Lipids (2016) 51:25–38
DOI 10.1007/s11745-015-4105-x

ORIGINAL ARTICLE

Dietary Omega-3 Fatty Acids Prevented Adipocyte Hypertrophy by Downregulating *DGAT-2* and *FABP-4* in a Sex-Dependent Fashion

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Received: 30 April 2015 / Accepted: 18 November 2015 / Published online: 11 December 2015
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Abstract Obesity is characterized by an increase in fat mass primarily as a result of adipocyte hypertrophy. Diets enriched in omega (*n*)-3 polyunsaturated fatty acids (PUFA) are suggested to reduce obesity, however, the mechanisms are not well understood. We investigated the effect of *n*-3 PUFA on adipocyte hypertrophy and the key genes involved in adipocyte hypertrophy. Female C57BL/6 mice were fed semi-purified diets (20 % w/w fat) containing high *n*-3 PUFA before mating, during pregnancy, and until weaning. Male and female offspring were continued on high *n*-3 PUFA (10 % w/w), medium *n*-3 PUFA (4 % w/w), or low *n*-3 PUFA (2 % w/w) diet for 16 weeks post-weaning. Adipocyte area was quantified using microscopy, and gonadal mRNA expression of acyl CoA:diacylglycerol acyltransferase-2 (DGAT-2), fatty acid binding protein-4 (FABP-4) and leptin were measured. The high *n*-3 PUFA group showed higher levels of total *n*-3 PUFA in gonadal TAG compared to the medium and low *n*-3 PUFA groups ($P < 0.001$). The high *n*-3 PUFA male group had a lower adipocyte area compared to the medium and low *n*-3 PUFA group ($P < 0.001$); however, no difference was observed in females. The high *n*-3 PUFA male group showed lower mRNA expression of FABP-4, DGAT-2 and leptin compared to the low *n*-3 PUFA group, with no difference in females. Plasma lipid levels were lower in the high *n*-3 PUFA group compared to the other groups. Our findings show for the first time that *n*-3 PUFA prevents adipocyte hypertrophy by downregulating FABP-4, DGAT-2 and leptin; the effects are however sex-specific.

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Keywords Adipocyte hypertrophy · DGAT-2 · FABP-4 · Leptin · *N*-3 PUFA · Obesity

Abbreviations

ARA	Arachidonic acid
ALA	Alpha linolenic acid
CREB	cAMP response element binding protein
COX	Cyclooxygenase
CVD	Cardiovascular disease
DGAT	Acyl CoA:diacylglycerol acyltransferase
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
FABP-4	Fatty acid binding protein-4
GLC	Gas liquid chromatography
HDL	High density lipoprotein
IL-6	Interleukin-6
LA	Linoleic acid
LDL	Low density lipoprotein
LDL-r	Low density lipoprotein receptor
LPL	Lipoprotein lipase
MUFA	Monounsaturated fatty acid
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
NEFA	Non-esterified fatty acids
PPARγ	Peroxisome proliferator-activated receptor-gamma
PUFA	Polyunsaturated fatty acid
SCD-1	Stearoyl-CoA desaturase-1
SFA	Saturated fatty acid
SREBP-1	Sterol regulatory element binding protein
TC	Total-cholesterol
TAG	Triacylglycerols
TNF-α	Tumor necrosis factor

Introduction

The incidence of obesity is on the rise in both developed and developing countries [1, 2]. Obesity is defined as the accumulation of excess fat, and is associated with pathophysiological conditions such as type-2 diabetes, cardiovascular disease (CVD), and dyslipidemia [3, 4]. The phenotype of obesity is characterized at the cellular level by an increase in adipose tissue mass, mainly as a result of the enlargement of adipocytes (hypertrophy) [5]. Adipocyte hypertrophy is accompanied by changes in metabolic activities such as lipid metabolism, which could exacerbate the development of obesity [6]. Larger adipocytes are known to both release more fatty acids and produce more triglycerides (TAG) relative to smaller adipocytes [7].

Adipocyte hypertrophy is a consequence of excess accumulation of TAG in the adipose tissue, and it is highly dependent on the nuclear receptor protein, peroxisome proliferator activator receptor-gamma (PPARγ). Therefore, elucidating the molecular process involved in the formation of TAG in the adipose tissue is important in understanding the development of obesity. Acyl CoA:diacylglycerol acyltransferase (DGAT) is responsible for the synthesis of TAG [8]; it catalyzes the covalent binding of acyl CoA to diacylglycerol (DAG), which is the final step of TAG synthesis [9]. DGAT-2, one of the two isoforms of the enzyme has been shown to be the main DGAT isoform involved in TAG synthesis [10]. Furthermore, the size of an adipocyte affects its endocrine functions. Larger adipocytes secrete more inflammatory cytokines and non-esterified fatty acids (NEFA), and increase lipolysis, which could lead to the development of metabolic syndrome [11]. Fatty acid binding protein-4 (FABP-4), also known as adipocyte fatty acid binding protein 2 (aP2), accounts for around 6 % of cellular protein [12] and has been implicated in the regulation of glucose and lipid metabolism [12]. Mice lacking the FABP-4 gene are genetically protected from developing obesity and its complications [13]. Adipose tissue also secretes adipokines such as leptin. Leptin is a hormone produced primarily by adipocytes and it plays a vital role in food intake and metabolism [14]. The secretion of leptin has been positively correlated with the size of adipocytes [15], and clinical studies have also shown a positive correlation between the size of adipocyte and circulating leptin concentration [16].

The etiology of obesity remains elusive; however, obesity has been suggested to arise as a result of an interplay between genetic and environmental factors [17]. The development of obesity is closely linked with nutrition; hence, nutritional intervention is a viable option of tackling the obesity epidemic. High dietary fat consumption has been suggested to be a major risk factor of obesity [18]. Besides the quantity of dietary fat, the quality of fat has also been suggested to play an important role in the development of obesity. Studies using rodents have shown that a high fat diet promotes adipose tissue differentiation and hypertrophy [19, 20]. Omega (n)-3 polyunsaturated fatty acid (PUFA) has been shown to elicit several health benefits, including but not limited to the prevention of type-2 diabetes, CVD, and alleviation of inflammation [21]. The Western diet has witnessed a transition to lower consumption of *n*-3 PUFA [22], which may be part of the reason for the rise in the prevalence of obesity. There is burgeoning evidence of the anti-obesity effects of *n*-3 PUFA. Rodent studies have shown that *n*-3 PUFA could prevent the development of insulin resistance and obesity [23, 24]. Studies in rats have also shown that *n*-3 PUFA could reduce obesity by reducing the size of an adipocyte without affecting the adipocyte number and the body weight [25].

The incidence of obesity and related complication are well established at the population level, however, there is still a lack of information on the mechanisms at the cellular level. Furthermore, the biological causes of obesity vary between both sexes [26, 27], and there are reported sex differences in adipose tissue storage and metabolism between males and females [28]. The pathological effects of obesity also differ by sex [29–31], and estrogen plays an important role in fat distribution in females [32]. Obesity has been identified as an independent risk factor of CVD in both males and females [33, 34]; however, most studies have excluded the effect of sex and an adequate amount of *n*-3 PUFA in the diet. In the present study, we hypothesized that high dietary *n*-3 PUFA will prevent adipocyte hypertrophy by downregulating the mRNA expression of DGAT-2 and FABP-4 in a sex dependent fashion in C57BL/6 mice. The specific objectives were to investigate the sex-specific effects of diets varying in the quantity of *n*-3 PUFA on adipocyte TAG fatty acid composition; on adipocyte size; on the mRNA expression of adipocyte DGAT-2, FABP-4, leptin, PPARγ, and on plasma lipids and lipoproteins of C57BL/6 mice. Our findings show for the first time that *n*-3 PUFA prevents adipocyte hypertrophy by downregulating the mRNA expression of DGAT-2, FABP-4, and leptin; however the effects were sex-dependent.

Materials and Methods

Experimental Diets

The experimental diets were prepared according to our previously published paper [35]. Briefly, a semi-synthetic base diet designed to allow the control of fat

Table 1 Composition of the semi-purified diet with 20 % (w/w) fat level

Ingredients	Semi-synthetic diets (g/kg)
Casein	200
DL-methionine	3
Sucrose	305
Corn starch	190
Alphacel non-nutritive bulk	50
Vitamin mix[a]	11
Mineral mix[b]	40
Fat	200

Supplied in quantities adequate to meet National Research Council requirements

[a] Vitamin Mix (1 kg): Thiamine hydrochloride, 0.6 g; riboflavin, 0.6 g; pyridoxine hydrochloride, 0.7 g; nicotinic acid, 3.0 g; d-calcium pantothenate, 1.6 g; folic acid, 0.2 g; d-biotin, 0.02 g; cyanocobalamin (vitamin B_{12}), 0.001 g; retinyl palmitate (vitamin A) premix (250,000 IU/g), 1.6 g; DL-α-tocopherol acetate (250 IU/g), 20 g; cholecalciferol (vitamin D_3, 400,000 IU/g), 0.25 g; menaquinone (vitamin K_2), 0.005 g; sucrose, finely powdered, 972.9 g

