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Role of lipid droplet proteins in liver steatosis

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Mini Review

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Abstract

Five proteins of the perilipin (Plin) family such as Plin1 (perilipin) Plin2 (adipose differentiation-related protein), Plin3 (tail-interacting protein of 47 kiloDaltons), Plin4 (S3-12) and Plin5 (myocardial lipid droplet protein) are characterized as lipid droplet (LD) proteins in adipocytes. Recent reports have demonstrated that fat-specific protein 27 (FSP27) and hypoxia-inducible protein 2 (HIG2) are also thought to be novel LD proteins in addition to proteins of the Plin family. Growing evidence have shown that LD proteins play a role in the pathophysiology in the fatty liver disease which is characterized by hepatocytes containing LD with excessive neutral lipid. Studies showed LD proteins such as Plin1, Plin2, Plin3, Plin5, FSP27 and HIG2 are expressed in the liver steatosis. Among them, high fat diet increases expression of Plin2 and/or FSP27 through activation of peroxisome proliferator activated receptor γ to develop fatty liver. In this article, recent advances on the role of LD proteins in pathophysiology of fatty liver diseases are summarized.

Introduction

Lipid droplets (LD) are spherical organelles found in many types of eukaryotic cells composed of a core of neutral lipids covered by a monolayer of phospholipids, free cholesterol and specific proteins. Proteomic studies have identified LD proteins involved in lipid metabolism and transport, intracellular trafficking, signaling, and cytoskeletal organization [13. 52, 62]. Accumulating evidence have shown that the perilipin (Plin) family consisted of five proteins such as Plin1 (perilipin), Plin2 (adipose differentiation-related protein: ADRP), Plin3 (tail-interacting protein of 47 kiloDaltons: TIP47), Plin4 (S3-12) and Plin5 (myocardial lipid droplet protein: MLDP) are characterized as LD proteins in adipocytes [3 31]. In addition, recent reports have demonstrated the characteristics of novel LD proteins, other than Plin family. These include mouse fat-specific protein 27 (FSP27) and its closely related human homolog cell death-inducing DFF45-like effector C (CIDEC), and hypoxia-inducible protein 2 (HIG2) [16, 36]. Fatty liver is characterized

by LD containing excessive neutral lipid in hepatocytes [1].   In addition to

a number of studies on roles of LD proteins in adipocytes, growing

evidence have shown the relationship between LD proteins and liver

steatosis.   This review summarizes the role of LD proteins in the

pathophysiology of liver steatosis.

Plin1 (Perilipin)

Plin1, the founding member of the Plin family of proteins that has

the ability to bind LD [38], is a marker of adipocyte differentiation and thus

has been used as a reporter gene to identify regulators of adipogenesis.

Expression of the Plin1 gene is regulated primarily by peroxisome

proliferator activated receptor γ (PPARγ) (Table 1) [9, 44].   Because Plin1

has been reported to be restricted to adipocytes and certain steroidogenic

cells [4, 21] and to be absent in normal (Table 1) and steatotic livers of

various species [6, 10], it had been considered that Plin1 would not be

implicated in the pathophysiology of fatty liver diseases.   However, it is

possible for Plin1 to be involved in the process of lipid accumulation in the

liver under certain pathological conditions in human as described below.

Straub et al. [49] examined the expression of three Plin family proteins

including Plin1 in human fatty liver diseases and characterized the LD

proteins in normal and diseased liver connected with LD accumulation.

According to the study, antibodies against Plin1 reacted positively at the

LD-cytoplasm interface in all steatotic liver tissues analyzed, whereas

nonsteatotic hepatocytes were negative (Table 1).　Plin1 staining also was

positively correlated with the degree of steatosis.　Thus, Plin1, which was

thought to be characteristic for LD of adipocytes and steroidogenic cells,

becomes *de novo* expression in hepatocytes of human steatotic liver.

Plin2 (Adipose differentiation-related protein: ADRP)

Plin2 is a protein that was first identified as an RNA transcript

significantly induced during differentiation of cultured adipocytes [28, 29].

