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Marine *n*-3 fatty acids promote size reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a high-fat diet

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We evaluated the effects of partly substituting lard with marine *n*-3 fatty acids (FA) on body composition and weight, adipose tissue distribution and gene expression in five adipose depots of male Wistar rats fed a high-fat diet. Rats were fed diets including lard (19·5 % lard) or *n*-3 FA (9·1 % lard and 10·4 % Triomar™) for 7 weeks. Feed consumption and weight gain were similar, whereas plasma lipid concentrations were lower in the *n*-3 FA group. Magnetic resonance imaging revealed smaller visceral (mesenteric, perirenal and epididymal) adipose depots in the *n*-3 FA-fed animals (35, 44 and 32 % reductions, respectively). *n*-3 FA feeding increased mRNA expression of cytokines as well as chemokines in several adipose depots. Expression of *Adipoq* and *Pparg* was enhanced in the mesenteric adipose depots of the *n*-3 FA-fed rats, and fasting plasma insulin levels were lowered. Expression of the lipogenic enzymes *Acaca* and *Fasn* was increased in the visceral adipose depots, whereas *Dgat1* was reduced in the perirenal and epididymal depots. *Cpt2* mRNA expression was almost doubled in the mesenteric depot and liver. Carcass analyses showed similar body fat (%) in the two feeding groups, indicating that *n*-3 FA feeding led to redistribution of fat away from the visceral compartment.

Marine *n*-3 fatty acids: Body composition: Visceral adipose depots: Gene expression

Numerous studies in animals, populations and clinical trials have revealed beneficial effects of *n*-3 very-long-chain PUFA in health and disease. Marine oils contain high proportions of the *n*-3 very-long-chain PUFA EPA and DHA. Dietary intake of these fatty acids (FA) may delay the development of atherosclerosis and reduce the risk of CVD. Moreover, dietary intake of *n*-3 FA decreases postprandial concentrations of NEFA and plasma VLDL concentrations[1–3]. Experiments in cell models have elucidated the mechanisms behind the lipid-lowering effects. Incubation of cultured rat hepatocytes with EPA reduces cholesterol and TAG esterification by inhibiting acyl coenzyme A:cholesterol acyltransferase and acyl coenzyme A:1,2 diacylglycerol acyltransferase, respectively[4,5]. This, in turn, inhibits synthesis and secretion of VLDL[5,6]. The TAG-lowering effect of marine *n*-3 FA is also mediated *via* stimulation of FA oxidation in liver and to a smaller extent in skeletal muscle[7]. Replacing dietary saturated fat with *n*-3 FA has been shown to promote decreased whole-body lipid utilisation and increased carbohydrate utilisation in rats[8].

The risk of developing type 2 diabetes mellitus and CVD is markedly enhanced with visceral adiposity as compared with subcutaneous distribution of fat[9]. There are regional differences between adipose tissue depots with respect to expression of enzymes in lipolytic and anti-lipolytic pathways, uptake and release of NEFA, as well as adipokine production[10]. Several studies have shown that *n*-3 FA feeding reduces the size of perirenal and epididymal white adipose depots[1,11–13]. Rustan *et al.* showed that this effect was associated with a reduction in adipocyte size in these depots[1]. Belzung *et al.* showed in high-fat-fed rats that *n*-3 FA selectively limited the hypertrophy of retroperitoneal and epididymal adipose depots, with no effect on other major depots and no hyperplasia in the retroperitoneal depot[11].

Abbreviations: AOAC, Association of Official Analytical Chemists; CRP, C-reactive protein; FA, fatty acid; I, intensity; MR, magnetic resonance; MRI, magnetic resonance imaging; ROI, region of interest; TZD, thiazolidinedione.

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More knowledge is needed about the effect of dietary fat on whole-body fat distribution and adipose tissue depot functions.

In the present study we report results from a feeding experiment with rats, where we aimed to elucidate the effects of long-term dietary supply of marine *n*-3 FA on whole-body composition, as well as sizes and functions of adipose tissues evaluated by gene expression analysis. The reference group was fed a lard-enriched high-fat diet, whereas the *n*-3 FA group had one-third of the lard substituted with concentrated EPA and DHA. We also provide gene expression analyses for forty-four genes involved in energy metabolism and inflammation for five different adipose depots (subcutaneous, mesenteric, perirenal, epididymal and interscapular), as well as the liver. Interscapular adipose tissue may under certain conditions contain a high proportion of brown adipose tissue[14], whereas the other depots primarily consist of white adipose tissue. We also performed some metabolic assays and plasma analyses of lipids and adipokines, and carcass analyses to evaluate the effect of *n*-3 FA feeding on whole-body composition. To our knowledge, we are the first to report a comprehensive study of genes involved in lipogenesis and lipid metabolism, as well as adipokines, in an *n*-3 FA feeding study.

Materials and methods

Animals

Male rats of the Wistar strain (SPF, Mol) were purchased from Møllegaard Breeding Centre (Ejby, Denmark). The rats were fed *ad libitum* a low-fat reference diet (chow) for 1 week, before a high-fat feeding regimen with two semi-synthetic diets (see below) for 49 d. The body weights of the animals were within the range 211–265 g at the start of the experimental feeding, approximately aged 7 weeks. The rats were randomly divided into two groups with ten animals in each group and housed in individual cages. The temperature in the animal quarters was 21 ± 1°C, the humidity was 55 ± 10 % and the dark period was from 19.00 to 07.00 hours. The rats were given free access to tap water. The protocol was approved by the National Animal Research Authority.

Diets

Each animal group was offered one of two semi-synthetic diets: lard (19·5 % lard, Erica Lard; Ten Kate Vetten BV, Musselkanaal, The Netherlands) or *n*-3 FA (9·1 % lard and 10·4 % Triomar™ (EPAX5500); Pronova Biocare, Lysaker, Norway). Triomar contained >55 % of total *n*-3 FA as TAG: EPA, 300 mg/g; DHA, 190 mg/g; total *n*-3 FA, 580 mg/g (total *n*-3: EPA, DHA, 18 : 3, 18 : 4, 20 : 4, 21 : 5, 22 : 5). This dose represents about 3·6 % of total energy intake of the rats and is comparable with traditional Inuit intakes of marine FA[15]. In addition, 1·5 % of soyabean oil (Mills Soyaolje; Denofa Lilleborg, Fredrikstad, Norway) was provided to both dietary groups to avoid essential FA deficiency. The dietary composition (g/100 g) was: maize starch, 31·5; fat, 21·5; sucrose, 20; casein, 20; salt mixture, 5; vitamin mixture, 1·5; cellulose, 1. The diets provided approximately 40 % of the energy from fat. The diets were kept at −20°C and given to the rats in portions sufficient for 1 d supply.

The FA composition of the experimental diets is given in Table 1. The *n*-3 FA diet included 17·4 % EPA and 10·1 % DHA, whereas the lard diet included 0·03 % or less of these very-long-chain *n*-3 PUFA. The lard diet was particularly rich in the MUFA oleic acid (18 : 1*n*-9; 36·2 % of the total FA), and also consisted of a high amount of the SFA palmitic acid (16 : 0; 25·3 %) and stearic acid (18 : 0; 13·1 %). For determination of FA composition, lipids were extracted by a mixture of chloroform and methanol[16]. The extracts were added heneicosanoic acid (21 : 0) as internal standard. To remove neutral sterols and non-saponifiable material, the extracts were heated in 0·5 M-KOH in an ethanol–water solution. Recovered FA were re-esterified using BF_3–methanol. The methyl esters were quantified by GLC as previously described[17].

