Molecular actions of hypocholesterolaemic compounds from edible mushrooms

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Abstract

Cholesterol levels are strictly regulated to maintain its homeostasis therefore, if it is not absorbed with the diet, the cholesterol biosynthetic pathway is enhanced and *vice versa*. Nowadays, the commonly prescribed therapeutic treatments for hypocholesterolemic patients are targeted toward the reduction of both cholesterol intestinal absorption and/or its endogenous biosynthesis. But, when hypercholesterolemia is still moderate the consumption of food products with cholesterol-lowering capacities is more desirable than drugs. The marketed food supplemented with hypocholesterolemic compounds are only inhibiting mechanisms for cholesterol absorption (i.e. phytosterols, cereal β-glucans). However, certain fungal extracts obtained from edible mushrooms might be able of modulating cholesterol levels by both strategies as pharmaceutical drugs and functional foods. *In vitro* and *in vivo* studies indicated that fungal sterols down-regulated genes involved in the cholesterol homeostasis (such as *Srebf2* and *Nr1h4* (FXR)) and other specific mushroom extracts (β-glucans and other water-soluble compounds) also stimulated transcriptional profiles similar to simvastatin or ezetimibe (two hypocholesterolemic drugs). These and other observations suggested that the hypocholesterolemic effect of

mushrooms extracts could be due to transcriptional and post-transcriptional modulations besides other indirect effects.

1. Introduction

Although cardiovascular diseases (CVDs) incidence is decreasing over the last decades due to medical advances and advise, they are still the second leading cause of premature death in Western world after cancer[1]. Already for many years, health authorities are reinforcing efforts to inform people about what CVD are, their main symptoms, their health consequences and the way to decrease the risk of suffering them.

CVD risks are influenced by genetic factors such as specific tendencies to obesity, hypertension, etc., gender or age however, many risk factors are also modulated by life style habits such as smoking, sedentary/sporting or in/adequate diets[2] and this is the reason that CVDs are considered as multifactorial diseases.

Several biomarkers are used to evaluate the real individual CVD risk *i.e.* elevated homocysteine, coronary artery calcium-phosphate calcification or elevated fibrinogen blood level but, the most useful marker for CVD risk is the LDL (low-density lipoprotein)-cholesterol concentration in serum, one of the major transport cholesterol complexes. The LDL-cholesterol constitutes 60-70% of total serum cholesterol and a blood concentration higher than 200 mg/dL is considered, nowadays, as risky level[3,4].

Due to the increasing interest of modern society for natural products as agents able to decrease the consumption of pharmaceutical drugs, new food products with high levels of hypocholesterolemic compounds are being explored. Nowadays, the cholesterol-lowering effect of compounds such as plant sterols (phytosterols, phytostanols and derivatives)[5,6]; dietary fibers such as β-glucans or chitosans[7]; peptides such as those derivate from soy proteins[8] or bovine milk β-lactoglobulins[9] have been studied and therefore, several functional food products supplemented with these compounds are available at the supermarkets[10-14].

Some edible mushrooms species are able of lowering cholesterol levels in serum since they are a good source of fungal sterols (with high structural similarity to phytosterols), dietary fibers such as β-glucans and chitins (fungal polysaccharides), β-lactones, specific proteo/glucan complexes or compounds such as eritadenine[10]. Recent studies pointed out that ergosterol-enriched extracts and other fungal compounds were also able of modulating cholesterol-related gene expression in animal intestine and liver[15].

Therefore, in this work, the molecular events occurring during cholesterol synthesis and absorption are reviewed analyzing the influence of some hypocholesterolemic compounds obtained from edible mushrooms.

2. Cholesterol metabolism

Cholesterol, together with phospholipids, modulates membrane fluidity influencing transport through them, permeability, configuration of membrane proteins or enzyme activities. Furthermore, cholesterol is involved in many metabolic pathways since it is a precursor of a wide range of biological molecules such as bile acids (*i.e.* cholic acid), steroids hormones (*i.e.* testosterone) and lipophilic vitamins (*i.e.* vitamin D_3)[7]. This sterol is synthesized mainly in the liver, besides other organs such as adrenal glands, intestine or ovaries. But, it can also be incorporated from the diet after the digestion process. Thus, well-balanced mechanisms of cholesterol synthesis, bile acids catabolism, cholesterol intake and excretion through faeces will maintain healthy and stable cholesterol values in serum (homeostasis). Until few years ago, liver was considered the main control center of cholesterol homeostasis however, more recent studies pointed intestine as a tissue highly involved in the regulation of plasma cholesterol levels and homeostasis[13,16,17].

2.1. Molecular events occurring during cholesterol digestion

Although food digestion in humans starts in mouth with the mechanical chewing and starch degradation by salivary enzymes (mastication), fat remains undigested until it reaches the stomach. Gastric digestion is mainly oriented toward protein degradation however, some lipid-degraded enzymes are also active at this step. Afterwards, the main fat digestion take place in duodenal lumen where all lipid compounds from gastric bolus (including free or esterified diet cholesterol) are mixed with the bile and pancreatic juices.

Cholesterol molecules from diet, bile, intestinal secretions and desquamated cells together with the bile secreted compounds such as phospholipids (lecithin) and bile acids (salts of taurocholic and deoxycholic acids, etc.) form small emulsified droplets. Then, pancreatic lipase, phospholipase A_2 and cholesterol esterase transform the emulsified particles in a series of colloidal structures including vesicles, micelles or dietary mixed micelles (DMM)[10].

2.2. Molecular events occurring during cholesterol absorption

Most of the micellated lipid-like compounds are incorporated into the organism through the second part of small intestine (jejunum), except bile acids that can be absorbed at jejunum and ileum levels by an apical sodium-dependent bile acid transporter (ASBT)[18,19]. The SR-B1 scavenger receptor (encoded by SCARB1 gen in humans) mainly located at both apical and basolateral membranes[17,20] of adrenal glands, hepatocytes and enterocytes (Figure 1), is involved in the regulation of the endocrine metabolism, vitamin absorption or bile secretion. It also plays a role in the cholesterol transport through membranes as a receptor of HDL (high-density lipoprotein)-cholesterol but not in the small intestine absorption context[20,21]. SR-B1 transport allows a passive bi-directional cholesterol efflux depending of concentration gradients[22] pointing SR-B1 as an important modulator of reverse cholesterol transport (RCT) (described later)[20]. However, although SR-B1 contribute to enterocytic cholesterol absorption, recent studies demonstrated that Niemann-Pick C1-like protein (NPC1L1) is the main sterol transporter from the intestinal lumen to the enterocyte cytoplasm, being imperative for non-esterified cholesterol absorption[23].

NPC1L1 is involved in this transmembrane sterol efflux due to a sterol-sensing domain (SSD) and it is co-localized at the cellular and intracellular vesicular membranes. The distribution of non-esterified cholesterol determines the main location of NPC1L1 proteins. At low intracellular concentrations, NPC1L1 will be mostly exposed at the brush-border enterocyte membrane and it will be translocate inside the cell at high levels of non-esterified cholesterol[24]. In human, NPC1L1 genes are not only expressed in enterocytes of small intestine but also in liver where they are expressed in large amounts. Human hepatocytic NPC1L1 protein is located at the canalicular membrane facilitating the uptake of newly secreted biliary cholesterol and therefore, showing a similar role that intestinal NPC1L1[20]. Transcriptional regulation of NPC1L1 is not yet elucidated but, it seems to be influenced by sterol regulatory element-binding protein or SREBP2 (encoded by SREBF2 gene in humans) that are sensors activating different answers depending on the

intracellular cholesterol concentrations. At low cholesterol levels, SCAP (integral membrane protein) goes along with SREBP2 from endoplasmic reticulum (ER) to Golgi body (GB) for subsequent processing and activation. On contrary, at high or enough cholesterol levels SCAP-SREBP2 complex is retained by INSIG proteins (Insulin induced gene 1 protein located in ER membrane) to avoid SREBP2 maturation impairing its transcription[25]. Moreover, other reports pointed PPARδ (peroxisome-proliferator-activated receptor δ) as another NPC1L1 modulator since down-regulation of the cholesterol transporter has been induced by PPARδ activation in mice[11].

Intracellular non-esterified cholesterol concentrations could also be modulated by ATP-binding cassette (ABC) transporters such as ABCG5/ABCG8 and ABCA1, located respectively at apical and basolateral enterocyte sides. ABCG5 and ABCG8 proteins, expressed in liver and small intestine, are involved in the reverse cholesterol transport (RCT) of sterols, from intracellular environment to lumen. Independent expression of both genes is necessary for the proper function of this heterodimer[26]. Over-expression of ABCG5/8 heterodimer increases non-esterified cholesterol excretion to the lumen reducing its internal concentration and consequently inducing activation of cholesterol synthesis rate[27]. In small intestine, the ABCG5/8 gene expression seems to be regulated by a LXR-dependent member of nuclear receptor family named RXR (retinoid X receptor)[26-28] while in liver, the heterodimer is directly modulated by LXR [30]. Apparently, the latter receptor, along with PPARδ, is also modulator of the ABCA1 expression[10,26]. ABCA1 is a transport protein directly involved in excretion of exceeding non-esterified cholesterol into HDL. LXR agonist administration or high cholesterol concentrations in the cytosol stimulate a direct effect on the transcriptional modulation of these transport proteins although its specific regulatory mechanisms remains still unclear. A protein-protein interaction with another transcription factor affecting the transcription rate of the ABC proteins have been hypothesized [29].

