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Regulation of hepatic gene expression by saturated fatty acids

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ABSTRACT

Diets rich in saturated fatty acids have long been associated with increased plasma cholesterol concentrations and hence increased risk of cardiovascular disease. More recently, they have also been suggested to promote the development of non-alcoholic fatty liver disease. While there is now considerable evidence to suggest that polyunsaturated fatty acids exert many of their effects through regulating the activity of transcription factors, including peroxisome proliferator activated receptors, sterol regulatory binding proteins (SREBPs) and liver X receptor, our understanding of how saturated fatty acids act is still limited. Here we review the potential mechanisms whereby saturated fatty acids modulate hepatic lipid metabolism thereby impacting on the synthesis, storage and secretion of lipids. Evidence is presented that their effects are, at least partly, mediated through modulation of the activity of the SREBP family of transcription factors.

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1. Introduction

It has now been well established that, through a variety of pathways, including peroxisome proliferator activated receptors (PPARs), sterol regulatory element binding proteins (SREBPs) and liver X receptors (LXRs), fatty acids can regulate the expression of a range of genes involved in lipid and lipoprotein metabolism within the liver [1–3]. However, much of the existing evidence relates specifically to the role of polyunsaturated fatty acids in regulating gene expression with much less understanding of the potential roles of saturated fatty acids. In this review, we will explore the impact of dietary saturated fatty acids on hepatic metabolism in the development of pathologies such as raised plasma cholesterol, metabolic syndrome (Met Syn) and non-alcoholic fatty liver disease (NAFLD) and existing evidence for direct effects on gene expression.

2. Role of the liver in regulating plasma lipoprotein concentrations

The liver plays a major role in regulating the availability of lipid to the other tissues of the body by modulating lipid synthesis and the uptake and secretion of lipoproteins [4,5]. It is a major site of synthesis of both cholesterol and triacylglycerol. A reciprocal relationship between dietary cholesterol intake and hepatic cholesterol synthesis ensures relatively modest changes in whole body cholesterol concentrations over a fairly wide range of dietary intakes [6]. Levels of hepatic cholesterol synthesis are very tightly regulated by a series of intricate negative feedback loops, all of which serve to keep overall body cholesterol levels very stable. In addition to distributing such cholesterol around the body through its secretion within very low density lipoprotein (VLDL), the liver represents the major site of cholesterol metabolism and excretion through its conversion to bile acids and subsequent loss in the faeces. The synthesis of triacylglycerol is largely regulated by the availability of fatty acids. These can arise from three major sources; *de novo* synthesis, diet and release from adipose tissue [6].

In terms of lipoprotein metabolism, the liver is the site of assembly and secretion of VLDL and the major site of removal of chylomicron remnants, intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) from the circulation. It synthesizes and secretes apolipoprotein (apo) A1, the major protein component of HDL, and actively removes cholesterol from the circulating HDL particles following interaction with cell surface receptors [7]. When lipid supply exceeds the ability to mobilize lipid the liver has the capacity to store relatively large amounts of both cholesterol ester and triacylglycerol.

The synthesis and secretion of VLDL from the liver is largely governed by lipid substrate supply [6]. The expression of apoB100 appears to be largely constitutive. When sufficient triacylglycerol is available the apoB is lipidated and a VLDL particle is formed. In the absence of sufficient triacylglycerol the emerging apoB is degraded. Transfer of triacylglycerol onto apoB is mediated by the microsomal triglyceride transfer protein (MTP) [8]. Evidence is

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emerging that certain polymorphisms in MTP may result in impaired functioning, reduced VLDL production and associated accumulation of lipid in the liver [9]. The synthesis of VLDL certainly appears more complex than originally proposed, and new genes (e.g. Cideb) have recently been found to play a role in the addition of triacylglycerol to the developing lipoprotein [10]. While there is evidence to suggest that cholesterol availability regulates the synthesis and secretion of VLDL, the mechanisms whereby free and esterified cholesterol are incorporated into the particle remain to be fully elucidated.

Cholesterol enters the liver from a variety of sources including chylomicron remnants, IDL, LDL and HDL. Chylomicron remnants can be taken up by the LDL receptor related protein and the LDL receptor [11]. IDL and LDL are taken up by the LDL receptor [10] and removal of cholesterol from HDL appears to be largely mediated by interaction with the Scavenger receptor 1B [12]. This uptake is regulated by intracellular cholesterol concentrations. Hepatic cholesterol concentration appears to be primarily regulated through a combination of modulation of LDL receptor expression and *de novo* synthesis. Over the last 30 years, the seminal work of Goldstein, Brown and co-workers have elucidated the mechanism whereby the transcription factor, SREBP2, regulates expression of the genes for many of the enzymes of the cholesterol synthesis pathway and the LDL receptor (see below), demonstrating one of the first examples of nutrient regulation of gene expression [13,14].

3. Dietary fatty acids and plasma cholesterol

For over 50 years it has been recognized that the amount and type of fatty acids in the diet have a major impact on plasma cholesterol concentrations and associated risk of developing atherosclerotic vascular disease [15]. Epidemiological observations, such as the 7 countries study, clearly showed that populations with high dietary intakes of saturated fatty acids had higher plasma cholesterol levels and an increased incidence of cardiovascular disease [16]. This was supported human feeding trials which directly demonstrated that increasing saturated fatty acid intake increases total plasma cholesterol concentration in a dose-dependent manner [17,18]. Such findings have underpinned dietary advice for reducing cardiovascular risk for several decades.

The development of techniques to separate individual lipoprotein fractions led to the understanding that cardiovascular risk not only relates to total plasma cholesterol concentration but also to the relative proportion of cholesterol carried in individual lipoprotein fractions. Thus, while a positive relationship exists between LDL cholesterol and CVD risk, HDL cholesterol has an inverse relationship and is hence viewed as protective. The major impact of saturated fatty acids has been shown to be in increasing LDL cholesterol partially offset by a lesser effects on HDL cholesterol [19,20]. It has also been recognized that the specific structure of fatty acids influences their impact on lipoprotein concentration. For example, the cholesterol raising effects of saturated fatty acids appears to be restricted to those with between 12 and 16 carbon atoms.

