Journal of Food and Nutrition Research, 2016, Vol. 4, No. 9, 621-632
Available online at http://pubs.sciepub.com/jfnr/4/9/10

DOI:10.12691/jfnr-4-9-10

Structured Triacylglycerols with Docosahexaenoic Acid (DHA) at the *sn*-2 Position Increase DHA Incorporation in Brown Adipose Tissue, but not in White Adipose Tissue, of Hamsters

Paula A. Lopes[1,#,*], Narcisa M. Bandarra[2,3,#], Susana V. Martins[1], Júlia Ferreira[2], José L. Guil-Guerrero[4], José A. M. Prates[1,*]

[1]CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal
[2]DIVAV, Instituto Português do Mar e da Atmosfera, Av. Brasília, 1449-006 Lisboa, Portugal
[3]CIIMAR, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal
[4]Departamento de Tecnología de Alimentos, Universidad de Almería, 04120 Almería, España
[#]Authors who contributed equally.
*Corresponding author: ampalopes@fmv.ulisboa.pt; japrates@fmv.ulisboa.pt

Abstract In this study, we hypothesized that incorporation of docosahexaenoic acid (DHA, 22:6*n*-3) across adipose tissues will be higher when it is ingested as triacylglycerols (TAG) structured at the *sn*-2 position, which enhances efficacy of DHA supplementation and protective actions against obesity. Ten-week old Golden Syrian male hamsters were randomly allocated to 4 dietary groups with 10 animals each: linseed oil (LSO; control group), fish oil (FO), fish oil ethyl esters (FO-EE) and structured DHA at the *sn*-2 position of TAG (DHA-SL). After 12 weeks, there were no variations in body composition parameters, including subcutaneous white adipose tissue (WAT), retroperitoneal WAT and brown adipose tissue (BAT) mass or plasma insulin, leptin and adiponectin across dietary treatments. In opposition to the large variations found for fatty acid composition in both depots of WAT, BAT was less responsive to diets. Even so, DHA and eicosapentaenoic acid (EPA, 20:5*n*-3) were not found in subcutaneous and retroperitoneal WAT in contrast to DHA, which was incorporated successfully in BAT reaching the highest percentage in DHA-SL fed hamsters. The principal component analysis of plasma hormones and fatty acids discriminated BAT from WAT and set apart 3 clusters matching 3 fat depots pointing toward an individual signature on fatty acids deposition. In turn, discrimination of dietary treatments within each adipose tissue was unattainable. Taken together, structured *sn*-2 position DHA-containing TAG, relative to commercially fish oils, had no impact neither on fat depots weight nor on systemic hormones, but improved DHA incorporation in BAT due to its higher bioavailability.

Keywords: *structured DHA, sn-2 TAG, n-3 PUFA, white adipose tissue, brown adipose tissue, fatty acid composition, hamster*

Cite This Article: Paula A. Lopes, Narcisa M. Bandarra, Susana V. Martins, Júlia Ferreira, José L. Guil-Guerrero, and José A. M. Prates, "Structured Triacylglycerols with Docosahexaenoic Acid (DHA) at the *sn*-2 Position Increase DHA Incorporation in Brown Adipose Tissue, but not in White Adipose Tissue, of Hamsters." *Journal of Food and Nutrition Research*, vol. 4, no. 9 (2016): 621-632. doi: 10.12691/jfnr-4-9-10.

1. Introduction

Excessive intake of a Western-style diet rich in saturated fatty acids (SFA) and *n*-6 polyunsaturated fatty acids (PUFA) and poor in *n*-3 PUFA is considered one of the major factors promoting obesity-associated chronic non-resolving inflammation [1]. In contrast, docosahexaenoic (DHA, 22:6*n*-3) and eicosapentaenoic (EPA, 20:5*n*-3) acids and their hydroxide products were demonstrated to have potent anti-inflammatory, hypolipidemic, and weight-reducing properties that counteract the onset of obesity [2,3,4]. This body of evidence supports the concept that increasing body *n*-3 fatty acid levels may be a valid strategy to prevent the development of obesity and/or attenuate associated chronic metabolic and inflammatory diseases [5].

