Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1740 (2005) 266–286

BIOCHIMICA ET BIOPHYSICA ACTA BBA

http://www.elsevier.com/locate/bba

Review

Regulation of adipocyte differentiation and function by polyunsaturated fatty acids

Lise Madsen[a,*], Rasmus Koefoed Petersen[a,b], Karsten Kristiansen[a]

[a]*Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*
[b]*BioLigands, International Science Park Odense, Denmark*

Received 1 October 2004; received in revised form 23 February 2005; accepted 2 March 2005
Available online 22 March 2005

Abstract

A diet enriched in PUFAs, in particular of the n-3 family, decreases adipose tissue mass and suppresses development of obesity in rodents. Although several nuclear hormone receptors are identified as PUFA targets, the precise molecular mechanisms underlying the effects of PUFAs still remain to be elucidated. Here we review research aimed at elucidating molecular mechanisms governing the effects of PUFAs on the differentiation and function of white fat cells. This review focuses on dietary PUFAs as signaling molecules, with special emphasis on agonistic and antagonistic effects on transcription factors currently implicated as key players in adipocyte differentiation and function, including peroxisome proliferator activated receptors (PPARs) (alpha, beta and gamma), sterol regulatory element binding proteins (SREBPs) and liver X receptors (LXRs). We review evidence that dietary n-3 PUFAs decrease adipose tissue mass and suppress the development of obesity in rodents by targeting a set of key regulatory transcription factors involved in both adipogensis and lipid homeostasis in mature adipocytes. The same set of factors are targeted by PUFAs of the n-6 family, but the cellular/physiological responses are dependent on the experimental setting as n-6 PUFAs may exert either an anti- or a proadipogenic effect. Feeding status and hormonal background may therefore be of particular importance in determining the physiological effects of PUFAs of the n-6 family.

Keywords: Polyunsaturated fatty acid; Adipocyte differentiation; Peroxisome proliferator-activated receptor; Sterol regulatory element-binding protein; Liver X receptor

1. Introduction

Obesity is the major factor predisposing individuals to different dyslipidemic conditions and is thus becoming an enormous challenge to health systems worldwide. Although the so-called obesity epidemic is now recognized by the World Health Organization as one of the top 10 global health problems, clinicians have had little success in the fight against obesity. During the last decade, several genetic defects have been associated with obesity in humans, among those a number of mutations in genes involved in appetite regulation [1]. However, such mutations have only been detected in a minor fraction of obese individuals and the dramatic increase in the number of obese individuals during the last decades is rather a result of changes in the environment and eating habits. Thus, habitual food intake in excess of energy expenditure is still the primary factor leading to obesity.

As dietary fat contains more calories than protein and carbohydrates, limiting the intake of fat has been recommended in order to prevent obesity. Moreover, ever since the

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; dex, dexamethasone; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ERK1/2, extracellular signal-regulated kinase 1/2; DMSO, dimethylsulfoxide; LA, linoleic acid; MAPK, mitogen-activated protein kinase; LOX, lipoxygenase; LXR, liver X receptor; mix, methylisobutylxanthine; MDI, methylisobutylxanthine, dexamethasone and insulin; Ole, oleic acid; Palm, palmitic acid; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; rosi, rosiglitazone; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein; TG, triacylglycerol

* Corresponding author. Tel.: +45 6550 2429; fax: +45 6550 2467.
E-mail address: lise.madsen@bmb.sdu.dk (L. Madsen).

0925-4439/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2005.03.001

relationship between elevated plasma cholesterol and coronary heart diseases was established, low-fat diets have been recommended by several official institutions, including the World Health Organization and the American Heart Association, as a prophylactic treatment for different lipid-related disorders. However, reducing the relative amount of fat in the diet alone apparently is not sufficient to prevent weight gain. In USA, energy intake from dietary fats dropped from 40% to 33% from the 1960s to 1995. In the same period, the number of obese adults (BMI>25) was steadily increasing, reaching 56% in the early 1990s and 65% at present [2–4]. Similarly, in Norway, the relative intake of dietary fats dropped from 40% to 34% from the middle of the 1970s to the middle of the 1990s [5]. In spite of this decrease in intake of dietary fats, the average male BMI increased from 24.8 to 26.6 (9.1 kg) and the average female BMI increased from 24.7 to 25.1 (3.7 kg) from 1960 to 1999 [6].

It is now clear that the effect of dietary fats on human health depends on the sources and nature of the fatty acids. Dietary fat associated with an increased risk of dyslipidemic disorders, including coronary heart disease, is primarily trans-fat, saturated fat and cholesterol. Generally, saturated fat of mammalian origin seems to be far more harmful to human health than unsaturated fat from plants and fish. A considerable number of studies have demonstrated the beneficial effects of PUFAs on lipid-related disorders in humans [7–11]. Thus, not only the quantity of ingested fats, but also the composition and nature of the fatty acids are of pivotal importance for human health.

The specific actions of different fatty acids are to a large extent determined by their metabolic properties. Chain-length, position and number of double bounds determine both physical and chemical properties of the fatty acids as well as their metabolic destinies. Most effects of fatty acids on cellular homeostasis are mediated by their metabolites. Inside the cell, fatty acids are elongated, desaturated, β-oxidized, peroxidized, incorporated into phospholipids and complex lipids such as ceramides and sphingolipids, or they participate in or interfere with eicosanoid synthesis. Enzymes in the eicosanoid pathway (cyclooxygenases, lipoxygenases, and P450 epoxygenases) normally use the major n-6 fatty acid, arachidonic acid, liberated from phospholipids by phospholipases, as substrates. Eicosanoids are not stored in the cells, but rather synthesized and immediately released (within 5–60 s) in response to a variety of hormones or cytokines. Arachidonic acid-derived eicosanoids have extremely short biological half-lives, and hence, the initial phases of the signaling cascades are stringently controlled. Of note, a number of pathologic states such as inflammation, asthma, hypertension and certain types of cancer are associated with the dysregulation of the eicosanoid pathway [12–14]. The structurally similar n-3 PUFAs may replace arachidonic acid in phospholipids. Some n-3 PUFAs are converted into products with properties distinct from those generated from arachidonic acid, while others, in particular DHA, are inhibitors of cyclo-oxygenases (and possibly lipoxygenases). The consumption of a diet enriched in n-3 PUFA (specifically EPA and DHA) may thereby affect eicosanoid biosynthesis [15–17].

Dietary fatty acids and their metabolites are able to modulate protein expression by several mechanisms. They may affect gene transcription, messenger RNA processing and modulate posttranslational modifications of proteins [18–21]. PUFAs are known to suppress lipogenic gene transcription by downregulating the expression of the sterol regulatory element-binding proteins (SREBPs) [22–25] and they may function as antagonists of liver X receptors (LXR) [26,27] and as activators/ligands for the peroxisome proliferator-activated receptors (PPARs) [28–32]. Knowledge regarding fatty acids as kinase cascade activators/inhibitors has also accumulated during the last decades [33–38].

Until now, far more attention has been focused on strategies for treating obesity-associated diseases rather than focusing on preventing or treating the major underlying risk factor, obesity. However, understanding the processes that lead to de novo differentiation of adipocytes and onset of obesity would be necessary for developing new rational modalities for the prevention and treatment of obesity. It has been demonstrated that diets enriched in n-3 PUFAs decrease adipose growth in rodents [39–48], but only few studies have addressed questions concerning the effects of different fatty acids on adipocyte differentiation. Here, we review research aimed at elucidating molecular mechanisms governing the effects of PUFAs on the differentiation and function of white fat cells, with special emphasis on dietary PUFAs as signaling molecules.

2. PUFAs and PPARγ

2.1. PUFAs as inducers of adipocyte differentiation and PPARγ activators

Feeding rodents a high-fat diet induces the replication and differentiation of preadipocytes as well as adipose tissue hypertrophy [49–55]. High-fat feeding leads to increased levels of expression of the transcription factors C/EBPα and PPARγ and a number of PPARγ-target genes involved in adipocyte differentiation and lipid storage [56]. The adipocyte differentiation process is strictly dependent on the activation of PPARγ [28,57–59], and the forced expression of PPARγ and/or administration of PPARγ-ligands induce adipocyte differentiation of fibroblasts as well as myoblasts in vitro [60,61]. PPARγ knock-out is embryonic lethal due to placental dysfunction [28,58,59]. By aggregation of PPARγ null embryos with tetraploid embryos, one PPARγ-deficient pup has been recovered, and this pup was completely devoid of adipose tissue [28]. Furthermore, adipose tissue-targeted knock-out of PPARγ results in severe lipodystrophic animals without adipose

tissue [57]. Heterozygous PPARγ-deficient mice are reported to have decreased fat mass and smaller sized adipocytes [58,62,63]. These animals were, with one exception [64], reported to be protected from high-fat diet induced adipocyte hypertrophy [58,62,63].

A surprising variety of compounds are able to activate the PPARs [30,65,65–69]. Most fatty acids activate all three members of the PPAR family at micromolar concentrations [29,31,32,70]. In general, coactivator-dependent receptor ligand assays, binding competition assays and transactivation assays indicate that PUFAs are better activators of PPARγ than MUFAs and saturated fatty acids [29,31,32,70]. An exception is erucic acid, that does not activate the PPARs [65,70]. The highest binding affinity is achieved with compounds containing 16–20 carbons [29], but also DHA activates the PPARs [29,30,32].

The administration of synthetic PPARγ-ligands to various strains of rats leads to a dose-dependent increase in fat mass [71–74] and the generation of new small fat cells [74,75]. Thus, the activation of PPARγ by ligands or high-fat feeding induces adipocyte differentiation also in vivo. Whereas synthetic PPARγ-ligands induce hyperplasia and not hyperthrophy, high-fat feeding induces both hypertrophy and hyperplasia. The type of fat, however, is important since fish oil feeding decreases body weight and fat mass in a dose-dependent manner [46], whereas PUFAs, in general, are able to limit hyperplasia as well as hypertrophy in high-fat fed rats [47,49,76–79]. Thus, available data clearly indicate that PPARγ is essential for adipose conversion. The effect of PPARγ is, however, highly dependent on the type of ligands that activate the receptor.

The 3T3-L1 cell-line is a widely used model system for the analysis of adipocyte differentiation. The characteristic of this cell line is its ability to differentiate into mature adipocytes upon stimulation with insulin, dexamethasone and methylisobutylxantine when maintained in fetal bovine serum. When 3T3-L1 cells in fetal bovine serum are treated with dexamethasone alone, a supply of an exogenous PPARγ-ligand is necessary for efficient conversion into mature adipocytes. In this system, we tested the ability of a selection of fatty acids, reported to activate PPARγ, to induce adipocyte differentiation. As demonstrated in Fig. 1, fatty acids are weak inducers of adipocyte differentiation at concentrations as high as 100 μM. Of note, the weak effects on adipocyte differentiation appear to mirror the ability of the individual fatty acids to activate PPARγ in transactivation assays (Fig. 1).

In the presence of both dexamethasone and insulin, several fatty acids, except erucic acid, more effectively stimulated the differentiation and increased the accumulation of triacylglycerol (Fig. 2). The saturated fatty acid palmitic acid and the MUFA oleic acid stimulated adipocyte

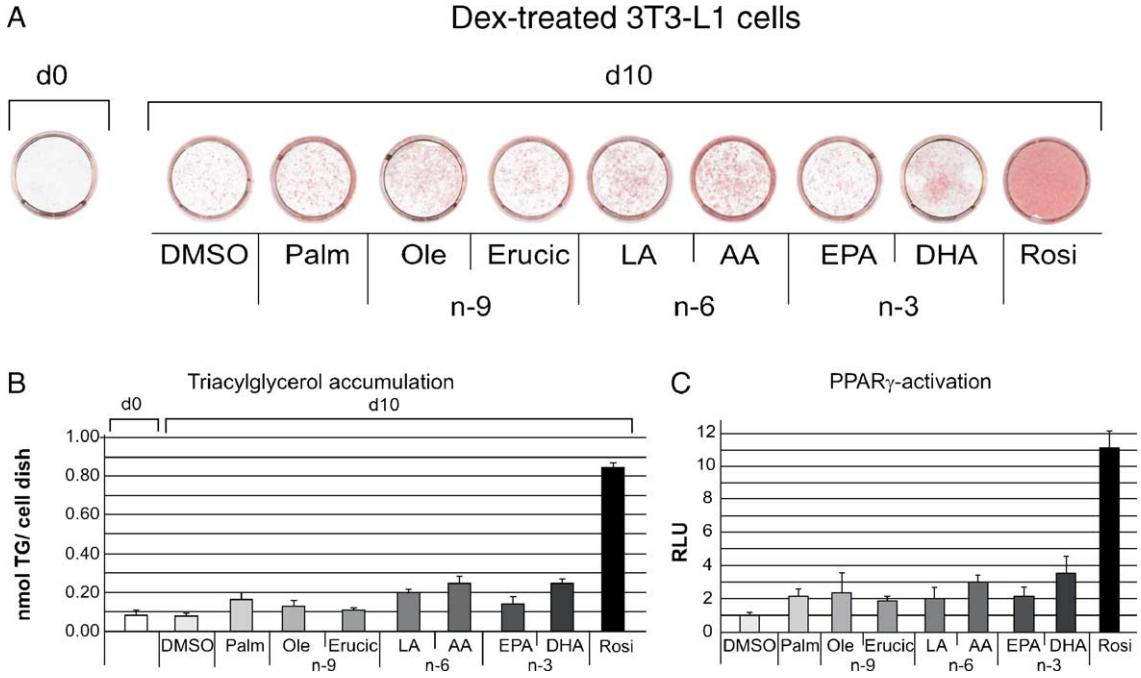

Fig. 1. 3T3-L1 cells were induced to differentiate at 2-day post confluence (designated day 0) with DMEM containing 10% fetal bovine serum (FBS) and 1 μM dexamethasone (Dex). From day 2, the medium consisted of DMEM with 10% FBS and was changed every second day. 100 μM palmitic acid (Palm), oleic acid (Ole), erucic acid, linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or 1 μM rosiglitazone (rosi) dissolved in DMSO were added when differentiation was induced at day 0 and were present throughout the differentiation period. (A) The cells were stained with oil-red-O and photographed on day 0 and day 10. (B) The levels of triacylglycerols were measured in whole cell extracts on day 0 and day 10. (C) 3T3-L1 cells were transiently transfected with the UASGalx4-TK-luc reporter, a vector expressing Gal4-DBD-PPARγ-LBD and a CMV-β-galactosidase vector. The cells were incubated for 24 h in the absence or presence of 100 μM fatty acids or 0.5 μM rosiglitazone dissolved in DMSO. Data are presented as luciferase activity normalized to β-galactosidase. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

differentiation and triacylglycerol accumulation to a similar extent, whereas PUFAs of both the n-3 and n-6 families were less effective (Fig. 2). It is also noteworthy that the lipid droplets in cells induced to differentiate in the presence of PUFAs were smaller in size than the lipid droplets formed in the presence of saturated fatty acids and MUFAs (Fig. 2). Thus, dietary PUFAs decrease lipid droplet size both in vitro and in vivo.

