REVIEW

Regulation of genes involved in lipid metabolism by dietary oxidized fat

Robert Ringseis and Klaus Eder

Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Gießen, Gießen, Germany

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Although oxidized fats are widely considered to have detrimental effects on human health, a large number of feeding studies with experimental animals have consistently demonstrated that oxidized fats, compared with fresh fats, cause a reduction in the concentrations of triacylglycerols and cholesterol in liver and plasma. The reason for these effects became clear when recently it was shown that thermo-oxidized fats contain characteristic substances such as hydroxylated fatty acids and cyclic fatty acid monomers which are potent ligands and activators of peroxisome proliferator-activated receptor α – a transcription factor controlling genes involved in fatty acid and lipoprotein metabolism. In addition, oxidized fats have also been reported to inhibit expression of genes involved in fatty acid synthesis and cholesterol homeostasis. These effects are mediated by inhibiting the maturation of sterol regulatory-element binding proteins, which are transcription factors regulating genes involved in fatty acid synthesis and cholesterol homeostasis. This review summarizes the phenotypical alterations of lipid metabolism observed in feeding studies dealing with oxidized fats and addresses the molecular mechanisms underlying these lipid metabolism alterations, in particular the lipid lowering effects of dietary oxidized fats.

Keywords:
Liver / Oxidized fat / Peroxisome proliferator-activated receptor α / Sterol regulatory-element binding protein

1 Introduction

In recent years, the contribution of oxidized fats to total energy intake has markedly increased in industrialized countries [1]. This is mainly due to the rising consumption of deep-fried products, which are very popular because of their desirable flavour, colour, and crispy texture. In a cohort from the European Prospective Investigation into Cancer and Nutrition study for instance, the percentage of energy intake from fried food was more than 23% in the highest quintile of consumption [2]. During deep frying, several chemical reactions occur within the frying oil resulting in the formation of a mixture of chemically distinct lipid peroxidation products. Large quantities of the frying oil are absorbed into the fried food during deep frying and therefore ingested during their consumption.

Feeding experiments with rats, guinea pigs, and pigs revealed that ingestion of oxidized fats provokes various effects such as depletion of antioxidants, increase in lipid peroxidation [3–9], impairment of glucose tolerance [10], and alterations of thyroid hormone homeostasis [11–13]. In addition, a great number of studies showed that oxidized fats influence lipid metabolism. In this regard, it is noteworthy that most of these studies dealing with the lipid metabolism alterations of oxidized fats reported reduced triacylglycerol (TAG) and cholesterol concentrations in liver and plasma of animals fed oxidized fat [7, 14–28]. Considering that elevated blood lipid concentrations are

Correspondence: Dr. Robert Ringseis, Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Gießen, Heinrich-Buff-Ring 26-32, 35392 Gießen, Germany
E-mail: robert.ringseis@ernaehrung.uni-giessen.de
Fax: +49-641-9939239

Abbreviations: ACO, acyl-CoA oxidase; **CFAM,** cyclic fatty acid monomers; **CPT,** carnitine-palmitoyltransferase; **CYP4A1,** cytochrome P450 isoenzyme 4A1; **HODE,** hydroxy octadecadienoic acid; **HPODE,** hydroperoxy octadecadienoic acid; **Insig,** insulin-induced gene; **PPARα,** peroxisome proliferator-activated receptor α; **OCTN2,** novel organic cation transporter 2; **SCAP,** SREBP cleavage activating protein; **SREBP,** sterol regulatory-element binding proteins; **TAG,** triacylglycerol

known risk factors for the development of atherosclerosis, the lipid lowering effect of oxidized fat indicates that oxidized fats not only induce adverse effects but obviously also exert potentially beneficial effects. In most of the earlier studies dealing with oxidized fats [29, 30], strongly oxidized fats were used which contained concentrations of lipid peroxidations products which were clearly above the limit allowed for used frying fats in restaurants according to EU legislation. Feeding of such fats not only reduced food intake and growth rate of the animals but also induced pronounced oxidative stress and even provoked toxic effects [5, 29–32]. Moreover, it could be shown that the digestibility of fat from such abused oxidized fats was reduced [5, 31, 32]. It has therefore been argued that the lipid lowering actions of oxidized fats were mainly due to these confounding effects, in particular the reduced food and energy intake and the decreased digestion and absorption of oxidized fats. To avoid these confounding effects of oxidized fats, in more recent studies only moderately oxidized fats were used and a controlled feeding system, in which animals of all treatment groups were fed identical amounts of diet, was applied [7, 15, 16, 19, 21–25, 27, 28]. Moreover, in these studies, the loss of antioxidants and PUFA occurring during thermo-oxidation of the fats was substituted by supplementing the oxidized fat diets with synthetic antioxidants (vitamin E) and PUFA-rich fats, respectively, therefore, excluding secondary effects due to a reduced intake of antioxidants or PUFA. The lipid lowering actions of oxidized fats, however, were still observed in these studies. These observations, therefore, strongly suggested that the lipid lowering effects of oxidized fats are not due to potentially confounding effects (reduced food intake, decreased nutrient digestibility, depletion of antioxidants, and essential fatty acids deficiency) but rather mediated directly by inducing alterations of lipid metabolism in the organism.

This review, first, summarizes the phenotypical alterations of lipid metabolism observed in feeding studies dealing with oxidized fats, and, second, addresses the molecular mechanisms underlying these lipid metabolism alterations, in particular the lipid lowering effects of dietary oxidized fats.