Due to confusions noticed in several publications, it is worthy to define the specific role of two widely mentioned enzymes involved in the transferring of acyl groups within the cholesterol metabolism. Acetyl-Coenzime A transferase (ACAT) and Sterol O-acyltransferase (SOAT) are two enzymes belonging to the acyltransferases family however, they do not catalyze the same reaction (Table 1). ACAT isoforms (ACAT1/ACAT2) are responsible of the reversible formation of acetoacetyl-CoA from two molecules of acetyl-CoA. The ACAT1 and ACAT2 genes are respectively located in chromosome 11 (11q22.3) and chromosome 6 (6q25.3)[30,31]. However, SOAT isoforms (SOAT1/SOAT2) catalyze the formation of fatty acid-cholesterol esters from cholesterol and acyl-CoA molecules and are encoded by two genes located respectively in loci 1q25.2 and 12q13.13[32,33]. Then, since some of the mentioned works along the text wrongly referred to ACAT when it should indicate SOAT, a personal advice (^{AvS} mark) was made pointing attention where required.

Once non-esterified cholesterol reaches the cytoplasm become a substrate of ACAT2$^{(AvS)}$, an integral membrane protein mainly expressed in small intestine and liver. ACAT2$^{(AvS)}$ decreases cytoplasmic amounts of non-esterified cholesterol promoting its esterification and integration into the ER pre-chylomicrons modulating the cholesterol transmembrane absorption rate from intestinal lumen[34]. It also plays an important role maintaining the dynamic equilibrium (homeostasis) between free-cholesterol and esterified-cholesterol[35]. More than 50% of sterols esterification within the enterocytes is carried out by ACAT2$^{(AvS)}$ with a higher affinity for cholesterol esterification rather than other non-cholesterol sterols.

The internal ER triglycerides re-assemblage is carried out by several enzymes such as lysophosphatidate acyltransferase (AGPAT), phosphatidate phosphatase (LPAP), 2-acylglycerol O-acyltransferase 2 (MGAT2) and diacylglycerol O-acyltransferase 1 (DGAT1). Then, esterified-cholesterol together with the triglycerides is packed into pre-chylomicrons by the microsomal triglyceride transfer protein (MTTP) and the apolipoprotein B48 (an isoform derived from APOB gene characteristic of enterocytes). MTTP and APOB48 proteins constitute an active heterodimeric complex linked by ionic interactions with a particular feedback assembly and

secretion system *i.e.* the larger the APOB48 subunit is, the lower binding capacity with MTTP is noticed and less APOB48 is secreted. Therefore, the APOB48-MTTP binding process plays an important role in the lipoprotein biogenesis[36-38].

The combined regulatory effect of NPC1L1, ABCA1, ABCG5/8 and ACAT2$^{(AvS)}$ activities play a critical role in modulating the amount of esterified cholesterol that will be integrated in the pre-chylomicrons with the assistance of the apolipoporotein B48 (APOB48), the microsomal triglyceride transfer protein (MTTP) and the diacylglicerol-o-acyltransferase (DGAT1/2)[39].

Once prechylomicrom structure is assembled, it is further transformed into chylomicron in the GB and excreted by exocytosis into the lymph system through enterocyte basolateral membrane. On the other hand, the non-esterified cholesterol remaining in the cytoplasm could bind to APOA1 protein for a further transport to the lymphatic vessels leading to nascent HDL lipoproteins. Thus, HDL as well as chylomicrons are released free into the blood stream and transported to the liver and peripheral organs such as adrenal glands[11].

2.3. Molecular events occurring during cholesterol synthesis

Total blood cholesterol levels are not only dependent on exogenous cholesterol absorption but also on endogenous cholesterol synthesis. Several tissues are involved in *de novo* cholesterol biosynthesis *i.e.* enterocytes, adrenal glands, ovaries or testicles but, mostly it is generated by hepatic cells. In fact, one of the liver´s main roles is the production of the bile salts from cholesterol as constitutive compounds of biliary fluids needed for the digestion processes while the cholesterol synthesized in adrenals glands or intestine is used respectively as hormone precursor and cholesterolemia modulator[11]. Cholesterol biosynthesis is carried out by a combination of mevanolate and steroid biosynthetic pathways [40](Figure 2).

The 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is considered the key enzyme of cholesterol synthesis although the activities of many enzymes involved in the biosynthetic pathway such as ACAT2, hydroxymethylglutaryl-CoA synthase (HMGCS), Delta24-

196 sterol reductase (DHCR24), farnesyl-diphosphate farnesyltransferase (FDFT1/SQS) or 7-
197 dehydrocholesterol reductase (DHCR7), are also susceptible of modulation. Recently, Gill *et al*.
198 (2011) suggested that squalene monoxygenase (SQLE) might be the second critical modulatory
199 point despite its lower specificity within the cholesterol metabolism compared to HMGCR (after *in*
200 *vitro* experiments)[41].

201 However, other enzymes such as SQS and SQLE are also gaining attention as a potential
202 *stop-point* of cholesterol biosynthesis because SQS it is involved in the transformation of farnesyl
203 pyrophosphate into squalene, being the first specific reaction at the branching point between
204 sterol and non-sterol biosynthesis. SQS transcriptional product and protein are modulated by
205 cholesterol since low levels of this sterol activate the SQS promoter by sterol regulatory element
206 binding proteins (mostly SREBP2). On the contrary, SQS mRNA concentration decreases as
207 response of cholesterol excess. SQLE is a monoxygenase (also named squalene epoxidase) that
208 catalyzes the next step after squalene transformation by SQS, a crucial oxygenation process
209 yielding squalene 2,3-epoxide (Figure 2). Modulation of SQLE activity seems also cholesterol-
210 dependent and apparently regulation is mediated by proteosome activity[41].

211 The HMGCR includes seven domains inserted in ER (endoplasmic reticulum) membrane
212 with an active carboxyl chain located at the cytosol. HMGCR is ubiquitously expressed *i.e.* immune
213 (white blood cells), nervous, muscle, small intestine or reproductive (ovary cells) human tissues[42].
214 HMGCR transcription and degradation depends of a sterol/non-sterol feedback regulation but not
215 directly controlled by cholesterol molecule. Under cholesterol depleted conditions, SREBP-SCAP
216 complex is formed in ER membrane (without any INSIG interaction) then, it is transported to GB
217 where SREBP is activated by proteolytic events facilitating its translocation into the nucleus to
218 activate HMGCR transcription (Figure 3a). On the contrary, in cholesterol exceeding conditions,
219 high amount of oxysterols are synthetized by the mitochondrial sterol 27-hydroxylase (CYP27A)
220 and several mechanisms reducing HMGCR transcriptional rates are activated[43]. The HMGCR

221 regulatory mechanisms could be classified in INSIG-dependent (modulating at transcriptional and
222 post-transcriptional levels), or INSIG-independent.

223 INSIG-dependent HMGCR regulation mechanisms (Figure 3b):

224 - INSIG can disrupt SREBP activation by binding to SCAP when high sterol concentrations are
225 noticed in the cytosol. Once INSIG-SCAP complex is formed, SCAP is structurally altered impairing
226 the SREBP recognition and stopping the assembly of SREBP-SCAP complex for his further transport
227 from ER (endoplasmic reticulum) to GB (Golgi body). In consequence, SREBP is not translocated to
228 the nucleus and HMGCR transcription is inactivated[17,44].

229 - Under similar conditions, INSIG can also binds to the N-terminal region of HMGCR and conjugate
230 it with ubiquitin. Ubiquitation is carried out by gp78 (membrane-bound ubiquitin E3 ligase) assisted
231 by Ubc7 (an E2 ubiquitin conjugating enzyme) providing active ubiquitins and a few other enzymes.
232 Then, ubiquitinated HMGCR is released into the cytosol and subsequently proteosome-degraded
233 with the participation of p97/VCP (ATPase associate to membrane).

234 However, the presence of high levels of sterols is not mandatory for the ubiquitination
235 process but it stimulate HMGCR degradation by enhancing INSIG-HMCR bindings[44].

236 INSIG-independent HMGCR regulation mechanisms (figure 3b):

237 - HMGCR activity could be also modulated in situations of cellular stress (low ATP levels) by
238 AMPkinase (AMP-activated protein kinase). In this case, HMGCR is inactivated by a serine
239 phosphorylation due to the AMPkinase activity. It is a reversible reaction and HMGCR can also be
240 activated by the protein phosphatase 2A (PP2A)[44,45].

241 - Non-sterol isoprenoids might also modulate HMGCR translation by a mechanism still unclear
242 according to Burg *et al*. (2011)[44].

2.4. Molecular events occurring during cholesterol excretion

For several decades, classical reverse cholesterol transport (RCT) was considered the main mechanism to eliminate cholesterol however, recent studies suggest another possible pathway called transintestinal cholesterol excretion (TICE)[17].

