

## Regulation of lipogenic genes in obesity

Stéphane Mandard, Sander Kersten

► **To cite this version:**

Stéphane Mandard, Sander Kersten. Regulation of lipogenic genes in obesity. Regina Brigelius-Flohé and Hans-Georg Joost. Nutritional Genomics: Impact on Health and Disease, Wiley-VCH, pp.208-219, 2006. <inserm-00576547>

**HAL Id: inserm-00576547**

**<http://www.hal.inserm.fr/inserm-00576547>**

Submitted on 14 Mar 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Regulation of lipogenic genes in obesity

**Stéphane MANDARD (PhD) and Sander KERSTEN<sup>+</sup> (PhD)**

<sup>+</sup>corresponding author:

Sander KERSTEN

Nutrition, Metabolism and Genomics group

Division of Human Nutrition

Bomenweg, 2

Wageningen University

6703 HD Wageningen

phone: (+31) 317 485787

fax: (+31) 317 483342

email: sander.kersten@wur.nl

Stéphane MANDARD

Nutrition, Metabolism and Genomics group

Division of Human Nutrition

Bomenweg, 2

Wageningen University

6703 HD Wageningen

phone: (+31) 317 485786

fax: (+31) 317 483342

e-mail: stephane.mandard@wur.nl

## **Abstract**

Lipogenesis describes the process of fatty acid and triglyceride synthesis. Lipogenesis mainly occurs in liver and fat tissue and is under the coordinated control of hormonal, nutritional and transcription factors. Several transcription factors have been identified as critical regulators that mediate the effect of hormones and nutrients on gene transcription. This includes the Sterol Regulatory Element Binding Protein-1c “SREBP-1c”, the CCAAT/Enhancer-Binding Protein-alpha “C/EBP $\alpha$ ”, the nuclear hormone receptors Liver X Receptors “LXRs”, the Peroxisome Proliferator-Activated Receptor gamma “PPAR $\gamma$ ”, the Estrogen Related Receptor alpha “ERR $\alpha$ ”. The role of these transcription factors in these processes is reviewed and discussed. Although lipogenesis may appear as an attractive target for pharmacological treatment of obesity, recent insights into the metabolic consequences of non-adipose triglyceride storage has shifted attention to alternative targets.

The growing prevalence of obesity worldwide has become an immediate public health concern.

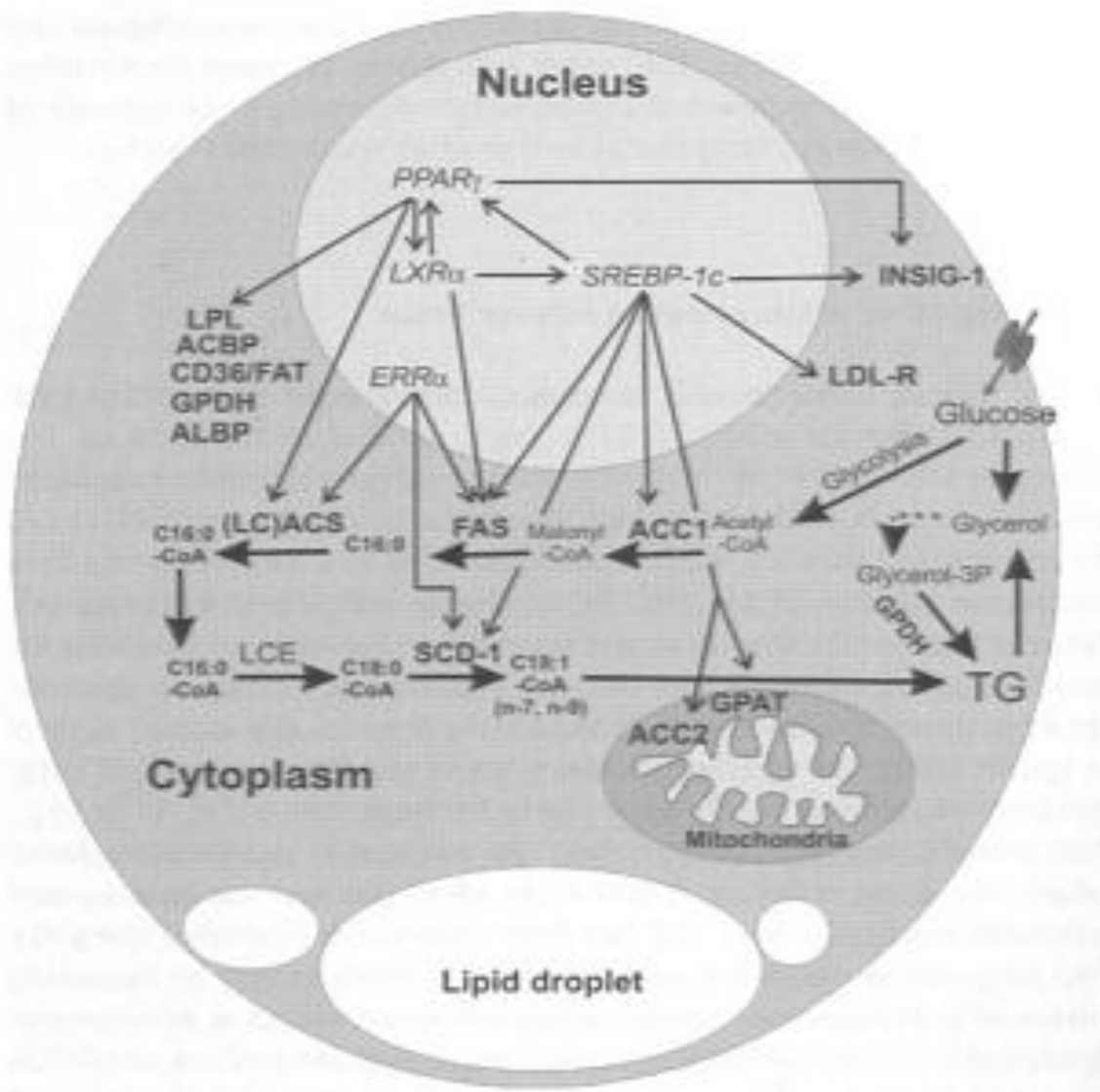
Nowadays, obesity occurs at a progressively younger age, which calls for urgent action to prevent a future epidemic of type 2 diabetes. The complexity of obesity as a metabolic disorder, often being associated with insulin resistance, dyslipidemia, and hypertension, and the poor response of this disease to treatment belie the notion that it is caused by a simple imbalance between energy consumption and energy expenditure. Accordingly, strategies aimed at reducing obesity should consider not only the complexity of regulation of energy storage and utilization, but also take into account the powerful evolutionary mechanisms that resist long term weight loss.

