Naissance d'une fédération

Oléagineux, Corps Gras, Lipides. Volume 8, Numéro 1, 3, Janvier - Février 2001, Editorial

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Résumé : Renouant avec la tradition des initiatives conjointes DGF1 et AFECG2, le colloque de Würzburg, en octobre dernier, a été l'occasion de présenter les bases de l'European federation for the science and technology of lipids, la future Fédération européenne pour l'étude des corps gras (Eurofedlipid)3.

ARTICLE

With the view on human lipid nutrition straight-chain fatty acids having 16 to 22 carbon atoms have attracted attention over 80 years by now and it has become common practice to group these nutrients into saturated, monounsaturated, n-6 and n-3 polyunsaturated fatty acid classes. Since the hallmark formulation of the "essential fatty acid concept" [1] health messages for these classes have been marshaled by scientists and public health authorities ever since [2]. They will not be repeated here, rather we draw attention to a class of isoprenoid branched-chain fatty acids of plant origin. They are minor lipid constituents of our food, but have not been investigated with regard to their nutritional value or non-value.

Isoprenoid branched-chain fatty acids are derived from phytol which is produced by ester hydrolysis of the phytyl side-chain linked to chlorophyll ring IV. This cleavage is carried out by microorganisms which are present in the stomach of ruminants. Humans and non-ruminant mammals can neither cleave off phytol from chlorophyll nor endogenously synthesize phytol, however, they can rapidly oxidize this alcohol to phytanic acid and subsequently degrade it to pristanic acid *(Figure 1)* and lower homologs. Thus one source of phytanic acid are dairy products and ruminant meat, a prominent example being butter which contains up to 0.1% (w/w) phytanic acid. Another source of phytanic and pristanic acids are liver oils from fish which feed on phytoplankton [3]. The mean amount of phytanic acid taken up by humans varies between 50 to 100mg per day [4].

Lately, fatty acids have been addressed as signaling and regulator molecules [5, 6]. Within this context isoprenoid branched-chain fatty acids are not known. Yet, they are very minor constituents of phospholipids and triglycerides and their degradation in peroxisomes has received attention with respect to underlying enzyme machinery and mechanisms [7] and in view of metabolic disorders pertaining to it [7, 8].

We have recently elaborated a signaling path for fatty acids to the nucleus which affects gene transcription and thus regulates gene expression [9]. Our findings provide a mechanism for nutrient fatty acid-gene interaction in which cooperation of a class of nuclear receptors, the peroxisome proliferator activated receptors (PPARs) with a class of cellular transport proteins, the fatty acid binding proteins (FABPs), is pivotal. We have learned in the course of these investigations that

phytanic acid and pristanic acid top straight-chain fatty acids in agonist function for a PPAR or in binding affinity for a FABP. Here we highlight this class of branched-chain fatty acids in nutrient - gene interaction and discuss consequences and aspects for lipid nutrition.

Degradation of chlorophyll-derived branched-chain fatty acids

In the mammalian cell isoprenoid branched-chain fatty acids are degraded in the peroxisome. After uptake of phytanic acid and pristanic acid into the cell, or of phytol which is subsequently quickly oxidized to phytanic acid, they are transported to the exofacial leaflet of the peroxisomal membrane where they are activated by phytanoyl-CoA ligase [10]. Upon transport of the activated branched-chain fatty acids into this organelle, C_{20} phytanoyl-CoA, due to the methyl branch in beta-position is first degraded *via* alpha-oxidation to C_{19} pristanoyl-CoA [11]. This compound enters the beta-oxidation cycle *(Figure 2)*, catalyzed by peroxisomal pristanoyl-CoA oxidase, D-bifunctional enzyme (D-PBE, carrying enoyl-CoA hydroxylase/beta-hydroxyacyl-CoA dehydrogenase activities), and sterol carrier protein x (SCPx, carrying branched-chain beta-ketoacyl thiolase activity). Branched-chain acyl-CoAs are the prevalent substrates for these enzymes, however, straight-chain acyl-CoAs are also accepted though to a lesser extent [12]. This set of enzymes is not subject to regulation by fatty acids, in contrast to another peroxisomal set of beta-oxidative enzymes which is responsible for the degradation of straight-chain fatty acids by means of 3 enzymes, namely straight-chain acyl-CoA oxidase, L-bifunctional enzyme (L-PBE) and beta-ketoacyl-CoA thiolase. The first and the last one are regulated by fatty acids *via* transcriptional activation by fatty acids [12].

On the basis that D-PBE could compensate for the absence of L-PBE, but not *vice versa*, it was suggested that the first set of enzymes is involved in basal degradation of branched- and straight-chain fatty acids, while the second set of enzymes is a highly regulated beta-oxidation system which responds to changes in lipid homeostasis [12, 13]. An additional important accessory protein is sterol carrier protein 2 (SCP2, formerly known as non-specific lipid transfer protein). This protein acts as an intraperoxisomal carrier for fatty acid-CoA esters and appears to be a prerequisite for their oxidation by interacting with the beta-oxidation enzyme complex [14].

Accumulation of phytanic acid in SCP2/SCPx deficient mice and consequences for gene expression

Deletion of the SCP2/SCPx gene in mice results in a complex phenotype. Although most major tissues of the SCP2/SCPx deficient mice did not show any morphological changes, an increased diaminobenzidine staining in liver tissue, which is specific for peroxisomes, was observed. Catalase activity was also elevated 2-fold in these mice. In addition to the morphological and biochemical abnormalities, the SCP2/SCPx deficient mice had markedly altered hepatic gene expression. Beside increased expression of liver fatty acid binding protein (L-FABP, see below), straight-chain acyl-CoA oxidase and beta-ketoacyl-CoA thiolase in the peroxisome, and mitochondrial beta-ketoacyl-CoA thiolase were upregulated [15].