Experimental protocol

The rats were offered 20 g/d of the experimental diets in a tray that allowed no spilling of the pasty diet, and individual daily feed intake was recorded. The intake of *n*-3 FA was on average 1·25 g/d in the *n*-3 FA group calculated from analysis of FA composition of the diet (Table 1) and an average feed intake of 18 g/animal per d. Body weight was registered twice weekly. At the end of the feeding period, five animals in each group were used for the estimation of adipose depot volumes by magnetic resonance imaging (MRI), dissection

Table 1. Fatty acid composition of the experimental diets (% total fatty acids)*

Fatty acid	Lard	*n*-3
14 : 0	1·6	0·9
15 : 0	0·07	0·06
16 : 0	25·3	14·5
16 : 1*n*-7	1·9	1·5
16 : 1*n*-9	0·2	0·1
17 : 0	0·3	0·4
18 : 0	13·1	8·8
18 : 1*n*-7	2·6	2·8
18 : 1*n*-9	36·2	22·4
18 : 2*n*-6	15	10·1
18 : 3*n*-3	1·3	1·3
18 : 3*n*-6	0·02	0·1
18 : 4*n*-3	0	1·4
20 : 0	0·2	0·2
20 : 1*n*-7	0·04	0·1
20 : 1*n*-9	0·6	0·9
20 : 1*n*-11	0·02	0·07
20 : 2*n*-6	0·3	0·3
20 : 3*n*-6	0·09	0·1
20 : 3*n*-9	0·03	0·06
20 : 4*n*-3	0	0·6
20 : 4*n*-6	0·2	1·0
20 : 5*n*-3	0·01	17·4
22 : 0	0·05	0·1
22 : 1	0	0·4
22 : 4*n*-6	0·08	0·1
22 : 5*n*-3	0·9	1·6
22 : 5*n*-6	0	0·3
22 : 6*n*-3	0·03	10·1
24 : 1*n*-9	0	0·8

*Data are presented as the average of three measurements.

and carcass analysis, whereas the other five rats were used for other analyses of plasma and several tissues.

Plasma analysis

The rats were anaesthetised with 20 mg pentobarbital intraperitoneally (50 mg/ml). Blood was collected by aortic puncture, mixed with 0·1 % EDTA and immediately chilled on ice. Plasma was prepared and stored at −70°C before analyses. Plasma lipids were measured enzymically on the Technicon Axon system (Miles, Tarrytown, NY, USA) using the following kits: TAG (Bayer, Tarrytown, NY, USA), phospholipids (PAP150; BioMerieux, Lyon, France), total cholesterol (Bayer) and NEFA (NEFA C; Wako Chemicals, Dalton, OH, USA). Plasma glucose was measured enzymically on the Technicon Axon system (Miles, NY) using the Gluco-quant kit (Roche, Mannheim, Germany). Plasma levels of TNFα, IL-6, IL-10, C-reactive protein (CRP) and insulin were measured using commercial ELISA. Samples were analysed in duplicates, and the intra-assay CV were as follows: TNFα (Bender MedSystems, Vienna, Austria), 12·4 %; IL-6 (Bender MedSystems), 8·1 %; CRP (Alpha Diagnostic International, San Antonio, TX, USA), 1·8 %; insulin (Linco Research, St Charles, MO, USA), 3·5 %. Plasma levels of IL-10 (BioSource International, Camarillo, CA, USA) were below the detection limit of the assay. Leptin and adiponectin were measured by competitive RIA (Linco Research) with the use of [^{125}I]leptin and [^{125}I]adiponectin, respectively, as tracers. The intra-assay CV were 4·4 % for leptin and 7·7 % for adiponectin.

Dissection

From killed rats, mesenteric adipose tissue was obtained by stripping out the whole mesenterium from the duodenum to the appendix. Subcutaneous fat was dissected from the lower abdominal part on the left side in an area of about 2 × 2 cm. Epididymal fat was taken from the region around the testis and epididymis on the right-hand side. Perirenal fat included the depot located around the right kidney and suprarenal gland in addition to the abdominal pelvic depot as described by Murano *et al.* [18]. The interscapular adipose depots were obtained by dissecting the white superficial and the deeper brown fat between the shoulder blades.

Magnetic resonance imaging

The rats were killed with pentobarbital intraperitoneal injections and mounted in a supine position in a plastic bed in a home-built, solenoid-type double Cu sheet induction coil, 30 cm long and 100 mm diameter with an unloaded Q-factor of 435. The coil was positioned transversely in the middle of the coil of a General Electric SIGNA 1.5 T clinical magnetic resonance (MR) scanner (General Electric Medical Systems, Milwaukee, WI, USA). An external attenuator was used in addition to the internal attenuation to reduce the transmission signal amplitude to a suitable value.

The rats were scanned in sagittal, coronal and axial planes with a fast spin echo (FSE) T_1 sequence, TE/TR = 13/100 ms (where TE is the echo time after excitation and TR the MR sequence repetition time). To enhance the signals from fat, the frequency was centred on the fat peak, f_{fat} about 63 880 220 Hz. The forty sagittal and coronal slices were interleaved with a thickness of 2 mm, an image acquisition matrix of 256 × 160 and a field of view (FOV) of 250 × 156 mm. The sixty-four axial image slices were interleaved with a thickness of 4 mm, an acquisition matrix of 256 × 256 and a FOV of 80 × 80 mm. Only one excitation (per specific MR sequence; number of excitations (NEX) = 1) was used, giving a total scan time of approximately 10 min per rat.

Magnetic resonance imaging analysis

Interactive data language (IDL) software (RSI International (UK) Ltd, Crowthorne, Berkshire, UK) was used to develop a program where calculations were carried out over voxels satisfying certain inclusion criteria within specified regions of interest (ROI). The calculations involved counting and averaging over the voxels (the three-dimensional analogue of a pixel), and the inclusion criteria were usually values above certain thresholds. The voxels satisfying the criteria were depicted through a coloured overlay region over the original MR image within the present ROI. Preliminary measurements over regions with essentially no fat or pure fat established an intensity (I) scale for fat content in the different fat depots. The width of the intensity distribution in the fat depot ROI was much larger than the width measured in homogeneous adipose tissues, the latter giving a quasi-Gaussian high-intensity peak with a width of only 3–4 % of the peak intensity value. We assumed the low-intensity tail above the fat threshold to be due mainly to partial volume effects, and evaluated the fat content by linear interpolation of the established intensity scale. We used a threshold, I_{25}, corresponding to approximately 25 % fat on this scale, to evaluate the number of voxels (N_{vox}) with intensity larger than I_{25} in the present ROI, each multiplied by the fat content, I/I_{fat}, in this low-intensity region. The fat threshold value I_{25} produced overlay images that seemed to coincide with regions characterised as fat by visual inspection. The total tissue (fat and non-fat) threshold was chosen to be the voxel intensity value giving an overlay image coinciding maximally with the outline of the MR image of the animal. Sagittal and coronal slices were imaged with identical MRI settings and threshold values. Because axial slices were imaged with different resolution and thickness as compared with the sagittal and coronal slices, we used different threshold values providing similar results for total tissue and total body fat as the evaluation in the other two planes.

In addition to the total fat content, MRI volumes of the following fat depots were evaluated: interscapular, perirenal, mesenteric, subcutaneous abdominal, and epididymal. The sagittal, coronal or axial plane images were chosen for evaluation depending on in which plane the boundaries of the fat depot were most clearly depicted. In some cases images from two, or even all three planes were analysed for comparison.

Carcass analysis

After MRI analysis, the five rats from each feeding group were separately autoclaved at 121°C for about 30 min and

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transferred to a custom-made homogeniser. Water containing a foam-reducing agent (Antifoam E100 conc.; Bayer Chemicals AG, Leverkusen, Germany) was added (1:1) before starting the homogeniser. After 2 min in the blender, the constituents were completely homogenised. Total fat percentage was determined by extracting the lipids from a part of the homogenate with petroleum ether at 100°C, and weighing the extracted material (Tecator™ application note AN 77/85 1985.03.15, Association of Official Analytical Chemists (AOAC) method 960.39 and AOAC method 945.16)[19]. Protein was determined by the Kjeldahl method (Tecator™ application note: Determination of Kjeldahl Protein in Fish and Fish-products using the Kjeltec Auto system 1983.02.01 ASN 56/83 (Cu catalyst), AOAC method 981.10[19]). Water was determined by desiccation of a freeze-dried part of each homogenate, and ash was determined by heating the dried material to 550°C for 18–20 h totally and weighing the remains.

Glucose transport and glycogen content in soleus muscle

Glucose uptake and glycogen content were measured in epitrochlearis muscles and in soleus muscle strips as described by Jensen *et al.* [20].