2.2. PUFA metabolites as inducers of adipocyte differentiation and PPARγ activators

The high concentrations of fatty acids required to activate PPARγ suggest that they are metabolized in the cell to active forms or induce the release or synthesis of the true endogenous PPAR-ligand(s). Indeed, several fatty acid metabolites activate PPARγ at lower concentrations than the fatty acids per se. Several products of the lipoxygenase and cyclooxygenase pathways are reported to activate PPARγ [70,80–86]. The most studied eicosanoid in this matter is 15-d-PGJ2 that was reported to bind and activate PPARγ and induce adipocyte differentiation [85,86]. Since then, 15-d-PGJ2 has often been referred to as the endogenous PPARγ-ligand. However 15-d-PGJ2 is produced in picomolar concentrations in 3T3-L1 adipocytes, several orders of magnitude below the levels required to induce differentiation [87]. Furthermore, upon induction of differentiation, production of 15-d-PGJ2 does not increase

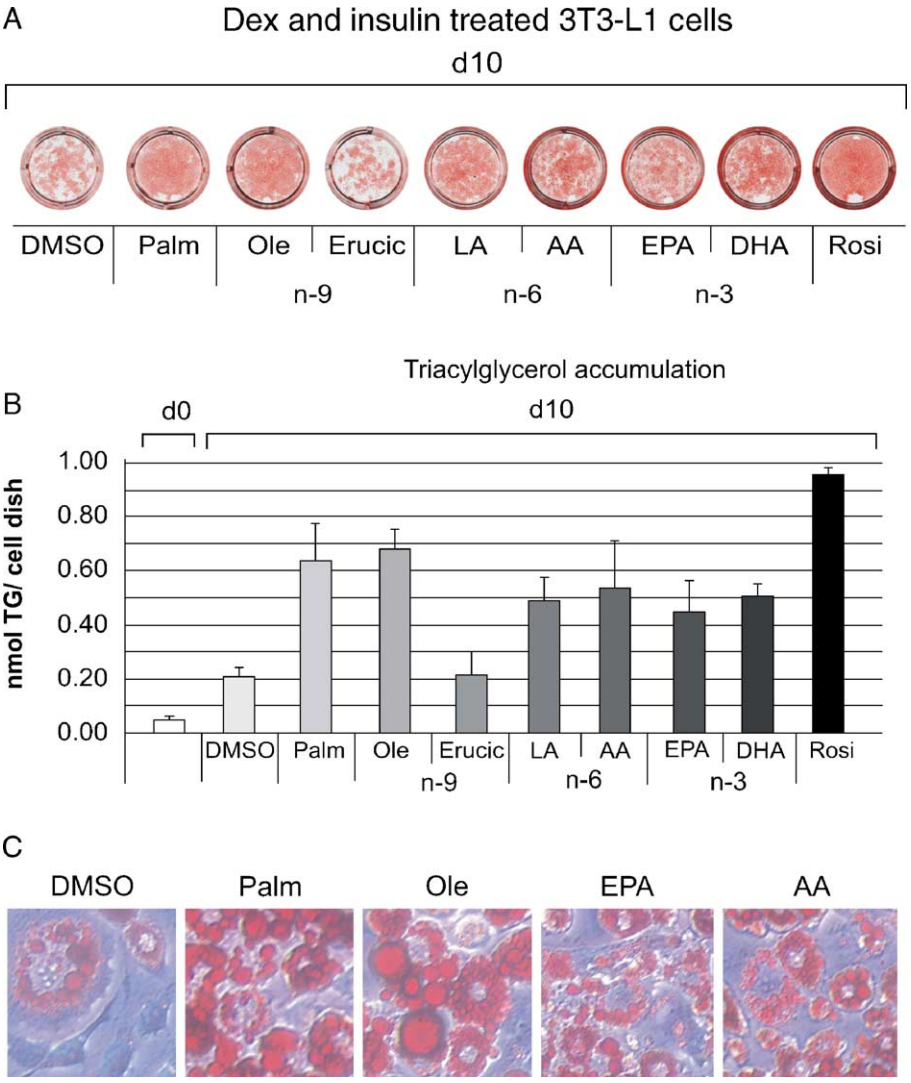

Fig. 2. 3T3-L1 cells were induced to differentiate at 2-day post confluence (designated day 0) with DMEM containing 10% fetal bovine serum (FBS), 1 μM dexamethasone (Dex) and 1 μg/ml insulin (ins). After 48 h, the cells were refed with DMEM containing 10% FCS and 1 μg/ml insulin. From day 4, the medium consisted of DMEM with 10% FBS and was changed every second day. 100 μM palmitic acid (Palm), oleic acid (Ole), erucic acid, linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or 1 μM rosiglitazone (rosi) dissolved in DMSO were added when differentiation was induced at day 0 and were present throughout the differentiation period. (A) The cells were stained with oil-red-O and photographed on day 0 and day 10. (B) The levels of triacylglycerols were measured in whole cell extracts on day 0 and day 10. (C) Micrographs of oil-red-O stained cells at day 10. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

[87,88]. Thus, 15-d-PGJ2 is unlikely to represent the true endogenous PPARγ-ligand mediating adipocyte differentiation [87].

Enzymes in the eicosanoid pathway, cyclo- and lipoxygenases and P450 epoxygenases normally use the major n-6 fatty acid, arachidonic acid, liberated from phospholipids by phospholipase A2, as substrate. Phospholipase A2 levels increase during differentiation, indicating increased substrate availability for eicosanoid synthesis [89]. The role of eicosanoid synthesis in adipose tissue development is, however, not clear cut and the role of cyclooxygenase in adipocyte differentiation has been a matter of dispute. Different prostaglandins have opposing effect on adipocyte differentiation. 15-d-PGJ2 and prostacyclin have been demonstrated to be proadipogenic [85,86,90–92], whereas $PGF_{2\alpha}$ inhibits the differentiation of primary preadipocytes [93–95], 1246 cells [94] and 3T3-L1 cells [96–98]. Secondly, as some of the commonly used cyclooxygenase inhibitors are themselves PPARγ activators, conflicting results using cyclooxygenase inhibitors have been obtained [99,100]. Cyclooxygenase-2 has been implicated in the regulation of body fat accumulation as haplo-insufficient mice are prone to develop obesity [101]. Low levels of arachidonic acid, especially in phospholipids, are observed in obese animals [102,103] and reduced prostaglandin synthesis has also been reported in adipocytes isolated from an animal model of obesity [104].

Recently, we initiated a comprehensive analysis of the expression of the different lipoxygenases and cyclooxygenases in adipose tissue. Seven different lipoxygenases are identified in mice. Using RT-PCR analysis, p12(S)-LOX, l12(S)-LOX and the novel eLOX-3 were detected in adipose tissue [105]. Generally, all these lipoxygenases were expressed at higher levels in the stromal vascular fraction than in adipocytes isolated from brown and white adipose tissues [105]. Cyclooxygenase-1 and -2 were expressed almost exclusively in the stromal vascular fraction (Philip Hallenborg, unpublished).

We and others have reported that endogenous PPARγ-ligands are produced rapidly upon methylisobutylxanthine, dexamethasone and insulin (MDI) treatment of 3T3-L1 cells, reaching a maximum during the first 4 days of differentiation [88,105]. In our hands, the inclusion of the general lipoxygenase-inhibitor NDGA (nordihydroguaiaretic acid) and the 12/15-LOX selective inhibitor baicalein, but not inhibitors of cyclooxygenases and P450, prevented the MDI-induced differentiation of 3T3-L1 cells and the production of endogenous PPARγ-ligands [105]. Using a stably integrated PPARγ-ligand-sensing reporter system in 3T3-L1 cells, Tzameli et al. reported that treatment with baicalein did not prevent the activation of the reporter, and thus concluded that lipoxygenases were not involved in the production of endogenous PPARγ-agonist in these cells [88]. However, baicalein itself is also able to enhance PPARγ-mediated transactivation and also increases rosiglitazone-induced PPARγ-mediated transactivation [105], and Tzameli et al. did not report whether treatment with baicalein abolished adipocyte differentiation. In our hands, baicalein-mediated inhibition of adipocyte differentiation was rescued by administration of rosiglitazone [105]. Accordingly, we concluded that the endogenous PPARγ-ligand(s) promoting adipocyte differentiation are generated by a baicalein-sensitive lipoxygenase-dependent pathway [105].

2.3. Effects of PUFAs mediated via prostaglandins

It is well documented that diets enriched in n-3 PUFAs decrease adipose growth in rodents [39–44], but concerns have been raised regarding the intake of n-6 fatty acids. Although a few studies demonstrate that a diet enriched in n-6 PUFAs decrease adipose tissue growth [44,106], n-6 PUFAs are often associated with an increased propensity to obesity [107–109]. The major n-6 PUFA, arachidonic acid, is required for the induction of differentiation of Ob1771 pre-adipose cells [91]. This proadipogenic effect of arachidonic acid is mediated by prostacyclin and is thus cyclooxygenase dependent [90–92]. In sharp contrast, however, another arachidonic acid-derived metabolite, PGF2α, potently inhibits the differentiation of primary preadipocytes [93,95], 1246 cells [94] and 3T3-L1 cells [96–98,110]. The effect of PGF2α is suggested to be mediated through an FP receptor-mediated increase in intracellular calcium and increased DNA synthesis [98] and through the activation of mitogen-activated protein kinase (MAPK), resulting in an inhibitory phosphorylation of PPARγ [111]. The role of PGE2, the third major prostaglandin produced in adipose tissue, in the regulation of adipocyte differentiation has been more controversial. It was reported that PGE2 only elicited an antilipolytic effect in mature adipocytes, but had no effect on preadipocytes [112]. However, it was recently demonstrated that functional EP4 receptors are present in 3T3-L1 preadipocytes and that the addition of PGE2 or a synthetic EP4 agonist suppressed MDI-induced adipocyte differentiation of 3T3-L1 cells [113,114].

The inclusion of 100 μM of n-6 PUFAs, arachidonic acid or linoleic acid, prevents adipocyte differentiation and accumulation of triacylglycerol when 3T3-L1 cells are induced to differentiate by MDI [96–98,110] (Fig. 3). On the other hand, differentiation proceeds in the presence of n-3 PUFAs, but less triacylglycerol accumulates compared with control cells (Fig. 3). Additionally, the lipid droplets in the adipocytes were very small (Fig. 3). Saturated and monounsaturated fatty acids did not affect differentiation (Fig. 3).

Non-selective cyclooxygenase inhibitors [98,110], as well as selective inhibitors of cyclooxygenase-1 and -2 [110], rescue the arachidonic acid-mediated inhibition of differentiation. The inhibitory effect of n-6 PUFAs on lipogenic gene expression is reported to be independent of cyclooxygenase activity in hepatocytes [115], but mediated through prostaglandins in differentiated 3T3-L1 adipocytes

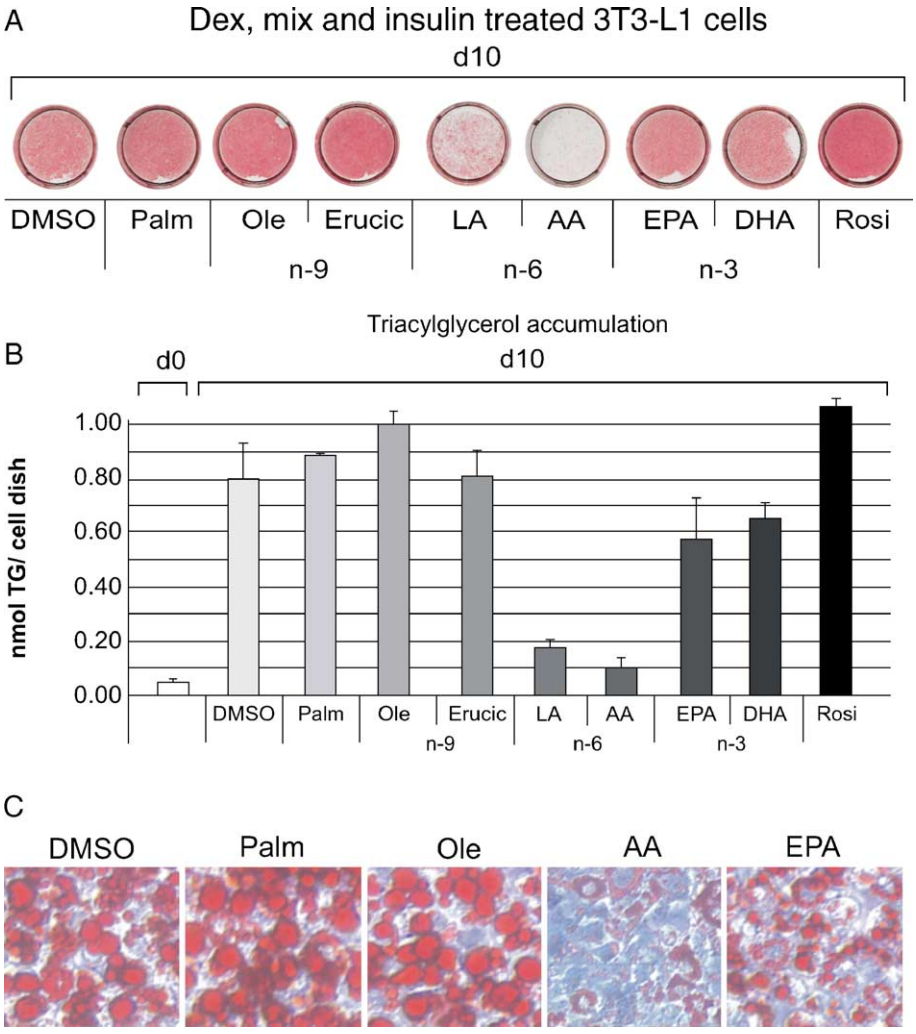

Fig. 3. 3T3-L1 cells were induced to differentiate at 2-day post confluence (designated day 0) with DMEM containing 10% fetal bovine serum (FBS), 0.5 mM methylisobutylxanthine (mix), 1 μM dexamethasone (Dex) and 1 μg/ml insulin (ins). After 48 h, the cells were refed with DMEM containing 10% FCS and 1 μg/ml insulin. From day 4, the medium consisted of DMEM with 10% FBS and was changed every second day. 100 μM palmitic acid (Palm), oleic acid (Ole), erucic acid, linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or 1 μM rosiglitazone (rosi) dissolved in DMSO were added when differentiation was induced at day 0 and were present throughout the differentiation period. (A) The cells were stained with oil-red-O and photographed on day 0 and day 10. (B) The levels of triacylglycerols were measured in whole cell extracts on day 0 and day 10. (C) Micrographs of oil-red-O stained cells at day 10. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

[116,117]. Compared with arachidonic acid and linoleic acid, EPA is a poor substrate for cyclooxygenases [118–120]. Additionally, n-3 fatty acids are able to inhibit cyclooxygenase activity [17,119]. The differential potency of n-3 and n-6 PUFAs to prevent adipocyte differentiation and the finding that the general cyclooxygenase-1 and -2 inhibitor, indomethacin, rescues adipocyte differentiation in the presence of arachidonic acid (Fig. 4) suggest that at least some effects of the n-6 PUFAs are mediated by prostaglandins. Interestingly, in the presence of indomethacin, the n-6 arachidonic acid and the n-3 EPA were equally effective in reducing triacylglycerol accumulation, and in both cases did the adipocytes possess small lipid droplets (Fig. 4).

Thus, n-6 PUFAs inhibit MDI-induced adipocyte differentiation of 3T3-L1 cells far more efficiently than n-3 PUFA does. This is in apparent conflict with feeding experiments in rodents showing that n-3 PUFA more effectively prevents high-fat diet-induced obesity than n-6 PUFA does [110,121]. Since the inhibitory effect of arachidonic acid on adipocyte differentiation requires prostaglandin synthesis, the effect of n-6 PUFAs is determined by the expression and activities of the cyclooxygenase. 3T3-L1 cells express both cyclooxygenase-1 and cyclooxygenase-2 [110,121]. Upon the induction of differentiation, cyclooxygenase-2 is transiently induced and then declines, whereas cyclooxygenase-1 expression declines gradually as differentiation progresses [110,121]. However, treatment with arachidonic acid leads to a sustained expression of cyclooxygenase-2 [110]. Moreover, as different prostaglandins are reported to have opposing effect on the differentiation process, the effect of n-6 PUFAs is also dependent on the expression and activities

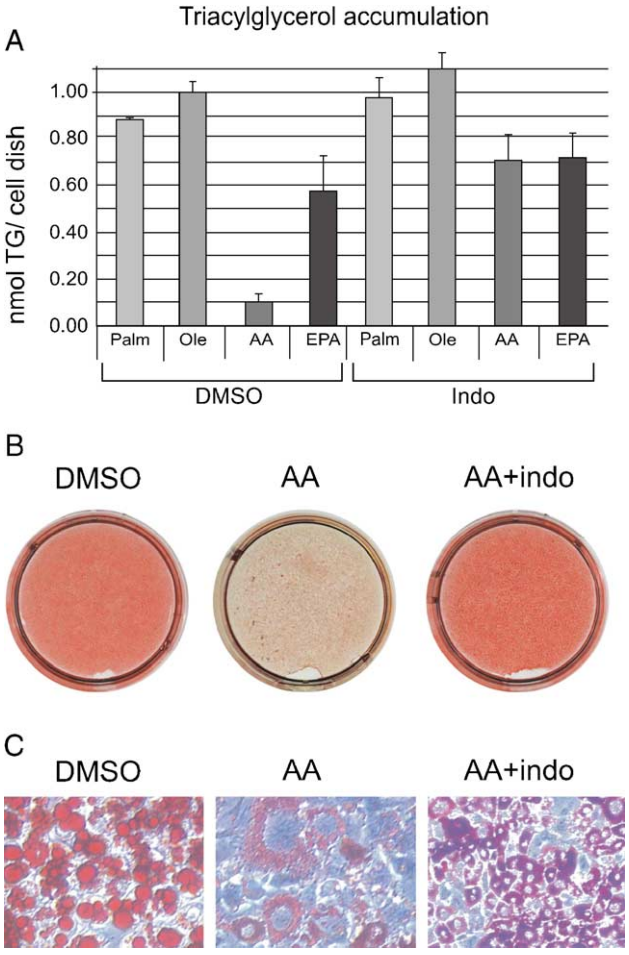

Fig. 4. 3T3-L1 cells were induced to differentiate as described in Fig. 3. 100 μM fatty acids and/or 1 μM indomethacin (indo) was added when differentiation was induced on day 0 and were present throughout the differentiation period. (A) The levels of triacylglycerols were measured in whole cell extracts on day 10. (B) The cells were stained with oil-red-O and photographed on day 10. (C) Micrographs of oil-red-O stained cells at day 10. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

of downstream enzymes that determine the type of prostaglandins produced. The effect of PUFAs in reducing the size of lipid droplets appears on the other hand to be related to the number of double bounds in the fatty acids, independent of prostaglandin synthesis.

