Impaired local production of pro-resolving lipid mediators in obesity and 17-HDHA as a potential treatment for obesity-associated inflammation

Running title: Role of 17-HDHA in obesity-driven inflammation

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Word count: 4394

Number of tables and figures: 7 figures and online supplemental material (1 supplemental figure and 2 supplemental tables)
ABSTRACT

Obesity-induced chronic low-grade inflammation originates from adipose tissue and is crucial for obesity-driven metabolic deterioration including insulin resistance and type 2 diabetes. Chronic inflammation may be a consequence of a failure to actively resolve inflammation, and could result from a lack of local specialized pro-resolving lipid mediators (SPM) such as resolvins and protectins, which derive from the n-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We assessed obesity-induced changes of n-3-derived SPM in adipose tissue and effects of dietary EPA/DHA thereon. Moreover, we treated obese mice with SPM precursors and investigated effects on inflammation and metabolic dysregulation. Obesity significantly decreased DHA-derived 17-hydroxydocosahexaenoic acid (17-HDHA, resolvin D1 precursor) and protectin D1 levels in murine adipose tissue. Dietary EPA/DHA treatment restored endogenous biosynthesis of n-3 derived lipid mediators in obesity while attenuating adipose tissue inflammation and improving insulin sensitivity. Notably, 17-HDHA treatment reduced adipose tissue expression of inflammatory cytokines, increased adiponectin expression and improved glucose tolerance parallel to insulin sensitivity in obese mice. These findings indicate that impaired biosynthesis of certain SPM and SPM precursors including 17-HDHA and protectin D1 contributes to adipose tissue inflammation in obesity and suggest 17-HDHA as a novel treatment option for obesity-associated complications.
INTRODUCTION

Obesity is associated with a chronic low-grade inflammation that plays a key role in the development of insulin resistance, leading the way to type 2 diabetes and cardiovascular disease (1,2). Obesity-driven low-grade inflammation originates from the adipose tissue and is characterized by increased accumulation of macrophages and other inflammatory cells (3,4) and a shift from an anti-inflammatory M2-like (CD206+) to an inflammatory M1-like (CD11c+) macrophage phenotype which expresses inflammatory cytokines and contributes to insulin resistance (5-9). Alteration of the macrophage phenotype significantly contributes to adipose tissue inflammation and its metabolic consequences such as insulin resistance (10-12).

Secretion of inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor α (TNF-α), interleukin (IL)-6 and osteopontin (OPN) is increased in obesity-induced adipose tissue inflammation while production of anti-inflammatory and insulin-sensitizing adiponectin is reduced (13,14). In addition to peptide mediators, adipose tissue produces considerable amounts of free fatty acids and fatty acid-derived bioactive lipid mediators with potent pro- and anti-inflammatory actions. Particularly the recently described n-3 polyunsaturated fatty acid (PUFA)-derived lipid mediators resolvins and protectins are of major interest as they have been characterized as a novel genus of potent anti-inflammatory and pro-resolving lipid mediators which are produced during self-limited acute inflammation in inflammatory exudates and promote resolution, i.e. active termination of inflammation (15,16). The biosynthesis of these locally acting specialized pro-resolving mediators (SPM) is regulated by the availability of the n-3 PUFA eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), and the spatial and temporal control of specific lipoxigenase pathways (17-19). Briefly, in murine tissues EPA-derived 18-hydroxyeicosapentaenoic acid (18-HEPE) and the DHA-derived leukocyte-type 12/15-lipoxygenase (12/15-LOX) products/intermediates 17-hydroxydocosahexaenoic acid (17-
HDHA) and 17-H(peroxy)DHA interact with 5-lipoxygenase (5-LOX) to generate the SPM resolvin E1 (RvE1), resolvin D1 (RvD1) or protectin D1 (PD1), respectively (Fig. 1A). Uncontrolled inflammation and failed resolution could be a critical component in the pathogenesis of many chronic inflammatory diseases including cardiovascular diseases, cancer as well as metabolic disorders (17,20).
n-3 PUFA treatment attenuates obesity-associated adipose tissue inflammation and insulin resistance but underlying mechanisms remain elusive (21,22). Recent studies demonstrated that n-3 PUFA feeding of mice elevates adipose tissue levels of 17-HDHA, PD1 and RvD1 (22,23). Transgenic restoration of n-3 PUFA (in fat-1 transgenic mice) increased tissue levels of n-3 PUFA-derived SPM and SPM precursors such as PD1 and 17-HDHA (24-26) and protected against obesity-linked insulin resistance (26). Accordingly, the impact of obesity itself on SPM biosynthesis in adipose tissue is of particular interest. Since obesity-induced alterations of SPM biosynthesis in adipose tissue could provide a clue for obesity-driven chronic inflammation and a rationale for novel potential treatment options, we characterized n-3 PUFA-derived lipid mediator profiles in adipose tissue of genetically (db/db) and high-fat (HF) diet-induced obese mice and respective lean controls. Based on these results, we treated obese mice with the SPM precursor 17-HDHA. Here we report obesity-induced decrease in endogenous biosynthesis of the DHA-derived lipid mediators 17-HDHA and PD1 as a potential mechanism involved in chronic adipose tissue inflammation further leading to metabolic complications. Treatment of obese mice with 17-HDHA, SPM precursor and marker of the local tissue PD1 and RvD1 biosynthetic pathways, attenuated adipose tissue inflammation and improved glucose tolerance. Hence, we could identify 17-HDHA as a potential treatment for obesity-induced adipose tissue inflammation and associated metabolic complications.
RESEARCH DESIGN AND METHODS

Animals and dietary interventions. Male C57BL/6J WT mice and male BKS.Cg-Dock7<sup>+/+</sup>Lepr<sup>db/db</sup> (db/db) mice as well as lean non-diabetic littermates (db/+) were purchased from Charles River Laboratories (Sulzfeld, Germany). At 8 weeks of age WT mice were placed on a high-fat (HF) diet (60% kcal from fat, D12492; Research Diets Inc., New Brunswick, NJ, USA) or a low-fat (LF) control diet (10% kcal from fat, D12450B; Research Diets Inc.) for 18 weeks or placed on a short-term HF diet for 4 or 14 days and compared with WT mice on normal standard chow. For genetic obesity, db/db and db/+ mice on normal chow were sacrificed at 16 weeks of age. For dietary treatment with different fatty acid compositions, db/db and db/+ mice were fed for 6 weeks either a LF control diet (3.2 kcal/g) or isocaloric HF diets (4.2 kcal/g) that included 40% kcal from lard oil (rich in saturated and monounsaturated fatty acids, HF/S), safflower oil (rich in n-6 PUFA, HF/6) or a HF/S diet with 30% (v/v) of lard oil being replaced by the n-3 PUFA concentrate EPAX6000TG (containing 290 mg EPA/g oil and 190 mg DHA/g oil; HF/S3 diet contained approximately 3% EPA- and 2.3% DHA-derived calories) generously provided by EPAX AS (Aalesund, Norway) as described (21,27). Detailed analysis of fatty acid composition of diets was performed previously (27). Diets were purchased from Altromin (Germany).