[b] Mineral Mix: Calcium phosphate dibasic, 500.0 g/kg; sodium chloride, 74.0 g/kg; potassium citrate monohydrate, 220.0 g/kg; potassium sulfate, 52.0 g/kg; magnesium oxide, 24.0 g/kg; manganese carbonate (43–48 % Mn), 3.50 g/kg; ferric citrate (16–17 % Fe), 6.0 g/kg; zinc carbonate (70 % ZnO), 1.6 g/kg; cupric carbonate (53–55 % Cu), 0.30 g/kg; potassium iodate, 0.01 g/kg; sodium selenite, 0.01 g/kg; chromium potassium sulfate, 0.55 g/kg; sucrose, finely powdered, 118.0 g/kg

level at 20 % w/w was purchased without fat (MP Biomedicals, USA). Fish oil (Menhaden), safflower oil, extra-virgin olive oil, and lard were used as sources of *n*-3 PUFA, *n*-6 PUFA, monounsaturated fatty acids (MUFA) and SFA respectively. The oils were used to make three different mixtures containing approximately 10 % (High *n*-3), 4 % (Medium *n*-3), and 2 % (Low *n*-3) *n*-3 PUFA of the total dietary fat, while keeping the amounts of total SFA and MUFA constant as per our previous publications [36, 37]. The medium and low *n*-3 PUFA diets were designed to contain an *n*-6:*n*-3 PUFA ratio of 15:1 and 30:1 respectively which approximately represent the current *n*-6:*n*-3 PUFA ratios in a typical Western diet; while the high *n*-3 PUFA diet contains an *n*-6:*n*-3 PUFA ratio of 5:1, which is suggested to be an optimal ratio for whole body homeostasis [22]. The diets were isocaloric and contained the same amount of protein, carbohydrate and fat; the only difference was the ratio of *n*-6 to *n*-3 PUFA. The diets were designed to test the quality of dietary fat consumed in regards to the ratio of *n*-6:*n*-3 PUFA composition. The composition of the semi-synthetic diet is provided in Table 1. The fatty acid composition of the experimental diet was analyzed as per our previous publications [35, 38] and is given in Table 2.

Animals and Experimental Design

All the experimental procedures were done in accordance with the principles and guidelines of the Canadian Council on Animal Care and were approved by Memorial University's Animal Care Committee (approval no: 10-09-SK). The C57BL/6 mouse model was used for this study as this model is susceptible to diet induced hyperlipidemia, atherosclerosis [39, 40], diabetes and obesity [41, 42], making it suitable for the study of lipid and lipoprotein metabolism. Furthermore, we chose the normal C57BL/6 mice over the obese C57BL/6 mice as we were interested in the cellular events that lead to obesity in normal physiology, and not in a disease state. The study was designed according to our previously published paper with modifications [35–37]. Seven week old male and female C57BL/6 mice were obtained from Charles Rivers Laboratories (MA, USA), and were housed in separate cages under regulated temperature (21 ± 1 °C) and humidity (35 ± 5 %) conditions with a 12-h light/12-h dark period cycle. Mice were acclimatized on standard rodent chow pellets (Prolab RMH 3000) (PMI nutrition, MO, USA) for 1 week. After this period, female mice were fed the high *n*-3 PUFA diet for 2 weeks before mating. Female mice were continued on this diet throughout gestation, lactation, and until weaning to ensure an optimum level of *n*-3 PUFA for maintaining whole body homeostasis [22]. It was important to put the mothers on the same diet in order to exclude the differential effects of *n*-3 and *n*-6 on adipogenesis at this critical stage of development [43, 44]. At weaning, the offspring (male and female) were divided into three separate groups. Each group was fed one of the three experimental diets that differed in *n*-3 PUFA levels, and designated as "High *n*-3", "Medium *n*-3", and "Low *n*-3" diets, for 16 weeks postweaning. At the end of the experimental period, the animals were sacrificed after an overnight fast using isoflurane. Blood was collected by cardiac puncture in tubes containing EDTA (4.5 mM, pH 7.4), and plasma was separated immediately. Tissues were removed and weighed at the time of sacrifice, snap frozen in liquid nitrogen and stored at −80 °C until further analyses. Animals were provided with fresh food ad libitum every other day during the period of the experiment, and water. Body weights of the animals were documented weekly, and food consumption was recorded every 2 days.

Fatty Acid Analyses of Adipocyte Triglycerides

Total lipids were extracted from gonadal fat pads using the method of Folch et al. [45] as per our previous publication [38]. Adipocyte TAG were separated on thin layer chromatography (TLC) plates using hexane: ethyl ether: acetic acid (70:30:2 v/v) [46]. The TAG spots were identified in comparison with known standard. The TAG spots were scraped and the fatty acid composition was determined

Table 2 Fatty acid composition of the experimental diets

Fatty acids (mol%)	Low *n*-3	Medium *n*-3	High *n*-3
14:0	0.13	0.48	1.56
16:0	6.91	8.13	9.58
18:0	5.27	4.47	2.65
∑SFA	12.31	13.08	13.79
16:1*n*-7	0.40	0.10	2.67
18:1*n*-9 + C18:1*n*-7	27.60	25.63	25.11
20:1*n*-9	ND	0.49	0.55
∑MUFA	28.10	26.22	28.34
18:2*n*-6	57.68	57.04	48.14
20:4*n*-6	0.10	0.13	0.21
18:3*n*-6	0.04	0.04	0.10
22:4*n*-6	0.08	ND	0.46
∑Omega-6	57.90	57.21	48.92
18:3*n*-3	0.55	0.64	0.79
20:5*n*-3	0.29	1.27	3.39
22:6*n*-3	0.33	0.99	2.74
18:4*n*-3	0.15	0.20	0.89
22:5*n*-3	0.39	0.27	0.57
20:4*n*-3	0.07	0.11	0.61
∑Omega-3	1.79	3.49	8.96

Data are expressed as mol% of the total extracted fatty acids

ΣSFA sum of saturated fatty acids, *ΣMUFA* sum of monounsaturated fatty acids, *ΣPUFA* sum of polyunsaturated fatty acids, *ΣOmega-6* sum of omega-6 fatty acids, *ΣOmega-3* sum of omega-3 fatty acids, *ND* not detected

using gas chromatography (GC) according to our previously published method [38].

Adipocyte Histology and Imaging

Adipocyte area was measured using computer image analysis according to the method of Chen and Farese [47] with minor modifications. Briefly, gonadal fat was harvested from mice fresh at time of sacrifice and fixed in 4 % paraformaldehyde for 24 h. Fats were collected at the same location to control for cell size variation. Fixed tissues were transferred to phosphate buffered saline (PBS), paraffin embedded and 5 μm sections were made. Sections were stained using standard hematoxylin and eosin (H&E) staining procedure. The stained sections were visualized using the Epifloures-ence microscope (E600) at 10X magnification. All the pictures were taken at the same time, using the same settings and magnification. The analyses of cell area were performed using the same scale bar (50 μm) to ensure uniformity. Adipocytes areas were determined using Image J software. A total of 4 images per animals were obtained, and the sizes of 40 cells in two different microscopic fields were obtained per image giving a total of 800 cells per group.

Table 3 Sequences of primers used for qPCR

Gene	Primers
DGAT-2 (S)	ctagctagttaggctaggtttcac
DGAT-2 (AS)	caggaggatatagcgccagag
FABP-4 (S)	cataaccctagataggcgggg
FABP-4 (AS)	ccagcttagtcaccatctcgt
Leptin (S)	accaggcacccttaggagggg
Leptin (AS)	tagtaggggccctcactccctag
PPARγ (S)	tagttatagggtagaaactctaggg
PPARγ (AS)	agagctagattccgaagttagg
RPLPO (S)	tcactagtagccagctcagaac
RPLPO (AS)	aatttcaataggtagcctctagg

All primers were designed using NCBI primer blast, and obtained from IDT technologies

S sense primer, *AS* anti-sense primer, *DGAT2* acyl CoA:diacylglycerol acyltransferase 2, *FABP4* fatty acid binding protein 4, *PPARγ* peroxisome proliferator activator receptor protein gamma, *RPLPO* ribosomal protein large (P0)

RNA Extraction and Real-Time qPCR

Total RNA was extracted from gonadal fat pads using Trizol method [48] and contaminating genomic DNA was removed by treating with DNAse enzyme (Promega, USA). RNA concentration and purity were measured using NanoDrop 2000 (Thermo Scientific, USA). Primers for DGAT-2, FABP-4, leptin, and PPARγ used for qPCR were designed using NCBI primer blast (www.ncbi.nlm.nih.gov/tools/primer-blast/), and obtained from IDT technologies (IA, USA); primer sequences are given in Table 3. Amplification was performed using iQ SYBER Green Supermix (Biorad, USA). The reactions were run in a reaction volume of 20 μl and 100 ng cDNA per reaction. The ΔCt was recorded for each gene of interest, and normalized with RPLPO (large ribosomal protein) as the house keeping gene. The expression levels between the two groups were compared using the Pfaffl's method [49]. The Pfaffl's method is based on the relative quantification of mRNA expression level of the target gene relative to a reference gene. This method takes into account the difference in the efficiency between the target and reference genes. The Ct value of the target gene is normalized to that of the reference gene, and the change in Ct is normalized to a change in Ct of chosen calibrator sample; the expression ratio is calculated by the published Pfaffl's equation [49].

