

Dynamics of hepatic and intestinal cholesterol and bile acid pathways: The impact of the animal model of estrogen deficiency and exercise training

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dynamics that involves transport lipoproteins which levels are tightly dependent on how the liver and the intestine regulate cholesterol and biliary acid metabolism. Regulation of cholesterol and biliary acids by the liver and the intestine is in turn coupled to a large array of enzymes and transporters that largely influence the inflow and the outflow of cholesterol and biliary acids through these organs. The activity of the key regulators of cholesterol and biliary acids may be influenced by several external factors such as pharmacological drugs and the nutritional status. In recent years, more information has been gathered about the impact of estrogens on regulation of cholesterol in the body. Exposure to high levels of estrogens has been reported to promote cholesterol gallstone formation and women are twice as likely as men to develop cholesterol gallstones. The impact of estrogen withdrawal, such as experienced by menopausal women, is therefore of importance and more information on how the absence of estrogens influence cholesterol regulation is started to come out, especially through the use of animal models. An interesting alternative to metabolic deterioration due to estrogen deficiency is exercise training. The present review is intended to summarize the present information that links key regulators of cholesterol and biliary acid pathways in liver and intestine to the absence of estrogens in an animal model and to discuss the potential role of exercise training as an alternative.

Key words: PSCK9; Low-density lipoprotein receptor; Very low-density lipoprotein; Sterol regulatory element binding proteins; Ovariectomy; High-density lipoprotein; Lipoproteins

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Abstract

Plasma cholesterol level is determined by a complex

Core tip: The liver is considered the master piece in regulation of plasma cholesterol levels. Together with

the intestine they control the influx and the efflux of cholesterol and biliary acids in the body. Cholesterol and its conversion into biliary acids are regulated by an extended network of enzymes and transporters that largely influence plasma cholesterol levels. The key regulators of cholesterol and biliary acids in liver and intestine are in turn affected by several factors including estrogens levels and more recently exercise training. Low estrogenic levels, such as seen in post-menopausal women, are associated with higher plasma cholesterol levels. In recent years more information has been accumulated on the extent to which low estrogenic levels, such as seen in an ovariectomized animal model, influence cholesterol and biliary metabolism at the molecular level. As an alternative to a deficiency in estrogens, exercise training has been reported to exert a beneficial effect on these key regulators of cholesterol and biliary acids.

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INTRODUCTION

The importance of estrogens in regulating cholesterol and biliary acid metabolism in liver is enlightened by clinical studies confirming that women are twice as likely as men to develop cholesterol gallstones^[1,2]. Oversaturation of biliary cholesterol is the requisite defect for the formation of gallstones^[1]. This pathophysiological state is induced by either hypersecretion of biliary cholesterol or decreased secretion of bile acids. Therefore, both the cholesterol secreted into bile and the bile acids synthesized from cholesterol in liver are involved in the disease^[3]. Exposure to high levels of estrogens has been reported to promote cholesterol gallstone formation^[4]. Similarly, the estrogen receptor α -selective agonist propylpyrazole and tamoxifen treatment, that have estrogen-like activity, augment biliary cholesterol secretion in mice^[4] and increase gallstone prevalence in women^[5]. On the whole, these findings indicate that there is a close relationship between estrogens, cholesterol and biliary acid metabolism in liver. This in turn raises the question of the extent to which a deficiency in estrogens, as happens with menopause, affects cholesterol and biliary acid regulation in liver. The first element to take into consideration is the fact that estrogen withdrawal in animals decreases gene expression of HMGCoA-reductase (-r), the rate-limiting enzyme in hepatic cholesterol biosynthesis^[6].

Estrogen-deficient state in Ovx animals has been repeatedly reported to result in substantial liver fat accumulation indicating that fat metabolism is perturbed

by the absence of estrogens^[7,8]. The information on the impact of the absence of estrogens on cholesterol metabolism, however, is scarce. An increase in plasma cholesterol levels in Ovx rats has been reported 30 years ago^[9,10]. This has been confirmed in more recent studies in Ovx animals^[6,11] as well as in post-menopausal women^[12]. The situation of liver cholesterol levels in Ovx animals is more controversial. Liver total cholesterol level was reported not to be affected by estrogen withdrawal in some studies^[11,13] while it has been found to be increased in rats ovariectomized for 5-8 wk^[14,15]. Large cholesterol accumulation has also been found in liver of Ovx rats fed with a high-fat diet, that was not observed in liver of Ovx rats fed a standard diet and in Sham rats fed a high-fat diet^[6]. The authors suggested a vulnerability to cholesterol accumulation in liver of Ovx animals fed a high fat diet. These findings have, at least, the merit of raising questions on the impact of the lack of estrogens on regulatory pathways involved in liver cholesterol metabolism. Cholesterol homeostasis in liver depends on cholesterol synthesis, uptake, and clearance. One of the aims of the present review is to summarize our present knowledge of the extent to which the lack of estrogens in an ovariectomized animal model affects the regulation of molecular pathways of cholesterol and bile acids in liver and intestine.

One of the best non-pharmacological strategies for the treatment of metabolic disturbances leading to coronary artery disease is exercise training^[16,17]. In recent years, there has been a fair amount of studies indicating that exercise training is also beneficial in circumventing the detrimental effects of estrogen removal on metabolic pathways involved in liver fat accumulation^[18]. Treadmill exercise for 12 wk has also been reported to reduce plasma low density lipoprotein (LDL)-cholesterol (-C) and total cholesterol in Ovx rats^[19] while plasma LDL-C was decreased in 6-wk trained Ovx rats fed a high fat diet for 10 mo^[20]. Although limited, there is recent information on the impact of exercise training on regulation of cholesterol pathways in liver and intestine in response to metabolic disturbances. For instance there are reports indicating an increased fecal cholesterol excretion in exercising animals^[21]. There is also a recent report of changes with exercise training in gene expression of intestinal nuclear receptors involved in the defense system against endobiotic and xenobiotic insults suggesting that regular exercise contributes to the intestinal maintenance of cholesterol and bile acid homeostasis^[22]. In the present review, a consideration will be given to the effects of exercise training on cholesterol and bile acids pathways, especially in the context of estrogens deficiency.