Hepatic enzyme activities

The livers were homogenised and fractionated[21], and the activities of acyl-CoA synthetase[22], carnitine palmitoyltransferase-II[23] and acyl-CoA oxidase[24] were determined in the post-nuclear fractions.

Adipose tissue and gene expression analyses

Mesenteric, subcutaneous, perirenal, epididymal and interscapular adipose tissue depots and liver were collected from each rat and snap-frozen in liquid N_2 before storage at −70°C. The tissues were pulverised with an ice-cold steel pestle and mortar. Total RNA was isolated from 100 mg tissue using the RNeasy Lipid Tissue Mini Kit from Qiagen (Venlo, The Netherlands). RNA was quantified by spectrophotometry (NanoDrop 1000; NanoDrop Technologies, Waltham, MA, USA), and the integrity was evaluated by capillary electrophoresis (Agilent 2100 Bioanalyser; Agilent Technologies, Inc., Santa Clara, CA, USA). Total RNA (400 ng) was reversely transcribed in 20 μl reactions using the High Capacity cDNA Reverse Transcription Kit with RNase inhibitor (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's directions. Real-time PCR was performed with custom-made 384-well microfluidic cards (TaqMan Low Density Arrays; Applied Biosystems). Forty-four genes of interest were selected, as well as four endogenous controls, and analysed in duplicates. Official symbols and full names of the genes, as well as Applied BioSystems' product codes, are given in Table 2. The expression values of each gene in all samples were normalised against the average of the endogenous controls. *18S* and *Arbp* varied significantly between the lard and *n*-3 groups in mesenteric fat, and were therefore excluded as endogenous controls in this depot.

Adipose tissue extraction and adipokine protein analyses

Approximately 0·1 g frozen, comminuted adipose tissue was mixed with 0·4 ml lysis buffer (1 M-2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-HCl, 1 M-NaCl, 85 % glycerol), 0·5 M-EDTA (pH 8) and Complete protease inhibitor cocktail (Roche, Basel, Switzerland), and immediately homogenised for 1 min using an Ultra-Turrax device. Samples were centrifuged for 15 min at 3000 ***g***, the floating fat layers were discarded and the aqueous portions of the samples were centrifuged for another 15 min at 15 000 ***g***. Total protein concentrations were measured using a Multiskan Plus reader (Titertek, Labsystem, Helsinki, Finland). All samples were diluted to a total protein concentration of 0·5 μg/μl. Concentrations of adiponectin, monocyte chemoattractant protein 1, leptin, IL-1β, IL-6, TNFα and plasminogen activator inhibitor-1 (total) were measured using a rat adipocyte LINCO*plex* kit (RADPCYT-82K; Linco Research) according to the manufacturer's protocol. The samples were analysed in tetra- or pentaplicates using a Bio-Plex 200 instrument (Bio-Rad, Richmond, CA, USA).

Statistics

Values are reported as mean values and standard deviations for ten animals per group in Fig. 1, and mean values with their standard errors from four or five animals per group in the remaining Figs. 2–6 and Tables 2 and 4. Independent-samples *t* tests were used to compare the lard and *n*-3 FA groups. Significant differences in MRI volumes between the two diet groups were found by *t* tests. Correlation coefficients were calculated between dissection weights and volumes (determined by MRI) of adipose depots (Table 3). A 5 % level of significance was applied in all analyses.

Results

Animals and diets

Both experimental diets contained the same amount of energy (per g), and the rats were individually offered 20 g/d of the respective diets throughout the 7-week feeding period. There were no differences in average weight gain in the two groups of animals (Fig. 1(a)). The average amount of feed consumed by the rats in the *n*-3 FA group and the reference lard group was also indistinguishable (Fig. 1(b)). To determine if there were differences in body composition in the two dietary groups, we performed carcass analyses with no significant differences in the content of fat, protein, ash or water between the two groups after 7 weeks of feeding (Fig. 1(c)).

Plasma analyses

Plasma concentrations of TAG, phospholipids and cholesterol were decreased by 56, 41 and 40 %, respectively, after 7 weeks of feeding in the *n*-3 FA-fed as compared with the lard-fed animals (Fig. 2(a)). Plasma NEFA were reduced non-significantly in the *n*-3 FA group. There were no significant differences in plasma levels of adiponectin, leptin, CRP, TNFα, IL-6 or IL-10 between the groups (data not shown). Plasma insulin concentrations were markedly lower (72 %) in the *n*-3 FA-fed animals (Fig. 2(b)), whereas plasma glucose concentrations were similar in both groups (data not shown).

Glucose uptake and glycogen content in skeletal muscle

To investigate the insulin response of skeletal muscle after the experimental feeding, glucose uptake was measured *in vitro* in epitrochlearis muscles and in soleus muscle strips. There were no significant differences in either basal or insulin-stimulated glucose uptake in soleus muscle strips (Fig. 3) and epitrochlearis (data not shown) between the two dietary groups. The amount of glycogen in the epitrochlearis muscle was measured as 155 (SEM 9) mmol/kg dry weight in the *n*-3 FA group and 173 (SEM 8) mmol/kg in the lard group. In soleus, the glycogen content was 122 (SEM 16) and 130 (SEM 12) mmol/kg dry weight in the *n*-3 FA-fed group and lard-fed group, respectively, with no significant differences in muscle glycogen content between the two groups.

Hepatic enzyme activity

The hepatic enzyme activities of acyl-CoA synthetase, acyl-CoA oxidase and carnitine palmitoyltransferase-II were significantly increased in the *n*-3 FA group as compared with the lard group, by 92, 17 and 68 %, respectively (Fig. 4).

Table 2. Official symbol, official full name and Applied Biosystems' product code for the forty-four genes selected, as well as the four endogenous controls

Official gene symbol	Official full name	Applied BioSystems' product code*
Acaca	Acetyl-coenzyme A carboxylase α	Rn00573474_m1 Acaca
Acbd3	Acyl-coenzyme A binding domain containing 3	Rn00788231_m1 Acbd3
Ace	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	Rn00561094_m1 Ace
Acsl1	Acyl-CoA synthetase long-chain family member 1	Rn00563137_m1 Acsl1
Adfp	Adipose differentiation related protein	Rn01472318_m1 Adfp
Adipoq	Adiponectin	Rn00595250_m1 Adipoq
Apln	Apelin, AGTRL1 ligand	Rn00581093_m1 Apln
Ccl2	Chemokine (C-C motif) ligand 2; also named MCP-1	Rn00580555_m1 Ccl2
Cpt1a	Carnitine palmitoyltransferase 1a, liver	Rn00580702_m1 Cpt1a
Cpt2	Carnitine palmitoyltransferase 2	Rn00563995_m1 Cpt2
Cxcl1	Chemokine (C-X-C motif) ligand 1; also named CINC-1; GRO1	Rn00578225_m1 Cxcl1
Dgat1	Diacylglycerol *O*-acyltransferase 1	Rn00584870_m1 Dgat1
Fabp4	Fatty acid binding protein 4, adipocyte	Rn00670361_m1 Fabp4
Fabp5	Fatty acid binding protein 5, epidermal	Rn00821817_g1 Fabp5
Fasn	Fatty acid synthase	Rn00569117_m1 Fasn
Hgf	Hepatocyte growth factor	Rn00566673_m1 Hgf
Hsd11b2	Hydroxysteroid 11-β dehydrogenase 2	Rn00492539_m1 Hsd11b2
Il10	Interleukin 10	Rn00563409_m1 Il10
Il1b	Interleukin 1β	Rn00580432_m1 Il1b
Il6	Interleukin 6	Rn00561420_m1 Il6
Lep	Leptin	Rn00565158_m1 Lep
Lipe	Lipase, hormone sensitive	Rn00563444_m1 Lipe
Lpl	Lipoprotein lipase	Rn00561482_m1 Lpl
Mt1a	Metallothionein 1a	Rn00821759_g1 Mt1a
Nr1h3	Nuclear receptor subfamily 1, group H, member 3; also named LXR α	Rn00581185_m1 Nr1h3
Pbef1	Pre-B-cell colony enhancing factor 1; also named visfatin	Rn00822046_m1 Pbef1
Pklr	Pyruvate kinase, liver and red blood cell	Rn00561764_m1 Pklr
Plin	Perilipin	Rn00558672_m1 Plin
Ppara	Peroxisome proliferator activated receptor α	Rn00566193_m1 Ppara
Pparg	Peroxisome proliferator activated receptor γ	Rn00440945_m1 Pparg
Prkaa1	Protein kinase, AMP-activated, α 1 catalytic subunit	Rn00569558_m1 Prkaa1
Prkaa2	Protein kinase, AMP-activated, α 2 catalytic subunit; also named AMPK	Rn00576935_m1 Prkaa2
Rbp4	Retinol binding protein 4, plasma	Rn01451318_m1 Rbp4
Retn	Resistin	Rn00595224_m1 Retn
RGD1652323	Similar to fatty acid translocase/CD36	Rn00580728_m1 RGD1562323_predicted Cd36
Scd1	Stearoyl-coenzyme A desaturase 1	Rn00594894_g1 Scd1
Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, member 1; also named PAI-1	Rn00561717_m1 Serpine1
Slc27a1	Solute carrier family 27 (fatty acid transporter), member 1; also named FATP-1	Rn00585821_m1 Slc27a1
Slc2a4	Solute carrier family 2 (facilitated glucose transporter), member 4; also named GLUT-4	Rn00562597_m1 Slc2a4
Tgfb1	Transforming growth factor, β 1	Rn00572010_m1 Tgfb1
Tnf	Tumour necrosis factor	Rn99999017_m1 Tnf
Ucp1	Uncoupling protein 1	Rn00562126_m1 Ucp1
Ucp2	Uncoupling protein 2	Rn00571166_m1 Ucp2
Ucp3	Uncoupling protein 3	Rn00565874_m1 Ucp3
Arbp†	Acidic ribosomal phosphoprotein P0	Rn00821065_g1 Arbp
Gapdh†	Glyceraldehyde-3-phosphate dehydrogenase	Rn99999916_s1 Gapdh
Ppif†	Peptidylprolyl isomerase F (cyclophilin F)	Rn00597197_m1 Ppif
18S†		Hs99999901_s1