RCT is a derivative branch of the hepatobiliary pathway. Lipoproteins such as HDL or LDL make available cholesterol for hepatic absorption as esterified or non-esterified molecules. Esterified cholesterol is transformed by the hepatic cholesteryl ester hydrolase (NCEH1) into the non-esterified form after hydrolysis of the ester linkage. Thus, the generated forms are directly excreted through the ABCG8/5 heterodimer protein or transformed into bile salts[46]. CYP7A1 (cholesterol 7alpha-monooxygenase) is the enzyme responsible for cholesterol transformation into primary bile salts (cholic and chenodeoxycholic acid) within the neutral bile acids pathway in liver. Synthesized bile salts are secreted to bile canaliculus by the bile salt export pump (BSEP) or the multidrug resistance-associated protein 2 (MRP2) and become part of bile fluids. Recent *in vivo* studies have suggested the involvement in RCT of other cholesterol transporters such as NPC1L1[20,47] although its role on biliary cholesterol excretion is not yet elucidated. But, it might involve adjustments in cholesterol balance to avoid excessive loss of the metabolite through the intestinal track.

TICE have been suggested as an alternative cholesterol excretion mechanism where the sterol is directly eliminated from blood through the intestinal mucosa and excreted via feces[17]**.** The hypothesis was drawn after the unexpected results obtained by several authors that noticed an unwarranted balance between cholesterol inputs and outputs in mouse models. They showed a higher amount of fecal cholesterol than the sum of dietary intake and biliary secretion[48] or an unaltered cholesterol excretion rate in knockout NPC1L1 mice with a decreasing of 90% biliary excretion[49].

These results questioned the complete RTC classical concept[50] as well as the contribution of biliary or non-biliary cholesterol to its RCT excretion[51]. Although the involvement of several

membrane transports in TICE have been studied (*i.e.* SR-B1[52], NPC1L1[53], APOA1[54], LDLR or ABCG5/8[55]), it is still unknown whether TICE is carried out through the basolateral or apical transporters or whether HDLs are involved[56] therefore, the mechanism is not yet elucidated. There is also scientific controversy about the TICE importance, some authors suggest that it could be only a compensatory cholesterol excretion mechanism in case of biliary cholesterol depletion but, other authors pointed it as the main mechanism in cholesterol excretion. Van der Velde *et al.* (2007, 2010) estimated TICE contribution as 70 and 30% of total cholesterol excretion respectively in mice and humans[48,56].

2.5. Molecular events occurring during cholesterol transport

HDL, IDL (intermediate-density lipoprotein) , LDL and VLDL (very low-density lipoprotein) are the connecting structures responsible for transporting of cholesterol molecules through the blood stream from one tissue to another until they are detected by cellular membrane receptors such as SR-B1 for HDL or LDLR (LDL-receptor) for both structures.

The non-esterified cholesterol eliminated by ABCA1 through the basolateral membrane of enterocytes is bound to APOA1 generating nascent-HDL and the esterified cholesterol is similarly assembled with APOB48 in ER synthetized pre-chylomicrons further transformed into chylomicrons in GA and excreted by exocytosis to intracellular space. Therefore, both structures become cholesterol transporters and they distribute it via the blood stream to the rest of the organism.

Nascent HDL is transformed into mature HDL by accumulation of non-esterified cholesterol molecules secreted by hepatocyte and enterocyte ABCA1 protein. Once mature HDLs are formed, some cholesterol molecules are esterified by action of lecithin—cholesterol acyltransferase (LCAT) along it blood transport. Esterified and non-esterified cholesterol are detected by the SR-B1 located in the basolateral membrane of cells from liver, small intestine or gland adrenals allowing the incorporation of esterified cholesterol inside the cell. Intracellular esterified cholesterol is transformed into non-esterified cholesterol (by SOAT action) and HDL is turned into LDL. The non-

esterified cholesterol can be used for bile salts synthesis and their further intestinal secretion by BSEP or to be directly excreted via ABCA1 (basolateral membrane) and ABCG5/ABCG8 (apical membrane) activity. In turn, secreted non-esterified cholesterol again could be attached to nascent HDL to create mature HDL and continue with cholesterol transport.

LDL molecules are recognized by liver, intestine and adrenal gland LDLRs as esterified cholesterol suppliers. LDL particles can be generated not only by VLDL transformation (by SR-B1 activity) but also by addition of esterified cholesterol to APOB100 (apolipoprotein isoform characteristic of hepatocytes) leading to very low density lipoproteins (VLDL). These structures are secreted to blood stream by hepatocytes. VLDL lipids are used by the muscle and peripheral tissues as they pass through the blood stream generating IDL and LDL by lipoprotein lipases (LPL) activity. Moreover, esterified cholesterol of chylomicron structures is also recognized by LDLR providing to the hepatocyte those cholesterol molecules assembled in enterocytic ER after digestion process.

The complex cholesterol biosynthesis and absorption enginery and the multifactorial regulation system make the control of cholesterol metabolism a difficult challenge for the scientific community. Particularly because some of the involved compounds are also intermediates of other metabolic pathways *i.e* LXR modulates ABCG5/8 activity but also DIO1, a selenoprotein involved in the thyroid hormone metabolism. However, these facts are also making it a flexible system that could be modulated from different critical points.

3. Fungal molecules modulating cholesterol metabolism

Many food compounds have been described as modulators of cholesterol homeostasis interacting with specific control points and inhibiting or altering cholesterol metabolism; even some *in vivo* studies suggest synergistic hypocholesterolaemic activities between several inhibitors[57-59]. Those compounds showed different targets depending if they modulate cholesterol absorption, synthesis, excretion or transport.

Intake of dietary fibers (DF) from cereals etc., increase cholesterol and bile acids excretion rates lowering their bioavailability because they might act as bile acid scavengers. This mechanism impair bile acids re-absorption stimulating the hepatic synthesis of new bile acids from cholesterol reducing its blood concentration[10,60,61] although for particular β-glucans, modulation of cholesterol-related gene expresions have been also noticed[62].

Plant sterols (phytosterols and phytostanols) are considered direct cholesterol competitors for their incorporation into DMMs because of their structural similarity and due to the limited capacity of DMMs to solubilize lipophilic and water-insoluble molecules[63-65]. Some reports also suggested that phytosterols might modulate SOAT activities although it still remains partially unclear. Cholesterol enterocytic esterification by ACAT$^{(AvS)}$ was decreased by sterols competition although the enzyme showed lower esterification efficiency for plant sterol than cholesterol. Other authors suggested that plant sterols influenced MTTP and APOB48 lipoprotein *in vitro* models[65].

Compounds such as novel amino β-lactams derivates[66] or curcuminoids polyphenols were described as potential NPC1L1 inhibitiors by showing high binding affinity or acting by indirect influence on SREBP1[37]. ABCG5/8 postranscriptional regulations were also described for spironolactones or polyphenols from *Aronia Melanocarpa*[67,68].

Other compounds from natural sources have been reported as ACAT$^{(AvS)}$ inhibitors reducing cholesterol esterification rates such as alkamides from *Piper nigrum*[69], shikonin derivatives from *Lithospermum erythrorhizon*[70], an isoprenyl flavonoid identified as grabol from licorice roots[71], ursolic acid (indirect inhibition via (PPAR)-α activation)[72] or flucoxanthin from marine plants[73].

*3.**1.*** ***Hypocholesterolemic compounds of edible mushrooms***

A few specific bioactive compounds are already pointed as responsible for the hypocholesterolaemic properties of mushrooms. The most extensively investigated are fungal sterols (ergosterol are its derivatives such as ergost-22-ene-1,3-diol, ergosta-5,7-dien-3b-ol, (22E)-ergosta-1,4,6,22-tetraen-3-one, etc.) and β-glucans together with other dietary fibres.

Ergosterol (ergosta-5,7,22-trien-3β-ol) is considered the major fungal sterol (from 53% up to 80% of the fungal sterols, w/w) followed by ergosterol derivatives such as ergosta-5,8,22-trien-3-ol, ergosta-7,22-dien-3-ol, ergosta-5,7dien-3-ol and ergosta-7-en-3-ol (fungisterol)[74]. However, their precise concentration in the different mushroom strains was dependent on environmental conditions (developmental stage, cultivation conditions etc.) or specie. For instance, *Chantharellus cibarius* and *Craterellus cornucopioides* showed almost exclusively ergosterol, while other species showed more ergosta-5,7-dienol than fungisterol (*Lyophyllum shimeji or Pleurotus ostreatus*). *Flammulina velutipes* contained high amounts of ergosta 5,8,22-trien3β-ol[10].

Apparently, the hypocholesterolaemic activities of fungal sterols were mainly due to their structural similarity with cholesterol as noticed for plant sterols. Therefore, these compounds were able to hinder cholesterol incorporation into DMMs as phytosterols and phytostanols. Ergosterol-enriched fractions obtained using green technologies such as supercritical fluids extraction (SFE), were more effective than β-sitosterol in displacing cholesterol from DMMs during an *in vitro* digestion model[59]. Moreover, fungal sterol-enriched extracts also showed HMGCR inhibitory activities *in vitro*[75] and ergosterol was also considered as a competitive inhibitor of C24-reductase due to its double bond at C-22 in the side chain of its structure (as also noticed for stigmasterol and brasicasterol). The C24-reductase is another enzyme involved in the cholesterol biosynthetic pathway down-stream from HMGCR[76].

Fungal β-glucans showed different molecular structures than cereal polysaccharides. Their branching profiles are (1→3) and (1→3)(1→6) conferring them many biological activities such as antitumoral, antioxidant, immunomodulatory etc. despite hypocholesterolaemic properties[10,77,78]. Some reports suggested that those bioactive properties are due to their different tridimensional configuration depending on their monomer composition, branching degree or conformation while other authors considered their water solubility (that is also partially dependent on their glycosidic linkages) a more relevant factor, considering those water-insoluble as the responsible for the cholesterol lowering activities by impairing cholesterol absorption. Moreover, other publications

370 pointed β-glucans degradation products (generated *i.e.* after a digestion process) as
371 hypocholesterolemic molecules more bioactive than their larger precursors[10,79]. However, until
372 now the precise structural requirements for the observed hypocholesterolemic action of fungal β-
373 glucans remains unclear awaiting further studies.

