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Mechanisms for Controlling the Synthesis of Lipids – Review

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Abstract

Lipids are synthesized, transported, and recognized by the concerted actions of numerous enzymes, binding proteins, and receptors. A comprehensive analysis of lipid molecules, "lipidomics," in the context of genomics and proteomics is crucial to understanding cellular physiology and pathology; consequently, lipid biochemistry has become a major research target of the postgenomic revolution and systems biochemistry. This review is aimed at studying the mechanisms involved in the regulation of lipids. These mechanisms can either be short term or long term. When the concentration of a particular lipid is high in the body, it inhibits the rate–limiting enzyme involves in its synthesis in a feedback mechanism thereby limiting its rate of synthesis. The synthesis of lipids can also be controlled through a negative feed-back mechanism. This is situation where the product when in a certain level, decreases the activities of the enzymes involved in its synthesis especially the rate–limiting enzymes. Diet also plays a major role in this regulation. When a diet is rich in a certain lipid, its synthesis is inhibited. Several hormones have been reported to regulate the synthesis of lipids. The transcription of the rate–limiting enzyme also plays a role in the regulation of the synthesis of lipids. This mechanism involves several transcriptional factors. The synthesis of lipids is well coordinated and regulated.

Keywords: Lipids, Synthesis, Regulation, Feedback, Transcriptional Factors.

Introduction

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment [1]. Lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water [2]. They constitute a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, etc. [3].

Although the term lipid is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides [4]. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as other sterol-containing metabolites such as cholesterol [5]. Although humans and other mammals use various biosynthetic pathways to synthesize and break down lipids, some essential lipids cannot be made this way and must be obtained from the diet [6].

Lipids have several roles in the body. They include:

- ✓ They provide energy for the body [7].
- ✓ They act as chemical messenger in the body [8].
- ✓ They are involved in the maintenance of body temperature [9].
- ✓ They are involved in membrane layer formation [10].
- ✓ They are also involved in the formation of prostaglandins and play vital role in inflammation [11].

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1. Classifications of Lipids

The biologically important lipids can be classified into three types, namely simple lipids, compound lipids and derived lipids.

1.1. Simple Lipids

These are esters of fatty acids with various types of alcohol. Fats are esters of fatty acids and glycerol. A fatty acid is an organic acid with a hydrocarbon chain ending in a carboxyl (COOH) group. Most fatty acids have an even number of carbon atoms ranging between 14 and 22 (most commonly 16 or 18). The carbon and hydrogen atoms form a long hydrocarbon tail that is hydrophobic (having no affinity for water). Simple lipids are futher classified into two: triacylglycerol (neutral fats) and waxes [12].

1.2. Compound Lipids

These are lipids which contain an inorganic or organic group in addition to fatty acids and glycerol. They are also referred to as complex lipids. On hydrolysis they give phosphoric acid, various sugars, sphingosine, ethanolamine, serine etc. in addition to fatty acids and glycerol (Feigenson, 2006). Compound lipids are further classified as phospholipid, glycolipids, lipoprotein, sphingolipids and sulfolipids.

1.3. Derived Lipids

This is the third major class of lipids. They are hydrolytic products of simple and compound lipids. They includes fatty acids and alcohols [14].

2. Synthesis of Lipids

In animals, when there is an oversupply of dietary carbohydrate, the excess carbohydrate is converted to triglycerides [15]. This involves the synthesis of fatty acids from acetyl-CoA and the esterification of fatty acids in the production of triglycerides, a process called lipogenesis. Fatty acids are made by fatty acid synthases that polymerize and then reduce acetyl-CoA units. The acyl chains in the fatty acids are extended by a cycle of reactions that add the acetyl group, reduce it to an alcohol, dehydrate it to an alkene group and then reduce it again to an alkane group [16]. The enzymes of fatty acid biosynthesis are divided into two groups, in animals and fungi all these fatty acid synthase reactions are carried out by a single multifunctional protein, while in plant plastids and bacteria separate enzymes perform each step in the pathway. The fatty acids may be subsequently converted to triglycerides that are packaged in lipoproteins and secreted from the liver [17].

Lipogenesis is the process by which acetyl-CoA is converted to fats. The former is an intermediate stage in metabolism of simple sugars, such as glucose, a source of energy of living organisms. Through lipogenesis, the energy can be efficiently stored in the form of fats. Lipogenesis encompasses the processes of fatty acid synthesis and subsequent triglyceride synthesis (when fatty acids are esterified with glycerol to form fats) [18]. The products are secreted from the liver in the form of very-low-density lipoproteins (VLDL) [19].