AGTRL1, angiotensin receptor-like 1; CINC-1; cytokine-induced neutrophil chemoattractant 1; GRO1, growth-related oncogene 1; MCP-1, monocyte chemoattractant protein 1; LXR, liver X receptor; AMPK, AMP-activated protein kinase; PAI-1, plasminogen activator inhibitor-1.

* Applied BioSystems, Foster City, CA, USA.

† Endogenous controls.

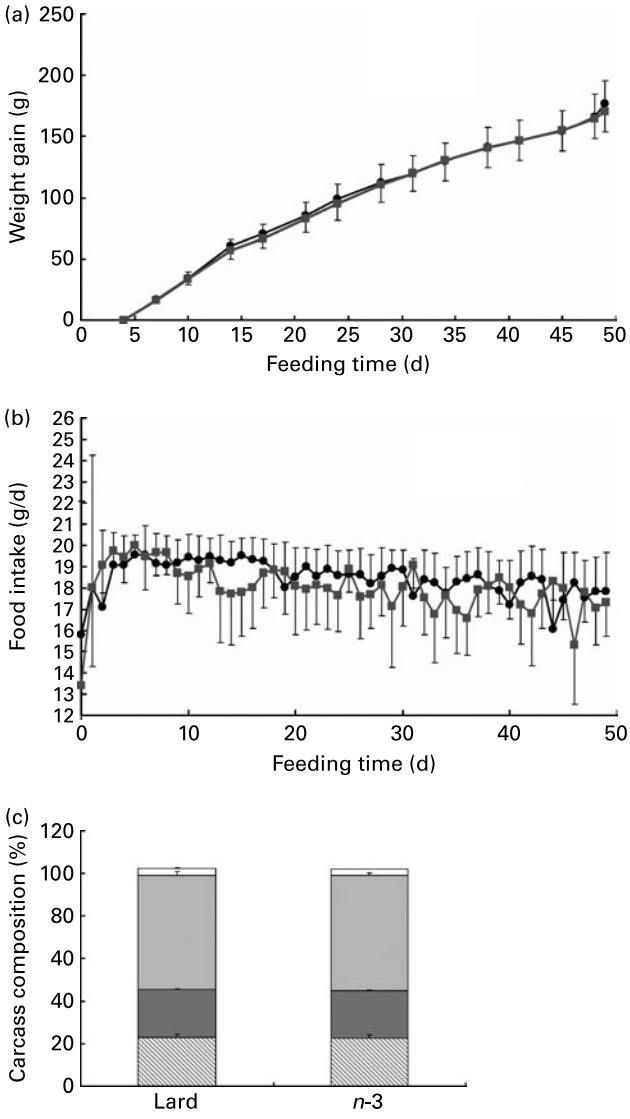

Fig. 1. (a) Body-weight gain in the two dietary groups (–●–, lard; –■–, *n*-3 fatty acids) during the 7 weeks of feeding. Values are means for ten rats per group, with standard deviations represented by vertical bars. The average start weight was 240 g. (b) Daily feed intake in the two dietary groups (–●–, lard; –■–, *n*-3 fatty acids) during the 7 weeks of feeding. Values are means for ten rats per group, with standard deviations represented by vertical bars. (c) Body composition in the two dietary groups determined by carcass analysis, shown as percentage of ash (□), water (▨), protein (■) and fat (▧) in autoclaved rats. Values are means for five rats per group, with standard errors represented by vertical bars.

Adipose tissue depots

Five adipose tissue depots were dissected and weighed, and volumes were estimated using MRI on killed whole animals (Fig. 5). Dissection weights of the perirenal and epididymal depots were significantly reduced by 51 and 31 % after *n*-3 FA feeding as compared with the lard feeding, respectively, whereas the volume estimated by MRI was reduced by 43 and 32 % in these depots. There was no significant difference in dissection weights of the mesenteric adipose depots. Estimated MRI volume of the mesenteric depot was, however, significantly reduced by 35 %. There were no significant differences between the two feeding groups in weight or volume of interscapular and subcutaneous adipose depots. The correlation coefficients between estimated volume and dissection weight of the fat depots were significant and in the range 0·67–0·84 for subcutaneous, perirenal, epididymal and interscapular fat, whereas it was 0·39 and non-significant for the mesenteric depot (Table 3). Representative MR images of the different adipose depots are shown in Fig. 6.

Table 3. Correlation coefficients between depot volume estimated by magnetic resonance imaging and depot weight obtained by dissection

Fat depot	Correlation coefficient	*P*
Subcutaneous	0·81	<0·001
Mesenteric	0·39	0·15
Perirenal	0·84	<0·001
Epidymal	0·82	<0·001
Interscapular	0·67	0·006

Adipose tissue extracts

The concentrations of several adipokines (adiponectin, monocyte chemoattractant protein 1, leptin, IL-1β, IL-6, TNFα and plasminogen activator inhibitor-1 (total)) were determined in aqueous extracts from the five different adipose tissue depots. No statistically significant differences were observed between the two dietary groups (data not shown).

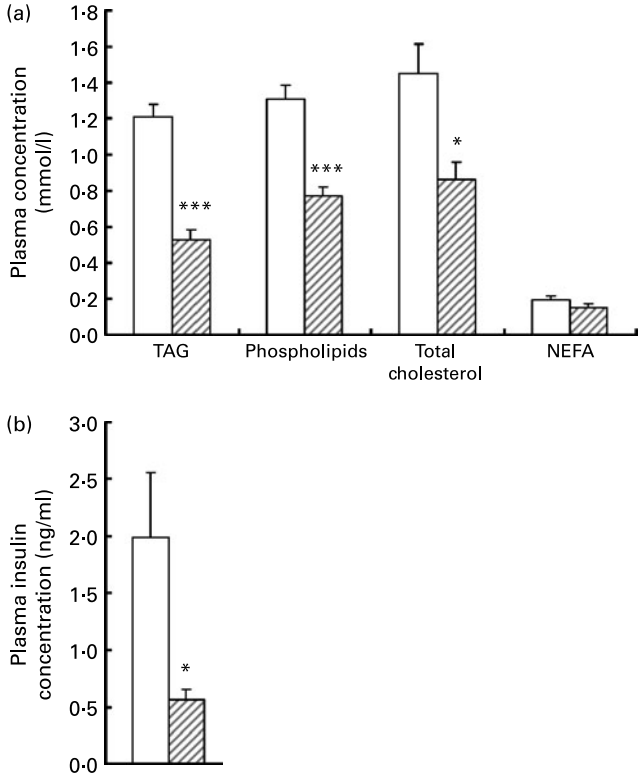

Fig. 2. Plasma concentrations of TAG, phospholipids, total cholesterol and NEFA (a) and insulin (b) in the two dietary groups (□, lard; ▨, *n*-3 fatty acids) after 7 weeks of feeding. Values are means for five rats per group, with standard errors represented by vertical bars. Mean value was significantly different from that of the lard group: * $P<0·05$, *** $P\leq0·001$ (*t* test).