A cAMP-responsive element in the cyclooxygenase-2 promoter has been shown to be essential for the induction of cyclooxygenase-2 by forskolin and dibutryl-cAMP in other cell lines [122,123]. In 3T3-L1 cells, sustained cyclooxygenase-2 expression by arachidonic acid requires the addition of a cAMP-elevating agent and is dependent of protein kinase A-activity [110]. Like sustained cyclooxygenase-2 expression, the general inhibition of adipocyte differentiation by arachidonic acid is dependent upon the addition of cAMP-elevating agents and protein kinase A-activity [110]. As observed for 3T3-L1 cell, arachidonic acid has also been shown to inhibit the differentiation of primary preadipocytes [93,95] and 1246 cells [94]. In contrast, arachidonic acid was reported to be proadipogenic in Ob17 [124], Ob1771 [90,91] and 3T3-F442A cells [91]. Interestingly, the differentiation of these latter cell lines is induced in media containing no cAMP-elevating agents, suggesting that hormonal status and cAMP signaling may account for some of the apparently contradictory results achieved when examining the effect of arachidonic acid on adipocyte differentiation.

2.4. PPARγ- and MAPK-induced phosphorylation

Over the past decade, evidence has accumulated that fatty acids may activate or inhibit various kinase cascades, including the ERK1/2 MAPK-pathway [33–38]. The effect of different fatty acids and their metabolites on ERK1/2 activation in 3T3-L1 cells is, however, largely unknown. In MDI-treated 3T3-L1 cells, the inclusion of arachidonic acid enhances ERK1/2 activation following induction and prolongs the period during which particularly ERK2 remains in the active state [110]. Moreover, the inhibition of MAPK-activation by MEK-inhibitors also restored adipogenesis in the presence of arachidonic acid [110] and $PGF_{2\alpha}$ [111]. Thus, MAPK-activity is necessary for arachidonic acid-mediated inhibition of adipocyte differentiation.

It is generally agreed that a transient upregulation of ERK1/2 MAPK-activity promotes adipogenesis and is necessary for clonal expansion [26,125–127], whereas the sustained activation of ERK1/2 has been demonstrated to inhibit adipogenesis [9,128–131]. Although recent findings clearly indicate that clonal expansion is an integral part of the differentiation process [132], it has also been reported that clonal expansion, at least under certain conditions, is dispensable [129,133]. Additionally, MAPK-activity is also able to downregulate PPARγ2 activity by the phosphorylation of serine 112 [134–136]. A serine-to-alanine substitution at this position of PPARγ prevents MAPK-phosphorylation [134–136]. However, when mice homozygous for such a mutation (S112A) are fed a high-fat diet, weight gain and adipose tissue mass are comparable to wild-type mice, but in contrast to wild-type mice, they remain insulin sensitive [137]. Interestingly, the mutant mice have smaller adipocytes and are more glucose tolerant than wild-type mice [137]. On the other hand, a mutation that increases PPARγ activity by preventing phosphorylation has been found in obese humans [138].

One of the main cyclooxygenase products in preadipocytes, $PGF_{2\alpha}$ [139], is reported to block adipogenesis by the phosphorylation of PPARγ through the activation of MAPK [111]. However, in our hands, arachidonic acid was unable to inhibit differentiation when 3T3-L1 cells were transduced with either the PPARγ wild-type or the S112A mutant (unpublished). Hence, even though MAPK activity is required for arachidonic acid-mediated inhibition of adipo-

cyte differentiation, the role of PPARγ phosphorylation in this process is never the less uncertain.

3. PUFAs as activators of PPARα

Whereas PPARγ is indispensable for adipogenesis, white adipose tissue develops normally in PPARα knock-out mice, but larger adipose stores and increased body weight are reported in aged mice [140,141]. PPARα serves as a receptor for a structurally diverse group of compounds, including hypolipidemic fibrates and a wide variety of saturated and unsaturated fatty acids. In agreement with transfection studies by Johnson et al. [31], Xu et al. found that saturated and unsaturated fatty acids bind to PPARα with approximately the same affinity using a scintillation proximity assay [142]. On the other hand, Lin et al. demonstrated that PPARα had a higher affinity for PUFAs than MUFAs using a fluorescence-based PPARα-ligand interaction assay [143]. Using transactivation assays, several groups have reported that PUFAs are better activators of PPARα than MUFAs and saturated fatty acids [29–31,65,70,82,144]. The latter is also supported by Forman et al., who used a conformation-based assay to determine binding of fatty acids to PPARα [29,65]. Optimal binding affinity was achieved with compounds containing 16–20 carbons [29]. However, unsaturated very long chain fatty acids, such as DHA, are good activators of PPARα compared with other fatty acids [29,30,32].

PPARα plays a pivotal role in the regulation of intermediary metabolism in the liver, particularly fatty acid oxidation, under conditions where plasma fatty acid concentrations are elevated. During fasting conditions, fatty acids are mobilized from adipose tissue and taken up by the liver where they are β-oxidized. The activation of PPARα has since long been known to increase hepatic fatty catabolism and reduce plasma triacylglycerols. The PPARα knock-out mice have reduced capacity to metabolize long-chain fatty acids [145,146] and the pleotropic effects of peroxisome proliferators are abolished [147]. Like the hypolipidemic fibrates, dietary PUFAs induce the expression of PPARα target genes, stimulate β-oxidation and reduce plasma triacylglycerol in rodents [148–156]. In agreement with most transfection and binding assays, dietary n-3 fatty acids are more effective than are n-6 fatty acids in activating genes controlled by PPARα in the liver of rodents [144,148,149,152,154]. Using 6 different doses of fish oil, Nakatani et al. [46] found a good correlation between decreased amount of white adipose tissue and hepatic PPARα activation. Studies in PPARα knock-out mice have also demonstrated that PPARα is necessary for n-3 PUFA to activate the expression of genes encoding β-oxidation enzymes [144,157].

Triacylglycerol stores in fat cells are largely derived from circulating triacylglycerols. Thus, the activation of PPARα by n-3 PUFAs increases hepatic β-oxidation and energy expenditure and thereby decreases the output of VLDL-particles from the liver and limits triacylglycerol supply to adipose tissue. Similar to dietary PUFAs, synthetic PPARα-agonists such as fenofibrate and bezafibrate as well as nonmetabolized fatty acids have been demonstrated to prevent high fat diet-induced increase of body weight and adipose tissue mass in mice and rats [73,158–160]. Also, both fibrates and n-3 PUFAs upregulate UCP2 expression in liver, possible via PPARα [161]. The activation of PPARα might therefore reduce obesity indirectly by increasing hepatic fatty acid oxidation.

A study by Guerre-Millo et al. would argue against this, as they reported that PPARα knock-out mice on either a pure Sv/129 background or 10 generations backcrossed C57BL/6N mice did not have significantly different amounts of adipose tissue compared with wild-type mice when fed a high-fat diet [162]. On the other hand, Kim et al. reported that PPARα KO mice on a mixed background (C57BL/6Nx129/Sv) have increased body weight when fed a high-fat diet, compared to their wild-type littermates [163]. A third study by Akiyama et al. using PPARα knock-out mice on a pure Sv/129 or C57BL/6N background reported an increased gonadal fat pad weight in the knock-out mice when the mice were subjected to a high-fat diet, but body weight did not differ between PPARα knock-out mice and wild-type mice [164]. Similar results were reported by Haluzik et al. using 120S4/SvJaePparatm^{1Gonz} PPARα knock-out mice from the Jackson Laboratory [165]. The different background of the mice might account for these differences.

PPARα is expressed at low levels in preadipocytes and the expression is induced during adipocyte differentiation [166]. In primary adipocytes, bezafibrate is reported to increase fatty acid oxidation accompanied by increased mRNA levels of UCP2 and UCP3 [167]. PUFAs are also able to increase UCP2 mRNA in 3T3-L1 preadipocytes [168]. When 3T3-L1 cells differentiate, fatty acid oxidation decreases dramatically (Fig. 5a). However, in the presence of PUFAs, β-oxidation is elevated compared with control cells on day 10 (Fig. 5b). In this respect, arachidonic acid was far more effective than EPA (Fig. 5b). This might, however, be a result of decreased differentiation. When PUFAs were added to fully differentiated cells on day 10, EPA and DHA were more effective than arachidonic acid in elevating β-oxidation (Fig. 5c).

Bezafibrate, known to reduce fat depots in rats [169], also increases acyl-CoA oxidase mRNA levels, fatty acid peroxisomal β-oxidation [170] and enzymes involved in mitochondrial β-oxidation [167] in rat white adipose tissue. Additionally, bezafibrate treatment of rats increases the expression of UCP3 in epididymal white adipose tissue [169]. Bezafibrate treatment has also been reported to induce UCP1 expression to detectable levels in epididymal white adipose tissue [169]. As the ATP-yield is reduced by the upregulation of these proteins, energy expenditure may be increased by PPARα activation also in adipose tissue.

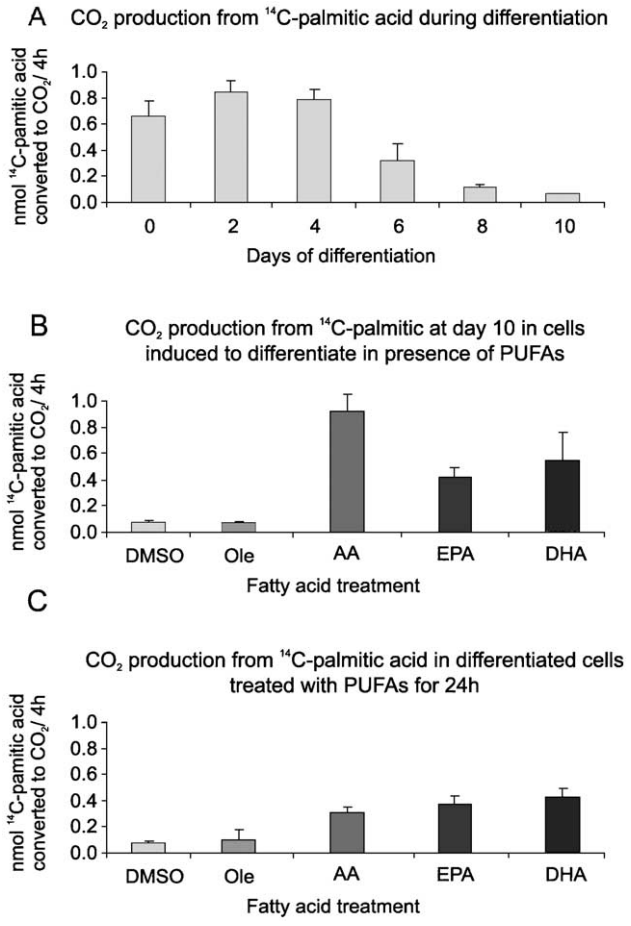

Fig. 5. 3T3-L1 cells were induced to differentiate as described in Fig. 3. To measure fatty acid β-oxidation cells were incubated with [U-^{14}C]palmitic acid for 4 h. Labeled CO_2 were trapped quantitated by scintillation as described earlier [110]. (A) Fatty acid β-oxidation was measured during differentiation at days 0, 2, 4, 6, 8 and 10. (B) 100 μM fatty acids were added when differentiation was induced on day 0 and was present throughout the differentiation period. Fatty acid β-oxidation was measured at day 10. (C) 100 μM fatty acids were added at day 9 and were present for 24 h. Fatty acid β-oxidation was measured at day 10. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

In conclusion, PUFAs may increase fatty acid oxidation and energy expenditure in adipose tissue by the activation of PPARα. Furthermore, by the activation of PPARα in the liver, PUFAs upregulate hepatic fatty acid oxidation and energy expenditure accompanied by decreased apolipoprotein expression and release of very-low density lipoprotein particles. Thereby, fatty acids are re-partitioned and drained from blood and extrahepatic tissues.

4. PUFAs as activators of PPARδ

PPARδ was cloned in 1994 [171] and was suggested to play a role in molecular signaling initiating adipocyte differentiation [172]. PPARδ is induced during the initial stages of adipocyte differentiation [172] but its role has been a matter of debate. As most PPAR-ligands activate more than one receptor subtype, the specific role of PPARδ in adipocyte differentiation has been difficult to determine. However, more specific ligands and the generation of PPARδ knock-out mice have been useful tools to explore its function.

The synthetic selective PPARδ-ligand L165041 has been shown to increase the expression of adipocyte specific markers and modestly promote terminal differentiation of preadipocytes [173]. The inclusion of L165041 in the differentiation medium enhanced terminal differentiation and lipid accumulation in wild-type but not PPARδ knock-out cells [174]. The overexpression of PPARδ enhanced the responsiveness of preadipocytes to fatty acids in terms of maximal response and sensitivity and promoted terminal differentiation by inducing PPARγ expression [175]. Additionally, the nonadipogenic fibroblast cell line 3T3-C2 became responsive to fatty acids and acquired preadipocyte characteristics when PPARδ was ectopically expressed [176]. However, a PPARγ-ligand was still necessary to trigger terminal differentiation suggesting an interaction between PPARδ and PPARγ in modulating differentiation [176]. In contrast, the overexpression of PPARδ and treatment with ligands were reported to be insufficient to stimulate differentiation of NIH-3T3 fibroblasts [177], and similarly, it was reported that the activation of PPARδ did not induce the differentiation of fibroblasts [174] and 3T3-L1 cells [178]. The lack of effect in these experiments might, however, be due to a lack of a cAMP-elevating agent. We have demonstrated that the addition of a cAMP-elevating agent is crucial for the ability of PPARδ-ligands to induce the expression of PPARγ and induce adipocyte differentiation in PPARδ-expressing NIH-3T3 cells [173].

A second possible role for PPARδ in adipogenesis is the modulation of clonal expansion as ligand activation of PPARδ causes increased cell proliferation [173]. Moreover, enforced expression of PPARδ also renders the nonadipogenic cell-line 3T3-C2 cells capable of resuming cell proliferation in response to the administration of fatty acids [179].

A role for PPARδ in the development of adipose tissue is further supported by the fact that adipose tissue stores are reduced in PPARδ null-mice [180,181]. Recently, it was also demonstrated that adipocyte differentiation of PPARδ-deficient cells is impaired even in the presence of a PPARγ-agonist [174]. The apparent importance of PPARδ in adipocyte differentiation might seem to contradict the fact that mice with adipose tissue targeted PPARδ ablation accumulate more fat than wild-type mice when fed a high-fat diet [182]. However, the recombination of the PPARδ locus was mediated via an aP2 promoter-controlled expression of the cre recombinase, and thus occurred relatively late in the differentiation process. Furthermore, these mice lack a functional PPARδ in both white and brown adipose tissue [182]. Thus, a significantly compromised UCP1 expression resulting in

impaired thermogenesis might also contribute to the rapid weight gain in these mice [182]. It has not yet been possible to generate mice with white adipose tissue specific ablation of PPARδ.

The adipose tissue-targeted expression of a constitutively active form of PPARδ was shown to reduce adiposity [182]. These transgenic animals have increased the expression of genes required for fatty acid oxidation and energy dissipation, and treatment with PPARδ-agonists caused severe lipid depletion [182]. The activation of PPARδ in adipose tissue also reverses obesity in the leptin-deficient $Lepr^{db/db}$ mice [182]. The increased expression of PPARδ in brown adipose tissue leads to increased β-oxidation and expression of UCP1 and UCP3 [182] and thus increased energy expenditure. Although UCP1 is a brand of brown adipose tissue, UCP1 expression was significantly induced also in the white fat of the PPARδ transgenic mice [182]. Of note, low levels of transgenic expression of UCP1 in white fat are sufficient to protect against obesity [183]. PPARδ-ligands also induce fatty acid β-oxidation [182,184] and expression of UCP3 in myotubes [182] and primary muscle cells [185]. UCP3 is also upregulated in L6 myocytes by the synthetic PPARδ-agonist GW742 [186]. Skeletal muscle PPARδ is indeed under nutritional regulation and its expression is induced under conditions such as fasting, when β-oxidation is high [184]. PPARδ might therefore act as a key metabolic regulator of fat burning in peripheral tissues.

