, $P < 0.050$). CYP7A1 is the main gene involved in bile acid biosynthesis from cholesterol.

CONCLUSION: These results suggest that voluntary wheel running modulates cholesterol metabolism in Ovx animals through up-regulation of SHP and bile acid formation.

Keywords: Exercise, Cholesterol 7 Alpha-Hydroxylase, Rat, Cholesterol, Low Density Lipoprotein Receptor

Date of submission: 28 Nov. 2016, ***Date of acceptance:*** 12 Mar. 2017

Introduction

Accumulated evidence from human and animal studies shows that estrogen deficient state leads to disturbances in fat and cholesterol metabolism.[1,2] While most studies were limited to assessment of plasma cholesterol levels, recent studies indicated that hepatic cholesterol metabolism is also affected by estrogen withdrawal.[3,4] Considering that the liver is a master regulator of cholesterol metabolism, there is a need for a better understanding of the liver response under estrogen withdrawal. Nutritional approaches have frequently been used to investigate the response of the liver to estrogen deficient conditions.[5,6] For instance, a large hepatic cholesterol accumulation was observed in ovariectomized (Ovx) animals when fed a high-fat and/or high-fat high-cholesterol diet.[4,7] In addition to hepatic cholesterol accumulation, it has been shown that the combined effect of cholesterol diet and ovariectomy resulted in suppression of transcripts of hepatic bile salt export pump (BSEP) and Na^+-taurocholate cotransporting polypeptide (NTCP), two transporters of bile acids in liver.[8] From this last study, it appears that a better

1- Department of Kinesiologie, University of Montreal, Montreal, Canada
2- Laboratory of Exercise Physiology, Department of Physiological Sciences, Federal University of Sao Carlos, Sao Carlos, Brazil
3- Associate Professor, Department of Kinesiologie, University of Montreal, Montreal, Canada
4- Assistant Professor, Institute of Research of Hospital Montfort, Institute of Savoir of Montfort AND School of Human Kinetics, Faculty of Health Sciences, University of Ottawa, Ottawa, Canada
5- Professor, Department of Kinesiologie, University of Montreal, Montreal, Canada
Correspondence to: Zahra Farahnak, Email: zahra.farahnak@umontreal.ca

knowledge of the contribution of bile acid transport/metabolism, which is the main way of elimination of excess cholesterol transiting through the liver, can shed some light on how liver regulates cholesterol metabolism in Ovx animal.

In addition to nutritional approaches, there is some evidence that exercise training may also affect hepatic cholesterol metabolism. For instance, it has been reported that voluntary wheel running increased cholesterol conversion into bile acids based on the observation of increased fecal bile acid excretion and consequently decreased atherosclerotic burden in low density lipoprotein receptor (LDL-R) deficient mice.[9] However the underlying molecular mechanisms for these observations have not been fully explored.

One of the key molecules involved in bile acid metabolism is small heterodimer partner (SHP). SHP interacts with several nuclear receptor family members. Through these interactions, SHP is involved in diverse metabolic pathways, including cholesterol, bile acid, triglyceride, and glucose homeostasis.[10,11] The interaction of SHP and farnesoid X receptor (FXR) is a well-known relationship that results in bile acid homeostasis. FXR is known as a bile acid receptor and bile acid-activated FXR induces SHP gene expression that results in inhibition of cholesterol 7 alpha-hydroxylase (CYP7A1) gene transcription.[12-14] CYP7A1 catalyzes the rate-limiting step in cholesterol conversion into bile acids in liver.[15] Moreover, it was also reported that SHP is down regulated in Ovx mice and alternatively, up-regulation of SHP by estrogens suggests that there is a link between estrogens and SHP.[16] Therefore, the main aim of the study was to determine the effect of training on key molecular markers of hepatic FXR/SHP/CYP7A1 pathway involved in bile acid metabolism in Ovx rats fed a cholesterol diet. We also targeted gene expression of the molecules involved in hepatic cholesterol metabolism including LDL-R and low-density lipoprotein receptor-related protein 1 (LRP1).

Materials and Methods

Female Sprague-Dawley rats (n = 49) weighing 180–200 g were obtained from Charles River (St-Constant, PQ, Canada) and housed individually to monitor food intake. The animals had ad libitum access to food and tap water. Their environment was controlled in terms of light (12 h light–dark cycle starting at 06:00 AM), humidity and room temperature (20–23 °C). Body weight and food intake were monitored bi-weekly from the start of experiment. All experimental procedures were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of the University of Montreal in agreement with the Canadian Council on Animal Care's rules (CCAC-CCPA).

