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Regulation of lipogenic genes in obesity

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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/EBPα", the nuclear hormone receptors Liver X Receptors "LXRs", the Peroxisome Proliferator-Activated Receptor gamma "PPARγ", the Estrogen Related Receptor alpha "ERRα". 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.

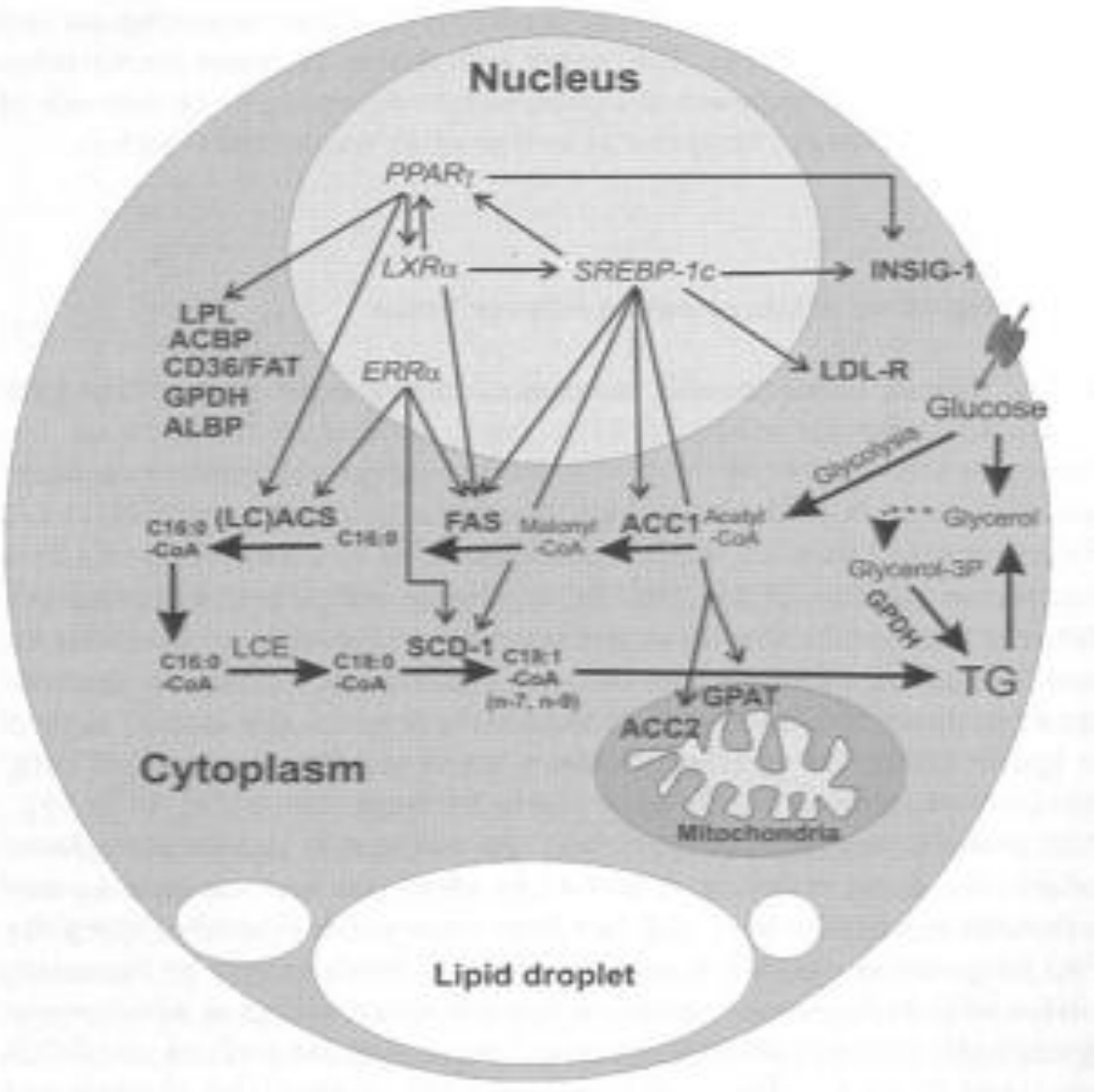
The growing prevalence of obesity worldwide has become an immediate public health concern. Nowadays, obesity occurs at a progressively younger age, which calls for urgent action to prevent a future epidemic of type 2 diabetes. The complexity of obesity as a metabolic disorder, often being associated 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 symplify the understanding. Abbreviations used are as follow: ACBP: Acyl-CoA Binding Protein; ACC: Acetyl-CoA Carboxylase; ERRα: Estrogen Related Receptor α; 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;