Plasma Biochemical Parameters

Plasma biochemical parameters were measured using commercially available kits according to the manufacturer's instruction as per our previous publication [35] (kit #236-17 and 234-60 for plasma triglycerides (TAG) and total

cholesterol (TC) respectively, Genzyme Diagnostics, PEI, Canada; and kit # 999-34691 for non-esterified fatty acids (NEFA), Wako Chemicals, VA, USA). Plasma high density lipoprotein cholesterol (HDL-c) was separated from non-HDL lipoproteins (LDL, VLDL, IDL, and chylomicrons) by precipitation using kit #200-26A (Diagnostic Chemicals Ltd, Canada); the HDL-c concentration in the resulting supernatant after the separation was determined using total cholesterol assay kit #234-60 (Genzyme Diagnostics, PEI, Canada). Plasma low density lipoprotein cholesterol (LDL-c) concentration was calculated according to the method of Friedewald et al. [50, 51]. Fasting blood glucose concentration was measured at the time of sacrifice from tail blood using commercially available glucometer (Lifescan Inc, CA, USA). The current study on the effects of high *n*-3 PUFA on adipocytes was conducted at the same time as our previous study describing the sex specific effects of perinatal and postnatal dietary *n*-3 PUFA on lipids and lipoproteins [35], and therefore share the same high *n*-3 PUFA group for plasma biochemical parameters.

Statistical Analysis

Data were analysed using IBM SPSS Statistics (version 20.0). Sample means were compared using two way analysis of variance (ANOVA) to determine main effects of sex and diet, and the interactions between them. Pairwise comparison using Bonferrroni correction was used to determine differences among the groups when there was an observed statistical significant difference. Results are expressed as mean ± standard deviation (SD). Gonadal fat TAG fatty acid compositions were expressed as mol% of the total extracted fatty acids. Fatty acid composition data were then arcsine transformed and real-time qPCR data were $\log_{10}$ transformed prior to statistical analyses. Differences were considered to be statistically significant if the associated *P* value was <0.05.

Results

No significant differences were observed in body weight, fat pad weight, and food intake (data not shown) amongst the dietary groups, and between males and females.

Fatty Acid Composition of Gonadal TAG of Mice Fed Varying Quantity of Dietary n-3 PUFA

The gonadal TAG fatty acid composition of both male and female mice fed varying amounts of dietary *n*-3 PUFA is presented in Table 4. There was a concentration dependent significant increase in the gonadal levels of myristic acid (C14:0), with the high *n*-3 PUFA diet showing the highest amount ($P < 0.0001$) in both male and female mice. There was however, no effect of sex on C14:0 or an interaction between sex and diet. The male mice showed a significant independent increase in the levels of palmitic acid (C16:0), stearic acid (C18:0), and total saturated fatty acids (SFA) compared to the female mice ($P < 0.001$). The levels of C16:0 and total SFA were significantly higher in the high *n*-3 PUFA group compared to the low and medium *n*-3 PUFA groups in male offspring ($P < 0.001$). The level of C18:0 was higher in the high *n*-3 PUFA group compared to the medium *n*-3 PUFA group, but was not different from the low *n*-3 PUFA group. On the other hand, females showed higher level of C18:0 in the high *n*-3 PUFA diet compared to both the low and medium *n*-3 PUFA groups ($P < 0.001$). Similar to the males, females in the high *n*-3 PUFA group also showed the highest total SFA level followed by the medium and the low *n*-3 PUFA groups ($P < 0.001$).

Female mice showed a significantly higher level of palmitoleic acid (C16:1n7) compared to male mice ($P < 0.001$); however, no effect of sex was observed in the TAG fatty acid levels of C18:1n9/C18:1n7, C20:1n9, and total MUFA. High *n*-3 PUFA group showed the lowest level of C18:1n9/C18:1n7 compared to the medium and low *n*-3 PUFA groups in both male and female mice. The medium *n*-3 PUFA group showed significantly lower level of C18:1n9/C18:1n7 than the low *n*-3 PUFA group ($P < 0.001$). No difference was observed in the level of C20:1n9 among the three dietary groups in male mice. The female mice fed the high *n*-3 PUFA diet however showed significantly lower levels of C20:1n9 compared to the medium and low *n*-3 PUFA groups ($P < 0.01$). A lower concentration of total MUFA was observed in the high *n*-3 PUFA mice compared to the low *n*-3 PUFA mice for both male and female mice ($P < 0.001$).

There was no effect of sex on individual and total gonadal *n*-6 PUFA levels, and no interaction between diet and sex was observed; however, there was a significant independent effect of diet in both male and female mice ($P < 0.01$). Interestingly, there was a significantly higher amount of linoleic acid (LNA; C18:2n6) and total *n*-6 PUFA in the high *n*-3 PUFA group compared to both medium and low *n*-3 PUFA groups in male mice ($P < 0.05$). A similar trend was also observed in female mice; however, there was no statistically significant difference in LNA between high and medium *n*-3 PUFA groups. Arachidonic acid (ARA; C20:4n6) was significantly lower in the high and medium *n*-3 PUFA groups compared to the low *n*-3 PUFA group in the male mice; however, no difference was observed in the female mice among the three dietary groups.

There was a significant interaction of sex and diet in the levels of eicosapentaenoic acid (EPA; C20:5n3) ($P < 0001$), docosahexaenoic acid (DHA; C22:6n3) ($P < 0.01$) and total

Table 4 Gonadal triglyceride fatty acid composition of male and female mice fed diets varying in the quantity of *n*-3 polyunsaturated fatty acids

Fatty acids (mol%)	Male			Female			Main effect		
	Low *n*-3	Medium *n*-3	High *n*-3	Low *n*-3	Medium *n*-3	High *n*-3	Diet	Sex	Diet × sex
C14:0	1.0 ± 0.04^{c}	1.28 ± 0.08^{b}	2.06 ± 0.31^{a}	1.0 ± 0.16^{z}	1.2 ± 0.09^{y}	2.0 ± 0.23^{x}	$P < 0.0001$	NS	NS
C16:0	14.8 ± 0.49^{b}	15.3 ± 0.82^{b}	16.9 ± 1.56^{a}	12.1 ± 1.44^{y}	13.74 ± 1.24^{xy}	15.0 ± 1.62^{x}	0.0002	$P < 0.0001$	NS
C18:0	2.7 ± 0.35^{ab}	2.4 ± 0.35^{b}	3.3 ± 0.81^{a}	1.86 ± 0.25^{y}	2.1 ± 0.18^{y}	2.62 ± 0.43^{x}	0.0007	0.0005	NS
∑SFA	18.5 ± 0.72^{b}	18.9 ± 1.00^{b}	22.2 ± 1.76^{a}	15.0 ± 1.76^{z}	17.0 ± 1.35^{y}	19.67 ± 1.82^{x}	$P < 0.0001$	$P < 0.0001$	NS
C16:1n7	4.4 ± 0.80	4.9 ± 0.62	$4.9 \pm 0.$	8.0 ± 1.38	6.78 ± 1.39	6.23 ± 0.75			
C18:1n9/18:1n7	49.23 ± 3.17^{c}	44.8 ± 4.4^{b}	34.4 ± 1.62^{a}	48.6 ± 5.51^{z}	43.1 ± 3.29^{y}	34.4 ± 1.93^{x}	$P < 0.0001$	NS	NS
C20:1n9	0.6 ± 0.07	0.5 ± 0.27	0.53 ± 0.03	0.84 ± 0.4^{y}	0.59 ± 0.09^{y}	0.37 ± 0.11^{x}	0.009	NS	NS
∑MUFA	54.2 ± 2.88^{b}	50.3 ± 4.53^{b}	39.8 ± 1.66^{a}	57.5 ± 5.82^{z}	50.5 ± 4.11^{y}	40.96 ± 2.63^{x}	$P < 0.0001$	NS	NS
C18:2n6	26.8 ± 3.18^{b}	30.2 ± 4.41^{b}	36.2 ± 2.18^{a}	26.53 ± 7.17^{y}	31.4 ± 3.16^{xy}	37.8 ± 2.72^{x}			
C20:4n6	0.2 ± 0.03^{b}	0.11 ± 0.03^{a}	0.14 ± 0.02^{a}	0.18 ± 0.06	0.14 ± 0.05	0.1 ± 0.06	0.001	NS	NS
∑Omega-6	27.0 ± 3.2^{b}	30.3 ± 4.43^{b}	36.4 ± 2.19^{a}	26.7 ± 7.17^{y}	31.6 ± 3.2^{y}	37.87 ± 2.73^{x}	$P < 0.0001$	NS	NS
C18:3n3	0.17 ± 0.03	0.14 ± 0.11	0.18 ± 0.03	0.12 ± 0.1	0.19 ± 0.11	0.18 ± 0.11			
C18:4n3	0.06 ± 0.06	0.08 ± 0.05	0.13 ± 0.05	0.3 ± 0.3	0.15 ± 0.11	0.3 ± 0.3	NS	NS	NS
C20:4n3	ND	ND	0.09 ± 0.02	0.15 ± 0.09	0.12 ± 0.09	0.07 ± 0.06	NS		
C20:5n3	ND	0.168 ± 0.07^{b}	0.44 ± 0.13^{a}	0.24 ± 0.12^{y}	0.27 ± 0.09^{y}	0.45 ± 0.15^{x}	$P < 0.0001$	0.003	$P < 0.0001$
C22:5n3	ND	ND	0.25 ± 0.04	ND	0.1 ± 0.1^{y}	0.24 ± 0.03^{x}	$P < 0.0001$		
C22:6n3	ND	0.1 ± 0.1^{b}	0.47 ± 0.07^{a}	0.1 ± 0.07^{y}	0.15 ± 0.06^{y}	0.41 ± 0.06^{x}	$P < 0.0001$	NS	0.01
∑Omega-3	0.3 ± 0.16^{c}	0.48 ± 0.19^{b}	1.57 ± 0.25^{a}	0.77 ± 0.49^{y}	0.81 ± 0.24^{y}	1.44 ± 0.35^{x}	$P < 0.0001$	$P < 0.02$	0.04

Data are expressed as mol% of the total extracted fatty acids. Values are expressed as mean ± SD, $n = 6$. Main effects and interactions were determined by two-way ANOVA after arcsine transformation. Letters (a, b, c) were used to denote significant differences between dietary groups in males, and letters (x, y, z) represent significant differences between dietary groups in females

ΣSFA sum of saturated fatty acids, *ΣMUFA* sum of monounsaturated fatty acids, *ΣPUFA* sum of polyunsaturated fatty acids, *ΣOmega-6* sum of omega-6 polyunsaturated fatty acids, *ΣOmega-3* sum of omega-3 polyunsaturated fatty acids, *ND* not detected, *NS* not significant

n-3 PUFA ($P < 0.05$). As expected, the high *n*-3 PUFA fed male and female mice had a significantly higher accretion of EPA, DPA, DHA and total *n*-3 PUFA compared to the low *n*-3 PUFA group ($P < 0.001$). There was however no difference in DHA and total *n*-3 PUFA between the medium and low *n*-3 PUFA group in the female mice. Interestingly, female mice had a significantly higher level of DHA and total *n*-3 PUFA compared to male mice ($P < 0.01$).