Adipose tissue is a master regulator in controlling whole-body lipid flux, thereby modulating both glucose and lipid homeostasis in humans [6]. It is probably among the most plastic organs in the body, constantly expanding and regressing depending on the metabolic status. Variations of response across fat depots suggest that the features of adipose tissue responsible for adipocyte proliferation and (trans) differentiation may not be homogenous, both in nature and distribution [7]. In this respect, subcutaneous adipose tissue and visceral adipose tissue are metabolically distinct [8]. Visceral fat expansion occurs mainly by hypertrophy with great infiltration of

macrophages [9] and regulated by a great number of glucocorticoid receptors β-adrenoceptors, and a lower number of insulin receptors [10] in opposition to subcutaneous fat, which expands predominantly by hyperplasia and appears protective through enhanced adipogenic capacity [11]. Hence, visceral fat accumulation is important in clinical terms because it is more closely linked to the metabolic syndrome. Conversely, brown adipocytes constitute a metabolically active tissue responsible for non-shivering thermogenesis and depletion of excess calories [12].

Brown adipose tissue (BAT) appears to be effective in the protection against metabolic disorders associated with obesity and diabetes, as brown adipocytes seem to be more "healthy" than white adipocytes [13,14].

Based on current research [15,16,17], the physiological activity of *n*-3 LCPUFA may depend on their specific lipid structure. Although not consensual, the results revealed that there seems to be a difference in the apparent bioavailability of *n*-3 LCPUFA, such that the triacylglycerols (TAG) form is more bioavailable than the ethyl esters (EE) form [18,19,20,21]. In this sense, much attention has been directed to the chemical synthesis of structured lipids (SL), in particular of triacylglycerols containing *n*-3 LCPUFA. Structured lipids are defined as chemically or enzymatically modified TAG that change fatty acid composition and/or positional distribution in the glycerol backbone. The molecular structure of different TAG could have implications regarding fatty acids attainability influencing their metabolic fate in the organism, namely digestion, intestinal absorption, and physiological properties, being enhanced for fatty acids located at the *sn*-2 position [15,22,23,24].

To further expand knowledge on body fat reduction properties of *n*-3 LCPUFA in the *in vivo* setting, we tested the hypothesis that incorporation of DHA in white adipose tissue (WAT, subcutaneous and visceral) and brown adipose tissue (BAT) in hamsters is greater when it is ingested as TAG structured at the *sn*-2 position. For comparison, fish oil (a commercial available oil rich in DHA, regardless the *sn* position of TAG) and fish oil fatty acid ethyl esters were used. In addition, we postulated on the variability of fatty acid deposition across subcutaneous WAT, retroperitoneal WAT and interscapular BAT, which might reflect differences in adipose tissue depot's location and metabolic function.

2. Methods and Materials

2.1. Ethics Statement

The experimental procedures were reviewed by the Ethics Commission of CIISA/FMV and approved by the Animal Care Committee of the National Veterinary Authority (Direcção-Geral de Alimentação e Veterinária, Portugal), following the appropriated European Union guidelines (2010/63/EU Directive) to minimize animals suffering.

2.2. Animals and Diets

Lipid metabolism of hamsters is more similar to humans than mice and rats [25,26] making it possible to extrapolate results to humans. Ten-week old Golden Syrian male hamsters were purchased from Charles River (Charles River Laboratories, L'Arbresle, France). Animals were housed individually upon arrival and maintained on a controlled environment (20-24ºC, 14:10-hour light-dark cycle). During the first week, all animals were fed on a standard diet to minimize stress and stabilize all metabolic conditions. After this period, 40 hamsters were assigned to four body weight-matched groups, with ten animals each: the linseed oil (LSO) group, rich in alpha linolenic acid (18:3*n*-3) without DHA or EPA, taken as the control group; the fish oil (FO) group, a commercial available oil rich in TAG with DHA and EPA, regardless the *sn* position of TAG; the fish oil ethyl esters (FO-EE) group; and the structured DHA (DHA-SL) at the *sn*-2 position of TAG. The final sum of DHA and EPA was identical across FO, FO-EE and DHA-SL dietary treatments.

Diets were manufactured by the Experimental Diets Unit from the University of Almeria. The proximate chemical composition of the diets was determined according to Association of Official Agricultural Chemists (AOAC) [27], and fatty acid composition was assessed as described by Bandarra et al. [28] (Table 1).