Lipid mediator treatment. After 17 weeks on HF diet WT mice were treated with DHA (4 µg/g body weight), 17S-HDHA (50 ng/g body weight) both purchased from Cayman Chemicals, or vehicle control (0.9% NaCl containing 3% delipidated faf-BSA and 2% ethanol) by intraperitoneal (i.p.) injection every 12 hours for 8 days or continuous application with osmotic (Alzet®) pumps (120 ng 17S-HDHA/g body weight per day) for 15 days. All mice were housed in pathogen-free facility on a 12-hour light/dark cycle with free access to food and water. Food intake and weight gain were monitored throughout the studies. Blood was drawn after a 3-hour fasting period immediately before mice were sacrificed. Tissues
were collected and immediately snap frozen in liquid nitrogen. The study protocols were approved by the Austrian Federal Ministry for Science and Research and followed the guidelines on accommodations and care of animals formulated by the European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

**Metabolic measurements.** Triglyceride, free fatty acid and cholesterol concentration was analyzed in plasma using an automated analyzer (Falcor 350, A. Menarini Diagnostics, Florence, Italy). ELISA kits were used to determine plasma insulin (Mercodia AB, Uppsala, Sweden), adiponectin and C-reactive protein (both Alpco Diagnostics, Salem, NH, USA). We calculated homeostasis model assessment of insulin resistance (HOMA-IR) as an index for insulin resistance (28). Insulin sensitivity was assessed by insulin tolerance test after a 5-hour fasting period. Briefly, i.p. injection of recombinant human insulin (Novorapid®, Novo Nordisk A/S, Denmark) at a dose of 2 U/kg and 0.75 U/kg body weight was given to db/db and WT HF mice, respectively. Blood glucose concentrations were determined before and 30, 60, 90 and 120 minutes after insulin injection. Glucose tolerance test was performed after overnight fasting and blood glucose was measured before and 15, 45, 75, 105 and 135 minutes after an i.p. injection of 20% glucose (0.75 g/kg body weight).

**Lipid mediator analysis of adipose tissue samples.** Lipid mediators were extracted from adipose tissue using solid phase extraction. From the lean groups, tissue of two animals was pooled for one sample, from obese animals tissue samples were analyzed in duplicates. Briefly, 300 mg tissue was homogenized in methanol after adding deuterated prostaglandin E₂ (PGE₂-d₄; Cayman Chemical, Ann Arbor, MI, USA) as an internal standard. Cleared supernatants were acidified to pH 3.0, loaded onto Oasis®-HLB Extraction Cartridges (Waters, Milford, MA, USA) and eluted with 1% ethyl acetate in methanol. Extracted samples were analyzed by HPLC-tandem mass spectrometry (LC-MS/MS) using a triple quadrupole
mass spectrometer (API5000 AB SCIEX, USA/Canada) equipped with a reversed phase column (ACE3 C18-AR, ACT, UK). Mass spectrometry analysis was conducted in electrospray negative ionization mode, and lipid mediators were identified by multiple reaction monitoring (MRM) using the following transitions: 17-HDHA (343.3:245.2 m/z), PD1 (359.3:136.1 m/z), 18-HEPE (317.3:215.2 m/z), RvE1 (349.3:195.1 m/z), RvD1 (375.3:164.1 m/z) and PGE2 (351.3:315.3 m/z). For enantiomeric separation of 17(R/S)-HDHA, a Chiralpak AD-RH (Daicel, Germany) was used. Calibration curves, retention times and MRM parameters were established and optimized as described in (29) using synthetic standards (Cayman Chemical).

Reverse transcription and gene expression analysis. Tissue samples were homogenized in TRIzol reagent (Invitrogen) and RNA was isolated according to the manufacturer’s protocol. One microgram of total RNA was treated with DNase I and transcribed to cDNA using Superscript II and random hexamer primers (all Invitrogen) as described (30). Gene expression of F4/80 (Emr1, Mm00802530_m1), MCP-1 (Ccl2, Mm00441242_m1), TNF-α (Tnf, Mm00443258_m1), IL-6 (Il6, Mm00446190_m1), Osteopontin (Spp1, Mm00436767_m1), GLUT-4 (Slc2a4, Mm00436615_m1), Adiponectin (Adipoq, Mm00456425_m1), peroxisome proliferator-activated receptor (PPAR) γ (PPARγ, Mm01184322_m1), PPARα (Ppara, Mm00440939_m1), NF-κB (Nfkb1, Mm00476361_m1), 12/15-LOX (Alox15, Mm01250458_m1) and 5-LOX (Alox5, Mm01182749_m1) was analyzed by quantitative real-time RT-PCR on an ABI Prism 7000 cycler using assays-on-demand kits (TaqMan® Gene Expression Assay, Applied Biosystems) and normalized to UbiquitinC mRNA (Ubc, Mm01198158_m1).

Isolation of adipose tissue macrophages and flow cytometry analysis. Stromal-vascular cells of gonadal adipose tissue were isolated by collagenase digestion and centrifugation to
remove adipocytes as described (7). For flow cytometry analysis of M1-like and M2-like macrophage phenotypes stromal-vascular cells were stained for three-colour immunofluorescence analysis by dye-labelled antibodies according to standard procedures using CD16/CD32 blocking Abs (BD Biosciences), F4/80-PE, CD206(MR)-AlexaFluor® 488 (both AbDSerotec), and CD11c-APC (BD Biosciences).

