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ENDOCRINOLOGY

Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3T3-L1 cells and C57BL/6J mice adipose tissue

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Abstract Obesity is positively correlated to dietary lipid intake, and the type of lipid may play a causal role in the development of obesity-related pathologies. A major protein secreted by adipose tissue is adiponectin, which has antiatherogenic and antidiabetic properties. The aim of this study was to evaluate the effects of four different high-fat diets (enriched with soybean oil, fish oil, coconut oil, or lard) on adiponectin gene expression and secretion by the white adipose tissue (WAT) of mice fed on a selected diet for either 2 (acute treatment) or 60 days (chronic treatment). Additionally, 3T3-L1 adipocytes were treated for 48 h with six different fatty acids: palmitic, linoleic, eicosapentaenoic (EPA), docosahexaenoic (DHA), lauric, or oleic acid. Serum adiponectin concentration was reduced in the soybean-, coconut-, and lard-enriched diets in both groups. Adiponectin gene expression was lower in retroperitoneal WAT after acute treatment with all diets. The same reduction in levels of adiponectin gene expression was observed in epididymal adipose tissue of animals chronically fed soybean and coconut diets and in 3T3-L1 cells treated with palmitic, linoleic, EPA, and DHA acids. These results indicate that the intake of certain fatty acids may affect serum adiponectin levels in mice and adiponectin gene expression in mouse WAT and 3T3-L1 adipocytes. The effects appear to be time dependent and depot specific. It is postulated that the downregulation of adiponectin expression by dietary enrichment with soybean oil or coconut oil may contribute to the development of insulin resistance and atherosclerosis.

Keywords White adipose tissues · Adiponectin · High-fat diets · Mice · 3T3-L1

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Introduction

Adiponectin is the transcriptional product of the apM1 gene and is the most abundantly secreted protein from adipose tissue in humans [2, 22]. This adipokine increases insulin sensitivity and has anti-inflammatory and antiatherogenic effects [9]. Decreased serum adiponectin levels have been observed in subjects with insulin resistance, obesity, type II diabetes mellitus, and heart disease [9, 18]. Serum adiponectin levels are inversely correlated with body mass index, blood pressure, fasting glycemia, insulin resistance, serum insulin levels, and uric acid levels [43].

Epidemiological studies suggest a correlation between the type of lipid consumed in the diet, the levels of these fatty acids in adipose tissue, serum concentrations of these

fatty acids, and the incidence of certain metabolic diseases [14, 39].

The types of lipid present in the diet may be implicated in the development of insulin resistance, the incidence of cardiovascular disease, and changes in the inflammatory response [19, 30, 41]. It has been shown that saturated fatty acids increase insulin resistance and the incidence of cardiovascular disease and that monounsaturated and polyunsaturated fatty acids (PUFA) are protective against the development of these pathologies [19, 30].

The aim of this study was to evaluate the body composition, serum adiponectin levels, and adiponectin gene expression in WAT of mice fed diets enriched with 17.5% (by weight) soybean oil, coconut oil, fish oil, or lard. The effect of different fatty acids on adiponectin gene expression by 3T3-L1 adipocytes was also studied.

Materials and methods

Animals and experimental conditions

The Experimental Research Committee of the São Paulo Federal University approved all procedures for the care of the animals used in this study. Thirty and 90-day-old male C57Bl6 mice were used in this study and were kept under controlled conditions of light (12:12 h light–dark cycle with lights on at 07:00) and temperature (22±1°C). During the experimental period, the animals were maintained in individual cages and received water and the specific diet ad libitum. The animals were chronically or acutely treated, according to the protocols that follow.

Chronic treatment with lipid-enriched diets

Thirty-day-old mice were divided into five groups and fed for 8 weeks with one of the following diets:

1. Control (C): commercial chow, containing approximately 4% lipid
2. Soybean (S): control diet enriched with 17.5% soybean oil
3. Fish (F): control diet enriched with 17.5% fish oil
4. Lard (L): control diet enriched with 17.5% lard
5. Coconut (CC): control diet enriched with 17.5% coconut oil

The food intake and body weight were measured weekly at 09:00.

Acute treatment with lipid-enriched diets

Ninety-day-old mice were divided into five groups and fed for 2 days using the same diets described above.