Table 1. Chemical and fatty acid composition of the diets fed to hamsters

	LSO	FO	FO-EE	DHA-SL
Chemical composition (g/100 g)				
Gross energy (kJ/100 g)	1410	1427	1443	1443
Crude protein	11.5	11.1	11.5	11.3
Crude fat	1.3	1.8	1.8	2.2
Carbohydrates	69.7	70.2	70.7	69.9
Crude ash	2.9	2.9	2.9	3
Fatty acid profile (g/100 g of total fatty acids)				
10:0	4.43	1.87	2.09	5.70
12:0	4.76	3.00	2.30	3.60
14:0	0.300	1.00	1.40	1.21
16:0	10.2	12.0	11.4	10.7
16:1*n*-7	0.100	1.20	2.10	0.60
18:0	3.44	3.70	3.60	3.60
18:1*n*-9	22.1	22.9	23.4	22.8
18:2*n*-6	41.6	41.2	38.9	40.3
18:3*n*-3	7.88	5.80	5.54	5.70
18:4*n*-3	0.800	0.300	0.500	ND
20:1*n*-9	ND	1.20	1.30	0.100
20:4*n*-3	ND	1.10	1.62	0.200
20:5*n*-3	ND	1.20	0.770	0.300
22:1*n*-11	ND	1.30	1.20	ND
22:6*n*-3	ND	1.70	1.80	2.6

LSO, the linseed oil group (the control group); FO, the fish oil group FO-EE, the fish oil ethyl esters group; DHA-SL, the structured DHA group. ND, not detected.

All diets were based on the AIN-93M formulation and contained (g/100 g feed): casein (14.0), corn starch (46.6), maltodextrin (15.5), sucrose (10.0), cellulose (5.0), soybean oil (4.0), L-cystine (0.18), AIN-93M mineral mix (3.5), AIN-93M vitamin mix (1.0), choline bitartrate (0.25), and *tert*-butylhydroquinone (0.0008) with modified lipid fraction. The LSO diet contained linseed oil (0.335), the FO diet contained fish oil (0.796), the FO-EE diet contained fish oil ethyl esters (0.796) and the DHA-SL diet contained structured DHA (0.421 g/100 g feed).

For DHA-SL, DHASCO® oil was obtained from DSM Nutritional Products (Columbia, MD, USA). *Pseudomonas* sp. lipase, solvents, and reagents used in the structured lipid preparation were purchased from Sigma

Chemical Co. (St. Louis, MO, USA). DHASCO® oil (5 g) was mixed with capric acid (10:0), at a 1:3 molar ratio of oil:capric acid in appropriate tubes, and then lipase (25% by weight of substrates) and water (3% by weight of substrates and enzyme) were added in hexane (30 mL). The mixture was incubated in an orbital shaker at 250 rpm at 45°C. After 24 hours, the reaction was stopped by addition of a mixture of acetone and ethanol (20 mL; 1:1 v/v). In order to neutralize the released and unused free fatty acids, the reaction mixture was titrated with a 0.5 M NaOH solution (using a phenolphthalein indicator) until the colour of the solution turned pink. The acylglycerols were then extracted into hexane (250 mL). The two layers (aqueous, hexane) were allowed to separate in a separator funnel, and the lower aqueous layer was discarded. The hexane layer was passed through a bed of anhydrous sodium sulphate to remove any residual water. The hexane was evaporated using a rotary evaporator at 45°C and the acylglycerol fraction was recovered and analyzed [29].

Throughout the trial, hamsters had free access to water and food. Body weight and feed intake were recorded twice a week. After 12 weeks of feeding trial, hamsters were fasted for 12 hours and euthanized by a mechanical-physical method. Hamsters were placed in a chamber and anesthetized using a mixture of 20% of isofluorane in propylene glycol (v/v) for 30 seconds [30] followed by decapitation with a small-animal guillotine. The trunk blood was collected into lithium heparin tubes and centrifuged (1500*g* for 10 minutes at room temperature) to obtain plasma. Following on blood collection, subcutaneous and visceral white adipose tissues from hamsters' pelvic and retroperitoneal anatomical regions, respectively as well as brown adipose tissue from hamsters' interscapular region were excised, weighed and stored at -80°C for subsequent fatty acid determination.