**Immunohistochemistry.** Adipose tissue was fixed with 4% paraformaldehyde and paraffin-embedded. After dewaxation and rehydration, immunohistochemical staining for MAC-2 (anti-MAC-2/galectin-3 antibody, Cedarlane Laboratories, Canada) was performed using Vectastain ABC Kit (Vector Laboratories) and Sigma Fast 3,3’-diaminobenzidine as substrate (Sigma) according to the manufacturer’s recommendations. Sections were counterstained with haematoxylin. Samples were analyzed with Nikon Eclipse E800 light microscope and digital images were captured with a DXM 1200 camera. Crown-like structure (CLS) density was obtained by counting total number of CLS in each section compared with total number of adipocytes (31).

**Western blotting.** Lysates of gonadal adipose tissue were prepared as previously described (21). Briefly, gonadal adipose tissue was homogenized and lysed on ice for 30 minutes in Tris-buffered saline, pH 7.4, containing 1% Triton X-100 (Pierce) and phosphatase and protease inhibitors. The tissue extract was cleared from fat, nuclei, and debris by centrifugation. Identical amounts of protein were separated by SDS-PAGE and blotted onto nitrocellulose membranes and probed for IκBα (Anti-IκBα Rabbit antibody, 400001, Calbiochem) and β-Tubulin (Anti-β-Tubulin mouse antibody, T4026, Sigma). Chemiluminescence was generated by a BM chemiluminescence substrate (Roche) and quantification of band intensities was performed using AlphaEase FC Software Version 3.2.1.
Statistics. Data are given as means ± SEM. Comparisons between lean and obese mice were assessed by unpaired two-tail Student's $t$-test. Treatment effects within a genotype were analyzed with univariate ANOVA using Dunnett’s $t$-test for post hoc analysis. For correlation analysis Spearman’s rank correlations was calculated. A $P$ value of $\leq 0.05$ was considered statistically significant.
RESULTS

Genetic and diet-induced obesity decreases adipose tissue levels of the n-3 PUFA-derived lipid mediators 17-HDHA and PD1. We first aimed to investigate the impact of obesity on n-3 PUFA-derived lipid mediator levels in murine adipose tissue. Therefore gonadal adipose tissue from two different mouse models of obesity, genetic (db/db) and diet-induced obesity was analyzed using LC-MS/MS. Mean body weight is presented in Fig. 1B. We identified significant levels of the n-3 PUFA lipid mediators 17-HDHA and PD1, which derive from DHA by 12/15-LOX activity, as well as the EPA-derived RvE1 precursor 18-HEPE in murine adipose tissue of lean and obese animals, whereas RvE1 and RvD1 were under the detection limit. Of note, obesity significantly reduced 17-HDHA and PD1 in gonadal adipose tissue of db/db mice compared to lean littermates (db/+ ) fed a normal standard chow while 18-HEPE was significantly increased (Fig. 1C-E). Obesity-induced alterations of n-3 PUFA-derived lipid mediators were confirmed in WT mice fed a HF diet for 18 weeks compared to LF-fed counterparts. These findings indicate that obesity per se as well as HF-feeding leads to a deficiency in DHA-derived mediators of resolution in adipose tissue.

In subcutaneous adipose tissue levels of 17-HDHA, PD1 and partly 18-HEPE (in WT HF mice) were either significantly lower compared to gonadal adipose tissue or even under the detection limit indicating predominant production in the metabolically more relevant gonadal adipose tissue (Fig. 1F-H).

Adipose tissue levels of 17-HDHA and PD1 are already affected after 4 days of HF diet. Considerable acute inflammatory alterations in adipose tissue may occur within few days of HF diet treatment (32,33). Since induction of SPM biosynthesis during acute inflammation is critical for subsequent resolution, we examined if altered lipid mediator levels can be found after a short-term HF diet compared to normal chow for 4 and 14 days in WT mice, when weight change was minimal and accumulation of macrophages did not yet occur (Fig. 2A and
In animals fed the HF diet for 4 days significantly lower concentrations of 17-HDHA and PD1 than in normal-chow fed animals were detected in gonadal adipose tissue, while 18-HEPE was not altered. Additionally, prostaglandin E$_2$ (PGE$_2$) tended to be elevated in adipose tissue indicating an acute inflammation after 4 days of HF diet (Fig. 2C-F). Differences between HF and normal chow-fed animals in adipose tissue 17-HDHA and PD1 levels were more pronounced after 14 days, when PGE$_2$ levels were also decreased and first inflammatory changes (increased MCP-1 expression, Fig. 2A and C) were identified. These findings show that changes in SPM or their precursors belong to the earliest alterations in diet-induced inflammation and hence inefficient biosynthesis of PD1 and 17-HDHA as well as other SPM might contribute to chronic-low grade inflammation in adipose tissue.

To assess whether obesity-induced decrease of 17-HDHA and PD1 could be due to altered relevant enzyme expression, we examined 12/15-LOX and 5-LOX gene expression in adipose tissue. We detected considerable mRNA levels of 12/15-LOX and 5-LOX in gonadal adipose tissue of $db/db$ and WT HF animals (Supplemental Figure 1A and B) and significantly lower levels in subcutaneous adipose tissue (P<0.05; data not shown) in concordance with the different 17-HDHA and PD1 concentrations within the different fat pads (Fig. 1F and G). However, there was a moderate decrease of 12/15-LOX in diet-induced obesity while genetic obesity had no impact (Supplemental Figure 1A).

**Adipose tissue 17-HDHA is associated with reduced adipose tissue inflammation.** In a different set of experiments we evaluated possible correlations of n-3 PUFA-derived lipid mediators with markers of adipose tissue inflammation in obese animals. Gonadal adipose tissue level of 17-HDHA negatively correlated with gene expression of TNF-$\alpha$ (Fig. 3A), F4/80 (Fig. 3B) and IL-6 (Fig. 3C) in diet-induced obesity. In contrast, adiponectin gene expression tended to correlate with 17-HDHA adipose tissue concentration (Fig. 3D) without reaching statistical significance. In genetic obesity 17-HDHA level negatively correlated with
IL-6 and fasting glucose (Fig. 3E and F). These results indicate a possible link between obesity, adipose tissue inflammation and 17-HDHA level.