Table 1 Fatty acid composition, as percent of total lipid content, of control diet (C) and control diet enriched with 17.5% soybean oil (S), fish oil (F), lard (L), or coconut oil (CC)

Fatty acid		C	S	F	L	CC
6:0	Caproic					6.1
8:0	Caprylic					4.9
10:0	Capric					3
12:0	Lauric	0.6				39
14:0	Mirystic	2.07		8.85	2	11
16:0	Palmitic	20.4	12.2	21.3	26	9.6
16:1 n-7	Palmitoleic			11.2		
18:0	Estearic	5.12	3.18	4.63	14	3.1
18:1 n-9	Oleic	27.4	25.1	17.1	45	13
18:2 n-6	Linoleic	41	55.1	11.1	9.2	9.8
18:3 n-3	Linolenic	2.91	4.35			
20:1 n-9	Eicosenoic			3.38		
20:5 n-3	EPA			14		
22:6 n-3	DHA			8.1		
SMCFA		0.6				53.4
SLCFA		27.6	15.4	34.8	42	23
MUFA		27.4	25.1	31.7	45	14
PUFA		43.9	59.5	33.2	9.2	9.8
n-6 PUFA		41	55.1	11.1	9.2	9.8
n-3 PUFA		2.91	4.35	22.1		
n-6/n-3		14	12.7	0.5		

SMCFA saturated medium-chain fatty acids, *SLCFA* saturated long-chain fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-6/n-3* relation n-6 over n-3.

Preparation of the diets

The diets were prepared in the Laboratory of Nutrition Physiology (Department of Physiology, São Paulo Federal University) using the commercial chow NUVILAB CR1 (Paraná, Brazil) with casein added to obtain a 20% protein content as described previously [13].

The standard chow was ground and enriched with 17.5% (w/w) soybean oil, fish oil, coconut oil, or lard according to the protocol and 0.013% butylated hydroxytoluene (w/w). Water was added to obtain the consistency necessary to allow homogenization of the mixture. After homogenization, the mixture was passed through a milling machine to produce pellets that were then dried in a forced ventilation oven at 60°C for 24 h.

The diets used in this study contained 22.4 g lipid/100 g and 1,683 kJ/100 g on average. The control diet contained 4.8 g lipid/100 g and 1,210 kJ/100 g. The fatty acid composition of the study diets as a percentage of total lipid content is shown in Table 1. The diets were stored in plastic containers at 4°C.

Sample collection

The animals were fed the respective diets for 2 days (acute treatment) or 8 weeks (chronic treatment) and then killed by decapitation without sedation. Trunk blood was collected and immediately centrifuged, and serum was stored at −70°C before determination of adiponectin concentration by enzyme-linked immunosorbent assay (Linco Research, USA). Samples of epididymal, retroperitoneal, and subcutaneous WAT were dissected and immediately frozen in liquid nitrogen. The samples were stored at −70°C.

Carcass lipid and protein content

A further group of mice treated chronically was used for determination of carcass lipid and protein content. Carcasses were eviscerated, weighed, and stored at −20°C. Lipid content was measured as described by Stansbie et al. [32] and standardized using the method described by Oller do Nascimento and Williamson [26]. Briefly, the eviscerated carcass was autoclaved at 120°C for 90 min and homogenized with double the mass of water. Triplicate aliquots of this homogenate were weighed and digested in 3 ml of 30% KOH and 3 ml of ethanol for at least 2 h at 70°C in capped tubes. After cooling, 2 ml of 12N H_2SO_4 were added, and the sample was washed three times with petroleum ether for lipid extraction. Results are expressed as grams of lipid/100 g of carcass. For protein measurements, aliquots of the same homogenate (approximately 1 g) were heated to 37°C for 1 h in 0.6N KOH with constant shaking. After clarification by centrifugation, protein content was measured according to Lowry et al. [21].

Cell culture

3T3-L1 cells were obtained from the American Type Culture Collection and cultured at 37°C in a humidified atmosphere of 5% CO2/95% air. The cells were maintained in a growth medium containing the following constituents: Dulbecco's modified Eagle's medium (Invitrogen) with 25 mmol/l glucose, 1 mmol/l pyruvate, 4.02 mmol/l L-alanyl-glutamine, and 10% fetal calf serum (Sigma). Differentiation of the cells was initiated 24 h after confluence by incubation for 2 days in the growth medium containing 0.25 μmol/l dexamethasone, 0.5 mmol/l 3-isobutyl-1-methyl-xanthine and 5 μg/ml insulin (Sigma). This was followed by 12 days in the growth medium containing 5 μg/ml insulin. At 10 days, specific agents were added, and the cells were harvested 48 h later. The following fatty acids were employed: palmitic, linoleic, eicosapentaenoic (EPA), docosahexaenoic (DHA), lauric, and oleic (Sigma) at a concentration of 250 μmol/l. In the control plates, the medium was changed, but no agent was added.

RNA preparation and Northern blot analysis

Total ribonucleic acid (RNA) was extracted from tissues and 3T3-L1 cells with Tri-Reagent (Sigma). The RNA concentration was determined from the absorbance at 260 nm. Fifteen-microgram aliquots of RNA were run on 1.3% agarose–formaldehyde gels, blotted onto a positively charged nylon membrane (Roche) overnight, cross-linked under UV light, and hybridized as previously described [15, 35].