Terpenes and isoprenoids, including the carotenoids, are made by the assembly and modification of isoprene units donated from the reactive precursors isopentenyl pyrophosphate and dimethylallyl pyrophosphate. These precursors can be made in different ways [20]. In animals and archaea, the mevalonate pathway produces these compounds from acetyl-CoA, while in plants and bacteria the non-mevalonate pathway uses pyruvate and glyceraldehyde 3-phosphate as substrates. One important reaction that uses these activated isoprene donors is steroid biosynthesis. Here, the isoprene units are joined together to make squalene and then folded up and formed into a set of rings to make lanosterol. Lanosterol can then be converted into other steroids such as cholesterol and ergosterol [21].

3. Mechanisms for Controlling the Synthesis of Lipids

3.1. Sterol Regulatory Element Binding Proteins (SREBPS)

The feedback regulation of cholesterol synthesis in animals has led to the identification of a unique family of membrane-bound transcription factors, sterol regulatory element binding proteins (SREBPs) [22]. In the presence of cholesterol, SREBPs are sequestered in the endoplasmic reticulum (ER). In the absence of a sterol signal, however, SREBPs undergo specific proteolytic events that lead to activation of

distinct sets of target genes that control lipid metabolism [23]. The N-terminal domain of SREBP is a basic helix-loop-helix leucine zipper transcription factor. The C-terminus forms a tight complex with SREBP cleavage- activating protein (Scap), which functions as the sterol sensor in this system. Two genes encode three SREBP isoforms (~1150 residues): SREBP-1a, SREBP-1c/ADD1 and SREBP-2 [22, 24]. The predominant forms in the liver are SREBP-2 and SREBP-1c/ADD1, which preferentially regulate genes involved in sterol biosynthesis and fatty acid synthesis, respectively [23]. SREBP-1a activates all SREBP responsive genes. To date, SREBPs are known to enhance directly transcription of more than 30 genes needed for uptake and synthesis of cholesterol, fatty acids, triglycerides, and phospholipids [25]. Despite acting in diverse biosynthetic pathways, the activity of each SREBP isoform is regulated by sterols and Scap [26].

In sterol-replete cells, Scap binds to cholesterol in the ER membrane and assumes a conformation that promotes binding to the ER-resident protein Insig (for insulin-induced gene) [27]. Scap escorts SREBP to the Golgi, where two sequential proteolytic cleavage events, mediated by the site 1 (S1P) and site 2 (S2P) proteases, release the N-terminal transcription factor domain from the membrane [28]. Released SREBP is transported into the nucleus as a dimer by importin through interactions with the helix-loop-helix domain [29]. In the nucleus, SREBP activates transcription by binding to sterol regulatory element (SRE) sequences in the promoters of target genes. The resultant increase in synthesis and uptake of cholesterol then feeds back to inhibit activation of SREBP. Finally, nuclear residence of SREBPs is limited by ubiquity independent proteasomal degradation.

Scap (1278 residues) contains two functional domains: the N-terminus consists of eight transmembrane segments and the C-terminus contains multiple WD repeats that mediate binding to SREBP. Genetic and biochemical experiments define transmembrane segments 2-6 (TM2-TM6) of Scap as a sterol-sensing domain. Point mutations in TM2-TM6 (Y298C, L315F or D443N) prevent binding of Scap to Insig, resulting in constitutive ER-to-Golgi transport of SREBP-Scap that is resistant to sterol inhibition [28]. In addition, recombinant Scap TM1-TM8 binds to cholesterol in vitro and undergoes a cholesterol-dependent conformational change [30, 31]. In the absence of sterols, Scap forms a complex with the COPII cargo-selection proteins Sec23 and Sec24 [32, 33]. COPII binding requires the sequence MELADL between TM6 and TM7 in Scap, whereas sterol inhibition of COPII binding requires Insig [34].

Interestingly, HMG-CoA reductase, which catalyzes the first committed step in sterol synthesis, also contains a sterolsensing domain and is negatively regulated by binding to Insig [35]. However, in the case of HMGCoA reductase, sterol-regulated binding to Insig accelerates ubiquitylation and proteasomal degradation of the enzyme [36]. Sterol-sensing domains are also found in other proteins, such as the Neimann-Pick type C disease gene *NPC1* and the Hedgehog receptor Patched. However, the role of Insig in regulation of these proteins remains to be tested [37].