4. Dietary fatty acids and non-alcoholic fatty liver disease (NAFLD)

NAFLD is a term used to describe the accumulation of lipid within the liver (steatosis) in the absence of significant alcohol intake, which cannot be attributed to viral, congenital or autoimmune factors [21–23]. It is now widely regarded as the most common form of chronic liver disease in many Western Countries and its prevalence appears to be increasing rapidly. This has been attributed to the ongoing 'epidemic' of obesity and associated insulin resistance, Met Syn and type 2 diabetes. Estimates of prevalence of NAFLD in the developed countries suggest that in excess of 30% of the adult population could be affected [21,23]. The risk of developing NAFLD increases with the degree of obesity with a reported incidence of 96% in subjects undergoing bariatric surgery [24]. There also appears to be a close association between the degree of insulin resistance and incidence of NAFLD [25].

Hepatic steatosis involves the accumulation of intracytoplasmic triacylglycerol within hepatocytes. This may be a result of the formation of large droplets of macrovasicular fat which displace other components of the cell, including the nucleus or a combination of large and small droplets [26]. This accumulation of lipid is often referred to as the first "hit" in a "two-hit" process that leads to non-alcoholic steatohepatisis (NASH), inflammation, cirrhosis and ultimately liver failure [27]. As such, simple steatosis is often thought to be relatively benign unless a second "hit" subsequently occurs. The nature of this second event remains to be established but factors such as oxidative stress and/or circulating inflammatory molecules have been implicated [26,28].

Diet is clearly closely associated with risk of developing NAFLD. Excessive energy intake results in accumulation of triacylglycerol in adipose tissue and appears to be central to the aetiology of the disease [22,23,25,26]. Excessive adiposity is frequently associated with insulin resistance which in turn leads to unrestrained lipolysis of adipose tissue triacylglycerol and inappropriate release of free fatty acids into the circulation. In many individuals this leads to the cluster of metabolic disturbances (high plasma TAG, low HDL cholesterol, hypertension and glucose intolerance) which make up Met Syn [29].

While dietary fat appears to directly provide a relative small proportion of the fatty acids incorporated into hepatic triacylglycerol, it does of course represent the primary source of adipose tissue fatty acids which may be subsequently released and re-esterified in the liver [30]. The specific nature of dietary fatty acids may also directly impact on hepatic *de novo* lipogenesis [31]. It has been reported that dietary fatty acids impact on lipogenesis by directly regulating the expression of key enzymes involved in fatty acid synthesis particularly, acetyl coenzyme A carboxylase (ACC), fatty acid synthase (FAS) and stearoyl CoA desaturase (SCD1) [31–33]. It is now well established, at least in animal models that polyunsaturated fatty acids can specifically down-regulate expression of the genes for these enzymes [1–3]. By contrast, it has been suggested that saturated fatty acids may actually stimulate the expression of these enzymes [31–33]. Evidence for these effects and potential mechanisms are discussed below.

5. Regulation of hepatic gene expression by fatty acids

It is clear that fatty acids have the ability to alter a number of pathways involved in lipid and lipoprotein metabolism within the liver. In recent years considerable progress has been made in understanding mechanisms whereby fatty acids can regulate the expression of genes for enzymes, apolipoproteins and receptors involved in lipid metabolism. Much of this has focussed on the impact of polyunsaturated fatty acids. The ability for polyunsaturated fatty acids to down-regulate the expression of genes for enzymes involved in fatty acid synthesis, including ACC, FAS and SCD, has been recognized for some time [1–3]. These genes appear to be repressed by both n-3 and n-6 PUFA though response to the former may be more profound.

There are at least three transcription factor families that play a major role in the fatty acid-induced regulation of hepatic gene transcription. These include PPARα, LXR, and SREBPs. PPARs and LXR are members of the nuclear hormone receptor superfamily of transcription factors. These ligand-activated intracellular transcription factors bind to specific motifs (response elements) within the promoters of genes as heterodimers with the retinoid X receptor (RXR). Ligand binding appears to initiate conformational changes which displace co-repressors and facilitate interaction with co-activators [34]. Through a reorganization of the chromatin and modification or recruitment of a range of factors involved in gene expression this leads to activation of transcription.

6. Peroxisome proliferator-activated receptor-alpha

PPARs are the most extensively characterised nuclear receptors that are regulated by fatty acids [35]. There are three isoforms, PPARα, PPARβ (also known as δ) and PPARγ, with PPARα being the predominant isoform in liver.

Ligand activation of PPARα is associated with transcriptional up-regulation of a wide range of genes for proteins associated with fatty acid oxidation and lipoprotein metabolism, these include; acyl coenzyme A oxidase, carnitine palmitoyl transferase 1, lipoprotein lipase, apolipoproteins AI and CIII [36]. A diverse range of compounds act as ligands for the various PPAR isoforms. This includes both saturated and unsaturated fatty acids, though in general PUFA tend to be more potent. While all three PPAR subtypes have been shown to bind n-3 and n-6 PUFA [37,38], affinity appears to be greatest for PPARα followed by PPARγ and PPARβ [37]. Furthermore, a range of eicosanoids, derived form n-3 and n-6 PUFA, have also been shown to be PPAR ligands, often with greater affinity than their parent molecules [37]. Interestingly, an endogenous ligand for PPARα has recently been described to be a phospholipid with a palmitate and an oleate moiety. The ability of this phospholipid to activate PPARα seems to require FAS [39], suggesting cellular rather than dietary-derived fatty acids may be more important. Furthermore, these observations suggest the different pools of fat within the cell itself may be important in determining fatty acid function.

7. Liver X receptors

LXRs have been described as sensors of cholesterol in the nucleus as they are activated by increased intracellular cholesterol concentrations. The naturally occurring oxysterols 22-R-hydroxycholesterol and 24, 25-epoxycholesterol and long chain fatty acids have been identified as ligands for LXRs [40]. There are two LXR family members, and LXRα is the most highly expressed isoform in the liver with LXRβ being ubiquitously expressed in most cell types, but not hepatocytes [41]. As with PPARs, LXRs bind to response elements as heterodimers with RXR. They regulate the expression of genes involved in sterol and fatty acid metabolism, particularly hepatic bile acid synthesis, including adenosine triphosphate-binding cassette protein A1 (ABCA1, involved in reverse cholesterol transport), apolipoprotein E, lipoprotein lipase, cholesterol ester transfer protein, and phospholipid transfer protein [42]. LXRs also play an important role in the regulation of genes involved in other aspects of metabolism including lipogenesis [43] and carbohydrate metabolism [44,45]. Recent evidence suggests that the regulation of lipogenesis by LXR is mediated primarily by its effects on SREBP1c expression (see below). A link between LXR activation and plasma LDL levels was also recently unravelled, with the identification of a novel LXR target gene termed IDOL, an E3 ubiquitin ligase that regulates LDL receptor at the protein level [46]. PUFA have been reported to inhibit the interaction of oxysterols with LXR and thereby inhibit its activation of target genes [47–49].