2.3. Plasma Hormones

Plasma insulin (Mercodia, Uppsala, Sweden), leptin (R&D Systems, Minneapolis, USA) and adiponectin (R&D Systems) were measured by ELISA following the supplier recommendations.

2.4. Fatty Acid Composition of Adipose Tissues

Fatty acids methyl esters (FAME) were prepared according to Bandarra et al. [31]. Samples were lyophilized (-60°C and 2.0 hPa) to constant weight. FAME were obtained through addition of 1 mL of anhydrous methanol, 0.5 mL of sodium methoxide (1 mol/L in methanol), swirling for 5 minutes, and 1 hour reaction in the dark, following the conditions referred by Christie [32]. The layer separation was improved through 10 minutes in an ultrasonic bath and centrifuged at 1500*g* for 5 minutes. The *n*-hexane layer was collected and the aqueous phase re-extracted with 2.5 mL of *n*-hexane, and centrifuged again. FAME were concentrated to a final volume of 25 µL in *n*-heptane and 2 µL of sample was injected on a capillary DB-Wax capillary column (30 m, 0.25 mm internal diameter and 0.25 µm film thickness, J&W Scientific, Agilent, USA) in a Varian CP-3800 gas chromatograph equipped with flame ionisation detector (Varian, Palo Alto, CA, USA). The temperature of injector and detector was set at 250°C. Adequate separation was obtained over a 40-minute period, with 5 minutes at 180°C, followed by an increase of 4°C/min until 220°C, and kept at this temperature for 25 minutes. Authentic standards were used for fatty acid identification. Individual fatty acids were expressed as percentage of the total fatty acids.

2.5. Statistical Analyses

Statistical analyses were carried out with the Statistical Analysis Systems (SAS) software package, version 9.3 (SAS Institute, Cary, NC, USA). Sample size was determined by the POWER procedure and it was based on the manuscript by Lee et al. [33] on the incorporation of structured DHA in the liver which provides an idea about the standard deviation and the magnitude of the effect. A sample size of 9 hamsters provided a statistical power of at least 80% for detecting 20% difference with a two-tailed probability of $p < 0.05$. However, we enrolled 10 hamsters *per* group to guarantee statistical power for all measurements. All data were checked for normality and variance homogeneity, and reported as means ± standard error of the means (SEM). The General Linear Model (GLM) procedure was used to perform variance analysis and differences between groups were calculated using the Tukey's *post hoc* test at $p < 0.05$.

The principal component analysis (PCA) is a multivariate technique that reduces the dimensionality of data by transforming a number of related variables into a set of uncorrelated variables, while retaining as much variation as possible [34], using the proportion of fatty acids from subcutaneous WAT, retroperitoneal WAT and interscapular BAT. Only fatty acids common to all tissues were included in the multivariate analysis. The PRINCOMP procedure of SAS was applied after data standardization and PC analyses were based on the correlation matrix.

3. Results

3.1. Baseline Characterization of the Dietary Groups

As previously published in a companion paper [35], the consumption of FO, FO-EE and DHA-SL diets had no impact on hamster's daily feed intake, total body weight gain and final body weight ($p > 0.05$).

3.2. Adipose Tissues Weight and Plasma Hormones

The variations in fat depots mass, following on normalization with hamster's final body weight, are illustrated in Figure 1.

The subcutaneous WAT was the heaviest, the retroperitoneal WAT was the intermediate, and the interscapular BAT was the lightest ($p < 0.001$), regardless the dietary group. Curiously, subcutaneous WAT, retroperitoneal WAT and interscapular BAT weights were unchanged by dietary treatments ($p > 0.05$). In line with this, Figure 2 presents the non-variations of plasma hormones, insulin (Figure 2A), leptin (Figure 2B) and adiponectin (Figure 2C) across dietary groups ($p > 0.05$).