Sequence similarity of Plin2 to the Plin family led to the discovery that

Plin2 coats lipid storage droplets in a variety of cell lines, including early

differentiating 3T3-L1 adipocytes [5].   Later analysis revealed that Plin2

is intrinsically associated with the surface of LD and is believed to function

in the intracellular mobilization and storage of neutral lipids [5, 23, 50].   It

has been demonstrated that Plin2 abundance is directly proportional to the

levels of intracellular lipid found within cells and Plin2 is increased in

specific diseases involving fat accumulation [23, 48, 55].   Thus, a role of

Plin2 in adipocytes has been established.   Additionally, recent evidence

has showed that Plin2 expression is increased in the hepatocytes of fatty

liver.   Among LD proteins reported, Plin2 seems to be the best

characterized protein in fatty liver diseases.

There are a couple of studies on Plin2 expression in human fatty

liver.   Immunohistochemical examination for detecting Plin2 protein in

human liver tissues was performed in 2006 (Table 1) [40].   Liver samples

were obtained from normal and fatty liver patients, respectively.   Plin2

protein was strongly expressed in liver samples obtained from patients with

fatty liver when compared with normal liver samples.   Plin2 protein was

detected in the surface of LD in the hepatocytes.   Increased expression of

Plin2 protein in liver tissues in patients with fatty liver was furthermore

confirmed by Straub et al. in 2008 [49] and Fujii et al. in 2009 (Table 1)

[15].   Thus, expression of Plin2 in the surface of LD in the hepatocytes is

a pathophysiological change in human fatty liver disease.

High fat diet in mice and leptin deficient *ob/ob* mice develop liver

steatosis.   Plin2 expression in the liver was also examined in these models.

Plin2 protein was higher expressed in the surface of LD in the hepatocytes

in the *ob/ob* mice when compared with control mice (Table 1) [40, 46].

Real-time PCR revealed an increased expression of Plin2 mRNA in the

liver of *ob/ob* mice, suggesting *de novo* expression of Plin2.   Plin2 mRNA

and protein expression was also increased in the liver by high fat diet

(Table 1) in a time-dependent manner.   Higher expression of Plin2 in

hepatocytes of mice fed with high fat diet is seen as early as 2 weeks on the

diet.   Since lipid accumulation in the liver which could be detected by

microscopy is significant within 2 weeks after high fat diet [27], high fat diet increased Plin2 expression in the hepatocytes that is going to accumulate lipid. Overexpression of Plin2 is associated with expansion of LD pools and increased cellular triacylglycerol mass even when the cells were cultured in delipidated serum [26], suggesting that increased expression of Plin2 by itself is capable of inducing lipid accumulation in cells. Chang et al. [6] showed that Plin2-deficient mice display a 60 % reduction in hepatic triglyceride and are resistant to diet-induced fatty liver, and Imai et al. [25] reported that a Plin2 antisense oligonucleotide reduced liver steatosis in *ob/ob* and diet-induced obese mice, strongly suggesting that Plin2 plays a key role in the development of diet-induced liver steatosis. These results suggest that Plin2 expression is likely related to the formation of lipid droplets in the hepatocytes by high fat diet.

Inoue et al. have demonstrated that lipid accumulation in the liver was observed under microscopy as early as 2 weeks after high fat diet containing approximately 80 % cholesterol and that high fat diet for 12

weeks developed a fatty liver phenotype [27].   In the fatty liver model, PPARγ mRNA and protein expression was specifically up-regulated in the liver.   With regard to the functional role of PPARγ expression in hepatic steatosis, a couple of reports demonstrated that liver-specific disruption of PPARγ in *ob/ob* mice improves fatty liver [37] and steatosis in the mouse liver was induced by PPARγ overexpression [61], indicating that PPARγ expression plays a key role in the development of lipid accumulation in the hepatocytes.   In other words, up-regulation of PPARγ gene in the liver would result in steatosis while lipid accumulation would be prevented by down-regulation of PPARγ gene in the hepatocytes.   Thus PPARγ should be considered as a key molecule in the process of accumulating lipid in the hepatocytes.   A couple of studies have shown that Plin2 gene expression is regulated by a PPARγ-dependent mechanism (Table 1).   Targett-Adams et al. [51] have found a PPAR response element (PPRE) with the sequence 5'-AGGTGA A AGGGCG-3' within promoter region of Plin2 gene. Mutational analysis revealed that Plin2 PPRE specifically mediated the

up-regulation of transcription in response to activation by agonists of PPAR

subtypes in both rat and human hepatocyte-derived cell lines, suggesting

that PPARs control transcription of Plin2 by means of a functional PPRE

located within its promoter.   In fact, troglitazone significantly

up-regulated Plin2 mRNA expression in a human hepatic cell line, HepG2

[40].   It was also demonstrated that hepatic cells overexpressing PPARγ2

accumulates LD with a corresponding increase in Plin2 expression [46],

suggesting that PPARγ activation in human hepatocytes up-regulates Plin2

gene expression.   Based on these evidence, we would speculate that high

fat diet induces Plin2 expression in hepatocytes probably through increased

expression of PPARγ, followed by induction of liver steatosis (Figure 1).