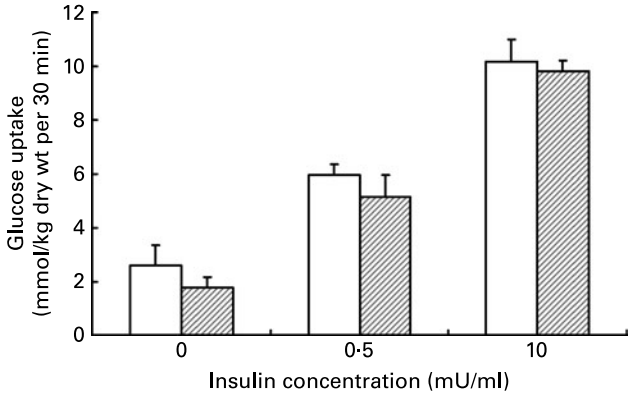

Fig. 3. Glucose uptake in dissected soleus skeletal muscle strips in the two dietary groups (□, lard; ▨, *n*-3 fatty acids) after 7 weeks of feeding. The muscle strips were incubated without insulin or with 0·5 and 10 mU insulin/ml, and glucose uptake was determined and calculated from the intracellular accumulation of 2-deoxy-D-[^{3}H]glucose. Values are means for five rats per group, with standard errors represented by vertical bars.

Gene expression analysis

The effects of *n*-3 FA feeding on mRNA expression of forty-four selected genes in five adipose depots and liver are presented in Table 4. Several cytokines and chemokines (*Il10*, *Il1b*, *Il6*, *Cxcl1*, *Ccl2*, *Mt1a*, *Retn*, *Tnf*) were significantly increased (1·5- to 13-fold) in the five depots in the *n*-3 FA-fed as compared with the lard-fed animals. The adipogenic transcription factor *Pparg* was increased 4-fold in the mesenteric depot in the *n*-3 FA group. The lipogenic enzymes *Acaca* and *Fasn*, as well as *Fabp5*, were enhanced in the mesenteric, perirenal (*Fasn* not significantly) and epididymal depots, whereas they were reduced in the interscapular depot containing significant amounts of brown adipose tissue. *Dgat1* was reduced in the perirenal and epididymal depots. *Scd1* was significantly reduced in the epididymal, perirenal and interscapular depots, as well in the liver of the *n*-3 FA-fed rats. *Ucp2* mRNA expression was doubled in the subcutaneous depots of *n*-3 FA-fed rats, whereas *Ucp3* was reduced in the perirenal adipose depots. mRNA expression of *Cpt2*, which is involved in FA transport and β-oxidation in mitochondria, was almost doubled in the mesenteric depot and liver, and reduced in the epididymal depot. Expression of *Adfp*, which is a lipid droplet-associated protein, was 2- to 3-fold increased in the epididymal and perirenal depots. The insulin-sensitising adipokine *Adipoq* (adiponectin) was 4-fold increased in the mesenteric depot. We observed that the RNA yield was lower, and the quality higher, from the mesenteric depots of the *n*-3 FA-fed rats compared with the lard-fed animals. This may reflect some contamination by pancreatic tissue in the lard-fed rats because we observed expression of the pancreatic markers *Ela1* and *Prss1* in some mesenteric adipose tissue samples[25].

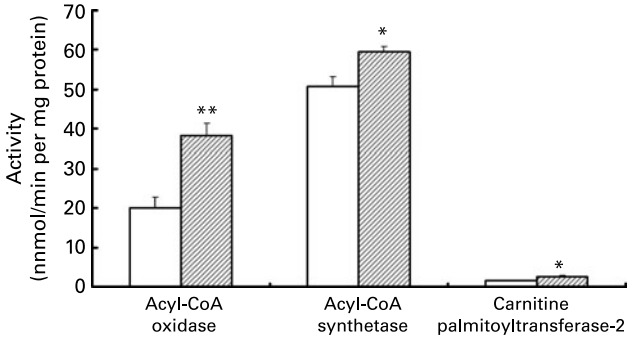

Fig. 4. Activities of acyl-CoA synthetase, acyl-CoA oxidase and carnitine palmitoyltransferase-II in livers from lard-fed (□) and *n*-3 fatty acid-fed (▨) rats after 7 weeks of feeding. The livers were homogenised and fractionated, and the enzyme activities were measured in post-nuclear fractions. Acyl-CoA oxidase was measured by a spectrophotometric assay, whereas acyl-CoA synthetase and carnitine palmitoyltransferase-II activities were measured utilising radioactive labelled substrates. Values are means for five rats per group, with standard errors represented by vertical bars. Mean value was significantly different from that of the lard group: * $P<0·05$, ** $P<0·01$ (*t* test).

Discussion

By using MRI, we showed that the mesenteric adipose depots are significantly smaller in the *n*-3 FA-fed animals as compared with lard-fed animals. Also epididymal and perirenal adipose depots were reduced in *n*-3 FA-fed animals in agreement with previous reports[1,12,13]. The mesenteric, perirenal and epididymal depots are all located in the visceral compartment inside the peritoneal cavity. A reduction in size of these depots is important because visceral adiposity is associated with the metabolic syndrome and is a risk factor for developing CVD and type 2 diabetes mellitus[9,26–28].

Waist:hip ratio or waist circumference is emerging as a better risk marker for CVD than BMI because the latter does not take into account the distribution of body fat. Results from the INTERHEART study show a protective effect of an increased hip circumference (reflecting subcutaneous storage of fat on the hips and thighs) related to risk of myocardial infarction[9,26]. Dietary marine oils limit the TAG accumulation in perirenal and epididymal adipose tissue, reducing hypertrophy of the adipocytes[11,13]. Rustan *et al.* [1] have previously shown that *n*-3 FA feeding of rats reduced adipocyte cell volume in perirenal and epididymal adipose depots, whereas the cell volume was unaltered in the mesenteric and

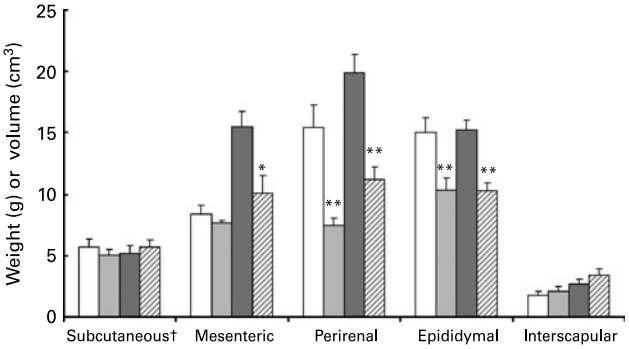

Fig. 5. Size of adipose tissue depots determined by dissection and weighing, and volume estimated by magnetic resonance imaging (MRI) of five depots in lard- and *n*-3 fatty acid-fed rats after 7 weeks of feeding. (□), Dissection weight, lard group; (▩), dissection weight, *n*-3 fatty acid group; (■), MRI volume, lard group; (▨), MRI volume, *n*-3 fatty acid group. Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that of the lard group: * $P<0·05$, ** $P<0·01$ (*t* test). † The lower abdominal part on the left side of the subcutaneous adipose depot in an area of about 2 × 2 cm.