The present review is divided in two large sections related respectively to the pathways involved into hepatic cholesterol influx and efflux and how estrogen deficiency affects key regulators of these pathways. This will be followed by a discussion of the known effects of exercise training on these pathways.

HEPATIC CHOLESTEROL INFLUX

Western-type diets provide approximately 400 mg of cholesterol per day while our body synthesizes approximately 1 g *de novo*^[23,24]. Hence, blood cholesterol levels reflect both dietary and endogenously synthesized cholesterol. The liver is a central component in regulation of cholesterol metabolism. This organ is able to acquire cholesterol through *de novo* synthesis and from all classes of circulating lipoproteins^[25].

Cholesterol biosynthesis

The total body content of cholesterol is approximately 100 g, of which approximately 90% are found at the cellular levels and 10% in circulation^[26]. Cholesterol is synthesised virtually in all nucleated cells^[27]. For instance, the central nervous system contains approximately 25% of the unesterified cholesterol present in the body and it comes almost entirely for *in situ* synthesis^[28]. It is assumed that approximately 24% of cholesterol synthesis occurs in small intestine of rats and a significant fraction of it is transported to liver where nearly 50% of total cholesterol synthesis occurs^[29]. Cholesterol synthesis starts, similarly to *de novo* lipogenesis, by the transfer of acetyl CoA from mitochondria to cytosol. The further condensation of three units of acetyl CoA forms an HMG-CoA that is transported to the endoplasmic reticulum (ER) where it is reduced to mevalonate by the enzyme HMGCoA-r follows by several steps leading to the formation of isoprene, squalene, lanosterol, and finally cholesterol. The action of the enzyme HMGCoA-r is the rate-limited step in endogenous cholesterol synthesis.

Regulation of cholesterol biosynthesis: The view that cholesterol is randomly distributed within cell membrane no longer holds. For instance the distribution of lipids and cholesterol in the outer leaflet is organized into domains so-called rafts and caveolae playing intricate roles to maintain cellular homeostasis^[30,31]. On the other hand, membranes of the endoplasmic reticulum and the Golgi apparatus contain comparatively little cholesterol, an important factor in cholesterol homeostasis^[32]. Maintenance of cholesterol homeostasis is orchestrated mainly by a feedback regulatory system that senses the level of cholesterol in cell membranes and modulates cholesterol biosynthesis and uptake from plasma lipoproteins^[33]. The molecular mechanism of how hepatocytes maintain cholesterol homeostasis has become more precise with the discovery of the transcription factors sterol regulatory element binding proteins (SREBPs)^[32].

Short-term regulation of the enzyme HMGCoA-r is operated by mechanisms such as phosphorylation/dephosphorylation of the catalytic domain (serine 871) by specific kinases (AMPK) and phosphatases (protein phosphatase 2A)^[34,35]. HMGCoA-r is physiologically present in the cell in unphosphorylated active form (30%) and phosphorylated inactive form (70%)^[36].

Long-term regulation of HMGCoA-r relies on synthesis and degradation rate of the enzyme. The cholesterol system is unique in that the regulated end-product, cholesterol, is sequestered entirely within cell membranes. Sterol regulatory elements (SREs) are nucleotidic sequences in the gene promoters, encoding proteins involved in cholesterol homeostasis such as HMGCoA-r and LDL receptor (LDL-R). These sequences are recognized by a family of transcription factors called SREBP^[37]. The SREBP family members, SREBP-1 (a and c) and SREBP-2, are synthesized as membrane protein in the endoplasmic reticulum.

SREBP-2 is considered to be largely involved in the regulation of cholesterol metabolism. In ER, SREBP interacts with a cargo protein called SREBP cleavage-activated protein (SCAP), which acts as a transporter and cholesterol sensor^[37,38]. The complex formation is essential for the exit of SREBPs from the ER and subsequent proteolytic activation^[39]. The SREBP/SCAP containing vesicles from the ER also contain a membrane anchored serine protease of the subtilisin family called Site-1 protease (SIP-1). Sip becomes activated only during its transport to the Golgi^[40]. SCAP escorts SREBP from the ER to the Golgi apparatus where the SREBPs are proteolytically processed by SIP-1 to yield active fragments that migrate to the nucleus encoding its target genes^[33]. To release active SREBP, another enzyme is required, Site-2 protease. Interestingly, the nuclear action of SREBP induces new SREBP mRNA through SREs located in the promoter regions of their own genes^[41]. When cholesterol builds up in the ER membrane, a conformational change in SCAP occurs through the direct cholesterol binding to the sterol domain and triggers SCAP to bind to Insig, another ER membrane protein^[42]. This association hampers the transport of the SREBP/SCAP complex to the Golgi apparatus, resulting in a reduced proteolytic activation of precursor SREBP. For instance, high dietary cholesterol prevents maturation of SREBPs and cuts off cholesterol and LDL receptor synthesis.

Estrogen deficiency and HMGCoA-r regulation: Since plasma cholesterol level is increased in Ovx animals^[6,11] one might expect an increase in cholesterol synthesis. However, HMGCoA-r mRNA secondary to Ovx was found to be decreased in several studies in rats^[6,11,43,44] and in mice fed a high-fat high-cholesterol diet^[13]. Along with HMGCoA-r, gene expression of SREBP-2, the transcription factor involved in the regulation of HMGCoA-r, was also decreased in Ovx animals^[14,43]. On the opposite, an increase in HMGCoA-r protein content has been reported in frog and rat after 5 d of estrogen administration^[45,46]. On the whole these results strongly suggest that an increased cholesterol biosynthesis is not responsible for the increased higher plasma cholesterol found with estrogen deficiency in animals and in post-menopausal women. They also suggest an accumulation of cholesterol in the ER membrane.

