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Fish oil and 3-thia fatty acid have additive effects on lipid metabolism but antagonistic effects on oxidative damage when fed to rats for 50 weeks☆

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Abstract

The 3-thia fatty acid tetradecylthioacetic acid (TTA) is a synthetic modified fatty acid, which, similar with dietary fish oil (FO), influences the regulation of lipid metabolism, the inflammatory response and redox status. This study was aimed to penetrate the difference in TTA's mode of action compared to FO in a long-term experiment (50 weeks of feeding). Male Wistar rats were fed a control, high-fat (25% w/v) diet or a high-fat diet supplemented with either TTA (0.375% w/v) or FO (10% w/v) or their combination. Plasma fatty acid composition, hepatic lipids and expression of relevant genes in the liver and biomarkers of oxidative damage to protein were assessed at the end point of the experiment. Both supplements given in combination demonstrated an additive effect on the decrease in plasma cholesterol levels. The FO diet alone led to removal of plasma cholesterol and a concurrent cholesterol accumulation in liver; however, with TTA cotreatment, the hepatic cholesterol level was significantly reduced. Dietary FO supplementation led to an increased oxidative damage, as seen by biomarkers of protein oxidation and lipoxidation. Tetradecylthioacetic acid administration reduced the levels of these biomarkers confirming its protective role against lipoxidation and protein oxidative damage. Our findings explore the lipid reducing effects of TTA and FO and demonstrate that these bioactive dietary compounds might act in a different manner. The experiment confirms the antioxidant capacity of TTA, showing an improvement in FO-induced oxidative stress.

Keywords: Reactive oxygen species; Oxidative damage; UCP; β-oxidation

Abbreviations: AASA, α-aminoadipic semialdehyde; *Acaca*, acetyl-coenzyme A carboxylase α; *Acadm*, acyl-coenzyme A dehydrogenase, medium chain; *Acadvl*, acyl-coenzyme A dehydrogenase, very long chain; ACOX1, acyl-CoA oxidase 1; *Arbp*, acidic ribosomal protein, P0; CPT, carnitine palmitoyltransferase; CEL, *N*ε-carboxyethyl-lysine; CML, *N*ε-carboxymethyl-lysine; DBI, double-bond index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; *Gapdh*, glyceraldehyde 3-phosphate dehydrogenase; GSA, γ-glutamic semialdehyde; *Hadha*, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, α-subunit; MDAL, *N*ε-malondialdehyde-lysine; PL, phospholipids; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TTA, tetradecylthioacetic acid; UCP, uncoupling protein.

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1. Introduction

The body's inability to handle excess energy intake leads to metabolic abnormalities such as hyperglycemia and dyslipidemia. The coexistence of these risk factors with overweight and hypertension represents the metabolic syndrome [1,2]. Thus, the discovery and testing of dietary supplements that can improve lipid metabolism and maintain homeostasis, and consequently, prevent the development of risk factors of metabolic syndrome, is of great importance.

Both fish oil (FO) and tetradecylthioacetic acid (TTA) have been widely used in *in vivo* experiments. FO has been shown to inhibit lipogenesis and exert a hypolipidemic effect by lowering plasma cholesterol and triacylglycerol (TAG) levels [3], as well as having an anti-inflammatory effect beneficial against both atherosclerosis [4] and arthritis [5,6]. The effects of FO are attributed to ω−3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are thought to bind and activate peroxisome proliferator-activated receptors (PPARs) [7]. They act upon ligand activation by controlling networks of target genes and therefore serve as lipid sensors because they can be triggered by metabolic derivates of fatty acids in the body.

Tetradecylthioacetic acid, a structurally modified 16-carbon saturated fatty acid (SFA) with a sulfur atom inserted in the third position of the alkyl chain, has been documented as a pan-PPAR ligand in several cell lines [8–10]. Tetradecylthioacetic acid has a particularly high affinity for PPARα [9–11] and exerts its hypolipidemic effect by inducing gene expression of enzymes involved in hepatic fatty acid β-oxidation. It thereby reduces the availability of fatty acids for very-low-density-lipoprotein synthesis and secretion and lowers plasma TAG and cholesterol levels. In addition, TTA has been shown to have an important role in diminishing of inflammation [12–16].

Both physiological processes and externally induced oxidative stress reactions can lead to formation of reactive oxygen species (ROS) in the body. It is well-known that dietary FO supplementation can increase lipoxidative damage due to the high amount of unsaturated fatty acids [17–19]. Tetradecylthioacetic acid has a potent capacity to attenuate the oxidative stress and protect the cellular membrane lipids from oxidative damage. Based on this, we hypothesized that a dietary TTA supplementation to the high-fat diet would be able to reduce the oxidative stress induced by FO.

Thus, the main focus in this 50-week-long *in vivo* experiment was to investigate the effects of TTA and FO, given separately or in combination, on body weight gain, plasma and liver lipid levels, as well as fatty acid composition in plasma and several genes encoding important enzymes involved in fatty acid metabolism. Further, despite the similar ability to act through PPAR-activation, we revealed principal differences in the mechanisms of action of TTA and FO. Finally, we confirmed that TTA can act as an antioxidant and prevent the FO-caused oxidative damage.