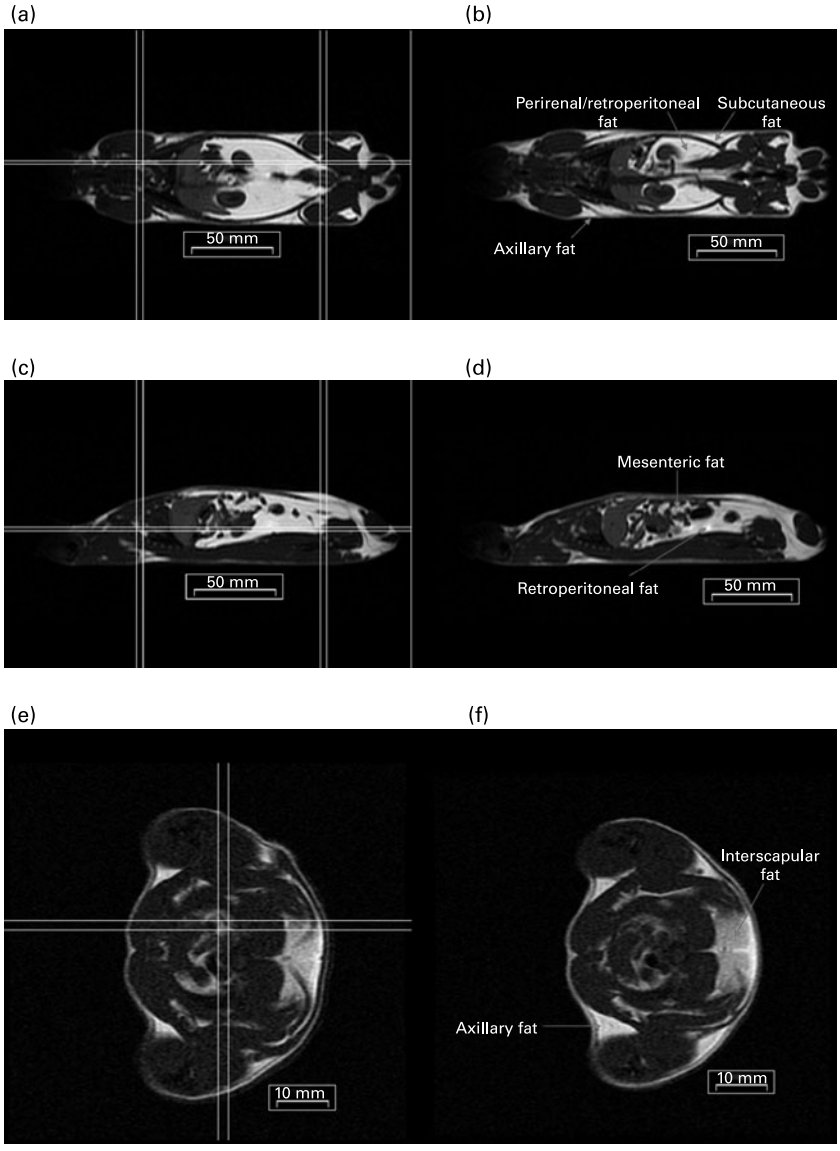

Fig. 6. Magnetic resonance (MR) images of adipose depots of representative rats in different planes after 7 weeks of feeding. Coronal MR images from the lard (a) and *n*-3 fatty acid (b) groups, showing perirenal/retroperitoneal, axillary and subcutaneous adipose depots. Sagittal MR images of left sections of the lard (c) and *n*-3 fatty acid (d) groups showing mesenteric and retroperitoneal adipose tissue depots. Axial MR images of the lard (e) and *n*-3 fatty acid (f) groups showing axillary and interscapular fat. Sections corresponding to the sagittal (2 mm) and axial (4 mm) slices are shown in (a), sections corresponding to the coronal (2 mm) and axial (4 mm) slices are shown in (c) and sections corresponding to the coronal (2 mm) and sagittal (2 mm) slices are shown in (e).

subcutaneous depots. Although we found the visceral adipose depots to be reduced in the *n*-3 FA-fed rats, we found no differences between the two groups in total weight gain, which is in agreement with Kusunoki *et al.*[29] and Pérez-Matute *et al.*[30]. Moreover, there were no differences between the two groups in body composition as determined by our whole-body carcass analysis.

By dissecting out and weighing the mesenteric, perirenal, interscapular and epididymal adipose depots, as well as subcutaneous adipose tissue on the left side of the lower abdominal part, we accounted for approximately 28 % of total body fat in the rats. Carcass analyses showed similar body fat percentage in the two groups. Thus, our finding of smaller mesenteric, perirenal and epididymal adipose depots in the *n*-3 FA-fed group suggests that *n*-3 FA feeding promoted a redistribution of adipose tissue, rather than a reduction in the total amount of fat. Both the dissection and MRI analysis included only the lower abdominal part on the left side of the subcutaneous depots, thereby providing low sensitivity for detecting differences in the two dietary groups in this depot. It is therefore possible that the animals in the *n*-3 FA group had more subcutaneous fat in total than the lard-fed group, because the MRI-estimated volume of the left abdominal subcutaneous adipose depot was higher (not statistically significant) in the *n*-3 FA-fed animals. In addition, expression of the lipolytic enzyme *Lipe* was reduced by 60 % in the subcutaneous depot. Studies on thiazolidinediones (TZD) have shown that the weight gain following treatment is due primarily to enhanced subcutaneous adiposity, accompanied by reduced visceral adiposity and intrahepatic TAG accumulation[31,32].

Table 4. Effects of 7 weeks of *n*-3 fatty acid (FA) feeding on mRNA expression levels in five adipose depots and liver†
(Mean values with their standard errors for four to five rats per group)