An antisense oligonucleotide (5′-CTCTCCAGGAGTGC CATCTCTGCCATCACGG) based on the mouse adiponectin complementary deoxyribonucleic acid sequence (PubMed) was synthesized as a hybridization probe and end-labeled (5′-end) with a digoxigenin ligand (MWG, USA). After post-hybridization washes, membranes were incubated with an antibody against digoxigenin (Fab-fragment; Roche) for 30 min and then with CDP-Star chemiluminescence substrate (Roche; 10 min at room temperature). Signals were collected by exposure to the film for 40–50 min at room temperature.

After probing for adiponectin messenger RNA (mRNA), blots were stripped and reprobed for 18S ribosomal RNA (rRNA). The sequences of the antisense oligonucleotides used as probes for 18S rRNA were as previously described [37]. Blots were quantified by densitometry using the Image J software.

Statistical analysis

The data are presented as mean±SEM. Adiponectin gene expression results are expressed using arbitrary units with

Table 2 Daily fatty acid intake (mg) of mice fed the control diet (C) or control diet enriched with 17.5% soybean oil (S), fish oil (F), lard (L), or coconut oil (CC), for 8 weeks

Fatty acid	C	S	F	L	CC
Caproic					40.57±1.53
Caprylic					32.59±1.23
Capric					19.95±0.75
Lauric	1.18±0.03^{a}				262.04±9.91^{b}
Mirystic	4.09±0.11^{a}		46.24±1.53^{b}	11.73±0.36^{c}	71.16±2.69^{d}
Palmitic	40.34±1.13^{a}	66.12±1.81^{b}	111.44±3.68^{c}	151.86±4.64^{d}	63.85±2.41^{b}
Palmitoleic			58.41±1.93		
Estearic	10.10±0.28^{a}	17.21±0.47^{b}	24.19±0.80^{c}	82.67±2.53^{d}	20.62±0.78^{bc}
Oleic	54.02±1.52^{a}	135.87±3.71^{b}	89.49±2.95^{c}	262.73±8.03^{d}	89.12±3.37^{c}
Linoleic	80.92±2.27^{a}	298.20±8.15^{b}	57.99±1.91^{c}	53.94±1.65^{c}	65.18±2.46^{ac}
Linolenic	5.74±0.16^{a}	23.54±0.64^{b}			
Eicosenoic			17.66±0.58		
EPA			73.35±2.42		
DHA			42.32±1.40		
SMCFA	1,18±0,03^{a}				355,15±13,43^{b}
SLCFA	54,53±1,53^{a}	83,33±2,28^{b}	181,86±6,00^{c}	246,26±7,52^{d}	155,63±5,88^{e}
MUFA	54.02±71.52^{a}	135.87±3.71^{b}	165.56±5.46^{c}	262.73±8.03^{d}	89.12±3.37^{e}
PUFA	86.66±2.43^{a}	321.74±8.79^{b}	173.66±5.73^{c}	53.94±1.65^{d}	65.18±2.46^{d}
n-6 PUFA	80.92±2.27^{a}	298.20±8.15^{b}	57.99±1.91^{c}	53.94±1.65^{c}	65.18±2.46^{ac}
n-3 PUFA	5.74±0.16^{a}	23.54±0.64^{b}	115.67±3.82^{c}		
Total	196.39±5.51^{a}	540.93±14.78^{b}	521.09±17.19^{b}	562.93±17.2^{b}	665.08±25.15^{c}

Data is presented as mean±SEM. Values in the same row with different superscript letters are different from one another at $p<0.05$. The number of samples for each group is 16.
SMCFA saturated medium-chain fatty acids, *SLCFA* saturated long-chain fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids.

100 as the control value and compared using Student's *t* test. The other results were analyzed using one-way analysis of variance followed by Tukey's test. Differences were considered significant when $p<0.05$.

Results

Table 2 shows the average daily consumption of fatty acids (mg/day). These data were derived from the fatty acid composition of each diet and the quantified food intake during the experimental period (data not shown).

Mice fed the coconut oil-enriched diet had a significantly increased intake of medium chain saturated fatty acids, especially lauric acid (typically found in high concentrations in coconut oil). With regard to long-chain saturated fatty acids, the CC group ingested more myristic acid than the other groups.