At 1 week after their arrival, rats underwent either a bilateral ovariectomy (Ovx, n = 34) or a bilateral sham-operation (Sham, n = 15) according to the technique described by Robertson et al. under isoflurane anaesthesia.[17] After surgery, all animals were injected with antibiotics (Tribrissen 48%; 0.125 cc/kg, subcutaneously) and analgesics (carprofen; 4.4 mg/kg, subcutaneously) for 3 days. Then sham and Ovx rats were given either a standard diet (SD) or a high-cholesterol diet (Chol). The Chol diet consisted of a standard diet (SD) supplemented with 0.25% cholesterol (SD + Chol). Our main experimental group was composed of Ovx rats fed a high-cholesterol diet (Ovx-Chol, n = 17) that was compared, on one hand, to a group of Ovx rats fed a standard diet (Ovx-SD, n = 17) to observe the effects of the diet and, on the other hand, compared to a group of sham rats fed the cholesterol diet (Sham-Chol, n = 15) to observe the effect of estrogen withdrawal. These three groups of Ovx and sham rats were also subdivided into either voluntary wheel running (Tr) or sedentary groups (Sed). Tr rats were placed in freely rotating wheel cages while Sed rats were placed in blocked running wheel cages. Each wheel cage was equipped with a sensor connected to a computerized data acquisition system enabling the continuous sampling of running data from individual rats. The rats were on diet and training for 5 weeks.

Rats were euthanized between 09:00 and 12:00 AM. Food was removed from the cage overnight before sacrifice. Rats refrained from exercising ~24 h before sacrifice. Immediately after complete anaesthesia with isoflurane, the abdominal cavity was opened following the median line of the abdomen. Approximately 4 ml of blood was collected from the abdominal vena cava (< 45 s) into syringes treated with ethylenediaminetetraacetic acid (15%; EDTA). Blood was centrifuged (3000 rpm; 4 °C; 10 min; Beckman GPR Centrifuge; Beckman Coulter) and the plasma was kept for further analyses. Immediately after blood collection, the liver median lobe was removed and freeze-clamped. This sample was used for cholesterol and mRNA determinations. Several organs and tissues were removed and weighed (Mettler AE 100) in the

following order: uterus, urogenital, retroperitoneal and mesenteric fat deposits. The urogenital fat pad included adipose tissue surrounding the kidneys, uterus and bladder as well as ovaries, oviducts and uterus. The retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles. The mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastroesophageal sphincter to the end of the rectum, with special care taken in distinguishing and removing pancreatic cells. All tissue samples were frozen in liquid nitrogen immediately after being weighed (Mettler AE-100). All tissue samples were stored along with plasma samples at −80 °C until analyses were performed.

Liver total cholesterol (TC) levels were determined with some adaptations of the procedure described by Folch et al.[18] Briefly, 0.1 g of liver was homogenized in a chloroform–methanol mixture (2:1, v/v). The chloroform layer was collected and evaporated overnight. After adding 10% Triton X-100 in isopropanol, the sample was assayed for total cholesterol using commercial kits according to the manufacturer's instructions (Wako Diagnostics and Chemicals USA, Richmond, VA, USA).

RNA isolation and quantitative real-time (RT) polymerase chain reaction (PCR): Total RNA was extracted from frozen liver using RNA extraction Mini kit (Invitrogen) according to the manufacturer's protocol. Then RNA was treated with DNase (Invitrogen) in order to avoid genomic contamination. Total RNA (2 µg) was reverse-transcribed into complementary DNA using high capacity complementary DNA reverse transcription kits (Applied Biosystems). RT samples were stored at -20 °C. Gene expression for β-actin was determined using a pre-validated Taqman Gene Expression Assay (Applied Biosystems, Rn01462661, Foster City, CA). Gene expression level for target genes was determined using assays designed with the Universal Probe Library from Roche. The primer sets used to generate amplicons are presented in (Table 1). To validate the efficiency of the qPCR assays, we used a mix of the samples tested in the study. The ABI PRISM® 7900HT (Applied Biosystems) was used to detect the amplification level and was programmed with an initial step of 3 min at 95 °C, followed by 40 cycles for 5 s at 95 °C and 30 s at 60 °C. All reactions were run in triplicate and the average values of threshold cycle (C_T) were used for quantification. β-actin was used as endogenous control. The relative quantification of target genes was determined using the $\Delta\Delta C_T$ method. Briefly, the C_T values of target genes were normalized to an endogenous control gene (β-actin) ($\Delta C_T = C_{T\ target} - C_{T\ \beta\text{-actin}}$) and compared with a calibrator: ($\Delta\Delta C_T = \Delta C_{T\ Sample} - \Delta C_{T\ Calibrator}$). Relative quantification (RQ) was calculated using the Sequence Detection System (SDS) 2.2.2 software (Applied Biosystems) through the following formula: $RQ = 2^{-\Delta\Delta C_T}$.

All data are presented as mean ± standard error. Statistical significance ($P < 0.050$) was determined using a 2-way analysis of variance (ANOVA) for non-repeated measures with exercise and surgery-diet as main factors. Interpretation of the comparisons was made only between the Ovx-Chol and the Ovx-SD groups on one hand and between the Ovx-Chol and the Sham-Chol groups on the other hand. Fisher's least significant difference (LSD) post hoc test was used in the event of a significant interaction effect. For a significant surgery-diet effect without interaction, Fisher's LSD from a one-way ANOVA was used.