8. Sterol regulatory element binding proteins

SREBPs are membrane-bound members of the basic helix-loop-helix leucine zipper (bHLHLZ) family of transcription factors. They play a major role in regulating the expression of genes associated with lipid and lipoprotein metabolism [50–52]. Two SREBP genes produce three separate proteins (SREBP1a and 1c from one and SREBP2 from the other) with SREBP1c and SREBP2 being the predominant isoforms in the liver. Each of the SREBP proteins is synthesized in an immature form which resides on the endoplasmic reticulum (ER) and is then activated by a two-step proteolytic process in the Golgi apparatus [50]. SREBPs are transported to the Golgi through the action of an escort protein called SCAP [53]. SCAP contains binding sites for COPII proteins which cluster the SCAP-SREBP complex into COPII coated vesicles [54]. These vesicles then bud from the ER and fuse with the Golgi. Increases in cellular cholesterol cause conformational changes in SCAP leading to the retention of SREBP in the ER [55] as a result of binding of SCAP to two ER anchor proteins called Insig 1 and 2 [56]. The binding of Insig to SCAP inhibits its interaction with COPII proteins and thus retards its movement to the Golgi [53]. In turn, this leads to reduced proteolysis and a decrease in the nuclear active form of SREBP. The ER contains surprisingly low levels of cholesterol [57] and therefore provides an appropriate location for the regulation of cellular cholesterol levels. Indeed, SREBP cleavage in the ER was shown to be very responsive to relatively small changes in ER cholesterol levels [58]. This sensitivity to changes in intracellular sterol concentrations may not apply to all three SREBP isoforms. While evidence from studies using cell lines indicate that both SREBP1a and SREBP2 display such sensitivity to sterols, *in vivo* studies indicate SREBP1c may not respond to sterol depletion [59].

The transcriptionally active SREBP translocates to the nucleus where it binds to sterol regulatory elements (SREs) on target genes. While there is a sizeable overlap in the specificities of the individual SREBP isoforms, studies in transgenic mice over-expressing truncated nuclear active forms of SREBP in liver have shown specific roles for each isoform (Table 1). Over-expression of SREBP2 results in a vast increase in cholesterol synthesis through the up-regulation a number of the enzymes of the cholesterol synthetic pathway [60]. As discussed below SREBP2 always plays a major role in the regulation of expression of the LDL receptor. Over-expressing the nuclear form of SREBP1a results in the accumulation of cholesterol and triglycerides in the liver [61] while over-expressing SREBP1c causes an increase in

Table 1
Effect of over-expression of SREBPs on gene expression.

Target gene	SREBP1c	SREBP1a	SREBP2
LDL receptor	0.9	2.6	5.8
HMGCoA synthase	1.0	3.1	13.0
HMGCoA reductase	1.0	3.9	75.0
Squalene synthase	0.7	3.1	10.0
ACC	2.1	9.4	7.4
FAS	3.9	16.0	15.0
SCD1	3.0	6.8	2.7

Data shows fold change in hepatic mRNA concentration in transgenic mice over-expressing each of the SREBPs compared to wild-type animals (compiled from Refs. [60–62]).

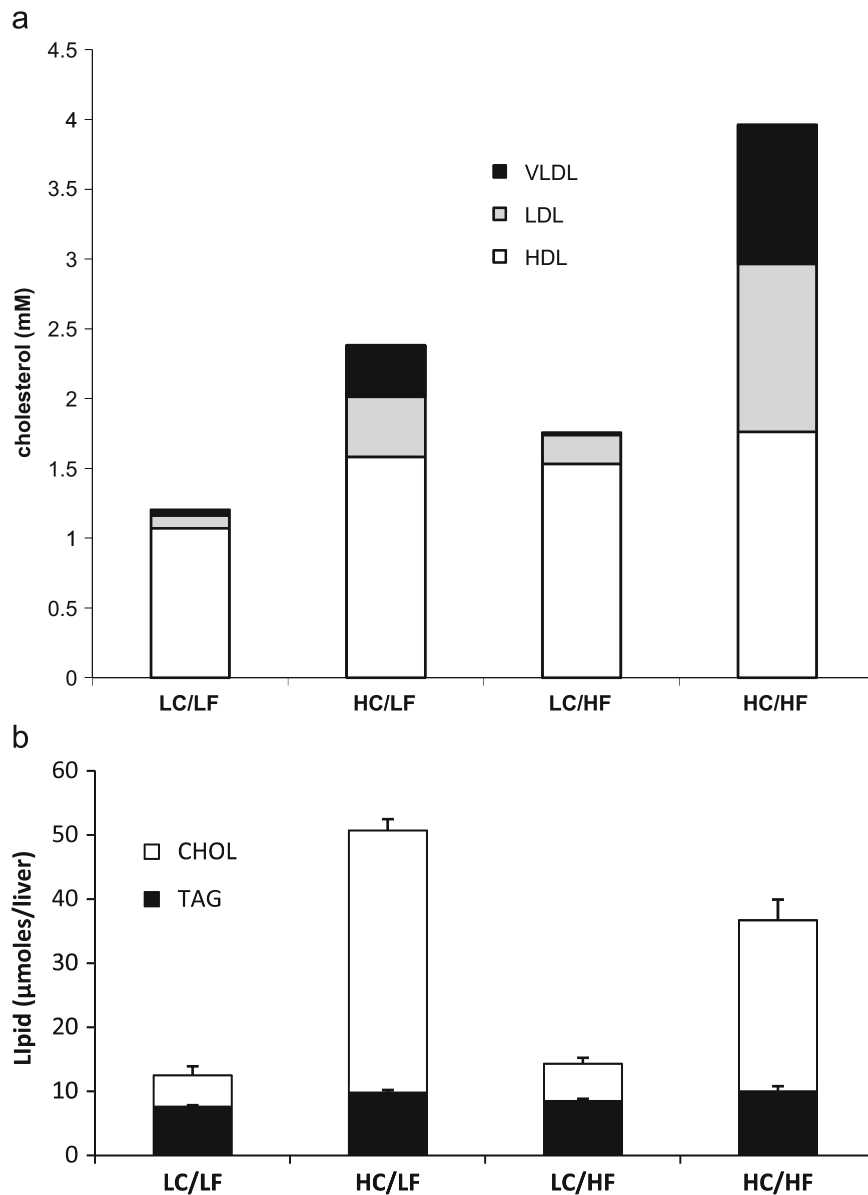

Fig. 1. Impact of dietary fat and cholesterol on (a) plasma lipoprotein cholesterol and (b) liver lipids in the hamster. Animals were fed a diet with (HF) or without (LF) 17.5% by weight fat formulated to mimic the fatty acid composition of a Western diet, in the absence (LC) or presence (HC) of 0.2% by weight cholesterol, for 4 weeks. Lipoproteins were separated by preparative ultracentrifugation and lipoprotein and liver lipids determined enzymatically.

liver triacylglycerol without a concomitant increase in cholesterol [62]. This suggests that while SREBP2 is predominantly involved in regulating cholesterol metabolism, SREBP1c primarily regulates fatty acid synthesis while SREBP1a can regulate enzymes of both the cholesterol and fatty acid synthetic pathways. However, as discussed below, there may not be as strict a demarcation of function of the SREBP isoforms as originally proposed.