Mice fed the lard-enriched diet consumed higher amounts of palmitic, stearic, and oleic acid compared to the other groups. Only the F mice had EPA and DHA in

Table 3 Body weight gain (g), mean daily energy intake (kJ/day), lipid and protein carcass content (g/100g), and adiponectin serum concentrations (μg/ml) of mice fed the control diet (C) or control diet enriched with 17.5% soybean oil (S), fish oil (F), lard (L), or coconut oil (CC), for 8 weeks (chronic treatment) and adiponectin serum concentrations of acutely treated mice

	C	S	F	L	CC
Body weight gain	10.57±0.73^{a}	8.02±0.51^{b}	9.28±0.52^{ab}	8.89±0.37^{ab}	8.64±0.42^{ab}
Daily energy intake	191.79±1.63^{a}	164.22±1.17^{b}	169.03±1.26^{b}	189.37±3.26^{a}	185.73±1.09^{a}
Carcass protein	31.90±1.80^{a}	31.91±2.00^{a}	34.76±0.90^{b}	32.80±1.40^{ab}	34.00±0.60^{ab}
Carcass lipid	9.73±0.67^{a}	14.82±0.75^{b}	14.92±0.73^{b}	13.04±0.64^{b}	13.33±1.11^{b}
Adiponectin chronic	14.33±0.24^{a}	13.13±0.34^{b}	14.04±0.33^{ab}	13.40±0.30^{b}	13.25±0.34^{b}
Adiponectin acute	14.33±0.24^{a}	10.48±0.83^{b}	13.08±0.30^{b}	12.12±0.09^{b}	12.46±0.31^{b}

Data is presented as mean±SEM. Values in the same row with different superscript letters are different from one another at $p<0.05$. The number of samples for each group for body weight gain and energy intake is 16, for carcass protein and lipid content is eight, and for adiponectin serum concentrations is nine.

Table 4 Adiponectin mRNA quantification in epididymal, retroperitoneal and subcutaneous white adipose tissues of mice fed with the control diet (C) or control diet enriched with 17.5% soybean oil (S), fish oil (F), lard (L), or coconut oil (CC), for 2 days (acute) or 8 weeks (chronic)

	C	S	F	L	CC
Epididymal adipose tissue					
Chronic	100.0±6.1	54.3±9.1*	73.4±9.0	92.2±3.5	67.2±5.3*
Acute		72.1±10.4	75.2±12.8	79.7±12.4	76.4±6.7
Retroperitoneal adipose tissue					
Chronic	100.2±6.4	69.0±6.0*	86.7±7.6	103.2±6.7	95.0±14.9
Acute		55.5±5.6*	53.0±8.9*	45.3±7.1*	54.7±5.5*
Subcutaneous adipose tissue					
Chronic	100.0±14.1	52.2±5.8*	104.8±9.4	104.1±8.7	74.6±6.8
Acute		96.4±6.5	85.7±12.5	76.5±9.6	93.8±4.7

Results are expressed as mean±SEM as arbitrary units, stipulating 100 as the control value. The numbers of samples varied between six and eight. *$p<0.05$ as compared to the control group

their diet. This group also ate greater quantities of palmitic acid compared to the C, S, and CC groups.

The largest consumption of linoleic acid was observed in the S mice, followed by the C and CC mice. The diets of the F, L, and CC groups did not contain detectable amounts of linolenic acid.

In total, the CC mice ingested more lipid than any other group. The S, F, and L mice had equivalent lipid intakes, all of which were higher than the C mice.

The five groups had similar body weights at the beginning of the study (data not shown). Body weight gain was lower in the S group, compared to C. The daily caloric intake was similar among C, L, and CC mice and was higher than in S and F mice. The fish oil-enriched diet increased the carcass protein content in relation to the control and soybean diets. The carcass lipid content was increased in all lipid-enriched diet groups as compared to the control (Table 3).

Serum adiponectin concentration was decreased in the four high-fat diet groups treated acutely. When treated chronically, only the S, L, and CC mice showed decreased serum adiponectin concentrations (Table 3). Acute treatment with high-fat diets decreased adiponectin gene expression in retroperitoneal WAT. No effect was observed in either epididymal or subcutaneous WAT (Table 4).

Chronic treatment with soybean oil-enriched diet reduced adiponectin gene expression in epididymal, retroperitoneal, and subcutaneous WAT. The same was observed in the epididymal WAT of CC mice (Table 4).

Adiponectin gene expression was decreased in 3T3-L1 cells treated with palmitic, linoleic, EPA, and DHA acids, but no changes were observed in cells treated with lauric and oleic acids (Fig. 1).