2 Phenotypical alterations of lipid metabolism by oxidized fats

2.1 Effect of oxidized fat on TAG concentrations in liver and plasma

TAG lowering effects have been consistently observed in numerous independent experiments most of which have been performed with rats. Table 1 summarizes an overview about published studies reporting an effect of oxidized fats on liver and plasma TAG concentrations in different species. Evidently, the TAG lowering effects of oxidized fats observed were clearly stronger in rats and mice than in guinea pigs and pigs indicating a species dependency of this effect. In rats and mice, the reported TAG lowering effects of oxidized fats in the liver were in the range between –21 and –85% (compared with the fresh fat group), whereas in pigs the hepatic TAG concentration was decreased by an oxidized fat only marginally by approximately 3% [27]. The strongest TAG lowering effect observed in the liver was reported in one study with lactating rats [23]. In this study, the hepatic TAG concentration was reduced by feeding an oxidized fat by 85%. The oxidized fat in this study was prepared by heating sunflower oil at 60°C for 25 days and was fed to the rats for a total of 21 days, from parturition until weaning. Noteworthy, in all other studies considered in Table 1, the oxidized fats were fed to the animals for longer time periods (up to 98 days) than in the study with lactating rats [23], in which the strongest TAG lowering effect has been observed. This indicates that the alteration of hepatic TAG content is rapidly induced in the liver by feeding an oxidized fat and that the duration of the administration of the oxidized fat is less important for the mediation of this effect. Also of great interest is the observation from a recent study [25] that feeding of an oxidized fat compared with a fresh fat is capable of preventing from hepatic TAG accumulation induced by simultaneous ethanol administration. This shows that the TAG lowering action of oxidized fat is obviously useful during conditions of disturbed hepatic lipid metabolism such as fatty liver disease.

In plasma, the degree of TAG lowering by oxidized fat reported in different studies was in a similar range as in the liver and ranged between –11 [17] and –76% [19] (Table 1). As in the liver, it was evident that the TAG lowering effect of oxidized fat was generally lower in guinea pigs or pigs than in rats or mice [27]. This again indicates a species dependency of this effect of oxidized fat.

Considering that the heating regime (temperature and time) influences the spectrum of lipid peroxidation products present in the oxidized fat (low temperature, long time: predominance of primary lipid peroxidation products; high temperature, short time: predominance of secondary lipid peroxidation products), it has also been studied whether the heating regime influences the degree of TAG lowering. In one study [19], in which the TAG lowering property of oxidized fats prepared by different heating regimes (either 50°C for 38 days, 105°C for 81 h or 190°C for 24 h) was investigated, a slightly stronger TAG lowering effect in the liver was observed in rats fed the oxidized fat prepared at the lowest temperature. However, strong TAG lowering effects in the liver were also found in rats fed oxidized fats prepared at 205°C [17, 20]. It is therefore likely that both primary and secondary lipid peroxidation products contribute to the TAG lowering effect of oxidized fats.

Interestingly, studies in lactating rats revealed that oxidized fats not only reduce hepatic and plasma TAG concentrations but also cause a reduction of TAG in the

Table 1. Effect on liver and plasma TAG concentrations by oxidized fat as observed in studies with different species

Species	Fat source, heating regime	Feeding period (days)	Percental change of liver TAG concentration (compared with control)	Percental change of plasma TAG concentration (compared with control)	Ref.
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	56	−36%	−38%	[14]
Rat	Soybean oil, 80°C, 5 days	56	−13%[b)]	+5%	[77]
Rat	Soybean oil, 130°C, 22 h	40	−22%	−21%	[15]
Rat	Lard:safflower oil (2:1, w/w), 150°C, 6 days	51	−21%	−32%	[16]
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	42	−72%	−11%	[17]
Rat	Sunflower oil, 98°C, 48 h	69	n.d.[a)]	−47%	[18]
Rat	Sunflower oil:lard (1:1, w/w), 50°C, 38 days	63	−41%	−68%	[7, 19]
Rat	Sunflower oil:lard (1:1, w/w), 105°C, 81 h	63	−24%	−63%	[7, 19]
Rat	Sunflower oil:lard (1:1, w/w), 190°C, 24 h	63	−32%	−76%	[7, 19]
Rat	Sunflower oil, 55°C, 42 days	56	−21%	−66%	[19]
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	42	−71% (male) −43% (female)	−27% (male) −9% (female)	[20]
Rat	Sunflower oil:lard (1:1, w/w), 50°C, 38 days	63	−41%	−63%	[21]
Rat	Sunflower oil:linseed oil (80:20, w/w), 50°C, 16 days	98	−36%	n.d.[a)]	[22]
Rat	Sunflower oil, 60°C, 25 days	32	−32%	−41%	[25]
Rat	Sunflower oil, 60°C, 25 days	21	−85%	−61%	[23]
Rat	Sunflower oil, 60°C, 25 days	22	−59%	n.d.[a)]	[24]
Mouse	Heated hydrogenated palm fat:fresh sunflower oil (92:8, w/w), 170°C, 48 h with repeated frying of French fries for 6 min every 30 min	112	n.d.[a)]	−53%	[76]
Guinea pig	Soybean oil, 205°C, 6 h with potato sticks in the oil, four consecutive days	60	n.d.[a)]	−21%	[26]
Pig	Sunflower oil, 180°C, 24 h	28	−3%	−12%	[27]

a) Not determined.
b) Total lipid content (g/100 g liver), TAGs are not specified.

milk [22, 23, 33]. As maternal milk is the only source of nutrients for the suckling pups, it was not surprising that the reductions in TAG and, concomitantly, energy in the milk by oxidized fat in these studies were accompanied by a significantly impaired development of litters during lactation [22, 23, 33]. Also noteworthy, when oxidized fat was fed to pregnant rats, a TAG lowering effect was even observed in the liver of the fetuses [24], suggesting that the components of oxidized fats responsible for TAG lowering apparently pass the placental barrier in sufficient amounts to exert this effect in the fetal liver.