While the SCP2/SCPx deficient animals did not exhibit significant differences regarding relative levels of straight-chain fatty acid classes in serum, phytanic acid levels were 10-fold elevated in comparison to that in control mice. Feeding experiments with semisynthetic diets supplemented with phytol (0.5% w/w) increased the level of phytanic acid in serum from 16 to 1163muM in SCP2/SCPx deficient mice, from 1.4 to 129muM in control mice. The concentrations in pristanic acid were not elevated in SCP2/SCPx deficient mice when fed a normal diet but rose up to 2-fold after feeding the phytol

enriched diet.

Prolonged feeding of phytol-enriched diet to SCP2/SCPx deficient mice reduced body weight down to 60% of the starting value at the end of the second feeding week. Already after 1 week these mice showed a pronounced decrease of lipid levels in serum and almost complete absence of fat tissue. In addition to these abnormalities, feeding of phytol or pristanic acid with the diet resulted in an additional increase in expression of the genes observed to be upregulated in SCP2/SCPx deficient mice fed a normal diet [15].

In conclusion, either lack of SCPx/SCP2 or phytol feeding in this animal model created high serum and tissue levels of branched-chain fatty acids, which induced enhanced expression of enzymes responsible for the degradation of straight-chain fatty acids. It became obvious that a direct link must exist between nutrient branched-chain fatty acids and gene expression.

Mediators for branched-chain fatty acid signaling

Peroxisome proliferator activated receptor

A decade ago it was recognized that drugs administered to rodents to elicit peroxisome proliferation target for orphan receptors, whose DNA binding domain interacted with the peroxisome proliferator responsive element (PPRE) in the promoter DNA of a whole set of enzymes [16]. These receptors, subsequently named peroxisome proliferator activated receptors (PPARs), belong to a large superfamily of nuclear hormone receptors which comprises various ligand activated transcription factors. PPARs have been found in all mammalian species searched for so far, and three distinct isoforms have been identified until today, namely PPARalpha, PPARbeta (or delta also called NUC-I or FAAR) and PPARgamma [17]. Soon it was realized that fatty acids, which in form of triglycerides and phospholipids are part of our daily food, are the natural ligands of these transcription factors [4, 18-20]. This provided first rationales to understand nutrient "fat"- gene interactions. Whereas regulation of gene expression is a consequence of this interaction in all mammals, peroxisome proliferation appears to be specific for rodents.

PPAR isoforms show a distinct tissue distribution pattern. PPARalpha is predominantly expressed in cells with high peroxisomal activities and high catabolic rates for fatty acids. It is most abundantly expressed in liver, but also present in kidney, heart and brown adipose tissue [21, 22]. With respect to the tissues analyzed the distribution pattern for this isoform is similar for humans and rodents, however, concentrations in the former are lower. The beta isoform is found in nearly every tissue [21], in contrast to the restricted occurrence of the gamma isoform of which three related subtypes exist, found mainly in adipose tissue [21, 23, 24].

The PPAR isoforms are defined by their different primary structures of the C-terminal ligand binding domain [25] and consequently this domain is responsible for ligand affinity. Interestingly, subtypes of PPARgamma differ in primary structure at the amino-terminus [26] and one can speculate this domain to be responsible for tissue specific interactions but not for specific ligand binding.

Lipid-lowering drugs of the clofibrate type were shown to target for PPARalpha, whereas insulin sensitizing thiazolidinediones target for PPARgamma. Of the natural ligands straight-chain fatty acids, with few exceptions, all bind and transactivate all PPAR isoforms. Interestingly, branched-chain phytanic acid binds and transactivates PPARalpha but not PPARgamma while pristanic acid was

shown to be a potent activator of PPARalpha as well [9, 27]. Thus isoprenoid branched-chain fatty acids appear to interact solely with the alpha isoform of PPARs for high potency induction of enzymes. *In vitro* experiments revealed a relationship between binding affinity and transactivating capability, *i.e.*, the potential to induce enzyme transcription. This relationship is not a strict quantitative correlation for reasons discussed later in this review, however, a good qualitative indicator. Taking the example of PPARalpha, *Table* shows that the binding affinity of saturated straight-chain fatty acids for this transcription factor is rather low in comparison to mono- and polyunsaturated fatty acids, whereas the affinity of branched-chain fatty acids is extremely high; this is matched by the higher transactivation potentials of these class of fatty acids to induce gene transcription [20].

Hep G2 cells, an immortalized human hepatoma cell line, offered the opportunity to test PPARalpha-mediated transactivation of straight- and branched-chain fatty acids by cloning the PPRE of the L-FABP gene in front of a reporter gene. In this model system phytanic and pristanic acids where the most potent natural transactivators *(Figure 3)* [27, 28].

Liver fatty acid binding protein

It has long been speculated how nutrient fatty acids, once taken up into the cell, reach their intracellular targets. Such targets for example are the cellular membranes where fatty acid are incorporated into phospholipids to fulfil structural needs, or is the endoplasmatic reticulum for deposit of fatty acids into triglycerides, or are the beta-oxidative peroxisomes and mitochondria to which fatty acids are delivered as fuels. Yet, a small proportion of fatty acids must be delivered to the nucleus to effect gene transcription which would classify them as signaling molecules. For the mammalian cell is generally assumed that a family of structurally highly conserved 14-15kDa transport proteins in the cytosol, the fatty acid binding proteins (FABPs), take up the role as central distributors of fatty acids [29]. Until now 13 different types of this protein family are known, which all share a common structural motif consisting of a beta-barrel and a alpha helix-turn-alpha helix motif, where the former harbors the ligand binding site. FABPs bind, with one exception (see below), fatty acids in a 1:1 ratio [29].

Since the discovery of FABPs in 1972 [30] a plethora of reports appeared that implicate a role for FABPs in intracellular transport and storage of fatty acids, in modulation of enzyme activity, in differentiation and growth regulation [31, 32]. The multifunctional properties of FABPs are matched by their cell specific expression in various mammalian organs. Thus in liver parenchymal cells liver (L-) FABP is expressed in amounts up to 5% of cytosolic proteins, but this protein is also found in cells lining the intestinal mucosa and in certain kidney cells [33]. Biochemical and immunochemical evidence revealed that L-FABP in rat hepatocytes was present not only in the cytosol, but also in the nucleus [34]. This evidence gave first clues that L-FABP might be involved in the mechanism by which straight- and branched-chain fatty acids as signaling molecules reach their nuclear destination for interaction with PPARalpha.