Receptors involved in hepatic uptake of cholesterol from lipoproteins

Lipoprotein remnant receptors: Upon completion of hydrolysis (approximately 50% of TG removal) chylomicrons and VLDL lose affinity for lipoprotein lipase (LPL) and dissociate^[47]. The apoproteins A1 and C are then transferred to high-density lipoprotein (HDL) in exchange for apo E upon what they are then called chylomicrons and VLDL remnants^[48,49]. The acquisition of apo E is crucial since it will serve eventually as ligands for receptor mediated clearance. Intermediate density lipoproteins (IDL) which are VLDLs that interact for prolonged period with LPL are also remnants particles. The remnant lipoproteins are then small enough to enter the space of Disse. Once into the space of Disse, remnant lipoproteins small enough to fit between the endothelial cells are sequestered by high-molecular-weight heparin proteoglycan (HSPG) molecules. Within the space of Disse the particles are remodeled by hepatic lipase. Final uptake by the hepatocytes is receptor mediated that include LDL-R, LDL related protein (LRP), a complex LRP-HSPG or HSPG alone^[25,50]. These mechanisms are efficient so that half-life of remnants in plasma is 30 min. The apoB-48 containing chylomicron remnants are completely cleared from the plasma. However the presence of apoB-100 in VLDL alters their metabolism so that only 50% of VLDL remnants are cleared by lipoproteins remnant receptors.

Receptors involved in hepatic uptake of LDL-cholesterol: VLDL remnants that are not taken up by the remnant receptors are metabolized to a greater extent by LPL, become increasing smaller, relatively deficient in TG and enriched in cholesterol esters. These particles are called IDL. Because IDL contains apoE a fraction of these particles may be taken up by the liver through the remnant receptors^[51]. However, the remainder will be changed to LDL following further hydrolysis of the TG by the hepatic lipase. The apoE and apoC- II molecules will then transfer to HDL and leave apoB as their only apolipoprotein^[52]. The LDL-R is the only receptor able to clear up LDL from the circulation. Because of the lack of apoE, the LDL particle is a relatively weak ligand for the LDL receptor^[53]. As a result, the half-life of the LDL particle is relatively long (two to four days) thus accounting for 65%-75% of total plasma cholesterol. Interaction of apoB with the LDL-R facilitates the internalisation and the further degradation of LDL^[53]. Inside the cell, the LDL particle is hydrolysed to release unesterified cholesterol. The LDL-R is expressed on the cell surface of several tissues including liver, macrophages, lymphocytes, adrenal cortex, gonads, and smooth muscle^[25].

Metabolism of the LDL-R: The LDL-R is a cell surface receptor that mediates specific uptake and catabolism of plasma lipoproteins containing apoB or apoE^[53]. The primary function of this receptor is the removal of highly atherogenic LDL particles from circulation^[53]. Since the liver contains approximately 70% of total

LDL-R found in the body^[54], hepatic LDL-R activity is an important contributor to regulation of plasma cholesterol LDL levels. The LDL-R activity is downregulated post-transcriptionally by a protease, proprotein convertase subtilisin kexin type 9 (PCSK9)^[55]. PCSK9 is highly expressed in liver and intestine^[56]. However, circulating PCSK9 originates exclusively from hepatocytes^[57]. The gene expressions of LDL-R and PCSK9 as well as HMGCoA-r are regulated by a transcription factor, SREBP-2^[58]. Within the endoplasmic reticulum, PCSK9 undergoes an auto catalytic cleavage^[56] that results in a tightly bound secretable heterodimeric complex^[59]. PCSK9 is, therefore, readily measured in plasma. PCSK9 binds to the LDL-R at the surface of the hepatocytes and/or within the cell^[60]. LDL-R is then directed from the cell surface recycling toward degradation in the endosome/lysosome pathway^[61]. Mutations leading to a loss of function or genetic invalidation of PCSK9 largely reduce circulating LDL-C levels and reduce cardiovascular events (88%) in humans (for a review see^[60]).

The co-regulation of PCSK9 and HMGCoA-r by the same transcription factor has consequences. As discussed by Poirier *et al*^[60], statins that lower LDL-C by inhibiting HMGCoA-r also increase the expression of PCSK9^[62] which decreases their capacity at increasing LDL-R. This may explain why LDL-C levels do not reach therapeutic goals in many patients with statins therapy. Hepatocyte nuclear factor 1 alpha, a key mediator of the effects of bile acids on gene expression, also regulates PCSK9^[63].

Estrogen deficiency and LDL-R: In line with the reduction in HMGCoA-r, gene expression of hepatic LDL-R has been repeatedly reported to be reduced in Ovx animals^[11,13,14,43,64]. Along with LDL-R, PCSK9 transcripts in liver and PCSK9 plasma levels have also been shown to be reduced in Ovx rats^[14]. These results concord with the reports that estrogens administration upregulates *LDL-R* gene expression in rat liver^[46,65]. In a recent study, Roubtsova *et al*^[66] showed, using PCSK9 KO mice, that the interaction between PCSK9 and LDL-R was sex-specific, thus depending on estrogens. The similar decrease in PCSK9 and LDL-R in Ovx animals is, however, puzzling considering that a decrease in PCSK9 should lead to an increase in LDL-R. It has been proposed that the rate of cycling of hepatic LDL-R on cell surface might be an explanation. When hepatic cholesterol increases, as it is observed in Ovx animals^[11,14], the transcriptional regulation of PCSK9 and LDL-R both mediated by SREBP-2 would be inhibited, and the rate of cycling of the hepatic LDL-R slowed down leading to higher levels in circulating LDL-cholesterol. The transcriptional regulation of the LDL-R is, however, paradoxical since SREBP-2 also regulates the transcription of PCSK9, thus leading to two opposing effects initiated by the same signal. In a recent publication, Starr *et al*^[67] proposed a more dynamic role for PCSK9, suggesting that phosphorylated PCSK9 promotes degradation of LDL-R, whereas nonphosphorylated PCSK9 is in an LDL-R-protective state. Taken together, these results emphasize

the need to a better understanding of the sex specific interaction between LDL-R and PCSK9, especially in view of a new class of cholesterol lowering drugs, the PCSK9 inhibitors^[68].