Table 1. Oligonucleotide primers used for quantitative real-time polymerase chain reaction

Gene	Forward primers	Reverse primers
CYP7A1	Ggagcttatttcaaatgatcagg	cactctgtaaagctccactcactt
FGFR4	Ttgaggcctctgaggaaatg	tcttgctgctccgagattg
FXR	Ccacgaccaagctatgcag	tctctgtttgctgtatgagtcca
HMG-CoAr	Caaccttctacctcagcaagc	acagtgccacacacaattcg
LDL-R	Tgctactggccaaggacat	ctgggtggtcggtacagtg
LRP1	Aatcgagggcaagatgacac	ccagtctgtccagtacatccac
NTCP	Aaaatcaagcctccaaaggac	ttgtgggtacctttttccaga
SHP	Cctcggtttgcatacagtgtt	aggttttgggagccatcaa
SREBP2	Gtgcagacagtcgctacacc	aatctgaggctgaaccagga
ActB	Cccgcgagtacaaccttct	cgtcatccatggcgaact
Cyclophilin B	Acgtggttttcggcaaagt	cttggtgttctccaccttcc

CYP7A1: Cholesterol 7 alpha-hydroxylase; FGFR4: Fibroblast growth factor receptor 4; FXR: Farnesoid X receptor; HMG-CoAr: 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL-R: Low-density lipoprotein-receptor; LRP1: Low density lipoprotein receptor-related protein 1; NTCP: Na+-taurocholate cotransporting polypeptide; SHP: Small heterodimer partner; SREBP2: Sterol regulatory element binding protein 2; ActB: Actin Beta

Table 2. Anthropometric parameters and food intake

Variables	Ovx-SD		Ovx-Chol		Sham-Chol	
	Sed	Tr	Sed	Tr	Sed	Tr
Final body weight (g)	438.70 ± 9.30	452.50 ± 14.10	433.20 ± 10.60	450.60 ± 11.60	372.30 ± 13.20$^{\dagger\dagger\dagger,\delta\delta\delta}$	346.60 ± 10.60$^{\dagger\dagger\dagger,\delta\delta\delta}$
Intra-abdominal fat pad weights (g)	37.80 ± 2.80	39.60 ± 3.90*	39.30 ± 3.80	34.30 ± 4.30*	30.20 ± 4.50$^{\dagger\dagger\dagger,\delta\delta\delta}$	14.30 ± 2.80$^{*,\dagger\dagger\dagger,\delta\delta\delta}$
Food intake (kcal/day)	100.10 ± 2.90	114.70 ± 3.70***	101.80 ± 2.20	113.00 ± 3.10***	92.60 ± 5.60$^{\dagger,\delta}$	101.80 ± 2.50$^{***,\dagger,\delta}$
Uterus (g)	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.54 ± 0.08$^{\dagger\dagger\dagger,\delta\delta\delta}$	0.55 ± 0.07$^{\dagger\dagger\dagger,\delta\delta\delta}$

Ovx: Ovariectomized; Sham: Sham operated; SD: Standard diet; Chol: Standard diet + 0.25% cholesterol; Sed: Sedentary group; Tr: Trained group

Values are mean ± standard error

* Significantly different from respective Sed group ($P < 0.050$); *** ($P < 0.001$); † Significantly different from respective Ovx-SD group ($P < 0.05$); ††† ($P < 0.001$); $^{\delta}$ Significantly different from respective Ovx-Chol group ($P < 0.05$); $^{\delta\delta\delta}$ ($P < 0.001$)

Results

Anthropometric parameters, food intake and total distance run: Running did not significantly impact on final body weight in any of the experimental groups; However, running increased ($P < 0.001$) food intake in all trained groups (Table 2). On the other hand, final body weight ($P < 0.001$) as well as food intake ($P < 0.050$) were lower in Sham-Chol group compared to both Ovx groups. Intra-abdominal fat pad weight, which was composed of urogenital, retroperitoneal and mesenteric fat deposits, was decreased ($P < 0.050$) by training in both sham and Ovx groups fed the cholesterol diet, whereas Ovx-SD group showed slightly higher intra-abdominal fat weight under training. Similar to body weight, intra-abdominal fat pad weight was significantly ($P < 0.001$) higher in the two Ovx groups compared to Sham-Chol group. The Chol diet as compared to the SD diet in Ovx animals had no effect on the final body weight, food intake and intra-abdominal fat pad weight. Uterus weight was higher in sham rats compared to Ovx groups confirming total ovariectomy in Ovx rats (Table 2). Total running distance was 6.09 ± 0.39 km/d in Sham-Chol rats. Ovx-SD and Ovx-Chol rats ran 2.79 ± 0.30 and 2.82 ± 0.33 km/d, respectively.