Adipocyte Area of Mice Fed Varying Quantity of Dietary n-3 PUFA

H&E staining of the gonadal fat pads revealed smaller adipocytes in male mice fed the high *n*-3 PUFA diet compared to other groups (Fig. 1a–c); however, no noticeable difference was observed among the three dietary groups in the female mice (Fig. 1d–f). Imaging and statistical analyses of the stained sections showed a significant interaction between sex and diet on adipocyte area ($P < 0.001$; Fig. 1d). The female mice in the medium and low *n*-3 PUFA groups had significantly smaller adipocytes ($P < 0.001$; Fig. 1g) compared to their male counterparts. Male mice on the high *n*-3 PUFA diet had significantly smaller adipocytes compared to male mice on both medium and low *n*-3 PUFA diet ($P < 0.001$; Fig. 1g); no difference was observed in the male mice between the medium and low *n*-3 PUFA groups. There was no difference in the size of adipocytes in female mice among the three dietary groups.

Gonadal mRNA Expressions of FABP-4, DGAT-2, Leptin, and PPARγ of Mice Fed Varying Quantity of Dietary n-3 PUFA

The mRNA expressions of key genes involved in adipocyte hypertrophy were measured using real time qPCR. No significant interaction was observed between sex and diet in the mRNA expression levels of FABP-4 and leptin; however, there were independent effects of sex and diet (Fig. 2a, b). The high *n*-3 PUFA diet revealed significantly lower mRNA expression of FABP-4 and leptin compared to low *n*-3 PUFA diet ($P < 0.05$; Fig. 2a, b). Interestingly, similar to the adipocyte data, the female mice fed the low and medium *n*-3 PUFA diets showed lower mRNA expression levels of FABP-4 and leptin compared to the male counterparts ($P < 0.05$; Fig. 2a, b). There was an interaction between sex and diet on the mRNA expression of DGAT-2 and PPARγ ($P < 0.05$; Fig. 2c, d), where female mice showed a lower expression. The high *n*-3 PUFA group had significantly lower mRNA expression of DGAT-2 and PPARγ compared to the low *n*-3 PUFA groups in male

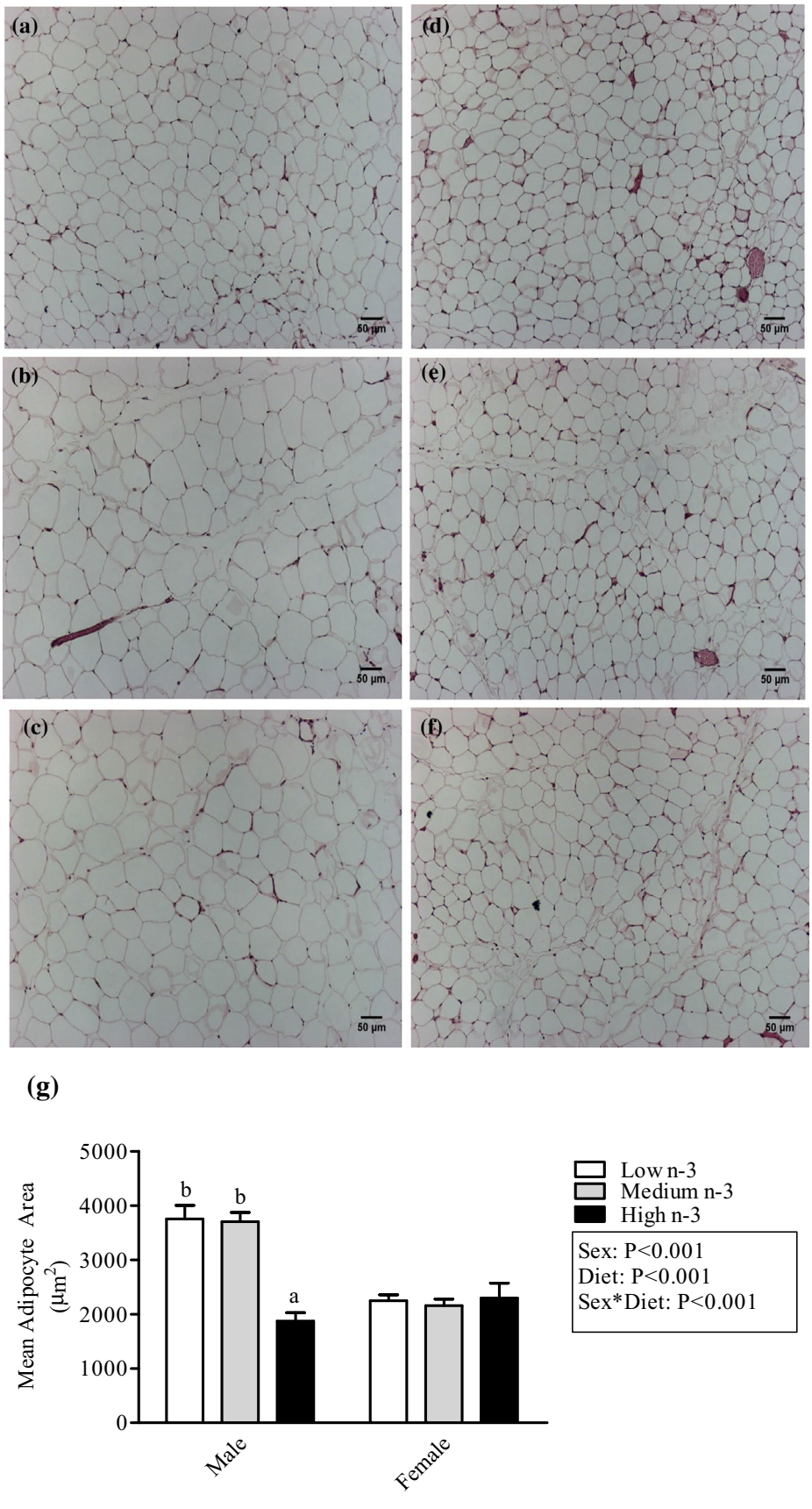

Fig. 1 Effect of diets varying in the quantity of *n*-3 PUFA on gonadal adipocyte size. Hematoxylin and eosin stained histological sections of gonadal fat of male mice (**a**–**c**) fed high, medium, and low *n*-3 PUFA diets respectively; and female mice (**d**–**f**) fed high, medium, and low *n*-3 PUFA diets respectively at image capture of ×10 magnification. **g** represents the mean surface area of gonadal adipocytes in male and female mice; *bar* represents 50 µm. Values are expressed as means ± SD, $n = 5$ per dietary group; 800 cells were measured per group. Data were assessed using two way ANOVA to determine the main effects and interactions of diets and sex; pairwise comparison using Bonferroni correction was used to determine differences when there was an observed statistical significant difference. *Letters* (*a*, *b*) represent significant differences between dietary groups in male mice where $P < 0.05$ was considered significant