PUFA have been shown to decrease nuclear SREBP-1 protein levels without affecting SREBP2 [63]. Thus, the role of SREBP1c in regulating lipogenic gene expression makes it an obvious candidate for mediating the effects of PUFA on lipogenesis. Cell culture studies have clearly shown that PUFA have the ability to reduce SREBP1a and 1c mRNA and SREBP-1 protein concentrations [64]. This effect of PUFA on SREBP mRNA concentrations has been attributed to both repression of gene transcription and increased turnover of the mRNA. As indicated above, at least part of this effect may be mediated through LXR. PUFA inhibition of the interaction of oxysterols with LXR may directly impact its ability to activate SREBP1c transcription [65–67]. Both the mouse and the human SREBP1c promoters have been shown to contain potential LXREs [68]. Thus, inhibition of the association of LXR with its oxysterol ligands by PUFA may repress activation of transcription of the SREBP1c gene by LXR. Another potential mechanism is the inhibition of LXR transcriptional activity by PUFA activation of PPARs. Over-expression of PPARα and PPARγ in HEK-293 cells has been shown to inhibit SREBP1c promoter activity and this effect was abolished by mutating the putative LXREs in the SREBP1c promoter [69]. It has been suggested that this was a result of increased competition between PPAR and LXR for association with its transcriptional partner RXR. There is also data to suggest PUFA may affect the nuclear abundance of SREBP isoforms by regulating proteasomal degradation of these proteins [70]. Thus, it appears that the regulation of lipogenic gene expression by dietary PUFA is a result of a complex interaction between a number of transcription factors including SREBPs, PPARs and LXR.

9. Saturated fatty acids and hepatic gene expression

So far, much of this discussion has focussed on the effects of PUFA on hepatic gene expression. However, as already discussed, it is clear that dietary saturated fatty acids have a major impact on the development of both raised plasma LDL concentrations and the genesis of NAFLD. Is there evidence that these effects are a result of modulation of hepatic gene expression by saturated fatty acids? Using the hamster as a model species, Dietschy and colleagues [71] clearly demonstrated that saturated fatty acids with between 12 and 16 carbon atoms reduced receptor mediated uptake of LDL by the liver. They concluded that reduced hepatic clearance of LDL (rather than increased production) was the predominant mechanism whereby such saturated fatty increased plasma LDL cholesterol concentrations [71,72]. Subsequently, in the same species we showed that diets enriched in trimyristin or tripalmitin reduced LDL receptor mRNA concentration in a dose-dependent manner [73,74]. More recently, we have been investigating the impact of a diet rich in saturated fat, formulated to mimic the fatty acid composition of a typical Western diet, on the expression of a range of genes in the liver (Vallim, Bennett and Salter, unpublished data). Fig. 1 indicates that, in the absence of added cholesterol such a diet modestly increases the concentration of VLDL and LDL cholesterol. When cholesterol is added to the diet the effect is potentiated. It was also of note that, in the absence of cholesterol, the high fat diet had little impact on hepatic lipid concentration. However, when cholesterol was added there was a modest increase in hepatic TAG concentration and a dramatic increase in storage of hepatic cholesterol ester. The lack of effect of the high fat diet (in the absence of cholesterol) on hepatic lipids is contrary to what is frequently seen in mice [75]. For example, Oosterveer et al. [31] fed male C57B1/6J mice a high saturated fat diet, similar to that used in our study, for a period of 6 weeks. At the end of the study hepatic TAG was approximately 80% higher than in control mice (16.4 vs. 9.2 μmol/g) fed a low fat chow. Total hepatic cholesterol (free+esterified) also increased but to a lesser extent (9.8 vs. 6.9 μmol/g).

We also examined the effect of dietary fat and cholesterol on the expression of a number of hepatic genes (Fig. 2). Fat and cholesterol feeding were both associated with a reduction in SREBP2 and LDL receptor mRNA concentrations. This is consistent with the well established role of SREBP2 in regulating LDL receptor expression through direct interaction with an SRE in the promoter of the receptor gene and is supported by the strong correlation seen between SREBP2 and LDL receptor mRNA across all of the diets (Fig. 3a). Cholesterol feeding and the associated accumulation of cholesterol in the liver are likely to inhibit the maturation of SREBP2 at the endoplasmic reticulum, thus limiting the availability of mature, nuclear SREBP2. As the SREBP2 gene contains an active SRE and has been shown to auto-regulate its own transcription this would lead to a reduction in transcription of the SREBP2 gene and further reduce nuclear SREBP availability. The mechanism whereby dietary fat is reducing SREBP2 mRNA remains to be established, but this is again likely to be the cause of the reduction in LDL receptor expression. It has been suggested that saturated fatty acids represent a poor substrate for cholesterol esterification [76] and that this may lead to a redistribution of cholesterol within the cell. This in turn may lead to a regulation of SREBP2 maturation by free cholesterol or its oxidized derivatives. The reduced storage of cholesterol in the liver of animals fed both cholesterol and fat (Fig. 1b) supports this hypothesis.