Molecular markers of bile acid metabolism: The most significant effects of training in the present study were found for hepatic gene expression of SHP and CYP7A1 with higher values measured in Tr compared to Sed rats in all experimental conditions ($P < 0.05$, Figure 1 a). The SHP and CYP7A1 responses were not significantly affected by the surgery and the diet. However, their transcription factor, FXR mRNA was decreased in cholesterol-fed rats. There was no impact of exercise training on FXR transcript (Figure 1 a). Hepatic gene expression of NTCP, involved in bile acids uptake at the basolateral membrane of hepatocytes, and fibroblast growth factor receptor 4 (FGFR4) were decreased ($P < 0.001$) following the cholesterol diet but their gene expressions were not affected by training (Figure 1 b). Hepatic FGFR4 mediates the effect of intestinal fibroblast growth factor 15 (FGF15) on suppression of CYP7A1 in liver.

Molecular markers of hepatic cholesterol synthesis and uptake: Gene expression levels of sterol regulatory element binding protein 2 (SREBP2), a key regulator of hepatic cholesterol content, as well as its target genes including 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAr) and LDL-R were not changed by training (Figure 2). Cholesterol feeding in both sham and Ovx rats led to lower ($P < 0.010$) gene expression of SREBP2 and all of its aforementioned target genes compared to Ovx rats fed the SD (Figure 2). HMG-CoAr is involved in cholesterol biosynthesis in liver. These results imply that the hepatic transcript of SREBP2 and all its target genes involved in hepatic cholesterol biosynthesis and cholesterol uptake from circulation were down regulated by the cholesterol diet.

In addition to LDL-R, LRP1 is also involved in hepatic cholesterol uptake form circulation. Running had no impact on the gene expression level of LRP1 similar to LDL-R. On the other hand, the expression level of LRP1 was lower ($P < 0.010$) in Ovx-Chol than in both Ovx-SD and Sham-Chol groups (Figure 2). These findings indicate that LRP1 gene expression was significantly reduced by the combined effect of cholesterol feeding and ovariectomy.

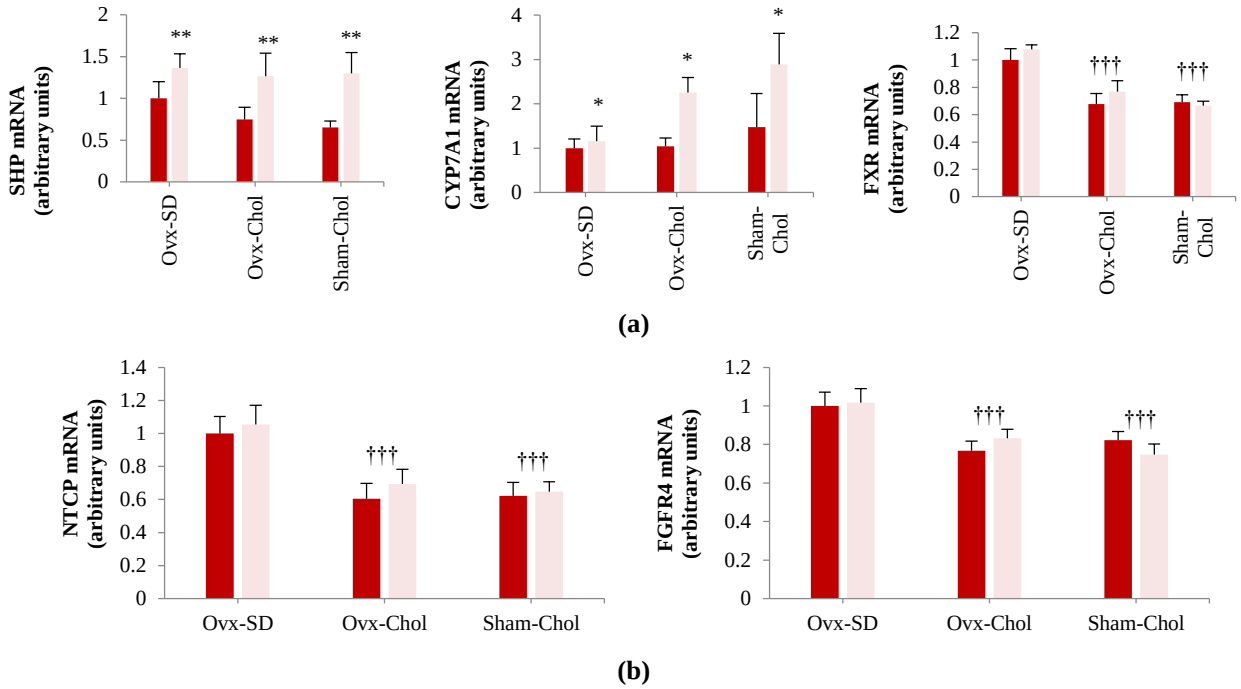

Figure 1. Hepatic mRNA expression of genes related to bile acid metabolism in ovariectomized (Ovx) rats fed a standard diet (SD) (Ovx-SD), Ovx rats fed a standard diet + 0.25% cholesterol (Chol) (Ovx-Chol) and sham operated (Sham) rats fed the Chol diet (Sham-Chol) in sedentary (■) or trained (□) state

Values are mean ± standard error

(a) * Significantly different from respective sedentary group ($P < 0.050$), ** ($P < 0.010$); ††† Significantly different from respective Ovx-SD group ($P < 0.001$)