2.2 Effect of oxidized fat on cholesterol concentrations in liver and plasma

Reports on the effects of oxidized fat on cholesterol concentrations in liver and plasma are somewhat less consistent than those on TAG concentrations. Nevertheless, the majority of studies also reported reduced cholesterol concentrations in liver and plasma following the administration of oxidized fats, as summarized in Table 2. No effect on hepatic cholesterol concentration was observed in one study with rats [15], which received either fresh soybean oil or oxidized soybean oil prepared by heating at 130°C. In this study, an effect of oxidized fat on plasma cholesterol concentrations was not reported. In three other experiments with rats [7, 19], only the concentrations of cholesterol in the liver were decreased by 22, 25, and 13%, respectively, by the oxidized fat, whereas the plasma cholesterol concentrations remained unchanged. In a study with guinea pigs, the concentration of cholesterol in the liver was increased by approximately 30% by feeding oxidized soybean oil [26]. In this study, an effect of the oxidized fat on plasma cholesterol concentrations was not reported. The increase of hepatic cholesterol concentration in guinea pigs, however, seems not to be a species-specific phenomenon because in another study with guinea pigs cholesterol concentrations in liver and plasma were reduced by 26 and 18%, respectively, [28]. Furthermore, one study in pigs revealed only negligible effects of oxidized fat on liver and plasma cholesterol concentrations [27]. The lack of effect in the study from Luci *et al.* [27] cannot also be attributed to a species-specific feature because oxidized fat

Table 2. Effect on liver and plasma cholesterol concentrations by oxidized fat as observed in studies with different species

Species	Fat source, heating regime	Feeding period (days)	Percental change of liver cholesterol concentration (compared with fresh fat control)	Percental change of plasma cholesterol concentration (compared with fresh fat control)	Ref.
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	56	−22%	−15%	[14]
Rat	Soybean oil, 130°C, 22 h	40	+2%	n.d.[a)]	[15]
Rat	Lard:safflower oil (2:1, w/w), 150°C, 6 days	51	−20%	−13%	[16]
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	42	−60%	−18%	[17]
Rat	Sunflower oil, 98°C, 48 h	69	n.d.[a)]	−23%	[18]
Rat	Sunflower oil:lard (1:1, w/w), 50°C, 38 days	63	−33%	−9%	[7, 19]
Rat	Sunflower oil:lard (1:1, w/w), 105°C, 81 h	63	−22%	+1%	[7, 19]
Rat	Sunflower oil:lard (1:1, w/w), 190°C, 24 h	63	−25%	−1%	[7, 19]
Rat	Sunflower oil:lard (1:1, w/w), 55°C, 49 days	56	n.d.[a)]	−15%	[7]
Rat	Sunflower oil, 55°C, 42 days	56	−13%	+2%	[19]
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	42	−55% (male) −22% (female)	−14% (male) −9% (female)	[20]
Rat	Sunflower oil:lard (1:1, w/w), 50°C, 38 days	63	−29%	−9%	[21]
Rat	Sunflower oil:linseed oil (80:20, w/w), 50°C, 16 days	98	−55%	n.d.[a)]	[22]
Rat	Sunflower oil, 60°C, 25 days	6	−19%	−19%	[34]
Mouse	Heated hydrogenated palm fat:fresh sunflower oil (92:8, w/w), 170°C, 48 h with repeated frying of French fries for 6 min every 30 min	112	n.d.[a)]	−15%	[76]
Guinea pig	Soybean oil, 205°C, 6 h with potato sticks in the oil, four consecutive days	60	n.d.[a)]	+30%	[26]
Guinea pig	Sunflower oil: lard (1:1, w/w), 55°C, 49 days	29	−26%	−18%	[28]
Pig	Sunflower oil, 110°C, 48 h	35	n.d.[a)]	−30%	[11]
Pig	Sunflower oil, 180°C, 24 hours	28	+6%	−7%	[27]

a) Not determined.

caused a strong cholesterol lowering effect in plasma of pigs in another study [11].

The feeding periods of the studies considered lasted from 6 [34] to 98 days [22]. Interestingly, the strength of the cholesterol lowering effect of oxidized fat in liver and plasma was independent of this; for instance, the percental changes of cholesterol concentrations in liver and plasma were similar when the oxidized fats were administered for either 6 [34], 29 [28], 51 [16], or 56 days [14]. This indicates that even short-term administration of oxidized fats causes a significant cholesterol lowering effect and that the duration of the oxidized fat administration is of minor importance for the cholesterol lowering effect of oxidized fat. It is, moreover, noteworthy that the type of the oxidized fat (fat source and heating regime) has obviously also little influence on the cholesterol lowering effect of oxidized fat. This was shown in one study from Eder *et al.* [19], where the effect of oxidized fats (1:1 – mixture of sunflower oil and lard) prepared by different heating regimes (either 50°C for 38 days, 105°C for 81 h, or 190°C for 24 h) was investigated. On the other hand, the strength of the cholesterol lowering effect in the liver markedly differed between two other studies [14, 17], although the oxidized fats administered in these two studies were prepared by the same conditions (soybean oil heated for 205°C with repeated frying of wheat flour dough sheets on four consecutive days). Although the reason for these divergent results between these two studies cannot be definitely explained, the observations from these studies and the abovementioned study from Eder *et al.* [19] suggest that the spectrum of lipid peroxidation products in the oxidized fat, which is influenced by the heating regime, is also of lower significance for the cholesterol lowering effect of oxidized fat.

2.3 Effect of oxidized fat on phospholipid concentrations in liver and plasma

Besides cholesterol and TAG concentrations, few studies have additionally reported an effect of oxidized fat on phospholipid concentrations. Three studies revealed that administration of an oxidized fat increases the concentration of total phospholipids in the liver [16, 17, 21]. Sülzle *et al.* [21], moreover, reported elevations in the hepatic concentrations of specific phospholipid subclasses such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and cardiolipin by oxidized fat. A further study in rats [20] did not reveal an effect of oxidized fat on phospholipid concentrations in the liver. In plasma, phosholipid concentrations were reported to be reduced by approximately 21 and 10% by the administration of oxidized fats in studies from Eder [16] and Chao *et al.* [20], respectively. No effect of oxidized fat on plasma phospholipid concentration was found in an earlier study from Chao *et al.* [17].