Insigs are ER-resident proteins that contain six transmembrane segments and negatively regulate Scap and HMG-CoA reductase [38]. Humans have two proteins, Insig-1 (277 amino acids) and Insig-2 (225 amino acids), that differ in the length of their cytosolic N-termini [38]. Studies using mice and cultured cells lacking Insig-1 as well as Insig-2 demonstrate that they are essential mediators of cholesterol feedback regulation, controlling both activation of SREBP through ER retention and sterol-accelerated degradation of HMG-CoA reductase [29, 39]. Although the two proteins appear functionally equivalent, expression of Insig-1 and expression of Insig-2 is inversely regulated by insulin in the liver [40]. Insig-1 is an SREBP target and is highly expressed in livers of mice fed a normal diet owing to elevated insulin and SREBP- 1c levels. Upon fasting, insulin falls, decreasing Insig-1 and increasing Insig-2 expression. The situation is reversed upon refeeding animals, when insulin levels rise, up-regulating Insig-1 and down regulating Insig-2 [40].

SREBP is sequentially processed by S1P (1052 residues) and S2P (519 residues) [28]. S1P (also called SKI-1), a member of the subtilisin/kexin family of serine proteases, cleaves after a leucine residue in the consensus sequence RxxL in the luminal loop of SREBPs [32, 41]. The zinc metalloprotease S2P cleaves a Leu-Cys bond predicted to lie within the lipid bilayer by a process known as regulated intramembrane proteolysis (RIP) [30]. SREBP is not the only substrate for these proteases: the two proteins function in tandem to

activate the stress response transcription factor ATF6 [42].

SREBPs function as master regulators of cholesterol and fatty acid synthesis. SREBP-2 upregulates expression of most cholesterol biosynthetic enzymes and the LDL receptor, whereas SREBP- 1c stimulates transcription of genes required for fatty acid synthesis, such as acetyl-CoA carboxylase and fatty acid synthase [23]. SREBPs cooperate with other DNA-binding transcription factors and coactivators. Maximal transcriptional activation requires additional DNA-binding proteins: NF-Y and CREB for the HMG-CoA reductase gene and Sp1 for the LDL receptor gene [43]. In addition, SREBPs recruit the coactivators CBP/p300 and the mediator complex to stimulate transcription [43]. The coactivator PGC-1, induced by a fat-rich diet, also binds SREBP-1c and is required for SREBP-mediated lipogenic gene expression [25]. Importantly, these factors permit modulation of SREBP activity independently of sterol-regulated proteolytic processing.

The central role that SREBPs play in control of lipid synthesis is highlighted by the multiple inputs to SREBP activity from other signaling pathways. The nuclear hormone receptors RXR and LXR function as a heterodimer to upregulate SREBP-1c in response to cholesterol overloading, possibly to increase the supply of unsaturated fatty acids needed for cholesterol esterification and storage [44]. In the liver, transcription of both

SREBP-1c and SREBP-2 is stimulated by SREBPs in a feed-forward mechanism that requires SRE sequences in the promoters of these genes [23]. One function of the liver is to convert excess carbohydrates to fatty acids for storage as triglycerides. Insulin stimulates this fatty acid synthesis in response to excess carbohydrate [23]. Importantly, these lipogenic effects of insulin in the liver are mediated by SREBP-1c [45]. Insulin increases SREBP-1c mRNA levels and SREBP-1c target gene expression in both the liver and tissue culture cells [46, 47]. Although a complete description of insulin action on SREBP-1c requires further experimentation, a recent study suggests that control of SREBP-1c transcription by insulin is mediated by RXR-LXR [21]. Interestingly, polyunsaturated fatty acids (PUFA) inhibit SREBP-1c and fatty acid synthesis activity by antagonizing LXR dependent activation of SREBP-1c. LXR may thus integrate these two dietary signals [48].

Mature nuclear SREBP is highly unstable owing to its ubiquit-independent degradation. Although this is not sterol regulated, phosphorylation of SREBP promotes its binding to the E3 ubiquitin ligase SCFFbw7 and thus its ubiquitylation and degradation. This can be mediated by GSK3, whose activity is inhibited by insulin signaling, which suggests a non-transcriptional mechanism by which insulin may stimulate SREBP activity [49]. Homologs of SREBP have been identified and characterized in fission yeast, flies and worms. In *S. pombe*, SREBP is activated in response to sterol depletion as a consequence of low oxygen levels [49].

Yeast SREBP is required for anaerobic growth and activates genes required for adaptation to low oxygen levels. In *D. melanogaster*, a cholesterol auxotroph, SREBP is not regulated by sterols, but instead activation is controlled by phosphatidylethanolamine [28]. In response to reduced levels of this lipid, *Drosophila* SREBP activates lipogenic enzymes. Lastly, *C. Elegans* SREBP is highly expressed in the intestine, where it is required for expression of lipogenic enzymes and fat production. What regulates *C. elegans* SREBP is unknown. Analysis of SREBP function in these organisms promises to give insights into the molecular mechanisms of sterol sensing and the evolution of this regulatory system [28].