SHP: Small heterodimer partner; CYP7A1: Cholesterol 7 alpha-hydroxylase; FXR: Farnesoid X receptor

(b) ††† Significantly different from respective Ovx-SD group ($P < 0.001$)

NTCP: Na^{+}-taurocholate cotransporting polypeptide; FGFR4: Fibroblast growth factor receptor 4

Liver TC content: Liver TC content was not affected by training in any of the nutritional conditions (Figure 3). Nevertheless, cholesterol feeding in both Sham and Ovx rats led to significantly ($P < 0.001$) higher hepatic cholesterol accumulation than in Ovx rats fed the SD. Moreover, liver TC was significantly higher in sham than in Ovx animals fed the cholesterol diet ($P < 0.050$, Figure 3).

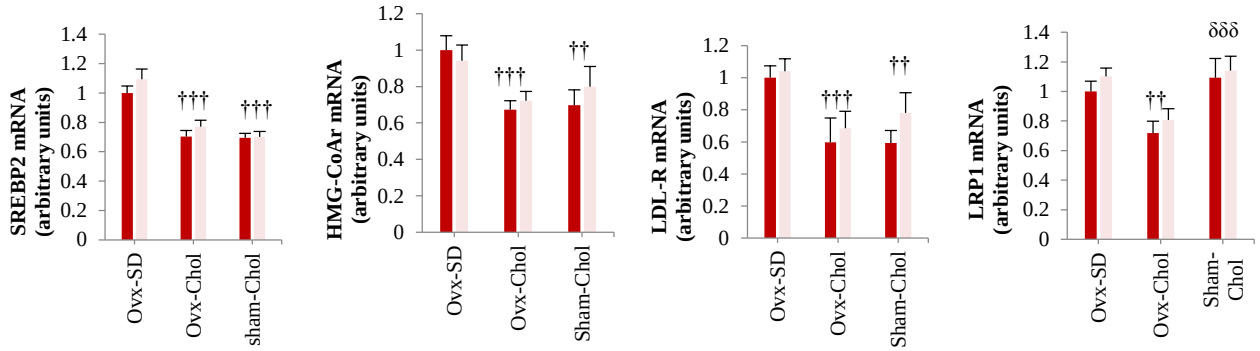

Figure 2. Hepatic mRNA expression of genes involved in hepatic cholesterol biosynthesis and cholesterol uptake from the circulation in ovariectomized (Ovx) rats fed a standard diet (SD) (Ovx-SD), Ovx rats fed a standard diet + 0.25% cholesterol (Chol) (Ovx-Chol) and sham operated (Sham) rats fed the Chol diet (Sham-Chol) in sedentary (■) or trained (□) state

Values are mean ± standard error

† Significantly different from respective Ovx-SD group ($P < 0.050$), †† ($P < 0.010$), ††† ($P < 0.001$) δδδ Significantly different from respective Ovx-Chol group ($P < 0.001$)

SREBP2: Sterol regulatory element-binding protein-2; HMG-CoAr: 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL-R: Low-density lipoprotein-receptor; LRP1: Low-density lipoprotein-receptor-related protein-1

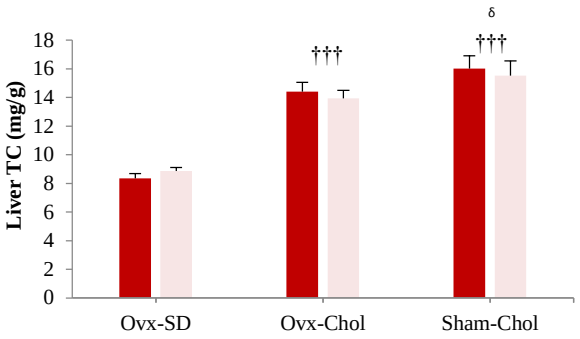

Figure 3. Liver total cholesterol (TC) content in ovariectomized (Ovx) rats fed a standard diet (SD) (Ovx-SD), Ovx rats fed a standard diet + 0.25% cholesterol (Chol) (Ovx-Chol) and sham operated (Sham) rats fed the Chol diet (Sham-Chol) in sedentary (■) or trained (□) state

Values are mean ± standard error

††† Significantly different from respective Ovx-SD group ($P < 0.001$); δ Significantly different from respective Ovx-Chol group ($P < 0.050$)

Discussion

The main finding of our study is an increased gene expression of two key markers of bile acid metabolism (SHP and CYP7A1) found in the liver of exercise-trained rats. This finding was observed in animals fed a standard diet but more importantly when rats were fed a high-cholesterol diet and that independently of estrogen levels. This observation suggests that exercise training may help overcome a cholesterol load in liver by stimulating bile acid metabolism. On the other hand, exercise training was associated with no change in SREBP2, HMG-CoAr, LDL-R, and LRP1 transcripts indicating an absence of molecular effects on key markers of cholesterol synthesis and hepatic cholesterol uptake from the circulation.