L-FABP binds two fatty acid molecules which is exceptional [35]. It has higher affinities for unsaturated than for saturated fatty acids, however, no differences were observed with respect to position and number of double bonds [36]. In addition to straight-chain fatty acids with K_d's in the range of 0.1-2muM, L-FABP binds branched-chain phytanic acid with affinities in the low nM range *(Table)*. The comparison of data for fatty acid binding to L-FABP with those for binding to PPARalpha

also infers a role for the former in targeting the signaling fatty acids to the latter: K_d's for ligand binding to the second site of L-FABP are generally higher, for the first binding site generally lower than those for binding to PPARalpha [20, 37]. These fatty acid/L-FABP equilibria preceding the fatty acid/PPAR binding equilibria have to be considered under *in vivo* conditions when PPAR transactivation is judged on the basis of fatty acid/PPAR affinity constants and *vice versa.*

Mechanism of fatty acid signaling

Summarizing the foregoing one can state: (1) feeding chlorophyll-derived branched-chain fatty acids to mice strongly induced enzymes of peroxisomal beta-oxidation and L-FABP in hepatocytes; (2) branched-chain fatty acids highly transactivated PPARalpha in the human HepG2 cell line; (3) phytanic acid was ligand for both PPARalpha and L-FABP; (4) L-FABP was found in the cytosol and nuclei of hepatocytes. Based on these observations we envisaged PPARalpha and L-FABP to be part of a signal transducing chain in hepatocytes that enable straight-chain fatty acids in general and branched-chain fatty acids in particular to induce gene expression.

To prove our hypothesis we made use of stably transfected antisense L-FABP HepG2 cell lines which differed in their intracellular L-FABP contents [38]. We reasoned that fatty acids taken up by the cell were to be transported by L-FABP into the nucleus for interaction with PPARalpha to affect gene transcription. Consequently transactivation should increase not only with the amount of fatty acids administered to the cell, but also with the amount of L-FABP in the cell. This was indeed the case as can be seen first in the upper part of *Figure 4*, which demonstrates that at fixed intracellular L-FABP concentration linoleic acid afforded a dose dependent enhancement of PPARalpha trans-activation. The lower part of *Figure 4* reveals not only that increased L-FABP content in the cells led to increased transactivation, but also that phytanic acid is a very potent agonists for transactivation [9, 27]. In accordance with our data it was shown by others that L-FABP enters the nucleus when loaded with fatty acids [39], which corroborates its function as a carrier for fatty acid signaling molecules to their respective nuclear receptors. Once inside the nucleus L-FABP interacts with PPARalpha by protein/protein interaction as we could demonstrate with the aid of a mammalian two-hybrid system, by immuno-coprecipitation of the L-FABP/PPARalpha complex from nuclear lysates, and by an *in vitro* pull down assay [9]. This interaction is independent of ligand present, but it should be noted that other nuclear proteins may be candidates for a ligand-dependent interaction of L-FABP [39]. Future research certainly has to identify these proteins.

The current view on the mechanism of nutrient fatty acid interaction with genes in hepatocytes is depicted in *Figure 5*. The signaling path starts when fatty acids enter the cell. This process may be a point of control exerted by fatty acid translocators in the membrane [40] and by intracellular L-FABP contents. Bound to cytosolic L-FABP a small proportion of the fatty acid/L-FABP complex enters the nucleus [9] where L-FABP interacts with PPARalpha. Upon transfer of the fatty acid to PPARalpha this receptor heterodimerizes with another receptor (RXR) for binding to a PPRE in promoters of genes [41]. Such elements are found in genes encoding peroxisomal beta-oxidation enzymes, *i.e.*, straight-chain acyl-CoA oxidase and beta-ketoacyl-CoA thiolase [12], and cytosolic/nuclear L-FABP [42]. Subsequently the expression of respective proteins is induced to promote degradation of all straight-chain fatty acids. Moreover, increased L-FABP expression establishes an autoregulatory mechanism to further enhance degradation of fatty acid by increased transport of agonists to PPARalpha for transactivation.

It was noted that L-FABP also interacts with PPARgamma, but not with PPARbeta [9]. As PPARgamma in comparison to PPARalpha is present only in minor amounts in hepatocytes and is not transactivated by phytanic acid, the contribution to fatty acid degradation might not be substantial. PPARgamma, however, is predominantly expressed in adipocytes in which adipocyte (A-) FABP is found in great proportions [33]. It remains to be seen whether the mechanism elucidated for L-FABP/PPARalpha in hepatocytes also applies for A-FABP/ PPARgamma in adipocytes, by signaling of straight-chain fatty acids possibly for the storage of fatty acids.

Implications for nutrition

The phenotype of increased phytanic acid concentration observed in SCP2/SCPx deficient mice reflects a pathophysiological situation observed in humans called Refsum's disease. Refsum's disease is an autosomal-recessively inherited disorder characterized clinically by several abnormalities like retinitis pigmentosa, peripheral neuropathy, and cerebellar ataxia [4]. All patients exhibit accumulation of phytanic acid in blood and tissues [43]. In comparison to SCP2/SCPx deficient mice, the disease in humans is caused by the deficiency of phytanoyl-CoA hydroxylase, the peroxisomal protein catalyzing the first step in the alpha-oxidation of phytanic acid [44]. Patients afflicted with Refsum's disease exhibit serum concentrations of this branched-chain fatty acid up to 1.4mM in comparison to normal subjects whose serum concentrations of this acid vary between 0.5 and 10muM [43]. The mechanism of how the increase in phytanic acid concentration leads to the observed phenotype has not been elucidated yet, but several hypotheses have been put forward. Since Refsum's patients eventually suffer from demyelinisation of the central nervous system, it has been suggested that incorporation of phytanic acid into lipid membranes and the concurrent increase in fluidity, due to the branched-chain character of this fatty acid, cause this effect. Another hypothesis proposes that phytanic acid, which has a structure similar to geranylgeranyl and farnesyl structures, inhibits modifications of geranyl anchored membrane proteins. This effect might explain deficiencies in sight as several geranylated ras proteins which are implicated in the sight process. Furthermore, it has been speculated that phytanic acid at abnormally high concentrations mimics 9-*cis*-retinoic acid, the physiological agonist of RXR, despite a 100-fold lower affinity. Activated RXR induces apoptosis in several cells including glioblastoma cells, thus unspecific activation of this nuclear receptor might lead to death of these cells which in turn could explain the neurological disorders of the patients. Although all this is speculation, the question is certainly valid on the width of the concentration window for branched-chain fatty acids in healthy persons to make good use of their potency to induce beta-oxidative enzymes.