374 β-Glucans from a large number of mushroom species have been studied such as lentinan from
375 shiitake mushrooms (*Lentinula edodes),* schizophyllan *from Schizophyllum commune*, grifolan from
376 *Grifola frondosa*, and many others from *Agaricus blazei*, *Ganoderma lucidum* (a medicinal
377 mushroom consumed as nutraceutical or dietary supplement), *P. ostreatus* as well as the (1→3) β-
378 glucans and glucuronoxylomannans from *Auricularia polytricha* and *Tremella fuciformis*[80,81]. They
379 all showed different conformations, solubility and therefore biological properties.

380 Chitin, a special type of water-insoluble β-(1→4)-glucan including N-acetylglucosamine
381 monomers, is also considered an hypocholesterolaemic polysaccharide while its derivative, named
382 chitosan, was more studied because of its antitumor and immunomodulation activities[10,77]. Several
383 polysaccharide fractions (containing high percentages of fungal β-, α-glucans and
384 chitooligosacharides among others) from *L. edodes*, *P. ostreatus* and *A. bisporus* extracted by using
385 pressurized technologies (PSE) showed bile acid binding capacities *in vitro* similar to those shown
386 by cereals fibers[82] indicating that might impair cholesterol absorption as previously suggested for
387 plant β-glucans.

388 According to Gunde-Cimerman *et al.* (1993, 1995) suggestions, lovastatin an inhibitor of
389 HMGCR (3-hydroxy-3methylglutaryl-Coenzime A reductase), was naturally present in some
390 *Pleurotus spp.* fruiting bodies such as *P. ostreatus* or *P. eryngii*[83,84] and in other species such as *A.*
391 *bisporus* or *B. edulis*. However, recent studies did not detect any statin in mushroom species
392 showing interesting HMGCR inhibitory activity pointing to some water soluble polysaccharides and
393 proteoglucans as the responsible for the noticed *in vitro* inhibition[10,75].

394 HMGCR inhibition was also mediated by an AMP kinase via phosphorylation and green and
395 black tea polyphenols induced a direct increase of HMGCR phosphorylation possibly via AMP

kinases phosphorylation. Their precise mechanism of action is still unclear but, it seems to involve activation of regulatory factors such as PPAR[85,86].

Only a few compounds are still nowadays pointed as potential inhibitors of the SQS such as resveratrol from wine[87]as well as zaragozic acids isolated from the liquid broth of certain ascomicetes[100].

24(S), 25-epoxycholesterol was also pointed as inhibitor of the DHCR24 activity. This enzyme catalyze the transformation of desmosterol into cholesterol. The inhibitor did not modify DHCR24 protein levels, but increased desmosterol accumulation decreasing cholesterol levels in *in vitro* studies due to its structural similarity with desmosterol[88].

3.2 Molecular events modulated by mushroom extracts

The presence of certain natural molecules in mushrooms do not only modify cholesterol absorption or metabolic pathway of consumers but can also modulate the expression of some genes related to cholesterol homeostasis. Recent studies carried out on particular edible mushrooms studied their influence as attempt to further identify the most interesting fungal compounds to treat moderate hypercholesterolemia.

Mushrooms such as *Pleurotus ostreatus, Grifola frondosa* and *Hypsizigus marmoreus* were able of differently modulating the gene expression patterns of mice livers fed with each mushroom (10-14%) for 4 weeks. Triglyceride levels in liver and plasma decreased in the mice fed with *P. ostreatus* compared with those in the control group. Moreover, liver cholesterol decreased while plasma total cholesterol increased probably due to HDL values that were also increased. Cholesterol in the liver was lower in the group fed with *G. grondosa* than in the control group but no changes were found in the *H. Marmoreus*-fed group. DNA microarrays analysis of the livers revealed that CTP1A and FABP families were upregulated in the *P. ostreatus*-fed group, which were considered to promote lipid transport and β-oxidation. In the *G. frondosa*-fed group, not only the gene involved in signal transduction of innate immunity via TLR3 and interferon but also virus resistance genes (such as MX1, RSAD2 and OAS1) were upregulated[89].

Administration of *Agaricus brasiliensis* (also known as *Agaricus blazei*) to hypercholesterolemic albino Fischer rats during 6 weeks lowered cholesterol levels in serum and induced significant changes in the expression of cholesterol-related genes. HMGCR mRNA expression was not influenced but LDLR upregulation was noticed together with upregulation of CYP7A1, the rate-limiting enzyme for bile acid synthesis, and mRNA levels of the ABCG5/8 carriers. These increases were accompanied by a significant increase in the content of cholesterol excreted in the faces and by a concomitant increase in NR1H3 (LXR) mRNA levels. However, in this case PPAR-α was not significantly upregulated compared with levels noticed in the hypercholesterolemic control group[90].

In a few reports specific extracts and not the whole fruiting body were studied. For instance, ethanol (95%) extracts obtained from *P. ostreatus* were administrated to hypercholesterolemic Male Wistar rats during 14 days and a reduction of triglycerides and HDL cholesterol was noticed in plasma. Moreover, the extract was able of up regulate the genes that were downregulated after hypercholesterolemia induction such as those related to fatty acids oxidation such as acyl CoA oxidase (ACO) and synthetase (ACS) together with carnityl palmityl transferase-1 (CPT-1) and PPAR-α. They also downregulated genes related to the fatty acids biosynthesis and cholesterol metabolism (FAS fatty acid synthase, SREBF1, APOC3) to similar expression than during normocholesterolemia[91].

Hericium erinaceus hot water and ethanol extracts were administrated together with a high-fat diet to C57BL76J mice for 4 weeks. Incorporation of the extracts in the diet resulted in a significant decrease in body weight, fat and serum and hepatic triglicerides levels compared to control. The ethanol extract was acting as an agonist of PPAR-α since it was able of up-regulating mRNAs usually modulated by PPAR-α (ACAT, ApoA1, LPL or SREBP1) in spite of the fact that the PPAR-α expression itself did not change[92].

An extract obtained from cauliflower mushroom (*Sparassis crispa*) significantly enhanced hepatic cholesterol catabolism when administrated to Male Sprague-Dawley rats fed

448 a cholesterol-rich diet for 4 weeks because they were able of up-regulating CYP7A1 mRNA
449 expression concomitant with HMGCR downregulation after. Additionally, the extract
450 supplementation resulted in cholesterol and bile acid fecal excretion[93].

451 ***3.3 Molecular events modulated by fungal sterols***

452 Since plant and fungal sterols share structural similarities, similar modulation of cholesterol
453 related genes might also be expected. However, in the few studies carried out using fungal sterols
454 different effects were noticed.

455 Ergosterol was able of regulating sterol regulatory element binding protein (SREBP)
456 cleavage in yeast as response to cellular oxygen levels[94] suggesting that it might also modulate
457 these elements in mammalians. Studies carried out using cell cultures revealed that DMMs isolated
458 from *in vitro* digestion of ergosterol and extracts containing ergosterol mixed with cholesterol were
459 not influencing transcriptional levels of SREBF1 differently than when only cholesterol was
460 administrated. Moreover, only a slight inhibition was noticed for SREBF2 mRNA of Caco2 cells when
461 treated with an ergosterol-containing extract but not with ergosterol itself. However, other
462 cholesterol related genes were also overexpressed such as the LDLR (when an ergosterol-enriched
463 extract from *Agaricus bisporus was applied*)[15]. When the lower compartment of the Caco2 cells
464 supplemented with the sterol-containing digested extracts were added to HepG2 cells, higher
465 modulation of genes related to the lipid metabolism was noticed than those more directly related
466 to the cholesterol homeostasis such as DGAT2 upregulation.

467 When similar extracts were given for 4 weeks to C57BL/6JRj mice previously fed for 4
468 weeks with hypercholesterolemic diets, inhibition of SREBF2 and NR1H4 (gene enconding FXR)
469 were noticed in jejunum up to similar levels than hypocholesterolemic drugs such as ezetimibe and
470 simvastatin[15]. This downregulation was due to their ergosterol content because supplementation
471 with purified ergosterol induced similar modulation. FXR is the farnesoid X receptor, a nuclear
472 receptor also involved in the regulation of cholesterol homeostasis[95]. FXR is involved in the
473 activation or inhibition of bile acids synthesis and transport acting as a sensor of their

concentration[96]. The extract induced downregulation of DGAT2 in liver as observed in cell cultures but upregulation of FDFT1, might be to compensate for the reduction on the ratio TC/HDL recorded. Reduction on the triglycerides levels were also noticed in liver, perhaps because of the observed modulation on DGAT2 gene transcription. Moreover, this extract induced overexpression of DIO1 mRNA in jejunum, the tissue where cholesterol absorption is higher[97]. Type I iodothyronine deiodinase is a selenoprotein encoded by DIO1 gene and play a major role in normal thyroid function, but it is also indirectly influencing cholesterol homeostasis by regulating the LDLR gene expression. Increased DIO1 activity in liver correlated with up-regulation of the LDLR mRNA and lowering of TC, TG and LDL levels in serum[15]. However, no significant effect was noticed by Gil-Ramirez *et al.*, 2015[97] on the latter gene, perhaps the supplemented concentration was sufficient to stimulate DIO1 but it did not revert on LDLR readjustments. On the contrary, ergosterol administrated as single compound induced downregulation of DIO1 mRNA therefore the effect of the ergosterol enriched extract could be due to some other sterol or compound present in the extract[97].

