

## Mini Review

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# Impact of Dietary Oxidized Lipids on Energy Metabolism

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## ABSTRACT

Consumption of dietary fat is known to influence metabolic rate and metabolic pathways. Dietary intake of unoxidized polyunsaturated fatty acids was shown to lead to an increased metabolic rate. Identification of the underlying mechanism revealed that modifications of the energy metabolism are associated with modifications of membrane lipid composition leading to the membrane pacemaker theory of metabolism. Mitochondrial membranes were shown to adapt their lipids to the dietary fat composition. Dietary fat is commonly prepared by applying heat treatment to increase palatability. Heat treatment of food lipids result in the formation of oxidized lipids. Intake of oxidized lipids might affect energy metabolism in a different way than their corresponding unoxidized lipids. However, scientific literature of the effects of individual oxidized lipids found in heat-treated dietary fats on the energy metabolism relevant for metabolic syndrome, diabetes and obesity research is scarce. This review comprises current knowledge of the impact of unoxidized and oxidized lipids on the energy metabolism.

**KEYWORDS:** Oxidized lipids; Energy metabolism; Membrane pacemaker theory of metabolism.

**ABBREVIATIONS:** ATP: Adenosine triphosphate; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; GIT: Gastro Intestinal Tract; LDLR: Low Density Lipoprotein Receptor; PUFA: Polyunsaturated fatty acids; ROS: Reactive Oxygen Species; TAG: Triacylglycerol.

## ORIGINS OF DIETARY OXIDIZED LIPIDS

Lipids are macronutrients which predominantly serve as constituents of all membranes, provide energy, and are involved in cellular signaling. The most abundant dietary lipids are Triacylglycerols (TAGs), comprising approximately 80-95% of all dietary lipids.<sup>1</sup> Other main dietary lipids are phospholipids and sterols. One of the most prominent representatives of sterols is cholesterol. Besides playing a key role in the physical characteristics of membranes, cholesterol is the precursor for steroid hormones and bile acids. Cholesterol is a monounsaturated lipid, which makes it prone to oxidation comparable to other mono- and poly-unsaturated fatty acyl chains in TAGs and phospholipids. The susceptibility of fatty acids to oxidation strongly depends on the degree of unsaturation. A high number of double bonds decreases the energy required for detachment of the bis-allylic hydrogen. While abstraction of the allylic hydrogen atom in oleic acid requires 322 kJ/mol, it only needs 171 kJ/mol in linoleic acid.<sup>2</sup> Once lipid oxidation is initiated, lipid radicals are rearranged to form conjugated diene radicals, which, in the presence of molecular oxygen, form peroxy radicals. By generating hydroperoxy lipids, autooxidation propagates. Fatty acid hydroperoxides can be further decomposed to volatile short-chain aldehydes, ketones or alcohols *via* scission of the carbon chain. Degradation of fatty acid hydroperoxides without scission of the carbon chain leads to the formation of triacylglycerides with keto, epoxy, hydroxyl and aldehyde groups, the so called oxidized monomers. Fatty acid hydroperoxides can also undergo condensation reactions resulting in the production of oxidized dimers and oligomers. Due to cyclization reactions and isomerizations cyclic fatty acid monomers and *trans* fatty acids could be identified as degradation products of fatty acid hydroperoxides. For cholesterol hydro-

peroxide, which is generated by the abstraction of allylic C-7 hydrogen, the most predominant decomposition products are 7-ketocholesterol, 25-hydroxycholesterol, 7 $\alpha$ -hydroxycholesterol, 7 $\beta$ -hydroxycholesterol, cholesterol-5 $\alpha$ ,6 $\alpha$ -epoxide, cholesterol-5 $\beta$ ,6 $\beta$ -epoxide and cholesterol-3,5,6-triol.<sup>3,4</sup> The amount of lipid oxidation products formed in foods depends on environmental factors, such as temperature, irradiation, oxygen availability, presence of (anti)oxidants, metals and enzymes (lipoxygenases).