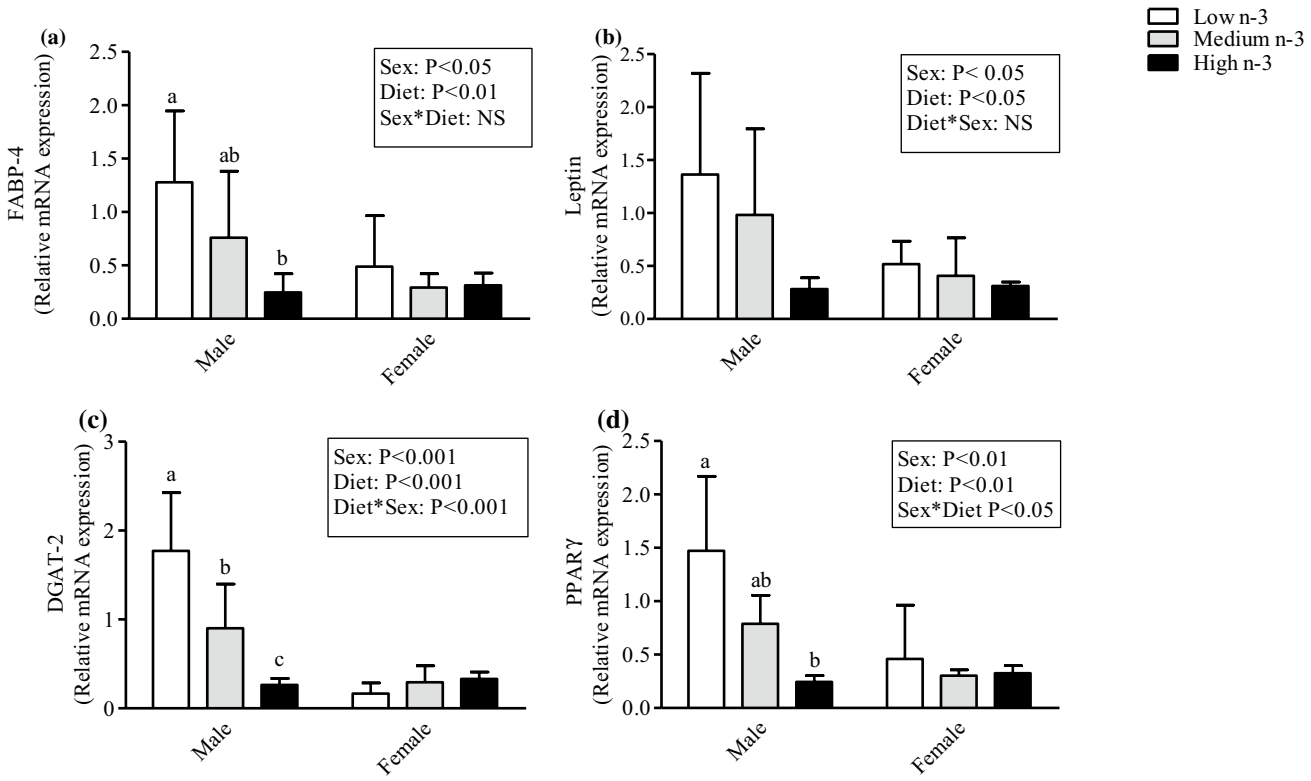

Fig. 2 Effect of diets varying in the quantity of *n*-3 PUFA on gonadal mRNA expression. The data represent male and female gonadal mRNA expression of: **a** fatty acid binding protein-4 (FABP-4); **b** leptin, **c** Acyl CoA:diacylglycerol acyltranferase-2 (DGAT-2), and **d** peroxisome proliferator activator receptor protei*n*-gamma (PPAR-γ) normalized with RPLPO as the house-keeping gene. Values are expressed as means ± SD, $n = 6$ per dietary group. Data were assessed using two way ANOVA to determine the main effects and interactions of diets and sex; pairwise comparison using Bonferroni correction was used to determine differences when there was an observed statistical significant difference. *Letters* (*a*, *b*, *c*) represent significant differences between dietary groups in males, where $P < 0.05$ was considered significant. *NS* not significant

mice ($P < 0.05$; Fig. 2c, d); however, no significant differences were observed among the three dietary groups in female mice.

Plasma Biochemical Parameters of Mice Fed Varying Quantity of Dietary n-3 PUFA

Obesity could result in hyperglycemia and dyslipidemia, characterized by an increase in plasma concentration of lipids and lipoproteins; thus we measured these parameters in mice fed the high, medium and low levels of *n*-3 PUFA (Table 5). The high *n*-3 PUFA male mice had significantly lower concentration of fasting plasma glucose compared to the medium and low *n*-3 PUFA groups ($P < 0.05$); however, no statistical significant difference was observed among different dietary groups in the females. There was no independent effect of sex on plasma concentration of NEFA; there was also no interaction between sex and diet. However, both male and female mice fed the low *n*-3 PUFA diet had a significantly higher concentration of NEFA compared to the medium and high *n*-3 PUFA groups ($P < 0.01$). However, there was no significant difference in plasma NEFA concentration between the high and medium *n*-3 PUFA groups in both males and females. Similar to the adipocyte area data, there was a sex dependent effect of diet on plasma concentration of TAG ($P < 0.001$). The female mice in the low and medium *n*-3 PUFA groups had a significantly lower plasma TAG concentration compared to their male counterparts ($P < 0.01$). The concentration of TAG was significantly lower in the high *n*-3 PUFA groups compared to the medium and low *n*-3 PUFA groups in male offspring ($P < 0.001$); however, no difference was observed between the low and medium *n*-3 PUFA groups. Interestingly, female mice showed no significant effect of the diet in any of the dietary groups. Plasma total cholesterol (TC) concentration was lower in female mice compared to male mice ($P < 0.01$). The high *n*-3 PUFA group had a significantly lower concentration of TC compared to the medium and low *n*-3 PUFA groups in both male and female mice ($P < 0.01$). There was no effect of sex on plasma low density lipoprotein cholesterol (LDL-c) levels; however, similar to TC data, LDL-c was lower in the high *n*-3

Table 5 Plasma biochemical parameters of mice fed diets varying in the quantity of *n*-3 polyunsaturated fatty acids

Lipids (mmol/L)	Male			Female			Main effect		
	Low *n*-3	Medium *n*-3	High *n*-3	Low *n*-3	Medium *n*-3	High *n*-3	Diet	Sex	Diet × sex
Glucose	11.0 ± 1.3^{a}	11.7 ± 2.0^{a}	9.3 ± 1.5^{b}	10.0 ± 1.43	9.6 ± 1.27	9.1 ± 2.27	0.03	0.03	NS
NEFA	0.49 ± 0.19^{a}	0.32 ± 0.07^{b}	0.27 ± 0.08^{b}	0.41 ± 0.12^{x}	0.3 ± 0.08^{y}	0.24 ± 0.1^{y}	$P < 0.0001$	NS	NS
TAG	0.41 ± 0.08^{a}	0.46 ± 0.1^{a}	0.23 ± 0.08^{b}	0.28 ± 0.05	0.28 ± 0.08	0.28 ± 0.08	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
TC	1.78 ± 0.23^{a}	1.86 ± 0.16^{a}	1.25 ± 0.15^{b}	1.63 ± 0.15^{x}	1.54 ± 0.24^{x}	1.1 ± 0.18^{y}	$P < 0.0001$	$P < 0.0001$	NS
LDL	1.22 ± 0.24^{a}	1.34 ± 0.2^{a}	0.77 ± 0.16^{b}	1.15 ± 0.17^{x}	1.13 ± 0.23^{x}	0.71 ± 0.16^{y}	$P < 0.0001$	NS	NS
HDL	0.38 ± 0.07	0.32 ± 0.1	0.41 ± 0.15	0.36 ± 0.13	0.28 ± 0.09	0.31 ± 0.08	NS	NS	NS

Values are expressed as means ± SD, $n = 8$. Data were assessed using two way ANOVA to determine the main effects and interactions of diets and sex; pairwise comparison using Bonferroni correction was used to determine differences when there was a significant difference. Letters (a, b) were used to denote significant differences between dietary groups in males, and letters (x, y) represent significant differences between dietary groups in females. $P < 0.05$ was considered significant

NEFA non-esterified fatty acids, *TAG* triglycerides, *TC* total cholesterol, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *NS* not significant

PUFA group compared to the medium and low *n*-3 PUFA groups in both male and female mice, and no difference was observed between medium and low *n*-3 PUFA groups ($P < 0.01$). Diet and sex had no effect on the plasma concentration of high density lipoprotein cholesterol (HDL-c) in both male and female mice.

Discussion

The underlying mechanism connecting obesity with the pathogenesis of type-2 diabetes and CVD is poorly understood; however, the role of adipose tissue has been implicated [52]. In the current study, the sex-dependent effect of diets varying in the quantity of *n*-3 PUFA was investigated on adipocyte hypertrophy, and the mRNA expression of key genes such as DGAT-2, FABP-4, leptin, and PPARγ involved in adipose tissue enlargement. We report a novel sex-specific effect of *n*-3 PUFA on the cellular events that lead to obesity by preventing adipocyte hypertrophy through the downregulation of the mRNA expression of DGAT-2 and FABP-4.

Adipose tissue composition of essential fatty acids is mainly determined by dietary intake [53]; it was thus imperative to assess the accretion of dietary *n*-3 PUFA in adipose tissue TAG. As expected, there was a dose dependent accretion of EPA, DHA, and total *n*-3 PUFA in adipose tissue. The high *n*-3 PUFA group had the highest accretion of EPA, DHA, and total *n*-3 PUFA compared to the two other groups; these findings are in line with previously reported data [53]. Interestingly, there was higher accretion of medium chain and saturated fatty acids compared to longer chain and highly unsaturated fatty acids in the high *n*-3 PUFA group. Studies have shown that the mobilization of fatty acids from adipose tissue is a function of their structure and not their composition in adipose tissue [54]. SFA was reported to be mobilized the least, followed by MUFA, and PUFA was mobilized the most, especially the 20 carbon PUFAs such as EPA and ARA because they serve as precursors to eicosanoids production [54]. Thus, the lower levels of EPA and ARA in our study could be due to rapid mobilization of these fatty acids for physiological functions. The high *n*-3 PUFA group, however, showed a lower concentration of ARA and higher concentration of EPA in the gonadal TAG. ARA is metabolized to pro-inflammatory, pro-aggregatory, and vasoconstrictive eicosanoids, which support the development of CVD [55]. Conversely, EPA-derived eicosanoids are anti-inflammatory and prevent the development CVD [56]. Higher amounts of *n*-3 PUFA in mice fed with a high *n*-3 PUFA diet suggests that these fatty acids and their bioactive metabolic products could be responsible for regulating key genes involved in adipocyte metabolism, thereby preventing obesity and associated complications. Another interesting observation is the increase in gonadal SFA and a decrease in gonadal MUFA in the high *n*-3 PUFA group compared to both the medium and low *n*-3 PUFA groups; this increase could be due to the reduction in the desaturation of SFA to MUFA in the high *n*-3 PUFA group. Stearoyl-CoA desaturase-1 (SCD-1) is the enzyme responsible for the synthesis of MUFA from SFA. Studies have reported a positive correlation between SCD-1 and obesity [57], and a high activity of SCD-1 increases TAG synthesis and decreases fatty acid oxidation [57], consistent with obesity. Although we did not measure the expression of SCD-1, however, we suspect a downregulaion of the gonadal fat expression of SCD-1 in the high n-3 PUFA group, which could be responsible for the high SFA observed in the high n-3 PUFA group.