LXRα: Liver-X-Receptor α; PPARγ: Peroxisome Proliferator-Activated Receptor γ; 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 PPARγ, 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 PPARγ expression, SREBP-1c is also able to induce PPARγ activity by the production of an endogenous ligand, leading to the stimulation of both adipogenesis and lipogenesis (4). Together with PPARγ, 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 PPARγ 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 PPARγ targets, including Lipoprotein Lipase, CD36, Adipocyte Lipid Binding Protein and cytosolic Glycerol 3-phosphate dehydrogenase. Recently, *INSIG-1* was reported as a novel PPARγ 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 PPARγ (9). Likely, fine-tuning of adipogenesis is achieved by balanced expression of several opposing factors that include PPARγ, SREBP-1c, and INSIG.

Another recently identified direct target gene of PPARγ 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 PPARγ in lipid storage in mature fat cells, it has been observed that both heterozygous PPARγ mutant mice and adipose specific PPARγ-deficient mice exhibit smaller fat stores on a high fat diet (12-14). In adipose specific PPARγ-deficient mice mRNA expression of genes involved in both lipogenesis and adipogenesis were strongly down-regulated, illustrating an absolute requirement for PPARγ (14, 15). Fibrosis, macrophage infiltration and hypertrophy, implying loss of more than 80% of adipocytes, were evident in mutant fat. Thus, PPARγ 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).