Fatty acids are able to activate PPARδ in transfection assays, but less is known about the potency of different fatty acids to activate PPARδ. Apparently, fatty acids that are unable to undergo fatty acid oxidation, such as 2-bromopalmitate [172] and tetradecylthioacetic acid [187], are effective activators of PPARδ. Using transactivation assays or ligand-induced complex formation assays, three different groups have reported that PUFAs are better activators than are saturated fatty acids [29,31,32]. Using a coactivator-dependent receptor ligand assay, Krey et al. reported that long-chain fatty acids were better activators than are very long-chain fatty acids, C>20 [82]. However, DHA activates PPARδ at least as well as linoleic acid in transfection assays [32].

Similar to PPARδ-agonists, dietary PUFAs, especially of the n-3 family, activate genes involved in β-oxidation [47,188–190] and induce the expression of UPC3 in skeletal muscle [42,188], the expression of UCP2 in white adipose tissue [191] and the expression of UCP1 in brown adipose tissue [43]. PPARδ may accordingly play dual roles in adipocyte differentiation and function. The activation of PPARδ seems to promote the early stages of adipogensis including mitotic clonal expansion and the induction of PPARγ2 expression, whereas in more mature adipocytes (and in muscle), the activation of PPARδ by PUFAs may reduce adiposity by inducing the expression of genes required for fatty acid oxidation and energy dissipation.

5. PUFAs as inhibitors of SREBP

Feeding status does not only affect adipose cells by controlling the number of adipose cells, but also determines the amount of lipid in each cell. When rats are fed a high-carbohydrate diet, or refed after fasting, several genes involved in de novo fatty acid synthesis and triacylglycerol synthesis in liver and adipose tissue are induced. The expression of lipogenic genes is controlled by the transcription factors SREBP-1a and -1c [192–194]. The nutritional induction of genes involved in lipogenesis is mainly controlled by the SREBP-1c isoform. During fasting, the expression of SREBP-1c and the expression of lipogenic genes are reduced dramatically in both the liver [195] and adipose tissue [196]. Refeeding a fat-free, high-carbohydrate diet induces de novo fatty acid synthesis and the expression of SREBP-1c and lipogenic genes to levels significantly higher than those observed in the normal fed state in both liver [195] and adipose tissue [196]. The effect is, however, less prominent in the adipose tissue than in the liver [197]. In fasted–refed SREBP-1c knock-out mice, the expression of lipogenic genes also rises, but not to the same extent as in the liver of wild-type animals [198]. The effect of refeeding is totally abolished in mice lacking the SREBP cleavage activating protein (SCAP), in which the activation of all SREBPs is absent [198]. Thus, it appears that SREBP-1a or -2 may substitute for SREBP-1c in permitting an insulin-mediated response of lipogenic gene expression [198].

The ability of PUFAs to inhibit lipogenesis in the liver has been known since the early studies by Allmann and Gibson almost 40 years ago [199]. Since then, several groups have demonstrated that dietary PUFAs suppress hepatic lipogenesis [150,200–204]. It is noticeable that several of these studies show that PUFAs of the n-3 and n-6 family are equally effective in inhibiting lipogenesis in the liver of rodents.

PUFAs are able to alter the expression of lipogenic genes by interfering with SREBP-1 expression at different levels in mouse liver. Firstly, dietary PUFAs are able to lower SREBP-1c mRNA levels [24,25,205]. Secondly, PUFAs inhibit the proteolytic maturation that is necessary for SREBP to exert transcriptional activity [23,46,206]. The possibility that PUFAs lower SREBP-1 expression by accelerating the degradation of mature SREBP-1 is, however, excluded by the finding that PUFAs do not reduce the amount of ectopically expressed mature SREBP-1 protein [207]. Dietary PUFAs are also known to repress the expression of lipogenic genes in adipose tissue [78,208], but far less is known concerning the underlying mechanism in peripheral tissue.

In hepatocytes, the ability of PUFAs to decrease the level of mature SREBP-1 and expression levels of lipogenic genes increases with increasing chain length and number of double bounds [22,144]. The placement of the double bounds appears, however, to be of less

importance, as both n-6 and n-3 PUFAs are equally effective. Neither does the effect of PUFAs on hepatic lipogenesis require cyclooxygenase activity [115]. In adipocytes, fatty acids of the n-6 family are far more potent than those of the n-3 family and require cyclooxygenase activity [116].

During adipocyte differentiation, SREBP-1 mRNA is highly induced [209] and proteolytically activated [210]. Several lines of evidence have suggested a role of SREBP-1c, also known as adipocyte determination and differentiation factor (ADD1), in adipocyte differentiation [209]. Forced expression of SREBP-1c induced an expression of adipocyte specific genes and increased the differentiation of the nonadipogenic fibroblast cell line NIH-3T3 [211]. It has also been demonstrated that SREBP-1c is able to directly transactivate the C/EBPβ promoter [212] and induce expression of PPARγ [213]. The forced expression of SREBP-1c also leads to the production of a putative endogenous PPARγ-ligand [214].

Although these findings strongly suggest a role of SREBP-1c in adipocyte differentiation, SREBP-1c knockout mice have normal adipose tissue [198,215] and mice that lack both the SERBP-1c and -1a isoforms also have normal amounts of adipose tissue and fully differentiated adipocytes expressing normal levels of adipocyte specific markers [216]. The ablation of SREBP-1 did not decrease the amount of white adipose tissue in C45BL/6J-129Sv/Ev hybrids [216] or obese leptin-deficient Lep$^{ob/ob}$ mice [217,218]. SREBP-1c is the predominant SREBP-1 isoform expressed in adipose tissue [193], but an adipocyte-specific overexpression of SREBP-1c in mice severely inhibits adipocyte differentiation and renders the mice lipodystrophic [219]. On the other hand, adipocyte specific transgenic expression of SREBP-1a in mice led to massively enlarged adipocytes with increased expression of lipogenic genes and increased rate of de novo fatty acid synthesis [220]. The nature of the SREPB-1 isoforms in 3T3-L1 cells is controversial. The 3T3-L1 cells studied in Spiegelmans laboratory were reported to predominantly express SREBP-1c [196], whereas 3T3-L1 cells from the laboratory of Goldstein and Brown [193] as well as Sato [210] exclusively expressed SREBP-1a. The reason and potential consequence of this discrepancy are not clear, but the expression of lipogenic genes is highly induced during the differentiation of both cell lines.

During differentiation, de novo fatty acid synthesis also increases tremendously (Fig. 6a). When 3T3-L1 cells are induced to differentiate in the presence of PUFAs, the rate of de novo fatty acid syntheses is decreased compared with control cells (Fig. 6b). A similar, but less pronounced, effect is seen when fatty acids are added to fully differentiated cells (Fig. 6c). Similar to the effect of PUFAs on differentiation and triacylglycerol accumulation (Fig. 3), n-6 PUFAs are more effective than n-3 PUFAs to inhibit de novo fatty acid synthesis (Fig. 6). However,

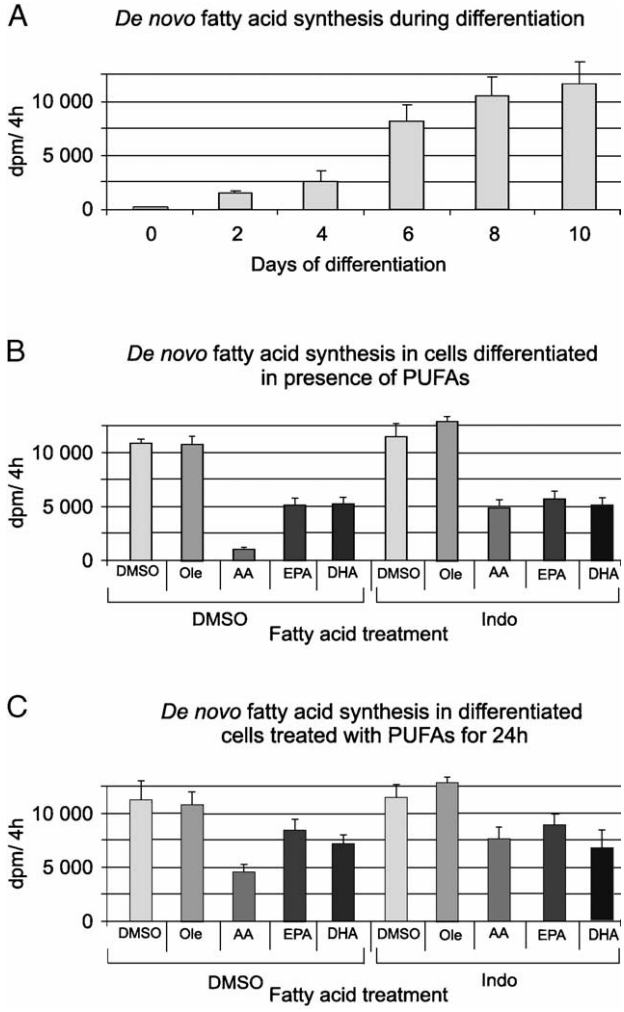

Fig. 6. 3T3-L1 cells were induced to differentiate as described in Fig. 3. To measure de novo fatty acid synthesis, cells were incubated with [^{14}C]acetic acid for 4 h. Labeled fatty acids were extracted and quantitated by scintillation as described earlier [110]. (A) De novo fatty acid oxidation was measured during differentiation at days 0, 2, 4, 6, 8 and 10. (B) 100 μM fatty acids and/or 1 μM indomethacin (indo) were added when differentiation were induced on day 0 and was present throughout the differentiation period. De novo fatty acid synthesis was measured on day 10. (C) 100 μM fatty acids and/or 1 μM indomethacin (indo) were added at day 9 and were present for 24h. De novo fatty acid synthesis was measured on day 10. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

while the inclusion of the non-selective cyclooxygenase inhibitor, indomethacin, completely rescued differentiation and triacylglycerol accumulation of arachidonic acid-treated cells (Fig. 3), the rate of de novo fatty acid synthesis was only partially restored (Fig. 6). This suggests that arachidonic acid reduces the rate of de novo fatty acid synthesis in both a prostaglandin-dependent and -independent manner. Part of the inhibitory action of arachidonic acid on fatty acid synthesis is probably due to its polyunsaturated nature, as also EPA and DHA were able to inhibit de novo fatty acid synthesis in a cyclooxygenase independent manner (Fig. 6). Interestingly, when cyclo-

oxygenase activity is inhibited, the efficiency of PUFAs to inhibit de novo fatty synthesis was related to the chain length and degree of unsaturation. It is also noteworthy that the reduction in the size of lipid droplets by PUFAs was not dependent of cyclooxygenase activity. The ability of fatty acids to reduce lipid droplet size is therefore probably related to their degree of unsaturation, whereas the ability of arachidonic acid to inhibit differentiation requires cyclooxygenase activity.

Two of the SREBP-regulated genes highly induced during adipocyte differentiation are the stearoyl-CoA desaturases (SCD) or Δ^9-desaturases [221] that catalyze the initial desaturation of long-chain fatty acids, primarily palmitic acid and stearic acid at the Δ^9 position. The induction of SCD1 and -2 in 3T3-L1 differentiation is paralleled by the increased expression of SREBP-1c [222]. SCD activity has been shown to be elevated in the adipose tissue of various animal models of obesity [223]. In contrast to SREBP-1 knock-out mice, the SCD1 knock-out mice have reduced adiposity and are resistant to diet-induced obesity [224]. The disruption of the SCD-1 gene in *ob*/*ob* mice also reduces obesity in this model [225]. Dietary PUFAs are major regulators of SCD expression in several organs including adipose tissue and PUFAs dowregulate the expression of SCD-1 in adipose tissue of rats and differentiated 3T3-L1 cells [223]. The downregulation of SCD expression might therefore contribute to the ability of PUFAs to reduce adipose tissue growth. The underlying mechanism by which reduced SCD1 expression leads to reduced adiposity remains, however, to be deciphered.

The roles of the different SREBPs in adipocyte differentiation and adipose tissue development are thus still not elucidated. Although PUFAs evidently decrease lipogenesis in adipose tissue, in an SREPB-dependent manner, one cannot yet determine the importance of this effect in relation to the effect of PUFAs on adipose tissue mass.

6. PUFAs as antagonists of LXR-activation

LXRα is a potent activator of SREBP-1c expression and, thereby, the expression of several lipogenic genes in both liver and adipose tissue [226,227]. The increased expression of lipogenic genes in mice treated with LXR agonists is blunted in SREBP-1c knock-out mice, indicating an essential role of SREBP-1c in the LXR response [198]. Also, LXRα/β-deficient mice have reduced expression of SREBP-1c and lipogenic genes [228,229]. Two different groups have suggested that LXRα is responsible for mediating the PUFA effect on SREBP-1 [26,27]. The activation of the SREBP-1c promoter by LXRα is suppressed by PUFAs [27]. n-6 PUFAs are more potent LXR antagonists than are n-3 PUFAs, whereas monounsaturated and saturated fatty acids had little effect [27]. Thus, it appears that PUFAs downregulate hepatic lipogenic gene expression by serving as antagonists for LXR.

Several lines of evidence indicate a role for LXR in adipose tissue as well. Firstly, both LXRα and LXRβ are highly expressed in adipose tissue [230–232]. Secondly, LXRα expression is induced during adipocyte differentiation [233]. Thirdly, LXRα is regulated by PPARγ [234] and C/EBPα [235]. Recently, it was also demonstrated that PPARγ is a LXRα target in adipocytes [227]. However, LXR is not indispensable, as the LXRα/β double knock-out mice develop adipose tissue [226,236]. Older double knock-out mice exhibit reduced adipose tissue stores [236], but the basis for this phenotype is not yet known.

The role of LXR as a modulator of adipogenesis has recently been a matter of debate. Juvet et al. reported increased lipid accumulation in 3T3-L1 cells by treatment with the synthetic ligand T0901317 or the endogenous ligand 22(*R*)-hydroxycholesterol [236]. These results were recently reproduced by Seo et al., who found increased lipid

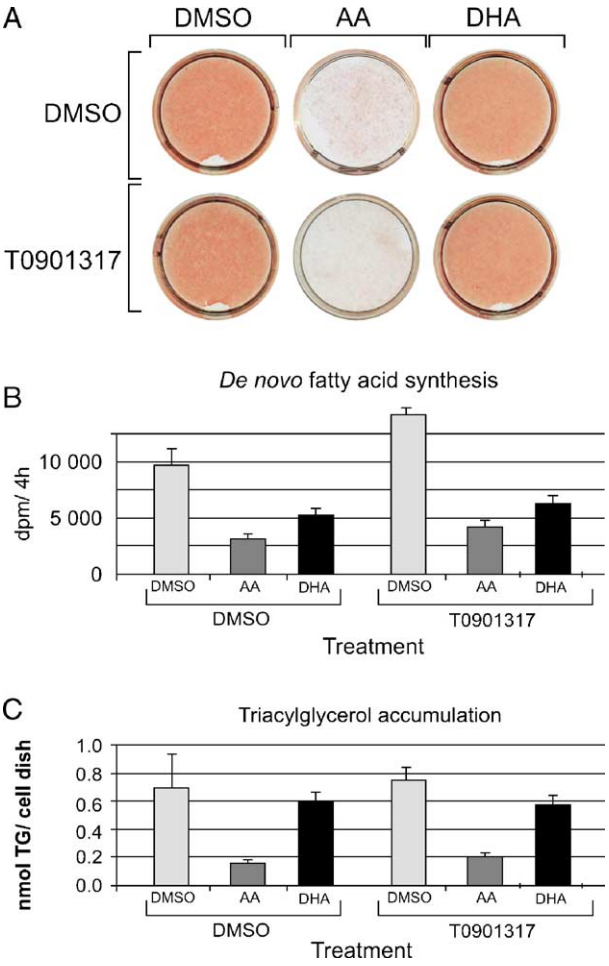

Fig. 7. 3T3-L1 cells were induced to differentiate as described in Fig. 3. 100 μM fatty acids and/or 1 μM T0901317 were added when differentiation was induced on day 0 and were present throughout the differentiation period. (A) The cells were stained with oil-red-O and photographed on day 8. (B) De novo fatty acid synthesis was measured on day 8. (C) The levels of triacylglycerols were measured in whole cell extracts on day 8. The experiments were performed in duplicate or triplicate and repeated at least twice. Data are presented as mean ± S.D. of at least three independent experiments.

accumulation and enhanced differentiation of 3T3-L1 cells treated with 22(*R*)-hydroxycholesterol or T0901317 [227]. A potential role of LXRα in adipocyte differentiation was also supported by the finding that the suppression of LXRα by siRNA attenuated differentiation in 3T3-L1 cells [227]. On the other hand, Hummasti et al. reported that the synthetic LXR agonist GW3965 had no significant effect on lipid accumulation and morphologic differentiation in 3T3-L1 or 3T3-442A cells [237]. The forced expression of LXRα in NIH-3T3 fibroblasts did not induce differentiation and had no effect in conjunction with PPARγ [237]. This is in accordance with our finding that in 3T3-L1 cells, the continuous presence of T0901317 had no effect on the differentiation and accumulation of triacylglycerol [233]. However, when LXRα was ectopically expressed in 3T3-L1 preadipocytes, the activation of LXRα by T0901317 prevented differentiation [233].