Metabolism of the LRP1 receptor: LRP1 is a member of the *LDL-R* gene family which also includes receptors such as LRP2 (megalin), LRP8 (apoE receptor 2), and the VLDL receptor (VLDLR)^[69]. LRP1 is expressed in several types of cells including hepatocytes, fibroblasts, smooth muscle cells, and neurons^[70]. This transmembrane protein displays both scavenging and signaling functions. LRP1 mediates removal of at least 30 different ligands, including VLDL remnants or IDL and chylomicron remnants from the circulation^[71], but also several molecules unrelated to lipid homeostasis including proteases, protease inhibitor complexes, extracellular matrix proteins, growth factors, toxins, and viral proteins^[72]. LRP1 also acts as an endocytic receptor for several intracellular proteins released by necrotic cells, which failure to be efficiently cleared may be associated with the onset of autoimmune disease^[73,74]. Interestingly, LRP1, by regulating cell signaling through several mechanisms, may change the activity of other receptors by controlling the abundance of these receptors in the plasma membrane^[75]. For instance, disruption of the LRP gene in adult normal mice resulted in a compensatory upregulation of the LDL-R in the liver^[76].

The gene expression of LRP1 is complex and appears to be regulated by hormones and growth factors^[77]. LRP1, as well as other members of the LDL-R family, are bound by a molecule called receptor-associated protein (RAP) that blocks the bindings of ligands to these receptors^[78]. RAP functions as a molecular chaperone that assists in the trafficking of the LRP1 to the cell surface^[79]. In different tissues, LRP1 gene expression has been reported to be affected by factors such as hypercholesterolemia, lipopolysaccharides, growth factors, and hypoxia (for a review see^[80]). Hepatic LRP1 expression has been reported to be negatively associated with intracellular cholesterol level and positively associated with expression of SREBP-2^[81]. On the whole, LRP1 may be seen as a complex biosensor allowing the cells to answer to micro-environmental variations^[80].

Estrogen deficiency and LRP1 receptor: A reduction in gene expression of LRP1 in Ovx rats was first reported by Ngo Sock *et al*^[14] and confirmed in recent studies at the protein levels^[15]. This decrease in LRP1 in Ovx animals may be associated with the decrease in the SREBP-2 transcription factor^[81]. Interestingly, it has been recently reported that LRP1 is also a target for PCSK9 in HepG2 cells^[82]. These authors postulated that LDL-R can effectively compete with LRP1 for PCSK9 activity. A reduction in *LRP1* gene expression could contribute to the increase in plasma cholesterol in Ovx rats by reducing the uptake of circulating lipoprotein remnants. Finally, inducible degrader of the low-density lipoprotein receptor an ubiquitin ligase that also me-

diates the degradation of the LDL-R was found not to be affected by an ovariectomy^[66].

VLDLR

In addition to LRP, the LDL-R gene family includes a further member that functions as receptor for VLDL^[83]. The VLDLR is expressed in several tissues including heart, muscle, adipose tissue, and macrophages but barely detectable in liver under normal conditions^[83,84]. This receptor has been suggested to be important for the metabolism of apoE-containing triacylglycerol-rich lipoproteins, such as VLDL and IDL.

Interestingly, circulating PCSK9 originating from liver can regulate VLDLR in adipose tissue, which tissue does not express PCSK9^[57]. In that manner, the absence of circulating PCSK9 resulted in an increase in the level of surface of VLDLR in the perigonadal tissue^[57]. Interestingly, the increase was 10 times higher in female than in male mice^[57]. This response was in line with the typical female pattern in mice that implies a high surface VLDLR levels in perigonadal fat and low surface LDLR levels in hepatocytes^[66].

Hepatic cholesterol uptake from HDL

HDL is a class of lipoproteins that is able to remove excess cholesterol from cells and transport it through plasma to the liver. The apoA1 is the major structural determinant of HDL. It is involved in the formation as well as in the interaction with its receptor, scavenger receptor class B, type 1 (SR-B1)^[85]. HDL formation occurs mainly in the liver and to a lesser extent in the intestine^[85]. The events start when lipid-poor apoA1 is secreted by the liver or the intestine^[86] or dissociates from lipoprotein particles in the plasma^[87]. ApoA1 interacts with the membrane-embedded ATP binding cassette A1 (ABCA1) and incorporates small amount of phospholipids and unesterified cholesterol into the apoA1 molecule^[88]. Maturation of these preβHDL in the plasma occurs due to two enzymes, lecithin: Cholesterol acyl transferase (LCAT) that esterifies cholesterol and phospholipid transfer protein (PLTP) that transfers phospholipids from remnant particles to HDL.

HDLs have the ability of removing excess cholesterol from cells. The first mechanism involved the action of preβHDL interacting with ABCA1 that in addition of forming a new HDL by the liver is used to remove excess cholesterol from macrophages^[89]. Spherical mature HDL may remove cholesterol from cells using several mechanisms. The particle may interact with SR-B1 on the plasma membrane. Macrophages also express ABCG1 transporters that mediate transfer of excess cholesterol to HDL. Finally excess cholesterol from cells may also efflux in absence of binding to transport protein, travels short distance through plasma and be taken up by HDL^[25]. The activity of LCAT and PLTP prevents the HDL from being saturated with cholesterol. The enzyme cholesterol ester transfer protein (CETP) that transfer cholesteryl ester molecules from HDL to remnant particles in exchange for TG also increases the capacity of HDL to accept

unesterified cholesterol from cells.

HDLs circulating to the liver interact with SR-B1 the main HDL receptor^[90]. SR-B1 in the liver facilitates the uptake of cholesterol and cholesterol esters from the HDL particle without the apoA1^[86]. ApoA1 may then be recycled to form a new pre β HDL. The action of SR-B1 is facilitated by the hydrolysis of TG by the hepatic lipase. The adrenal gland and gonads also highly express SR-B1 most likely due to their requirement in cholesterol^[86].

HDLs are considered limiting for the reverse cholesterol transport because it is assumed that they deliver peripheral cholesterol to the liver for biliary secretion and eventually fecal excretion^[91,92]. As discussed by Temel and Brown^[93], however, there is evidence that HDL-driven cholesterol efflux does not correlate with how much is lost in bile or in the feces. Mice genetically lacking ApoA1 or ABCA1 and, therefore having very low circulating levels of HDL, or showing different steady-state concentrations of HDL-C have normal biliary and fecal cholesterol loss^[94,95]. Some authors argue that apoB-containing lipoproteins and particularly the activity of CETP play a substantial role in reverse cholesterol transport^[96].