## AMOUNT OF DIETARY OXIDIZED LIPIDS

Considerable amounts of dietary oxidized lipids have been quantified in Western diet due to processing of food. The amount of cholesterol oxidation products varies from 0.1  $\mu\text{g/g}$  beef<sup>5</sup> to 18.7  $\mu\text{g/g}$  mortadella<sup>6</sup> in meat and can reach up to 33.6  $\mu\text{g/g}$  anchovies<sup>7</sup> as in sea food. In butter, the content of oxysterols ranges from 13.7 to 27.3  $\mu\text{g/g}$ .<sup>8</sup> Formation of lipid hydroperoxides in corn oil heated at 100 °C for 36 h in the presence of air was calculated to be 243 mmol/L, while in the untreated oil solely 0.9 mmol/L lipid hydroperoxides could be detected.<sup>9</sup> Thus, heating promotes lipid oxidation. Heating of safflower oil by frying potato chips seven times for 10 min with one hour storage at room temperature between each frying resulted in an approximately 15-fold increase of the peroxide value.<sup>10</sup> Cholesterol oxidation products were also shown to be elevated in roasted salmon treated at 200 °C for 30 min yielding 7.38  $\mu\text{g/g}$  compared to fried samples treated at 180 °C for 3 min in olive oil yielding solely 2.98  $\mu\text{g/g}$ .<sup>3</sup> Besides elevated temperatures, cold fluorescent light was shown to induce lipid oxidation.<sup>11</sup> Recently, we could demonstrate an increase of the peroxide value by 1473 $\pm$ 1.79% ( $p \leq 0.001$ ) after household-representative storage of soybean oil in the presence of cold fluorescent light for 56 days.<sup>11</sup> During the household-representative storage of the study oil an increasing oxygen-containing headspace was considered to mimic consumer handling. Due to the multiple environmental factors determining the kind and amount of lipid oxidation products formed during food processing quantitative exposure of lipid oxidation products to humans is hard to generalize. However, under defined processing conditions the susceptibility of each lipid-containing food product to oxidation can be determined, leading to quantifiable amounts of ingested lipids.

## ABSORPTION OF OXIDIZED LIPIDS

The absorption and metabolism of 1-<sup>14</sup>C-methyl linoleate hydroperoxide was studied in rats.<sup>12</sup> It could be shown that the labeled methyl linoleate hydroperoxide and its labeled decomposition products were chiefly recovered from the stomach (48.0%), the expired <sup>14</sup>CO<sub>2</sub> (30.5%) and the small intestine (9.3%) 24 h after intubation of the labeled compound. Another study with rats, which received 17  $\mu\text{mol}$  labeled linoleic acid hydroperoxide and 18  $\mu\text{mol}$  unoxidized linoleic acid, confirmed the high recovery of approximately 65% of linoleic acid hydroperoxide in the gastric lumen immediately after ingestion.<sup>13</sup> It could be shown that the decomposition products, linoleic acid hydroxide, epoxyketones, 9-oxononanoic acid and hexanal in-

creased several minutes after administration of the linoleic acid hydroperoxides, suggesting that linoleic acid hydroperoxide decomposed over time to these products in the gastric lumen. A small percentage of 15.4% of the ingested linoleic acid hydroperoxide and its decomposition products was recovered in the gastric tissue 30 min after administration. Hexanal was shown to enter the small intestine and be absorbed into the blood. To this end, Kanazawa and Ashida<sup>13</sup> suggested that trilinoleoylglycerol hydroperoxides are cleaved in the stomach by gastric lipases to the free oxidized fatty acid, which are partly absorbed by the gastric tissue and partly decomposed to secondary reaction products. Subsequently, the decomposition products are partially absorbed by the intestine.

Cholesterol oxidation products were also reported to be absorbed in the intestines by different species. However, the degree of absorption in rats, rabbits and humans differed among the cholesterol oxidation products, with 7 $\beta$ -hydroxycholesterol, cholesterol-5 $\alpha$ ,6 $\alpha$ -epoxide and 7-ketocholesterol being chiefly absorbed.<sup>14-16</sup> After absorption of oxysterols in the upper intestinal tract, the oxysterols are transferred in the blood within chylomicrons. The chylomicrons carry TAGs to tissues. The activity of endothelial lipoprotein lipase leads to the formation of chylomicron remnants, which are rapidly cleared by the liver. Thus, lipid oxidation products have been shown to be bioavailable.