When we induce 3T3-L1 cells to differentiate in the presence of the synthetic LXRα agonist T0901317, we observed that differentiation and triacylglycerol accumulation were comparable to control cells, whereas a slight but reproducible increased de novo fatty acid synthesis was observed (Fig. 7). The inhibitory effect of arachidonic acid and DHA on de novo fatty acid synthesis and triacylglycerol accumulation was similar in the absence and presence of the LXR-agonist (Fig. 7). The basis for these different observations remains to be established. It is worth to note, however, that the differentiation of 3T3-L1 cells in the absence of an LXR-ligand was very poor in the papers, where LXR-ligands were reported to induce differentiation [227,236] compared with those that reported no effect [233,237]. Also, as LXRα appears to mediate its action by the SREBP-1c isoform, and thereby the expression of several lipogenic genes in both liver and adipose tissue [198,226,227], differences in SREBP-1 isoform expression in different cell lines, as discussed above, might influence the results. Consequently, further studies are needed to determine the precise function of LXR in adipose tissue.

7. Conclusion

It is evident that a diet enriched in PUFAs, in particular of the n-3 family, decreases adipose tissue mass and suppresses the development of obesity in rodents. The precise underlying mechanism is, however, still not elucidated. A number of key regulatory transcription factors involved in adipogensis are clearly targeted by PUFAs. One specific transcription factor may theoretically be responsible for mediating the action of PUFAs alone, but it is more likely that more than one is implicated and that feeding status of the animal determines the specific set of factors that are involved (Fig. 8).

Frequently, the effects of PUFAs are studied in animals fed a standard laboratory chow diet supplemented with the fatty acids for a various periods of time and then fasted overnight before being killed. In rats fasted overnight, plasma glucose and insulin levels are relatively low. Thus, the expression of LXRα and SREBP-1 is low, whereas PPARα and PPARδ are expressed at higher levels. Under such conditions, fatty acids are mobilized from adipose tissue and fatty acid oxidation in both the liver and peripheral tissue is high. The effects of PUFAs on PPARα and PPARδ target genes are prominent in such an experimental setup, whereas the effects on SREBP-target

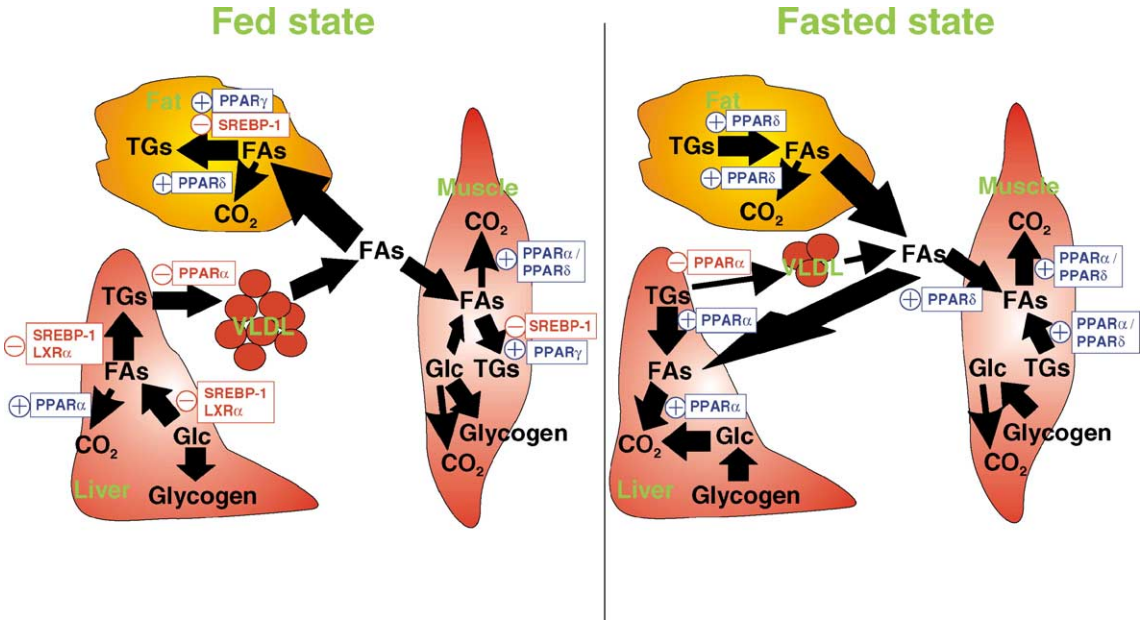

Fig. 8. A simplified scheme of lipid homeostasis in the fed and the fasted states. The size of the arrows indicates the magnitude of the flux of fatty acid and fatty acid metabolites. The potential stimulatory (+) or inhibitory (−) effects of dietary PUFAs on transcription factors are indicated.

genes are severely blunted. To unmask the inhibitory effects of PUFAs on SREBP-target genes, experiments must be performed when animals are in the fed state. When animals are refed after fasting, these effects are especially prominent. Under such conditions, the plasma levels of glucose and insulin are high. Fatty acid synthesis is high, whereas fatty acid oxidation and the expression of PPARα and PPARδ are low. Fatty acids are secreted from the liver into circulation and taken up by adipose tissue. These effects are even more pronounced when the diets are enriched in carbohydrates. Under such conditions, the expression of PPARα is low and the effect of PPARα activators are blunted [238,239].

For obesity studies, different animal models of genetic obesity, such as Zucker rats, *ob*/*ob* and *db*/*db* mice, or high fat diet-induced obesity are most commonly used. These models do not recapitulate human obesity. Firstly, similar monogenic causes of human obesity (lep- and lepR-), as well as others such as MC4R defect, Prader-Willi and Bardet-Biedel syndromes, are rare. Secondly, obesity in humans is not comparable to a high fat diet-induced obesity in animal models, as the relative intake of dietary fat has dropped, whereas average BMI has increased. By focusing almost exclusively on fat during the last decades, food companies have removed fat from several products. However, the fat has, to a large extent, been substituted by energy-dense refined carbohydrates. Furthermore, the intake of refined sugar from soda and other soft-drinks has increased tremendously the last decades. In Norway, the average intake per year increased from 41 l in 1970 to 127 l (representing 9 kg refined sugar) in 2001 [5]. Among teenagers, the situation is even worse, as the daily intake in this group in average amounts to 0.4–0.5 l sugar containing drinks. Furthermore, the consumption is often evenly distributed during the day. This constant ingestion of sugar results in a chronically elevated level of blood sugar and insulin and thus renders the body in a constant lipogenic state.

Feeding status may be of particular importance concerning the effect of PUFAs of the n-6 family. Fundamental opposite effects of n-6 fatty acids are reported on both adipose tissue development in animals and adipocyte differentiation in vitro. Firstly, different arachidonic acid metabolites have opposing effects on adipocyte differentiation. The metabolism of arachidonic acid may thus determine its effect. Secondly, the inhibitory effect of arachidonic acid and metabolites on adipocyte differentiation requires the elevation of cellular cAMP. In the absence of elevated cAMP-levels, arachidonic acid acts proadipogenic. The adipogenic potential of arachidonic acid is also potentiated by insulin. One could therefore postulate that the adipogenic effect of arachidonic acid on adipose tissue development in animals, and possible also in humans, would be particular marked if a high arachidonic intake is combined with a high intake of carbohydrates.

Acknowledgements

Work in the authors' laboratory has been supported by The Danish Biotechnology Program, The Danish Natural Science Research Council and the European Community (contract QLK1-CT-2001-00183).

References

[1] I.S. Farooqi, S. O'Rahilly, Monogenic human obesity syndromes, Recent Prog. Horm. Res. 59 (2004) 409–424.
[2] W.E. Connor, S.L. Connor, M.B. Katan, S.M. Grundy, W.C. Willett, Should a low-fat, high-carbohydrate diet be recommended for everyone? N. Engl. J. Med. 337 (1997) 562–567.
[3] J.O. Hill, H.R. Wyatt, G.W. Reed, J.C. Peters, Obesity and the environment: where do we go from here? Science 299 (2003) 853–855.
[4] S.L. Weinberg, The diet-heart hypothesis: a critique, J. Am. Coll. Cardiol. 43 (2004) 731–733.
[5] Sosial og helsedirektoratet, Utviklingen i Norsk kosthold 2002, (2003).
[6] Sosial og helsedirektoratet, Vekt-helse Rapport 1/2000, (2000).
[7] C.E. Roynette, P.C. Calder, Y.M. Dupertuis, C. Pichard, n-3 Polyunsaturated fatty acids and colon cancer prevention, Clin. Nutr. 23 (2004) 139–151.
[8] M. Hirafuji, T. Machida, N. Hamaue, M. Minami, Cardiovascular protective effects of n-3 polyunsaturated fatty acids with special emphasis on docosahexaenoic acid, Pharm. Sci. 92 (2003) 308–316.
[9] M.Y. Abeywardena, R.J. Head, Longchain n-3 polyunsaturated fatty acids and blood vessel function, Cardiovasc. Res. 52 (2001) 361–371.
[10] H.C. Bucher, P. Hengstler, C. Schindler, G. Meier, N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials, Am. J. Med. 112 (2002) 298–304.
[11] S.C. Larsson, M. Kumlin, M. Ingelman-Sundberg, A. Wolk, Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms, Am. J. Clin. Nutr. 79 (2004) 935–945.
[12] S.G. Harris, J. Padilla, L. Koumas, D. Ray, R.P. Phipps, Prostaglandins as modulators of immunity, Trends Immunol. 23 (2002) 144–150.
[13] P.E. Christie, W.R.J. Henderson, Lipid inflammatory mediators: leukotrienes, prostaglandins, platelet-activating factor, Clin. Allergy Immunol. 16 (2002) 233–254.
[14] J.M. Wallance, Nutritional and botanical modulation of the inflammatory cascade — eicosanoids, cyclooxygenases, and lipoxygenases — as an adjunct in cancer therapy, Integr. Cancer Ther. 1 (2002) 7–37.
[15] C.N. Serhan, S. Hong, K. Gronert, S.P. Colgan, P.R. Devchand, G. Mirick, R.L. Moussignac, Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals, J. Exp. Med. 196 (2002) 1025–1037.
[16] H. Grimm, K. Mayer, P. Mayser, E. Eigenbrodt, Regulatory potential of n-3 fatty acids in immunological and inflammatory processes, Br. J. Nutr. 87 (2002) S59–S67.
[17] T. Ringbom, U. Huss, A. Stenholm, S. Flock, L. Skattebøl, P. Perera, L. Bohlin, Cox-2 inhibitory effects of naturally occurring and modified fatty acids. J. Nat. Prod. 2001 Jun; 64(6): 745–9 64 (2001) 744–749.
[18] S.D. Clarke, Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome, J. Nutr. 131 (2001) 1129–1132.
[19] S.D. Clarke, The multi-dimensional regulation of gene expression by fatty acids: polyunsaturated fats as nutrient sensors, Curr. Opin. Lipidol. 15 (2004) 13–18.

[20] S. Kersten, Effects of fatty acids on gene expression: role of peroxisome proliferator-activated receptor alpha, liver X receptor alpha and sterol regulatory element-binding protein-1c, Proc. Nutr. Soc. 61 (2002) 371–374.
[21] K. Wahle, D. Rotondo, SD. Heys, Polyunsaturated fatty acids and gene expression in mammalian systems, Proc. Nutr. Soc. 62 (2003) 349–360.
[22] T.S. Worgall, S.L. Sturley, T. Seo, T.F. Osborne, R.J. Deckelbaum, Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein, J. Biol. Chem. 273 (1998) 25537–25540.
[23] V.C. Hannah, J. Ou, A. Luong, J.L. Goldstein, M.S. Brown, Unsaturated fatty acids down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells, J. Biol. Chem. 276 (2001) 4365–4372.
[24] M.K. Mater, A.P. Thelen, D.A. Pan, D.B. Jump, Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription, J. Biol. Chem. 274 (1999) 32725–32732.
[25] J. Xu, M.T. Nakamura, H.P. Cho, S.D. Clarke, 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 (1999) 23577–23583.
[26] J. Ou, H. Tu, B. Shan, A. Luk, R.A. DeBose-Boyd, Y. Bashmakov, J.L. Goldstein, M.S. Brown, 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. 98 (2001) 6027–6032.
[27] T. Yoshikawa, 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.I. Osuga, T. Gotoda, S. Ishibashi, N. Yamada, Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements, J. Biol. Chem. 277 (2002) 1705–1711.
[28] Y. Barak, M.C. Nelson, E.S. Ong, Y.Z. Jones, P. Ruiz-Lozano, K.R. Chien, A. Koder, R.M. Evans, PPARγ is required for placental, cardiac, and adipose tissue development, Mol. Cell 4 (1999) 585–595.
[29] B.M. Forman, J. Chen, R.M. Evans, Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta, Proc. Natl. Acad. Sci. 94 (1997) 4312–4317.
[30] M. Gottlicher, A. Demoz, D. Svensson, P. Tollet, R.K. Berge, J.-Å. Gustafsson, Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor, Biochem. Pharmacol. 46 (1993) 2177–2184.
[31] T.E. Johnson, M.K. Holloway, R. Vogel, S.J. Rutledge, J. Perkins, G. Rodan, A. Schmidt, Structural requirements and cell-type specificity for ligand activation of peroxisome proliferator-activated receptors, J. Steroid Biochem. Mol. Biol. 63 (1997) 1–18.
[32] K. Yu, W. Bayona, C.B. Kallen, H.P. Harding, C.P. Ravera, G. McMahon, M. Brown, M.A. Lazar, Differential activation of peroxisome proliferator-activated receptors by eicosanoids, J. Biol. Chem. 270 (1995) 23975–23983.
[33] A. Denys, A. Hichami, N.A. Khan, Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase enzyme activity in human T-cells, Mol. Cell. Biochem. 232 (2002) 143–148.
[34] X. Fan, X. Huang, C. Da Silva, M. Castagna, Arachidonic acid and related methyl ester mediate protein kinase C activation in intact platelets through the arachidonate metabolism pathways, Biochem. Biophys. Res. Commun. 169 (1990) 933–940.
[35] Y.H. Jiang, J.R. Lupton, W.C. Chang, C.A. Jolly, H.M. Aukema, R.S. Chapkin, Dietary fat and fiber differentially alter intracellular second messengers during tumor development in rat colon, Carcinogenesis 17 (1996) 1227–1233.
[36] T. Kawaguchi, K. Osatomi, H. Yamashita, T. Kabashima, K. Uyeda, Mechanism for fatty acid sparing effect on glucose-induced transcription. Regulation of carbohydrate-responsive element-binding protein by amp-activated protein kinase, J. Biol. Chem. 277 (2002) 3829–3835.
[37] M. Murata, H. Kaji, K. Iida, Y. Okimura, K. Chihara, Dual action of eicosapentaenoic acid in hepatoma cells. Up-regulation of metabolic action of insulin and inhibition of cell proliferation, J. Biol. Chem. 276 (2001) 31422–31428.
[38] S. Madani, A. Hichami, M. Charkaoui-Malki, N.A. Khan, Diacylglycerols containing omega 3 and omega 6 fatty acids bind to RasGRP and modulate MAP kinase activation, J. Biol. Chem. 279 (2004) 1176–1183.
[39] Y. Shimomura, Y. Tamura, M. Suzuki, Less body fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet, J. Nutr. 120 (1990) 1291–1296.
[40] H. Wang, L.H. Storlien, X.F. Huang, Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression, Am. J. Physiol., Endocrinol Metabol. 282 (2002) E1352–E1359.
[41] A. Minami, N. Ishimura, S. Sakamoto, E. Takishita, K. Mawatri, K. Okada, Y. Nakaya, Effect of eicosapentaenoic acid ethyl ester v. oleic acid-rich safflower oil on insulin resistance in type 2 diabetic model rats with hypertriacylglycerolaemia, Br. J. Nutr. 87 (2002) 157–162.
[42] S.H. Cha, A. Fukushima, K. Sakuma, Y. Kagawa, Chronic docosahexaenoic acid intake enhances expression of the gene for uncoupling protein 3 and affects pleiotropic mRNA levels in skeletal muscle of aged C57BL/6NJcl mice, J. Nutr. 131 (2001) 2636–2642.
[43] Y. Takahashi, T. Ide, Dietary *n*-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat, Br. J. Nutr. 84 (2000) 175–184.
[44] M. Okuno, K. Kajiwara, S. Imai, T. Kobayashi, N. Honma, T. Maki, K. Suruga, T. Goda, S. Takase, Y. Muto, H. Moriwaki, Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation, J. Nutr. 127 (1997) 1752–1757.
[45] I.S. Jang, D.Y. Hwang, K.R. Chae, J.E. Lee, Y.K. Kim, T.S. Kang, J.H. Hwang, C.H. Lim, Y.B. Huh, J.S. Cho, Role of dietary fat type in the development of adiposity from dietary obesity-susceptible Sprague–Dawley rats, Br. J. Nutr. 89 (2003) 429–438.
[46] T. Nakatani, H.J. Kim, Y. Kaburagi, K. Yasuda, O. Ezaki, A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: relationship to anti-obesity, J. Lipid Res. 44 (2003) 369–379.
[47] J. Ukropec, J.E. Reseland, D. Gasperikova, E. Demcakova, L. Madsen, R.K. Berge, A.C. Rustan, I. Klimes, C.A. Drevon, E. Sebokova, The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression, Lipids 38 (2003) 1023–1029.
[48] M. Pellizzon, A. Buison, F. Ordiz Jr., L. Santa Ana, K.-L.C. Jen, Effects of dietary fatty acids and exercise on body-weight regulation and metabolism in rats, Obes. Res. 10 (2002) 947–955.
[49] F. Belzung, T. Raclot, R. Groscolas, Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets, Am. J. Physiol. 246 (1993) R1111–R1118.
[50] J.J. Berger, R.J. Barnard, Effect of diet on fat cell size and hormone-sensitive lipase activity, J. Appl. Physiol. 87 (1999) 227–232.
[51] R.J. Ellis, R.B. McDonald, J.S. Stern, A diet high in fat stimulates adipocyte proliferation in older (22 month) rats, Exp. Gerontol. 1990 25 (1990) 141–148.
[52] B.J. Klyde, J. Hirsch, Increased cellular proliferation in adipose tissue of adult rats fed a high-fat diet, J. Lipid Res. 20 (1979) 705–715.
[53] W.H. Miller Jr., I.M. Faust, J. Hirsch, Demonstration of de novo production of adipocytes in adult rats by biochemical and radioautographic techniques, J. Lipid Res. 25 (1984) 336–347.