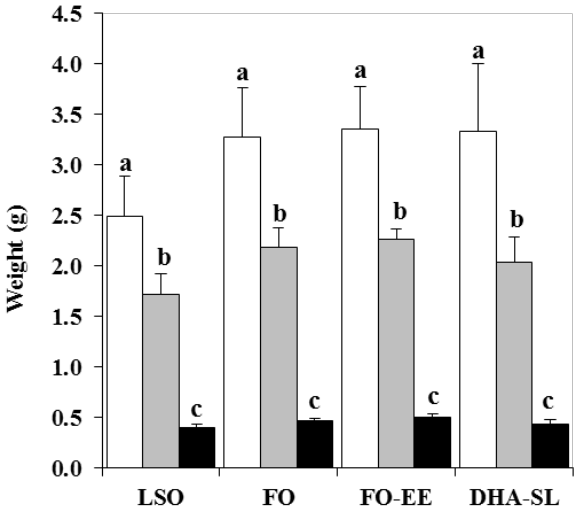

Figure 1. Subcutaneous white adipose tissue (white bars), retroperitoneal white adipose tissue (gray bars) and interscapular brown adipose tissue (black bars) weight from hamsters fed on LSO, FO, FO-EE and DHA-SL diets

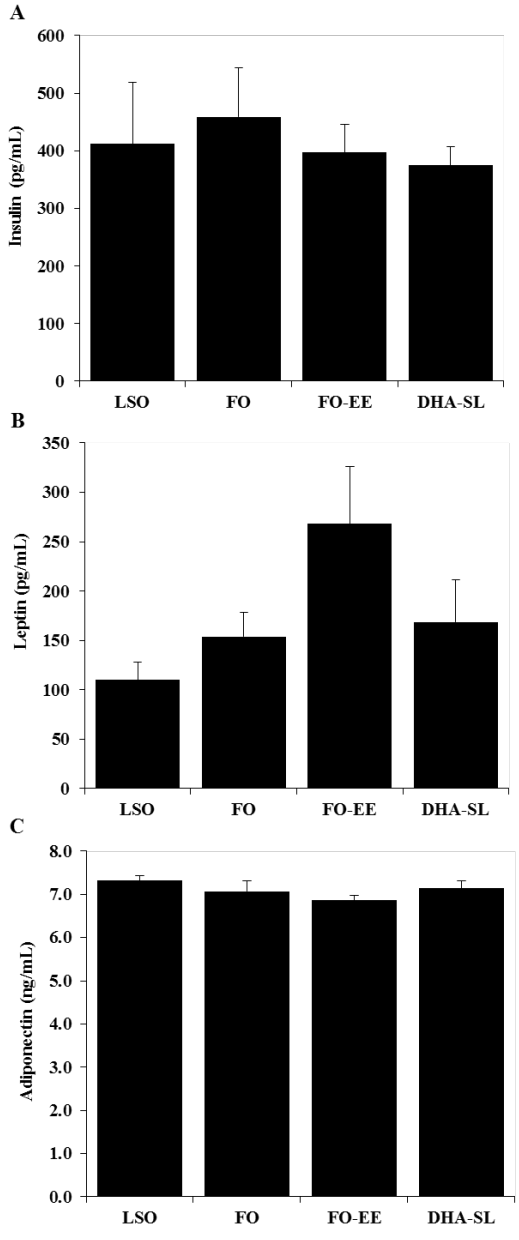

Figure 2. Plasma hormones, insulin (A), leptin (B) and adiponectin (C) from hamsters fed on LSO, FO, FO-EE and DHA-SL diets

3.3. Fatty Acid Composition of Subcutaneous WAT

The fatty acid composition of subcutaneous WAT across dietary treatments is shown in Table 2.