2.4 Effect of oxidized fat on carnitine concentrations in the liver

A further interesting finding with respect to phenotypical alterations of lipid metabolism by oxidized fats is that rats treated with oxidized fat have increased hepatic carnitine concentrations [35]. Carnitine is a water-soluble quaternary amine (3-hydroxy-4-*N,N,N*-trimethylaminobutyric acid) which plays an important role in lipid and energy metabolism by acting as a shuttling molecule for the translocation of long-chain fatty acids (acyl groups) from the cytosol into the mitochondrial matrix, where β-oxidation occurs. Hence, all tissues that use fatty acids as a fuel source such as liver, skeletal muscle, heart, and placenta require carnitine for normal function. Consequently, energy metabolism is critically impaired in these tissues of patients with primary or secondary carnitine deficiency and these patients develop severe pathological abnormalities such as cardiomyopathy or hepatomegaly. The finding that oxidized fat increases hepatic carnitine concentration is therefore indicative of an increased capacity of the hepatocytes for the import of long-chain fatty acids into the mitochondrium and subsequent oxidative degradation of these fatty acids within the mitochondrium.

2.5 Effect of oxidized fat on concentrations of free fatty acids and ketone bodies in plasma

Also noteworthy, feeding experiments with rats [25] and pigs [27] revealed that oxidized fats increase plasma concentrations of ketone bodies like β-hydroxybutyrate. Ketogenesis occurs physiologically in the liver during fasting to provide alternative energy substrates to the brain due to reduced availability of glucose. During this state, nonesterified fatty acids are released from white adipose tissue by the action of hormone-sensitive lipase, transferred to the tissues *via* the blood, taken up into tissues by fatty acid transporters, and degraded *via* β-oxidation to acetyl-CoA which is the precursor for ketone bodies. Hence, the findings in rats and pigs indicate that oxidized fats stimulate ketogenesis in the liver.

Reported effects of oxidized fat on free fatty acid concentrations in plasma are less consistent. Two studies revealed a reduction of plasma free fatty acids by oxidized fat by approximately 13 and 26% in female weanling rats [20] and lactating rats [23], respectively. In another study with male weanling rats [17], no effect of oxidized fat on plasma free fatty acids could be demonstrated.

It is important to note that in the design of most of the recent studies reporting the lipid metabolism alterations of oxidized fats [7, 15, 16, 19, 21–25, 27, 28], several precautions have been taken (use of moderately oxidized fats, controlled feeding systems, substitution of antioxidant, and PUFA losses during fat oxidation) to avoid potentially confounding effects such as reduced food intake, decreased nutrient digestibility, depletion of antioxidants, essential fatty acids deficiency, which might be causative for the observed phenotypical alterations of lipid metabolism by oxidized fats. It is therefore likely that these alterations are mediated by affecting lipid metabolism directly in the liver, which will be discussed in the subsequent chapters.

3 Effects of oxidized fat on molecular regulators of lipid metabolism

Observations from well-controlled studies [7, 15, 16, 19, 21–25, 27, 28] have led to suggest that the pronounced lipid metabolism alterations induced by feeding of oxidized fats are mediated directly by primary and secondary lipid peroxidations products which are absorbed from the ingested fats in the intestine and transported *via* the blood to the liver. Hepatic lipid metabolism in mammals is regulated critically by two transcription factors acting as master regulators of genes involved in lipid catabolism and lipid synthesis – peroxisome proliferator-activated receptor α (PPARα) and sterol regulatory element-binding protein (SREBP)-1 and -2. Due to their fundamental role in the regulation of lipid metabolism, they have been considered in several feeding experiments dealing with oxidized fats to elucidate the lipid metabolism alterations induced by the administration of such fats.

PPARα is a ligand-activated transcription factor that is abundantly expressed in tissues with high rates of fatty acid oxidation such as the liver [36], and predominantly controls genes involved in all aspects of lipid catabolism including cellular fatty acid uptake, intracellular fatty acid transport, fatty acid transport through the mitochondrial membrane, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, as well as gluconeogenesis [36, 37]. In addition, genes involved in carnitine uptake and carnitine biosynth-

esis were also recently shown to be regulated by PPARα [38–40]. PPARα can be activated by both endogenous and synthetic ligands. Endogenous ligands of PPARα are fatty acids and their derivatives (eicosanoids) [41, 42]. Endogenous ligand activation of PPARα is observed during fasting [37, 43], since free nonesterified fatty acids are released from adipose tissue and taken up into tissues at increased levels during this state. Consequently, in the liver, where PPARα is most abundant, genes involved in fatty acid β-oxidation, ketogenesis, gluconeogenesis, carnitine uptake, and carnitine biosynthesis are strongly upregulated during fasting [36–38]. In addition to endogenous ligands, PPARα is also activated by a heterogenous group of synthetic compounds including the fibrate class of lipid lowering drugs (clofibrate, fenofibrate, bezafibrate, and gemfibrozil) [41, 42], which have been in clinical use for the treatment of hypertriacylglycerolemia and hypercholesterolemia since about 40 years.

SREBPs are a family of transcription factors that regulate the coordinated expression of genes involved in lipid synthesis and uptake [44]. Three isoforms of SREBP are known in mammals, SREBP-1a, SREBP-1c, and SREBP-2. Although SREBP-1c, the predominant isoform in adult liver preferentially activates genes required for fatty acid synthesis and their incorporation into TAG and phospholipids, SREBP-2 preferentially activates the low-density lipoprotein receptor gene and various genes required for cholesterol synthesis such as 3-hydroxy-3-methylglutaryl-CoA reductase [45]. SREBP-1a is an activator of both the cholesterol and the fatty acid biosynthetic pathway, but it is present in much lower amounts in liver than the other two forms [46]. SREBPs are synthesized as 120-kDa integral membrane proteins of the endoplasmic reticulum and form a complex with SREBP cleavage activating protein (SCAP). When sterol concentrations in cells are high, the SCAP/SREBP complex is retained in the endoplasmic reticulum. When cells are depleted of sterols, SCAP escorts SREBP to the Golgi for proteolytic processing. In the Golgi, sequential cleavages occur releasing the mature *N*-terminal domain of SREBP which then translocates to the nucleus and activates transcription of sterol regulatory element-containing genes [45, 47, 48]. Retention of the SCAP/SREBP complex in the endoplasmic reticulum is mediated by sterol-dependent binding of the complex to one of the two endoplasmic reticulum retention proteins designated insulin-induced gene (Insig)-1 and -2 [49, 50].