[54] C.K. Roberts, R.J. Barnard, K.H. Liang, N.D. Vaziri, Effect of diet on adipose tissue and skeletal muscle VLDL receptor and LPL: implications for obesity and hyperlipidemia, Atherosclerosis 161 (2002) 133–141.
[55] G. Shillabeer, D.C. Lau, Regulation of new fat cell formation in rats: the role of dietary fats, J. Lipid Res. 35 (1994) 592–600.
[56] I.P. Lopez, A. Marti, F.I. Milagro, M.D.L.A. Zulet, M.J. Moreno-Aliaga, J.A. Martinez, C. De Miguel, DNA microarray analysis of genes differentially expressed in diet-induced (cafeteria) obese rats, Obes. Res. 11 (2003) 188–194.
[57] H. Koutnikova, T.A. Cock, M. Watanabe, S.M. Houten, M.F. Champy, A. Dierich, J. Auwerx, Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPARγ hypomorphic mice, Proc. Natl. Acad. Sci. 100 (2003) 14457–14462.
[58] N. Kubota, Y. Terauchi, H. Miki, H. Tamemoto, T. Yamauchi, K. Komeda, S. Satoh, R. Nakano, C. Ishii, T. Sugiyama, PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance, Mol. Cell 4 (1999) 597–609.
[59] E.D. Rosen, P. Sarraf, A.E. Troy, G. Bradwin, K. Moore, D.S. Milstone, B.M. Spiegelman, R.M. Mortensen, PPAR[gamma] is required for the differentiation of adipose tissue in vivo and in vitro, Mol. Cell 4 (1999) 611–617.
[60] E. Hu, P. Tontonoz, B.M. Spiegelman, Transdifferentiation of myoblasts by the adipogenic transcription factors PPARγ and C/EBPα, Proc. Natl. Acad. Sci. 92 (1995) 9856–9860.
[61] X. Wu, Y. Xie, N.L. Bucher, S.R. Farmer, Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis, Genes Dev. 9 (1995) 2350–2363.
[62] T. Kadowaki, K. Hara, N. Kubota, K. Tobe, Y. Terauchi, T. Yamauchi, K. Eto, H. Kadowaki, M. Noda, R. Hagura, Y. Akanuma, The role of PPARgamma in high-fat diet-induced obesity and insulin resistance, J. Diabetes Complicat. 16 (2002) 41–45.
[63] T. Yamauchi, J. Kamon, H. Waki, K. Murakami, K. Motojima, K. Komeda, T. Ide, N. Kubota, Y. Terauchi, K. Tobe, H. Miki, A. Tsuchida, Y. Akanuma, R. Nagai, S. Kimura, T. Kadowaki, The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance, J. Biol. Chem. 276 (2001) 41245–41254.
[64] P.D.G. Miles, Y. Barak, R.M. Evans, J.M. Olefsky, Effect of heterozygous PPARgamma deficiency and TZD treatment on insulin resistance associated with age and high-fat feeding, Am. J. Physiol., Endocrinol Metabol. 284 (2003) E618–E626.
[65] H. Keller, C. Dreyer, J. Medin, A. Mahfoudi, K. Ozato, W. Wahli, Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor–retinoid X receptor heterodimers, Proc. Natl. Acad. Sci. 90 (1993) 2160–2164.
[66] I. Issemann, S. Green, Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators, Nature 347 (1990) 645–650.
[67] C. Jobin, O. Morteau, D. Han, R. Balfour Sartor, Specific NF-kappaB blockade selectively inhibits tumour necrosis factor-alpha-induced COX-2 but not constitutive COX-1 gene expression in HT-29 cells, Immunology 95 (1998) 537–543.
[68] C. Dreyer, G. Krey, H. Keller, F. Givel, G. Helftenbein, W. Wahli, Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors, Cell. 68 (1992) 887–897.
[69] M. Gottlicher, E. Widmark, Q. Li, J. Gustafsson, Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor, Proc. Natl. Acad. Sci. 89 (1992) 4653–4657.
[70] S.A. Kliewer, S.S. Sundseth, S.A. Jones, P.J. Brown, G.B. Wisely, C.S. Koble, P. Devchand, W. Wahli, T.M. Willson, J.M. Lenhard, J.M. Lehmann, Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma, Proc. Natl. Acad. Sci. 94 (1997) 4318–4323.
[71] P. De Vos, A.M. Lefebvre, S.G. Miller, M. Guerre-Millo, K. Wong, R. Saladin, L.G. Hamann, B. Staels, M.R. Briggs, J. Auwerx, Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma, J. Clin. Invest. 98 (1996) 1004–1009.
[72] K. Hallakou, L. Doare, F. Foufelle, M. Kergoat, M. Guerre-Millo, M.F. Berthault, I. Dugail, J. Morin, J. Auwerx, P. Ferre, Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker *fa/fa* rat, Diabetes 46 (1997) 1393–1399.
[73] E. Chaput, R. Saladin, M. Silvestre, A.D. Edgar, Fenofibrate and rosiglitazone lower serum triglycerides with opposing effects on body weight, Biochem. Biophys. Res. Commun. 271 (2000) 445–450.
[74] C.J. de Souza, M. Eckhardt, K. Gagen, M. Dong, W. Chen, D. Laurent, B.F. Burkey, Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance, Diabetes 50 (2001) 1863–1871.
[75] A. Okuno, H. Tamemoto, K. Tobe, K. Ueki, Y. Mori, K. Iwamoto, K. Umesono, Y. Akanuma, T. Fujiwara, H. Horikoshi, Y. Yazaki, T. Kadowaki, Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats, J. Clin. Invest. 101 (1998) 1354–1361.
[76] J.O. Hill, J.C. Peters, D. Lin, F. Yakubu, H. Greene, L. Swift, Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats, Int. J. Obes., Relat. Metab. Disord. 17 (1993) 223–236.
[77] C.C. Parrish, D.A. Pathy, A. Angel, Dietary fish oils limit adipose tissue hypertrophy in rats, Metabolism 39 (1990) 217–219.
[78] T. Raclot, R. Groscolas, D. Langin, P. Ferre, Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues, J. Lipid Res. 38 (1997) 1963–1972.
[79] A.C. Rustan, B.E. Hustvedt, C.A. Drevon, Dietary supplementation of very long-chain n-3 fatty acids decreases whole body lipid utilization in the rat, J. Lipid Res. 34 (1993) 1299–1309.
[80] J.T. Huang, J.S. Welch, M. Ricote, C.J. Binder, T.M. Willson, C. Kelly, J.L. Witztum, C.D. Funk, D. Conrad, C.K. Glass, Interleukin-4-dependent production of PPARγ ligands in macrophages by 12/15-lipoxygenase, Nature 400 (1999) 378–382.
[81] K.R. Kozak, R.A. Gupta, J.S. Moody, C. Ji, W.E. Boeglin, R.N. DuBois, A.R. Brash, L.J. Marnett, 15-lipoxygenase metabolism of 2-arachidonylglycerol. Generation of a peroxisome proliferator-activated receptor alpha agonist, J. Biol. Chem. 277 (2002) 23278–23286.
[82] G. Krey, O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M.G. Parker, W. Wahli, Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay, Mol. Endocrinol. 11 (1997) 779–791.
[83] J.B. Nixon, H. Kamitani, S.J. Baek, T.E. Eling, Evaluation of eicosanoids and NSAIDs as PPARγ ligands in colorectal carcinoma cells, Prostaglandins Leukot. Essent. Fat. Acids 68 (2003) 323–330.
[84] S.B. Shappell, R.A. Gupta, S. Manning, R. Whitehead, W.E. Boeglin, C. Schneider, T. Case, J. Price, G.S. Jack, T.M. Wheeler, R.J. Matusik, A.R. Brash, R.N. DuBois, 15*S*-Hydroxyeicosatetraenoic acid activates peroxisome proliferator-activated receptor γ and inhibits proliferation in PC3 prostate carcinoma cells, Cancer Res. 61 (2001) 497–503.
[85] B.M. Forman, P. Tontonoz, J. Chen, R.P. Brun, B.M. Spiegelman, R.M. Evans, 15-deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma, Cell 83 (1995) 803–812.
[86] S.A. Kliewer, J.M. Lenhard, T.M. Willson, I. Patel, D.C. Morris, J.M. Lehmann, A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation, Cell 83 (1995) 813–819.
[87] L.C. Bell-Parikh, T. Ide, J.A. Lawson, P. Mc Namara, M. Reilly, G.A. Fitz Gerald, Biosynthesis of 15-deoxy- Δ12,14-PGJ2 and the ligation of PPARγ, J. Clin. Invest. 112 (2003) 945–955.

[88] I. Tzameli, H. Fang, M. Ollero, H. Shi, J.K. Hamm, P. Kievit, A.N. Hollenberg, J.S. Flier, Regulated production of a peroxisome proliferator-activated receptor-γ ligand during an early phase of adipocyte differentiation in 3T3-adipocytes, J. Biol. Chem. 279 (2004) 36093–36102.
[89] G. Gao, G. Serrero, Phospholipase A2 is a differentiation-dependent enzymatic activity for adipogenic cell line and adipocyte precursors in primary culture, J. Biol. Chem. 265 (1990) 2431–2434.
[90] R.M. Catalioto, D. Gaillard, J. Maclouf, G. Ailhaud, R. Negrel, Autocrine control of adipose cell differentiation by prostacyclin and PGF2 alpha, Biochim. Biophys. Acta 1091 (1991) 364–369.
[91] D. Gaillard, R. Negrel, M. Lagarde, G. Ailhaud, Requirement and role of arachidonic acid in the differentiation of pre-adipose cells, Biochem. J. 257 (1989) 389–397.
[92] R. Negrel, D. Gaillard, G. Ailhaud, Prostacyclin as a potent effector of adipose-cell differentiation, Biochem. J. 257 (1989) 399–405.
[93] G. Serrero, N.M. Lepak, Prostaglandin F2 α receptor (FP receptor) agonists are potent adipose differentiation inhibitors for primary culture of adipocyte precursors in defined medium, Biochem. Biophys. Res. Commun. 233 (1997) 200–202.
[94] G. Serrero, N.M. Lepak, S.P. Goodrich, Paracrine regulation of adipose differentiation by arachidonate metabolites: prostaglandin F2 alpha inhibits early and late markers of differentiation in the adipogenic cell line 1246, Endocrinology 131 (1992) 2545–2551.
[95] G. Serrero, N.M. Lepak, S.P. Goodrich, Prostaglandin F2 alpha inhibits the differentiation of adipocyte precursors in primary culture, Biochem. Biophys. Res. Commun. 183 (1992) 442.
[96] D.A. Casimir, C.W. Miller, J.M. Ntambi, Preadipocyte differentiation blocked by prostaglandin stimulation of prostanoid FP_2 receptor in murine 3T3-L1 cells, Differentiation 60 (1996) 203–210.
[97] J. Kamon, T. Naitoh, M. Kitahara, N. Tsuruzoe, Prostaglandin F2 alpha enhances glucose consumption through neither adipocyte differentiation nor GLUT1 expression in 3T3-L1 cells, Cell. Signal. 13 (2001) 105–109.
[98] C.W. Miller, D.A. Casimir, J.M. Ntambi, The mechanism of inhibition of 3T3-L1 preadipocyte differentiation by prostaglandin F2alpha. Endocrinology. 137 (12) (1996 Dec) 5641–5650; 137 (1996) 5641–5650.
[99] M.S. Jaradat, B. Wongsud, S. Phornchirasilp, S.M. Rangwala, G. Shams, M. Sutton, K.J. Romstedt, D.J. Noonan, D.R. Feller, Activation of peroxisome proliferator-activated receptor isoforms and inhibition of prostaglandin H2 synthases by ibuprofen, naproxen, and indomethacin, Biochem. Pharmacol. 62 (2001) 1587–1595.
[100] J.M. Lehmann, J.M. Lenhard, B.B. Oliver, G.M. Ringold, S.A. Kliewer, Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs, J. Biol. Chem. 272 (1997) 3406–3410.
[101] J.N. Fain, L.R. Ballou, S.W. Bahouth, Obesity is induced in mice heterozygous for cyclooxygenase-2, Prostaglandins Other Lipid Mediat. 65 (2001) 199–209.
[102] P. Guesnet, J. Bourre, M. Guerre-Millo, G. Pascal, G. Durand, Tissue phospholipid fatty acid composition in genetically lean (Fa/−) or obese (*fa*/*fa*) Zucker female rats on the same diet, Lipids 25 (1990) 517–522.
[103] K. Wahle, L. Milne, J. Fletcher, G. McIntosh, Effect of dietary lipids and endocrine changes on polyunsaturated fatty acids in phospholipids of pancreas and brown adipose tissue of obese and lean rats, Comp. Biochem. Physiol. Physiol. 109 (1994) 235–244.
[104] H.D. Gaskins, D.B. Hausman, R.J. Martin, G.J. Hausman, Evidence for abnormal prostaglandin synthesis in obese Zucker rat adipose cell cultures, J. Nutr. 119 (1989) 458–462.
[105] L. Madsen, R.K. Petersen, M.B. Sørensen, C. Jørgensen, P. Hallenborg, L. Pridal, J. Fleckner, E.-Z. Amri, P. Krieg, G. Furstenberger, R.K. Berge, K. Kristiansen, Adipocyte differentiation of 3T3-L1 preadipocytes is dependent on lipoxygenase activity during the initial stages of the differentiation process, Biochem. J. 375 (2003) 539–549.
[106] T. Matsuo, H. Takeuchi, H. Suzuki, M. Suzuki, Body fat accumulation is greater in rats fed a beef tallow diet than in rats fed a safflower or soybean oil diet, Asia Pac. J. Clin. Nutr. 11 (2002) 302–308.
[107] M. Cleary, F. Phillips, R. Morton, Genotype and diet effects in lean and obese Zucker rats fed either safflower or coconut oil diets, Proc. Soc. Exp. Biol. Med. 220 (1999) 153–161.
[108] F. Massiera, P. Saint-Marc, J. Seydoux, T. Murata, T. Kobayashi, S. Narumiya, P. Guesnet, E.Z. Amri, R. Negrel, G. Ailhaud, Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? J. Lipid Res. 44 (2003) 271–279.
[109] A.M. Prentice, Overeating: the health risks, Obes. Res. 9 (2001) 234–238.
[110] R.K. Petersen, C. Jorgensen, A.C. Rustan, L. Froyland, K. Muller-Decker, G. Furstenberger, R.K. Berge, K. Kristiansen, L. Madsen, Arachidonic acid-dependent inhibition of adipocyte differentiation requires PKA activity and is associated with sustained expression of cyclooxygenases, J. Lipid Res. 44 (2003) 2320–2330.
[111] M.J. Reginato, S.L. Krakow, S.T. Bailey, M.A. Lazar, Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor gamma, J. Biol. Chem. 273 (1998) 1855–1858.
[112] G. Vassaux, D. Gaillard, C. Darimont, G. Ailhaud, R. Negrel, Differential response of preadipocytes and adipocytes to prostacyclin and prostaglandin E2: physiological implications, Endocrinology 131 (1992) 2393–2398.
[113] Y. Sugimoto, H. Tsuboi, Y. Okuno, S. Tamba, S. Tsuchiya, G. Tsujimoto, A. Ichikawa, Microarray evaluation of EP4 receptor-mediated prostaglandin E(2) suppression of 3T3-L1 adipocyte differentiation, Biochem. Biophys. Res. Commun. 322 (2004) 911–917.
[114] H. Tsuboi, Y. Sugimoto, T. Kainoh, A. Ichikawa, Prostanoid EP4 receptor is involved in suppression of 3T3-L1 adipocyte differentiation, Biochem. Biophys. Res. Commun. 322 (2004) 1066–1072.
[115] M. Mater, A.P. Thelen, D.B. Jump, Arachidonic acid and PGE2 regulation of hepatic lipogenic gene expression, J. Lipid Res. 40 (1999) 1045–1052.
[116] M.K. Mater, D. Pan, W.G. Bergen, D.B. Jump, Arachidonic acid inhibits lipogenic gene expression in 3T3-L1 adipocytes through a prostanoid pathway, J. Lipid Res. 39 (1998) 1327–1334.
[117] F. Haugen, N. Zahid, K.T. Dalen, K. Hollung, H.I. Nebb, C.A. Drevon, Resistin expression in 3T3-L1 adipocytes is reduced by arachidonic acid, J. Lipid Res. 46 (2005) 143–153.
[118] O. Laneuville, D.K. Breuer, N. Xu, Z.H. Huang, D.A. Gage, J.T. Watson, M. Lagarde, D.L. DeWitt, W.L. Smith, Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and -2, J. Biol. Chem. 270 (1995) 19330–19336.
[119] L.N. Larsen, K. Horvik, H.I. Sørensen, J. Bremer, Polyunsaturated thia- and oxa-fatty acids: incorporation into cell lipids and their effects on arachidonic acid- and eicosanoid synthesis, Biochim. Biophys. Acta 1348 (1997) 346–354.
[120] M.G. Malkowski, E.D. Thuresson, K.M. Lakkides, C.J. Rieke, R. Micielli, W.L. Smith, R.M. Garavito, Structure of eicosapentaenoic and linoleic acids in the cyclooxygenase site of prostaglandin endoperoxide H synthase-1, J. Biol. Chem. 276 (2001) 37547–37555.
[121] H. Yan, A. Kermouni, M. Abdel-Hafez, D.C.W. Lau, Role of cyclooxygenases COX-1 and COX-2 in modulating adipogenesis in 3T3-L1 cells, J. Lipid Res. 44 (2003) 424–429.
[122] R.E. Maldve, Y. Kim, S.J. Muga, S.M. Fischer, Prostaglandin E2 regulation of cyclooxygenase expression in keratinocytes is mediated via cyclic nucleotide-linked prostaglandin receptors, J. Lipid Res. 41 (2000) 873–881.