However, when the ergosterol containing extracts were incorporated into lard and the mixture supplemented together with the diet to induce hypercholesterolemia, insignificant effects were noticed on any SREBP, other cholesterol regulatory nuclear receptor or genes involved in the cholesterol metabolism except for ABCG5 and 8 mRNAs expression in cecum that were up-regulated. Perhaps the addition of the extract as ingredient inside a lipid matrix prevented their proper bioavailability (because it was also unable of lowering cholesterol levels in serum, HDLc or LDLc) or perhaps the beneficial properties of ergosterol were not sufficient to overcome the detrimental effect of lard and the hypercholesterolemic diet when administrated simultaneously[98].

Transcription of some ABC transporters (ABCG5/8 etc.) was induced by LXR factor in enterocytes (but not in hepatocytes)[65] although, there is a controversy about its influence on other transporters (ABCA1). Oxysterols such as 22(R)-hydroxycholesterol, 24(S),25-epoxycholesterol or 27-hydroxycholesterol etc., are considered as endogenous natural LXR agonist however, plant

500 sterols derivatives showed higher LXR agonist activity. Brasicasterols from unicellular algae and
501 *Brassiceas* sp (*i.e.* rapeseed) induced large variations in the gene expression of ABC transporters
502 due to their ability to act as the LXR factor in mice. Moreover, sitostanol (in mice), sitosterol (in
503 Caco2 cells) and a few 4-desmethylsterol derivatives were also able of inducing ABCA1 up-
504 regulation using their LXR agonist activity[5,11,13,65]. However, no direct effects of phytosterols or
505 fungal sterols were noticed on the transcriptional levels of the transporters because on the one
506 hand, in ABCA1 and ABCG5/8-deficient mice decreasing of cholesterol intestinal absorption was
507 also noticed after phytosterol administration indicating that ABC transporters were not their direct
508 targets. On the other hand, no significant overexpression or repression was neither noticed in
509 hypercholesterolemic C57BL/6JRj mice treated with ergosterol-containing extracts[15].

510 Other reports indicated that phytosterol/stanols modulated HMGCR expression via
511 $ACAT^{(AvS)}$ inhibition. Apparently, lower $ACAT^{(AvS)}$ activity led to higher free cholesterol amounts,
512 inhibiting the cholesterol biosynthetic pathway and HMGCR expression beside others. It also
513 reduced chylomicron assembling, and promoted the back efflux of non-esterified sterols to the
514 lumen[11,65,99]. However, their influence on SOAT seemed to be by chemical inhibition more than by
515 molecular modulation. Direct down-regulation of HMGCR mRNA (together with inhibition of the
516 enzyme) was only noticed in organic extracts obtained from the Reishi mushroom (*Ganoderma*
517 *lucidum*) containing oxygenated lanosterol derivatives. They also inhibited cholesterol synthesis in
518 T9A4 hepatocytes and reduced total cholesterol in hamsters[100].

519 Several *in vitro* studies pointed out that SREBP2, NPC1L1 and SR-B1 gene expression was
520 modulated by plant sterols such as stigmasterol and β-sitosterol toward the reduction of
521 cholesterol absorption. Surprisingly, HepG2 cells treated with these sterols showed simultaneous
522 down-regulation of NPC1L1 and SR-B1 when an opposite effect on these two molecules could be
523 expected. This modulation was not noticed when fungal sterols were applied to the hepatic cells
524 [15] neither when administrated to mice together with a hypercholesterolemic diet[98]. Studies using
525 homozygous and heterozygous knockout mice ($NPC1L1^{-/-}$ and $NPC1L1^{+/-}$) showed a lower

cholesterol absorption in homozygous animals however, heterozygous mice showed higher HMGCR mRNA levels in gut and liver tissues than wild-type animals without changes in ABC transporters expression rates. Authors explained their results by compensatory effects: difficulties for cholesterol absorption were compensated by a stimulation of endogenous cholesterol synthesis to maintain physiological plasma levels[101,102]. Moreover, when the knockout mice were treated with plant sterols or ezetimibe, the wild-type and heterozygous animals showed a reduction in cholesterol and TG levels. Absorption of campesterol and β-sitosterol was reduced in NPC1L1$^{+/-}$ and almost absent in NPC1L1$^{-/-}$ mice indicating that certain modulation of NPC1L1 took place[101,102]. However, no modulation of NPC1L1 expresion was noticed in C57BL/6 mice fed a fungal sterol extract together with a hypercholesterolemic diet[98].

Moreover, β-Sitosterol addition to Caco2 cultures induced down-regulation of HMGCR expression although this effect was not noticed in mice. Only sitosterolemic individuals showed reduction in HMGCR activity in ileum so apparently only large amounts of plan sterols or long-term 2% (w/w) plant sterol administration can induce such effect[11,103].

Other reports indicated that phytosterols might modulate the expression of other closely related genes such as those encoding the hepatic cholesterelogenic farnesyl phosphate synthase (FFPS), liver CYP7A1 (cytochrome P450 family 7 subfamily A polypeptide 1) or anexin 2-Caveolin 1 (ANXA2-CAV1) protein complex[11]. Therefore, fungal sterols might be involved in the modulation of CYP7A1 noticed during the administration of the complete mushroom fruiting bodies to hypercholesterolemic mice[90].

3.4 Molecular events modulated by fungal polysaccharides

At the present, not many studies have been carried out concerning the molecular effect of dietary fibers independly of their plant or fungal origen. However, already in 1996, Cheung reports suggested that fungal polysaccharides extracts could modulate cholesterol related enzymes activity (HMGCR) by several mechanisms and not only via direct inhibition. Later on, several

551 studies were carried out to investigate the molecular role of fungal polysaccharides on cholesterol
552 metabolism[78].

553 Fukushima *et al.* (2000)[104] examined the effects of a fiber extract obtained from *Agaricus*
554 *bisporus* on the LDLR mRNA expression after feeding rats with cholesterol-free diets during 4
555 weeks. Despite a higher relative liver weight in control group (cellulose supplementation)
556 comparing with those animals fed with the mushroom fiber extract as supplement, serum total
557 cholesterol, VLDL and LDL concentrations in the control group were significantly greater than
558 animals fed the *A. bisporus* fiber. Added to this, once LDLR mRNA hepatic levels were analyzed,
559 results showed that mushroom LDLR up-regulation was significantly higher than that induced by
560 cellulose fiber supplement[104]. One year later, the same group reported that maitake (*Grifola*
561 *frondosa*) and enokitake (*Flammulina velutipes*) fiber extracts lowered total serum cholesterol
562 levels by two mechanisms, by scavenging cholesterol molecules inducing their fecal excretion and
563 by enhancement of LDLR mRNA expression in rat liver[105]. However, no up-regulation was
564 significantly noticed when rats were fed shiitake (*Lentinula edodes*) fiber extracts.

565 Dietary fibres fractions from *P. ostreatus*, *L. edodes* and *A. bisporus* fruiting bodies obtained
566 as described by Jeurink *et al.* (2008)[106] including a low α-glucans amount and high concentrations
567 of chitin derivatives and other β-glucans[82] were also added to Caco2 cells to study their effect on
568 the most interesting cholesterol-related genes. The transcriptomic profile was studied after 1 and
569 24h application and results indicated that the fiber extracts obtained from *P. ostreatus* were able
570 of modulating transcription of more genes related to the cholesterol metabolism than the other
571 two mushroom studied at longer incubation times. Up-regulation of FDFT1 and NPC1L1 was
572 noticed together with slight modulation of a few others mRNAs therefore, *in vivo* experiments
573 were carried out using *P. ostreatus* fiber extract using two different experimental settings. In the
574 first one, C57BL/6J mice were firstly administrated hypercholesterolemic diets and then,
575 supplemented with the fiber extract (as palliative treatment) and in the second experiment, mice
576 were simultaneously fed with hypercholesterolemic diet plus fiber extract (preventive treatment).

577 Results indicated that their molecular responses were completely dependent on the
578 supplementation setting. In the palliative setting, administration of the fiber extract reduced
579 hepatic triglyceride levels and it might be because of the DGAT1 downregulation also recorded. In
580 the preventive setting, the fiber extract modulated cholesterol-related genes expression similarly
581 to simvastatin and ezetimibe in liver (*i.e.* by inhibiting FDFT1, NR1H3 (LXR) and NR1H4 (FXR) mRNA
582 expression) although no changes in plasma and liver biochemical data was recorded[107]. Later on,
583 when similar extract was applied in a higher concentration and mixed with lard, the
584 hypocholesterolemic effect was noticed (as reduction of TC and LDLc levels) but no relevant
585 modulation of cholesterol-related gene transcription was noticed despite overexpression of
586 NPC1L1 in mice liver and jejunum[98].