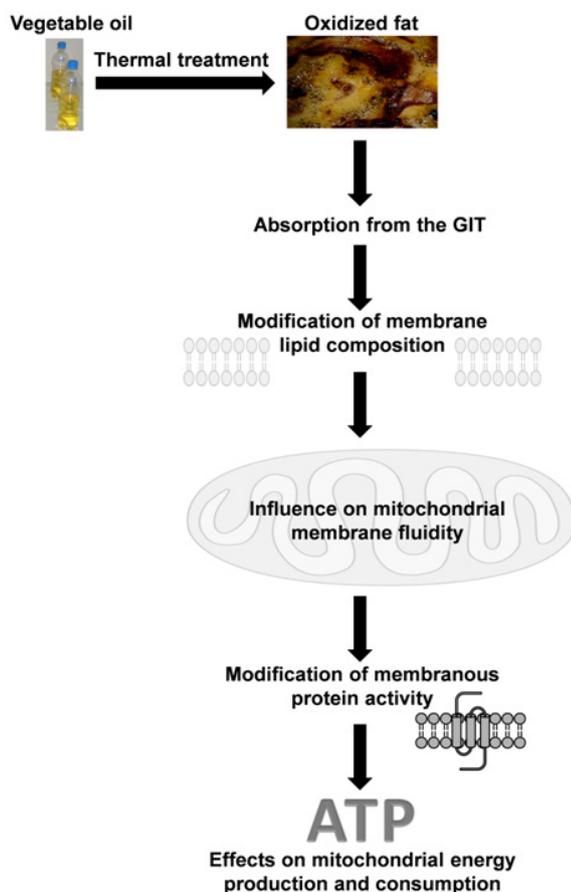
## IMPACT OF (OXIDIZED) LIPIDS ON ENERGY METABOLISM

Once absorbed fatty acids can be stored as triglyceride in adipose tissue, transferred to extra-hepatic tissue within lipoproteins and used for energy supply in any tissue containing mitochondria with oxygen availability. As mitochondria are the site of  $\beta$ -oxidation of fatty acids and ATP production, it plays a key role in energy supply. Total energy expenditure at rest is measured as basal metabolic rate in humans and animals. Several animal studies reported a correlation between dietary fatty acid profile and the basal metabolic rate in animals.<sup>17-19</sup> It could be shown that feeding omega-3 or omega-6 enriched diets to rats led to an increase of the metabolic rate compared to rats fed a saturated fat diet. Human studies confirmed that increasing PUFA content in the diet was associated with an elevated metabolic rate.<sup>20,21</sup> In a human cross-over study six healthy volunteers received a control diet ad libitum for 3 weeks and after a break of 10-12 weeks the subjects were administered the same control diet where 6 g visible fat per day was replaced by 6 g fish oil per day for 3 weeks.<sup>22</sup> Dietary intake of fish oil significantly reduced the body fat mass by -0.88 $\pm$ 0.16 kg compared to the intake of visible fat which led to an alteration of the body fat mass by -0.3 $\pm$ 0.34 kg. In addition, an increase of the rate of lipid oxidation to 1.06 $\pm$ 0.17  $\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1}$  was obtained when fish oil was consumed compared to the intake of the visible fat (0.87 $\pm$ 0.13  $\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ ).

A recent study with LDLR<sup>-/-</sup> mice fed a Western diet for 16 weeks revealed that liver metabolites associated with lipid and amino acid pathways were chiefly affected by the diet as

determined by a non-targeted metabolomic approach.<sup>23</sup> Supplementation of the Western diet with EPA or DHA reduced the Western diet-induced effects, whereby feeding the mice with a DHA-supplemented Western diet reversed the Western diet-induced effects more pronouncedly. Hepatic C<sub>20-22</sub> omega 3 fatty acids and their oxidation products were demonstrated to be enhanced after administration of DHA-supplemented Western diet, whereas monounsaturates, omega 6 fatty acids and its corresponding oxidation products were significantly decreased. Metabolomic analyses identified, for instance, 18-hydroxy-5Z, 8Z, 11Z, 14Z, 16E-eicosapentaenoic acid and 17, 18-dihydroxy-eicosa-5, 8, 11, 14-tetraenoic acid, two omega 3 fatty acids-derived oxidation products. DHA-supplemented diet was shown to affect the hepatic lipid metabolism, explaining the protective effect of DHA against Western-diet-induced nonalcoholic steatohepatitis in mice.<sup>23</sup>

So, dietary fat has an impact on the energy metabolism by modifying the metabolic rate and the metabolic pathways. However, the impact of processed food-derived lipid oxidation products on energy metabolism has not yet been addressed, despite the fact that many foods undergo processes before consumption to increase palatability (Figure 1).



**Figure 1:** Proposed mechanism of the potential effects of oxidized lipids on energy metabolism after ingestion of heat-treated lipids.