The effect of these diets on SREBP1c mRNA was very different to SREBP2. While dietary cholesterol modestly increased SRBBP1c mRNA concentrations, dietary fat had no effect. Analysis of the concentrations of immature and mature SREBP1c suggested that there was no significant difference in expression of maturation with any of the diets studied (data not shown). Despite the lack of effect on this regulator of lipogenic gene expression, major effects were seen in the mRNA concentrations for the potential target genes: ACC, FAS and SCD1. Both cholesterol and fat reduced ACC and FAS mRNA concentration but the effect was not additive. By contrast, while cholesterol on its own had very little effect on SCD1 expression, dietary fat dramatically reduced SCD1 mRNA. Adding cholesterol to the diet appeared to markedly attenuate the effect of fat on SCD1 expression. No significant correlation was seen between SREBP1c mRNA and either ACC mRNA (r=0.255, p=0.174) or FAS mRNA (Fig. 3b, r=0.182, p=0.336) but there was with SCD1 mRNA (r=0.508, p=0.004). However, while both ACC mRNA (r=0.692, $p<0.001$) and FAS mRNA (Fig. 3c, r=0.703, $p<0.001$) strongly correlated with SREBP2 mRNA, SCD1 mRNA

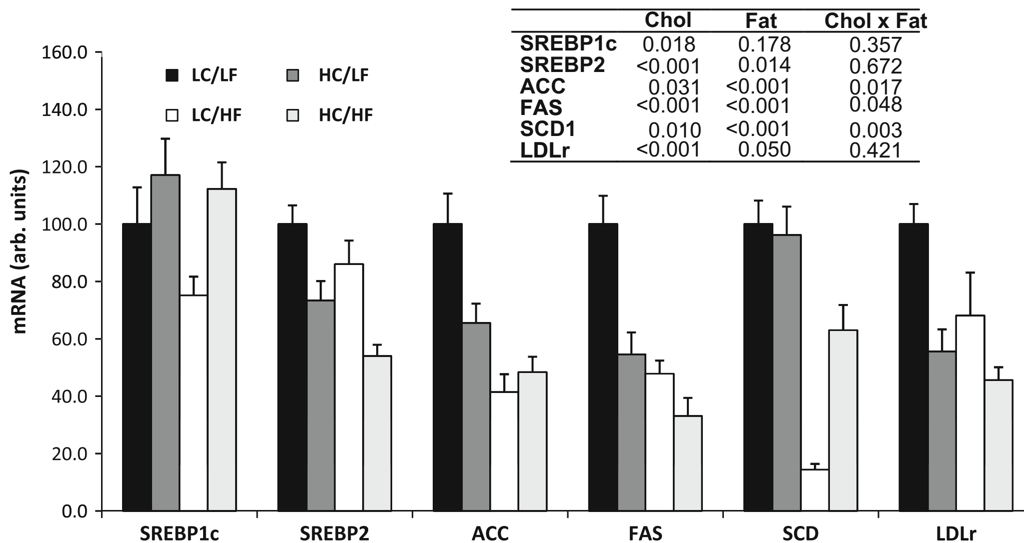

Fig. 2. Impact of dietary fat and cholesterol on hepatic mRNA concentrations in the hamster. Animals were treated as described in Fig. 1. Concentrations of mRNA for SREBP1c and 2, ACC and FAS were determined by quantitative real-time PCR and are normalized to mRNA concentrations for the house-keeping gene TATA box-binding protein. Data are expressed relative to the mean value for animals on the LC/LF diet. Data were analysed by 2-way analysis of variance with dietary cholesterol and fat as independent variables (p-values for independent or interactive effects are presented).

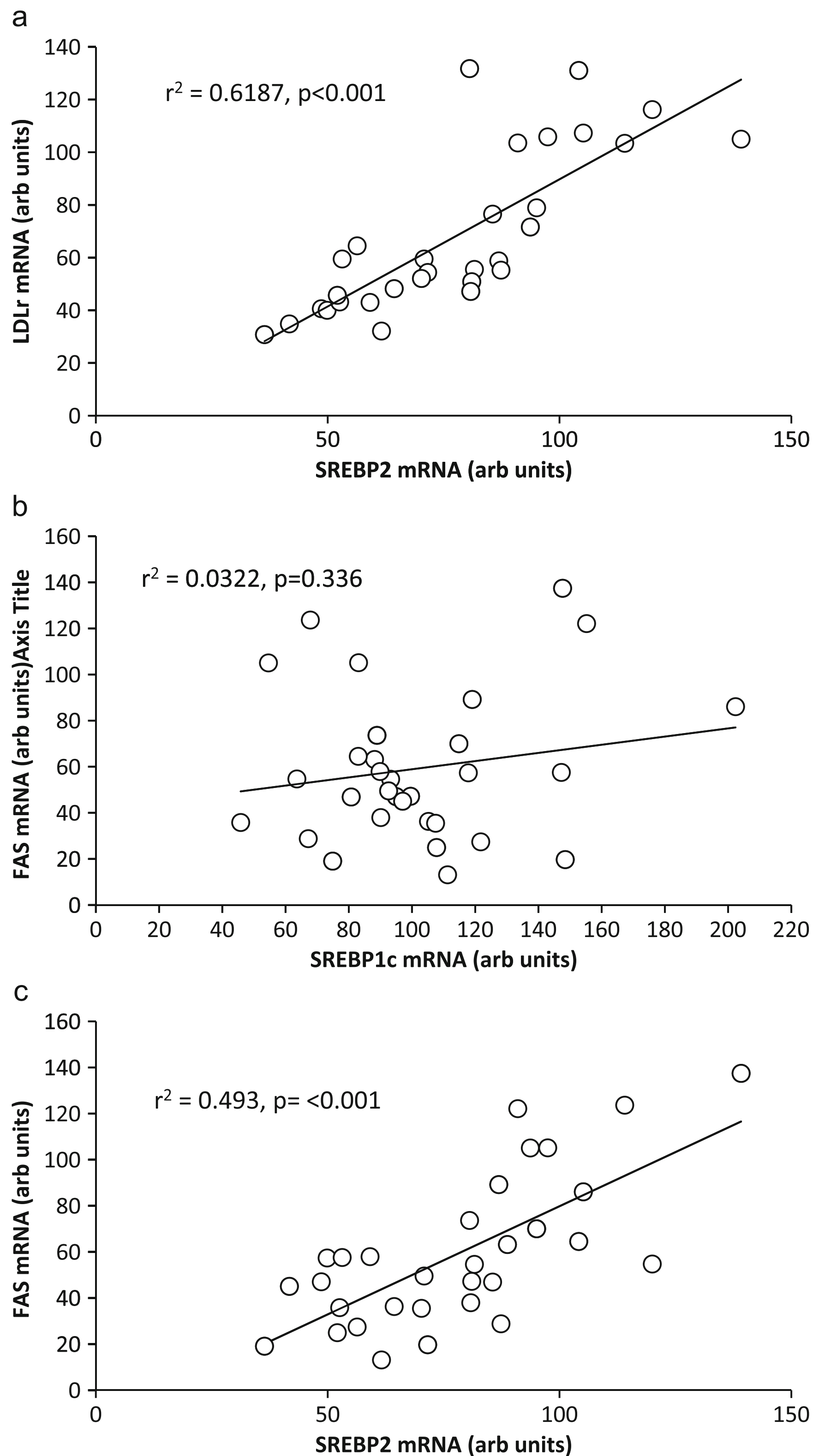

Fig. 3. Correlation of hepatic SREBP mRNA with LDL receptor and fatty acid synthase mRNA. Data represent hepatic mRNA values for individual animals as described in Fig. 2: (a) SREBP2 mRNA vs. LDRr mRNA, (b) SREBP1c mRNA vs. FAS mRNA and (c) SREBP2 mRNA vs. FAS mRNA.