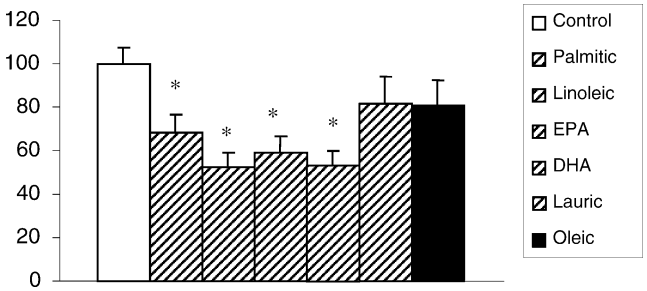

Fig. 1 Adiponectin gene expression by 3T3-L1 adipocytes treated for 48 h with 250 µmol/l of different fatty acids. Cells were treated for 48 h with palmitic, linoleic, eicosapentaenoic (EPA), DHA, lauric or oleic acids, 250 µmol/l. Results are expressed as percentage in relation to the control group. Mean±SEM. *Asterisk*, $p<0.05$ as compared to the control group. The numbers of samples varied between six and eight

Discussion

In the present study, we demonstrated that adiponectin gene expression and secretion in mice fed for either 2 (acute treatment) or 60 days (chronic treatment) are affected by high-fat diets and these responses are dependent on duration of treatment, adipose tissue depot, and on the fatty acid composition of the diet.

The treatment with high-fat diets caused increased ingestion of fatty acids among all groups compared to the control (Table 2). However, the daily energy intake was lower in S and F as compared to C (Table 3). These findings are in accordance with previous data [13, 17, 42]. The C, L, and CC mice had similar energy intakes.

Previous studies have reported that high-fat diets increase cholecystokinin secretion by the pancreas, which is a stimulus for satiety and reduction in food intake [23, 29]. The mechanisms underlying this process are not completely understood, as diet palatability and fatty acid composition may vary.

It has been shown that soybean oil- and fish oil-enriched diets increase serum leptin levels in rats [42]. Treatment of primary cultured rat adipocytes with EPA

increases leptin secretion [28]. It has also been shown that rats fed ad libitum with a beef tallow-enriched diet had lower serum leptin concentrations than rats treated with safflower- or fish oil-enriched diets [6]. These different effects of certain high-fat diets on serum leptin concentrations may explain the differences observed among the daily energy intake of the F and S mice, as compared to the CC and L mice.

In relation to the control group, the S group had lower daily energy intake and body weight gain, while the L and CC group had similar. Furthermore, these three groups had higher carcass lipid content. The F group had similar body weight gain and higher carcass lipid content but lower energy intake (Table 3). Gaiva et al. [13] obtained similar results regarding the fish oil-enriched diet treatment.

An increase in carcass protein content was observed in the F group (Table 3). Other studies have reported similar findings [13, 33]. It has been previously shown that high-fat diets increase body fat content even without higher energy intake [4, 40].

Diets rich in saturated fatty acids decrease uncoupling protein-1 (UCP-1) activity in brown adipose tissue [10]. In our study, the mice treated with lard or coconut presented similar body weight gain and energy intake in comparison to the control group, but their carcass lipid content was higher. It is possible that in these animals, the excess of energy is being stored, instead of being dissipated as heat.

On the contrary, it has been demonstrated that the ingestion of an n-3 PUFA-rich diet increased brown adipose tissue UCP-1 mRNA levels compared to an n-6 PUFA-rich diet [34]. Additionally, rats fed a diet enriched with EPA and DHA, in comparison to a control diet, displayed higher thermogenic activity of brown adipose tissue because of hyperplasia and an increase in thermogenic activity of mitochondria [27].

The similar body weight gain and lower energy intake observed in the F group indicate that these mice have higher energy efficiency as compared to the S, L, and CC, which in their turn have a higher energy efficiency than the control. In our study, although the fish oil used is an important source of n-3 PUFA, which increases UCP-1 mRNA levels and thermogenesis, it is also an important source of saturated fatty acids. Regarding the daily fatty acid intake, the fish oil-enriched diet is the second highest source of mirystic acid (coconut is the first) and the second highest source of palmitic acid (lard is the first; Table 2). In this case, the effects of n-3 have been possibly overcome by the effect of saturated fatty acids.

Both acute and chronic treatment with high-fat diets decreased the serum adiponectin levels in all groups but to a lesser extent in the mice chronically treated with the fish oil-enriched diet (Table 3).

The observed falls in serum adiponectin concentration were reflected to varying extents by decreased adiponectin gene expression in the different WAT depots. The greatest effect on adiponectin expression was observed in the retroperitoneal WAT of animals acutely treated with high-fat diets (Table 4). This may suggest both tissue- and time-specific effects of high-fat diets on adiponectin expression. Previous studies have been conducted to investigate changes in adipose tissue metabolism in the obese state. Milan et al. [24] observed that adiponectin gene expression was lower in visceral WAT of genetically obese rats compared to lean ones but similar in the subcutaneous depots of both groups. In the present study, adiponectin gene expression in subcutaneous WAT was less affected by the high-fat diet than in the retroperitoneal and epididymal depots (Table 4).