3.2.Inhibition of Lipid Synthesis through Activation of Amp Kinase: an Additional Mechanism for the Hypolipidemic Effects of Berberine

The alkaloid drug berberine (BBR) was recently described to decrease plasma cholesterol and triglycerides (TGs) in hypercholesterolemic patients by increasing expression of the hepatic low density lipoprotein receptor (LDLR). Using HepG2 human hepatoma cells, BBR inhibits cholesterol and TG synthesis in a similar manner to the AMP-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide 1-*b*-ribofuranoside (AICAR) [50]. Significant increases in AMPK phosphorylation and AMPK activity occurs when the cells were incubated with BBR. Activation of AMPK is observed by measuring the phosphorylation of acetyl-CoA carboxylase, a substrate of AMPK, correlated with a

subsequent increase in fatty acid oxidation. All of these effects were abolished by the mitogen-activated protein kinase inhibitor. Treatment of hyperlipidemic hamsters with BBR decreases plasma LDL cholesterol and strongly reduced fat storage in the liver [51].

Coronary heart disease is the most important cause of morbidity and mortality in developed countries. Among the different risk factors, increased LDL cholesterol level has been identified as a major cause of coronary heart disease, and it has been extensively demonstrated in clinical trials that treatment of dyslipidemic patients with drugs that decrease LDL cholesterol levels significantly reduces the risk for coronary heart disease [52]. Statins represent the major class of hypolipidemic drugs on the market. They act through the inhibition of HMG-CoA reductase, a pivotal enzyme in the cholesterol biosynthetic pathway, thus leading to a reduction of cholesterol concentration and a subsequent increase in expression of the low density lipoprotein receptor (LDLR), the main receptor involved in the hepatic clearance of LDL cholesterol. Recently, ezetimibe, a new LDL cholesterol-lowering therapy described as a cholesterol absorption inhibitor, was developed, and it is now commercialized as a monotherapy or in combination with statins [53]. One interest of combination therapy is to reach the goals recommended by the National Cholesterol Education Program Adult Treatment Panel III and to limit the potential side effects observed with high doses of statins. The discovery of new drugs that could be developed in combination with statins is still of interest, especially compounds targeting other lipid fractions, such as HDL cholesterol and triglycerides (TGs), or other risk factors, such as type II diabetes and hypertension. Berberine (BBR), an alkaloid isolated from the Chinese herb Coptis chinensis, has been widely used as a drug to treat gastrointestinal infections. Recently, BBR has been described as a new cholesterol-lowering drug [50]. BBR treatment of 32 type IIb dyslipidemic patients led to a 25% decrease in LDL cholesterol and a 35% decrease in TGs. This LDL cholesterol-lowering effect was attributed to the activity of BBR on hepatic LDLR expression via a new mechanism distinct from that of statins. Indeed, in a human hepatoma cell line (HepG2) as well as in hyperlipidemic hamsters, BBR upregulated the expression of LDLR through stabilization of its mRNA involving an extracellular regulated kinase (ERK)-dependent mechanism. However, statin treatment of hypercholesterolemic patients with TG levels, 200 mg/dl often shows a more pronounced effect on LDL cholesterol than on TGs. Thus, even if the mechanism of action is consistent with a LDL cholesterol-lowering effect, it is more difficult to explain the TG-lowering effect [38].

In HepG2 cells, BBR inhibits TG and cholesterol synthesis by a signaling pathway involving the MAPK/ERK cascade. BBR activates AMPK. The AMPK has been proposed to act as a fuel gauge in mammalian cells. Among the large number of AMPK protein targets, HMG-CoA reductase and ACC are well identified [54]. These two enzymes, involved in cholesterol and fatty acids synthesis, are inactivated by AMPK-mediated phosphorylation, leading to cholesterol and TG synthesis inhibition. AICAR is phosphorylated in cytosol by adenine kinase and then is converted in AICA-ribotide, which mimics AMP and activates AMPK in various cells, including HepG2 (Nakamaru et al., 2005). TG synthesis was determined using [14C]acetate or [14C]glycerol incorporation, whereas TG assembly was assessed by the incorporation of [14C]oleate [54].