did not (r=0.093, p=0.624). While such statistical associations must be viewed with caution they do suggest differences in the regulation of these lipogenic enzymes by SREBP isoforms and that, in certain situations ACC and FAS could possibly be regulated by SREBP2 as well as SREBP1c. This is supported by early data looking at the impact of over-expression of individual SREBP isoforms on gene expression in the mouse (Table 1). Over-expression of SREBP2 increased ACC and FAS (but not SCD) mRNA to a greater extent than SREBP1c [60]. Interaction of SREBP2 (either as a homodimer or heterodimers with SREBP1a or 1c) with

the FAS promoter has been shown to activate reporter gene expression [77]. Furthermore, increasing SREBP2 expression *in vivo*, by severely reducing cholesterol availability in mice, has been shown to increase FAS expression [78]. This was shown to be associated with an increased binding of SREBP2 to the FAS gene promoter.

The effects of the high fat diet on ACC, FAS and SCD expression in the hamster are very different from those in the mouse. In the study by Oosterveer et al. [31], mRNA for each of these three genes, and indeed for SREBP1c, was reported to increase in response to the high fat diet. Earlier studies by Lin et al. [32] have suggested that this may be due to an increase in expression of the SREBP1c activator, PGC-1β, although this was a very short-term (48 h) feeding study. Interestingly, despite the changes in ACC and FAS expression, Oosterveer et al. [31] reported that the accumulation of hepatic lipid, in mice fed the high fat diet, was associated with chain elongation and desaturation rather than *de novo* fatty acid synthesis. The reduction in SCD1 expression in hamsters (as compared to the increase in mice) on the saturated fat-rich diet (Fig. 2) may explain why in this species we saw no lipid accumulation.

10. Conclusions

High intakes of dietary saturated fatty acids increase plasma cholesterol, particularly that within the LDL fraction. This appears to be through down-regulation of hepatic LDL receptor expression, probably through modulation of activity of SREBP2. The exact mechanism by which saturated fatty acids regulated SREBP2 activity still remain to be established. The effects of dietary saturated fat on hepatic steatosis appear to be species dependent. In the mouse, such diets increase SREBP1c expression and its downstream targets. Recent evidence suggest that an increase in elongation and desaturation of fatty acids in the liver produces increased amounts of oleic acid and may be central to this effect [31]. By contrast, a diet rich in saturated fat was shown to down-regulate lipogenic gene expression in the hamster (including SCD1) which appeared resistant to the effects of such fatty acids on hepatic lipid accumulation. These experiments also provide preliminary *in vivo* evidence that, at least in some circumstances, the SREBP isoforms may be more promiscuous than generally believed and that SREBP2 can play a role in regulating lipogenic gene expression.

Conflicts of interest

The authors report no other relevant affiliations or financial involvement with any organization relating to the subject matter of this manuscript.

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References

[1] D.B. Jump, n-3 polyunsaturated fatty acid regulation of hepatic gene transcription, Curr. Opin. Lipidol. 19 (2008) 242–247.

[2] A.M. Salter, E.J. Tarling, Regulation of gene transcription by fatty acids, Animal 1 (2007) 1314–1320.

[3] H. Sampath, J. Ntambi, Polyunsaturated fatty acid regulation of genes of lipid metabolism, Annu. Rev. Nutr. 25 (2005) 317–340.

[4] C.J. Packard, J. Shepherd, Physiology of the lipoprotein transport system: an overview of lipoprotein metabolism, in: D.J. Betteridge, D.R. Illingworth, J. Shepherd (Eds.), Lipoproteins in Health and Disease, Arnold, London, 1999, pp. 3–316.

[5] A.M. Salter, E.H. Mangiapane, The lipoproteins, Diet, Lipoprotein and Coronary Heart Disease: A Biochemical Perspective, Nottingham University Press, Nottingham, 1999, pp. 41–66.

[6] P.J. Babin, G.F. Gibbons, The evolution of plasma cholesterol: direct utility or a "spandrel" of hepatic lipid metabolism, Prog. Lipid Res. 48 (2009) 73–91.

[7] K.-A. Rye, C.A. Bursill, G. Lambert, F. Tabet, P.J. Barter, The metabolism and ant-atherogenic properties of HDL, J. Lipid Res. 50 (2009) S195–S200.

[8] D.A. White, A.J. Bennett, M.A. Billett, A.M. Salter, The assembly of triacylglycerol-rich lipoproteins; an essential role for the microsomal triacylglycerol transfer protein, Br. J. Nutr. 80 (1998) 219–229.

[9] R. Gambino, M. Cassader, G. Pagano, M. Durazzo, G. Musso, Polymophism in microsomal triglyceride transfer protein: a link between liver disease and atherogenic postprandial lipid profile in NASH, Hepatology 45 (2007) 1097–1107.

[10] J. Ye, J.Z. Li, Y. Liu et al., Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B, Cell Metab. 9 (2009) 177–190.

[11] M.M. Hussain, D.K. Strickland, A. Bakillah, The mammalian low-density lipoprotein receptor family, Annu. Rev. Nutr. 19 (1999) 141–172.

[12] T.B. Trigatti, M. Kreiger, A. Rigotti, Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1732–1738.

[13] M.S. Brown, J.L. Goldstein, A proteolytic pathway that controls the cholesterol content of membranes, cells and blood, Proc. Natl. Acad. Sci. USA 96 (1999) 11041–11048.

[14] J.D. Horton, J.L. Goldstein, M.S. Brown, SREBPs: activators of the complete programme of cholesterol and fatty acid synthesis in the liver, J. Clin. Invest. 109 (2002) 1125–1131.