FXR/SHP/CYP7A1 pathway: Exercise training has been for a long time associated with changes in plasma cholesterol levels favouring a decrease in LDL-cholesterol and an increase in high-density lipoprotein (HDL) levels, the latter being in line with an increase in the so-called reverse cholesterol transport.[19,20] However, the basic pathways responsible for these exercise-induced beneficial effects are poorly understood. In recent years, Meissner et al.[9] and Meissner et al.[21] reported an increased fecal bile acid excretion in healthy and especially in hypercholesterolemic mice suggesting that bile acid metabolism may be involved in the action of exercise training on cholesterol metabolism. An important novel finding of our study is the increase in mRNA expression of SHP and CYP7A1with exercise training in all experimental conditions. The importance of the present SHP up-regulation by exercise training is enlighten by findings of previous studies indicating that missense mutations and polymorphisms in the promoter and coding regions of SHP in human were associated with severe early-onset obesity and diabetes.[22,23] SHP is, therefore, an excellent candidate that may link cholesterol/bile acid regulation to glucose and lipid adaptations known to occur with exercise training. In contrast to SHP and CYP7A1, their nuclear receptor FXR was not affected by exercise training. Hepatic FXR transcript was decreased by the cholesterol diet in both Sham and Ovx rats suggesting that there is no bile acid accumulation in liver and as a result there is not inhibition on CYP7A1 gene expression. Bile acids function as natural ligands for the transcription factor FXR.[24] Bile acids induce FXR activation which leads to stimulation of SHP gene expression and subsequently results in inhibition of CYP7A1 gene transcription.[12] CYP7A1 is the main enzyme involved in bile acid biosynthesis from cholesterol in liver.[15] In line with the FXR transcript reduction in cholesterol fed rats, increased hepatic TC accumulation suggests that there is a need to convert the extra cholesterol to bile acids as a way to eliminate excess cholesterol in the form of bile acids from the liver. The observed increase in the hepatic CYP7A1 transcript is in line with the concept that exercise training may regulate excess cholesterol through bile acid metabolism. In concert with our finding, an increase in hepatic gene expression of CYP27A1, a second important hepatic enzyme of bile acid formation from cholesterol, has been previously reported in trained mice fed a lithogenic diet for 12 weeks, thus favouring the catabolism of cholesterol to bile acids and reducing gallstone formation.[25] On the other hand, Meissner et al.[9] and Meissner et al.[21] did not find any changes in gene expression of CYP7A1 in mice assigned to voluntary wheel running in spite of an increase in fecal bile acid excretion. However, Pinto et al.[26] recently reported an increase in hepatic CYP7A1 gene expression in the liver of mice trained for 6 weeks while there were no differences in the [3H] cholesterol excretion into feces between the sedentary and exercise groups. In the present study, CYP7A1 transcripts were increased with exercise training in Ovx as well as in sham rats fed the cholesterol diet indicating that this exercise effect takes place independently of estrogen levels. Taken together, the present data support the

concept that increased bile acid biosynthesis following exercise training may contribute to elimination of cholesterol accumulation in liver.

Relationship between SHP and CYP7A1: As mentioned earlier, SHP is known to suppress the gene expression of CYP7A1, the rate-limiting enzyme involved in conversion of cholesterol into bile acids.[12,14,15] It has been recently reported that mRNA levels of hepatic SHP were reduced in Ovx mice while estrogen administration up-regulated SHP expression through binding to its proximal promoter. SHP promoter has an estrogen receptor responsive element (ERE) site.[16,27,28] Moreover, it was shown that this estrogen receptor α binding site on the SHP overlaps with the known FXR binding site on the SHP promoter. The combination of ethinyl estradiol plus FXR agonists did not produce an additive induction of SHP expression in Ovx mice, suggesting that simultaneous occupancy of this site by both estrogen receptor and FXR could not happen. Surprisingly, it has been also reported that induction of SHP by ethinyl estradiol did not inhibit expression of the well-known SHP target genes, CYP7A1 or CYP8B1.[27] It is expected that activation of SHP inhibits expression of CYP7A1,[12] which was not observed under estrogen treatment. Furthermore, it seems that SHP may also act independently of FXR. Lack of inverse relationship between SHP and CYP7A1 in the present study is in line with preceding mentioned finding that suggests that stimulation of SHP by estrogens may not result in suppression of CYP7A1 transcripts.[27] It seems that exercise training in our study imitated the effect of estrogen therapy on SHP transcripts. In fact, the present finding that exercise training also up-regulated SHP expression in Ovx animals extends previous findings showing that exercise training provokes estrogen-like effects on the expression of several genes involved in the regulation of lipid metabolism in liver.[29]

In contrast to SHP and CYP7A1, gene expressions of other markers of bile acid metabolism in liver, including NTCP and FGFR4 were all decreased by the cholesterol diet in sham and Ovx rats but not influenced by the training state. Down-regulated gene expression of NTCP suggests that there is less bile acid influx to the liver from the enterohepatic circulation. Decreased transcript levels of FGFR4 by cholesterol feeding may indicate that the inhibitory effect of intestinal FGF15 on CYP7A1 which acts through hepatic FGFR4 might be reduced. This lack of inhibitory effect on CYP7A1 would reinforce the interpretation that increased CYP7A1 gene expression following exercise training might be the mechanism to eliminate extra cholesterol from the liver and ultimately from the body.