Intraperitoneal injection of lipopolysaccharide (LPS) transiently

induced lipid accumulation in the mouse liver [41].   Microscopic

observation revealed that lipid accumulation started 12 h after LPS

injection.   In this model, up-regulation of Plin2 was observed in the liver

before detection of steatosis by microscopic observation.   It was also

demonstrated that LPS failed to increase PPARγ expression while LPS

potently inhibited expression of PPARα and its target genes involved in

fatty acid oxidation in the liver, indicating that PPARα but not PPARγ plays

a vital role in the LPS-induced lipid accumulation in the liver through

inhibition of fatty acid oxidation.   These results indicate that up-regulation

of Plin2 expression may be a common molecular event in lipid

accumulation in the liver in spite of the causes/mechanisms.

In addition to PPARs, it has been suggested that other nuclear

receptors such as liver X receptor (LXR) [24] might regulate expression of

LD proteins.   Kotokorpi et al. [32] have shown that the human Plin2 gene

is a direct liver X receptor (LXR) target gene in hepatocytes and the partial

LXR agonist, GW3965, significantly induces Plin2 expression in human

primary hepatocytes, suggesting that LXR is involved in the regulation of

gene expression of Plin2 in hepatocytes.   Thus, not only PPARs but also

other transcriptional factors including LXR might play a role in gene

expression of LD proteins. As shown by Grefhorst et al. [23],

pharmacological LXR activation by its ligand in mice leads to development of hepatic steatosis.　Because the expression of Plin2 correlates to fat storage in the liver [23, 35, 48, 55], these findings suggest that LXR would be implicated in the process of the accumulation of lipid through increasing Plin2 expression in hepatocytes.

Plin3 (Tail-interacting protein of 47 kiloDaltons: TIP47)

Plin3 was identified as a relative of Plin1 and Plin2 by sequence similarity of their amino-termini [23, 58]. Whereas Plin1 and Plin2 are both regulated by PPARs as described above, Plin3 is not PPAR-regulated (Table 1) [9].　In contrast to Plin1 and similar to Plin2, Plin3 is expressed in almost tissues ubiquitously and is indeed expressed at significant levels in the liver [6].　With regard to the expression of Plin3 in fatty liver, Straub et al. [49] have examined the expression of lipid-droplet-related proteins in human liver samples.　In representative normal and steatotic liver specimens, all cases were positive for Plin2 and Plin3, and the

staining intensity for Plin2, but not Plin3, positively correlated with the amount of LD. Thus, Plin3 is indeed expressed in not only normal liver but also fatty liver.   However, Plin3 failed to be associated with the amount of LD in the liver as described above, suggesting a minor role in the development of fatty liver diseases.   It has been also showed that Plin3 was associated with a minor subset of very small-size hepatocellular LD, whereas Plin2 and Plin1 stained larger size LD [49], suggesting Plin3 plays a different role in biology of LD when compared with Plin2.

Plin4 (S3-12)

Plin4 is expressed primarily in the white adipose tissue, and to lesser degree in skeletal muscle and heart [9, 47, 59], indicating adipocyte-restricted expression of Plin4 as well as Plin1.   Transcription of Plin4 in adipocytes is stimulated by PPARs as similarly as Plin1 and Plin2 (Table 1) [9, 45].   Plin4 mRNA was undetectable in normal liver (Table 1) [45].   Although we do not know at this moment whether Plin4 would be

involved in the process of the formation of liver steatosis, further studies

should be performed because of the following.   The overexpression of

PPARγ1 in transgenic mouse liver is capable of inducing hepatic lipid

accumulation and increasing gene expression of Plin4 in the liver [61].