**Attenuated inflammation and improved insulin sensitivity induced by dietary n-3 PUFA treatment are linked to increased adipose tissue levels of SPM and their precursors.** In a previous study we showed that PUFA-supplemented diets lead to an enrichment of respective fatty acids in the adipose tissue (27). Here, we further investigated if the known beneficiary effects of n-3 PUFA on adipose tissue inflammation and metabolic control in obese mice (21,34-36) are linked to an alleviation of the obesity-induced perturbations in endogenous n-3 PUFA-derived lipid mediator synthesis. Obese diabetic mice (db/db) and lean (db/+littermates were either fed a LF control diet or three different types of HF diets rich in saturated/monounsaturated fatty acids (HF/S), n-6 PUFA (HF/6), and a HF/S diet including long-chain n-3 PUFA (HF/S3) for 6 weeks similar to our previous study (21). Dietary n-3 PUFA treatment (HF/S3) increased plasma adiponectin, reduced overnight fasting glucose concentration compared to db/db animals fed a HF/S diet (Supplemental Table 1) and significantly decreased gene expression of F4/80 and MCP-1 in gonadal adipose tissue of db/db mice, whereas dietary n-6 PUFA treatment had no significant impact (Fig. 4A and B). Additionally, HF/S diet-induced increase of crown-like structures (CLS) which are formed by macrophages around adipocytes (31) was reduced after n-3 PUFA rich HF diet (HF/S3; Fig. 4C). Of note, flow cytometric analysis revealed a reduction in the proportion of inflammatory CD11c+ adipose tissue macrophages (F4/80+CD11c+CD206−; \( P:0.004 \)) and an increase of anti-inflammatory M2-like (F4/80+CD11c−CD206+) macrophages (\( P:0.07 \)) leading to a significantly decreased CD11c+/CD206+ ratio after HF/S3 compared to HF/S diet (Fig. 4D). Moreover, dietary n-3 PUFA treatment was associated with significant up-regulation of genes involved in insulin sensitivity (adiponectin and PPARγ) and glucose transport (GLUT-4) compared to HF/S and HF/6 fed animals (Fig. 4E-G) in adipose tissue. As shown by insulin
tolerance test, dietary n-3 PUFA treatment significantly improved insulin sensitivity compared to HF/S and HF/6 diets to a similar extent as seen in LF-fed db/db animals (Fig. 4H and I).

In parallel with decreased adipose tissue inflammation and improved insulin sensitivity, HF/S3 diet dramatically increased concentration of 17-HDHA, PD1 and 18-HEPE in gonadal adipose tissue of db/db mice (Fig. 5A-C) and RvE1 became detectable in adipose tissue after HF/S3 diet (Fig. 5D). Of note, db/db mice fed a HF/S3 diet showed significantly lower levels of 17-HDHA and PD1 compared to lean (db/+ ) counterparts fed the same diet (P:<0.01; Fig. 5A and B), indicating an inhibitory effect of obesity on SPM synthesis despite the n-3 PUFA enriched diet that favors SPM synthesis. In addition, HF/S diet increased percentage of the R-isoform of 17-HDHA, whereas HF/S3 diet enhanced adipose levels of both 17-HDHA enantiomers, with 17S-HDHA remaining the predominant isoform (Fig. 5E).

Altogether, these findings strongly suggest that dietary n-3 PUFA treatment reduces obesity-associated adipose tissue inflammation and insulin resistance by increasing endogenous SPM biosynthesis.

**Treatment with the DHA product 17-HDHA reduces obesity-induced adipose tissue inflammation.** As obesity strongly affected DHA-derived lipid mediators in adipose tissue we next investigated whether direct treatment with DHA and its metabolite 17-HDHA attenuates obesity-induced adipose tissue inflammation and its associated metabolic complications. To this end, WT mice were fed a HF or LF control diet for 17 weeks and HF fed animals were treated either with DHA (4 µg/g body weight), 17-HDHA (50 ng/g body weight) or vehicle via i.p. injection every 12 hours during 8 days. As shown in Supplemental Table 2 no differences in body weight, gonadal adipose tissue weight and fasting glucose were observed between treatments. Treatment with 17-HDHA and, less pronounced DHA reduced adipose tissue inflammation as determined by gene expression of the inflammatory
cytokines MCP-1, TNF-α, IL-6 and OPN concomitant with decreased gene expression of NF-κB in diet-induced obese animals compared to vehicle control (Fig. 6A-E). To investigate potential effects of 17-HDHA on inflammatory signaling we analyzed impact on NF-κB pathway in gonadal adipose tissue. Notably, 17-HDHA treatment increased protein level of IκBα, the major inhibitor of NF-κB signaling in adipose tissue (Fig. 6F and G). Immunohistochemical staining for MAC-2 revealed a trend towards decreased CLS formation after 17-HDHA and DHA treatment in gonadal adipose tissue (Fig. 6H and I) whereas mean adipocyte size was not altered (not shown). Moreover, DHA and 17-HDHA treatment reduced ratio of CD11c+ to CD206+ adipose tissue macrophages (Fig. 6J). Thus 17-HDHA markedly attenuated adipose tissue inflammation in obese mice.

**Treatment with 17-HDHA improves metabolic regulation in obesity.** We further analyzed whether 17-HDHA treatment also affects markers of metabolic control. 17-HDHA enhanced obesity-impaired gene expression of PPARγ, PPARα, GLUT-4 and adiponectin (Fig. 7A-D), while leptin expression was not significantly altered (data not shown). Additionally 17-HDHA treatment moderately improved glucose tolerance in obese mice (Fig. 7E) concomitant with decreased plasma insulin levels in response to the glucose tolerance test at the 45-minute time point (Fig. 7F).

Although not statistically significant fasting insulin concentration was reduced by approximately 30% and insulin resistance as estimated by HOMA-IR was approximately 40% lower after 17-HDHA and DHA treatment (Fig. 7G and H). Insulin tolerance test was in trend improved after 60 (P:0.1), 90 (P:0.08) and 120 minutes (P:0.1) upon 17-HDHA and DHA application for 8 days (Fig. 7I). Additionally, 17-HDHA treatment for 15 days via osmotic pumps resulted in increased insulin sensitivity as illustrated by significantly decreased fasting insulin concentration and HOMA-IR and a trend of reduced blood glucose concentration after 30 minutes (P:0.09) during an insulin tolerance test (Fig. 7J-L). Glucose concentration in
glucose tolerance test was significantly lower after 15 minutes by 17-HDHA treatment compared to vehicle control (Fig. 7M). Additionally, the 17-HDHA treated group revealed lower plasma insulin levels before and during the glucose tolerance test (after 45 minutes, Fig. 7N).