The distribution pattern of the main fatty acid classes across dietary groups showed a highest occurrence of MUFA (51 to 53%), followed by total PUFA (24 to 26%), and lastly by SFA and *n*-6 PUFA, both sharing a prevalence around 20% (21 to 23%). SFA were unchanged under this experimental design ($p > 0.05$), due to the non-variations of the main saturated fatty acids, 14:0, 15:0, 16:0, 17:0, 18:0 and 19:0 ($p > 0.05$). In a similar trend, the MUFA sum presented no differences ($p > 0.05$) among groups, although small changes were found for the 20:1*n*-9 ($p < 0.001$), 16:1*n*-7 ($p < 0.01$), 16:1*n*-9 ($p < 0.05$) and 18:1*n*-9 ($p < 0.05$) fatty acids. Even if total PUFA did not change ($p > 0.05$), the *n*-3 PUFA sum was increased in the LSO group in comparison to the others ($p < 0.001$), at the expenses of the alpha-linolenic acid (18:3*n*-3) ($p < 0.001$). EPA (20:5*n*-3) and DHA (22:6*n*-3) were not found. For the *n*-6 PUFA sum, the variations observed reflect the ones of the linoleic acid (18:2*n*-6) with higher percentages found in the DHA-SL group in relation to the FO ($p < 0.05$). Arachidonic acid (AA, 20:4*n*-6), the most prevalent LCPUFA from the *n*-6 family was not found in any dietary treatment. All in all, these individual changes on *n*-6 and *n*-3 fatty acids produced variations on the *n*-3/*n*-6 ratio; in fact, these ratios were identically across FO, FO-EE and DHA-SL diets but higher in the LSO diet ($p < 0.001$).

3.4. Fatty Acid Composition of Retroperitoneal WAT

The fatty acid profile of retroperitoneal WAT is presented in Table 3. The distribution pattern of the main fatty acid classes across dietary groups showed a highest occurrence of MUFA (48 to 51%), followed by SFA (25 to 27%), closely followed by total PUFA (23 to 24%), and lastly by *n*-6 PUFA (20 to 22%). Except for the increased percentage of 11:0 fatty acid in the DHA-SL group ($p < 0.001$), the main saturated fatty acids, 14:0, 15:0, 16:0, 17:0, 18:0 and 19:0, as well as total SFA, presented no variations across dietary groups ($p > 0.05$). The sum of MUFA was decreased in the DHA-SL group in comparison to the LSO ($p < 0.05$), mostly due to the 18:1*n*-9 ($p < 0.01$) fatty acid. Moreover, the 16:1*n*-7 percentage was higher in the FO group when compared to the LSO group ($p < 0.05$). 18:1*n*-7 fatty acid was higher in the FO, intermediate in the LSO and FO-EE, and lower in the DHA-SL ($p < 0.01$). In turn, higher percentages of the 20:1*n*-9 fatty acid were observed in the FO and FO-EE groups whereas lower percentages were observed in the LSO and DHA-SL ($p < 0.001$). Total and *n*-6 PUFA contents were higher in the DHA-SL ($p < 0.05$) due to the variations found in linoleic acid (18:2*n*-6) ($p < 0.05$), whereas *n*-3 PUFA sum was higher in the LSO group ($p < 0.001$) at the expenses of alpha-linolenic acid (18:3*n*-3) ($p < 0.001$). In resemblance to the subcutaneous WAT, arachidonic acid (20:4*n*-6) was not found in any dietary treatment. The *n*-3/*n*-6 ratio reached the highest value in the LSO being the values similar for FO, FO-EE and DHA-SL ($p < 0.001$) groups. EPA (20:5*n*-3) and DHA (22:6*n*-3) were once again, not found in white adipose tissue.

Table 2. Fatty acid composition (% of total fatty acids) of subcutaneous white adipose tissue