These results suggest that Plin4 gene expression would be related to the

pathophysiology of the liver steatosis induced by PPARγ overexpression.

Plin5 (Myocardial lipid droplet protein: MLDP)

Plin5 was reported in 2006 and 2007 by three independent groups

as a LD protein [8, 57, 60].   These reports were in agreement that Plin5 is

expressed in tissues that exhibit high levels of fatty acid oxidation,

including heart, skeletal muscle and liver (Table 1).   Plin5 mRNA and/or

protein were inducible by fasting in the heart, liver and skeletal muscle [8,

57, 60]. A physiological role for Plin5 induction during fasting is suggested

by its enrichment on fractionated LD in the fasted liver.   Although Plin5

was induced by high fat diet in the skeletal muscle [57], whether Plin5

expression in the liver is increased by high fat diet is not known.   Plin5

expression was stimulated in mouse tissues by a pharmacological ligand for

PPAR α (Wy-14643) in the liver, heart, and skeletal muscle [8, 57, 60]

and by a PPARγ agonist (rosiglitazone) in mouse epididymal adipose tissue

[57]. Thus, PPARs may be involved in the Plin5 gene expression (Table 1).

To evaluate the role of Plin5 in lipid metabolism in the liver, Wang et al.

[53] used AML12 mouse liver cells and demonstrated that cells expressing

Plin5 released lower amounts of fatty acids in basal conditions.   In

addition, Granneman et al. [20] have shown that Plin5 expression in the

liver was increased in a mice model of acute hepatic steatosis induced by

β3 adrenergic receptor agonist.   These results suggest that increased

expression of Plin5 is capable of inducing liver steatosis possibly through

inhibition of release of fatty acid in LD.

Hypoxia-inducible protein 2 (HIG2)

Gimm et al. [16] have very recently demonstrated that HIG2, a

HIF-1 target gene, is a novel LD protein and stimulates intracellular lipid

accumulation, which may have important clinical implication.   HIG2 co

localizes with the LD proteins such as Plin2 and Plin3.   In normal liver,

diffuse HIG2 signals could be founded in perivenous hepatocytes (Table 1),

which may be caused by the physiological oxygen gradient in the liver

lobule.   It has been also shown that HIG2 could be detected in

atherosclerotic arteries and fatty liver disease (Table 1), suggesting that this

ubiquitously inducible HIF-1 target gene may play an important functional

role in diseases associated with pathological lipid accumulation.   Thus,

HIG2 could be considered as a novel LD protein which may affect

pathogenesis in fatty liver disease.   Since HIG2 is a ubiquitously

expressed HIF-1-inducible LD protein and HIF-1 is the central regulators

of cellular metabolism under hypoxic conditions [30], HIG2 might play a

role in the pathophysiology of fatty liver especially under hypoxic

condition.   Further studies should be needed to clarify the role of HIG2 in

the pathophysiology of lipid accumulation in the hepatocytes.

Fat specific protein 27 (FSP27)

FSP27 was originally isolated by screen for genes specifically expressed in fully differentiated mouse adipocytes [11, 56]. The human homologue of FSP27 was isolated from a human liver cDNA library as cell death-inducing DFF45-like effector (CIDE) -C, also referred to as CIDEC [34]. The subcellular localization of the CIDE family proteins was originally determined to be in the mitochondria [7, 63]. However, recent studies revealed that FSP27 localizes to LD in 3T3L1 adipocytes [17, 43], suggesting that FSP27 is considered to be a novel LD protein that promotes the formation of LD. FSP27 mRNA was undetectable in normal liver in mice (Table 1). Interestingly enough, FSP27 was however expressed in the steatotic liver in *ob/ob* mice [36] and in mice with PPARγ overexpression [37]. Forced expression of FSP27 in hepatocytes *in vitro* or *in vivo* led to an increase of LD through increased triglyceride levels [36]. On the other hand, knockdown of FSP27 in the *ob/ob* mouse liver

partially improved the fatty liver [36], suggesting that FSP27 is a novel LD

protein which plays a vital role in the development of liver steatosis.

FSP27 expression is regulated by PPARγ as following.   The

downstream target genes of hepatic PPARγ were determined by subtractive

cDNA cloning between *ob/ob-* PPARγ wild type and *ob/ob*-PPARγ

knockout livers and among the genes identified were isolating FSP27 [36].