3.1 Evidence for the activation of PPARα by oxidized fat

In a study from Chao *et al.* [17], in which an oxidized soybean oil prepared under frying conditions was fed to growing rats for 6 wk, it was reported for the first time that oxidized fat causes activation of PPARα in the liver and the kidney. From their findings, the authors concluded that dietary oxidized fat, by activating PPARα, alters lipid metabolism in rats. In subsequent studies performed by different groups, the PPARα-activating effect of oxidized fat could be confirmed [20, 21, 23–25, 27, 34, 35, 51]. In all of these studies, activation of PPARα has been evaluated by determining the transcript levels of typical PPARα target genes such as acyl-CoA oxidase (ACO), cytochrome P450 isoenzyme 4A1 (CYP4A1), or carnitine-palmitoyltransferase I (CPT I), which are involved in peroxisomal and mitochondrial fatty acid oxidation, respectively. Determination of transcript levels of PPARα target genes is a widely accepted approach to evaluate PPARα activation because they are elevated in response to PPARα activation. On the contrary, expression of the PPARα nuclear receptor itself remains largely unchanged in response to PPARα agonists. Using a transcript profiling approach, Sülzle *et al.* [21] provided further evidence for the activation of the PPARα pathway by oxidized in showing that oxidized fat upregulates a comprehensive set of PPARα target genes including ACO, CYP4A1, CPT I, CPT II, medium-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, and 3-ketoacyl-CoA dehydrogenase. Moreover, liver enlargement (hepatomegaly) due to peroxisome proliferation which is a typical response to PPARα agonist [52, 53] was also observed in experimental animals fed oxidized fat [17, 21, 23], and is therefore a further indication for PPARα activation by oxidized fat.

In Table 3, an overview is given about the degree of PPARα activation by oxidized fat as evaluated by the upregulation of the PPARα target genes ACO and CYP4A1 in the liver of different species. This overview shows that activation of PPARα by oxidized fat is stronger in the liver of rodents than in pigs. This difference is probably explained by variations in the expression level of PPARα in tissues between these species. In rodents, PPARα is extremely high expressed in tissues, and activation of PPARα not only induces many genes involved in various metabolic pathways but also causes severe peroxisome proliferation in the liver [52], wherefore rodents belong to the proliferating species. In contrast to rodents, PPARα agonists like fibrates do not induce peroxisome proliferation in the liver of nonproliferating species, such as guinea pigs, pigs, monkeys, and humans. The nonproliferating species have a lower expression of PPARα in the liver and the response of many genes to PPARα activation is much weaker than in proliferating species [53, 54]. Therefore, effects related to PPARα activation observed in rodents cannot be directly applied for nonproliferating species such as humans. We have recently shown that pigs have a similar mRNA concentration of PPARα in the liver as humans [55], which is approximately tenfold lower than in rats. Considering this and the similarity between pig and human liver cells in the gene response to PPARα agonists [56], it is not unlikely that a similar PPARα response as observed in pigs fed oxidized fats is induced in the liver of humans consuming oxidized fats.

Table 3. Activation of PPARα by oxidized fat as evaluated by the upregulation of the PPARα target genes ACO and CYP4A1 in the liver of different species

Species	Fat source, heating regime	Fold induction of ACO (compared with control)	Fold induction of CYP4A1 (compared with control)	Ref.
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	1.7	2.8	[17]
Rat	Soybean oil, 205°C, 6 h with wheat flour dough sheets in the oil, four consecutive days	1.7 (male) 3.0 (female)	3.0 (male) 2.1 (female)	[20]
Rat	Sunflower oil:lard (1:1, w/w), 50°C, 38 days	3.3	14.0	[21]
Rat	Sunflower oil, 60°C, 25 days	3.0	4.2	[35]
Rat	Sunflower oil, 60°C, 25 days	3.8	6.4	[34]
Rat	Sunflower oil, 60°C, 25 days	5.3	2.4	[23]
Rat	Sunflower oil, 60°C, 25 days	4.0	4.2	[24]
Pig	Sunflower oil, 180°C, 24 h	1.3	n.d.[a)]	[27]

a) Not determined.

A further indication for PPARα activation by oxidized fat was provided in a more recent study showing that administration of oxidized fat increases the hepatic transcript level of novel organic cation transporter 2 (OCTN2) [35]. This gene has been recently identified as a novel PPARα target gene [40]. OCTNs operate on the intestinal absorption and renal reabsorption of carnitine and play a major role in tissue distribution by catalyzing the uptake of carnitine into body cells. Due to its high binding affinity for carnitine and its wide expression, OCTN2 is the physiologically most important carnitine transporter, operating for the reabsorption of carnitine from the urine as well as playing a major role in tissue distribution. Due to these functions of OCTN2, it is likely that upregulation of OCTN2 is responsible for the observation that administration of oxidized fat caused an increase in the carnitine concentration in the liver of rats [35].