Lipogenesis describes the process of fatty acid and triglycerides synthesis and is obviously of great relevance to obesity. An increase in lipogenesis will lead to fat accumulation, at least if it is not associated with elevated fat utilization. Thus, it appears that in principle the process of lipogenesis is an attractive target for obesity. This paper will review the most recent information on the molecular regulation of lipogenesis, with emphasis on the role of nuclear hormone receptors and other transcription factors.

## **1. Transcriptional regulation of lipogenesis in adipose tissue**

The effects of various nutrients and hormones on the expression of lipogenic genes in adipose tissue are mediated by a small number of transcription factors, including SREBP-1c. SREBP-1c (also known as Adipocyte Determination and Differentiation-1) belongs to the basic helix-loop-helix-leucine zipper family of transcription factors. It was initially discovered as a key player of adipocyte differentiation (1). In addition, SREBP-1c plays a critical role in lipogenesis (2). SREBP-1c is first synthesized as a precursor form (110 kDa) anchored in the endoplasmic reticulum, which later undergoes a proteolytic cleavage to generate the mature active form of SREBP-1c (50 kDa). In the nucleus, the mature form of SREBP-1c is able to bind to specific sequences (sterol-regulatory elements SRE and E-boxes) located in the promoter gene of SREBP target genes (Figure 1.1).

**Fig. 1.1: Regulation of lipogenic gene expression in adipocyte.**



**Genes involved in *de novo* fatty acid biosynthesis in adipocyte and regulated at the transcription level by transcription factors are listed.** Arrows (purple) indicate an upregulation of the gene expression. While gene transcription takes place in the nucleus, cytosolic and mitochondrial enzymes have been placed outside the nucleus to simplify the understanding. Abbreviations used are as follow: ACBP: Acyl-CoA Binding Protein; ACC: Acetyl-CoA Carboxylase; ERR $\alpha$ : Estrogen Related Receptor  $\alpha$ ; FAS: Fatty Acid Synthase; Glycerol 3-P: Glycerol-3-phosphate; GPAT: Glycerol-3 Phosphate Acyl-Transferase; INSIG-1: Insulin Induced Gene-1; LCACS: Long Chain Acyl-CoA Synthetase; LCE: Long Chain fatty acid Elongase; LDL-R: Low Density Lipoprotein Receptor;

LXR $\alpha$ : Liver-X-Receptor  $\alpha$ ; PPAR $\gamma$ : Peroxisome Proliferator-Activated Receptor  $\gamma$ ; SCD-1: Stearoyl-CoA desaturase-1; SREBP-1c: Sterol Responsive Element Binding Protein-1c; TG: Triglycerides.

SREBP-1c probably works in tandem with the adipogenic transcription factor PPAR $\gamma$ , which is a direct target of SREBP-1c in adipocytes and contains a Sterol Response Element in its promoter (3). It has been proposed that together with an up-regulation of PPAR $\gamma$  expression, SREBP-1c is also able to induce PPAR $\gamma$  activity by the production of an endogenous ligand, leading to the stimulation of both adipogenesis and lipogenesis (4). Together with PPAR $\gamma$ , important lipogenic genes such as Acetyl-CoA Carboxylase-1 and -2 (ACC), Fatty Acid Synthase (FAS), Stearoyl-CoA Desaturase-1 (SCD-1), Glycerol-3-Phosphate Acyltransferase (GPAT) and Low Density Lipoprotein Receptor (LDL-R) have been identified as direct targets of SREBP-1c in mature 3T3-L1 adipocytes (5). Another gene identified as a SREBP-1c target in adipocytes is INSIG-1, which is one of two recently discovered polytopic membrane proteins of the reticulum endoplasmic (6-8). In the presence of sterols, INSIGs bind to the SREBP cleavage-activating protein (SCAP), a critical escort protein required for the cleavage and activation of the SREBP family of membrane-bound transcription factors. It appears that INSIG tethers the SCAP-SREBP complex to the endoplasmic reticulum. Recent *in vitro* studies have shown that stable over-expression of *INSIG-1* in 3T3-L1 preadipocytes results in defective adipocyte differentiation, which is likely due to impaired fatty acid and triglyceride synthesis (9). Although the relevance of the SREBP-1c mediated upregulation of INSIG-1 in adipose tissue remains to be demonstrated, it can be speculated that it provides a feedback mechanism by which nuclear SREBP-1c can modulate its maturation and, by limiting the processing of SREBPs precursors, maintain a check on lipogenesis.

Apart from SREBP, several other nuclear factors play a critical role in the transcriptional control of adipose lipogenesis. Numerous loss and gain of function experiments have shown that the nuclear hormone receptor PPAR $\gamma$  plays a pivotal role in adipocyte differentiation (reviewed in (10)), and is

also an important activator of lipogenesis. Several lipo- and/or adipogenic genes have already been identified as direct PPAR $\gamma$  targets, including Lipoprotein Lipase, CD36, Adipocyte Lipid Binding Protein and cytosolic Glycerol 3-phosphate dehydrogenase. Recently, *INSIG-1* was reported as a novel PPAR $\gamma$  target gene (Fig.1) (6). Considering that INSIG-1 appears to inhibit lipo- and adipogenesis, it is hard to reconcile this observation with the pro-adipogenic role of PPAR $\gamma$  (9). Likely, fine-tuning of adipogenesis is achieved by balanced expression of several opposing factors that include PPAR $\gamma$ , SREBP-1c, and INSIG.