Both BBR and AICAR induce similar profiles on lipid synthesis, suggesting that both compounds could regulate lipid metabolism by a common mechanism. Therefore, there is the possibility that BBR could activate AMPK. It is currently accepted that ACC phosphorylation levels into the cells represent a marker of AMPK activity. BBR dose-dependently increased ACC phosphorylation, with a half-maximal effect obtained at 2 mg/ml. AICAR also increased ACC phosphorylation by 2.5-fold. AMPK activation leads to an increase in fatty acid oxidation, dependent on the inhibition of ACC by AMPK [54]. BBR can achieve a significant dose-dependent increase in fatty acid oxidation (half-maximal effect obtained at 10 mg/ml), and the phosphorylation of ACC, is totally inhibited in the presence of the MAPK inhibitor. AICAR also increases fatty acid oxidation by 16-fold in HepG2 cells, BBR (but not AICAR), activates AMPK through a MAPK/ERK-dependent pathway, leading to the activation of fatty acid oxidation. This latter effect, associated with a decrease in TG synthesis, could explain the TG-lowering effect of BBR observed in patients and suggests pleiotropic effects of BBR on lipid metabolism. In cell activity of AMPK, after incubation of HepG2 cells with BBR,

both the phosphorylation of the AMPK on threonine 172 and AMPK using specific antibodies, BBR significantly increases the phosphorylation of AMPK without altering the expression of AMPK Furthermore, preincubation of the cells with the MAPK inhibitor at 10 mM suppressed the effects of BBR on AMPK phosphorylation [55].

To reinforce the activation of AMPK by BBR, HepG2 cells were treated with BBR and the enzyme was immunoprecipitated. AMPK activity was quantified using SAMS peptides. The assay was done in the presence or absence of 50 mM AMP. Addition of AMP in the in vitro with assay elicites a 2.3-fold increase in AMPK basal activity. Furthermore, cells treated with BBR exhibited an increase in AMPK activity, by using the AMPK assay performed with or without AMP. Berberine inhibits lipid synthesis AMPK and is phosphorylated by the enzyme, leading to cholesterol and TG synthesis inhibition. When compared with the well-known AMPK activator AICAR, BBR induced a similar profile on lipid synthesis, with inhibition of cholesterol and TG synthesis without any impact on TG assembly [56].

3.3. Control of Cholesterol Synthesis Through Regulated Er-Associated Degradation of HMG CoA Reductase

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase catalyzes the conversion of HMG CoA to mevalonate, a rate-determining step in the synthesis of not only cholesterol, but also of nonsterol isoprenoids that are essential for normal cell function [57]. These molecules include ubiquinone and hemeA, which participate in aerobic cellular respiration, dolichol, which is required for the synthesis of N-linked glycoproteins, and the farnesyl and geranylgeranyl groups that become attached to various cellular proteins, increasing their membrane association. As the rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase is the target of a complex, multivalent feedback regulatory system that is mediated by sterol and nonsterol end-products of mevalonate metabolism [57]. This complex regulatory system operates at transcriptional and post-transcriptional levels and guards against the over accumulation of cholesterol while ensuring that essential nonsterol isoprenoids are constantly produced.

The complexity of the multivalent control of reductase was first revealed through the use of compactin (also known as ML-236B), a founding member of the statin family of competitive reductase inhibitors that was first isolated from the fungus *Penicillium citrinum* by Endo and co-workers in the 1970s. The activity of reductase is largely suppressed when cells are cultured under normal culture conditions (i.e., medium supplemented with fetal calf serum) and, as a result, cholesterol and nonsterol isoprenoids are produced at low rates. This suppression results from the receptor-mediated uptake of cholesterol-rich low-density lipoproteins (LDLs) present in the fetal calf serum of culture medium [57]. Internalized cholesterol is utilized in the synthesis of cell membranes; excess cholesterol becomes esterified and stored in cytoplasmic lipid droplets as cholesterol esters. The sterol also suppresses reductase activity by inhibiting the enzyme's expression through the multivalent regulatory system. Subjecting cells to cholesterol deprivation through incubation in medium supplemented with lipoprotein-deficient serum plus compactin triggers a massive increase in the amount of reductase protein [30]. This compensatory increase in reductase results from the combined effect of three regulatory events: enhanced transcription of the reductase gene, enhanced translation of the reductase mRNA, and extended half-life of the reductase protein [22]. Complete suppression of reductase in compactin-treated cells requires the addition of exogenous mevalonate together with LDL or oxysterols, oxygenated forms of cholesterol that are readily taken up by cells [57]. Together, these findings formed the basis for the concept that multiple feedback mechanisms mediated by sterol and nonsterol end-products of mevalonate metabolism control the levels and activity of reductase.