[15] A.M. Salter, D.A. White, Effects of dietary fat on cholesterol metabolism: regulation of plasma LDL concentrations, Nutr. Res. Rev. 9 (1996) 241–257.

[16] Anon, The diet and all-causes death rate in the Seven Countries Study, Lancet 318 (8237) (1981) 57–61.

[17] A. Keys, J.T. Anderson, F. Grande, Serum cholesterol response to changes in diet IV. Particular saturated fatty acids in the diet, Metabolism 14 (1965) 776–787.

[18] D.M. Hegsted, R.B. McGandy, M.L. Myers, F.J. Stare, Quantitative effects of dietary fat on serum cholesterol in man, Am. J. Clin. Nutr. 17 (1965) 281–295.

[19] R.P. Mensink, M.B. Katan, Effects of dietary fatty acids on serum lipds and lipoproteins. A meta-analysis of 27 trials, Arterioscler. Thromb. 12 (1992) 911–919.

[20] R.P. Mensink, P.L. Zock, A.D.M. Kester, M.B. Katan, Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins,: a meta-analysis of 60 controlled trials, Am. J. Clin. Nutr. 77 (2003) 1146–1155.

[21] D. Preiss, N. Sattar, Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations, Clin. Sci. 115 (2008) 141–150.

[22] G. Musso, R. Gambino, M. Cassader, Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD), Prog. Lipid Res. 48 (2009) 1–26.

[23] C.D. Byrne, R. Olufadi, K.D. Bruce, F.R. Cagampang, M.H. Ahmed, Metabolic disturbances in non-alcoholic fatty liver disease, Clin. Sci. 116 (2009) 539–564.

[24] J.B. Dixon, P.S. Bhathal, P.E. O'Brien, Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese, Gastroenterology 121 (2001) 91–100.

[25] R.S. Rector, J.P. Thyfault, Y. Wei, J.A. Ibdah, Non-alcoholic fatty liver disease and the metabolic syndrome:an update, World J. Gastroenterol. 14 (2008) 185–192.

[26] D.G. Tiniakos, M.B. Vos, E.M. Brunt, Nonalcoholic fatty liver disease: pathology and pathogenesis, Annu. Rev. Pathol. Mech. Dis. 5 (2010) 145–171.

[27] C.P. Day, O.F. James, Steatohepatitis: a tale of two "hits"?, Gastroenterology 114 (1998) 842–845.

[28] M. Malaguarnera, M. Di Rosa, F. Nicoletti, L. Malaguarnera, Molecular mechanisms involved in NAFLD progression, J. Mol. Med. 87 (2009) 679–695.

[29] S.M. Grundy, H.B. Brewer, J.I. Cleeman, S.C. Smith, C. Lenfant, Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/ American Heart Association Conference on scientific issues related to definition, Circulation 109 (2004) 433–438.

[30] K.L. Donnelly, C.I. Smith, S.J. Schearzenberg, J. Jessurun, M.D. Boldt, E.J. Parks, Sources of fatty acids stored in liver and secreted via lipoproteins in patients with non-alcoholic fatty liver disease, J. Clin. Invest. 115 (2005) 1343–1351.

[31] M.H. Oosterveer, T.H. van Dijk, U.J.F. Tietge, et al., High fat feeding induces hepatic fatty acid elongation in mice, PLoS ONE 4 (6) (2009) e6066.

[32] J. Lin, R. Yang, P.T. Tarr, et al., Hyerlipidemic effects of dietary saturated fats mediated through PGC-1β coactivation of SREBP, Cell 120 (2005) 261–273.

[33] H. Sampath, M. Miyazaki, A. Dobrzyn, J.M. Ntambi, Stearoyl-CoA desaturase-1 mediates the prolipogenic effects of dietary saturated fat, J. Biol. Chem. 282 (2007) 2483–2493.

[34] C.K. Glass, M.G. Rosenfeld, The coregulator exchange in transcriptional functions of nuclear receptors, Genes Dev. 14 (2000) 121–141.

[35] B. Desvergne, W. Wahli, Peroxisome proliferators-activated receptors: nuclear control of metabolism, Endocr. Rev. 20 (1999) 649–688.

[36] J.D. Brown, J. Plutzky, Peroxisome proliferator-activated receptors as transcriptional nodal points and therapeutic targets, Circulation 115 (2007) 518–533.

[37] G. Krey, O. Braissant, F. L'Horset, et al., Fatty acids, eicosanoids, and hypolipidemic agents identified a sligands of peroxisome proliferator-activated