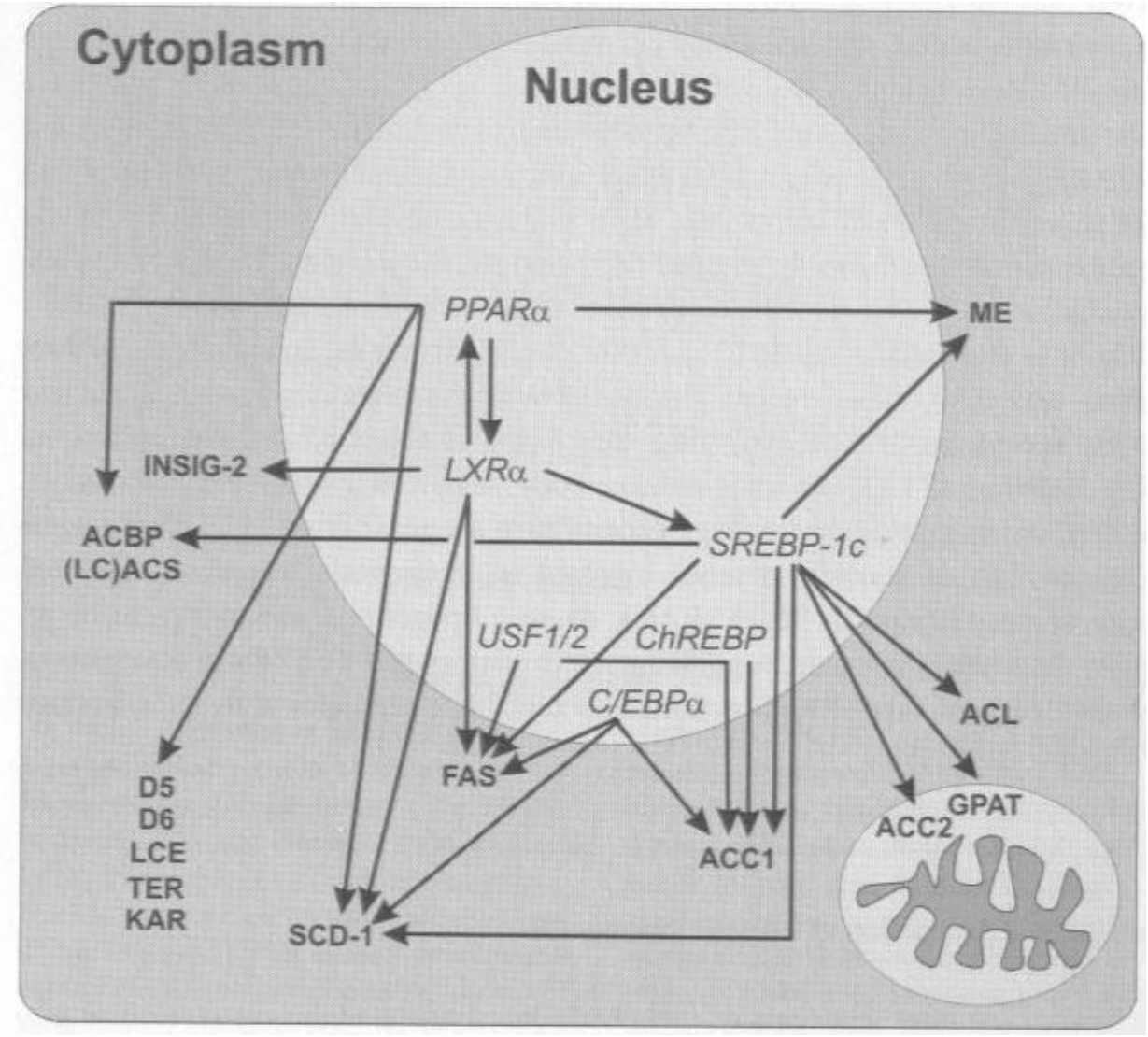
While it is clear that SREBP-1c and PPARγ are extremely important regulators of adipo- and lipogenesis, recent studies have drawn attention to other nuclear hormone receptors. One of these receptors is the Estrogen-Related Receptor alpha ERRα. Deletion of ERRα in mice reduces body weight and peripheral 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 ERRα on lipogenesis. These data suggest that ERRα 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 symplify 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/EBPα: 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; LXRα: Liver-X-Receptor α; ME: Malic Enzyme; PPARα: Peroxisome Proliferator-Activated Receptor α; 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-/- or USF2-/- 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-/- 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/EBPα in hepatic lipogenesis. Deletion of C/EBPα 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/EBPα null mice. Thus, in addition to its functional role in adipogenesis, C/EBPα 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 PPARγ, 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 LXRα mRNA, which in turn increases LXRα protein.

Although both insulin and LXRα stimulate SREBP-1c gene expression, they do so by different pathways. According to Hegarty et al. LXRα 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 LXRα. 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 PPARα 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 PPARα 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 PPARα would stimulate both fatty acid oxidation and fatty acid elongation/desaturation. It has been proposed that up-regulation of desaturases expression by PPARα may generate unsaturated fatty acids, which are agonists for PPARα. 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 PPARα (39).

PPARα 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 PPARα-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 LXRα 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 LXRα (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 PPARγ 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 PPARγ in hepatic steatosis (47). Indeed, specific deletion of hepatic PPARγ 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 PPARγ1 in liver of PPARα-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 PPARγ2 in hepatocytes was shown to lead to both hepatic lipid accumulation and induction of lipogenic and adipogenic gene expression (51). Thus, over-expression of PPARγ appears

to be both necessary and sufficient for hepatic steatosis. Accordingly, in certain pathological conditions, over-expression of PPARγ 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-α "TNF-α"and tumor growth factor-β. 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-α mRNA levels are increased in several models of genetically induce 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-α 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, TNFα 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 PPARγ (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 PPARγ, expression of which is upregulated by TZDs in both mouse and human adipocytes (59 and unpublished data). Visfatin might be regulated by PPARγ ligands as well, although this remains to be demonstrated. The possible stimulatory role of PPARγ 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, PPARγ 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 tighly 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.

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