Sterol and nonsterol isoprenoids inhibit reductase at different levels. For example, sterols inhibit the activity of sterol regulatory element-binding proteins (SREBPs), a family of membrane-bound transcription factors that enhance the uptake and synthesis of cholesterol by activating transcription of the genes encoding reductase and other cholesterol biosynthetic enzymes as well as the LDL-receptor [23]. Translation of reductase mRNA is blocked by a nonsterol isoprenoid. Although the identity of this regulatory product and its mechanism of action is unknown, the reaction may be mediated by the

complex 5′-untranslated region of the reductase mRNA. Sterol and nonsterol isoprenoids combine to reduce the half-life of reductase protein in compactin-treated cells from 11-12 hours to less than 1 hour by accelerating its ER-associated degradation (ERAD) from membranes through a mechanism mediated by the ubiquitin-proteasome system [37].

Mammalian HMG CoA reductase consists of 887 or 888 amino acids that can be separated into two domains [58]. The N-terminal domain of reductase encompasses 339 amino acids; the region is embedded into ER membranes through eight membrane-spanning segments separated by short hydrophilic loops. The 548 amino acid C-terminal domain projects into the cytosol where it exerts all of the enzymatic activity. The membrane domain of reductase is highly conserved across mammalian species and the region plays a key role in sterol-accelerated degradation of the enzyme as indicated by two early observations. First, the truncated, cytosolic C-terminal domain of reductase restores cholesterol synthesis when expressed in reductase-deficient Chinese hamster ovary (CHO) cells [58]. However, this protein is very stable and does not become rapidly degraded in the presence of sterols. The second observation stemmed from studies of a fusion protein between the membrane domain of reductase and soluble β-galactosidase. This reductase membrane domain-β-galactosidase fusion protein exhibits sterol-accelerated degradation that is similar to the wild type, full-length reductase. Considered together, these key observations are consistent with a mechanism whereby the membrane domain of reductase senses levels of membrane-embedded sterols, triggering reactions that render the enzyme susceptible to proteolytic degradation.

The membrane domain of reductase contains a stretch of ~ 180 amino acids called the sterol-sensing domain. This evolutionarily conserved domain comprises five of the eight membrane-spanning segments of reductase and is found in several other polytopic membrane proteins that are postulated to interact with sterols [37]. These proteins include the sterol-regulated escort protein Scap, the lipid transport proteins Niemann Pick C1 (NPC1) and NPC1L1, the Patched receptor for the cholesterol-modified morphogen Hedgehog, and Dispatched, which mediates release of Hedgehog from cells [59]. The function of the sterol-sensing domain was first demonstrated for Scap, which binds to the SREBP transcription factors in the ER. In sterol-deprived cells, Scap facilitates transport of SREBPs from the ER to the Golgi where active fragments of the transcription factor are released from membranes by proteolysis. The processed forms of SREBPs migrate to the nucleus and activate target gene expression, which leads to increased synthesis and uptake of cholesterol and other lipids [23]. When sterols accumulate in ER membranes, the membrane domain of Scap binds to one of two ER membrane proteins called Insig-1 and Insig-2 [38]. Insig binding blocks incorporation of Scap-SREBP into COPII-coated vesicles that bud from ER membranes and deliver proteins to the Golgi [60, 61]. Sequestration of Scap-SREBP complexes in the ER prevents proteolytic activation of SREBPs; expression of SREBP target genes declines and consequently, cholesterol synthesis and uptake is suppressed.

The topology of Scap in ER membranes is similar to that of reductase. The protein is anchored to membranes through its N-terminal domain, which includes eight membrane-spanning segments . The C-terminal domain projects into the cytosol and mediates association with SREBPs [43]. The sterol-sensing domain of Scap comprises transmembrane helices 2-6 and exhibits 55% amino acid similarity and 25% identity with the corresponding region of reductase. The importance of the sterol-sensing domain in the regulation of Scap is highlighted by findings that three point mutations (Tyr-298 to Cys, Leu-315 to Phe, and Asp-443 to Asn) within the region abolish sterol-regulated Insig binding, thereby relieving sterol-mediated ER-retention of mutant Scap-SREBP complexes [38, 61].