Another recently identified direct target gene of PPAR $\gamma$  in adipocytes is Acyl-CoA Binding Protein (ACBP) (11). It was proposed that ACBP would repress PPAR-mediated transactivation induced by exogenous fatty acids, thereby inhibiting 3T3-L1 adipogenesis. However, additional research is necessary to establish the potential effects of ACBP on PPAR transactivation.

With respect to the role of PPAR $\gamma$  in lipid storage in mature fat cells, it has been observed that both heterozygous PPAR $\gamma$  mutant mice and adipose specific PPAR $\gamma$ -deficient mice exhibit smaller fat stores on a high fat diet (12-14). In adipose specific PPAR $\gamma$ -deficient mice mRNA expression of genes involved in both lipogenesis and adipogenesis were strongly down-regulated, illustrating an absolute requirement for PPAR $\gamma$  (14, 15). Fibrosis, macrophage infiltration and hypertrophy, implying loss of more than 80% of adipocytes, were evident in mutant fat. Thus, PPAR $\gamma$  is not only essential for the early steps of the adipogenesis program but also for post-differentiation and survival of mature white adipocytes *in vivo* (14, 16).

While it is clear that SREBP-1c and PPAR $\gamma$  are extremely important regulators of adipo- and lipogenesis, recent studies have drawn attention to other nuclear hormone receptors. One of these receptors is the Estrogen-Related Receptor alpha ERR $\alpha$ . Deletion of ERR $\alpha$  in mice reduces body weight and peripheral fat deposits, while food consumption and energy expenditure are unaffected (17). At the gene level, expression of several enzymes involved in lipogenesis was down-regulated in white adipose tissue. In line with this, *de novo* lipogenesis was shown to be reduced in the mutant

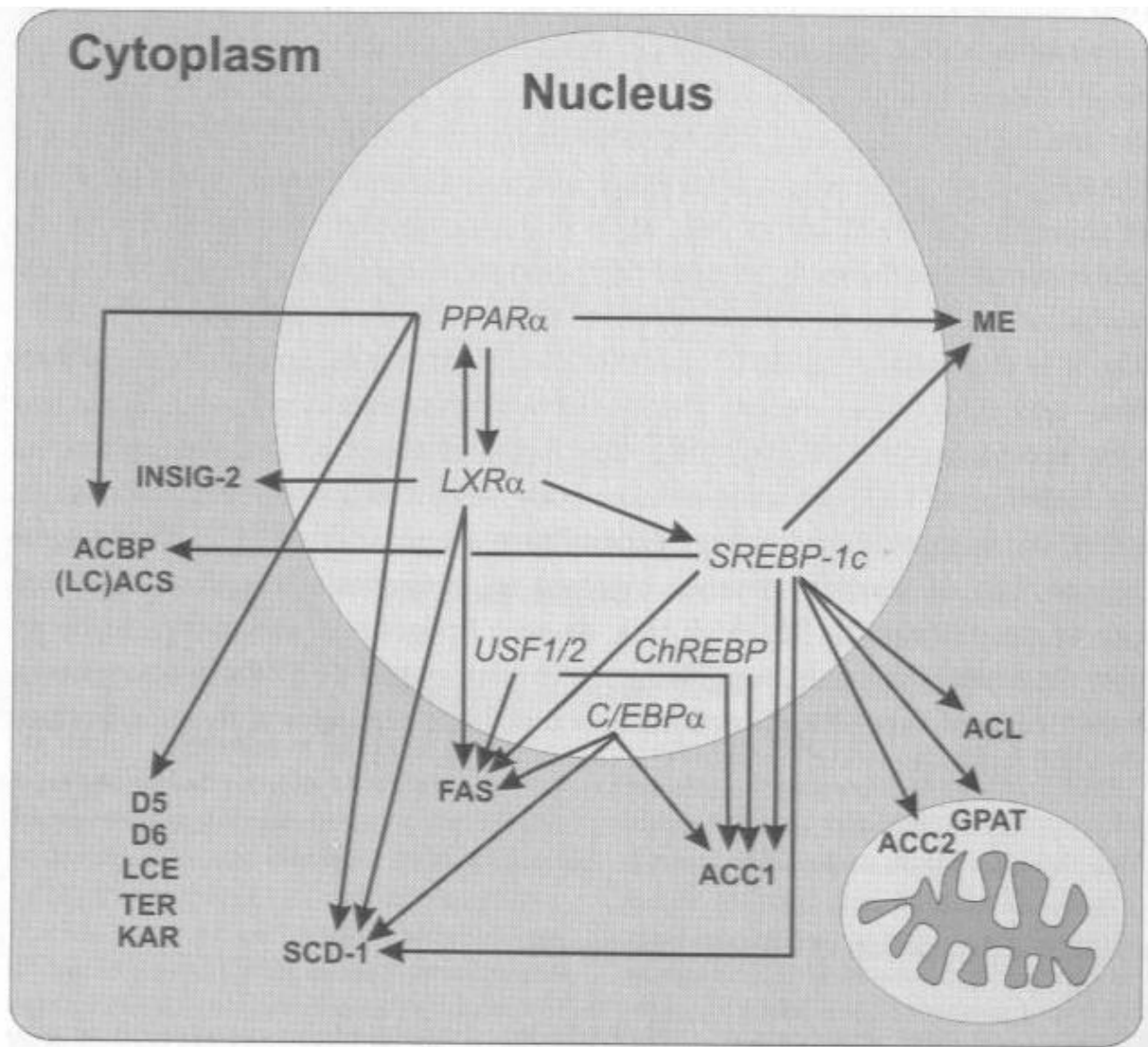
animals, suggesting a stimulatory role of  $ERR\alpha$  on lipogenesis. These data suggest that  $ERR\alpha$  functions as a metabolic regulator with an important effect on fat synthesis.