Because of this impact on fatty acid metabolism phytanic acid and its degradation products represent a nutritional opportunity. Although differences exist between mouse and human physiology it can be envisaged that consumption of phytanic acid would lead to an increase in beta-oxidation of fatty acids and thus to a decrease in triglyceride concentration as well as in number of adipocytes. Analysis of the benevolent and malevolent effects of nutrients, known to contain phytanic acid, might give a clue about the nutritional effects of phytanic acid. One way to directly assess the nutritional value of phytanic acid would be to analyze whether Refsum's patients show differences in triglyceride and adipocyte content which might be explained by alteration of their lipid metabolism due to signaling by phytanic acid in unphysiologically high doses.

The other way would be nutritional trials meals enforced with phytanic acid, with safety being ascertained by monitoring phytanic acid levels in the blood.

Recent studies demonstrate that other beneficial effects of phytanic acid beside induction of lipid metabolism might exist. It was demonstrated that phytanic acid in concentations of 100muM which is 10 fold less than the concentration observed in serum of Refsum's patients is able to induce apoptosis in proliferating smooth muscle cells [45]. Since proliferating smooth muscle cells contribute to the formation of arteriosclerotic plaques it can be hypothesized that phytanic acid prevents this formation by directing the cells toward cell death.

CONCLUSION

In conclusion, the basic mechanism of isoprenoid branched-chain fatty acid action in lipid homeostasis has been elucidated. A stage can now be set for careful valuation of nutritional and therapeutic opportunities.

Acknowledgements

The work carried out in the authors' laboratory and reviewed here has been supported by grants from the Deutsche Forschungsgemeinschaft (SFB 310/A4 and SP 135/12-1). C.W. gratefully acknowledges support by the Stiftungsfonds Unilever.

REFERENCES

1. BURR GO, BURR MM (1930). On the nature and role of fatty acids essential in

nutrition. *J Biol Chem*, 86: 587-622.

2. SIMOPOULOS AP (1999). Essential fatty acids in health and chronic disease. *Am J Clin Nutr*, 70: S560-9.

3. RATNAYAKE WM, OLSSON B, ACKMAN RG (1989). Novel branched-chain fatty acids in certain fish oils. *Lipids*, 24: 630-7.

4. STEINBERG D, VROOM FQ, ENGEL WK, CAMMERMEYER J, MIZE CE, AVIGAN J (1967). Refsum's disease: a recently characterized lipidosis involving the nervous system. *Ann Intern Med*, 66: 365-95.

5. WOLFRUM C, SPENER F (2000). Fatty acids as regulators of lipid metabolism. *Eur J Lipid Sci Technol*, 102: 746-62.

6. JUMP DB, CLARKE SD (1999). Regulation of gene expression by dietary fat. *Annu Rev Nutr*, 19: 63-90.

7. VERHOEVEN NM, WANDERS RJ, POLL-THE BT, SAUDUBRAY JM, JAKOBS C (1998). The metabolism of phytanic acid and pristanic acid in man: a review. *J Inherit Metab Dis*, 21: 697-728.

8. MOSER HW, BERGIN A, CORNBLATH D (1991). Peroxisomal disorders. *Biochem Cell Biol*, 69: 463-74.

9. WOLFRUM C, BORRMANN CM, BÖRCHERS T, SPENER F (2001). Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha- and gamma-mediated gene expression *via* liver fatty acid binding protein: a signaling path to the nucleus. *Proc Natl Acad Sci USA*, 98: 2323-8.

10. PAHAN K, SINGH I (1995). Phytanic acid oxidation: topographical localization of phytanoyl-CoA ligase and transport of phytanic acid into human peroxisomes. *J Lipid Res*, 36: 986-97.

11. VAN VELDHOVEN PP, MANNAERTS GP, CASTEELS M, CROES K (1999). Hepatic alpha-oxidation of phytanic acid: a revised pathway. *Adv Exp Med Biol*, 466: 273-81.

12. HASHIMOTO T (1999). Peroxisomal beta-oxidation enzymes. *Neurochem Res*, 24: 551-63.

13. WATKINS PA, CHEN WW, HARRIS CJ, HOEFLER G, HOEFLER S, BLAKE DC Jr , BALFE A, KELLEY RI, MOSER AB, BEARD ME (1989). Peroxisomal bifunctional enzyme deficiency. *J Clin Invest*, 83: 771-7.

14. WOUTERS FS, BASTIAENS PI, WIRTZ KW, JOVIN TM (1998). FRET microscopy demonstrates molecular association of non-specific lipid transfer protein (nsL-TP) with fatty acid oxidation enzymes in peroxisomes. *EMBO J*, 17: 7179-89.

15. SEEDORF U, RAABE M, ELLINGHAUS P, KANNENBERG F, FOBKER M, ENGEL T, DENIS S, *et al.* (1998). Defective peroxisomal catabolism of branched fatty acyl coenzyme A in mice lacking the sterol carrier protein-2/sterol carrier protein-x gene function. *Genes Dev*, 12: 1189-201.

16. ISSEMANN I, GREEN S (1991). Cloning of novel members of the steroid hormone receptor superfamily. *J Steroid Biochem Mol Biol*, 40: 263-9.