In addition, overexpression of PPARγ in mouse liver induced liver steatosis

with increased expression of FSP27 in liver.   Identified several putative

transcription factor binding sites in the 5'-upstream region of FSP27

include a functional PPAR response element.   Expression of FSP27 was

markedly increased by the treatment with a thiazolidinedione (TZD),

rosiglitazone [36].   Induction of the FSP27 gene by TZD is due to the

direct binding of TZD-activated PPARγ through the PPAR response

element on the FSP27 promoter, suggesting expression of FSP27 is directly

regulated by PPARγ (Table 1).   Based on these evidence, we would make

a hypothesis that high fat diet increases FSP27/CIDEC as similarly as Plin2

gene expression through increased expression of PPARγ in the liver, followed by induction of liver steatosis (Figure 1). Flach et al. [14] have very recently demonstrated in mice that loss of mitogen-activated protein kinase phosphatase-1 (MKP-1) protects from hepatic steatosis by repression of FSP27/CIDEC through a PPARγ-dependent pathway, furthermore supporting that FSP27/CIDEC plays an important role in the formation of liver steatosis.

Relationship between adipose triglyceride lipase (ATGL) and LD proteins

Hydrolysis of triacylglycerol (TAG) stored in LD compartment provides a convenient source of cellular fuel for energy production. On the other hand, lipolysis is an important process by which cells release energy stored in LD [12]. Therefore, TAG hydrolysis must be carefully controlled to meet tissue-specific requirements for energy or lipid substrates in both adipose and non-adipose tissues including the liver.

Complete lipolysis requires enzyme reactions to break down TAG into fatty acids and glycerol.   First, adipose triglyceride lipase (ATGL) hydrolyzes TAG to produce fatty acid and diacylglycerol in adipocytes [2, 18, 33, 39], indicating that activity of ATGL plays a vital role in lipolysis in adipocytes.   In the liver, Ong et al. [42] have very recently shown that ATGL is a major hepatic lipase that regulates TAG turnover and fatty acid signaling and partitioning. With regard to the relationship between ATGL and LD proteins, a couple of groups [19, 54] established Plin5 as a novel ATGL partner and provide evidence that the protein composition of Plins at the LD surface regulates lipolytic activity of ATGL in mouse liver cells. Thus, Plin5 plays a role in lipolysis through interacting with lipolytic enzymes such as ATGL, followed by regulation of lipid storage in the liver.

Conclusion

As shown in this review, recent studies are clarifying roles of LD proteins in the pathophysiology of fatty liver diseases.   Among 7 LD

proteins listed in this review, increased expression of Plin1, Plin2, Plin3, Plin5, HIG2, and FSP27 was observed in liver steatosis.   Among them, Plin2 and/or FSP27 might be involved in the diet-induced fatty liver probably through activation of PPARγ.   Further studies are required to draw a whole picture on the roles of LD proteins in lipid accumulation in the liver.