A change in the mass of the adipose tissue is characterized by an increase in size and/or number of adipocytes

[58]. Mature adipocytes are post-mitotic and can only undergo hypertrophy, as a result, new adipocytes arise from a population of preadipocytes [59]. The progression of obesity does not affect the death or production of adipocyte, however there is enlargement of adipose tissue mass primarily by adipocyte hypertrophy [60]. Our data show a prevention of adipocyte hypertrophy in response to high dietary *n*-3 PUFA in male mice; however, this response was not observed in female mice. Furthermore, there was no effect of any of the diets on body weight of male or female mice. Only very few rodent studies have reported that *n*-3 PUFA could reduce obesity by reducing the size of an adipocyte without affecting the adipocyte number and the body weight [25]. Given the gender disparity in the development of obesity and CVD, no study has investigated the sex-specific effect of *n*-3 PUFA on adipocyte hypertrophy. We are the first to report the sex-specific effects of diets varying in the amount of *n*-3 PUFA on adipocyte hypertrophy. The mechanism for the observed sex-specific differences in adipocyte hypertrophy between male and female is unclear; our speculation is that the differences are mediated by sex hormones. Body composition differs between male and females, and fat deposition is driven by testosterone and estrogen differently in both genders [61]. The female sex hormone estrogen has been shown to drive adipose tissue proliferation [62]. Furthermore, the metabolism of *n*-3 PUFA is affected by estrogen [63]; thus there could be a possible interplay in the metabolism of *n*-3 PUFA, sex hormones, and adipose tissue hypertrophy.

Adipocyte hypertrophy is mainly caused by excess storage of TAG in the adipose tissue; therefore, regulating the storage of TAG in adipose tissue could be a potentially viable mechanism for preventing obesity. DGAT catalyzes the final step of TAG synthesis [9], which makes it an important enzyme in TAG accumulation in adipose tissue. Our study demonstrates that high *n*-3 PUFA diet reduced the mRNA expression of DGAT-2 in a sex-dependent fashion; with smaller adipocytes showing the lowest mRNA expression of DGAT-2. Two isoforms of DGAT have been identified [64, 65]; the genes that code for DGAT-1 and DGAT-2 belong to different families, however, they have comparable substrate specificity [66]. Increased expression of DGAT-2 is associated with the formation of large lipid droplets, on the contrary overexpression of DGAT-1 produces small lipid droplets [10], emphasizing the importance of DGAT-2 in TAG synthesis. Furthermore, DGAT-2 can compensate for the function of DGAT-1, however, DGAT-1 cannot compensate for DGAT2. *In-vitro* downregulation of DGAT-2 results in reduction of TAG synthesis, and inhibition of DGAT-2 alleviates markers of hyperlipidaemia, obesity and CVD by inhibiting the enzymes involved in lipogenesis [67]. Our finding showing the inhibition of DGAT-2 mRNA expression by a high *n*-3 PUFA diet is novel, and to the best of our knowledge, no study has linked adipocyte hypertrophy to the regulation of DGAT-2 by *n*-3 PUFA. Dietary *n*-3 PUFA could serve as a promising therapy for downregulating the expression of DGAT-2 and consequently preventing the cellular events that lead to obesity.

DGAT-2 requires the presence of free fatty acids to catalyze the covalent binding of acyl CoA to DAG, and larger adipocytes are known to release more fatty acids into circulation compared to smaller adipocytes [68, 69]. FABP are proteins found in the cytoplasm that binds strongly with a variety of fatty acids, and are evolutionarily conserved between species and are involved in the transport of fatty acids for utilization by enzymes and tissues [70]. Under normal conditions, FABP-4 is responsible for regulating the release and transport of fatty acids [71], and is largely expressed in adipocytes and macrophages [72, 73]. It has been reported that the production and secretion of FABP-4 in the adipose tissue result in lipogenesis and excess secretion of free fatty acids and TAG, which could lead to ectopic lipid accumulation and consequent development of metabolic syndrome [74]. We report for the first time, a reduction in the mRNA expression of FABP-4 in response to high *n*-3 PUFA diet and adipocyte hypertrophy; however, this effect was sex-specific, where female mice showed a lower mRNA expression of FABP-4 compared to male mice. The role of FABP-4 in obesity is not well studied; however, it has been suggested that FABP-4 could be a novel adipokine just like leptin and adiponectin whose expression levels correlate with the size of the adipocyte [75]. Obese individuals have been shown to have higher expression of FABP-4 [12, 76] and individuals who lost weight were reported to have a reduced expression of FABP-4.

FABP-4 is transcriptionally regulated [77]; it has been suggested that FABP4 has a peroxisome proliferator receptor element (PPRE) in its promoter region [78]. PPARγ is highly expressed in the adipose tissue and FABP-4 may act closely with PPARγ to elicit its biological function [79]. Upon interacting with PPARγ ligand, cytosolic FABP-4 translocates to the nucleus where it interacts with PPARγ thereby stimulating its transcriptional activity [79]. Interestingly, similar to our findings with downregulation of FABP-4 mRNA expression by a high *n*-3 PUFA diet, the mRNA expression of PPARγ was also significantly lower in the high *n*-3 PUFA group compared to the low *n*-3 PUFA group. Furthermore, the effect was sex-specific with female mice showing a lower expression of PPARγ compared to male mice. Targeting the expression of FABP4 by *n*-3 PUFA could be a potential mechanism to prevent adipocyte hypertrophy and diet induced obesity.

The function of the adipose tissue is not only limited to the storage of TAG, it also functions as an endocrine organ, and release a number of adipokines such as leptin

into circulation [80]. The mRNA expression of leptin has been shown to be reduced by *n*-3 PUFA in both in vitro and in vivo studies [81]. We found a sex-dependent reduction in the mRNA expression of leptin in response to high *n*-3 PUFA diet, with the female mice showing a lower expression compared to male mice that correspond with the size of the adipocytes. Leptin is mostly expressed following the differentiation of pre-adipocytes to adipocytes which marks the onset of lipid accumulation in the cell [82]; and the expression is increased postweaning in rodents [83]. As the adipose tissue grows, the secretion of leptin also increases [84]. A strong association has been reported between leptin secretion and adipocyte size [85] which confirms our observations. We are however reporting for the first time that the effect of *n*-3 PUFA on leptin mRNA expression is sex-specific. There is a direct correlation between the mRNA expression of leptin and PPARγ; a reduction in leptin mRNA expression also leads to a reduction in the mRNA expression of PPARγ [81]. Our findings demonstrate a reduction in leptin and PPARγ mRNA expression on a high *n*-3 PUFA diet, further supporting the regulation of leptin by PPARγ. It has also been suggested that leptin controls circulating lipid concentration and regulates the size of an adipocyte by influencing the expression of DGAT through the central nervous system thereby controlling the level of TAG synthesis [86], supporting our observations on similar changes in leptin, DGAT and adipocyte hypertrophy.

Obesity is closely associated with hyperglycemia and dyslipidemia, a disorder of lipid and lipoprotein metabolism, involving hypercholesterolemia, hypertriglyceridemia, and elevated LDL-cholesterol concentrations [87, 88]. One of the major causes of dyslipidemia is the alteration of fatty acid metabolism in the adipose tissue [89, 90]. It has been reported that the production and secretion of FABP-4 in the adipose tissue result in lipogenesis and excess secretion of free fatty acids and TAG, which could lead to ectopic lipid accumulation and consequent development of metabolic syndrome [74]. The plasma biochemical parameters in our study showed that the high *n*-3 PUFA diet had reduced blood glucose and plasma concentrations of TAG and NEFA in the male offspring; however, the female mice showed no difference in plasma TAG concentration, which is similar to the trend we observed in the adipocyte cell size. Furthermore, *n*-3 PUFA has been shown to reduce TAG by stimulating β-oxidation of NEFA among other hypotriglyceridemic effects of *n*-3 PUFA [91]. N-3 PUFA has also been shown to decrease the expression of srebp-1c a key regulator of lipogenesis [92]; it is possible that changes in the expression of srebp-1c in response to the high n-3 PUFA diet could be responsible for the low TG in males observed in our setting; this needs to be investigated. An increase in the release of NEFA from adipocytes leads to the accelerated uptake of these fatty acids by the liver and channeled towards the production of TAG, cholesterol and LDL [93]. This was confirmed in our study, as the low *n*-3 PUFA group had higher plasma LDL-c and NEFA concentration compared to the low *n*-3 PUFA groups.