Based on the knowledge that oxidized fats markedly activate hepatic PPARα and reduce hepatic TAG concentrations, a study has been recently conducted investigating whether dietary oxidized fat is useful in the prevention of alcoholic fatty liver using an established rat model [25]. This study clearly revealed that TAG accumulation in response to ethanol feeding is markedly reduced in rats by simultaneous administration of oxidized fat when compared with a fresh fat. Moreover, this study showed that, in agreement with the previous studies [57, 58], the function of PPARα was impaired by ethanol feeding [25]. However, administration of the oxidized fat, but not fresh fat, during ethanol feeding resulted in the upregulation of PPARα target genes indicating that PPARα is activated by oxidized fat even in the presence of ethanol. Thus, these findings strongly suggested that prevention from alcoholic fatty liver development by oxidized fat is due to restoring the impaired PPARα function.

The components of oxidized fats which are supposed to be responsible for PPARα activation are oxidized fatty acids such as hydroxy and hydroperoxy fatty acids (*e.g.* 9-hydroxy octadecadienoic acid (9-HODE), 13-HODE, and 13-hydroperoxy octadecadienoic acid (13-HPODE)) (Fig. 1). Fats heated at moderate temperatures of below 100°C for a long period (several days or weeks) usually contain high amounts of these primary lipid peroxidation products, whereas fats heated at high temperatures have low levels of these compounds because hydroperoxides are relatively unstable and easily decompose at these temperatures. This largely explains that PPARα activation is usually stronger in experimental animals fed oxidized fats heated at moderate temperatures compared with those heated at high temperatures (Table 3). Cell-culture studies using different cell types clearly revealed that addition of pure hydroxy or hydroperoxy fatty acids to the incubation medium caused an activation of PPARα as determined in transactivation experiments and by expression analysis of PPARα target genes [59–62]. Besides hydroxy and hydroperoxy fatty acids, cyclic fatty acid monomers (CFAM) have also been suggested to be potential mediators of the activation of PPARα by oxidized fat. CFAM contain five- and six-membered rings in different positions in the original alkyl chain with two substituents in either cis- or trans-conformation relative to the ring. The main precursor fatty acids of CFAM are the essential fatty acids linoleic acid and α-linolenic acid, and cyclization occurs when vegetable oils rich in these fatty acids are heated at temperatures above 200°C [63]. Such temperatures are reached when foods are fried and during the refining of oils, especially in the deodorization step. Martin *et al.* [64] observed that rats fed TAGs containing purified CFAM generated from linseed oil developed a peroxisome proliferator-like response including hepatomegaly and elevated activities of ACO, CYP4A, and CPT I. In a subsequent study by the same group, 5 g/kg diet of an isolated CFAM fraction was administered for 3 wk to wild-type and PPARα-null mice to elucidate the role of PPARα in mediating these effects [65]. Among others, the authors found that dietary CFAM enhanced ACO and CYP4A activities two and threefold, respectively, in the liver of wild-type mice, whereas no effect was observed in the liver of PPARα-null mice, indicating that CFAM indeed induce a PPARα response.

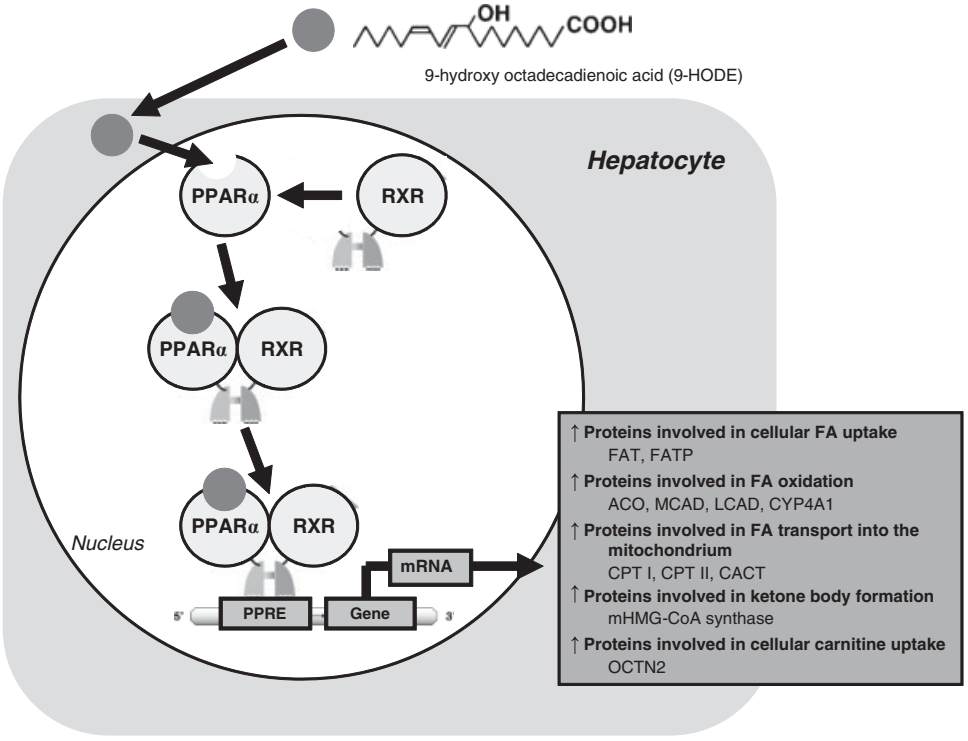

Figure 1. Activation of hepatic PPARα by oxidized fat is mediated by binding of hydroxy or hydroperoxy fatty acids such as 9-HODE, 13-HODE, and 13-HPODE contained in the oxidized fats to the PPARα protein which subsequently forms a complex with RXR. The PPARα/RXR complex binds to specific DNA sequences, called peroxisome proliferator-response elements, present in and around the promoter of PPAR target genes, and, thereby, stimulates their transcription. ACO, acyl-CoA oxidase; CACT, carnitine/acyl-carnitine translocase; CPT I/II, carnitine-palmitoyltransferase I/II; CYP4A1, cytochrome P450 isoenzyme 4A1; FAT, fatty acid translocase; FATP, fatty acid transport protein; 9-HODE, 9-hydroxy octadecadienoic acid; LCAD, long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; mHMG-CoA synthase, mitochondrial HMG-CoA synthase; OCTN2, novel organic cation transporter-2; PPARα, peroxisome proliferator-activated receptor α; PPRE, peroxisome proliferator-response elements; RXR, retinoid X receptor.