Estrogen deficiency and hepatic HDL receptor:

SR-B1 mRNA in liver that allows the return of cholesterol to liver *via* HDL was reported to be higher in Ovx compared to Sham rats^[14]. Interestingly, *ABCA1* gene expression, involved in biosynthesis of nascent HDL was also found to be increased in Ovx rats^[14]. An increase in gene expression of *ABCA1* was also found in jejunum of Ovx rats^[14]. Although limited, these findings point to the direction as if the hepatic contribution to HDL metabolism was increased with estrogen withdrawal.

HEPATIC CHOLESTEROL EFFLUX

There are essentially two ways by which liver can excrete cholesterol: (1) secretion of unmodified cholesterol or after its transformation in bile salts into bile caniculi; and (2) through VLDL secretion.

Hepatic cholesterol-bile acid metabolism

The liver is the only organ that has ability to eliminate cholesterol through its secretion into bile or its transformation into bile salts. Bile acids synthesis from cholesterol is stimulated by the nuclear factor liver X receptor (LXR) through its target gene cytochrome P450, family 7, subfamily a, polypeptide 1 (CYP7A1), the main enzyme in the conversion of cholesterol into bile acids^[97]. The synthesis of a full complement of bile acids requires 17 enzymes^[98]. The bile acid pool size is reduced by 75% in mice deficient in CYP7A1^[99]. An alternative biosynthetic pathway is initiated by the enzyme cholesterol 27 α -hydroxylase (Cyp27 α 1^[99]). Bile salts are highly soluble in water. They form aggregate with phospholipids derived from hepatocyte membranes and solubilize cholesterol in bile for transport from liver to intestine^[100]. Nuclear factor farnesoid X receptor (FXR) activated by bile acids, stimulates bile and cholesterol

efflux from liver. Opposite to LXR, FXR suppresses bile acids synthesis by inhibiting Cyp7A1. At the canalicular membrane of the hepatocytes, bile salts are pump into bile by a membrane transporter, ABCB11, also referred to as bile salt export pump (BSEP) and to a lesser extent by the multidrug resistance-associated protein 2 (MDR2; ABCC2^[101]), which activates two other transporters, ABCB4 involved in the transport of phospholipids and ABCG5/G8 a heterodimer involved in the secretion of cholesterol^[102-104]. Alternative mechanisms to ABCG5/G8 cholesterol secretion involve ATP8B1 and diffusion^[105]. Altogether bile salts and phospholipids form micelles which are stored in the gall bladder during fasting. In addition, bile salts may be exported to the blood at the sinusoidal membrane mediated by MRP3 (ABCC3) and MRP4 (ABCC4), as well as the organic solute transporter OST α/β ^[106]. Conversion of cholesterol to bile salts accounts for about 50% of daily cholesterol excretion^[107].

Estrogen deficiency and hepatic cholesterol-bile acid metabolism:

Cyp7A1 and Cyp8b1 transcripts have been reported to be decreased in Ovx rats and mice^[6,11,13,43] suggesting a reduction in cholesterol elimination *via* bile acid formation. This decrease has been found in Ovx rats fed a standard diet and even more so when Ovx rats were fed a high-fat (42%) diet^[6]. On the opposite, estrogen treatment has been reported to result in an increase in biliary cholesterol hypersecretion in mice^[4].

Estrogen deficiency was associated with lower transcript levels of BSEP and MDR2 suggesting that, in addition to synthesis, excretion of bile acids from hepatocytes to caniculi was decreased in Ovx rats^[15,43]. Furthermore, the gene expression of nuclear receptors FXR and LXR was found to be lower in Ovx compared to Sham animals^[43]. The decrease in gene expression of FXR suggests that bile acids did not accumulate in liver of Ovx rats. FXR mRNA levels are controlled by bile acids^[108]. The specific role of hepatic FXR is to prevent bile acid hepato-toxicity by initiating the expression of a gene network involved in the synthesis and excretion of bile acids. Accordingly, FXR-null mice show massive accumulation of cholesterol in hepatocytes^[109]. The indication that bile acid metabolism is disrupted in Ovx rats may in turn favours cholesterol accumulation in liver since bile acid secretion exerts a driven force for biliary cholesterol excretion^[110]. Supporting the hypothesis that biliary metabolic pathways are indeed disrupted in Ovx animals is the finding of a decrease in total bile production in Ovx rats^[111].

Gene expression of ABCG5/G8 transporters involved in exportation of cholesterol from the liver to the bile ducts was unchanged in Ovx compared to Sham rats^[6,15,43] and in aromatase knockout mice^[112] suggesting that these transporters are not regulated by estrogens.

Hepatic excretion of cholesterol through VLDL

VLDL assembly in liver is initiated by the entry of apoB100 in the lumen of the endoplasmic reticulum^[113].

The apoB protein is lipidated by the action of microsomal transfer protein (MTP) accumulating TG as well as cholesterol esters molecules. Besides MTP and apoB100, other molecular markers of VLDL assembly include diacylglycerol acyltransferase 2 (DGAT2), involved in the reesterification of TG^[114], and acyl-CoA: Cholesterol acyltransferase 2 (ACAT2) that converts free cholesterol into cholesterol esters^[115]. Further lipidation of the VLDL particles after they exit the endoplasmic reticulum compartment is carried on by a lipid droplet-associated protein, cell death-inducing DNA fragmentation factor alpha-like-effector B (Cideb)^[116]. The importance of Cideb has been enlightened by the finding of a reduction in plasma LDL levels in Cideb-null mice^[117]. However, hepatic cholesterol storage was increased in liver of these animals due to its increased LDL-R and ACAT expression. Finally, small GTP binding protein (Sar1a), an intracellular vesicular trafficking protein, facilitates the movements of VLDL particles between the endoplasmic reticulum and the Golgi apparatus where they are secreted in the plasma.