Taken together, these results indicate that 17-HDHA not only reduced adipose tissue inflammation but also improved glucose tolerance and insulin sensitivity in obese mice.
DISCUSSION

In this study we demonstrate an obesity-induced deficiency of PD1 and 17-HDHA, which is a PD1 pathway biomarker and RvD1 precursor, in adipose tissue. Our data indicate that the deficiency of these n-3 PUFA-derived SPM is linked to the development and perpetuation of obesity-driven adipose tissue inflammation that promotes type 2 diabetes. Moreover, we show that treatment with 17-HDHA mitigates obesity-driven inflammation and its metabolic consequences.

The obesity-associated disturbance of 17-HDHA and PD1 biosynthesis may result from decreased substrate availability and/or altered enzyme activities. HF feeding has been shown to reduce n-3 PUFA availability by elevating long chain n-6/n-3 PUFA ratio in adipose tissue membranes compared to chow fed counterparts (26). Accordingly, we demonstrate an impact of HF diet on lipid mediator synthesis in adipose tissue, which is independent of obesity since 17-HDHA and PD1 levels were affected also by short-term dietary treatment prior to the onset of obesity-induced adipose tissue inflammation (Fig. 2). On the other hand, our results on decreased SPM and SPM precursor levels in normal chow-fed db/db mice compared to lean littermates show that also obesity itself decreases adipose tissue levels of DHA-derived 17-HDHA available for SPM biosynthesis, while EPA-derived 18-HEPE was even increased (Fig. 1).

We show here that adipose tissue level of 12/15-LOX mRNA, a pivotal enzyme for 17-HDHA and PD1 biosynthesis in mice (37,38) is significantly decreased in diet-induced obesity corroborating previous data that impaired 12/15-LOX activity results in a resolution-deficient phenotype (39-41). In addition to 17-HDHA, obesity also decreased 12/15-LOX-derived 12-hydroxyeicosatetraenoic acid (12-HETE) and 15-hydroxyeicosatetraenoic acid (15-HETE) levels in adipose tissue (Supplemental Figure 1C and D) further indicating diminished 12/15-LOX activity. The fact that 18-HEPE synthesis in spite of decreased 17-HDHA and PD1 formation is not impaired by obesity (Fig. 1E) or HF-feeding *per se* (Fig. 2F)
might indicate that obesity but also HF diet especially affects synthesis of 12/15-LOX-derived lipid mediators. Alternatively the significant increase of 18-HEPE could represent a lack of 5-LOX activity for conversion to RvE1 and RvE2 (42) as well as an impaired 12/15-LOX pathway for conversion to the recently characterized RvE3 (43). However, the decrease in 17-HDHA and PD1 levels in obesity cannot clearly be related to altered enzyme expression on the mRNA level, in particular in db/db animals (Supplemental Fig. 1A). A precise determination of relevant enzyme activity may shed light on this issue but was beyond the present scope of this study.

Our study suggests that n-3 PUFA-derived SPM present in adipose tissue mediate crucial effects of n-3 PUFA treatment such as decreasing obesity-induced adipose tissue inflammation including a shift of macrophage polarization from M1-like to M2-like phenotype and improving metabolic parameters like insulin sensitivity (Fig. 4 and Fig. 5) and glucose tolerance (22,23,26,44). Previous studies demonstrated that both the number of M1-like adipose tissue macrophages expressing CD11c and the M1/M2 macrophage ratio is associated with insulin resistance (11,12) while the ablation of CD11c-positive cells resulted in the normalization of insulin sensitivity and glucose tolerance in obese mice (10,45). Of note, the doses used in the animals are high similar to other feeding experiments with n-3 PUFA (35,36,46) and cannot be reached in humans. Moreover, treatment with long-chain n-3 PUFA in high doses that are known to have anti-inflammatory effects (≥4 g/d) is rarely successful because of low adherence of patients that in part may be due to the unpleasant capsule size. Thus, similar beneficial effects with substances to be applied at much lower doses are highly desirable. The obesity-induced reduction of the SPM precursor 17-HDHA (Fig. 1C) and its negative correlation with markers of adipose tissue inflammation (Fig. 3) prompted us to focus on this compound. Treatment with 17-HDHA elicited an anti-inflammatory response in diet-induced obesity by significantly reducing inflammatory gene expression probably via attenuating NF-κB activation (Fig. 6) and increasing expression of
anti-inflammatory and insulin-sensitizing adiponectin as well as PPARγ in adipose tissue of obese mice. Notably, the anti-inflammatory effects of 17-HDHA were comparable to those of DHA at a dose that was nearly 100-fold lower. In addition to the anti-inflammatory and insulin sensitizing effects, 17-HDHA but not DHA treatment increased glucose tolerance, albeit moderately, concomitant with lower plasma insulin concentration (Fig. 7D and E) suggesting that 17-HDHA improves insulin action.

Our results with 17-HDHA and those achieved with RvE1 and RvD1 treatment of hepatic steatosis and insulin resistance (22,47) encourage to follow-up SPM precursor-based therapies in obesity.

DHA treatment affects M1-like /M2-like macrophage polarization as evaluated here by the ratio of CD11c+/CD206+ macrophages (Fig. 6E and (44)). In in vitro studies, 17-HDHA inhibits cytokine release of human microglial cells (48) and decreases TNF-α secretion of murine RAW macrophages (49-50) indicating that 17-HDHA or SPM derived from it exert potent anti-inflammatory action on macrophage-like cells. In our study, 17-HDHA treatment decreased M1/M2 ratio of adipose tissue macrophages (Fig. 6G) in a similar manner as DHA. Hence 17-HDHA could substitute for n-3 PUFA with respect to its potent anti-inflammatory effects in obesity-associated inflammation, which is mainly driven by macrophages (51).

17-HDHA might exert its anti-inflammatory activity and beneficial effects on metabolic regulation through different mechanisms. Definitely, 17-HDHA and its precursor 17-H(peroxy)DHA serve as SPM precursors for local conversion to RvD1 or PD1 that is cell type dependent (17). On the other hand 17S-HDHA, but not 17R-HDHA, directly activates PPARγ (49), which could contribute to increased adiponectin and GLUT-4 expression in adipocytes as well decreased inflammatory cytokine expression in macrophages (52) while possible action of 17-HDHA through PPARα remains to be elucidated. Additionally, anti-inflammatory effects of 17-HDHA treatment in obesity-induced inflammation might be due to decreased NF-κB activity which could be in part mediated through PPAR activation (53,54).
However, since PPARα target genes such as acyl-CoA oxidase were not altered by the treatment (data not shown), these data might argue against a dominant role of this nuclear receptor in 17-HDHA action to attenuate obesity-induced inflammation.