## **2. Transcriptional regulation of hepatic lipogenesis**

Consumption of large amounts of carbohydrates stimulates the conversion of glucose to fatty acids by upregulating glycolytic and lipogenic enzymes. The effects of carbohydrate feeding are mediated by insulin and glucose, which activate distinct signalling pathways. Upregulation of lipogenic genes by insulin partially occurs via the Upstream Stimulatory Factor (USF). USFs are ubiquitous bHLH-leucine zipper transcription factors that are able to form homo and/or heterodimers. They modulate expression of genes such as FAS and ACC by direct binding to promoter gene sequences called E-Boxes.



**Fig. 2.1: Regulation of lipogenic gene expression in hepatocyte.**



Genes involved in *de novo* fatty acid biosynthesis in hepatocyte and regulated at the transcription level by transcription factors are listed. Arrows (purple) indicate an upregulation of the gene expression. While gene transcription takes place in the nucleus, cytosolic and mitochondrial enzymes have been placed outside the nucleus to simplify the understanding. Abbreviations used are as follow: ACBP: Acyl-CoA Binding Protein; ACC: Acetyl-CoA Carboxylase; ACL: ATP Citrate Lyase; ALBP: Adipocyte Lipid-Binding Protein; CD36: Scavenger Receptor CD36; C/EBP $\alpha$ : CCAAT/Enhancer-Binding Protein-alpha; ChREBP: Carbohydrate Response Element Binding Protein; D5: Delta-5-desaturase; D6: Delta-6-desaturase; FAS: Fatty Acid Synthase; GPAT: Glycerol-3 Phosphate Acyl-Transferase; INSIG-2: Insulin Induced Gene-2; KAR: microsomal 3-KetoAcyl-CoA

Reductase; (LC)ACS: Long Chain Acyl-CoA Synthetase; LCE: Long Chain Fatty Acid Elongase; LPL: Lipoprotein Lipase; LXR $\alpha$ : Liver-X-Receptor  $\alpha$ ; ME: Malic Enzyme; PPAR $\alpha$ : Peroxisome Proliferator-Activated Receptor  $\alpha$ ; SCD-1: Stearoyl-CoA Desaturase-1; SREBP-1c: Sterol Responsive Element Binding Protein-1c. TER: *trans*-2,3-enoyl-CoA reductase; USF: Upstream Stimulatory Factor.

Studies with mice lacking USF1 and/or USF2 have provided compelling evidence for their role in the stimulatory effect of insulin and glucose on lipogenesis. In USF1<sup>-/-</sup> or USF2<sup>-/-</sup> mice, FAS expression in liver was strongly impaired after a fasting/refeeding cycle, demonstrating that USFs are critical factors for the transcriptional activation of FAS by diet (18). At the molecular level, USFs exerted their stimulatory effect on FAS transcription via an E-box motif located in the promoter of the FAS gene. This motif is likely shared with SREBP-1, which also is a potent activator of FAS gene expression. Studies with mice lacking or over-expressing SREBP-1c have indicated that this transcription factor is responsible for the coordinate induction of numerous lipogenic genes in liver, including ACC, FAS, SCD-1, GPAT, ACL, Malic Enzyme, Glucose-6 Phosphate Dehydrogenase, Spot 14, and Long Fatty Acid Elongase. Accordingly, over-expression SREBP-1c was associated with a dramatic build-up of hepatic triglycerides (19). At the present time, SRE have been identified in the promoter gene of FAS (20), ACC (21), ACL (22), GPAT (23), Spot 14 (24), SCD-1 (25), and ACBP (26). The last gene encodes a small intracellular protein that is able to bind long chain fatty acyl-CoAs. Although the role of ACBP *in vivo* is not very clear, it can be speculated that ACBP participates in *de novo* fatty acid biosynthesis. Indeed, high expression levels of ACBP have been observed in both hepatocytes and adipocytes and in liver ACBP expression is stimulated by insulin and repressed by fasting.

While the expression of many lipogenic enzymes is enhanced by insulin, optimal transcription of most lipogenic genes requires high carbohydrate levels as well. In concordance with this observation,

glucose has been identified as a potent activator of lipogenesis not only by acting as substrate, but also as an important regulatory molecule. Recent data indicate that increased glucose metabolism activates an intracellular signaling pathway, probably involving xylulose 5-phosphate, that transcriptionally regulates genes encoding lipogenic enzymes *via* the Carbohydrate Response Element Binding Protein “ChREBP”, a basic helix-loop-helix/leucine zipper transcription factor. ChREBP was identified and purified by taking advantage of its binding to the carbohydrate response element “ChRE” within the promoter of the L-type pyruvate kinase gene. ChREBP is stimulated by high concentrations of glucose that promote both translocation of ChREBP from the cytosol to the nucleus coupled with binding of ChREBP to a ChRE (27). Importantly, ChREBP becomes active in response to high glucose concentrations in liver independently of the insulin level. Mice with a targeted disruption of the ChREBP gene were recently generated, showing reduced expression of several glycolytic genes (28). In addition, the mRNA levels of ACC1, ACC2, LCE, ME, SCD1 and FAS were significantly lower in the ChREBP<sup>-/-</sup> mice compared to the wild-type littermates. Follow-up studies indicated that FAS, ACC and Spot 14 are direct targets of ChREBP with a ChRE present in their promoters (29, 30). These results suggest that ChREBP, apart from its predictable role in glucose utilization, is also of importance for fatty acid biosynthesis.

Knock-out mice have also turned out to be an invaluable tools to demonstrate a critical role for C/EBP $\alpha$  in hepatic lipogenesis. Deletion of C/EBP $\alpha$  in combination with leptin deficiency caused diminished lipogenic gene expression and was associated with a significant decrease in hepatic triglyceride content (31). Development of the fatty liver was exacerbated by high fat feeding but much less so in liver-specific C/EBP $\alpha$  null mice. Thus, in addition to its functional role in adipogenesis, C/EBP $\alpha$  stimulates hepatic lipogenesis (reviewed in (32)).