Estrogen deficiency and hepatic VLDL metabolism:

The observation that plasma cholesterol level is increased in OvX animals^[6,11] might suggest an increased cholesterol excretion through VLDL. On the opposite, a decrease in VLDL-TG production has been reported in estrogen-deficient animals^[118,119]. Supporting such a decrease in VLDL production at the molecular level is the repeatedly reported decrease in gene expression of MTP, the rate-limiting molecule for VLDL assembly and secretion, in OvX animals^[15,43,118]. Transcripts of other genes involved in VLDL synthesis, including apoB, DGAT2, ACAT2, Cideb, and Sar1a have also been reported to be decreased in OvX rats fed a standard diet^[15,43] and even more so for some genes (MTP and apoB100) in OvX rats fed an enriched-cholesterol diet^[15]. The additive effect of estrogen withdrawal and high-cholesterol diet on reducing markers of VLDL production was corroborated by an accumulation of total cholesterol and TG in liver and lower levels of these two forms of lipids in plasma^[15]. In search of an explanation for the postulated reduced VLDL production in OvX rats fed the cholesterol diet, it has been suggested that cholesterol may induce ER stress through cholesterol accumulation^[120] and that ER stress limits VLDL assembly and secretion through apoB degradation^[121]. Collectively, these results points toward the interpretation that VLDL assembly is disrupted upon ovariectomy leading to reduced excretion of TG and cholesterol from the liver, thus contributing to exacerbate liver fat and cholesterol accumulation^[14,15].

Molecular mechanisms by which estrogens regulate transcription of target genes involved in VLDL pathway are not well known. The classical genomic mechanism of estrogen action involves activation of its nuclear receptor (ER α and β) and subsequent binding to estrogen response elements located in the promoters of target genes^[122,123]. Estrogens have also been shown to have non-genomic actions mediated through a subpopulation

of ER α and β located at the plasma membrane^[124]. It is thus possible that estrogens affect expression of target genes involved in different metabolic pathways through interaction in the nucleus and/or activation of signal transduction pathways at the plasma membrane.

Intestinal excretion of biliary cholesterol

As mentioned above, hepatic cholesterol is secreted into bile unmodified or after its conversion into bile salts. These bile salts participate in cholesterol transport and eventually in fat digestion in the intestine. However, rather than being lost in the feces, most of the bile salts are recycled when they are taken up by transport proteins in the distal ileum. FXR controls the absorption of bile acids in the intestine through the regulation of bile acid transporters from the intestine to the portal system^[125]. These include apical sodium-dependent bile acid transporter, the ileal bile acid binding protein, and at the basolateral membrane of enterocytes the heterodimeric organic solute transporters α and β (OST α , OST β)^[126,127]. Bile salts picked up by these transporters enter the portal circulation and are transported back to the liver where they are eventually re-secreted into bile. This process of recycling back the bile salts between the intestine and the liver is called the enterohepatic circulation^[128]. The Na⁺-taurocholate cotransporting polypeptide (NTCP) is the major uptake system to transport bile salts from the blood into parenchymal cells^[129]. Together with several organic anions transporting polypeptide, it controls bile salt uptake at the sinusoidal membrane^[130]. Bile salt accumulation down-regulates NTCP at the transcriptional level mediated by FXR and the short heterodimer partner 1^[131].

Less than 10% of transported bile salts are lost in the feces (0.4 g/d)^[132]. Therefore, dietary cholesterol (0.4 g/d) constitutes only 25% compared to endogenous cholesterol (1.2 g/d) that passes through intestine in one day^[133]. Coordination between intestinal bile acids levels and hepatic bile acids biosynthesis is assured through the intestinal secretion of fibroblast growth factor 15/19 that inhibits Cyp7 α 1 in liver under FXR activation^[134].

Excretion of intestinal absorbed cholesterol: The cellular mechanisms by which chylomicrons in the intestine and VLDL in the liver are assembly are very similar. Their assembly depends of the availability of apoB, triglycerides, and the TG transfer protein MTP. However, opposite to liver, enterocytes express a protein called apoB editing complex-1^[135]. As a result of the action of this enzyme, translation of apoB comes to a premature stop making intestinal apoB in the intestine 48% as long as the protein expressed in the liver (apoB100). Cholesteryl esters added to the core molecule of chylomicrons come from biliary acids (75%) and from dietary sources. During digestion, cholesteryl esters in food are hydrolyzed to form unesterified cholesterol^[136]. Dietary and biliary cholesterol from micelles enter the enterocytes mainly (80%) *via* a protein channel, Neimann-Pick C-1 like 1 protein (NPC1L1)^[137]. Some of

this cholesterol is immediately pumped back into the lumen by the heterodimer transporter ABCG5/G8^[138]. A portion of cholesterol is also transferred to apoA1 by the ABCA1 transporter to form a nascent HDL. The fraction of cholesterol remaining is esterified to a long-chain fatty acid by ACAT2^[139].

Estrogen deficiency and intestinal bile acid-cholesterol metabolism: The information is rather limited in regard to biliary cholesterol metabolism in the intestine. A greater faecal excretion of bile acids has been reported in Ovx rats^[11]. The authors explain this response by suggesting a decreased reabsorption of bile acids from the ileum through a decrease in bile acid transporters. Gene expression of NTCP, the major uptake system to transport bile salts from the blood into parenchymal cells, was found to be unchanged in Ovx compared to Sham rats^[15]. On the other hand, gene expression of ABCA1 was reported to be increased in jejunum of Ovx rats, suggesting an increased efflux of intestinal cholesterol through HDL synthesis in Ovx animals^[14].

Transintestinal cholesterol excretion

The hepatobiliary pathway also referred to as the reverse cholesterol transport pathway is considered the major elimination cholesterol route. Nevertheless, fecal cholesterol excretion was observed in several states of disturbances in cholesterol biliary excretion supporting the existence of a new route for cholesterol excretion^[140-142]. In other words, a large part of the cholesterol found in the feces originates from a source other than bile and diet. The non-biliary alternative called the transintestinal cholesterol excretion pathway implies the direct secretion of plasma lipoprotein-derived cholesterol by the small intestine^[94,143,144]. Among the numerous studies on transintestinal cholesterol excretion (TICE), there is some agreement that under normal conditions TICE contributes to less than 30% of cholesterol found in the feces (for a review see^[93]). However, the TICE pathway may be stimulated under pathophysiological or pharmacological conditions. For instance, intestinal cholesterol excretion is inducible by a high-fat diet^[145] or pharmacologically by ligands of LXR^[146]. The importance of the role of TICE has been recently highlighted by the demonstration that TICE is essential to macrophage reverse cholesterol transport in mice^[142].