It has previously been described that treatment of mice with an EPA/DHA-enriched high-fat diet led to increased serum adiponectin levels but no alteration in adiponectin gene expression, either in subcutaneous, dorsolumbar, or epididymal fat pads, as compared to a control high-fat diet [12]. In another study, db/db mice treated with n-6 and n-3 PUFA-enriched diet had similar serum adiponectin levels and gonadal WAT adiponectin gene expression, as compared to animals treated with a low-fat diet [35].

Recently, it was demonstrated a tissue-specific response to adiponectin gene expression subsequent to treatment with high-fat diets [25]. That study reported that mice treated with a fish oil-enriched diet had increased serum adiponectin levels and raised adiponectin gene expression in retroperitoneal but not epididymal WAT, compared to animals fed a control diet or a diet rich in sunflower oil (rich in n-6 PUFA).

The differences between these results and those of the present study may be partly explained by the duration of treatment and the diet composition. The above study utilized a treatment lasting 15 days with a 59% fat-derived calories diet that contained 25% n-3 PUFA. In the present study, the mice were treated for 60 days, with a 47% fat-derived calories diet containing approximately 10% n-3 PUFA. This may suggest that the amount of n-3 PUFA in the diet might be an important factor for the stimulation of adiponectin gene expression.

In this study, the fish oil diet did not affect adiponectin gene expression or serum adiponectin concentration, in contrast to the other high-fat diet chronic treatments (Tables 3 and 4). This may suggest a protective effect of n-3 high-fat diets on serum adiponectin levels. However, the 2 days fish oil diet treatment and in vitro treatment with EPA and DHA decreased adiponectin mRNA levels in retroperitoneal white adipose tissue and 3T3-L1 adipocytes, respectively (Table 4 and Fig. 1), which could indicate a direct effect of the fish oils on adiponectin gene expression.

However, it is also possible to suggest that lipoperoxidation of n-3 fatty acids in vitro and in vivo acute treatment could be an important factor to promote a decrease in adiponectin expression. These fatty acids are extremely susceptible to lipid peroxidation [15]. It has been previously demonstrated that hydroxyalkenal 4-hydroxynonenal, derived from peroxidation of n-6 PUFA, reduces adiponectin gene expression in 3T3-L1 adipocytes [31].

The serum adiponectin concentration was reduced after acute treatment with fish oil-enriched diet. After chronic treatment with the fish oil diet, no difference was observed either in adiponectin serum concentration and gene expression in white adipose depots as compared to the control diet (Tables 3 and 4). It is possible to suggest that the acutely treated mice have not had time enough for the development of antioxidant properties caused by DHA and EPA, and on the contrary, in the chronically treated mice, the time of 8 weeks was enough for the overexpression of antioxidant enzymes. It has been demonstrated in vivo that the fish oil-enriched diet promotes an increase in antioxidant enzyme activities, which would reduce lipid peroxidation [3].

A reduction in adiponectin gene expression was observed after both acute and chronic treatment with soybean- and coconut oil-enriched diets (Table 4). Coconut oil is rich in medium-chain fatty acids, especially lauric acid, which are ketogenic [7]. Studies have shown that hyperketonemia increases serum tumor necrosis factor (TNF) α concentration [20], which is a potent inhibitor of adiponectin expression in adipose tissue and cultured adipocytes [5, 8, 38]. Additionally, it has been shown that the addition of lauric acid to the culture medium of 3T3-L1 adipocytes does not affect adiponectin gene expression (Fig. 1). This reinforces the concept that the effect of diets enriched with medium-chain fatty acids in reducing adiponectin gene expression may be dependent on ketone body production.

It has been shown that treatment for 7 weeks with the same soybean oil-enriched diet used in this study increased plasma corticosterone concentrations [42]. The reduced adiponectin gene expression observed in the chronically treated S group may be attributed, at least in part, to an increase in glucocorticoid levels, which, according to Fasshauer et al. [11], decrease adiponectin gene expression.

Mice fed chronically on a lard-enriched diet displayed decreased serum adiponectin levels, although adiponectin gene expression in WAT was similar to the control group (Tables 3 and 4). The lard-enriched diet is the richest in palmitic and oleic acids (Table 1). In 3T3-L1 adipocytes, treatment with palmitic acid alone decreased adiponectin gene expression, but oleic acid treatment had no effect (Fig. 1). It may therefore be suggested that the oleic acid present in the lard-enriched diet impairs the reduction in adiponectin gene expression that might otherwise be expected to occur with palmitic acid in vivo. It has been demonstrated in 3T3-L1 adipocytes that palmitate treatment induced gene expression but that the concentration of TNF-α in the medium was not increased [1]. In the present study, it may be the case that adiponectin mRNA is synthesized by the lard-fed animals, but the protein is not secreted into the bloodstream.