2. Methods

2.1. Animals and diets

This animal study was conducted according to the Guidelines for the Care and Use of Experimental Animals, and the protocol was approved by the Norwegian State Board of Biological Experiments with Living Animals. Eight- to 10-weeks-old male Wistar rats, weighing 200–250 g, were obtained from Taconic Europe (previously Möllegaard and Bomholtgaard, Ry, Denmark). Throughout the experiment, the rats were housed in Makrolon III cages in an open system and kept under standard laboratory conditions with temperature 22°C±1°C, dark/light cycles of 12/12 h, relative humidity 55%±5% and 20 air changes per hour. The animals were housed five per cage and had free access to food and water during the study. They were acclimatized under these conditions with standard chow for 1 week before the experiment started. All rats were divided into four groups. The first group of animals (control group) was fed a high-fat diet with 25% fat, consisting of 23% lard and 2% soybean oil. The second group (TTA group) was fed a high-fat diet supplemented with TTA (0.375%). The third group (FO group) was fed a diet supplemented with 10% FO (EPAX 4020 TG) (12.6% lard and 2% soy oil). The fourth group (TTA+FO group) was fed both diet supplements. The amount of ω−3 fatty acids in FO-containing diets was 8% (where the EPA content was 4.5% and DHA was 2.3%). All diets were isocaloric in their energetic value (4900 kcal).

The animals were part of a larger study described elsewhere, and all animals underwent a jejunogastric reflux surgical procedure (manuscript in preparation). An additional 2-month feeding experiment on male Wistar rats with and without surgery was performed to determine if the procedure had an effect on the nutritional state of the animals. There was no difference in body weight or plasma lipids between the groups (data not shown), and thus it could be assumed that the surgery had no adverse effect on nutritional uptake.

Tetradecylthioacetic acid was synthesized as previously described [20]. The rats were anaesthetized with isofluorane (Forane; Abbott Laboratories, Abbott Park, IL) inhalation under nonfasting conditions. Blood was drawn by cardiac puncture and collected in BD Vacutainer tubes containing EDTA (Becton-Dickinson, Plymouth, UK), and the organs were immediately removed and frozen in liquid nitrogen.

2.2. Quantification of lipids and fatty acids

Plasma and liver lipids were measured on the Hitachi 917 system (Roche Diagnostics, Mannheim, Germany). Quantification of TAGs and total cholesterol in plasma and liver were obtained by using kits from Roche Diagnostics. Choline-containing phospholipids (PLs) in plasma and liver were measured by PAP150 from bioMérieux (Lyon, France). Hepatic lipids were analyzed in cytoplasmic extracts. Lipids from plasma and liver were extracted before the fatty acid composition was analyzed as described previously [21,22].

From the fatty acid profile, the double-bond index (DBI) of lipid susceptibility to oxidative modification [23] was calculated: DBI=[(1×∑mol% monoenoic)+(2×∑mol% dienoic)+(3×∑mol% trienoic)+(4×∑mol% tetraenoic)+(5×∑mol% pentaenoic)+(6×∑mol% hexaenoic)].

2.3. Enzyme activities

Fresh liver tissue samples were homogenized in ice-cold sucrose medium and centrifuged. The resulting three postnuclear fractions, a mitochondrial-enriched fraction (M), a peroxisome-enriched fraction (L) and a cytosolic fraction (S), were isolated as previously described and frozen at −80°C [24]. Then the activities of fatty acyl-CoA oxidase 1 (ACOX1) [25], carnitine palmitoyltransferase II (CPT-II) [26], 3-hydroxy-3-methylglutaryl-coenzyme A synthase (HMG-CoA synthase) [27] and fatty acid synthase (FAS) [28,29] were measured.

2.4. Gene expression analysis

Liver samples were frozen in liquid nitrogen immediately after dissection and stored at −80°C. Total cellular RNA was purified from 20- to 30-mg tissue using RNeasy Mini Kit (Qiagen). RNA was quantified spectrophotometrically (NanoDrop 1000; NanoDrop Technologies, Boston, MA), and the quality was evaluated by capillary electrophoresis (Agilent 2100 Bioanalyzer; Agilent Technologies, Palo Alto, CA). For each sample, 400 ng total RNA was reversely transcribed in 20-μl reactions using Applied Biosystem's High Capacity cDNA Reverse Transcription Kit with RNase inhibitor according to the manufacturer's description. Real-time polymerase chain reaction was performed with custom-made 384-well microfluidic plates [Taq-Man

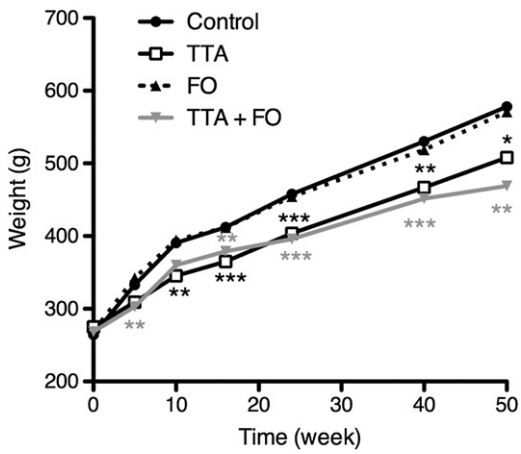

Fig. 1. Average weight of rats on high-fat diets supplemented with 3-thia fatty acid TTA and/or FO for 50 weeks. Data represent means (n=10). Values that were significantly different from control by t test are indicted by black (TTA) or gray (TTA+FO) asterisks (*P<.05, **P<.01, ***P<.001).