It seems that the liver initiates the activation of the TICE^[93]. Findings in mice with impaired hepatobiliary cholesterol excretion indicate that cholesterol is first transported to the liver before being delivered to the intestine^[93]. Temel and Brown^[93] summarized evidence that indicate that it is the subsequent steps within the liver that determine the amount of cholesterol eliminated through the biliary and non-biliary excretory mechanism. The excess cholesterol is most likely repacked into apoB rich lipoproteins secreted by the liver. These liver-derived apoB-containing lipoproteins are recognized by the proximal small intestine through LDL-R and probably

other mechanisms^[147]. Le May *et al*^[147] provided data suggesting that PCSK9 is a repressor of TICE dependent on the LDL-R. They also demonstrated that both LDL and HDL (possibly through SR-B1 transporter) provided cholesterol to TICE. Once the free cholesterol is liberated from the TICE lipoproteins, it may efflux from the apical side of the enterocyte through the ABCG5/G8 transporters or the multidrug transporter ABCG1a/b^[93].

EFFECTS OF EXERCISE TRAINING ON LIVER AND INTESTINAL CHOLESTEROL METABOLISM

The main finding supporting the contention that exercise training improves lipid and cholesterol metabolism is the reported increase in plasma HDL levels and the concomitant decrease in LDL-cholesterol and triglycerides in human studies^[148,149]. In animals, positive effects of exercise training on the outcome of disturbances in lipid and cholesterol metabolism has been demonstrated by Ramachandran *et al*^[150] who reported a 50% reduction in pre-existing atherosclerotic lesions in LDL-R KO mice. Similarly, Matsumoto *et al*^[151] reported that exercise training in LDL-R KO mice prevented aortic valve sclerosis. These authors specified that exercise exerted several numerous favourable effects that include preservation of valvular endothelial integrity, reduced recruitment of inflammatory cells, and oxidative stress. A decrease in aortic lesion size was also reported by Meissner *et al*^[21] after 12 wk of voluntary running wheel in LDL-R deficient mice.

However, as mentioned by Meissner *et al*^[152], the molecular pathways behind such exercise-induced improvements in plasma lipids are not well defined. In addition, the analysis of the effects of exercise training on the molecular components of cholesterol metabolism in liver is complicated by the variety of animal models used.

HMGCoA-r and exercise training

There is a paucity of information on the effects of exercise training on cholesterol biosynthesis. Ngo Sock *et al*^[14] reported that training (8 wk) did not appear to have any effect on HMGCoA-r as well as on SREBP-2 transcripts whether in Sham or in Ovx rats. Previously, Meissner *et al*^[152] reported an increase in lanosterol/cholesterol ratio in mice submitted to two weeks of voluntary exercise suggesting an increase in cholesterol biosynthesis. However, the same group of authors reported a decrease in HMGCoA-r after 12 wk of voluntary wheel running in LDL-R deficient mice^[21]. On the whole, there is no clear indication that hepatic cholesterol biosynthesis is changed with exercise training.

LDL-R and exercise training

Using CETP transgenic mice, an animal model that simulates reverse cholesterol transport (RCT) in human, Rocco *et al*^[153] found an increase in hepatic LDL-R protein

levels following 6 wk of treadmill exercise. Using this animal model they also found that exercise training improved macrophage RCT. An increase in *LDL-R* gene expression in liver of normal mice exercised for two weeks had been previously found^[154]. At the same time, Wilund *et al*^[155] reported an increase in *LDL-R* gene expression and a reduction in gallstone development in gallstone-sensitive mice fed a lithogenic diet after 12 wk of exercise training.

In a recent study, Wen *et al*^[156] found that treadmill exercise for 8 wk resulted in an increase in PCSK9, *LDL-R*, and SREBP-2 mRNA in high-fat fed mice. On the other hand, they found a reduction in plasma PCSK9 levels and no difference in *LDL-R* protein abundance. They attributed these latter responses to the lower levels of circulating *LDL-C* in trained animals.

In other respects, exercise training (8 wk) did not alter *LDL-R*, *PCSK9*, and *LRP1* gene expression in Sham rats as well as being ineffective in correcting reductions in these molecular markers in Ovx rats^[14]. On the opposite, Pinto *et al*^[157] recently reported an increase in *LDL-R* protein levels in male mice trained for 6 wk. Taken together, there is indication that exercise training may favour liver cholesterol uptake from circulation through *LDL-R* thus, supporting the general finding of a reduction in circulating *LDL-C* in human^[149].

HDL metabolism and exercise training

Exercise training (8 wk) did not influence SR-B1 and ABCA1 responses in Sham as well as in Ovx rats^[14]. On the other hand, an increase in ABCA1 mRNA had previously been reported following 6 wk of treadmill exercise in rats accompanied by an increase in plasma HDL-C concentration^[158].

Two weeks of exercise training resulted in an increase in SR-B1 in livers of exercised mice^[154]. Wilund *et al*^[155] also reported an increase in *SR-B1* gene expression and a reduction in gallstone development in gallstone-sensitive mice fed a lithogenic diet after 12 wk of exercise training. An increase in SR-B1 protein level in liver has also been reported in male mice trained for 6 wk along with the demonstration of an increased macrophage cholesterol flux to the liver^[157].

In CETP transgenic mice, Rocco *et al*^[153] found an increase in hepatic ABCA1 protein levels following 6 wk of treadmill exercise but no effects on SR-B1. On the whole, it appears that exercise training stimulates positive adaptations of molecular markers of HDL metabolism that would tend to support the finding of an increase circulating HDL levels with exercise training in human^[149].

Bile acids and exercise training

Wilund *et al*^[155] reported an increase in gene expression of Cyp27A1 in mice fed a lithogenic diet after 12 wk of exercise training. On the opposite, Meissner *et al*^[21,152] did not observe any effects of exercise on key genes expression involved in bile acid synthesis (*CYP7A1*, *CYP8B1*, and *CYP27A1*) in mice despite an increased fecal bile acid and cholesterol excretion, leading the authors to assume

a posttranscriptional regulation of these genes. The authors hypothesized that physical activity might increase bile acid synthesis to increase the capacity for micelle formation, thus increasing fatty acid absorption^[21]. More recently, Pinto *et al*^[157] reported an increase in *CYP7A1* gene expression in male mice trained for 6 wk. On the whole the existing molecular data would tend to support the physiological finding of an increase in fecal bile acid and cholesterol excretion in exercise trained animals.