These results demonstrate that dependent on the type of fatty acid present in the diet, a reduction in serum adiponectin levels and WAT adiponectin gene expression may occur. This effect would appear to be both time- and depot-specific. Enrichment of the diet with 17.5% fish oil resulted in a transitory reduction in serum adiponectin concentration, whereas enrichment with 17.5% lard, soybean oil, or coconut oil all reduced serum adiponectin levels. This effect could be also related to the increase in body fat mass observed in these groups. Several studies have examined plasma adiponectin levels and have found decreased levels in obese and diabetic subjects and significant inverse associations with some measure of insulin resistance [18].

In view of the effects of adiponectin on reducing insulin resistance and atherosclerosis, it is suggested that these diets, by inhibiting adiponectin production, may promote these pathogenic states associated with the metabolic syndrome.

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Conflict of interest
The authors of this research disclose any potential conflict of interest.

References

1. Ajuwon KM, Spurlock ME (2005) Palmitate activates the NF-kappaB transcription factor and induces IL-6 and TNFalpha expression in 3T3-L1 adipocytes. J Nutr 135:1841–1846
2. Arita Y, Kihara S, Ouchi N et al (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79–83
3. Bhattacharya A, Lawrence RA, Krishnan A, Zaman K, Sun D, Fernandes G (2003) Effect of dietary n-3 and n-6 oils with and without food restriction on activity of antioxidant enzymes and lipid peroxidation in livers of cyclophosphamide treated autoimmune-prone NZB/W female mice. J Am Coll Nutr 22:388–399
4. Boozer CN, Schoenbach G, Atkinson RL (1995) Dietary fat and adiposity: a dose–response relationship in adult male rats fed isocalorically. Am J Physiol 268:E546–E550
5. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B (2003) Regulation of adiponectin by adipose