17. LEMBERGER T, DESVERGNE B, WAHLI W (1996). Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu Rev Cell Dev Biol*, 12: 335-63.

18. GÖTTLICHER M, DEMOZ A, SVENSSON D, TOLLET P, BERGE RK, GUSTAFSSON JA (1993). Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor. *Biochem Pharmacol*, 46: 2177-84.

19. FORMAN BM, CHEN J, EVANS RM (1997). Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci USA*, 94: 4312-7.

20. ELLINGHAUS P, WOLFRUM C, ASSMANN G, SPENER F, SEEDORF U (1999). Phytanic acid activates the peroxisome proliferator-activated receptor alpha (PPARalpha) in sterol carrier protein 2-/ sterol carrier protein x-deficient mice. *J Biol Chem,* 274: 2766-72.

21. BRAISSANT O, WAHLI W (1998). Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. *Endocrinology,* 139: 2748-54.

22. KLIEWER SA, FORMAN BM, BLUMBERG B, ONG ES, BORGMEYER U, MANGELSDORF DJ, UMESONO K, EVANS RM (1994). Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA*, 91: 7355-9.

23. TONTONOZ P, HU E, GRAVES RA, BUDAVARI AI, SPIEGELMAN BM (1994). mPPAR gamma2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev*, 8: 1224-34.

24. FAJAS L, FRUCHART JC, AUWERX J (1998). PPARgamma3 mRNA: a distinct PPARgamma mRNA subtype transcribed from an independent promoter. *FEBS Lett*, 438: 55-60.

25. ESCHER P, WAHLI W (2000). Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat Res,* 448: 121-38.

26. ELBRECHT A, CHEN Y, CULLINAN CA, HAYES N, LEIBOWITZ M, MOLLER DE, BERGER J (1996). Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma 1 and gamma 2. *Biochem Biophys Res Commun*, 224: 431-7.

27. HANHOFF T, WOLFRUM C, ELLINGHAUS P, SEEDORF U, SPENER F (2001). Pristanic acid is activator of peroxisome proliferator activated receptor alpha. *Eur J Lipid Sci Technol*, 103: 75-80.

28. WOLFRUM C, ELLINGHAUS P, FOBKER M, SEEDORF U, ASSMANN G, BÖRCHERS T, SPENER F (1999). Phytanic acid is ligand and transcriptional activator of murine liver fatty acid binding protein. *J Lipid Res*, 40: 708-14.

29. HOHOFF C, SPENER F (1998). Fatty acid binding proteins and mammary-derived growth inhibitor. *Fett/Lipid*, 100: 252-63.

30. OCKNER RK, MANNING JA, POPPENHAUSEN RB, HO WK (1972). A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. *Science*, 177: 56-8.

31. STORCH J, THUMSER AE (2000). The fatty acid transport function of fatty acid-binding proteins. *Biochim Biophys Acta*, 1486: 28-44.

32. BÖRCHERS T, HOHOFF C, BUHLMANN C, SPENER F (1997). Heart-type fatty acid binding protein - involvement in growth inhibition and differentiation. *Prostaglandins Leukot Essent Fatty Acids*, 57: 77-84.

33. VEERKAMP JH, PAULUSSEN RJ, PEETERS RA, MAATMAN RG, VAN MOERKERK HT, VAN KUPPEVELT TH (1990). Detection, tissue distribution and (sub)cellular localization of fatty acid-binding protein types. *Mol Cell Biochem,* 98: 11-8.

34. BORDEWICK U, HEESE M, BÖRCHERS T, ROBENEK H, SPENER F (1989). Compartmentation of hepatic fatty-acid-binding protein in liver cells and its effect on microsomal phosphatidic acid biosynthesis. *Biol Chem Hoppe-Seyler*, 370: 229-38.

35. HAUNERLAND N, JAGSCHIES G, SCHULENBERG H, SPENER F (1984). Fatty-acid-binding proteins. Occurrence of two fatty-acid-binding proteins in bovine liver cytosol and their binding of fatty acids, cholesterol, and other lipophilic ligands. *Hoppe-Seyler's Z Physiol Chem*, 365: 365-76.

36. WOLFRUM C, BÖRCHERS T, SACCHETTINI JC, SPENER F (2000). Binding of fatty acids and peroxisome proliferators to orthologous fatty acid binding proteins from human, murine, and bovine liver. *Biochemistry*, 39: 1469-74.

37. LIN Q, RUUSKA SE, SHAW NS, DONG D, NOY N (1999). Ligand selectivity of the peroxisome proliferator-activated receptor alpha. *Biochemistry*, 38: 185-90.

38. WOLFRUM C, BUHLMANN C, ROLF B, BÖRCHERS T, SPENER F (1999). Variation of liver-type fatty acid binding protein content in the human hepatoma cell line HepG2 by peroxisome proliferators and antisense RNA affects the rate of fatty acid uptake. *Biochim Biophys Acta*, 1437: 194-201.

39. LAWRENCE JW, KROLL DJ, EACHO PI (2000). Ligand-dependent interaction of hepatic fatty acid-binding protein with the nucleus. *J Lipid Res*, 41: 1390-401.

40. DUTTA-ROY AK (2000). Cellular uptake of long-chain fatty acids: role of membrane-associated fatty-acid-binding/transport proteins. *Cell Mol Life Sci,* 57: 1360-72.

41. KREY G, MAHFOUDI A, WAHLI W (1995). Functional interactions of peroxisome proliferator-activated receptor, retinoid-X receptor, and Sp1 in the transcriptional regulation of the acyl-coenzyme-A oxidase promoter. *Mol Endocrinol*, 9: 219-31.

42. ISSEMANN I, PRINCE R, TUGWOOD J, GREEN S (1992). A role for fatty acids and liver fatty acid binding protein in peroxisome proliferation? *Biochem Soc Trans*, 20: 824-7.

43. KAHLKE W, WAGENER H, DIECKMANN H (1965). Studies on the phytol metabolism in lipoidosis heredopathia atactica polyneuritiformis (Refsum's syndrome). The transformation of 3H-phytol into the 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) of the plasma lipoid fractions. *Klin Wochenschr*, 43: 1345-7.