Insigs also bind to the sterol-sensing domain of reductase in a sterol-regulated fashion [35]. This binding is disrupted by mutation of the tetrapeptide sequence YIYF, which is located in the second transmembrane segment of reductase. Mutation of the YIYF sequence to alanine residues abolishes Insig binding and the mutant enzyme is no longer subjected to sterol-accelerated degradation [35]. The first tyrosine of the YIYF tetrapeptide (Tyr-75) is equivalent to Tyr-298 of Scap, which is required for Insig-Scap binding. When overexpressed, the sterol-sensing domain of Scap blocks sterol-accelerated degradation of reductase [35]. This effect is ablated by the Tyr-298 to Cys mutation in the Scap sterol-sensing domain, indicating that Scap and reductase binding sites on Insigs overlap. At least three

additional amino acids (Ser-60, Gly-87, and Ala-333) within the membrane domain of reductase are also required for Insig binding [29]. Even though Ser-60 and Gly-87 localize to the sterol-sensing domain of reductase, these residues are not present in the corresponding region of the Scap sterol-sensing domain. These observations emphasize the importance of detailed structural analyses of Scap-Insig and reductase-Insig complexes in future studies.

Two major differences exist between the Insig-mediated regulation of Scap and that of reductase. Insig binding to Scap leads to its retention in the ER, whereas Insig binding to reductase causes it to become rapidly ubiquitinated and degraded. This discrepancy can be rationalized when considering the other major difference between Insig-mediated regulation of Scap and reductase: sterol specificity. Cholesterol directly binds to the membrane domain of Scap, triggering a conformational change in the protein that allows for Insig binding. In contrast, cholesterol does not potently induce rapid ubiquitination of reductase, even when added to sterol-deprived membranes *in vitro*. Instead, the reaction is potently stimulated by the cholesterol synthesis intermediate 24,25-dihydrolanosterol both *in vitro* and in intact cells. It should be noted that lanosterol, the immediate precursor of 24,25-dihydrolanosterol, was also found to stimulate ubiquitination of reductase. However, it was subsequently determined that this activity was attributable to small amounts of contaminating 24,25-dihydrolanosterol in the preparations of lanosterol used in the initial studies [62]. The specificity of reductase ubiquitination is remarkable considering that lanosterol and 24, 25-dihydrolanosterol only differ in the degree of side-chain saturation. This suggests that the mechanism through which 24,25-dihydrolanosterol stimulates ubiquitination of reductase likely involves its direct binding to the enzyme. However, attempts to demonstrate direct binding of 24,25-dihydrolanosterol to reductase have so far been unsuccessful. Thus, the possibility that some other protein binds 24,25-dihydrolanosterol and induces reductase to bind Insigs cannot be excluded.

The findings described above not only help to explain how Insigs mediate sterol regulation of Scap and reductase through distinct mechanisms, but they also point to the production of 24,25-dihydrolanosterol as a key focal point in sterol regulation. The demethylation of lanosterol and 24,25-dihydrolanosterol has been implicated as a rate-limiting step in the sterol branch of the mevalonate pathway [63]. 24,25-Dihydrolanosterol suppresses its own synthesis by reducing flux through the mevalonate pathway via Insig-mediated degradation of reductase. Accumulation of lanosterol and 24,25-dihydrolanosterol is avoided because these sterols do not inhibit ER-to-Golgi transport of Scap-SREBP [36]. Thus, mRNAs encoding the enzymes that catalyze reactions subsequent to lanosterol synthesis remain elevated and lanosterol and 24,25-dihydrolanosterol are efficiently converted to cholesterol. As cholesterol begins to accumulate, Scap-SREBP transport to the Golgi is blocked, SREBP processing becomes inhibited, and the entire pathway is shut down. The physiologic relevance of 24,25-dihydrolanosterol as a regulator of reductase degradation is highlighted by the finding that oxygen deprivation causes the sterol to accumulate in cells [64]. At the same time, expression of both Insigs is enhanced through the action of the oxygen-sensitive transcription factor hypoxia-inducible factor (HIF)-1α. The accumulation of 24,25-dihydrolanosterol, coupled with HIF-mediated induction of Insigs, leads to rapid degradation of reductase, providing a link between oxygen sensing and cholesterol metabolism.

3.4. Mechanisms of Nutritional and Hormonal Regulation of Lipogenesis

Lipogenesis is very responsive to changes in the diet. Polyunsaturated fatty acids decrease lipogenesis by suppressing gene expression in liver, including that of fatty acid synthase, spot14 and stearoyl-CoA desaturase [64]. Conversely, a diet rich in carbohydrates stimulates lipogenesis in both liver and adipose tissue, leading to elevated postprandial plasma triglyceride levels. Fasting reduces lipogenesis in adipose tissue, which, combined with an increased rate of lipolysis, leads to net loss of triglycerides from fat cells. In contrast, in liver, because of the large amounts of fatty acids arriving from the adipose tissue, triglyceride synthesis is increased, resulting in a mild form of hepatosteatosis (fatty liver). This happens despite a reduced rate of fatty acid synthesis and decreased expression of numerous genes involved in lipogenesis [46].