The liver X receptor is a nuclear hormone receptor that is highly expressed in liver and that is activated by oxysterols. It was first shown to play an important role in the feed-forward control of bile acid synthesis from cholesterol by upregulating cholesterol 7- $\alpha$  hydroxylase expression. More

recent studies indicate that LXRs are also potent stimulators of lipogenesis in liver in mice. Functional LXR Response Elements were identified in the promoters of the PPAR $\gamma$ , SREBP-1c and FAS gene (33, 34). Accordingly, activation of LXRs by synthetic agonists leads to a robust induction of lipogenic genes that is translated into an increase of both plasma triglyceride and phospholipid levels (34). Studies with LXRs mutant null mice further established the key role of LXRs as factors that mediate the effect of insulin on lipogenesis. (35). In cultured primary hepatocytes insulin increases the half-life of LXR $\alpha$  mRNA, which in turn increases LXR $\alpha$  protein.

Although both insulin and LXR $\alpha$  stimulate SREBP-1c gene expression, they do so by different pathways. According to Hegarty et al. LXR $\alpha$  activation stimulates the production of the precursor SREBP-1c protein, yet this effect is not translated into the mature nuclear form (36). In contrast, insulin was shown to efficiently and rapidly stimulate the cleavage and maturation of the precursor SREBP-1c form. The molecular explanation for this differential effect may lie with INSIG-2, which is upregulated by LXR $\alpha$ . According to this scenario, INSIG-2 retains the mature form of SREBP-1c in the endoplasmic reticulum, which would thus escape the maturation process.

The nuclear hormone receptor PPAR $\alpha$  plays a pivotal role in the adaptive response to fasting by upregulating many genes involved in hepatic fatty acid oxidation, ketogenesis and gluconeogenesis. In this context, it is remarkable that PPAR $\alpha$  upregulates several genes involved in fatty acid elongation and desaturation, including SCD-1, SCD-2, delta 5 desaturase, delta 6 desaturase, LCE, TER and KAR (reviewed in (37, 38) and unpublished data). The enzymes SCD-1 and SCD-2 catalyze the conversion of stearic acid into oleic acid and are thus essential for the synthesis of mono- and poly-unsaturated fatty acids. An important question that arises is why a single factor such as PPAR $\alpha$  would stimulate both fatty acid oxidation and fatty acid elongation/desaturation. It has been proposed that up-regulation of desaturases expression by PPAR $\alpha$  may generate unsaturated fatty acids, which are agonists for PPAR $\alpha$ . Alternatively, it is possible that the high turn-over of plasmatic phospholipids membrane requires a constant level of (unsaturated) free fatty acids to prevent cell death. The story is

even more complex, since delta-5, -6 and -9 desaturase expressions are also under the transcriptional control of SREBP-1c, which is generally considered to have a function completely opposite to that of PPAR $\alpha$  (39).

PPAR $\alpha$  mediates the stimulatory effect of poly-unsaturated fatty acids (PUFAs) on hepatic fatty acid oxidation. However, PUFAs also inhibit hepatic lipogenesis by a process that is PPAR $\alpha$ -independent. Studies by a variety of groups subsequently showed that PUFA suppress lipogenesis by inhibiting expression of SREBP-1c (40-43). At the molecular level, it has been reported that PUFA are able to act as competitive LXR $\alpha$  antagonists, which would result in the downregulation of SREBP-1c and other lipogenic genes (44) (45). However, others have argued that down-regulation of SREBP-1 by PUFAs is independent of LXR $\alpha$  (46). Further studies are required to determine the precise molecular mechanisms responsible for this discrepancy.

### **3. Hepatic lipogenesis in steatosis**

A major metabolic consequence of obesity is insulin resistance, which is usually accompanied by storage of triglycerides in the liver. It is believed that the excess release of free fatty acids from adipose tissue lipolysis accounts for the triglycerides accumulation in liver. While PPAR $\gamma$  is barely expressed in liver under basal conditions, its expression is markedly increased in animals models of insulin resistance and fatty liver, suggesting a role for PPAR $\gamma$  in hepatic steatosis (47). Indeed, specific deletion of hepatic PPAR $\gamma$  improves fatty liver in two mouse models of hepatic steatosis (*ob/ob* and AZIP), yet worsens hyperglycemia and insulin resistance (48, 49). In addition, forced expression of PPAR $\gamma$ 1 in liver of PPAR $\alpha$ -deficient mice using adenovirus leads to the strong induction of both lipogenic and adipogenic genes (including aP2, adipsin, LPL, CD36, FAS, SCD-1), indicating transformation of hepatocytes towards adipocyte-like cells (50). Similarly, forced expression of PPAR $\gamma$ 2 in hepatocytes was shown to lead to both hepatic lipid accumulation and induction of lipogenic and adipogenic gene expression (51). Thus, over-expression of PPAR $\gamma$  appears

to be both necessary and sufficient for hepatic steatosis. Accordingly, in certain pathological conditions, over-expression of PPAR $\gamma$  in liver may contribute to steatosis.

#### **4. Nuclear hormone receptors and the control adipocytokine gene expression**

In the past few years it has become clear that fat tissue is not merely a storage depot for excess fat but also is an active endocrine tissue, which secretes a number of biologically active proteins with putative role in metabolic syndrome. Several proteins secreted by adipocytes that affect satiety and/or energy homeostasis have been identified. These so-called adipocytokines include resistin, adiponectin, acylation-stimulating protein, FIAF, visfatin, leptin, adipsin, plasminogen activator inhibitor-1, renin angiotensin system, metallothioneins and the inflammatory cytokines interleukin-6, tumor necrosis factor- $\alpha$  “TNF- $\alpha$ ” and tumor growth factor- $\beta$ . These molecules have been linked to a wide range of clinical abnormalities such as obesity, atherosclerosis, insulin resistance and type 2 diabetes mellitus (reviewed in (52)). For example, adipose TNF- $\alpha$  mRNA levels are increased in several models of genetically induced obesity and the same is true for the plasma resistin level. Both proteins seem to play a role in obesity-induced insulin resistance, at least in mice. While resistin and TNF- $\alpha$  diminish insulin sensitivity, a large body of evidence in mice and human indicates that adiponectin improves insulin sensitivity. Indeed, plasma levels of adiponectin were found to be decreased in obese patients and were positively correlated with insulin resistance (53) (54).