587 The molecular modulations noticed for fungal β-glucans seemed to be different than other
588 dietary fibers obtained from plants *i.e.* high viscosity oat or barley β-glucan extracts demonstrated
589 their ability to down-regulate SREBF2 gene expression in intestinal cells (NCl-H716) [62] but no with
590 *in vivo* testing. However, according to Hu, Wang & Xu (2008) corn bran dietary fiber up-regulated
591 the expression of other genes such as FXR in ileal cells or PPAR in liver[108].

592 Hepatic HMGCR up-regulation was observed after administration of hydroxylpropylmethycellulose
593 or inulin-oligofructose (1:1) to animal models[109,110] however, barley β-glucans administration
594 induced no changes on HMGCR expression[111]. Jones (2008)[111] noticed hepatic SREBF2 up-
595 regulation when soluble dietary fiber (guar gum, a galactomannan) was administrated to pigs. In
596 consequence, LDLR expression was enhanced and reduced the LDLs from the blood stream. Fungal
597 fibers instead seemed to modulate directly LDLR mRNA expression[104,105]. Hepatic ABCG5/8 gene
598 expression was also enhanced by guar gum consumption leading a higher cholesterol efflux from
599 the liver to intestinal lumen[111]. A similar enhancement together with ABCA1 mRNA was noticed in
600 jejunum from mice administrated *P. ostreatus* fibers (palliative treatment) but without significant
601 cholesterol reduction[107].

602 Other studies indicated that the scavenging of bile acids by plant fiber (β-glucans) activated
603 CYP7A1 to convert cholesterol into new bile acids[108,112] provoking an hepatic cholesterol decrease
604 that up-regulated LDLR expression and reduced cholesterol blood levels. Due to these changes, the
605 biosynthetic pathway was also activated via HMGCR up-regulation to compensate the lack of
606 hepatic cholesterol[112,113]. However, the effect of fungal fibers on CYP7A1 is still at the present
607 unknown.

608 Other studies were carried out using extracts containing the water soluble
609 polysaccharides from *L. edodes*. This fraction contained α- and β-glucans and fucomannogalactans
610 that when digested (*in vitro*) were applied to Caco2 cell cultures and no significant effect was
611 noticed on the modulation of cholesterol-related gene expression. But, when the lower
612 compartment of the cell monolayer was applied to HepG2 modulation of some mRNAs were
613 noticed after 24h incubation. However, the modulatory pattern fitted more with a low cholesterol
614 level response since enzymes such as HMGCR, FDFT1 and ACAT1 were overexpressed, perhaps
615 the HMGCR inhibitory activity showed by this extract was inducing a posttranscriptional
616 cholesterol reduction that activated the biosynthetic pathway after 24h[114]. Later on, when this
617 extract was administrated to normocholesterolemic mice for 4 weeks similar overexpression of
618 HMGCR (in liver) and FDFT1 (in jejunum) were noticed. However, when the extract was
619 administrated to hypercholesterolemic mice the modulation profile showed similarities with
620 those generated after administration of ezetimibe, simvastatin or both such as for instance down-
621 regulation of FDFT1 (in ileum), SREBF2 or NR1H4 (in jejunum) or up-regulation of ABCG8 or ACAT1
622 in jejunum.

623 Other polysaccharides extracted from 3 different strains of *Pleurotus tuber-regium*, when
624 administrated to obese-diabetic rats for 8 weeks, also showed and hypolipidemic effect that was
625 associated with up-regulated liver PPAR-alpha mRNA expression and protein levels. Moreover,
626 hyperglycemia was aslo attenuated by the polysaccharides, the elevated serum total cholesterol,
627 triglycerides and low-density lipoprotein (LDL) concentrations were controlled, and parallel

restoration of decreased high-density lipoprotein (HDL) levels were noticed after their supplementation[115].

3.5 Molecular events modulated by other fungal compounds

An adenosine analogue alkaloid (eritadenine) is another compound from *L. edodes* able of inhibiting the S-adenosylhomocysteine hydrolase, a key enzyme for the hepatic phospholipid metabolism. This inhibition could be related to the lowering of cholesterol levels in serum noticed in animal studies[116,117]. Eritadenine increases the hepatic microsomal phosphatidylethanolamine (PE) concentration and decreases the liver microsomal Δ6-desaturase activity, altering the fatty acid and molecular species profile in liver and plasma. When it was administrated to rats, suppression of Δ6-desaturase activity was accompanied by a significant reduction in the abundance of mRNA for the enzyme suggesting that dietary eritadenine might suppress the activity of liver microsomal Δ6-desaturase by altering the microsomal phospholipid profile and this effect was mediated by the regulation of the enzyme transcription[118]. Eritadenine was also involved in the up-regulation of the CYP7A1 expression noticed in liver of hypercholesterolemic mice fed the standard compound or 5, 10 or 20 % *L. edodes*[119].

Water extracts obtained by PWE (pressurized water extractions) from normal and selenium-enriched *A. bisporus* were tested *in vitro* as HMGCR inhibitors. Selenium supplementation enhanced the inhibitory activity of statins and therefore, the latter extracts might improve their HMGCR inhibitory capacity. However, no significant differences were found, they both similarly inhibited the enzyme only if the extracts were not thermally treated. When the extracts were applied to HepG2 cells to study their effect at the molecular level they also induced similar responses in most of the cholesterol-related genes. However, after 1h application overexpression of LDLR mRNA was noticed in non Se-fortified extracts. After 24h the LDLR mRNA was downregulated together with the FDFT1. Similar downregulation of the SQS mRNA was also noticed in Se-fortified extracts indicating that non-thermal water extracts from *A. bisporus* could inhibit transcription of this enzyme that is also a key enzyme downstream the cholesterol

metabolic pathway[120]. Reduced expression of GPX3 (glutathione peroxidase, a selenoprotein largely influenced by the presence of selenium in the media) was shown to increase cell-mediated oxidation of LDL and selenium supplementation (1 ppm) also induced downregulation of APOB and HMGCR expression during hypercholesterolemia in rat models. However, no significant modulation of the GPX3[97] and APOB[120] expression was noticed neither in non- or Se-fortified extracts only a slight up-regulation of HMGCR after 1h for non-fortified extracts and after 24h for Se-fortified ones perhaps to compensate the inhibition of the FDFT1 transcription noticed[120].

4 Conclusion

Consumption of edible mushrooms or functional foods containing specific fungal extracts should be encouraged within people with low to moderate hypercholesterolemia (before the use of pharmaceutical drugs) to lower their cholesterol levels in serum. As noticed for plants, cereals and other food derivatives, they contain specific compounds that can modulate cholesterol homeostasis via different transcriptional and post-traslational mechanisms that are nowadays, not completely understood but that they could be different than those described for plants. Thus, more studies are necessary to broad the knowledge toward the molecular effect of fungal compounds on human health since the results obtained up to now are promising, suggesting that their use as hypocholesterolemic foods might be more effective than those products actually offered in the markets.

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Figure 1: Cholesterol and fat digestion and absorption pathway. PL: phospholipids, FA: fatty acids, BA: bile acids, TAG: triacylglycerols, CL: cholesterol, LPA: lysophosphatidic acid, MAG: monoacylglycerols, LPA: lysophosphatidic acid, CE: cholesterol esters, ABCG5: ATP-binding cassette subfamily G member 5, ABCG8: ATP-binding cassette subfamily G member 8, ABCA1: ATP-binding cassette subfamily A member 1, ACAT2: Acetyl-CoA Acetyltransferase 2, FABPpm: Plasma membrane fatty acid-binding protein, NPC1L1: Niemann-Pick C1-like protein, SRB1: Scavenger receptor class B member 1, AGPAT: 1-acylglycerol-3-phosphate O-acyltransferase, LPAT: lysophosphatidate acyltransferase , MGAT2: monoacylglycerol acyltransferase-2, DGAT1: diacylglycerol O-acyltransferase 1, MTTP: microsomal triglyceride transfer protein large subunit, APOB48: apolipoprotein B-48, APOA1: apolipoprotein A-1, ER: endoplasmatic reticle, GB: Golgi body, PCM: pre-chylomicron; CM: chylomicron.

Figure 2: Cholesterol biosynthetic pathway from glycolysis product, Acetyl coenzyme A and Acetoacetyl coenzyme A. Ac-CoA: Acetyl coenzyme A; AcAc-CoA: Acetoacetyl coenzyme A; CoA: coenzyme A; HMG-CoA: 3-Hydroxy-3-methylglutaryl-CoA; A: Cholesta-7,24-dien-3β-ol; B: Cholesta-8-en-3β-ol; C: 7-Dehydro-desmosterol; D: Lathosterol; E: Desmosterol; F: 7-Dehydrocholesterol; ACAT2: acetyl-CoA acetyltransferase 2; HMGCS: hydroxymethylglutaryl-CoA synthase; HMGCR: 3-hydroxy-3-methylglutaryl coenzyme A reductase; SQS: farnesyl-diphosphate farnesyltransferase or squalene synthetase; SQLE: squalene monooxygenase; DHCR24: delta24-sterol reductase; DHCR7: 7-dehydrocholesterol reductase; SOAT: Sterol O-acyltransferase.