44. JANSEN GA, FERDINANDUSSE S, SKJELDAL OH, STOKKE O, DE GROOT CJ, JAKOBS C, WANDERS RJ (1998). Molecular basis of Refsum disease: identification of new mutations in the phytanoyl-CoA hydroxylase cDNA. *J Inherit Metab Dis*, 21: 288-91.

45. IDEL S, ELLINGHAUS P, WOLFRUM C, NOFER JR, ASSMANN G, SPENER F, SEEDORF U (2001). Dietary branched-chain fatty acids induce apoptosis in vascular smooth muscle cells. *J Biol Chem*, submitted.

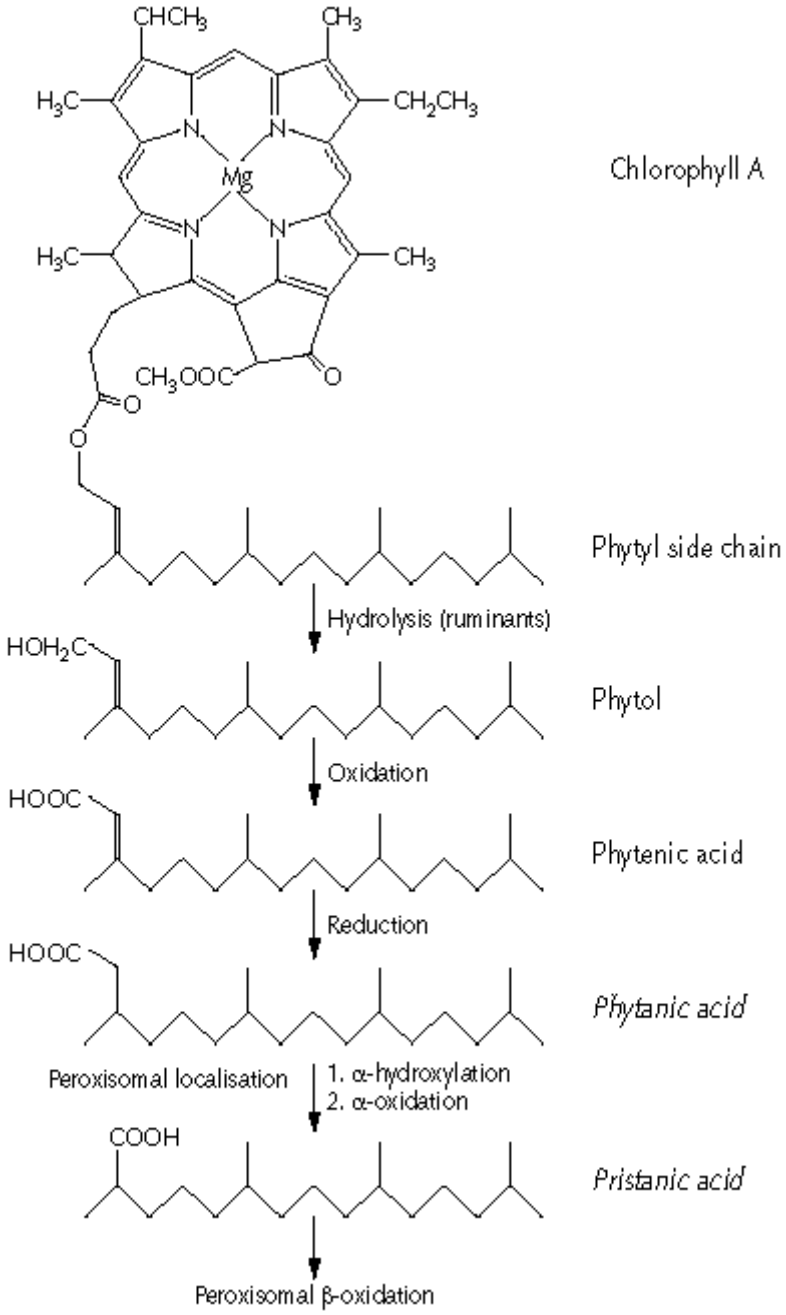

Figure 1. Degradation of the phytyl side chain of chlorophyll.