tissue-derived cytokines: in vivo and in vitro investigations in humans. Am J Physiol Endocrinol Metab 285:E527–E533
6. Cha MC, Jones PJ (1998) Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats. J Lipid Res 39:1655–1660
7. Crozier G, Bois-Joyeux B, Chanez M, Girard J, Peret J (1987) Metabolic effects induced by long-term feeding of medium-chain triglycerides in the rat. Metabolism 36:807–814
8. Degawa-Yamauchi M, Moss KA, Bovenkerk JE, Shankar SS, Morrison CL, Lelliott CJ, Vidal-Puig A, Jones R, Considine RV (2005) Regulation of adiponectin expression in human adipocytes: effects of adiposity, glucocorticoids, and tumor necrosis factor alpha. Obes Res 13:662–669
9. Diez JJ, Iglesias P (2003) The role of the novel adipocyte-derived hormone adiponectin in human disease. Eur J Endocrinol 148:293–300
10. Dube MG, Beretta E, Dhillon H, Ueno N, Kalra PS, Kalra SP (2002) Central leptin gene therapy blocks high-fat diet-induced weight gain, hyperleptinemia, and hyperinsulinemia: increase in serum ghrelin levels. Diabetes 51(6):1729–1736 Jun
11. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R (2002) Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun 90:1084–1089
12. Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ruzickova J, Kopecky J (2006) Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. Diabetologia 49:394–397
13. Gaiva MH, Couto RC, Oyama LM, Couto GE, Silveira VL, Ribeiro EB, Nascimento CM (2001) Polyunsaturated fatty acid-rich diets: effect on adipose tissue metabolism in rats. Br J Nutr 86:371–377
14. Garland M, Sacks FM, Colditz GA, Rimm EB, Sampson LA, Willett WC, Hunter DJ (1998) The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. Am J Clin Nutr 67:25–30
15. Gonzalez MJ, Gray JI, Schemmel RA, Dugan L Jr, Welsch CW (1992) Lipid peroxidation products are elevated in fish oil diets even in the presence of added antioxidants. J Nutr 122:2190–2195
16. Haugen F, Jorgensen A, Drevon CA, Trayhurn P (2001) Inhibition by insulin of resistin gene expression in 3T3-L1 adipocytes. FEBS Lett 507:105–108
17. Himaya A, Fantino M, Antoine JM, Brondel L, Louis-Sylvestre J (1997) Satiety power of dietary fat: a new appraisal. Am J Clin Nutr 65:1410–1418
18. Hotta K, Funahashi T, Arita Y et al (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 20:1595–1599
19. Hu FB, Van Dam RM, Liu S (2001) Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. Diabetologia 44:805–817
20. Jain SK, Kannan K, Lim G, McVie R, Bocchini JA Jr (2002) Hyperketonemia increases tumor necrosis factor-alpha secretion in cultured U937 monocytes and Type 1 diabetic patients and is apparently mediated by oxidative stress and cAMP deficiency. Diabetes 51:2287–2293
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
22. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 221:286–289
23. Maggio CA, Haraczkiewicz E, Vasselli JR (1988) Diet composition alters the satiety effect of cholecystokinin in lean and obese Zucker rats. Physiol Behav 43:485–491
24. Milan G, Granzotto M, Scarda A, Calcagno A, Pagano C, Federspil G, Vettor R (2002) Resistin and adiponectin expression in visceral fat of obese rats: effect of weight loss. Obes Res 10:1095–1103
25. Neschen S, Morino K, Rossbacher JC, Pongratz RL, Cline GW, Sono S, Gillum M, Shulman GI (2006) Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor-gamma-dependent mechanism in mice. Diabetes 55:924–928
26. Oller do Nascimento CM, Williamson DH (1988) Tissue-specific effects of starvation and refeeding on the disposal of oral [1–14C] triolein in the rat during lactation and on removal of litter. Biochem J 254:539–546
27. Oudart H, Groscolas R, Calgari C, Nibbelink M, Leray C, Le Maho Y, Malan A (1997) Brown fat thermogenesis in rats fed high-fat diets enriched with n-3 polyunsaturated fatty acids. Int J Obes Relat Metab Disord 21:955–962
28. Perez-Matute P, Marti A, Martinez JA, Fernandez-Otero MP, Stanhope KL, Havel PJ, Moreno-Aliaga MJ (2005) Eicosapentaenoic fatty acid increases leptin secretion from primary cultured rat adipocytes: role of glucose metabolism. Am J Physiol Regul Integr Comp Physiol 288:R1682–R1688
29. Rayner DV, Miller S (1993) Voluntary intake and gastric emptying in pigs: effects of fat and a CCK inhibitor. Physiol Behav 54:917–922
30. Sacks FM, Katan M (2002) Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am J Med 113:13S–24S
31. Soares AF, Guichardant M, Cozzone D, Bernoud-Hubac N, Bouzaidi-Tiali N, Lagarde M, Geloen A (2005) Effects of oxidative stress on adiponectin secretion and lactate production in 3T3-L1 adipocytes. Free Radic Biol Med 38:882–889
32. Stansbie D, Brownsey RW, Crettaz M, Denton RM (1976) Acute effects in vivo of anti-insulin serum on rates of fatty acid synthesis and activities of acetyl–coenzyme A carboxylase and pyruvate dehydrogenase in liver and epididymal adipose tissue of fed rats. Biochem J 160:413–416
33. Su W, Jones PJ (1993) Dietary fatty acid composition influences energy accretion in rats. J Nutr 123:2109–2114
34. Takahashi Y, Ide T (2000) Dietary n-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. Br J Nutr 84:175–184
35. Todoric J, Loffler M, Huber J, Bilban M, Reimers M, Kadl A, Zeyda M, Waldhausl W, Stulnig TM (2006) Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by n-3 polyunsaturated fatty acids. Diabetologia 49:2109–2119
36. Trayhurn P, Duncan JS, Nestor A, Thomas ME, Rayner DV (1994) Chemiluminescent detection of mRNAs on northern blots with digoxigenin end-labeled oligonucleotides. Anal Biochem 222:224–230
37. Trayhurn P, Thomas ME, Duncan JS, Rayner DV (1995) Effects of fasting and refeeding on ob gene expression in white adipose tissue of lean and obese (oblob) mice. FEBS Lett 368:488–490
38. Wang B, Jenkins JR, Trayhurn P (2005) Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-alpha. Am J Physiol Endocrinol Metab 288:E731–E740
39. Warensjo E, Sundstrom J, Lind L, Vessby B (2006) Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. Am J Clin Nutr 84:442–448

40. West DB, York B (1998) Dietary fat, genetic predisposition, and obesity: lessons from animal models. Am J Clin Nutr 67:505S–512S
41. Wohlers M, Nascimento CM, Xavier RA, Ribeiro EB, Silveira VL (2003) Participation of corticosteroids and effects of indomethacin on the acute inflammatory response of rats fed n-6 or n-3 polyunsaturated fatty acid-rich diets. Inflammation 27:1–7
42. Wohlers M, Xavier RA, Oyama LM, Ribeiro EB, do Nascimento CM, Casarini DE, Silveira VL (2005) Effect of fish or soybean oil-rich diets on bradykinin, kallikrein, nitric oxide, leptin, corticosterone and macrophages in carrageenan stimulated rats. Inflammation 29:81–89
43. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K, Saruta T (2002) Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin Sci (Lond) 103:137–142