	LSO	FO	FO-EE	DHA-SL	SEM	Significance
11:0	0.053^{b}	0.065^{b}	0.053^{b}	0.126^{a}	0.012	***
14:0	1.13	1.45	1.23	1.43	0.126	NS
15:0	0.487	0.540	0.464	0.524	0.037	NS
16:0	17.5	19.2	18.6	19.0	0.655	NS
16:1*n*-9	0.581^{a}	0.429^{b}	0.501^{ab}	0.525^{ab}	0.031	*
16:1*n*-7	5.58^{b}	7.76^{a}	6.57^{ab}	6.96^{ab}	0.418	**
17:0 isobr	0.072	0.071	0.078	0.074	0.005	NS
17:0	0.325	0.312	0.297	0.305	0.014	NS
16:3*n*-4	0.502^{b}	0.580^{a}	0.496^{b}	0.494^{b}	0.023	*
18:0	1.56	1.38	1.49	1.45	0.067	NS
18:1*n*-9	44.8^{a}	41.8^{b}	43.4^{ab}	41.7^{b}	0.816	*
18:1*n*-7	1.66	1.74	1.54	1.25	0.144	NS
18:2*n*-6	22.2^{ab}	21.4^{b}	22.2^{ab}	23.1^{a}	0.423	*
19:0	0.078	0.065	0.079	0.068	0.016	NS
18:3*n*-4	0.220	0.213	0.208	0.196	0.012	NS
18:3*n*-3	1.98^{a}	1.55^{b}	1.49^{b}	1.59^{b}	0.048	***
18:4*n*-3	0.107	0.089	0.110	0.105	0.011	NS
20:1*n*-9	0.230^{b}	0.319^{a}	0.318^{a}	0.180^{b}	0.020	***
20:2*n*-6	ND	ND	ND	ND	-	-
20:4*n*-6	ND	ND	ND	ND	-	-
20:5*n*-3	ND	ND	ND	ND	-	-
22:6*n*-3	ND	ND	ND	ND	-	-
Others	0.862	1.083	0.885	0.900	0.063	NS
Partial sums and ratios						
Total SFA	21.2	23.1	22.3	22.9	0.806	NS
Total MUFA	52.9	52.0	52.3	50.7	0.591	NS
Total PUFA	25.0	23.8	24.5	25.5	0.440	NS
Total *n*-3	2.09^{a}	1.64^{b}	1.60^{b}	1.69^{b}	0.048	***
Total *n*-6	22.2^{ab}	21.4^{b}	22.2^{ab}	23.1^{a}	0.423	*
n-3/*n*-6	0.094^{a}	0.077^{b}	0.072^{b}	0.073^{b}	0.002	***

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group; DHA-SL, the structured DHA group. n = 10 per group. ND, not detected.

Values are means ± SEM. Statistical significance: NS = not significantly different, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. ^{a,b,c} Means in the same row with different superscripts are statistically different (Tukey's *post hoc*, $p < 0.05$).

Table 3. Fatty acid composition (% of total fatty acids) of retroperitoneal white adipose tissue

	LSO	FO	FO-EE	DHA-SL	SEM	Significance
11:0	0.082^{b}	0.084^{b}	0.084^{b}	0.186^{a}	0.009	***
14:0	1.59	1.74	1.73	1.70	0.133	NS
15:0	0.634	0.652	0.617	0.645	0.033	NS
16:0	20.3	21.8	21.9	21.9	0.533	NS
16:1*n*-9	0.652	0.547	0.564	0.561	0.030	NS
16:1*n*-7	5.42^{b}	7.23^{a}	6.36^{ab}	6.62^{ab}	0.379	*
17:0 isobr	0.106	0.096	0.103	0.115	0.005	NS
17:0	0.401	0.405	0.370	0.368	0.023	NS
16:3*n*-4	0.522^{ab}	0.591^{a}	0.505^{ab}	0.493^{b}	0.024	*
18:0	1.82	1.68	1.72	1.70	0.081	NS
18:1*n*-9	43.0^{a}	40.2^{b}	41.2^{ab}	40.1^{b}	0.624	**
18:1*n*-7	1.22^{ab}	1.58^{a}	1.29^{ab}	0.818^{b}	0.129	**
18:2*n*-6	20.7^{ab}	20.1^{b}	20.5^{ab}	21.7^{a}	0.334	*
19:0	0.113	0.100	0.094	0.101	0.007	NS
18:3*n*-4	0.225^{a}	0.182^{b}	0.175^{b}	0.176^{b}	0.008	***
18:3*n*-3	2.03^{a}	1.55^{b}	1.44^{b}	1.52^{b}	0.048	***
18:4*n*-3	0.101	0.092	0.106	0.087	0.007	NS
20:1*n*-9	0.201^{b}	0.306^{a}	0.321^{a}	0.174^{b}	0.024	***
20:2*n*-6	ND	ND	ND	ND	-	-
20:4*n*-6	ND	ND	ND	ND	-	-
20:5*n*-3	ND	ND	ND	ND	-	-
22:6*n*-3	ND	ND	ND	ND	-	-
Others	0.839	1.04	0.921	1.04	0.103	NS
Partial sums and ratios						
Total SFA	25.1	26.5	26.6	26.7	0.645	NS
Total MUFA	50.5^{a}	49.9^{ab}	49.7^{ab}	48.3^{b}	0.497	*
Total PUFA	23.6^{ab}	22.5^{b}	22.7^{ab}	24.0^{a}	0.349	*
Total *n*-3	2.13^{a}	1.64^{b}	1.55^{b}	1.61^{b}	0.048	***
Total *n*-6	20.7^{ab}	20.1^{b}	20.5^{ab}	21.7^{a}	0.334	*
n-3/*n*-6	0.103^{a}	0.082^{b}	0.075^{b}	0.074^{b}	0.002	***

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group; DHA-SL, the structured DHA group. n = 10 per group. ND, not detected.