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Figure legends

Figure 1

Role of lipid droplet proteins in the mechanism of high fat

diet-induced liver steatosis.   In normal liver, lipid droplet proteins such as

Plin2 (ADRP), Plin3 (TIP47), Plin5 (MLDP) and HIG2 express at

significant levels while expression of Plin1, Plin4 and FSP27/CIDEC is

undetectable (not shown).   High fat diet induces PPARγ expression in the

liver, which increases Plin2 and/or FSP27/CIDEC expression, followed by
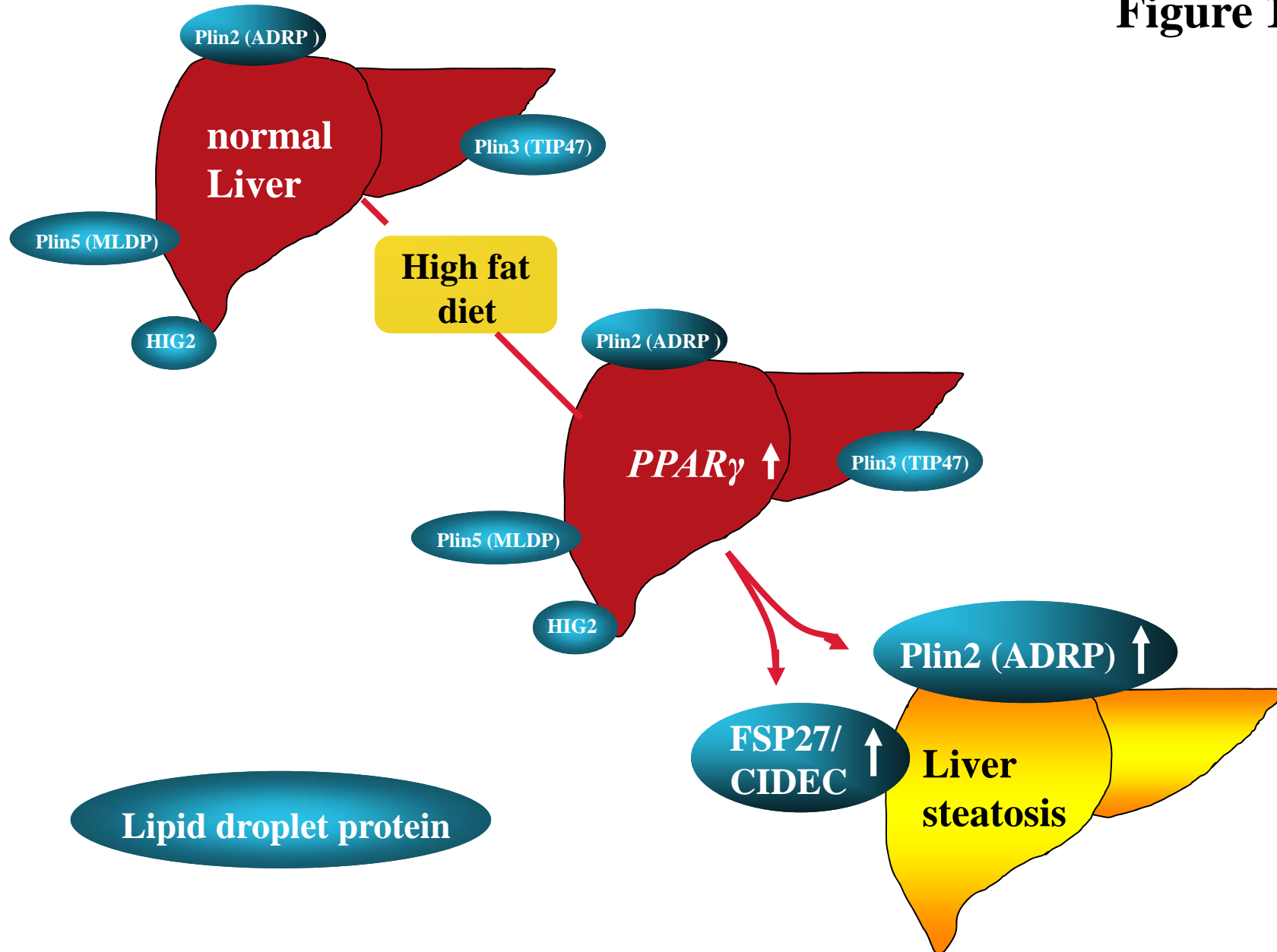
induction of liver steatosis.

Figure 1

Table 1 Expression of lipid droplet proteins in normal and fatty liver

lipid droplet protein	normal liver	fatty liver			regulation by PPARs
		human	ob/ob mice	HFD mice	
Plin1 (Perilipin)	undetectable [6, 9, 57]	↑ [49]			+ [9, 44]
Plin2 (ADRP)	+ [5, 9, 49]	↑ [15, 40, 49]	↑ [40, 46]	↑ [40]	+ [40, 46, 51]
Plin3 (TIP47)	+ [9, 49, 57]	↑ [49]			- [9]
Plin4 (S3-12)	undetectable [5, 9]				+ [9, 45]
Plin5 (MLDP)	+ [8, 57, 60]				+ [8, 57, 60]
HIG2	+ [16]	↑ [16]			
FSP27/CIDEC	undetectable [36]		↑ [36]		+ [14, 36, 61]

Perilipin (Plin); ADRP, adipose differentiation-related protein; TIP47, tail-interacting protein of 47 kiloDaltons (TIP47); MLDP, myocardial lipid droplet protein; HIG2, hypoxia-inducible protein 2;FSP27, fat-specific protein 27; CIDEC; death-inducing DFF45-like effector (CIDE) -C, HFD; High fat diet.