An adipocytokine that has major effects on triglyceride synthesis in adipocytes is acylation stimulating protein “ASP” (55). The uptake and re-esterification of nonesterified fatty acids by adipose tissue is enhanced by ASP, probably by stimulating the activity of diacylglycerol acyltransferase “DGAT”, a key enzyme in triglyceride synthesis. Moreover, ASP stimulates glucose uptake and transport in human adipocytes, thereby providing the substrates for further triglyceride synthesis.

An interesting newly (re)discovered adipocytokine is visfatin (56). Injection of recombinant visfatin was shown to decrease blood glucose in insulin resistant or deficient mice. Furthermore, like insulin,

visfatin stimulated glucose uptake by cultured adipocyte and muscle cells and decreased glucose output by hepatocytes. Interestingly, visfatin was found to stimulate triglyceride synthesis and accumulation in adipocytes to a similar extent as insulin. The precise molecular mechanism behind this observation is unclear but is likely linked to the insulin pathway, since visfatin was shown to bind the insulin receptor.

Thiazolidinediones influence the expression of many of these adipocytokines both positively and negatively. Leptin, TNF $\alpha$  and resistin are negative targets of TZDs (57) Resistin might mediate the effect of TZD on insulin sensitivity, at least in mice. Adiponectin, in contrast, is a direct positive target gene of PPAR $\gamma$  (58), which provides a molecular explanation for the induction of adiponectin gene expression by TZDs. The fasting-induced adipose factor FIAF/ANGPTL4 is also a direct positive target of PPAR $\gamma$ , expression of which is upregulated by TZDs in both mouse and human adipocytes (59 and unpublished data). Visfatin might be regulated by PPAR $\gamma$  ligands as well, although this remains to be demonstrated. The possible stimulatory role of PPAR $\gamma$  on visfatin gene expression would be of interest in the context of type 2 diabetes, since recombinant visfatin protein was shown to promote glucose uptake by adipocytes and to suppress glucose release by hepatocytes (56). In summary, PPAR $\gamma$  not only has a role in adipose lipogenesis and adipogenesis but also controls different endocrine pathways that are critical for whole body energy homeostasis.

## **5. Targeting lipogenesis for obesity ?**

In principle, the process of lipogenesis appears as an attractive target for the treatment of obesity. However, two major issues likely impose severe restrictions on the applicability of lipogenesis inhibitors in obesity management. The first issue is that conversion of carbohydrates and amino acids to fatty acids appears to be of minor significance in human individuals consuming a regular diet that is high in fat. Whereas in mice conversion of carbohydrates to fatty acids is very active, detailed metabolic studies have shown that this situation is not analogous in humans. Thus, inhibiting an

enzyme such as FAS in liver and adipose tissue is unlikely to have major consequences in humans. This is not true for enzymes involved in synthesis of triglycerides from fatty acids, which are highly active in humans in a variety of organs. The second issue that questions the usefulness of targeting lipogenesis is that inhibition of lipogenesis in fat tissue, without concomitantly activating energy expenditure, will merely cause a redistribution of fat storage from adipose tissue to other tissues such as liver and pancreas, a process which is now considered highly undesirable. Indeed, it seems that the effectiveness of thiazolidinediones toward ameliorating insulin resistance is positively correlated with their effect on subcutaneous fat gain, suggesting that at least for diabetes management strategies should promote preferential storage of fat in subcutaneous stores.

## **6. Conclusions and perspectives**

In conclusion, lipogenesis is tightly controlled by nutritional, hormonal and transcription factors. In the last few years, major advances have been made to identify the complex regulatory networks involved in the regulation of lipogenesis. One of the most pressing challenges that researchers will have to deal with is to extend their findings from rodents to humans. Finally, although lipogenesis may appear as an attractive target for the pharmacological treatment of obesity, it has become increasingly clear that inhibiting triglyceride storage in subcutaneous fat tissue is more likely to do harm than any good.

**Acknowledgements:** The work of the authors is supported by the Netherlands Organization for Scientific Research (NWO), the Dutch Diabetes Foundation, the Wageningen Centre for Food Sciences (WCFS), and the Royal Netherlands Academy of Arts and Sciences. The authors would like to thank Rinke Stienstra for critical reading of the manuscript.

## **Bibliography**



1. Tontonoz, P., J.B. Kim, R.A. Graves, and B.M. Spiegelman. 1993. ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. *Mol Cell Biol* 13:4753-4759.
2. Horton, J.D., and I. Shimomura. 1999. Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. *Curr Opin Lipidol* 10:143-150.
3. Fajas, L., K. Schoonjans, L. Gelman, J.B. Kim, J. Najib, G. Martin, J.C. Fruchart, M. Briggs, B.M. Spiegelman, and J. Auwerx. 1999. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol Cell Biol* 19:5495-5503.
4. Kim, J.B., H.M. Wright, M. Wright, and B.M. Spiegelman. 1998. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. *Proc Natl Acad Sci U S A* 95:4333-4337.
5. Le Lay, S., I. Lefrere, C. Trautwein, I. Dugail, and S. Krief. 2002. Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein beta as an SREBP-1C target. *J Biol Chem* 277:35625-35634.
6. Kast-Woelbern, H.R., S.L. Dana, R.M. Cesario, L. Sun, L.Y. de Grandpre, M.E. Brooks, D.L. Osburn, A. Reifel-Miller, K. Klausning, and M.D. Leibowitz. 2004. Rosiglitazone induction of Insig-1 in white adipose tissue reveals a novel interplay of peroxisome proliferator-activated receptor gamma and sterol regulatory element-binding protein in the regulation of adipogenesis. *J Biol Chem* 279:23908-23915.
7. Yang, T., P.J. Espenshade, M.E. Wright, D. Yabe, Y. Gong, R. Aebersold, J.L. Goldstein, and M.S. Brown. 2002. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110:489-500.
8. Yabe, D., M.S. Brown, and J.L. Goldstein. 2002. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc Natl Acad Sci U S A* 99:12753-12758.
9. Li, J., K. Takaishi, W. Cook, S.K. McCorkle, and R.H. Unger. 2003. Insig-1 "brakes" lipogenesis in adipocytes and inhibits differentiation of preadipocytes. *Proc Natl Acad Sci U S A* 100:9476-9481.
10. Rosen, E.D., and B.M. Spiegelman. 2001. PPARgamma : a nuclear regulator of metabolism, differentiation, and cell growth. *J Biol Chem* 276:37731-37734.
11. Helledie, T., L. Grontved, S.S. Jensen, P. Kiillerich, L. Rietveld, T. Albrektsen, M.S. Boysen, J. Nohr, L.K. Larsen, J. Fleckner, H.G. Stunnenberg, K. Kristiansen, and S. Mandrup. 2002. The gene encoding the Acyl-CoA-binding protein is activated by peroxisome proliferator-activated receptor gamma through an intronic response element functionally conserved between humans and rodents. *J Biol Chem* 277:26821-26830.
12. Kubota, N., Y. Terauchi, H. Miki, H. Tamemoto, T. Yamauchi, K. Komeda, S. Satoh, R. Nakano, C. Ishii, T. Sugiyama, K. Eto, Y. Tsubamoto, A. Okuno, K. Murakami, H. Sekihara, G. Hasegawa, M. Naito, Y. Toyoshima, S. Tanaka, K. Shiota, T. Kitamura, T. Fujita, O. Ezaki, S. Aizawa, T. Kadowaki, and et al. 1999. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 4:597-609.
13. Miles, P.D., Y. Barak, W. He, R.M. Evans, and J.M. Olefsky. 2000. Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency. *J Clin Invest* 105:287-292.
14. He, W., Y. Barak, A. Hevener, P. Olson, D. Liao, J. Le, M. Nelson, E. Ong, J.M. Olefsky, and R.M. Evans. 2003. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 100:15712-15717.