VLDL and exercise training

There is a report that VLDL-TG secretion rate is reduced in human following exercise training^[159]. A decrease in VLDL-TG accumulation and apoB mRNA after exercise training has also been reported in male Wistar rats^[160]. Accordingly, liver MTP protein content has been found to be decreased with exercise training in mice^[21] and in standard and high-fat fed female Sprague-Dawley strain rats^[161]. Since liver fat accumulation is reduced with exercise training^[162], the latter authors argue that the reduced liver VLDL production induced by regular exercise is a consequence of an increased lipid disposal through oxidation^[163]. It is also possible that an increased hepatic insulin sensitivity following exercise training may have resulted in a decrease in VLDL-TG synthesis and secretion. It is well documented that insulin suppresses the secretion of VLDL particles by the liver^[164] and *MTP* gene expression has been reported to be reduced by insulin in culture liver cells^[165].

Plasma VLDL-TG levels have also been reported to be reduced following exercise training in Ovx rats for which VLDL-TG levels were already reduced^[118]. This suggests that the effects of exercise training and estrogen withdrawal on VLDL-TG synthesis and/or secretion are additive and most likely take place through different pathways. On the other hand, the reduction in VLDL-TG production with exercise training in Ovx rats did not result in an accumulation of liver TG^[118]. This was explained by the fact that exercise training increases the use of lipids, therefore, reducing fat delivery to the liver.

Intestinal markers and exercise training

Gene expression of ABCA1 was reported to be increased in jejunum of Ovx rats but unchanged by exercise training (8 wk)^[14]. On the other hand, the same group of authors found an increase in ABCA1 in ileum of 8-wk trained rats^[22]. An increase in ABCA1 mRNA in the upper part of the small intestine in Wistar rats trained for 6 wk had been previously reported^[166]. Although limited, these findings concord with what has been found in liver and suggest that HDL synthesis from the intestine is increased following exercise training.

Wilund *et al*^[155] found a decrease in *NPC1L1* and *ABCG5/G8* gene expression in duodenum of mice after 12 wk of exercise training. The authors explain that the reduction in *ABCG5/G8* might have been the consequence of the reduction in *NPC1L1* and less cholesterol transported into the enterocytes. A decrease in *NPC1L1* and *ABCG5/G8* was also recently reported in the ileum of

8-wk trained rats^[22].

On the other hand, Meissner *et al*^[152] reported an increase in fecal bile and cholesterol loss and a decrease in jejunal NPC1L1, suggesting a decrease intestinal cholesterol absorption, in male mice submitted to voluntary exercise for two weeks. Running mice also displayed lower ileal OST α , OST β , and NTCP transporters, all involved in the enterohepatic circulation of bile acids. However, running did not affect mRNA levels of cholesterol efflux ABCG5/G8 in jejunum. On the whole these authors^[152] reached the conclusion of an increase cholesterol turnover with regular exercise. In a subsequent study, Meissner *et al*^[21] found a massive fecal bile acid loss in hypercholesterolemic LDL-R deficient mice trained for 12 wk. Decreases in ileal OST α and OST β mRNA have also been reported in 8-wk trained rats along with a decrease in FXR transcription factor indicating that the need to protect the intestine against bile acid overload is reduced in trained animals^[22]. Finally, Ngo Sock *et al*^[22] found a decrease in pregnane X receptor (PXR) mRNA in ileum of trained rats. Since PXR receptors protect organisms from exogenous chemical insults, and several endobiotics such as lipids, steroids, and bile acids^[167], the authors advocate that exercise training contributes to the maintenance of cholesterol and bile acid homeostasis^[22].

On the whole it appears that, at the molecular level, exercise training would contribute to the maintenance of normal circulating cholesterol levels by increasing hepatic LDL-R and HDL metabolism and by favouring adaptations to bile acid metabolism that stimulate fecal bile and cholesterol excretion. When discussing the effects of exercise training on cholesterol metabolism one has to consider that on contrary of fatty acids and glucose or glycogen, cholesterol is not metabolized during exercise. Therefore, it might be an interesting avenue to look at the impact of exercise training on cholesterol metabolism through its link with lipid and glucose metabolism such as intestinal lipid absorption or hepatic *de novo* lipogenesis.

IN SUMMARY (ESTROGEN DEFICIENCY EFFECTS)

HMGCoA-r gene expression in liver along with its transcription factor SREBP-2 is decreased in Ovx animals suggesting a decrease in cholesterol synthesis. There are also indications that bile acid synthesis (*i.e.*, CYP7A1) and transporters of bile acid excretion into caniculi (*i.e.*, BSEP) are also decreased with estrogen deficiency. The reduction in hepatic bile acid metabolism would support the finding that total bile production is reduced in Ovx rats^[111].

Although it has been shown that hepatic PCSK9 as well as SREBP-2 and LDL-R mRNA levels are reduced in estrogen deficient animals, there is on the whole data supporting the contention that LDL-R protein levels are increased in Ovx animals most likely associated with a reduction in *PCSK9* gene expression. Although it is

difficult at the present time to reconcile clearly the impact of the absence of estrogens on the dynamics of hepatic PCSK9 and LDL-R and its consequence on plasma LDL-cholesterol, it is evident that estrogen levels play a critical role. The sex specific interaction between LDL-R and PCSK9 would be particularly relevant to post-menopausal women, especially in view of a new class of cholesterol lowering drugs, the PCSK9 inhibitors^[68].

There are also data supporting the finding that VLDL and HDL metabolism are changed with the absence of estrogens. VLDL production and its main regulatory factor (MTP) have been repeatedly reported to be decreased in Ovx animals. On the other hand, increases in SR-B1 and ABCA1 mRNA in liver of Ovx animals support the contention that HDL metabolism is increased in these animals. An increase in ABCA1 in intestine suggesting an increase in HDL biosynthesis has also been reported^[14].

Although it is obvious that more work has to be done to clearly understand the changes in cholesterol and bile acid metabolism in liver and intestine with the absence of estrogens, the data actually available in Ovx models tend to indicate an increase in cholesterol influx into the liver and a decrease in cholesterol efflux.

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