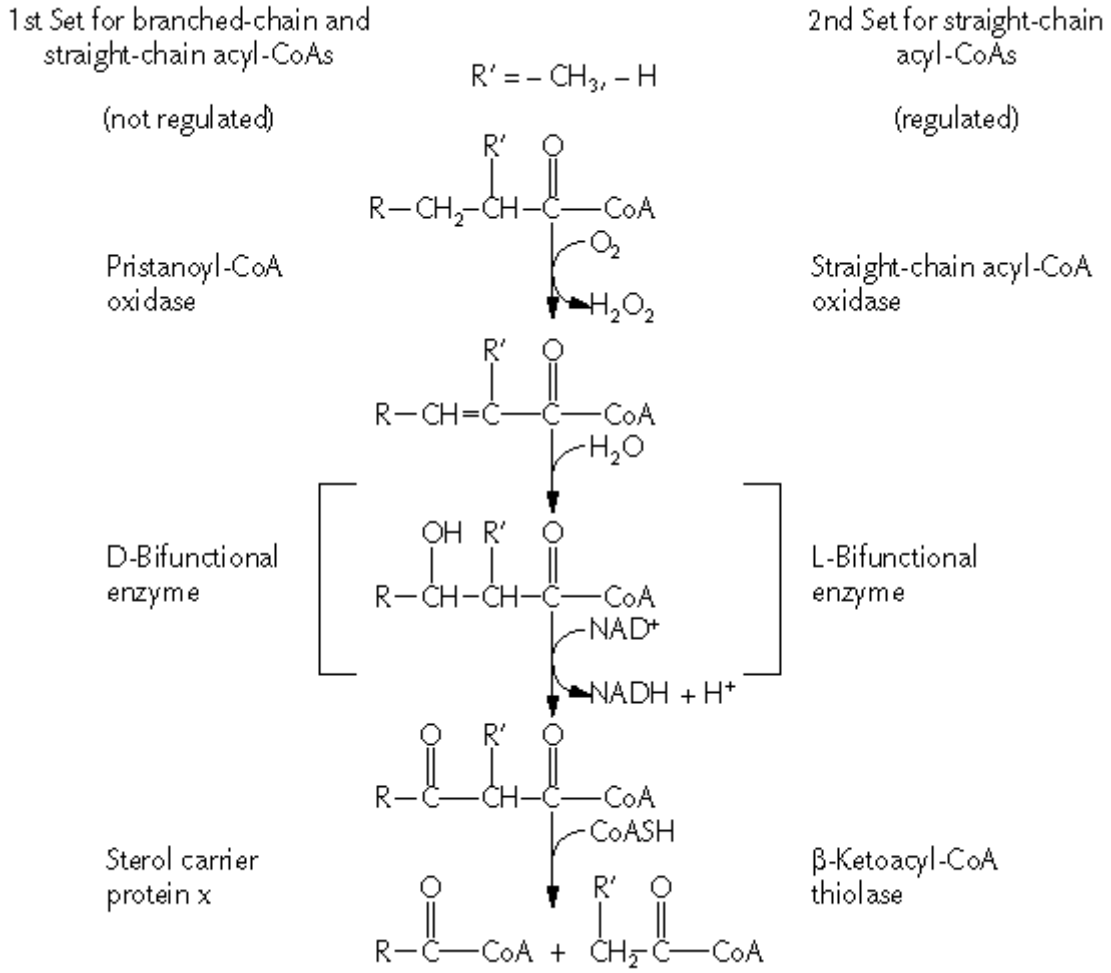

Figure 2. Enzymes of peroxisomal beta-oxidation. Straight-chain fatty acids degraded are of the very long- and long-chain type.

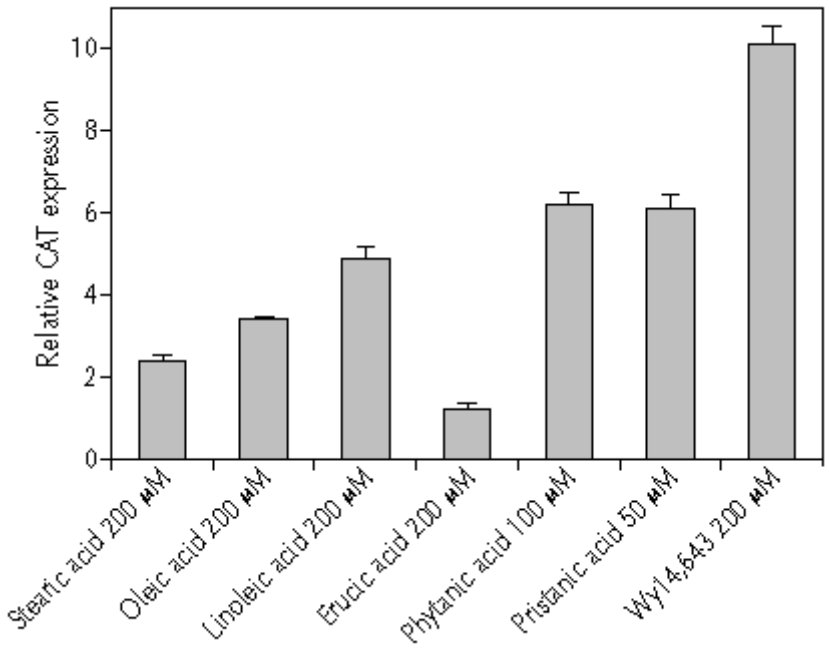

Figure 3. Transactivation of murine PPARalpha in HepG2 cells [20]. HepG2 cells were transfected with the expression vector for murine PPARalpha, pSV-beta-Gal and the CAT-reporter gene vector under the control of ideal PPRE. Cells were treated for 24h with fatty acids and Wy14,643 in the indicated amount. beta-Gal and CAT concentration were determined by ELISAs, DMSO control was set as one. Each value represents the mean of 6 independent experiments ± SD.