15. Zhang, J., M. Fu, T. Cui, C. Xiong, K. Xu, W. Zhong, Y. Xiao, D. Floyd, J. Liang, E. Li, Q. Song, and Y.E. Chen. 2004. Selective disruption of PPARgamma 2 impairs the development of adipose tissue and insulin sensitivity. *Proc Natl Acad Sci U S A* 101:10703-10708.
16. Imai, T., R. Takakuwa, S. Marchand, E. Dentz, J.M. Bornert, N. Messaddeq, O. Wendling, M. Mark, B. Desvergne, W. Wahli, P. Chambon, and D. Metzger. 2004. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proc Natl Acad Sci U S A* 101:4543-4547.
17. Luo, J., R. Sladek, J. Carrier, J.A. Bader, D. Richard, and V. Giguere. 2003. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol Cell Biol* 23:7947-7956.
18. Casado, M., V.S. Vallet, A. Kahn, and S. Vaulont. 1999. Essential role in vivo of upstream stimulatory factors for a normal dietary response of the fatty acid synthase gene in the liver. *J Biol Chem* 274:2009-2013.
19. Shimomura, I., Y. Bashmakov, and J.D. Horton. 1999. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem* 274:30028-30032.
20. Bennett, M.K., J.M. Lopez, H.B. Sanchez, and T.F. Osborne. 1995. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. *J Biol Chem* 270:25578-25583.
21. Magana, M.M., S.S. Lin, K.A. Dooley, and T.F. Osborne. 1997. Sterol regulation of acetyl coenzyme A carboxylase promoter requires two interdependent binding sites for sterol regulatory element binding proteins. *J Lipid Res* 38:1630-1638.
22. Sato, R., A. Okamoto, J. Inoue, W. Miyamoto, Y. Sakai, N. Emoto, H. Shimano, and M. Maeda. 2000. Transcriptional regulation of the ATP citrate-lyase gene by sterol regulatory element-binding proteins. *J Biol Chem* 275:12497-12502.
23. Ericsson, J., S.M. Jackson, J.B. Kim, B.M. Spiegelman, and P.A. Edwards. 1997. Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1- and sterol regulatory element-binding protein-responsive gene. *J Biol Chem* 272:7298-7305.
24. Koo, S.H., A.K. Dutcher, and H.C. Towle. 2001. Glucose and insulin function through two distinct transcription factors to stimulate expression of lipogenic enzyme genes in liver. *J Biol Chem* 276:9437-9445.
25. Tabor, D.E., J.B. Kim, B.M. Spiegelman, and P.A. Edwards. 1999. Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. *J Biol Chem* 274:20603-20610.
26. Sandberg, M.B., M. Bloksgaard, D. Duran-Sandoval, C. Duval, B. Staels, and S. Mandrup. 2004. The gene encoding acyl-CoA binding protein is subject to metabolic regulation by both SREBP and PPARalpha in hepatocytes. *J Biol Chem*.
27. Yamashita, H., M. Takenoshita, M. Sakurai, R.K. Bruick, W.J. Henzel, W. Shillinglaw, D. Arnot, and K. Uyeda. 2001. A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. *Proc Natl Acad Sci U S A* 98:9116-9121.
28. Iizuka, K., R.K. Bruick, G. Liang, J.D. Horton, and K. Uyeda. 2004. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc Natl Acad Sci U S A* 101:7281-7286.
29. Ishii, S., K. Iizuka, B.C. Miller, and K. Uyeda. 2004. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. *Proc Natl Acad Sci U S A* 101:15597-15602.
30. Shih, H.M., and H.C. Towle. 1992. Definition of the carbohydrate response element of the rat S14 gene. Evidence for a common factor required for carbohydrate regulation of hepatic genes. *J Biol Chem* 267:13222-13228.