In conclusion, we show for the first time that obesity significantly reduced endogenous production of 17-HDHA and PD1 in adipose tissue which probably impairs resolution of adipose tissue inflammation. This lack of anti-inflammatory and pro-resolving lipid mediators could contribute to chronic adipose tissue inflammation and metabolic complications in obesity. Application of pro-resolving lipid mediators or 17-HDHA as a critical precursor reconstitute endogenous resolution capacity and may thus provide a novel option for treatment and prevention of obesity-related diseases such as type 2 diabetes.
Acknowledgements

This work was supported by the European Community’s 7th Framework Programme (FP7/2007-2013) under grant agreement no. 201608, the Austrian Science Fund (as part of the CCHD doctoral program, W1205-B09), the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (all to T.M.S.). C.N.S. acknowledges support from NIH PO1 GM095467. No potential conflicts of interest relevant to this article were reported.

A.N. planned and conducted experiments, researched and analyzed data and wrote the manuscript. M.Z. supervised experiments, researched data and reviewed/edited the manuscript. D.M. established and performed LC-MS/MS of lipid mediators. B.K.I., L.L., E.E.H. and P.F. conducted research and reviewed manuscript. I.M. and S.C. performed immunohistochemistry and reviewed manuscript. C.N.S. contributed to discussion, critically reviewed/edited manuscript and provided some synthetic lipid mediators. T.M.S. designed research, raised grants, supervised experiments and reviewed/edited the manuscript. T.M.S and A.N. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

The authors thank Elisabeth Matzner, Barbara Legerer, Bernhard Wernly, Eva Ratzinger, Erika Nowotny, Liliana Ionasz, Sandra Haiderer, Alexander Juerets and Sandra Pferschy (all Medical University of Vienna) for excellent technical assistance and support and EPAX AS (Aalesund, Norway) for kindly providing EPAX 6000 TG.

Parts of this study were presented as abstracts at the 70th Scientific Sessions of the American Diabetes Association, 25-29 June 2010, Orlando, Florida, USA and the 11th International Congress on Obesity (ICO), 11-15 July 2010, Stockholm, Sweden.
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Figure legends

FIG 1. Obesity significantly reduces levels of n-3 docosanoid lipid mediator 17-HDHA and PD1 in gonadal adipose tissue. A: Simplified biosynthetic pathway of EPA- and DHA-derived SPM and their precursors. B: Mean body weight of lean (white bars) and obese (black bars; \( n = 10 \) animals per group) mice. Genetically obese animals developed similar obesity compared to WT mice fed a HF-diet for 18 weeks. C-E: n-3 PUFA-derived 17-HDHA, PD1 and 18-HEPE were analyzed in murine gonadal adipose tissue of lean (white bars, \( n = 20 \) animals per group, pooled for \( n = 10 \) samples) and obese mice (black bars, \( n = 10 \) animals per group) in two different mouse models of obesity using solid-phase extraction and HPLC-tandem mass spectrometry. Genetically obese \( db/db \) mice were compared to lean \( db/+ \) littermates both fed a normal standard chow, while HF diet-induced obese WT mice were compared to lean WT mice fed a LF diet. F-H: Lipid mediator concentration in gonadal adipose tissue (black bars) was compared to subcutaneous adipose tissue (hatched bars) of obese \( db/db \) and WT HF mice (\( n = 10 \) animals per group). All data are mean ± SEM. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \). COX-2, cyclooxygenase-2; CYP450, cytochrome P450 enzymes

FIG. 2. Adipose tissue reduction of 17-HDHA and PD1 represents one of the earliest alterations in diet-induced inflammation. WT mice were fed a normal standard chow (NC; white bars) or a HF diet (black bars) for 4 or 14 days. A: Gene expression of macrophage marker F4/80 and MCP-1 was determined in gonadal adipose tissue using quantitative real-time RT-PCR (\( n = 12 \) animals per group). B: Mean body weight after normal chow (white bars) or HF diet (black bars) for 4 and 14 days (\( n = 12-15 \) animals per group). C-F: Lipid mediator levels were analyzed in gonadal adipose tissue. PGE\(_2\) was increased after 4 days but significantly lower after 14 days of HF diet. HF diet decreased adipose tissue levels of 12/15-LOX-derived 17-HDHA and PD1 after 4 days and demonstrated an even stronger impact after
14 days (n = 12-15 animals per group). All data are mean ± SEM. *P: 0.088; *P < 0.05; **P < 0.01; ***P < 0.001.

FIG. 3. Adipose tissue 17-HDHA concentration negatively correlates with markers of adipose tissue inflammation in murine obesity. In obese animals (WT HF mice) gonadal adipose tissue level of 17-HDHA negatively correlated with gene expression of A: TNF-α (rho = -0.88; P: 0.002), B: F4/80 (rho = -0.87; P: 0.002) and C: IL-6 (rho = -0.73; P: 0.026) while gene expression of D: adiponectin tended to correlate with 17-HDHA adipose tissue level (rho = 0.63; P: 0.067). In db/db animals E: IL-6 gene expression (rho = -0.68; P: 0.04) and F: fasting glucose (rho = -0.77; P: 0.016) negatively correlated with 17-HDHA level in adipose tissue (n = 9 animals per group).

FIG. 4. Dietary n-3 PUFA treatment attenuates adipose tissue inflammation and improves insulin sensitivity. Lean (db/+ ) mice were fed a LF diet (white bars) and obese (db/db) animals were either fed a LF diet or three different isocaloric HF diets: 1. HF/S diet rich in saturated/monounsaturated fatty acids, 2. HF/6 diet rich in n-6 PUFA (all black bars) and 3. a HF/S diet supplemented with n-3 PUFA (HF/S3; hatched bars) for 6 weeks. A-B: Gene expression of macrophage marker F4/80 and MCP-1 after dietary treatment in gonadal adipose tissue (n = 10 animals per group). C: CLS formation, a hallmark of obesity-associated inflammation was assessed by MAC-2 staining and number of CLS in gonadal adipose tissue was calculated per 100 adipocytes (n = 5 animals per group). D: Flow cytometry analysis of the CD11c⁺CD206⁻/CD11c⁻CD206⁺ ratio of adipose tissue macrophages (F4/80⁺-cells) obtained from stromal-vascular fractions of db/+ LF control and db/db animals after dietary treatment with indicated diets (n = 8 animals per group). E-G: Gene expression of PPARγ, adiponectin and GLUT-4 after dietary treatment in gonadal adipose tissue (n = 10 animals per group). H: Insulin sensitivity was determined in db/db mice after LF control, HF/S, HF/6 or
HF/S3 diet. Blood glucose was measured before and 30, 60, 90 and 120 minutes after i.p. injection of insulin (2.0 U/kg body weight; \( n = 7-9 \) animals per group). \( I \): Area under the curve was calculated (\( n = 7-9 \) animals per group). For statistical analysis \( db/db \) mice were compared with those fed a HF/S diet. All data are mean ± SEM. \#P: 0.067; \*P < 0.05; \**P < 0.01; \***P < 0.001.