It can be reasoned that, somehow, the signal of reduced or excess food intake has to be translated into altered expression levels of lipogenic genes. This concept can be illustrated by examining the effects of

fasting, which is associated with a decrease in plasma glucose and an increase in plasma-free fatty acids. Plasma glucose levels stimulate lipogenesis via several mechanisms. First, glucose itself is a substrate for lipogenesis. By being glycolytically converted to acetyl-CoA, glucose promotes fatty acid synthesis. Secondly, glucose induces the expression of lipogenic genes. Finally, glucose increases lipogenesis by stimulating the release of insulin and inhibiting the release of glucagon from the pancreas [52].

Fasting is associated with significant changes in plasma hormone concentrations, such as a decrease in plasma insulin and leptin, and an increase in plasma growth hormone and glucagon. Insulin is probably the most important hormonal factor influencing lipogenesis. By increasing the uptake of glucose in the adipose cell via recruitment of glucose transporters to the plasma membrane, as well as activating lipogenic and glycolytic enzymes via covalent modification, insulin potently stimulates lipogenesis. These effects are achieved by the binding of insulin to the insulin receptor at the cell surface, thus activating its tyrosine kinase activity and inducing a plethora of downstream effects via tyrosine phosphorylation [66, 67]. Insulin also has long-term effects on the expression of lipogenic genes, probably via the transcription factor sterol regulatory element binding protein-1 (SREBP-1). In addition, insulin causes SREBP-1 to induce the expression and activity of glucokinase, thereby increasing the concentration of a glucose metabolite that supposedly mediates the effects of glucose on lipogenic gene expression [47].

Another hormone that has an important influence on lipogenesis is growth hormone (GH). GH dramatically reduces lipogenesis in adipose tissue, resulting in significant fat loss, with a concomitant gain of muscle mass [68]. These effects appear to be mediated by two pathways. In one case, GH decreases insulin sensitivity, resulting in down-regulation of fatty acid synthase expression in adipose tissue [69]. The details of this mechanism are still unknown, but GH probably interferes with insulin signaling at the post-receptor level. In the second case, GH may decrease lipogenesis by phosphorylating the transcription factors Stat5a and 5b. The loss of Stat5a and 5b in a knock-out model is shown to decrease fat accumulation in adipose tissue. The mechanism by which Stat5 proteins enhance fat storage remains to be determined [70].

Leptin is another hormone that may be involved in lipogenesis. There is a growing consensus that leptin limits fat storage not only by inhibiting food intake, but also by affecting specific metabolic pathways in adipose and other tissues. Leptin stimulates the release of glycerol from adipocytes, by both stimulating fatty acid oxidation and inhibiting lipogenesis. The latter effect is achieved by down-regulating the expression of genes involved in fatty acid and triglyceride synthesis, as was nicely demonstrated recently by oligonucleotide micro-array analysis [71]. Interestingly, another negative target of leptin is probably SREBP-1, suggesting that this transcription factor may be involved in mediating the inhibitory effect of leptin on lipogenic gene expression [71, 72].

A final endocrine/autocrine factor connected with triglyceride synthesis is acylation stimulating protein (ASP). ASP is a small peptide that is identical to C3adesArg, a product of the complement factor C_3 [73]. ASP is produced by adipose tissue and supposedly acts via an autocrine loop. Numerous *in vitro* studies have shown that ASP stimulates triglyceride accumulation in adipose cells [73]. This is achieved by an increase in triglyceride synthesis, as well as by a simultaneous decrease in adipose tissue lipolysis. Intraperitoneal injection of ASP has been reported to stimulate triglyceride clearance from plasma, indicating that ASP has a similar effect *in vivo* [74]. This is supported by studies with female ASP null mice, which display a pronounced reduction in adipose tissue mass despite an increased energy intake [75]. However, whereas Murray *et al.* [76] reported a delayed postprandial triglyceride clearance in ASP null mice, another group failed to find any differences between null and wild-type mice [77]. The reason for this discrepancy is unclear. Very little is known about how ASP stimulates triglyceride synthesis. It probably binds to some kind of cell surface receptor, thereby activating a signaling cascade that involves phosphodiesterase [78].

4. Conclusion

Controlling the synthesis of lipids involves both simple and complex mechanisms. These mechanisms can either be short term or long term. When the

concentration of a particular lipid is high in the body, it inhibits the rate–limiting enzyme involves in its synthesis in a feedback mechanism thereby limiting its rate of synthesis. Diet also plays a major role in this regulation. When a diet is rich in a certain lipid, its synthesis is inhibited. The transcription of the rate–limiting enzyme also plays a role in the regulation of the synthesis of lipids.

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