Interestingly, activation of hepatic PPARα is also observed in the fetal organism when oxidized fat is fed to pregnant rats [24], suggesting that the abovementioned components of oxidized fat decisive for PPARα activation pass the placental barrier and activate PPARα in the fetal liver. Although no literature data are available on the transplacental transport of oxidized fatty acids, it is well known that the transplacental transport of fatty acids in general is highly selective for individual fatty acids [66, 67]. The observation that the upregulation of transcript levels of PPARα-responsive genes by oxidized fat in the fetal liver was even more pronounced than in the liver of pregnant rats [24], and therefore, indicates that components of oxidized fat responsible for PPARα activation are presumably transported through the placenta with high preference.

Oxidized fatty acids such as 9-HODE, 13-HODE, and 13-HPODE were also shown to activate other PPAR isotypes such as PPARγ and PPARβ/δ [68, 69]. The two PPARγ proteins encoded by the PPARγ gene, PPARγ1 which has a low expression level in the liver and the nearly adipose tissue-specific PPARγ2 [70], are less likely candidates for the mediation of the lipid lowering actions of oxidized fats because PPARγ primarily triggers the expression of genes responsible for adipogenesis [71] and triglyceride storage in adipose cells [72]. In contrast to PPARγ, the PPARβ/δ protein has partially overlapping functions with PPARα and also plays a role in the regulation of fatty acid oxidation. Unlike PPARα, however, the PPARβ/δ protein is less expressed in the liver but strongly expressed in skeletal muscle [73]. Thus, the upregulation of genes involved in fatty acid oxidation in the liver by oxidized fat is probably largely explained by activation of the PPARα isotype. Nevertheless, future studies have to address whether oxidized fats are also capable of activating PPARβ/δ in skeletal muscle which might also contribute to the lipid lowering actions of oxidized fats.

3.2 Evidence for the inhibition of SREBPs by oxidized fat

As earlier studies showed that oxidized fats reduce the concentrations of liver and plasma lipids and inhibit the activity of lipogenic enzymes [15, 16, 19], studies have also

investigated an effect of oxidized fat on important regulators of lipid biosynthesis, like SREBPs. Koch *et al.* [34] were the first to test the hypothesis that the reduced concentrations of cholesterol in liver and plasma observed in rats fed an oxidized fat are mediated by an inhibition of SREBP-2, which in turn leads to a reduced expression of its target genes involved in hepatic cholesterol synthesis and cholesterol uptake. This study, indeed, revealed that oral administration of the oxidized fat lowered the concentration of the transcriptionally active SREBP-2 in the nucleus and reduced hepatic transcript levels of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-CoA reductase genes, which provides a plausible explanation for the cholesterol lowering effect of oxidized fat. Koch *et al.* [34] proposed that inhibition of SREBP-2 activation by oxidized fat is due to the observed upregulation of Insig-1 – the protein which prevents the translocation of SREBP from the endoplasmic reticulum to the Golgi, where proteolytic activation of SREBP and subsequent release of transcriptionally active forms of SREBP occur [49, 50]. The authors, moreover, proposed that the effect on Insig-1 is due to the activation of PPARα because it was recently shown that synthetic PPARα activators also inhibit hepatic cholesterol synthesis through an upregulation of Insig-1 [74]. In contrast to the findings in rats, a study in pigs [27] revealed that feeding an oxidized fat does not reduce the transcript levels of the SREBP-2 target genes 3-hydroxy-3-methylglutaryl-CoA reductase and low-density lipoprotein receptor in the liver. The exact reason for the lack of an inhibitory effect of oxidized fat in this regard in pigs is unknown. One might speculate that the observed activation of PPARα by the oxidized fat was to weak to exert an inhibitory effect on SREBP-2 activation as observed in rats [34].

Similar as the activation of SREBP-2, administration of oxidized fat to rats is also supposed to inhibit activation of SREBP-1c in the liver, although direct evidence for this assumption is still due. In contrast to SREBP-2, this SREBP isoform is mainly responsible for the transcriptional activation of genes involved in fatty acid synthesis, the so-called lipogenic enzymes such as fatty acid synthase, acetyl