		Subcutaneous		Mesenteric		Perirenal		Epididymal		Interscapular		Liver	
Gene	Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acaca	Lard	1·00	0·05	1·00	0·11	1·00	0·15	1·00	0·09	1·00	0·08	1·00	0·18
	n-3 FA	1·72***	0·15	3·60*	0·91	1·97*	0·30	1·78*	0·31	0·55**	0·11	0·56	0·04
Acbd3	Lard	1·00	0·12	1·00	0·06	1·00	0·16	1·00	0·06	1·00	0·08	1·00	0·05
	n-3 FA	0·92	0·12	1·22	0·14	1·07	0·06	0·81*	0·05	0·75*	0·04	0·88	0·05
Ace	Lard	1·00	0·12	1·00	0·61	1·00	0·18	1·00	0·24	1·00	0·09	1·00‡	0·01
	n-3 FA	1·11	0·47	1·24	0·48	0·62	0·08	0·52	0·02	0·69*	0·05	1·69	0·33
Acsl1	Lard	1·00	0·23	1·00	0·22	1·00	0·19	1·00	0·11	1·00	0·05	1·00	0·08
	n-3 FA	0·71	0·09	3·41*	1·05	0·72	0·06	0·70*	0·03	1·00	0·10	1·16	0·05
Adfp	Lard	1·00	0·24	1·00	0·20	1·00	0·16	1·00	0·08	1·00	0·15	1·00	0·19
	n-3 FA	2·55	0·71	2·39	0·64	2·52**	0·27	2·27***	0·19	1·08	0·17	0·76	0·17
Adipoq	Lard	1·00	0·21	1·00	0·20	1·00	0·13	1·00	0·09	1·00	0·06	1·00‡	0·07
	n-3 FA	0·53	0·14	4·11*	1·30	0·83	0·08	0·85	0·03	1·05	0·06	2·09	1·11
Apln	Lard	1·00	0·17	1·00	0·24	1·00	0·15	1·00	0·24	1·00	0·26	1·00‡	0·06
	n-3 FA	0·75	0·14	2·40*	0·48	0·51*	0·03	0·52	0·09	1·32	0·16	1·03	0·08
Ccl2	Lard	1·00	0·48	1·00	0·22	1·00	0·05	1·00	0·15	1·00	0·36	1·00‡	0·19
	n-3 FA	0·58	0·16	7·33*	2·67	2·49**	0·39	1·69*	0·18	3·62	1·89	0·70	0·07
Cd36§	Lard	1·00	0·19	1·00	0·26	1·00	0·15	1·00	0·07	1·00	0·10	1·00	0·27
	n-3 FA	0·46*	0·09	2·91	0·85	0·92	0·04	0·93	0·10	1·08	0·13	1·64	0·10
Cpt1a	Lard	1·00	0·17	1·00	0·08	1·00	0·16	1·00	0·17	1·00	0·27	1·00	0·20
	n-3 FA	1·73	0·63	0·96	0·05	1·04	0·06	0·84	0·05	0·85	0·12	1·28	0·06
Cpt2	Lard	1·00	0·13	1·00	0·09	1·00	0·06	1·00	0·06	1·00	0·10	1·00	0·04
	n-3 FA	1·34	0·37	1·83*	0·34	0·83	0·12	0·80*	0·03	0·89	0·12	1·78***	0·06
Cxcl1	Lard	1·00	0·43	1·00	0·17	1·00	0·23	1·00	0·22	1·00‡	0·14	1·00	0·63
	n-3 FA	0·91	0·66	11·56*	4·39	1·46	0·47	1·14	0·15	1·34	0·20	0·38	0·17
Dgat1	Lard	1·00	0·15	1·00	0·05	1·00	0·11	1·00	0·07	1·00	0·08	1·00	0·05
	n-3 FA	0·70	0·07	3·22	1·08	0·71*	0·03	0·80*	0·03	1·03	0·13	1·16	0·05
Fabp4	Lard	1·00	0·20	1·00	0·19	1·00	0·16	1·00	0·10	1·00	0·04	1·00	0·09
	n-3 FA	0·53	0·11	3·89	1·33	0·94	0·07	0·87	0·05	0·97	0·13	1·21	0·39
Fabp5	Lard	1·00	0·17	1·00	0·24	1·00	0·16	1·00	0·13	1·00	0·11	1·00	0·24
	n-3 FA	8·47	7·06	1·82*	0·28	1·51*	0·07	1·66**	0·14	0·60**	0·03	0·46	0·05
Fasn	Lard	1·00	0·30	1·00	0·19	1·00	0·20	1·00	0·14	1·00	0·06	1·00	0·46
	n-3 FA	2·68	0·98	3·77*	1·13	1·98	0·40	2·10*	0·43	0·40***	0·08	0·15	0·03
Hgf	Lard	1·00	0·21	1·00	0·23	1·00	0·13	1·00	0·10	1·00‡	0·26	1·00	0·15
	n-3 FA	0·40*	0·10	1·67	0·25	1·16	0·09	1·30	0·10	1·23	0·16	1·01	0·04
Hsd11b2	Lard	1·00	0·22	1·00‡	0·12	1·00	0·27	1·00	0·09	1·00‡	0·12	1·00	0·15
	n-3 FA	0·54	0·22	3·68*	1·05	0·69	0·09	0·99	0·10	1·98	1·24	0·77	0·23
Il10	Lard	1·00	0·32	1·00‡	0·18	1·00	0·19	1·00	0·22	1·00‡	0·41	1·00‡	0·22
	n-3 FA	0·38	0·05	13·55	9·98	1·78	0·44	1·73*	0·20	0·90	0·46	1·07	0·15
Il1b	Lard	1·00	0·34	1·00	0·26	1·00	0·24	1·00	0·28	1·00‡	0·12	1·00	0·14
	n-3 FA	0·65	0·25	2·23*	0·35	4·89***	0·71	2·02	0·40	1·08	0·06	1·45*	0·04
Il6	Lard	1·00‡	0·81	1·00	0·32	1·00	0·26	1·00	0·22	1·00‡	0·28	1·00‡	0·26
	n-3 FA	0·57	0·48	13·13*	4·36	3·55	1·33	1·96*	0·24	3·28	1·08	1·63	0·96
Lep	Lard	1·00	0·22	1·00	0·27	1·00	0·09	1·00	0·13	1·00	0·14	1·00‡	0·25
	n-3 FA	0·50	0·12	3·35	1·23	0·70	0·07*	0·65	0·09	0·67	0·16	7·25**	0·20
Lipe	Lard	1·00‡	0·11	1·00‡	0·10	1·00	0·18	1·00	0·24	1·00‡	0·25	NQ‡	
	n-3 FA	0·40**	0·05	3·30*	1·00	0·93	0·12	0·84	0·09	0·63	0·06	NQ	
Lpl	Lard	1·00	0·12	1·00	0·22	1·00	0·13	1·00	0·09	1·00	0·13	1·00	0·18
	n-3 FA	0·97	0·09	3·31*	1·00	0·89	0·10	0·77*	0·05	1·01	0·08	0·87	0·17
Mt1a	Lard	1·00	0·16	1·00	0·34	1·00	0·16	1·00	0·14	1·00	0·49	1·00	0·28
	n-3 FA	1·70	0·34	1·29	0·35	2·28**	0·26	1·52*	0·17	0·81	0·15	2·66	0·70
Nr1h3	Lard	1·00	0·17	1·00	0·13	1·00	0·12	1·00	0·08	1·00	0·29	1·00	0·12
	n-3 FA	0·46*	0·06	2·31	0·58	1·03	0·07	0·95	0·04	0·52	0·04	0·90	0·07
Pbef	Lard	1·00	0·12	1·00	0·24	1·00	0·06	1·00	0·07	1·00	0·12	1·00	0·08
	n-3 FA	1·96	0·57	2·58	0·68	0·92	0·03	1·02	0·10	0·82	0·16	1·99	0·33
Pklr	Lard	1·00‡	0·39	1·00‡	0·45	1·00‡	0·25	1·00‡	0·55	1·00‡	0·30	1·00	0·05
	n-3 FA	0·33	0·07	0·24	0·08	1·72	0·77	0·04	0·02	1·82	1·13	0·47***	0·01
Plin	Lard	1·00	0·21	1·00	0·17	1·00	0·17	1·00	0·10	1·00	0·03	1·00‡	0·12
	n-3 FA	0·50	0·13	3·73	1·32	0·70	0·05	0·73*	0·03	1·04	0·12	1·97	1·14
Ppara	Lard	1·00	0·09	1·00	0·16	1·00	0·10	1·00	0·06	1·00	0·19	1·00	0·13
	n-3 FA	0·91	0·13	1·24	0·39	0·93	0·30	0·70**	0·06	0·98	0·10	0·81	0·08
Pparg	Lard	1·00	0·27	1·00	0·14	1·00	0·17	1·00	0·11	1·00	0·17	1·00	0·28
	n-3 FA	0·36	0·10	3·42*	1·05	0·77	0·02	0·83	0·03	0·89	0·08	1·94	0·28
Prkaa1	Lard	1·00	0·13	1·00	0·17	1·00	0·10	1·00	0·08	1·00	0·07	1·00	0·15
	n-3 FA	0·71	0·03	1·70	0·31	0·89	0·05	0·89	0·03	0·94	0·16	1·18	0·11
Prkaa2	Lard	1·00	0·19	1·00	0·27	1·00	0·19	1·00	0·10	1·00	0·24	1·00	0·04
	n-3 FA	3·53*	1·19	1·14	0·33	0·48*	0·03	0·72*	0·04	0·58	0·07	1·37*	0·10