Figure 3: Molecular regulation pathway of HMGCR transcription under low (a) and high (b) intracellular cholesterol levels. INSIG: Insulin induced gene 1 protein; SREBP: Sterol regulatory element-binding protein; SCAP: SREBP cleavage-activating protein; COP-II: Coat complex protein II; SPH2: Sphingosine kinase II; TMUB1: Transmembrane and ubiquitin like domain containing 1; gp78: Membrane-bound ubiquitin E3 ligase; Ubc2: Ubiquitin-conjugating enzyme E2 2; HMGCR: 3-

Hydroxy-3-methylglutaryl-CoA reductase; SRE:Sterol regulatory element; RXR: retinoic X receptor; LXR: liver X receptor; ABCA1: ATP-binding cassette subfamily A member 1; ABCG5/8: ATP-binding cassette subfamily G member 5/8; SOAT: Sterol O-acyltransferase; UBQ: Ubiquitin; AMPK: AMP-activated protein kinase; PP2A: Phosphatase 2A.

Figure 1

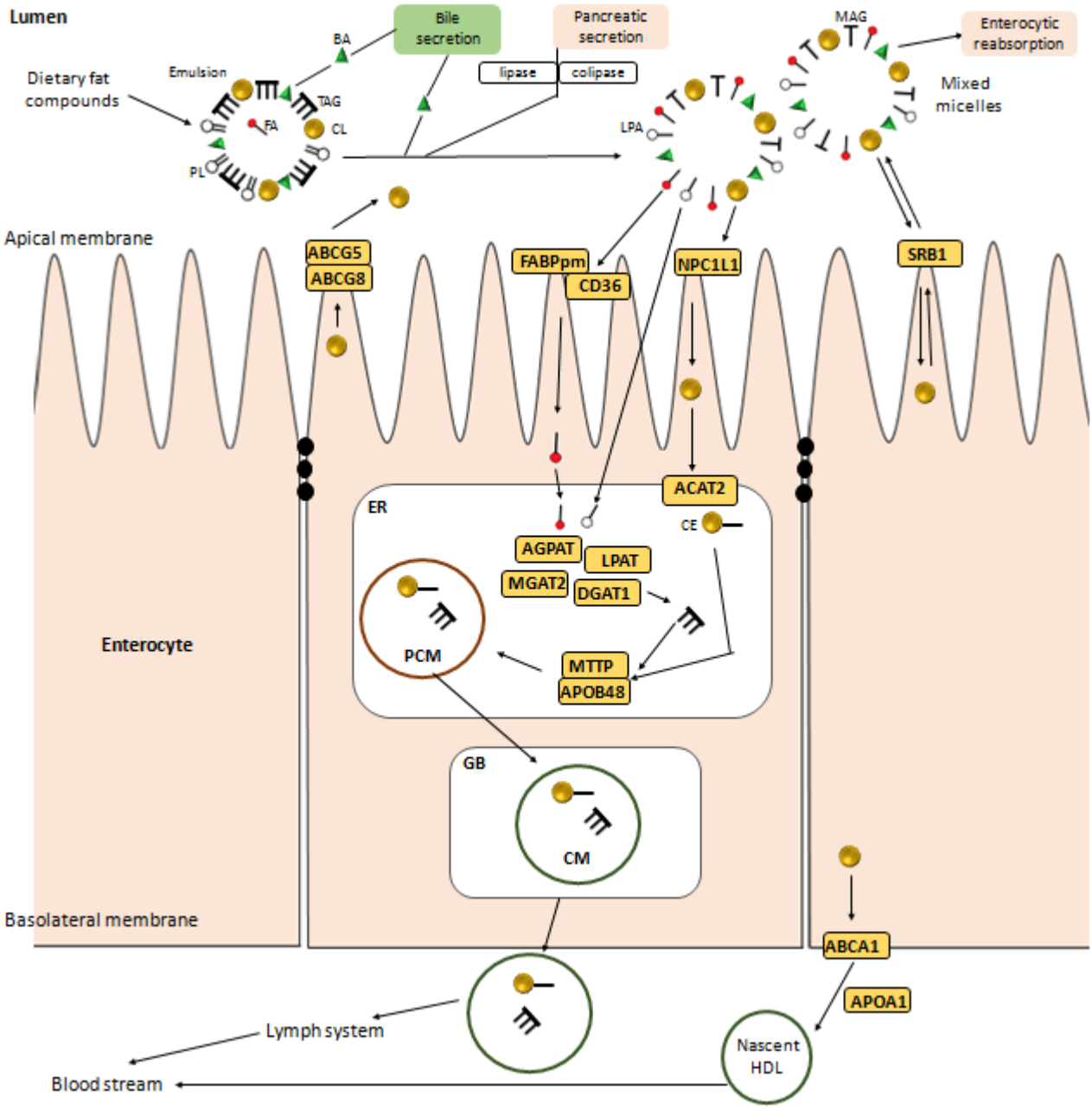

Lumen
Bile secretion
Pancreatic secretion
BA
Dietary fat compounds
Emulsion
TAG
FA
CL
PL
lipase
colipase
LPA
MAG
Enterocytic reabsorption
Mixed micelles
Apical membrane
ABCG5
ABCG8
FABPpm
CD36
NPC1L1
SRB1
ACAT2
ER
CE
AGPAT
LPAT
MGAT2
DGAT1
Enterocyte
PCM
MTTP
APOB48
GB
CM
Basolateral membrane
ABCA1
APOA1
Lymph system
Nascent HDL
Blood stream

Figure 2

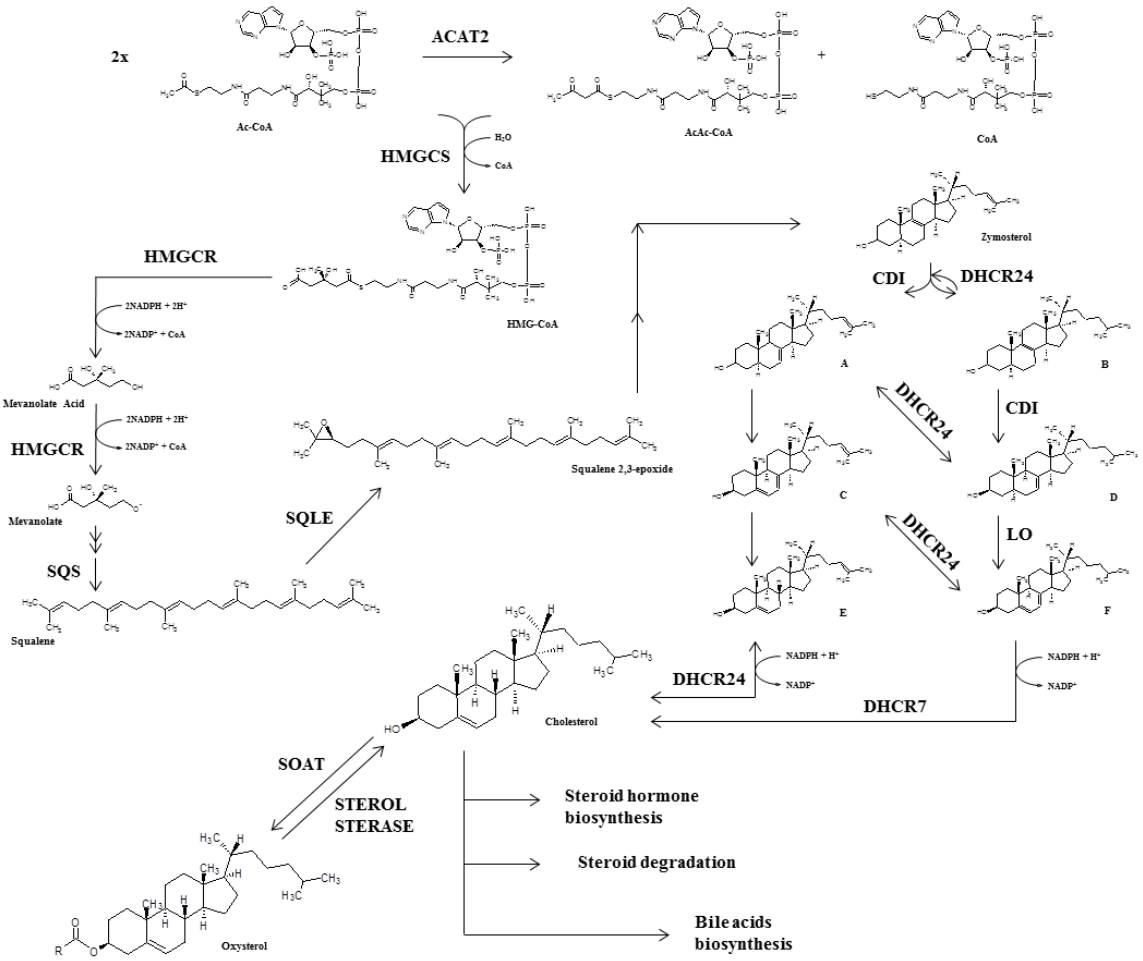

2x
ACAT2
Ac-CoA
AcAc-CoA
CoA
HMGCS
H2O
CoA
HMG-CoA
HMGCR
2NADPH + 2H+
2NADP+ + CoA
Mevanolate Acid
HMGCR
2NADPH + 2H+
2NADP+ + CoA
Mevanolate
SQS
Squalene
SQLE
Squalene 2,3-epoxide
Zymosterol
CDI
DHCR24
A
B
DHCR24
CDI
C
D
DHCR24
LO
E
F
NADPH + H+
NADP+
DHCR24
NADPH + H+
NADP+
DHCR7
Cholesterol
SOAT
STEROL
STERASE
Oxysterol
Steroid hormone biosynthesis
Steroid degradation
Bile acids biosynthesis