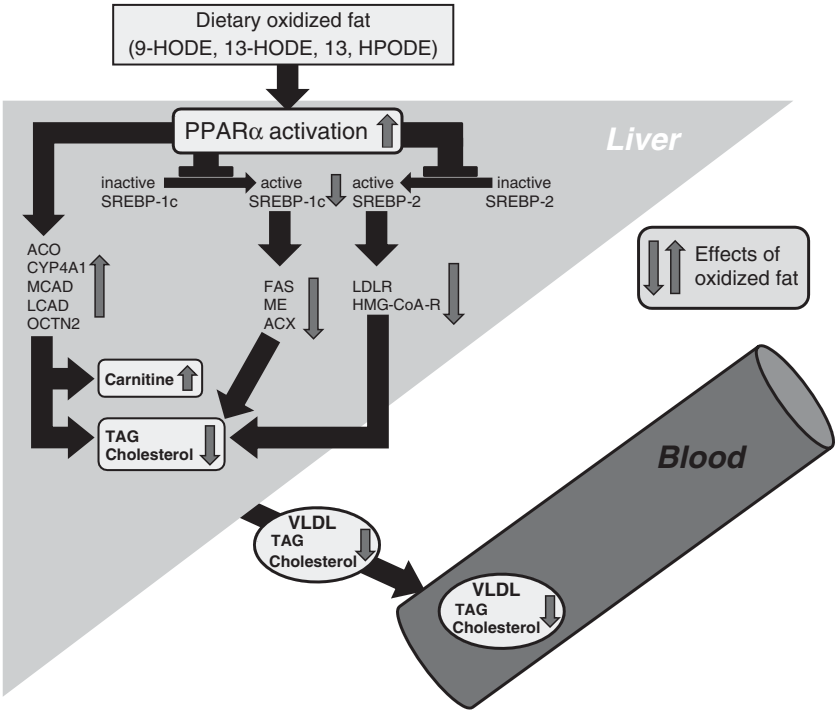

Figure 2. Proposed model explaining the lipid lowering effect of oxidized fat. Dietary oxidized fat containing hydroxy and hydroperoxy fatty acids (9-HODE, 13-HODE, and 13-HPODE) causes PPARα-mediated upregulation of various genes involved in fatty acid catabolism in the liver (ACO, CYP4A1, MCAD, LCAD, and OCTN2), and inhibition of activation of sterol regulatory element-binding protein (SREBP)-1c and SREBP-2 in the liver leading to reduced transcript levels of genes involved in fatty acid synthesis (FAS, ME, and ACX) and cellular cholesterol synthesis and uptake (LDLR, HMG-CoA-R), respectively. Both effects, stimulation of fatty catabolism and inhibition of fatty acid, TAG, and cholesterol synthesis contribute to the lipid lowering effect of oxidized fat in liver and plasma. Upregulation of the carnitine transporter OCTN2 is responsible for the elevated hepatic carnitine concentrations in response to dietary oxidized fat. ACX, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; CYP4A1, cytochrome P450 isoenzyme 4A1; FAS, fatty acid synthase; HMG-CoA-R; HMG-CoA reductase; HODE, hydroxy octadecadienoic acid; HPODE, hydroperoxy octadecadienoic acid; LCAD, long-chain acyl-CoA dehydrogenase; LDLR, low-density lipoprotein; MCAD, medium-chain acyl-CoA dehydrogenase; ME, malic enzyme; OCTN2, novel organic cation transporter-2; PPARα, peroxisome proliferator-activated receptor α; SREBP, sterol regulatory element-binding protein; VLDL, very low-density lipoprotein.

CoA-carboxylase, glucose-6-phosphate dehydrogenase, malic enzyme, and ATP citrate lyase. In a previous study with growing rats, it could be shown that feeding an oxidized soybean oil, prepared by heating the oil at 130°C for 22 h, reduced the activities of fatty acid synthase, acetyl CoA-carboxylase, and ATP citrate lyase in the liver compared with feeding fresh soybean oil [15]. These findings could be confirmed in a further study [19], in which the influence of different oxidized fats, prepared by heating at either 50°C for 38 days, 105°C for 81 h or 190°C for 24 h, was investigated. The observation from this study that the reduction of lipogenic enzyme activities by oxidized fat was stronger in rats receiving the oxidized fat prepared at 50 and 105°C than in those receiving the oxidized fat prepared at 190°C suggests that primary lipid peroxidation products, whose concentrations are lower in fats treated at high temperatures, are largely responsible for this effect. However, CFAM might also be involved because the activities of lipid synthesizing enzymes, namely Δ9-desaturase and phosphatidate phosphohydrolase, which are similarly regulated as lipogenic enzymes, were also demonstrated to be reduced in the liver of rats administered CFAM [64]. The abovementioned study [19], moreover, showed that gene expression of lipogenic enzymes in the liver is reduced by oxidized fats. Due to the fundamental role of SREBP-1c in the transcriptional regulation of lipogenic enzymes, the authors of that study [19] proposed that reduction of gene transcription of lipogenic enzymes by oxidized fats involves inhibition of SREBP-1 activation. This assumption is strongly supported by a recent study from König *et al.* [75], demonstrating that activation of either PPARα or PPARγ is accompanied by reduced levels of nuclear SREBP-1 and a decreased rate of fatty acid synthesis – an effect which probably involves upregulation of Insig-1 and Insig-2a. Due to its PPAR-activating properties, it is therefore very likely that oxidized fats inhibit fatty acid synthesis through the activation of either PPARα or PPARγ or both of them, and subsequent reduction of SREBP-1 maturation.

4 Conclusions and future perspectives

The most striking observation from a large number of feeding experiments dealing with oxidized fats is that administration of such fats reduces TAG and cholesterol concentrations in liver and plasma. Mechanistic studies dealing with this topic indicated that these effects of oxidized fat are mediated by activation of PPARα and, consequently, stimulation of fatty acid catabolism and inhibition of SREBP-1 and -2-dependent lipid synthesis in the liver. A model for the proposed mode of action of oxidized fat is shown in Fig. 2. Although it is difficult to ascribe the PPARα activating property of oxidized fat to individual chemical compounds due to the complex nature of oxidized fats, several candidate compounds, such as hydroxy and hydroperoxy fatty acids as well as CFAM, have been suggested. Future studies have to clarify whether other components of oxidized fats also contribute to their lipid lowering effects.

The lipid lowering action of oxidized fats indicates that oxidized fats may be beneficial during states where lipid metabolism is disturbed. This indication is supported by a recent study demonstrating that oxidized fat is useful in the prevention from the development of alcoholic fatty liver [25], a pathological state where excessive TAG accumulation occurs in the liver due to an impaired function of PPARα and a strong activation of SREBP. A more recent study, moreover, demonstrated that an oxidized fat prepared under deep-frying conditions causes activation of PPARα in the liver and the vasculature and inhibits atherosclerotic plaque development in the low-density lipoprotein receptor deficient mouse model of atherosclerosis [76]. Although these findings show that oxidized fats exert some beneficial effects, they must not be interpreted in that way that oxidized fats are generally regarded as a health-promoting component of the diet because oxidized fats were also reported to cause adverse effects [3–6, 8, 10]. They rather suggest that oxidized fats are a mixture of chemically distinct substances, some of which exhibit potent regulatory activity on lipid metabolism.

The authors have declared no conflict of interest.

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