The molecular mechanisms explaining the role of di-

etary (unoxidized) fat on energy metabolism have been under thorough investigations.<sup>24-27</sup> The energy metabolism has been suggested to be associated with membrane lipid composition.<sup>27</sup> In the field of comparative biology it could be demonstrated that species with high metabolic rates (endotherms) have highly polyunsaturated membranes while ectotherms with low metabolic rates are linked to cellular membranes which consists of more monounsaturated fatty acid acyl chains.<sup>27</sup> This finding led to the development of the so called membrane pacemaker<sup>7</sup> theory of metabolism.<sup>27</sup> In particular, membrane composition affects Na<sup>+</sup>/K<sup>+</sup> antiporter activity, which accounts for 10-60% of the resting metabolic rate.<sup>24-26</sup> The activity of the membrane-bound Na<sup>+</sup>/K<sup>+</sup>-ATPase was strongly correlated with the DHA content of the surrounding phospholipids.<sup>28</sup> It was suggested that a decrease in the degree of membrane lipid polyunsaturation might reduce energy-consuming processes such as the activity of ion transporters.<sup>29</sup> The membrane fatty acid composition might affect membrane-bound proteins, thereby modifying intracellular signaling. One of the integral membrane proteins, the glucose transporter 1, for instance, covers an area of approximately 17 molecules of a phosphatidylcholine bilayer consisting of saturated fatty acid chains.<sup>30</sup> Thus, high membrane fluidity is required for the insertion of the glucose transporter into the membrane. Membrane fluidity is primarily determined by the membrane composition. Unsaturated hydrocarbon tails cause a greater surface of the cross-section of the cylindrical hydrocarbon part of the phospholipid molecule compared to saturated tails. As a consequence, the interaction energy between the two unsaturated fatty acid chains is reduced, leading to an enhanced membrane fluidity.<sup>30</sup> The mechanism of fatty acid uptake was found to be similar to the mechanism of glucose uptake.<sup>31</sup> The membrane-located fatty acid transporters were reported to regulate lipid metabolism. As for the activity of the glucose transporter, an impact of the membrane flexibility, and thus degree of membrane lipid polyunsaturation, on the activity of the fatty acid transporters might be conceivable. The impact of oxidized lipids on the fatty acid and glucose uptake and any correlation to the membrane flexibility has not yet been studied.

The extent to which dietary lipids are incorporated into cellular membrane has been investigated previously.<sup>32</sup> The physiological conformer-regulator paradigm was applied to quantitate the incorporation of dietary lipids into the membrane, whereby the membrane lipids were plotted against the dietary lipids. Even though dietary lipid composition was changed this change could not be reflected in plasma membrane (average slope 0.07). Membranes are, thus, homeostatically regulated independent of the dietary fatty acids. However, a conforming response to dietary fat was obtained when the PUFA balance of the diet was below 10% of the membrane composition.<sup>33</sup> An average slope of the relationship between dietary fats and membrane lipids was determined to be 0.95 for membrane lipids from heart, liver, muscle, brain and red blood cells.

More specifically, mitochondrial membrane phospholipids were shown to conform to dietary fatty acids. Hepatic mitochondrial membrane lipid composition of rats fed a rapeseed

oil-rich diet for 11, 22 and 33 days was changed compared to the mitochondrial membrane of rats fed a standard diet.<sup>34</sup> The modified diet induced a decrease in the saturated to unsaturated molar ratio and an increased incorporation of oleic acid in the major mitochondrial tetra-acyl phospholipid, cardiolipin. It was reported that cardiolipin, which comprises 10-20% of total mitochondrial phospholipids, is essential for mitochondrial ATP formation.<sup>35</sup> Several studies showed that dietary lipids can modify mitochondrial respiration and ROS formation.<sup>36-39</sup> Polyunsaturated fatty acids as well as lipid oxidation products are known to activate uncoupling proteins leading to proton leak across the inner mitochondrial membrane without using the electrochemical gradient for ATP production.<sup>40,41</sup> Brookes, et al.<sup>42</sup> demonstrated that membrane unsaturation was positively correlated with proton permeability and metabolic rate suggesting that mitochondrial fatty acid composition might affect mitochondrial inner membrane proteins. Battino, et al.<sup>43</sup> investigated the effect of feeding fried oil to rats on their liver mitochondrial respiratory proteins. Intake of fried extra virgin olive oil rich in polar lipid oxidation products enhanced the hydroperoxide and the thiobarbituric acid reactive substances contents of mitochondrial membranes. In addition, it induced a stimulatory effect on the cytochrome c oxidase activity and increased the cytochrome c+c1 and cytochrome a+a3 content compared to the administration of non-fried extra virgin olive oil.

## CONCLUSION

The impact of dietary unoxidized fatty acids on the energy metabolism has been under thorough investigation. However, the bioenergetic effect of oxidized lipids still needs to be elucidated in mechanistic studies. So far, there is only little evidence that oxidized lipids might exert differential effects on mitochondrial respiratory chain. A systematic approach of the impact of lipid oxidation products from differently processed dietary fats on the bioenergetic pathways would be of great importance for the general public and especially for patients suffering from metabolic disorders.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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