31. Matsusue, K., O. Gavrilova, G. Lambert, H.B. Brewer, Jr., J.M. Ward, Y. Inoue, D. LeRoith, and F.J. Gonzalez. 2004. Hepatic CCAAT/enhancer binding protein alpha mediates induction of lipogenesis and regulation of glucose homeostasis in leptin-deficient mice. *Mol Endocrinol* 18:2751-2764.
32. Rosen, E.D., C.J. Walkey, P. Puigserver, and B.M. Spiegelman. 2000. Transcriptional regulation of adipogenesis. *Genes Dev* 14:1293-1307.
33. Repa, J.J., G. Liang, J. Ou, Y. Bashmakov, J.M. Lobaccaro, I. Shimomura, B. Shan, M.S. Brown, J.L. Goldstein, and D.J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev* 14:2819-2830.
34. Schultz, J.R., H. Tu, A. Luk, J.J. Repa, J.C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D.J. Mangelsdorf, K.D. Lustig, and B. Shan. 2000. Role of LXRs in control of lipogenesis. *Genes Dev* 14:2831-2838.
35. Tobin, K.A., S.M. Ulven, G.U. Schuster, H.H. Steineger, S.M. Andresen, J.A. Gustafsson, and H.I. Nebb. 2002. Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis. *J Biol Chem* 277:10691-10697.
36. Hegarty, B.D., A. Bobard, I. Hainault, P. Ferre, P. Bossard, and F. Foufelle. 2005. From The Cover: Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory elementbinding protein-1c. *Proc Natl Acad Sci U S A* 102:791-796.
37. Mandard, S., M. Muller, and S. Kersten. 2004. Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci* 61:393-416.
38. Kersten, S., J. Seydoux, J.M. Peters, F.J. Gonzalez, B. Desvergne, and W. Wahli. 1999. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* 103:1489-1498.
39. Matsuzaka, T., H. Shimano, N. Yahagi, M. Amemiya-Kudo, T. Yoshikawa, A.H. Hasty, Y. Tamura, J. Osuga, H. Okazaki, Y. Iizuka, A. Takahashi, H. Sone, T. Gotoda, S. Ishibashi, and N. Yamada. 2002. Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPARalpha. *J Lipid Res* 43:107-114.
40. Sekiya, M., N. Yahagi, T. Matsuzaka, Y. Najima, M. Nakakuki, R. Nagai, S. Ishibashi, J. Osuga, N. Yamada, and H. Shimano. 2003. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology* 38:1529-1539.
41. Xu, J., M.T. Nakamura, H.P. Cho, and S.D. Clarke. 1999. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J Biol Chem* 274:23577-23583.
42. Yahagi, N., H. Shimano, A.H. Hasty, M. Amemiya-Kudo, H. Okazaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, and N. Yamada. 1999. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. *J Biol Chem* 274:35840-35844.
43. Clarke, S.D., and D.B. Jump. 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu Rev Nutr* 14:83-98.
44. Ou, J., H. Tu, B. Shan, A. Luk, R.A. DeBose-Boyd, Y. Bashmakov, J.L. Goldstein, and M.S. Brown. 2001. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. *Proc Natl Acad Sci U S A* 98:6027-6032.
45. Yoshikawa, T., H. Shimano, N. Yahagi, T. Ide, M. Amemiya-Kudo, T. Matsuzaka, M. Nakakuki, S. Tomita, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, A. Takahashi, H. Sone, J. Osuga Ji, T. Gotoda, S. Ishibashi, and N. Yamada. 2002. 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* 277:1705-1711.

46. Pawar, A., D. Botolin, D.J. Mangelsdorf, and D.B. Jump. 2003. The role of liver X receptor-alpha in the fatty acid regulation of hepatic gene expression. *J Biol Chem* 278:40736-40743.
47. Chao, L., B. Marcus-Samuels, M.M. Mason, J. Moitra, C. Vinson, E. Arioglu, O. Gavrilova, and M.L. Reitman. 2000. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest* 106:1221-1228.
48. Matsusue, K., M. Haluzik, G. Lambert, S.H. Yim, O. Gavrilova, J.M. Ward, B. Brewer, Jr., M.L. Reitman, and F.J. Gonzalez. 2003. Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J Clin Invest* 111:737-747.
49. Gavrilova, O., M. Haluzik, K. Matsusue, J.J. Cutson, L. Johnson, K.R. Dietz, C.J. Nicol, C. Vinson, F.J. Gonzalez, and M.L. Reitman. 2003. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J Biol Chem* 278:34268-34276.
50. Yu, S., K. Matsusue, P. Kashireddy, W.Q. Cao, V. Yeldandi, A.V. Yeldandi, M.S. Rao, F.J. Gonzalez, and J.K. Reddy. 2003. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. *J Biol Chem* 278:498-505.
51. Schadinger, S.E., N.L. Bucher, B.M. Schreiber, and S.R. Farmer. 2005. PPAR{gamma}2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. *Am J Physiol Endocrinol Metab*.
52. Faraj, M., H.L. Lu, and K. Cianflone. 2004. Diabetes, lipids, and adipocyte secretagogues. *Biochem Cell Biol* 82:170-190.
53. Lihn, A.S., S.B. Pedersen, and B. Richelsen. 2005. Adiponectin: action, regulation and association to insulin sensitivity. *Obes Rev* 6:13-21.
54. Trujillo, M.E., and P.E. Scherer. 2005. Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med* 257:167-175.
55. Cianflone, K., Z. Xia, and L.Y. Chen. 2003. Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta* 1609:127-143.
56. Fukuhara, A., M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa, and I. Shimomura. 2004. Visfatin: A Protein Secreted by Visceral Fat that Mimics the Effects of Insulin. *Science*.
57. Steppan, C.M., S.T. Bailey, S. Bhat, E.J. Brown, R.R. Banerjee, C.M. Wright, H.R. Patel, R.S. Ahima, and M.A. Lazar. 2001. The hormone resistin links obesity to diabetes. *Nature* 409:307-312.
58. Iwaki, M., M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima, and I. Shimomura. 2003. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 52:1655-1663.
59. Mandard, S., F. Zandbergen, N.S. Tan, P. Escher, D. Patsouris, W. Koenig, R. Kleemann, A. Bakker, F. Veenman, W. Wahli, M. Muller, and S. Kersten. 2004. The direct peroxisome proliferator-activated receptor target fasting-induced adipose factor (FIAF/PGAR/ANGPTL4) is present in blood plasma as a truncated protein that is increased by fenofibrate treatment. *J Biol Chem* 279:34411-34420.