**FIG. 5. Dietary n-3 PUFA treatment increases synthesis of n-3 PUFA-derived SPM and their precursors in adipose tissue of genetically obese mice.** Lipid mediator concentration was determined in gonadal fat pads of \( db/+ \) and \( db/db \) mice fed a LF, HF/S, HF/6 or HF/S3 diet using tandem mass spectrometry. \( A-D \): Dietary n-3 PUFA treatment (HF/S3) of \( db/db \) animals significantly increased adipose tissue concentration of DHA-derived 17-HDHA and PD1 as well as EPA-derived 18-HEPE and RvE1 (\( n = 10 \) animals per group). Dietary effects within \( db/db \) and \( db/+ \) animals were compared with respective HF/S groups. \( E \): Stereoselective analysis using chiral based LC-MS/MS revealed 17S-HDHA as the main naturally occurring stereoisomer in gonadal adipose tissue of \( db/+ \) and \( db/db \) animals. Percentage of 17R-HDHA calculated from total 17-HDHA in adipose tissue was increased after HF feeding (\( n = 10 \) animals per group). All data are mean ± SEM. \*P < 0.05; \**P < 0.01; \***P < 0.001.

**FIG. 6. 17-HDHA significantly decreases obesity-associated adipose tissue inflammation.** HF-fed WT mice were treated with DHA, 17-HDHA (both hatched bars) or vehicle (VE) control (black bars) via i.p. injection every 12 hours during 8 days. \( A-E \): 17-HDHA treatment reduced gene expression of MCP-1, IL-6, TNF-\( \alpha \), OPN and NF-\( \kappa \)B in gonadal adipose tissue of WT HF animals compared to VE treated control group (\( n = 12-14 \) animals per group). \( F-G \): Immunoblot analysis and quantification of I\( \kappa \)B\( \alpha \) in gonadal adipose tissue of WT HF animals after VE and 17-HDHA treatment. The diagram shows means of the chemiluminescence
intensity ratios from IκBα versus β-Tubulin which was used as loading control (n = 6 animals per group). H: Representative images of CLS formation in gonadal adipose tissue (scale bar = 50 µm). I: Number of CLS counts per 100 adipocytes in gonadal adipose tissue after VE, DHA or 17-HDHA treatment (n = 5-6 animals per group). J: Flow cytometry analysis of CD11c⁺CD206⁺/CD11c⁺CD206⁻ ratio of adipose tissue macrophages (F4/80⁺cells) obtained from stromal-vascular fractions after DHA and 17-HDHA treatment compared to VE control (n = 5-6 animals per group). For statistical analysis DHA and 17-HDHA treated groups were compared with VE treated control group. All data are mean ± SEM. #P < 0.08; §P: 0.088; *P < 0.05.

FIG. 7. 17-HDHA treatment increases anti-inflammatory and insulin-sensitizing markers and improves glucose tolerance. A-D: Gene expression of PPARγ, PPARα, GLUT-4 and adiponectin in gonadal adipose tissue after vehicle (VE; black bars), DHA and 17-HDHA treatment (both hatched bars) via i.p. injection for 8 days every 12 hours (n = 12-14 animals per group). E: Glucose tolerance test was performed (i.p. injection of 0.75 g 20% glucose/ kg body weight; n = 12 animals per group) and F: plasma insulin concentration was measured at the indicated time points (n = 8 animals per group). Determination of G: 3-hour fasting plasma insulin (n = 10 animals per group), H: HOMA-IR (n = 10 animals per group) and I: insulin sensitivity performing an insulin tolerance test (0.75 U/kg body weight; n = 12 animals per group) upon VE, 17-HDHA and DHA i.p. injection for 8 days. J: 3-hour fasting plasma insulin (n = 10 animals per group), K: HOMA-IR (n = 10 animals per group), L: insulin sensitivity (n = 14 animals per group) and M: glucose tolerance (n = 12 animals per group) as well as N: plasma insulin levels before and in response to glucose tolerance test (n = 6 animals per group) were determined after prolonged 17-HDHA administration for 15 days via osmotic pumps (body weight: 50.9±0.8 g and 49.5±0.8 g after VE and 17-HDHA
treatment, respectively). For statistical analysis DHA and 17-HDHA treated animals were compared with VE treated control group. All data are mean ± SEM. *$P < 0.05$, **$P < 0.01$. 
Figure 1

A

\[
\begin{align*}
\text{EPA} & \xrightarrow{\text{COX-2/CYP450}} 18-\text{HEPE} \\
18-\text{HEPE} & \xrightarrow{5-\text{LOX}} \text{RvE1} \\
\text{DHA} & \xrightarrow{12/15-\text{LOX}} 17-\text{H(p)DHA/17-HDHA} \\
17-\text{H(p)DHA/17-HDHA} & \xrightarrow{5-\text{LOX}} \text{PD1} \quad \text{PD1} \quad \text{RvD1}
\end{align*}
\]

B

Body weight (g)

<table>
<thead>
<tr>
<th></th>
<th>Genetic obesity</th>
<th>Diet-induced obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Obese group</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

C

\[
\begin{align*}
\text{17-HDHA} & \quad \text{ng/g tissue} \\
\text{Genetic obesity} & \quad \text{Lean} \quad \text{Obese} \\
\text{Diet-induced obesity} & \quad \text{Lean} \quad \text{Obese}
\end{align*}
\]

D

\[
\begin{align*}
\text{PD1} & \quad \text{ng/g tissue} \\
\text{Genetic obesity} & \quad \text{Lean} \quad \text{Obese} \\
\text{Diet-induced obesity} & \quad \text{Lean} \quad \text{Obese}
\end{align*}
\]