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Table 4. *Continued*

Gene	Diet	Subcutaneous		Mesenteric		Perirenal		Epididymal		Interscapular		Liver	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Rbp4	Lard	1·00	0·21	1·00	0·22	1·00	0·13	1·00	0·13	1·00	0·07	1·00	0·09
	n-3 FA	0·40	0·12	3·74*	1·16	0·89	0·10	0·83	0·05	0·92	0·16	0·95	0·02
Retn	Lard	1·00	0·49	1·00	0·29	1·00	0·20	1·00	0·13	1·00	0·06	1·00‡	0·28
	n-3 FA	0·92	0·38	2·97*	0·81	0·95	0·09	0·96	0·11	0·63**	0·08	0·97	0·37
Scd1	Lard	1·00	0·27	1·00	0·19	1·00	0·11	1·00	0·08	1·00	0·20	1·00	0·15
	n-3 FA	0·54	0·10	0·87	0·38	0·18***	0·05	0·17***	0·07	0·20**	0·04	0·28**	0·08
Serpine1	Lard	1·00	0·26	1·00	0·21	1·00	0·26	1·00	0·15	1·00	0·15	1·00	0·17
	n-3 FA	1·11	0·42	6·99	2·94	0·91	0·16	0·85	0·11	0·59	0·19	1·49	0·48
Slc27a1	Lard	1·00	0·08	1·00	0·12	1·00	0·13	1·00	0·10	1·00	0·11	1·00	0·07
	n-3 FA	0·77	0·14	2·17*	0·36	1·16	0·14	1·25	0·11	0·48**	0·08	0·91	0·03
Slc2a4	Lard	1·00	0·21	1·00	0·16	1·00	0·12	1·00	0·09	1·00	0·03	1·00‡	0·12
	n-3 FA	0·45*	0·05	4·08	1·42	0·95	0·07	1·05	0·08	0·52***	0·04	0·69	0·10
Tgfb1	Lard	1·00	0·18	1·00	0·33	1·00	0·10	1·00	0·10	1·00	0·11	1·00	0·08
	n-3 FA	0·56	0·04	1·16	0·18	2·04***	0·05	1·74***	0·06	0·93	0·05	1·05	0·03
Tnf	Lard	1·00‡	0·10	1·00	0·27	1·00	0·13	1·00	0·11	1·00‡	0·14	1·00‡	0·13
	n-3 FA	1·23	0·13	1·57	0·43	1·86**	0·16	1·27	0·09	1·18	0·19	1·87	0·44
Ucp1	Lard	1·00‡	0·43	1·00‡	0·30	1·00	0·64	1·00‡	0·54	1·00	0·09	1·00‡	0·07
	n-3 FA	0·09	0·03	2·25	0·88	4·30	3·92	1·43	0·70	1·03	0·25	0·09	0·00
Ucp2	Lard	1·00	0·15	1·00	0·35	1·00	0·15	1·00	0·06	1·00	0·19	1·00	0·07
	n-3 FA	2·13*	0·52	1·17	0·19	1·28	0·06	1·08	0·06	1·20	0·18	0·94	0·04
Ucp3	Lard	1·00	0·28	1·00	0·29	1·00	0·11	1·00	0·12	1·00	0·15	NQ‡	
	n-3 FA	1·39	0·44	2·74	1·17	0·50**	0·04	0·75	0·11	0·96	0·10	NQ	

NQ, gene expression level not quantifiable.
Mean value was significantly different from that of the lard group: * $P<0·05$, ** $P<0·01$, *** $P<0·001$ (*t* test).
† The fold increase or reduction in the *n*-3 FA group as compared with the lard group is shown. Expression levels of target genes were normalised against the endogenous controls *18S*, *Arbp*, *Ppif* and *Gapdh*. In the mesenteric adipose depot, *18S* and *Arbp* varied significantly between the animals fed lard and *n*-3 FA, and were therefore excluded as endogenous controls. For explanation of gene symbols, see Table 2.
‡ Genes expressed at very low levels (Ct > 30).
§ The official gene symbol for *Cd36* is *RGD1652323*.

Because *n*-3 FA also activate *Pparg*, we suggest that there might be redistribution of adipose tissue from the visceral depots to the subcutaneous depot in the *n*-3 FA-fed animals.

The lower correlation coefficients between dissection weight and volume estimated by MRI for the mesenteric fat as compared with the other adipose tissue depots may reflect the difficulty of dissecting out this depot precisely. Fissoune *et al.* reported MRI measurements of two adipose tissue depots in mice, but did not validate against dissection weights as we have done[33]. MRI might represent a reliable non-invasive method and a more precise alternative to dissection in certain situations.

Expression of the adipogenic transcription factor *Pparg* was increased 3·4-fold in the mesenteric depot of *n*-3 FA-fed animals. As observed for the TZD, the *n*-3 FA EPA and DHA are good agonists for PPARγ[34,35], contrary to SFA predominantly found in lard. Activation of PPARγ is important for adipocyte differentiation[36]. Our finding that *Pparg* expression is increased in response to dietary *n*-3 FA is supported by Chambrier *et al.* who showed that EPA induced PPARγ gene expression in isolated human adipocytes[37]. This has also been shown in human skeletal muscle cells[38]. PPARγ activation may promote fat accumulation in subcutaneous depots, with reduced or unchanged visceral storage[39]. Also, some *ex vivo* preadipocyte studies have shown that abdominal subcutaneous preadipocytes differentiate in response to TZD more readily than cells from visceral depots of the same subjects[39]. A point to consider for all genes, and nuclear receptors in particular, is that gene expression levels provide limited information on their activities. The presence of cofactors, heterodimerisation, ligand availability and translocation to the nucleus are also of importance.

It is unexpected that *Acaca* (encoding acetyl-coenzyme A carboxylase α) expression, was increased in all four white adipose depots in the *n*-3 FA-fed animals, and *Fasn* (encoding FA synthase) was increased in the mesenteric and epididymal adipose depots. This may suggest enhanced synthesis of FA in these depots. However, *Dgat1* expression (encoding diacylglycerol acyltransferase) was reduced in the perirenal and epididymal adipose depots of the *n*-3 FA-fed animals. This is in line with decreased fat accumulation in these depots. It is possible that the simultaneous increase in the expression of the lipogenic enzymes *Acaca* and *Fasn*, with reduced *Dgat1* expression, reflects increased turnover with futile cycling of FA. Guan *et al.* have shown that glycerol kinase, which is normally not expressed in adipocytes, was induced by TZD in adipocytes, and propose a futile fuel cycle as a mechanism for TZD action[40] although this is controversial[41].

The hepatic activities of carnitine palmitoyltransferase-II and acyl-CoA oxidase were increased in the animals fed the *n*-3 FA diet, which might suggest that FA oxidation was elevated in these animals as compared with lard-fed rats. In addition, hepatic *Cpt2* mRNA was increased in the *n*-3 FA group in accordance with Halvorsen *et al.* [42]. Increased hepatic mitochondrial oxidation of FA may partially explain the reduction in plasma TAG observed in the *n*-3 FA group[7,43].

We observed that the mRNA levels of several cytokines and chemokines such as *Il1b*, *Tnfa*, *Tgfb1*, *Il6*, *Cxcl1*, *Ccl2* and *Retn*, and *Il10*, were increased in the mesenteric, perirenal

and/or epididymal adipose depots of the *n*-3 FA-fed animals as compared with the lard-fed animals. The metabolism of cytokines and chemokines is complex, and several of these proteins have both pro- and anti-inflammatory properties[44]. The biological effect of these findings is therefore difficult to interpret. For example, we do not know if cytokines secreted from skeletal muscle during exercise, such as IL-6, are beneficial or harmful[45,46]. The effect of *n*-3 FA feeding appears to be autocrine or paracrine in adipose tissue because we did not observe altered concentrations in plasma of TNFα, IL-6, IL-10, nor of the acute-phase protein CRP, after *n*-3 FA feeding. This could also be due to a low contribution by adipose tissue to the plasma pool of these factors. Moreover, it is possible that the increased expression of cytokines and chemokines reflects a lower proportion of adipocytes relative to leucocytes located in the adipose tissue[47,48], as well as a dilution of nuclear material in hypertrophic adipose tissue.

The effects observed in the present study may be due to an increased proportion of EPA and/or DHA, or to the reduction in content of SFA, although it is most likely that the effects are due to *n*-3 FA.

In conclusion, by substituting some dietary lard with very-long-chain *n*-3 FA, the volumes of the visceral adipose depots (mesenteric, perirenal and epididymal) in rats were markedly reduced. This occurred without affecting total body weight and body composition, suggesting that *n*-3 FA feeding redistributed fat within the body. The gene expression of several cytokines and chemokines was increased in different adipose depots, with unaltered plasma concentrations of the corresponding proteins.

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The contributions of the authors were as follows: M. H. R.-A. conducted the plasma analyses of adipokines and insulin, isolated and quality checked total RNA, conducted gene expression analyses and wrote the manuscript. A. C. R. planned and carried out the feeding experiment. A. J. W. isolated and quality checked total RNA, conducted gene expression analyses and carcass analyses. O. K. and B. A. G. performed the MRI analysis. H. W. and T. H. R. conducted the enzyme activity assays and plasma analyses of lipids and glucose. J. J. conducted the glucose uptake and glycogen content assays. R. C. conducted the adipose tissue extraction and adipokine analysis in tissue extracts. C. A. D. planned and carried out the feeding experiment, and coordinated the project. All authors contributed to revision of the manuscript.

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