Hepatic HMG-CoAr, LDL-R, and LRP1: Cholesterol feeding in our study resulted in lower hepatic transcripts of SREBP2 and its target gene, HMG-CoAr in both sham and Ovx animals. Moreover, hepatic expression of LDL-R and LRP1, two other target genes of SREBP2 which are involved in hepatic cholesterol uptake from circulation, were suppressed by the cholesterol diet. It seems that hepatic cholesterol accumulation might be a reason for the suppression of hepatic cholesterol biosynthesis and cholesterol uptake from plasma. It might be a protective response to prevent more cholesterol accumulation in the liver. These findings are in concert with the previous study that showed cholesterol feeding led to a reduction in SREBP2, LDL-R and LRP1 expression in the liver.[4] Meissner et al.[21] reported that fecal excretion of bile acids and neutral sterols in running mice was a reflection of elevated endogenous hepatic cholesterol synthesis in running group compared to sedentary mice. This is hardly the case in the present study since cholesterol feeding resulted in lower expression of HMG-CoAr in both sham and Ovx rats regardless of exercise intervention. Therefore, higher CYP7A1 mRNA expression under training is likely a consequence of hepatic dietary cholesterol accumulation. It thus seems that exercise does not modulate a cholesterol load by reducing cholesterol synthesis, but rather by stimulating bile acid metabolism. Moreover, a training effect was not observed in mRNA expression of SREBP2, LDL-R, and LRP1 in liver. Previously it was reported that exercise training increased mRNA expression of SREBP2 in Ovx rats but had no effect on its target genes in liver.[4] These data, therefore, do not provide any evidence that exercise training affects hepatic cholesterol uptake from the circulation through the LDL-R pathway. On the other hand, Wen et al. reported that a high fat diet plus exercise for 8 weeks led to an increase in SREBP2 protein with elevated levels of hepatic LDL-R mRNA in mice due to a reduction in cholesterol accumulation in liver.[30]

Liver TC: Higher CYP7A1 expression in response to training suggests higher bile acid synthesis and more excretion of the cholesterol from the liver in the form of bile acid. Therefore, less cholesterol accumulation might be expected within the liver. However, liver TC content was not

changed by training under all the experimental conditions. On the other hand, exercise running in other studies resulted in decreased hepatic cholesterol content in male mice.[21,30] It is possible that the reported effects of training in Ovx rats under the present duration of observation are only at the molecular levels. Considering the rats were fed a diet rich in cholesterol, hepatic TC content response to a training stimulus may need a longer time course of study.

Conclusion

In conclusion, the results of the present study indicate that exercise training modulates hepatic cholesterol metabolism through the up-regulation of SHP and bile acid metabolism. It seems that increased mRNA expression of SHP and subsequently higher gene expression levels of CYP7A1 is a positive response triggered by exercise to alleviate hepatic cholesterol accumulation and help to drive cholesterol out of the liver. Elevated cholesterol turnover induced by exercise training may contribute to improve hepatosteatosis and decrease the risk of atherosclerosis.

Acknowledgments

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC; 7594).

Conflict of Interests

Authors have no conflict of interests.