[123] W. Xie, B. Fletcher, R. Andersen, H.R. Herschman, v-src Induction of the TIS10/PGS2 prostaglandin synthase gene is mediated by an ATF/CRE transcription response element, Mol. Cell. Biol. 14 (1994) 6531–6539.
[124] R. Negrel, D. Gaillard, Metabolism of arachidonic acid and prostaglandin synthesis in the preadipocyte clonal line Ob17, Biochem. Biophys. Res. Commun. 98 (1981) 768–777.
[125] K. Liu, Y. Guan, M.C. MacNicol, A.M. MacNicol, J. McGehee, Early expression of p107 is associated with 3T3-L1 adipocyte differentiation, Mol. Cell. Endocrinol. 194 (2002) 51–61.
[126] D. Prusty, B.H. Park, K.E. Davis, S.R. Farmer, Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARgamma) and C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes, J. Biol. Chem. 277 (2002) 46226–46232.
[127] H. Zhang, J. Nohr, C.H. Jensen, R.K. Petersen, E. Bachmann, B. Teisner, L.K. Larsen, S. Mandrup, K. Kristiansen, Insulin-like growth factor-1/insulin bypasses Pref-1/FA1-mediated inhibition of adipocyte differentiation, J. Biol. Chem. 278 (2003) 20906–20914.
[128] C.M. Boney, P.A. Gruppuso, R.A. Faris, A.R. Frackelton Jr., The critical role of Shc in insulin-like growth factor-I-mediated mitogenesis and differentiation in 3T3-L1 preadipocytes, Mol. Endocrinol. 14 (2000) 805–813.
[129] J. Font de Mora, A. Porras, N. Ahn, E. Santos, Mitogen-activated protein kinase activation is not necessary for, but antagonizes, 3T3-L1 adipocytic differentiation, Mol. Cell. Biol. 17 (1997) 6068–6075.
[130] J.B. Hansen, R.K. Petersen, C. Jorgensen, K. Kristiansen, Deregulated MAPK activity prevents adipocyte differentiation of fibroblasts lacking the retinoblastoma protein, J. Biol. Chem. 277 (2002) 26335–26339.
[131] S. Shimba, T. Wada, M. Tezuka, Arylhydrocarbon receptor (AhR) is involved in negative regulation of adipose 3T3-L1 cells: AhR inhibits adipose differentiation independently of dioxin, J. Cell. Sci. 114 (2001) 2809–2817.
[132] Q.Q. Tang, T.C. Otto, M.D. Lane, Mitotic clonal expansion: a synchronous process required for adipogenesis, Proc. Natl. Acad. Sci. 100 (2003) 44–49.
[133] Z. Qiu, Y. Wei, N. Chen, M. Jiang, J. Wu, K. Liao, DNA synthesis and mitotic clonal expansion is not a required step for 3T3-L1 preadipocyte differentiation into adipocytes, J. Biol. Chem. 276 (2001) 11988–11995.
[134] M. Adams, M.J. Reginato, D. Shao, M.A. Lazar, V.K. Chatterjee, Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site, J. Biol. Chem. 272 (1997) 5128–5132.
[135] H.S. Camp, S.R. Tafuri, Regulation of peroxisome proliferator-activated receptor gamma activity by mitogenactivated protein kinase, J. Biol. Chem. 272 (1997) 10811–10816.
[136] E. Hu, J.B. Kim, P. Sarraf, M. Bruce, N. Spiegelman, Inhibition of adipogenesis through map kinase-mediated phosphorylation of PPARgamma, Science 274 (1996) 2100–2103.
[137] S.M. Rangwala, B. Rhoades, J.S. Shapiro, A.S. Rich, J.K. Kim, G.I. Shulman, K.H. Kaestner, M.A. Lazar, Genetic modulation of PPARγ phosphorylation regulates insulin sensitivity, Dev. Cell 5 (2003) 657–663.
[138] M. Ristow, D. Muller-Wieland, A. Pfeiffer, W. Krone, C.R. Kahn, Obesity associated with a mutation in a genetic regulator of adipocyte differentiation, N. Engl. J. Med. 339 (1998) 953–959.
[139] B.T. Hyman, L.L. Stoll, A.A. Spector, Prostaglandin production by 3T3-L1 cells in culture, Biochim. Biophys. Acta 713 (1982) 375–385.
[140] P. Costet, C. Legendre, J. More, A. Edgar, P. Galtier, T. Pineau, Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis, J. Biol. Chem. 273 (1998) 29577–29585.
[141] M.E. Poynter, R.A. Daynes, Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappa B signaling, and reduces inflammatory cytokine production in aging, J. Biol. Chem. 273 (1998) 32833–32841.
[142] H.E. Xu, M.H. Lambert, V.G. Montana, D.J. Parks, S.G. Blanchard, P.J. Brown, D.D. Sternbach, J.M. Lehmann, G.B. Wisely, T.M. Willson, S.A. Kliewer, M.V. Milburn, Molecular recognition of fatty acids by peroxisome proliferator-activated receptors, Mol. Cell 3 (1999) 397–403.
[143] Q. Lin, S.E. Ruuska, N.S. Shaw, D. Dong, N. Noy, Ligand selectivity of the peroxisome proliferator-activated receptor alpha, Biochemistry 38 (1999) 185–190.
[144] B. Ren, A.P. Thelen, J.M. Peters, F.J. Gonzalez, D.B. Jump, Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha, J. Biol. Chem. 272 (1997) 26827–26832.
[145] T. Aoyama, J.M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto, F.J. Gonzalez, Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha, J. Biol. Chem. 273 (1998) 5678–5684.
[146] F. Djouadi, C.J. Weinheimer, J.E. Saffitz, C. Pitchford, J. Bastin, F.J. Gonzalez, D.P. Kelly, A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor alpha-deficient mice, J. Clin. Invest. 102 (1998) 1083–1091.
[147] S.S. Lee, T. Pineau, J. Drago, E.J. Lee, J.W. Owen, D.L. Kroetz, P.M. Fernandez-Salguero, H. Westphal, F.J. Gonzalez, Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators, Mol. Cell. Biol. 15 (1995) 12–22.
[148] S.H. Wong, P.J. Nestel, R.P. Trimble, G.B. Storer, R.J. Illman, D.L. Topping, The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver, Biochim. Biophys. Acta 792 (1984) 103–109.
[149] A.C. Rustan, E.N. Christiansen, C.A. Drevon, Serum lipids, hepatic glycerolipid metabolism and peroxisomal fatty acid oxidation in rats fed omega-3 and omega-6 fatty acids, Biochem. J. 283 (1992) 333–339.
[150] N. Willumsen, J. Skorve, S. Hexeberg, A.C. Rustan, R.K. Berge, The hypotriglyceridemic effect of eicosapentaenoic acid in rats is reflected in increased mitochondrial fatty acid oxidation followed by diminished lipogenesis, Lipids 28 (1993) 683–690.
[151] M.S. Thomassen, E.N. Christiansen, K.R. Norum, Characterization of the stimulatory effect of high-fat diets on peroxisomal beta-oxidation in rat liver, Biochem. J. 206 (1982) 195–202.
[152] L. Berthou, R. Saladin, P. Yaqoob, D. Branellec, P. Calder, J.C. Fruchart, P. Denefle, J. Auwerx, B. Staels, Regulation of rat liver apolipoprotein A-I, apolipoprotein A-II and acyl-coenzyme A oxidase gene expression by fibrates and dietary fatty acids, Eur. J. Biochem. 232 (1995) 179–187.
[153] N.D. Lalwani, M.K. Reddy, S.A. Qureshi, C.R. Sirtori, Y. Abiko, J.K. Reddy, Evaluation of selected hypolipidemic agents for the induction of peroxisomal enzymes and peroxisome proliferation in the rat liver, Human Toxicol. 2 (1983) 27–48.
[154] H. Takeuchi, T. Nakamoto, Y. Mori, M. Kawakami, H. Mabuchi, Y. Ohishi, N. Ichikawa, A. Koike, K. Masuda, Comparative effects of dietary fat types on hepatic enzyme activities related to the synthesis and oxidation of fatty acid and to lipogenesis in rats, Biosci. Biotechnol. Biochem. 65 (2001) 1748–1754.
[155] L. Froyland, L. Madsen, H. Vaagenes, G.K. Totland, J. Auwerx, H. Kryvi, B. Staels, R.K. Berge, Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism, J. Lipid Res. 38 (1997) 1851–1858.
[156] L. Madsen, A.C. Rustan, H. Vaagenes, K. Berge, E. Dyroy, R.K. Berge, Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference, Lipids 34 (1999) 951–963.

[157] J. Dallongeville, E. Bauge, A. Tailleux, J.M. Peters, F.J. Gonzalez, J.C. Fruchart, B. Staels, Peroxisome proliferator-activated receptor alpha is not rate-limiting for the lipoprotein-lowering action of fish oil, J. Biol. Chem. 276 (2001) 4634–4639.
[158] M. Guerre-Millo, P. Gervois, E. Raspe, L. Madsen, P. Poulain, B. Derudas, J.M. Herbert, D.A. Winegar, T.M. Willson, J.C. Fruchart, R.K. Berge, B. Staels, Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity, J. Biol. Chem. 275 (2000) 16638–16642.
[159] F.P. Mancini, A. Lanni, L. Sabatino, M. Moreno, A. Giannino, F. Contaldo, V. Colantuoni, F. Goglia, Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats, FEBS Lett. 491 (2001) 154–158.
[160] L. Madsen, M. Guerre-Millo, E.N. Flindt, K. Berge, K.J. Tronstad, E. Bergene, E. Sebokova, A.C. Rustan, J. Jensen, S. Mandrup, K. Kristiansen, I. Klimes, B. Staels, R.K. Berge, Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance, J. Lipid Res. 43 (2002) 742–750.
[161] N. Tsuboyama-Kasaoka, M. Takahashi, H. Kim, O. Ezaki, Up-regulation of liver uncoupling protein-2 mRNA by either fish oil feeding or fibrate administration in mice, Biochem. Biophys. Res. Commun. 257 (1999) 879–885.
[162] M. Guerre-Millo, C. Rouault, P. Poulain, J. Andre, V. Poitout, J.M. Peters, F.J. Gonzalez, J.C. Fruchart, G. Reach, B. Staels, PPARα-null mice are protected from high-fat diet-induced insulin resistance, Diabetes 50 (2001) 2809–2814.
[163] B.H. Kim, Y.S. Won, E.Y. Kim, M. Yoon, K.T. Nam, G.T. Oh, D.Y. Kim, Phenotype of peroxisome proliferatoractivated receptor-alpha(PPARalpha) deficient mice on mixed background fed high fat diet, J. Vet. Sci. 4 (2003) 239–244.
[164] T.E. Akiyama, C.J. Nicol, C. Fievet, B. Staels, J.M. Ward, J. Auwerx, S.S.T. Lee, F.J. Gonzalez, J.M. Peters, Peroxisome proliferator-activated receptor-alpha regulates lipid homeostasis, but is not associated with obesity. Studies with congenic mouse lines, J. Biol. Chem. 276 (2001) 39088–39093.
[165] M. Haluzik, O. Gavrilova, D. LeRoith, Peroxisome proliferator-activated receptor-α deficiency does not alter insulin sensitivity in mice maintained on regular or high-fat diet: hyperinsulinemic-euglycemic clamp studies, Endocrinology 145 (2004) 1662–1667.
[166] A. Chawla, M.A. Lazar, Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival, Proc. Natl. Acad. Sci. 91 (1994) 1786–1790.
[167] A. Cabrero, M. Alegret, R.M. Sanchez, T. Adzet, J.C. Laguna, M. Vazquez, Bezafibrate reduces mRNA levels of adipocyte markers and increases fatty acid oxidation in primary culture of adipocytes, Diabetes 50 (2001) 1883–1890.
[168] J.M. Reilly, M.P. Thompson, Dietary fatty acids up-regulate the expression of UCP2 in 3T3-L1 preadipocytes, Biochem. Biophys. Res. Commun. 277 (2000) 541–545.
[169] A. Cabrero, G. Llaverias, N. Roglans, M. Alegret, R. Sanchez, T. Adzet, J.C. Laguna, M. Vazquez, Uncoupling protein-3 mRNA levels are increased in white adipose tissue and skeletal muscle of bezafibrate-treated rats, Biochem. Biophys. Res. Commun. 260 (1999) 547–556.
[170] M. Vazquez, N. Roglans, A. Cabrero, C. Rodriguez, T. Adzet, M. Alegret, R.M. Sanchez, J.C. Laguna, Bezafibrate induces acyl-CoA oxidase mRNA levels and fatty acid peroxisomal beta-oxidation in rat white adipose tissue, Mol. Cell. Biochem. 216 (2001) 71–78.
[171] S.A. Kliewer, B.M. Forman, B. Blumberg, E.S. Ong, U. Borgmeyer, D.J. Mangelsdorf, K. Umesono, R.M. Evans, Differential expression and activation of a family of murine peroxisome proliferator-activated receptors, Proc. Natl. Acad. Sci. 91 (1994) 7355–7359.
[172] E.Z. Amri, F. Bonino, G. Ailhaud, N.A. Abumrad, P.A. Grimaldi, Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes, J. Biol. Chem. 270 (1995) 2367–2371.
[173] J.B. Hansen, H. Zhang, T.H. Rasmussen, R.K. Petersen, E.N. Flindt, K. Kristiansen, Peroxisome proliferatoractivated receptor delta (PPARdelta)-mediated regulation of preadipocyte proliferation and gene expression is dependent on cAMP signaling, J. Biol. Chem. 276 (2001) 3175–3182.
[174] K. Matsusue, J.M. Peters, F.J. Gonzalez, PPARβ/δ potentiates PPARγ-stimulated adipocyte differentiation, FASEB J. 18 (2004) 1477–1479.
[175] C. Bastie, S. Luquet, D. Holst, C. Jehl-Pietri, P.A. Grimaldi, Alterations of peroxisome proliferator-activated receptor delta activity affect fatty acid-controlled adipose differentiation, J. Biol. Chem. 275 (2000) 38768–38773.
[176] C. Bastie, D. Holst, D. Gaillard, C. Jehl-Pietri, P.A. Grimaldi, Expression of peroxisome proliferator-activated receptor PPARdelta promotes induction of PPARgamma and adipocyte differentiation in 3T3C2 fibroblasts, J. Biol. Chem. 274 (1999) 21920–21925.
[177] R.P. Brun, P. Tontonoz, B.M. Forman, R. Ellis, J. Chen, R.M. Evans, B.M. Spiegelman, Differential activation of adipogenesis by multiple PPAR isoforms, Genes Dev. 10 (1996) 974–984.
[178] J. Berger, M.D. Leibowitz, T.W. Doebber, A. Elbrecht, B. Zhang, et al., Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARdelta ligands produce distinct biological effects, J. Biol. Chem. 274 (1999) 6718–6725.
[179] C. Jehl-Pietri, C. Bastie, I. Gillot, S. Luquet, P.A. Grimaldi, Peroxisome-proliferator-activated receptor delta mediates the effects of long-chain fatty acids on post-confluent cell proliferation, Biochem. J. 350 (2000) 93–98.
[180] Y. Barak, D. Liao, W. He, E.S. Ong, M.C. Nelson, J.M. Olefsky, R. Boland, R.M. Evans, Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer, Proc. Natl. Acad. Sci. 99 (2002) 303–308.
[181] J.M. Peters, S.S.T. Lee, W. Li, J.M. Ward, O. Gavrilova, C. Everett, M.L. Reitman, L.D. Hudson, F.J. Gonzalez, Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta (delta), Mol. Cell. Biol. 20 (2000) 5119–5128.
[182] Y.X. Wang, C.H. Lee, S. Tiep, R.T. Yu, J. Ham, H. Kang, R.M. Evans, Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity, Cell 113 (2003) 159–170.
[183] J. Kopecky, Z. Hodny, M. Rossmeisl, I. Syrovy, L.P. Kozak, Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution, Am. J. Physiol. 270 (1996) E768–E775.
[184] D. Holst, S. Luquet, V. Nogueira, K. Kristiansen, X. Leverve, P.A. Grimaldi, Nutritional regulation and role of peroxisome proliferator-activated receptor δ in fatty acid catabolism in skeletal muscle, Biochim. Biophys. Acta 1633 (2003) 43–50.
[185] J. Chevillotte, M. Rieusset, M. Roques, M. Desage, H. Vidal, The regulation of uncoupling protein-2 gene expression by omega-6 polyunsaturated fatty acids in human sceletal muscle cells involves multiple pathways including peroxisome proliferator activated receptor beta, J. Biol. Chem. 276 (2001) 10853–10860.
[186] D.M. Muoio, P.S. MacLean, D.B. Lang, S. Li, J.A. Houmard, J.M. Way, D.A. Winegar, J.C. Corton, G.L. Dohm, W.E. Kraus, Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR-delta, J. Biol. Chem. 277 (2002) 26089–26097.
[187] M. Westergaard, J. Henningsen, M.L. Svendsen, C. Johansen, U.B. Jensen, H.D. Schroder, I. Kratchmarova, R. Berge, L. Iversen, L. Bolund, K. Kragballe, K. Kristiansen, Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid, J. Invest. Dermatol. 116 (2001) 702–712.
[188] R.A. Baillie, R. Takada, M. Nakamura, S.D. Clarke, Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition, Prostaglandins Leukot Essent Fatty Acids 60 (1999) 351–356.
[189] G.W. Power, E.A. Newsholme, Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyl-