A higher concentration of TC was also observed in low and medium *n*-3 PUFA groups compared to the high *n*-3 PUFA group in male mice. It has been reported that the higher concentration of cholesterol observed in obese individuals leads to the production of more LDL-c resulting in the downregulation of the expression of LDL-receptor-(r) and reduced clearance of LDL-c [94, 95]. It was interesting however to notice that the plasma NEFA and TC cholesterol concentrations were lower in the high *n*-3 PUFA group compared to the low *n*-3 PUFA group in female mice, despite no significant difference in adipocyte size, suggesting a sex specific effect on lipid and lipoprotein metabolism. We have previously reported the sex-specific effects of high *n*-3 PUFA diet on plasma lipids and lipoprotein concentration [35] likely due to a clear distinction in hormonal regulation between male and female [96].

Our findings suggest that targeting the expression of FABP-4 and DGAT-2 by *n*-3 PUFA may be a mechanism to prevent adipocyte hypertrophy and the cellular events that lead to obesity in males. Although we did not measure markers of type-2 diabetes, ectopic fat deposition and adipose tissue dysfunction which are common in obese individuals; we can infer from the plasma lipid and lipoprotein data that the dyslipidemia associated with obesity is reduced by the administration of high *n*-3 PUFA diet. Furthermore, body composition differs between male and females, and fat deposition is driven by testosterone and estrogen differently in both sexes [61]. We acknowledge that we only studied gonadal fat depot which is one of a number of fat depots that may affect obesity. Future studies could compare the effects of dietary *n*-3 PUFA on the different major adipose tissue depot (subcutaneous and visceral) and adipocyte hypertrophy; this will give vital information on the depot specific effect of *n*-3 PUFA on adipose tissue and how it relates to the cellular events that lead to obesity.

Taken together, our data suggest that *n*-3 PUFA could prevent the cellular events that lead to obesity in males. We are proposing that the downregulation of DGAT-2, FABP-4 and leptin by *n*-3 PUFA is facilitated by PPARγ. Our findings also demonstrate that although female mice had lower expression of the key genes involved in adipocyte hypertrophy, lower levels of lipids and lipoproteins, and smaller adipocytes compared to male mice, males appeared to me more responsive to high *n*-3 PUFA diet. Thus, our findings emphasize that sex differences should be incorporated in the dietary recommendation of *n*-3 PUFA for the prevention of obesity and associated complications.

Acknowledgments This research was supported by funds from the Natural Sciences and Engineering Research Council (NSERC) and Canada Foundation for Innovation (CFI). We acknowledge the technical support provided by Sophie Gagnon for her help with GC. We would also like to acknowledge Dr. Sherri Christian for her assistance with measuring the adipocyte size. The authors declare no conflict of interest.

Compliance with Ethical Standards

Conflict of interest The authors report no conflict of interest.

References

1. Dunstan DW, Zimmet PZ, Welborn TA, De Courten MP, Cameron AJ, Sicree RA, Dwyer T, Colagiuri S, Jolley D, Knuiman M, Atkins R, Shaw JE (2002) The rising prevalence of diabetes and impaired glucose tolerance: the Australian Diabetes, Obesity and Lifestyle Study. Diabetes Care 25:829–834
2. Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL (1998) Overweight and obesity in the United States: prevalence and trends, 1960–1994. Int J Obes Relat Metab Disord 22:39–47
3. Grundy SM (2004) Obesity, metabolic syndrome, and cardiovascular disease. J Clin Endocrinol Metab 89:2595–2600
4. Zou C, Shao J (2008) Role of adipocytokines in obesity-associated insulin resistance. J Nutr Biochem 19:277–286
5. van Harmelen V, Skurk T, Rohrig K, Lee YM, Halbleib M, Aprath-Husmann I, Hauner H (2003) Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. Int J Obes Relat Metab Disord 27:889–895
6. Engfeldt P, Arner P (1988) Lipolysis in human adipocytes, effects of cell size, age and of regional differences. Horm Metab Res Suppl 19:26–29
7. Jamdar SC (1978) Glycerolipid biosynthesis in rat adipose tissue. Influence of adipose-cell size and site of adipose tissue on triacylglycerol formation in lean and obese rats. Biochem J 170:153–160
8. Bell RM, Coleman RA (1980) Enzymes of glycerolipid synthesis in eukaryotes. Annu Rev Biochem 49:459–487
9. Chen HC, Smith SJ, Tow B, Elias PM, Farese RV Jr (2002) Leptin modulates the effects of acyl CoA:diacylglycerol acyltransferase deficiency on murine fur and sebaceous glands. J Clin Invest 109:175–181
10. Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, Elias PM, Farese RV Jr (2004) Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. J Biol Chem 279:11767–11776
11. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T et al (1999) PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol Cell 4:597–609
12. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS (2006) Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem 52:405–413
13. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS (2000) Improved glucose and lipid metabolism in genetically obese mice lacking aP2. Endocrinology 141:3388–3396
14. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252
15. Guo KY, Halo P, Leibel RL, Zhang Y (2004) Effects of obesity on the relationship of leptin mRNA expression and adipocyte size in anatomically distinct fat depots in mice. Am J Physiol Regul Integr Comp Physiol 287:R112–R119
16. Couillard C, Mauriege P, Imbeault P, Prud'homme D, Nadeau A, Tremblay A, Bouchard C, Despres JP (2000) Hyperleptinemia is more closely associated with adipose cell hypertrophy than with adipose tissue hyperplasia. Int J Obes Relat Metab Disord 24:782–788
17. Orio F Jr, Palomba S, Cascella T, Savastano S, Lombardi G, Colao A (2007) Cardiovascular complications of obesity in adolescents. J Endocrinol Invest 30:70–80
18. Astrup A, Dyerberg J, Selleck M, Stender S (2008) Nutrition transition and its relationship to the development of obesity and related chronic diseases. Obes Rev 9(Suppl 1):48–52
19. Ellis JR, McDonald RB, Stern JS (1990) A diet high in fat stimulates adipocyte proliferation in older (22 month) rats. Exp Gerontol 25:141–148
20. Shillabeer G, Lau DC (1994) Regulation of new fat cell formation in rats: the role of dietary fats. J Lipid Res 35:592–600
21. Connor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71:171S–175S
22. Gomez Candela C, Bermejo Lopez LM, Loria Kohen V (2011) Importance of a balanced omega 6/omega 3 ratio for the maintenance of health: nutritional recommendations. Nutr Hosp 26:323–329
23. Ruzickova J, Rossmeisl M, Prazak T, Flachs P, Sponarova J, Veck M, Tvrzicka E, Bryhn M, Kopecky J (2004) Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. Lipids 39:1177–1185
24. Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, Hensler M, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, Kus V, Polak J, Mohamed-Ali V, Burcelin R, Cinti S, Bryhn M, Kopecky J (2009) n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. Diabetologia 52:941–951
25. Parrish CC, Pathy DA, Angel A (1990) Dietary fish oils limit adipose tissue hypertrophy in rats. Metabolism 39:217–219
26. Lovejoy JC (2003) The menopause and obesity. Prim Care 30:317–325
27. Wardle J, Haase AM, Steptoe A, Nillapun M, Jonwutiwes K, Bellisle F (2004) Gender differences in food choice: the contribution of health beliefs and dieting. Ann Behav Med 27:107–116
28. Power ML, Schulkin J (2008) Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. Br J Nutr 99:931–940
29. Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S (2009) Relationship between body fat mass and free fatty acid kinetics in men and women. Obesity (Silver Spring) 17:1872–1877
30. Menegoni F, Galli M, Tacchini E, Vismara L, Cavigioli M, Capodaglio P (2009) Gender-specific effect of obesity on balance. Obesity (Silver Spring) 17:1951–1956
31. Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, Mauriege P, Despres JP (1999) Gender difference in postprandial lipemia : importance of visceral adipose tissue accumulation. Arterioscler Thromb Vasc Biol 19:2448–2455
32. Morita Y, Iwamoto I, Mizuma N, Kuwahata T, Matsuo T, Yoshinaga M, Douchi T (2006) Precedence of the shift of body-fat distribution over the change in body composition after menopause. J Obstet Gynaecol Res 32:513–516
33. Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE, Hennekens CH (1995) Weight, weight change, and coronary heart disease in women. Risk within the 'normal' weight range. JAMA 273:461–465

34. Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Monson RR, Speizer FE, Hennekens CH (1990) A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med 322:882–889
35. Balogun KA, Randunu RS, Cheema SK (2014) The effect of dietary omega-3 polyunsaturated fatty acids on plasma lipids and lipoproteins of C57BL/6 mice is age and sex specific. Prostaglandins Leukot Essent Fatty Acids 91:39–47
36. Balogun KA, Cheema SK (2014) The expression of neurotrophins is differentially regulated by omega-3 polyunsaturated fatty acids at weaning and postweaning in C57BL/6 mice cerebral cortex. Neurochem Int 66:33–42
37. Balogun KA, Albert CJ, Ford DA, Brown RJ, Cheema SK (2013) Dietary omega-3 polyunsaturated fatty acids alter the fatty acid composition of hepatic and plasma bioactive lipids in C57BL/6 mice: a lipidomic approach. PLoS One 8:e82399
38. Chechi K, Herzberg GR, Cheema SK (2010) Maternal dietary fat intake during gestation and lactation alters tissue fatty acid composition in the adult offspring of C57Bl/6 mice. Prostaglandins Leukot Essent Fatty Acids 83:97–104
39. Paigen B, Mitchell D, Reue K, Morrow A, Lusis AJ, LeBoeuf RC (1987) Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc Natl Acad Sci USA 84:3763–3767
40. Paigen B, Ishida BY, Verstuyft J, Winters RB, Albee D (1990) Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. Arteriosclerosis 10:316–323
41. Surwit RS, Seldin MF, Kuhn CM, Cochrane C, Feinglos MN (1991) Control of expression of insulin resistance and hyperglycemia by different genetic factors in diabetic C57BL/6J mice. Diabetes 40:82–87
42. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN (1988) Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37:1163–1167
43. Muhlhausler BS, Cook-Johnson R, James M, Miljkovic D, Duthoit E, Gibson R (2010) Opposing effects of omega-3 and omega-6 long chain polyunsaturated Fatty acids on the expression of lipogenic genes in omental and retroperitoneal adipose depots in the rat. J Nutr Metab 2010:927836
44. Vaidya H, Cheema SK (2015) Arachidonic acid has a dominant effect to regulate lipogenic genes in 3T3-L1 adipocytes compared to omega-3 fatty acids. Food Nutr Res 59:25866
45. Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
46. Keenan MHJ RASB (1982) Effect of plasma-membrane phospholipid unsaturation on solute transport into saccharomyces cerevisiae NCYC 366. J Gen Microbiol 128:2547–2556
47. Chen HC, Farese RV Jr (2002) Determination of adipocyte size by computer image analysis. J Lipid Res 43:986–989
48. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
49. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
50. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
51. Fraulob JC, Ogg-Diamantino R, Fernandes-Santos C, Aguila MB, Mandarim-de-Lacerda CA (2010) A mouse model of metabolic syndrome: insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet. J Clin Biochem Nutr 46:212–223
52. Ferroni P, Basili S, Falco A, Davi G (2004) Inflammation, insulin resistance, and obesity. Curr Atheroscler Rep 6:424–431
53. Hodson L, Skeaff CM, Fielding BA (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 47:348–380
54. Conner WE, Lin DS, Colvis C (1996) Differential mobilization of fatty acids from adipose tissue. J Lipid Res 37:290–298
55. Farooqui AA, Horrocks LA, Farooqui T (2007) Modulation of inflammation in brain: a matter of fat. J Neurochem 101:577–599
56. Adkins Y, Kelley DS (2010) Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J Nutr Biochem 21:781–792
57. Hulver MW, Berggren JR, Carper MJ, Miyazaki M, Ntambi JM, Hoffman EP, Thyfault JP, Stevens R, Dohm GL, Houmard JA, Muoio DM (2005) Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. Cell Metab 2:251–261
58. Hausman DB, DiGirolamo M, Bartness TJ, Hausman GJ, Martin RJ (2001) The biology of white adipocyte proliferation. Obes Rev 2:239–254
59. Gray SL, Vidal-Puig AJ (2007) Adipose tissue expandability in the maintenance of metabolic homeostasis. Nutr Rev 65:S7–S12
60. Bjorntorp P (1974) Effects of age, sex, and clinical conditions on adipose tissue cellularity in man. Metabolism 23:1091–1102
61. Guo SS, Chumlea WC, Roche AF, Siervogel RM (1998) Age- and maturity-related changes in body composition during adolescence into adulthood: the Fels longitudinal study. Appl Radiat Isot 49:581–585
62. Roncari DA, Van RL (1978) Promotion of human adipocyte precursor replication by 17beta-estradiol in culture. J Clin Invest 62:503–508
63. Childs CE, Romeu-Nadal M, Burdge GC, Calder PC (2008) Gender differences in the n-3 fatty acid content of tissues. Proc Nutr Soc 67:19–27
64. Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E, Novak S, Collins C, Welch CB, Lusis AJ, Erickson SK, Farese RV Jr (1998) Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc Natl Acad Sci USA 95:13018–13023
65. Lardizabal KD, Mai JT, Wagner NW, Wyrick A, Voelker T, Hawkins DJ (2001) DGAT2 is a new diacylglycerol acyltransferase gene family: purification, cloning, and expression in insect cells of two polypeptides from Mortierella ramanniana with diacylglycerol acyltransferase activity. J Biol Chem 276:38862–38869
66. Cases S, Stone SJ, Zhou P, Yen E, Tow B, Lardizabal KD, Voelker T, Farese RV Jr (2001) Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. J Biol Chem 276:38870–38876
67. Yu XX, Murray SF, Pandey SK, Booten SL, Bao D, Song XZ, Kelly S, Chen S, McKay R, Monia BP, Bhanot S (2005) Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. Hepatology 42:362–371
68. Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306:1383–1386
69. Holm G, Jacobsson B, Bjorntorp P, Smith U (1975) Effects of age and cell size on rat adipose tissue metabolism. J Lipid Res 16:461–464
70. Zimmerman AW, Veerkamp JH (2002) New insights into the structure and function of fatty acid-binding proteins. Cell Mol Life Sci 59:1096–1116
71. Baar RA, Dingfelder CS, Smith LA, Bernlohr DA, Wu C, Lange AJ, Parks EJ (2005) Investigation of *in vivo* fatty acid metabolism in AFABP/aP2(−/−) mice. Am J Physiol Endocrinol Metab 288:E187–E193

72. Boord JB, Fazio S, Linton MF (2002) Cytoplasmic fatty acid-binding proteins: emerging roles in metabolism and atherosclerosis. Curr Opin Lipidol 13:141–147
73. Pelton PD, Zhou L, Demarest KT, Burris TP (1999) PPARgamma activation induces the expression of the adipocyte fatty acid binding protein gene in human monocytes. Biochem Biophys Res Commun 261:456–458
74. Rasouli N, Molavi B, Elbein SC, Kern PA (2007) Ectopic fat accumulation and metabolic syndrome. Diabetes Obes Metab 9:1–10
75. Kralisch S, Fasshauer M (2013) Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease? Diabetologia 56:10–21
76. Reinehr T, Stoffel-Wagner B, Roth CL (2007) Adipocyte fatty acid-binding protein in obese children before and after weight loss. Metabolism 56:1735–1741
77. Chmurzynska A (2006) The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. J Appl Genet 47:39–48
78. Schachtrup C, Emmler T, Bleck B, Sandqvist A, Spener F (2004) Functional analysis of peroxisome-proliferator-responsive element motifs in genes of fatty acid-binding proteins. Biochem J 382:239–245
79. Tan NS, Shaw NS, Vinckenbosch N, Liu P, Yasmin R, Desvergne B, Wahli W, Noy N (2002) Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. Mol Cell Biol 22:5114–5127
80. Kershaw EE, Flier JS (2004) Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–2556
81. Reseland JE, Haugen F, Hollung K, Solvoll K, Halvorsen B, Brude IR, Nenseter MS, Christiansen EN, Drevon CA (2001) Reduction of leptin gene expression by dietary polyunsaturated fatty acids. J Lipid Res 42:743–750
82. Mitchell SE, Rees WD, Hardie LJ, Hoggard N, Tadayyon M, Arch JR, Trayhurn P (1997) ob gene expression and secretion of leptin following differentiation of rat preadipocytes to adipocytes in primary culture. Biochem Biophys Res Commun 230:360–364
83. Rousseau V, Becker DJ, Ongemba LN, Rahier J, Henquin JC, Brichard SM (1997) Developmental and nutritional changes of ob and PPAR gamma 2 gene expression in rat white adipose tissue. Biochem J 321(Pt 2):451–456
84. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL et al (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292–295
85. Zhang Y, Guo KY, Diaz PA, Heo M, Leibel RL (2002) Determinants of leptin gene expression in fat depots of lean mice. Am J Physiol Regul Integr Comp Physiol 282:R226–R234
86. Suzuki R, Tobe K, Aoyama M, Sakamoto K, Ohsugi M, Kamei N, Nemoto S, Inoue A, Ito Y, Uchida S, Hara K, Yamauchi T, Kubota N, Terauchi Y, Kadowaki T (2005) Expression of DGAT2 in white adipose tissue is regulated by central leptin action. J Biol Chem 280:3331–3337
87. Misra A, Wasir JS, Vikram NK (2005) Waist circumference criteria for the diagnosis of abdominal obesity are not applicable uniformly to all populations and ethnic groups. Nutrition 21:969–976
88. Misra A, Vikram NK, Gupta R, Pandey RM, Wasir JS, Gupta VP (2006) Waist circumference cutoff points and action levels for Asian Indians for identification of abdominal obesity. Int J Obes (Lond) 30:106–111
89. Smith J, Al-Amri M, Dorairaj P, Sniderman A (2006) The adipocyte life cycle hypothesis. Clin Sci (Lond) 110:1–9
90. Sniderman AD, Cianflone K, Arner P, Summers LK, Frayn KN (1998) The adipocyte, fatty acid trapping, and atherogenesis. Arterioscler Thromb Vasc Biol 18:147–151
91. Pegorier JP, Le May C, Girard J (2004) Control of gene expression by fatty acids. J Nutr 134:2444S–2449S
92. Yoshikawa T, Shimano H, Yahagi N, Ide T, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Tomita S, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Takahashi A, Sone H, Osuga Ji J, Gotoda T, Ishibashi S, Yamada N (2002) Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements. J Biol Chem 277:1705–1711
93. Julius U (2003) Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. Exp Clin Endocrinol Diabetes 111:246–250
94. Cox C, Mann J, Sutherland W, Ball M (1995) Individual variation in plasma cholesterol response to dietary saturated fat. BMJ 311:1260–1264
95. Katan MB, Beynen AC (1987) Characteristics of human hypo- and hyperresponders to dietary cholesterol. Am J Epidemiol 125:387–399
96. McNamara RK, Able J, Jandacek R, Rider T, Tso P (2009) Gender differences in rat erythrocyte and brain docosahexaenoic acid composition: role of ovarian hormones and dietary omega-3 fatty acid composition. Psychoneuroendocrinology 34:532–539