E

\[
\begin{align*}
\text{18-HEPE} & \quad \text{ng/g tissue} \\
\text{Genetic obesity} & \quad \text{Lean} \quad \text{Obese} \\
\text{Diet-induced obesity} & \quad \text{Lean} \quad \text{Obese}
\end{align*}
\]

F

\[
\begin{align*}
\text{17-HDHA} & \quad \text{ng/g tissue} \\
\text{Genetic obesity} & \quad \text{Lean} \quad \text{Obese} \\
\text{Diet-induced obesity} & \quad \text{Lean} \quad \text{Obese}
\end{align*}
\]

G

\[
\begin{align*}
\text{PD1} & \quad \text{ng/g tissue} \\
\text{Genetic obesity} & \quad \text{Lean} \quad \text{Obese} \\
\text{Diet-induced obesity} & \quad \text{Lean} \quad \text{Obese}
\end{align*}
\]

H

\[
\begin{align*}
\text{18-HEPE} & \quad \text{ng/g tissue} \\
\text{Genetic obesity} & \quad \text{Lean} \quad \text{Obese} \\
\text{Diet-induced obesity} & \quad \text{Lean} \quad \text{Obese}
\end{align*}
\]

Legend:

- Lean
- Obese

Significance levels:

- * p < 0.05
- ** p < 0.01
- *** p < 0.001

ND: Not detected
Figure 3

Diabetes

Panel A: WT HF

- Tnf-α - relative mRNA expression
- 17-HDHA (ng/g tissue)

Panel B: WT HF

- F4/80 - relative mRNA expression
- 17-HDHA (ng/g tissue)

Panel C: WT HF

- IL-6 - relative mRNA expression
- 17-HDHA (ng/g tissue)

Panel D: WT HF

- Adiponectin - relative mRNA expression
- 17-HDHA (ng/g tissue)

Panel E: db/db

- IL-6 - relative mRNA expression
- 17-HDHA (ng/g tissue)

Panel F: db/db

- Fasting glucose (mg/dl)
- 17-HDHA (ng/g tissue)
Supplemental FIG. 1. A-B: Gene expression of 12/15-LOX and 5-LOX was determined in gonadal adipose tissue of obese mice (black bars) and appropriate lean controls (white bars) using quantitative real-time RT-PCR ($n = 10$ animals per group). C-D: Analysis of 12-HETE and 15-HETE in murine gonadal adipose tissue of lean (white bars, $n = 20$ animals per group, pooled for $n = 10$ samples) and obese (black bars, $n = 10$ animals per group) mice in two different mouse models of obesity using solid-phase extraction and HPLC-tandem mass spectrometry. Genetically obese $db/db$ mice were compared to lean $db/+\$ littermates both fed a normal standard chow, while HF diet-induced obese WT mice were compared to lean WT mice fed a LF diet. Data are mean ± SEM. #$P < 0.08; \*P < 0.05; \**P < 0.01; \***P < 0.001.$
Supplemental Table 1: Body characteristics and plasma parameters after dietary treatment with different fatty acids

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (db/+)</th>
<th>Obese (db/db)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF</td>
<td>LF</td>
</tr>
<tr>
<td>Food intake (g)/day</td>
<td>3.4 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Body weight before diet (g)</td>
<td>25.8 ± 0.3</td>
<td>41.4 ± 0.8</td>
</tr>
<tr>
<td>Body weight after diet (g)</td>
<td>29.3 ± 0.5***</td>
<td>46 ± 0.7***</td>
</tr>
<tr>
<td>GWAT weight (g)</td>
<td>0.41 ± 0.03***</td>
<td>2.0 ± 0.1**</td>
</tr>
<tr>
<td>Fasting glucose, on§ (mg/dl)</td>
<td>71 ± 2***</td>
<td>228 ± 31***</td>
</tr>
<tr>
<td>Fasting glucose, 5 hours (mg/dl)</td>
<td>102 ± 8***</td>
<td>261 ± 20***</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>3.7 ± 0.1***</td>
<td>2.2 ± 0.2***</td>
</tr>
<tr>
<td>C-reactive protein (ng/ml)</td>
<td>0.20 ± 0.02***</td>
<td>0.41 ± 0.02*</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>10 ± 2***</td>
<td>73 ± 13**</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>78 ± 3</td>
<td>112 ± 20**</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>58 ± 2***</td>
<td>107 ± 6***</td>
</tr>
<tr>
<td>Free fatty acids (µmol/l)</td>
<td>331 ± 50</td>
<td>332 ± 27</td>
</tr>
</tbody>
</table>

Blood parameters were analyzed in plasma, except fasting glucose was measured in total blood samples. Within db/db mice all diets were compared with animals fed a HF/S diet, while asterisks in the lean (db/+ ) group indicate differences to db/db mice on LF diet (n = 10 animals per group except plasma data of HF/6 - n = 5 animals per group). Results are expressed as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. #GWAT, gonadal adipose tissue; §on, overnight.
Supplemental Table 2: Body characteristics and plasma parameters after Vehicle, DHA and 17-HDHA and treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT LF</th>
<th>WT HF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>DHA</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>30.2 ± 0.7</td>
<td>47.1 ± 0.5</td>
</tr>
<tr>
<td>GWAT# weight (g)</td>
<td>0.5 ± 0.03</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Fasting Glucose, on§ (mg/dl)</td>
<td>122 ± 11</td>
<td>211 ± 9</td>
</tr>
<tr>
<td>Fasting Glucose, 5 hours (mg/dl)</td>
<td>132 ± 9</td>
<td>192 ± 4</td>
</tr>
<tr>
<td>C-reactive protein (ng/ml)</td>
<td>0.23 ± 0.01</td>
<td>0.49 ± 0.05</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>48 ± 2</td>
<td>58.9 ± 3</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>56 ± 5</td>
<td>146 ± 8</td>
</tr>
<tr>
<td>Free fatty acids (µmol/l)</td>
<td>302 ± 27</td>
<td>330 ± 23</td>
</tr>
</tbody>
</table>

Blood parameters were analyzed in plasma, except fasting glucose was measured in total blood samples. WT HF mice treated with DHA and 17-HDHA were compared with vehicle treated group (n = 12 animals per group, except plasma data of WT LF - n = 8 animals per group). Results are expressed as mean ± SEM. There were no statistically significant differences between the WT HF groups for all analyzed parameters, except for a trend reduction of CRP in the 17-HDHA treated group (P<0.1). #GWAT, gonadal adipose tissue; §on, overnight.