receptors by coactivator-dependent receptor ligand assay, Mol. Endocrinol. 11 (1997) 779–791.
[38] S.A. Kliewer, S.S. Sundeth, S.A. Jones, et al., Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and β, Proc. Natl. Acad. Sci. USA 94 (1997) 4318–4323.
[39] M.V. Chakravarthy, I.J. Lodhi, L. Yin, et al., Identification of a physiologically relevant endogenous ligand for PPARalpha in liver, Cell 138 (2009) 476–488.
[40] B.A. Janowski, M.J. Grogan, S.A. Jones SA, et al., Structural requirements of ligands for the oxysterol liver X receptors, LXRα and LXR®, Proc. Natl. Acad. Sci. 96 (1999) 266–271.
[41] J.J. Repa, D.J. Mangelsdorf, The role of orphan nuclear receptors in the regulation of cholesterol homeostasis, Annu. Rev. Cell Dev. Biol. 16 (2000) 459–481.
[42] R. Geyeregger, M. Zeyda, T.M. Stulnig, Liver X receptors in cardiovascular and metabolic disease, Cell. Mol. Life Sci. 63 (2006) 524–539.
[43] D.J. Peet, S.D. Turley, W. Ma, et al., Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRα, Cell 93 (1998) 693–704.
[44] B.A. Laffitte, L.C. Chao, J. Li, et al., Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue, Proc. Natl. Acad. Sci. USA 100 (2003) 5419–5424.
[45] G. Cao, Y. Liang, C.L. Broderick, et al., Antidiabetic action of a liver X receptor agonist mediated by inhibition of hepatic gluconeogenesis, J. Biol. Chem. 278 (2003) 1131–1136.
[46] N. Zelcer, C. Hong, R. Boyadjian, P. Tontonoz, LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor, Science 325 (2009) 100–104.
[47] V.C. Hannah, J. Ou, A. Luong, J.L. Goldstein, M.S. Brown, Unsaturated fatty acids downregulate SREBP isoforms 1a and 1c by two mechanisms, J. Biol. Chem. 276 (2001) 4365–4372.
[48] T. Yoshikawa, H. Shimano, M. Amemiya-Kudo, et al., Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter, Mol. Cell. Biol. 21 (2001) 2991–3000.
[49] T. Yoshikawa, H. Shimano, N. Yahagi, et al., Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements, J. Biol. Chem. 277 (2002) 1705–1711.
[50] M.S. Brown MS, J.L. Goldstein, A proteolytic pathway that controls the cholesterol content of membranes, cells and blood, Proc. Natl. Acad. Sci. USA 96 (1999) 11041–11048.
[51] J.D. Horton, J.L. Goldstein, M.S. Brown, SREBPs: activators of the complete programm of cholesterol and fatty acid synthesis in the liver, J. Clin. Invest. 109 (2002) 1125–1131.
[52] D. Eberlé, B. Hegarty, P. Bossard, P. Ferré, F. Foufelle, SREBP transcription factors: master regulators of lipid homeostasis, Biochimie 86 (2004) 839–848.
[53] J.L. Goldstein JL, R.A. DeBose-Boyd, M.S. Brown, Protein sensors for membrane sterols, Cell 124 (2006) 35–46.
[54] L.-P. Sun L-P, L. Li, J.L. Goldstein, M.S. Brown, Insig required for sterol-mediated inhibition of Scap/SREBP binding to COPII proteins in vitro, J. Biol. Chem. 280 (2005) 26483–26490.
[55] A. Nohturfft, D. Yabe, J.L. Goldstein, M.S. Brown, P.J. Espenshade, Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes, Cell 102 (2000) 315–323.
[56] T. Yang, P.J. Espenshade, M.E. Wright, D. Yabe, Y. Gong, R. Aebersold, J.L. Goldstein, M.S. Brown MS, Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs to the ER, Cell 110 (2002) 489–500.
[57] Y. Lange, T.L. Steck, J. Ye, M.H. Lanier, V. Molugu, D. Ory, Regulation of fibroblast mitochondrial 27-hydroxycholesterol production by active plasma membrane cholesterol, J. Lipid Res. 50 (2009) 1881–1888.
[58] A. Radhakrishnan, J.L. Goldstein, J.G. McDonald, M.S. Brown, Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance, Cell Metab. 8 (2008) 512–521.
[59] Z. Sheng, H. Otani, M.S. Brown, J.L. Goldstein, Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver, Proc. Natl. Acad. Sci. USA 92 (1995) 935–938.
[60] J.D. Horton, I. Shimomura, M.S. Brown, R.E. Hammer, J.L. Goldstein, N. Shimano, Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2, J. Clin. Invest. 101 (2000) 2331–2339.
[61] H. Shimano, J.D. Horton, R.E. Hammer, I. Shimomura, M.S. Brown, J.L. Goldstein, Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a, J. Clin. Invest. 98 (1996) 1575–1584.
[62] H. Shimano, J.D. Horton, I. Shimomura, R.E. Hammer, M.S. Brown, J.L. Goldstein, Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells, J. Clin. Invest. 99 (1997) 846–854.
[63] J.D. Horton, Y. Bashmakov, I. Shimomura, H. Shimano H, Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice, Proc. Natl. Acad. Sci. USA 95 (1998) 5987–5992.
[64] T.S. Worgall, S.L. Sturley, T. Soe, T.F. Osborne, R.J. Deckelbaum, Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of sterol regulatory element binding protein, J. Biol. Chem. 273 (1998) 23537–23540.
[65] V.C. Hannah, J. Ou, A. Luong, J.L. Goldstein, M.S. Brown, Unsaturated fatty acids downregulate SREBP isoforms 1a and 1c by two mechanisms, J. Biol. Chem. 276 (2001) 4365–4372.
[66] T. Yoshikawa, H. Shimano, M. Amemiya-Kudo, et al., Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter, Mol. Cell. Biol. 21 (2001) 2991–3000.
[67] T. Yoshikawa, H. Shimano, N. Yahagi, et al., Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements, J. Biol. Chem. 277 (2002) 1705–1711.
[68] E. Tarling, A. Salter, A. Bennett, Transcriptional regulation of human SREBP-1c (sterol-regulatory-element-binding protein-1c): a key regulator of lipogenesis, Biochem. Soc. Trans. 32 (2004) 107–109.
[69] T. Yoshikawa, T. Ide, H. Shimano, et al., Cross-talk between peroxisome proliferator-activated receptor (PPAR) alpha and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signalling, Mol. Endocrinol. 17 (2003) 1240–1254.
[70] D. Botolin, Y. Wang, B. Christian, D.B. Jump, Docosahexaenoic acid (22:6,n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk- and 26S proteasome-dependent pathways, J. Lipid Res. 47 (2006) 181–192.
[71] L.A. Woollett, D.K. Spady, J.M. Dietschy, Regulatory effects of the saturated fatty acids 6:0 through 18:0 on hepatic low density lipoprotein receptor activity in the hamster, J. Clin. Invest. 89 (1992) 1133–1141.
[72] D.K. Spady, L.A. Woollett, J.M. Dietschy, Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids, Annu. Rev. Nutr. 13 (1993) 355–381.
[73] A.J. Bennett, M.A. Billett, A.M. Salter, et al., Modulation of hepatic apolipoprotein-b, 3-hydroxy-3-methylglutaryl-coa reductase and low-density-lipoprotein receptor messenger-RNA and plasma-lipoprotein concentrations by defined dietary fats—comparison of trimyristin, tripalmitin, tristerin and triolein, Biochem. J. 311 (1995) 167–173.
[74] A.M. Salter, E.H. Mangiapane, A.J. Bennett, et al., The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids, Br. J. Nutr. 79 (1998) 195–202.
[75] Q.M. Anstee, R.D. Goldin, Mouse models in non-alcoholic fatty liver disease and steatohepatisis research, Int. J. Exp. Pathol. 87 (2006) 1–16.
[76] C.M. Daumerie, L.A. Woollett, J.M. Dietschy, Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools, Proc. Natl. Acad. Sci. USA 89 (1992) 10797–10801.
[77] S. Datta, T.F. Osborne, Activation domains from both monomers contribute to transcriptional stimulation of sterol regulatory element-binding protein dimmers, J. Biol. Chem. 280 (2005) 3338–3345.
[78] M.K. Bennett, Y.-K. Seo, S. Datta, D.-J. Shin, T.F. Osborne, Selective binding of sterol regulatory element-binding protein isoforms and co-regulatory proteins to promoters for lipid metabolic genes in liver, J. Biol. Chem. 283 (2008) 15628–15637.