References

1. Chaudhuri A, Borade NG, Hazra SK. A study of heart rate variablity tests and lipid profile in postmenopausal women. J Indian Med Assoc 2012; 110(4): 228, 230-28, 232.
2. Kaur A, Jindal S, Kaur IP, Chopra K. Effect of sesamol on the pathophysiological changes induced by surgical menopause in rodents. Climacteric 2013; 16(4): 426-37.
3. Ngo Sock ET, Cote I, Mentor JS, Prud'homme D, Bergeron R, Lavoie JM. Ovariectomy stimulates hepatic fat and cholesterol accumulation in high-fat diet-fed rats. Horm Metab Res 2013; 45(4): 283-90.
4. Ngo Sock ET, Chapados NA, Lavoie JM. LDL receptor and Pcsk9 transcripts are decreased in liver of ovariectomized rats: Effects of exercise training. Horm Metab Res 2014; 46(8): 550-5.
5. Cote I, Ngo Sock ET, Levy E, Lavoie JM. An atherogenic diet decreases liver FXR gene expression and causes severe hepatic steatosis and hepatic cholesterol accumulation: Effect of endurance training. Eur J Nutr 2013; 52(5): 1523-32.
6. Savard C, Tartaglione EV, Kuver R, Haigh WG, Farrell GC, Subramanian S, et al. Synergistic interaction of dietary cholesterol and dietary fat in inducing experimental steatohepatitis. Hepatology 2013; 57(1): 81-92.
7. Cote I, Chapados NA, Lavoie JM. Impaired VLDL assembly: A novel mechanism contributing to hepatic lipid accumulation following ovariectomy and high-fat/high-cholesterol diets? Br J Nutr 2014; 112(10): 1592-600.
8. Farahnak Z, Cote I, Ngo Sock ET, Lavoie JM. High dietary cholesterol and ovariectomy in rats repress gene expression of key markers of VLDL and bile acid metabolism in liver. Lipids Health Dis 2015; 14: 125.
9. Meissner M, Lombardo E, Havinga R, Tietge UJ, Kuipers F, Groen AK. Voluntary wheel running increases bile acid as well as cholesterol excretion and decreases atherosclerosis in hypercholesterolemic mice. Atherosclerosis 2011; 218(2): 323-9.
10. Lee YS, Chanda D, Sim J, Park YY, Choi HS. Structure and function of the atypical orphan nuclear receptor small heterodimer partner. Int Rev Cytol 2007; 261: 117-58.
11. Zhang Y, Hagedorn CH, Wang L. Role of nuclear receptor SHP in metabolism and cancer. Biochim Biophys Acta 2011; 1812(8): 893-908.
12. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell 2000; 6(3): 517-26.
13. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999; 3(5): 543-53.
14. Lee YK, Moore DD. Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner. J Biol Chem 2002; 277(4): 2463-7.
15. Jelinek DF, Andersson S, Slaughter CA, Russell DW. Cloning and regulation of cholesterol 7 alpha-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. J Biol Chem 1990; 265(14): 8190-7.
16. Wang X, Lu Y, Wang E, Zhang Z, Xiong X, Zhang H, et al. Hepatic estrogen receptor alpha improves hepatosteatosis through upregulation of small heterodimer partner. J Hepatol 2015; 63(1): 183-90.
17. Robertson MC, Owens RE, Klindt J, Friesen HG. Ovariectomy leads to a rapid increase in rat placental lactogen secretion. Endocrinology 1984; 114(5): 1805-11.

18. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; 226(1): 497-509.

19. Durstine JL, Grandjean PW, Cox CA, Thompson PD. Lipids, lipoproteins, and exercise. J Cardiopulm Rehabil 2002; 22(6): 385-98.

20. Halverstadt A, Phares DA, Wilund KR, Goldberg AP, Hagberg JM. Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. Metabolism 2007; 56(4): 444-50.

21. Meissner M, Nijstad N, Kuipers F, Tietge UJ. Voluntary exercise increases cholesterol efflux but not macrophage reverse cholesterol transport in vivo in mice. Nutr Metab (Lond) 2010; 7: 54.

22. Nishigori H, Tomura H, Tonooka N, Kanamori M, Yamada S, Sho K, et al. Mutations in the small heterodimer partner gene are associated with mild obesity in Japanese subjects. Proc Natl Acad Sci U S A 2001; 98(2): 575-80.

23. Hung CC, Farooqi IS, Ong K, Luan J, Keogh JM, Pembrey M, et al. Contribution of variants in the small heterodimer partner gene to birthweight, adiposity, and insulin levels: Mutational analysis and association studies in multiple populations. Diabetes 2003; 52(5): 1288-91.

24. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: Natural ligands for an orphan nuclear receptor. Science 1999; 284(5418): 1365-8.

25. Wilund KR, Feeney LA, Tomayko EJ, Chung HR, Kim K. Endurance exercise training reduces gallstone development in mice. J Appl Physiol (1985) 2008; 104(3): 761-5.

26. Pinto PR, Rocco DD, Okuda LS, Machado-Lima A, Castilho G, da Silva KS, et al. Aerobic exercise training enhances the in vivo cholesterol trafficking from macrophages to the liver independently of changes in the expression of genes involved in lipid flux in macrophages and aorta. Lipids Health Dis 2015; 14: 109.

27. Lai K, Harnish DC, Evans MJ. Estrogen receptor alpha regulates expression of the orphan receptor small heterodimer partner. J Biol Chem 2003; 278(38): 36418-29.

28. Evans MJ, Lai K, Shaw LJ, Harnish DC, Chadwick CC. Estrogen receptor alpha inhibits IL-1beta induction of gene expression in the mouse liver. Endocrinology 2002; 143(7): 2559-70.

29. Pighon A, Gutkowska J, Jankowski M, Rabasa-Lhoret R, Lavoie JM. Exercise training in ovariectomized rats stimulates estrogenic-like effects on expression of genes involved in lipid accumulation and subclinical inflammation in liver. Metabolism 2011; 60(5): 629-39.

30. Wen S, Jadhav KS, Williamson DL, Rideout TC. Treadmill exercise training modulates hepatic cholesterol metabolism and circulating PCSK9 concentration in high-fat-fed mice. J Lipids 2013; 2013: 908048.

How to cite this article: Farahnak Z, Tomaz LM, Bergeron R, Chapados N, Lavoie JM**. The effect of exercise training on upregulation of molecular markers of bile acid metabolism in the liver of ovariectomized rats fed a cholesterol-rich diet.** ARYA Atheroscler 2017; 13(4): 184-92.