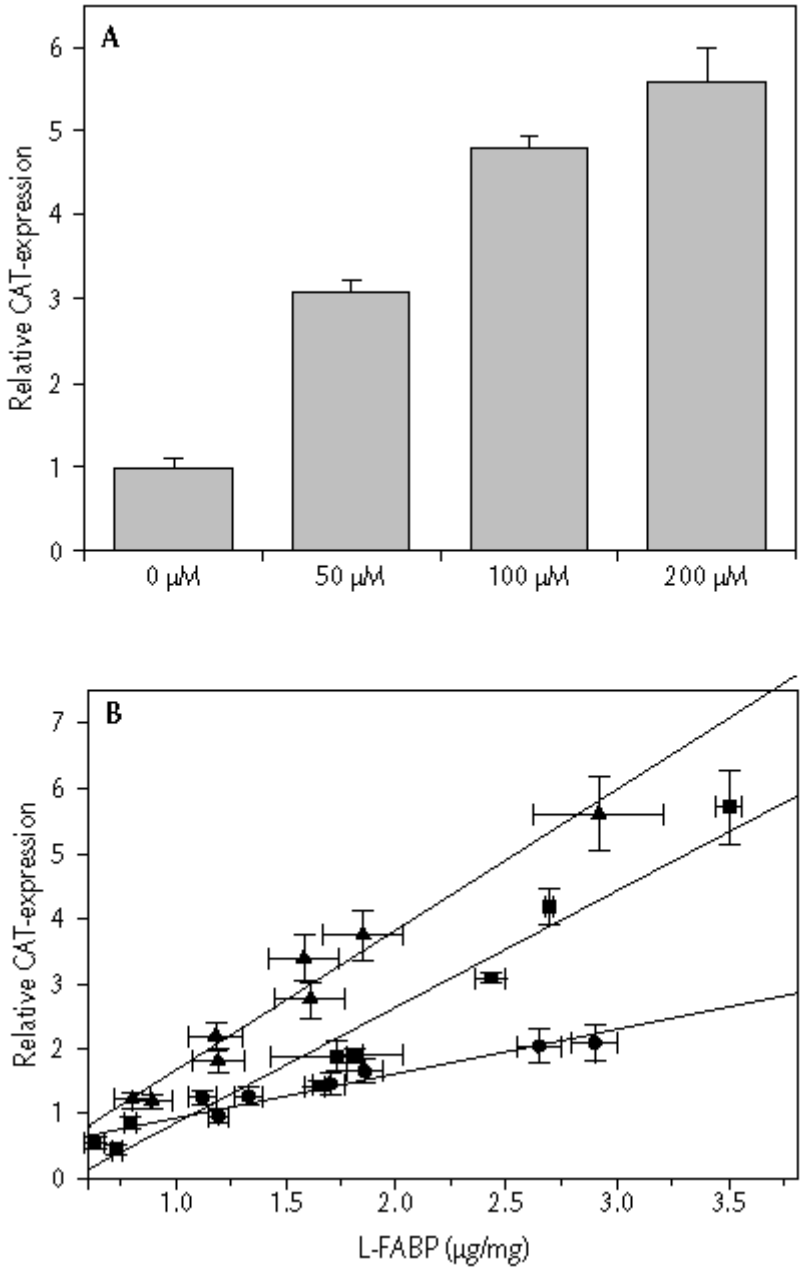

Figure 4. Transactivation of murine PPARalpha in HepG2 cells is dependent on L-FABP concentration [9]. A, HepG2 cells were transfected as described for Figure 3. Cells (L-FABP content 3.5mg/mg) were treated for 24h with linoleic acid in the indicated amount. B, Antisense L-FABP mRNA HepG2 cells [38], were transfected as described for Figure 3. Each data point represents a single clone (from a total of 8 clones differing in L-FABP contents). Cells were treated for 24h with 200mM stearic acid (●), 200muM linoleic acid (▲) and 100muM phytanic acid (■). beta-Gal, CAT and L-FABP concentrations were determined by ELISAs, DMSO control was set as one. Each data point represents the mean of 6 independent experiments ± SD.

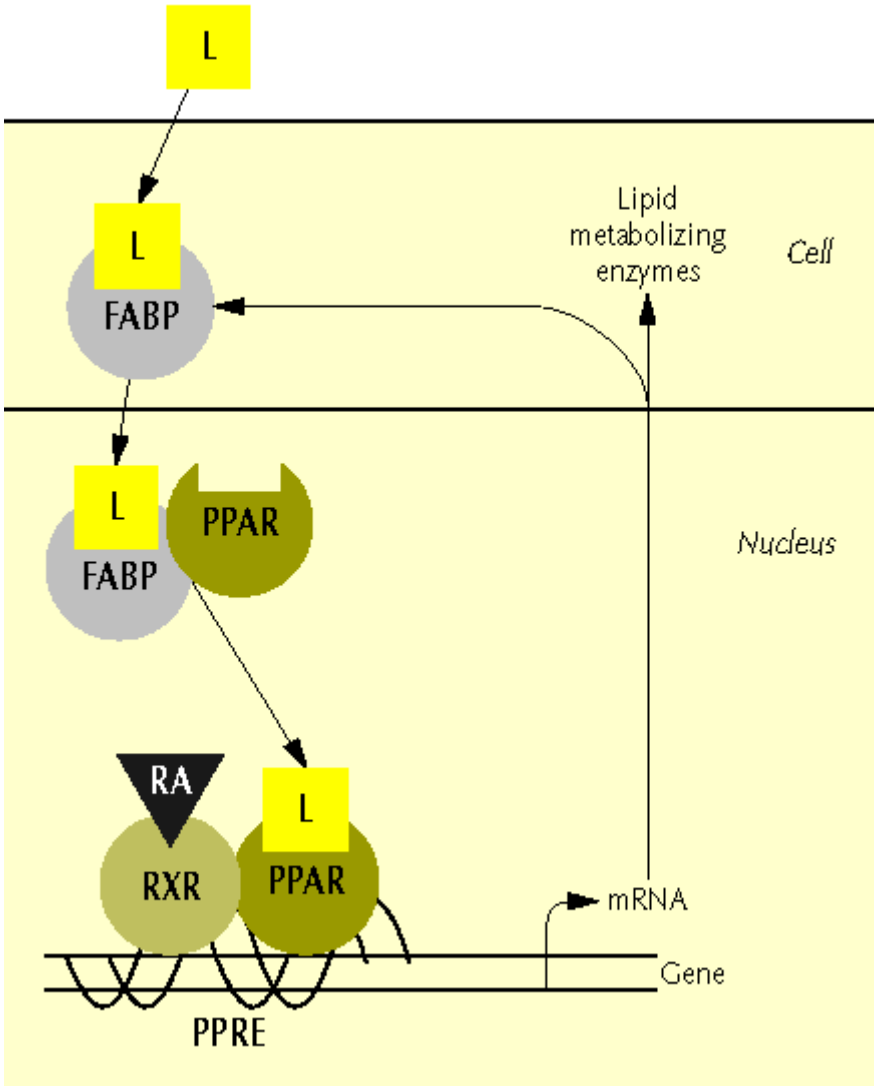

Figure 5. Scheme for L-FABP action in PPAR-mediated gene regulation. L, ligand (fatty acid, peroxisome proliferator); RA, cis-9 retinoic acid.

Table. *Affinities of fatty acids and peroxisome proliferators for human L-FABP and PPARalpha [20, 36]. ND indicates not measured.*

Binding protein	Palmitic acid	Stearic acid	Oleic acid	Phytanic acid	Beza-fibrate	Wy14,643
	Dissociation constant [muM]					
L-FABP 1st binding site	ND	1.9	0.006	0.015	6.32	3.3
L-FABP 2nd binding site	ND	13	0.94	0.282	112.6	low
PPARalpha	0.103	ND	0.099	0.021	0.045	0.016