transferase I in rat heart and skeletal muscle, J. Nutr. 127 (1997) 2142–2150.
[190] G.K. Totland, L. Madsen, B. Klementsen, H. Vaagenes, H. Kryvi, L. Froyland, S. Hexeberg, R.K. Berge, Proliferation of mitochondria and gene expression of carnitine palmitoyltransferase and fatty acyl-CoA oxidase in rat skeletal muscle, heart and liver by hypolipidemic fatty acids, Biol. Cell 92 (2000) 317–329.
[191] C.S. Hun, K. Hasegawa, T. Kawabata, M. Kato, T. Shimokawa, Y. Kagawa, Increased uncoupling protein2 mRNA in white adipose tissue, and decrease in leptin, visceral fat, blood glucose, and cholesterol in KK-AyMice fed with eicosapentaenoic and docosahexaenoic acids in addition to linolenic acid, Biochem. Biophys. Res. Commun. 259 (1999) 85–90.
[192] X. Hua, J. Wu, J.L. Goldstein, M.S. Brown, H.H. Hobbs, Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13, Genomics 25 (1995) 667–673.
[193] I. Shimomura, H. Shimano, J.D. Horton, J.L. Goldstein, M.S. Brown, Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells, J. Clin. Invest. 99 (1997) 838–845.
[194] C. Yokoyama, X. Wang, M.R. Briggs, A. Admon, J. Wu, X. Hua, J.L. Goldstein, M.S. Brown, SREBP-1, a basichelix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene, Cell 75 (1993) 187–197.
[195] J.D. Horton, Y. Bashmakov, I. Shimomura, H. Shimano, Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice, Proc. Natl. Acad. Sci. 95 (1998) 5987–5992.
[196] J.B. Kim, P. Sarraf, M. Wright, K.M. Yao, E. Mueller, G. Solanes, B.B. Lowell, B.M. Spiegelman, Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1, J. Clin. Invest. 101 (1998) 1–9.
[197] H. Shimano, N. Yahagi, M. Amemiya-Kudo, A.H. Hasty, J.I. Osuga, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, T. Gotoda, S. Ishibashi, N. Yamada, Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes, J. Biol. Chem. 274 (1999) 35832–35839.
[198] G. Liang, J. Yang, J.D. Horton, R.E. Hammer, J.L. Goldstein, M.S. Brown, Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory elementbinding protein-1c, J. Biol. Chem. 277 (2002) 9520–9528.
[199] D.W. Allmann, D.M. Gibson, Fatty acid synthesis during early linoleic acid deficiency in the mouse, J. Lipid Res. 79 (1965) 51–62.
[200] I. Ikeda, J.Y. Cha, T. Yanagita, N. Nakatani, K. Oogami, K. Imaizumi, K. Yazawa, Effects of dietary alphalinolenic, eicosapentaenoic and docosahexaenoic acids on hepatic lipogenesis and beta-oxidation in rats, Biosci. Biotechnol. Biochem. 62 (1998) 675–680.
[201] S.D. Clarke, D.R. Romsos, G.A. Leveille, Time sequence of changes in hepatic fatty acid synthesis in rats meal-fed polyunsaturated fatty acids, J. Nutr. 107 (1977) 1468–1476.
[202] M.J. Toussant, M.D. Wilson, S.D. Clarke, Coordinate suppression of liver acetyl-CoA carboxylase and fatty acid synthetase by polyunsaturated fat, J. Nutr. 111 (1981) 146–153.
[203] R.S. Schwartz, S. Abraham, Effect of dietary polyunsaturated fatty acids on the activity and content of fatty acid synthetase in mouse liver, Biochim. Biophys. Acta 711 (1982) 316–326.
[204] N. Iritani, M. Komiya, H. Fukuda, T. Sugimoto, Lipogenic enzyme gene expression is quickly suppressed in rats by a small amount of exogenous polyunsaturated fatty acids, J. Nutr. 128 (1998) 967–972.
[205] H.J. Kim, M. Takahashi, O. Ezaki, Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for downregulation of lipogenic enzyme mRNAs, J. Biol. Chem. 274 (1999) 25892–25898.
[206] D.P. Thewke, S.R. Panini, M. Sinensky, Oleate potentiates oxysterol inhibition of transcription from sterol regulatory element-1-regulated promoters and maturation of sterol regulatory element-binding proteins, J. Biol. Chem. 273 (1998) 21402–21407.
[207] N. Yahagi, H. Shimano, A.H. Hasty, M. Amemiya-Kudo, H. Okazaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, J.I. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, N. Yamada, 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 (1999) 35840–35844.
[208] G. Shillabeer, J. Hornford, J.M. Forden, N.C. Wong, D.C. Lau, Hepatic and adipose tissue lipogenic enzyme mRNA levels are suppressed by high fat diets in the rat, J. Lipid Res. 31 (1990) 623–631.
[209] P. Tontonoz, J.B. Kim, R.A. Graves, B.M. Spiegelman, ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation, Mol. Cell. Biol. 13 (1993) 4753–4759.
[210] J. Inoue, H. Kumagai, T. Terada, M. Maeda, M. Shimizu, R. Sato, Proteolytic activation of SREBPs during adipocyte differentiation, Biochem. Biophys. Res. Commun. 283 (2001) 1157–1161.
[211] J. Kim, BM. Spiegelman, ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism, Genes Dev. 10 (1996) 1096–1107.
[212] S.L. Lay, I. Lefrere, C. Trautwein, I. Dugail, S. Krief, 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 (2002) 35625–35634.
[213] L. Fajas, K. Schoonjans, L. Gelman, J.B. Kim, J. Najib, G. Martin, J.C. Fruchart, M. Briggs, B.M. Spiegelman, J. Auwerx, Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications foradipocyte differentiation and metabolism, Mol. Cell. Biol. 19 (1999) 5495–5503.
[214] J.B. Kim, H.M. Wright, M. Wright, B.M. Spiegelman, ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand, Proc. Natl. Acad. Sci. 95 (1998) 4333–4337.
[215] H. Shimano, J.D. Horton, I. Shimomura, R.E. Hammer, M.S. Brown, J.L. Goldstein, Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells, J. Clin. Invest. 99 (1997) 846–854.
[216] H. Shimano, I. Shimomura, R.E. Hammer, J. Herz, J.L. Goldstein, M.S. Brown, J.D. Horton, Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene, J. Clin. Invest. 100 (1997) 2115–2124.
[217] I. Shimomura, Y. Bashmakov, J.D. Horton, Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of *Diabetes mellitus*, J. Biol. Chem. 274 (1999) 30028–30032.
[218] N. Yahagi, H. Shimano, A.H. Hasty, T. Matsuzaka, T. Ide, T. Yoshikawa, M. Amemiya-Kudo, S. Tomita, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, J.I. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, N. Yamada, Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lepob/Lepob mice, J. Biol. Chem. 277 (2002) 19353–19357.
[219] I. Shimomura, R.E. Hammer, J.A. Richardson, S. Ikemoto, Y. Bashmakov, J.L. Goldstein, M.S. Brown, Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy, Genes Dev. 12 (1998) 3182–3194.
[220] J.D. Horton, I. Shimomura, S. Ikemoto, Y. Bashmakov, R.E. Hammer, Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver, J. Biol. Chem. 278 (2003) 36652–36660.
[221] D.A. Casimir, J.M. Ntambi, cAMP activates the expression of stearoyl-CoA desaturase gene 1 during early preadipocyte differentiation, J. Biol. Chem. 271 (1996) 29847–29853.
[222] D.E. Tabor, J.B. Kim, B.M. Spiegelman, P.A. Edwards, Identifica-

tion of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2, J. Biol. Chem. 274 (1999) 20603–20610.
[223] B. Jones, M. Maher, W. Banz, M. Zemel, J. Whelan, P. Smith, N. Moustaid, Adipose tissue stearoyl-CoA desaturase mRNA is increased by obesity and decreased by polyunsaturated fatty acids, Am. J. Physiol. 271 (1996) E44–E99.
[224] J.M. Ntambi, M. Miyazaki, J.P. Stoehr, H. Lan, C.M. Kendziorski, B.S. Yandell, Y. Song, P. Cohen, J.M. Friedman, A.D. Attie, Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity, Proc. Natl. Acad. Sci. 99 (2002) 11482–11486.
[225] P. Cohen, M. Miyazaki, N.D. Socci, A. Hagge-Greenberg, W. Liedtke, A.A. Soukas, R. Sharma, L.C. Hudgins, J.M. Ntambi, J.M. Friedman, Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss, Science 297 (2002) 240–243.
[226] J.R. Schultz, H. Tu, A. Luk, J.J. Repa, J.C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D.J. Mangelsdorf, K.D. Lustig, B. Shan, Role of LXRs in control of lipogenesis, Genes Dev. 14 (2000) 2831–2838.
[227] J.B. Seo, H.M. Moon, W.S. Kim, Y.S. Lee, H.W. Jeong, E.J. Yoo, J. Ham, H. Kang, M.G. Park, K.R. Steffensen, T.M. Stulnig, J.A. Gustafsson, S.D. Park, J.B. Kim, Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor γ expression, Mol. Cell. Biol. 24 (2004) 3430–3444.
[228] D.J. Peet, S.D. Turley, W. Ma, B.A. Janowski, J.M. Lobaccaro, R.E. Hammer, D.J. Mangelsdorf, Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear receptor LXRα, Cell 93 (1998) 693–704.
[229] T.M. Stulnig, K.R. Steffensen, H. Gao, M. Reimers, K. Dahlman-Wright, G.U. Schuster, J.A. Gustafsson, Novel roles of liver X receptors exposed by gene expression profiling in liver and adipose tissue, Mol. Pharmacol. 62 (2002) 1299–1305.
[230] P.J. Willy, K. Umesono, E.S. Ong, R.M. Evans, S. Heyman, D.J. Mangelsdorf, LXR, a nuclear receptor that defines a distinct retinoid response pathway, Genes Dev. 9 (1995) 1033–1045.
[231] J.-S. Annicotte, K. Schoonjans, J. Auwerx, Expression of the liver X receptor α and β in embryonic and adult mice, Anat. Rec., Part A 277A (2004) 312–316.
[232] K.R. Steffensen, M. Nilsson, G.U. Schuster, T.M. Stulnig, K. Dahlman-Wright, J.A. Gustafsson, Gene expression profiling in adipose tissue indicates different transcriptional mechanisms of liver X receptors α and β, respectively, Biochem. Biophys. Res. Commun. 310 (2003) 589–593.
[233] S.E. Ross, R.L. Erickson, I. Gerin, P.M. DeRose, L. Bajnok, K.A. Longo, D.E. Misek, R. Kuick, S.M. Hanash, K.B. Atkins, S.M. Andresen, H.I. Nebb, L. Madsen, K. Kristiansen, O.A. MacDougald, Microarray analyses during adipogenesis: understanding the effects of Wnt signaling on adipogenesis and the roles of liver X receptor α in adipocyte metabolism, Mol. Cell. Biol. 22 (2002) 5989–5999.
[234] A. Chawla, W.A. Boisvert, C.H. Lee, B.A. Laffitte, Y. Barak, S.B. Joseph, D. Liao, L. Nagy, P.A. Edwards, L.K. Curtiss, R.M. Evans, P. Tontonoz, A PPAR gamma-LXR-ABCA1 pathway in macreophages is involved in cholesterol efflux and atherogenesis, Mol. Cell 7 (2001) 161–171.
[235] K.R. Steffensen, G.U. Schuster, P. Parini, E. Holter, C.M. Sadek, T. Cassel, W. Eskild, J.A. Gustafsson, Different regulation of the LXRα promoter activity by isoforms of CCAAT/enhancer-binding proteins, Biochem. Biophys. Res. Commun. 293 (2002) 1333–1340.
[236] L. Juvet, S.M. Andersen, G.U. Schuster, K.T. Dalen, K.A. Tobin, K. Hollung, F. Haugen, S. Jacinto, S.M. Ulven, K. Bamberg, J.-Å. Gustafsson, H.I. Nebb, On the role of liver X receptors in lipid accumulation in adipocytes, Mol. Endocrinol. 17 (2003) 172–182.
[237] S. Hummasti, B.A. Laffitte, M.A. Watson, C. Galardi, L.C. Chao, L. Ramamurthy, J.T. Moore, P. Tontonoz, Liver X receptors are regulators of adipocyte gene expression but not differentiation: identification of apoD as a direct target, J. Lipid Res. 45 (2004) 616–625.
[238] I. Inoue, K. Takahashi, S. Katayama, Y. Harada, K. Negishi, A. Itabashi, J. Ishii, Effect of troglitazone (CS-045) and bezafibrate on glucose tolerance, liver glycogen synthase activity, and beta-oxidation in fructose-fed rats, Metabolism 44 (1995) 1626–1630.
[239] J. Skorve, R.K. Berge, The hypocholesterolemic effect of sulfur-substituted fatty acid analogues in rats fed a high carbohydrate diet, Biochim. Biophys. Acta 1167 (1993) 175–181.