Figure 3a

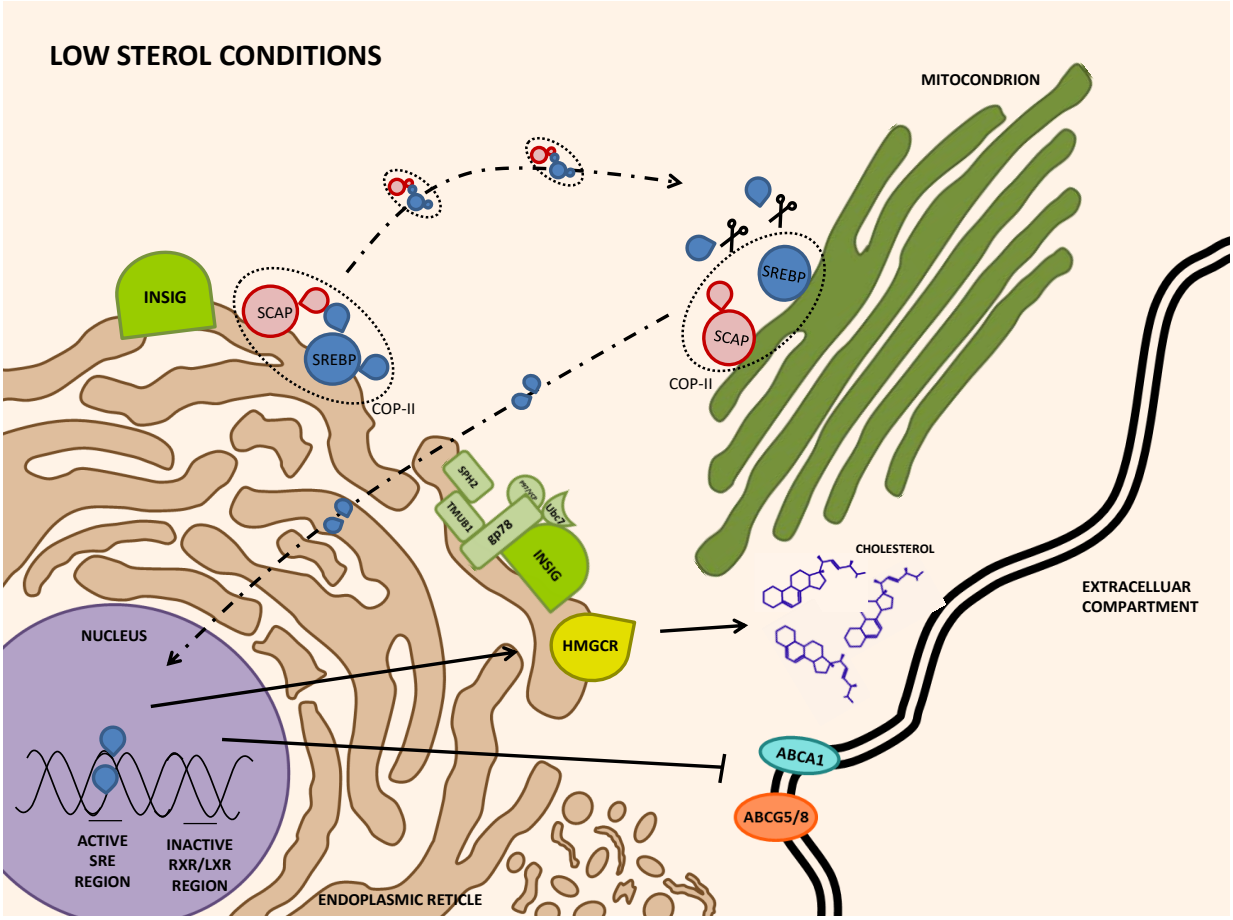


Figure 3b

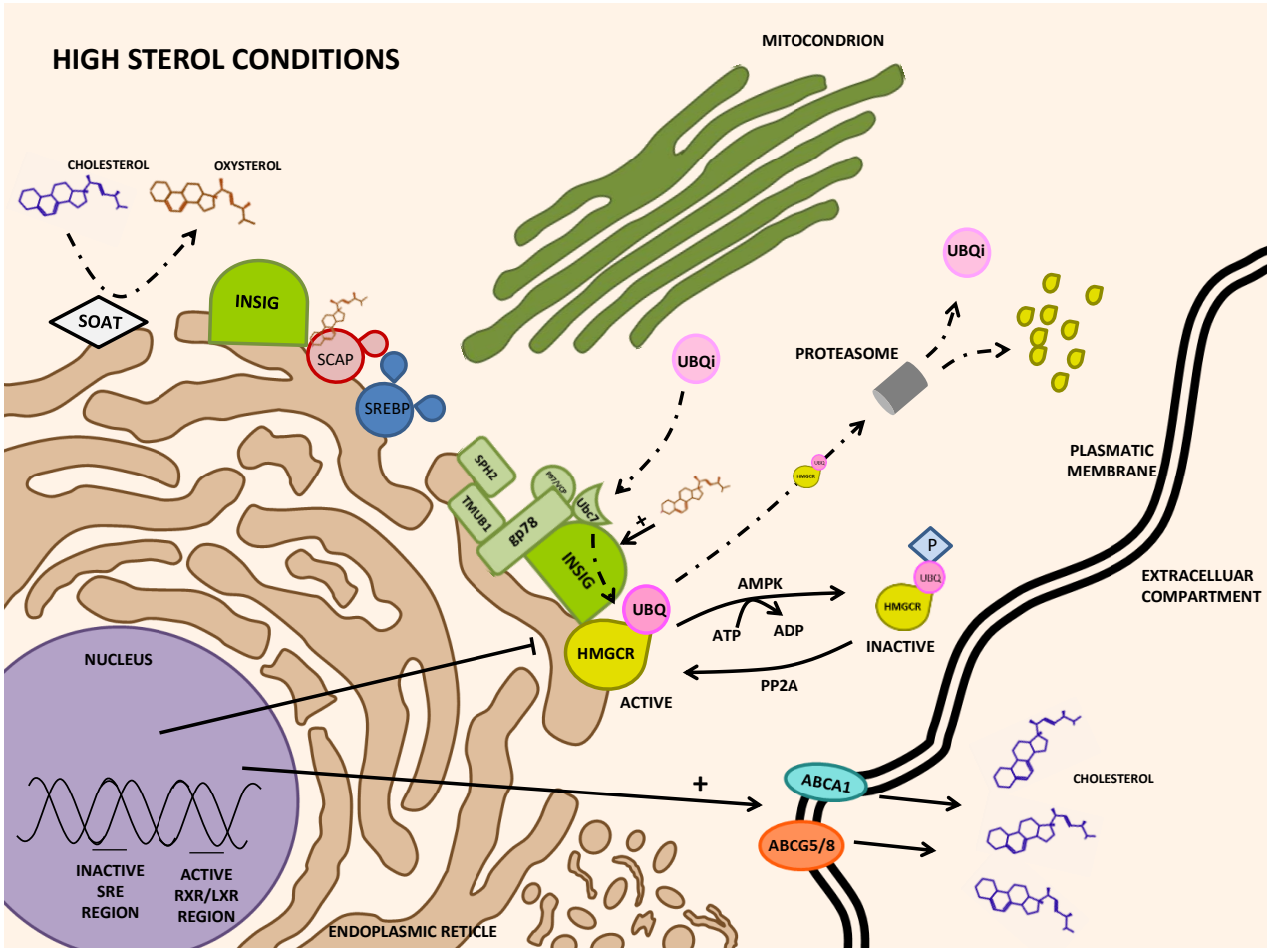
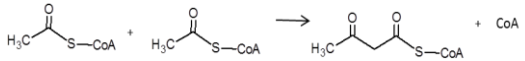
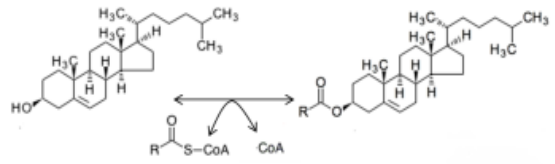

HIGH STEROL CONDITIONS
MITOCONDRION
CHOLESTEROL
OXYSTEROL
SOAT
INSIG
SCAP
SREBP
UBQi
PROTEASOME
UBQi
PLASMATIC MEMBRANE
EXTRACELLUAR COMPARTMENT
SPH2
TMUB1
gp78
UBC7
INSIG
UBQ
HMGCR
ACTIVE
AMPK
ATP
ADP
PP2A
P
UBQ
HMGCR
INACTIVE
NUCLEUS
INACTIVE SRE REGION
ACTIVE RXR/LXR REGION
ENDOPLASMIC RETICLE
ABCA1
ABCG5/8
CHOLESTEROL

Table 1: Chemical reaction, location within the metabolic pathway and within the cell of ACAT and SOAT, enzymes involved in cholesterol metabolism. M: mitochondrion; ER: endoplasmic reticle; Cy: cytoplasm; PM: plasmatic membrane; ExC: extracellular compartment. +: relative genes abundance

		ACAT/SOAT are involved in			mRNA subcellular location				
		Cholesterol synthesis		Cholesterol absorption					
	Reaction	Pre-HMGCR	Post-HMGCR	Small intestine (ER membrane)	M	ER	Cy	PM	ExC
ACAT [EC 2.3.1.9]		yes	no	no	+++	+	++	ND	ND
SOAT [EC 2.3.1.26]		no	yes	yes	ND	+++	ND	++	+