Statistical significance: NS = not significantly different, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. ^{a,b,c} Means in the same row with different superscripts are statistically different (Tukey's *post hoc*, $p < 0.05$).

Table 4. Fatty acid composition (% of total fatty acids) of interscapular brown adipose tissue

	LSO	FO	FO-EE	DHA-SL	SEM	Significance
11:0	0.043^{b}	0.054^{b}	0.057^{b}	0.088^{a}	0.006	***
14:0	1.11	1.33	1.28	1.30	0.062	NS
15:0	0.339	0.335	0.320	0.342	0.018	NS
16:0	18.4	20.1	20.3	19.5	0.546	NS
16:1*n*-9	0.556	0.504	0.542	0.496	0.024	NS
16:1*n*-7	3.36	4.24	3.84	3.76	0.342	NS
17:0 isobr	0.084	0.099	0.100	0.089	0.007	NS
17:0	0.447	0.455	0.445	0.468	0.024	NS
16:3*n*-4	0.389	0.441	0.407	0.403	0.024	NS
18:0	4.88	4.98	4.82	4.82	0.278	NS
18:1*n*-9	43.2^{a}	41.2^{ab}	41.6^{ab}	40.9^{b}	0.555	*
18:1*n*-7	1.13^{ab}	1.18^{a}	1.16^{a}	0.931^{b}	0.056	*
18:2*n*-6	22.2	21.0	21.3	23.0	0.579	NS
19:0	0.169	0.151	0.135	0.154	0.012	NS
18:3*n*-4	0.235	0.217	0.216	0.213	0.010	NS
18:3*n*-3	2.01^{a}	1.49^{b}	1.33^{b}	1.46^{b}	0.055	***
18:4*n*-3	0.067	0.050	0.075	0.057	0.011	NS
20:1*n*-9	0.338^{bc}	0.479^{a}	0.432^{ab}	0.296^{c}	0.027	***
20:2*n*-6	0.122	0.115	0.145	0.122	0.017	NS
20:4*n*-6	0.520	0.446	0.454	0.412	0.041	NS
20:5*n*-3	ND	ND	ND	ND	-	-
22:6*n*-3	ND	0.184^{b}	0.223^{b}	0.470^{a}	0.052	***
Others	0.477^{b}	0.961^{a}	0.863^{ab}	0.779^{ab}	0.109	*
Partial sums and ratios						
Total SFA	25.5^{b}	27.5^{a}	27.4^{ab}	26.8^{ab}	0.521	*
Total MUFA	48.5	47.6	47.6	46.3	0.635	NS
Total PUFA	25.5	24.0	24.1	26.1	0.636	NS
Total *n*-3	2.07^{a}	1.72^{bc}	1.63^{c}	1.98^{ab}	0.081	**
Total *n*-6	22.8	21.6	21.9	23.5	0.601	NS
n-3/*n*-6	0.091^{a}	0.080^{ab}	0.074^{b}	0.084^{b}	0.003	**

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group; DHA-SL, the structured DHA group. n = 10 per group. ND, not detected.
Values are means ± SEM. Statistical significance: NS = not significantly different, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. ^{a,b,c} Means in the same row with different superscripts are statistically different (Tukey's *post hoc*, $p < 0.05$).

3.5. Fatty Acid Composition of Interscapular BAT

The fatty acid profile of retroperitoneal WAT is presented in Table 4.

The distribution pattern of the main fatty acid classes across dietary groups showed a highest occurrence of MUFA (46 to 49%), followed by approximately equal prevalence of SFA (26 to 28%) and total PUFA (24 to 26%), and closely by *n*-6 PUFA (22 to 24%). In contrast to the large variations described for white fat depots, interscapular BAT presented fewer changes for fatty acids deposition across treatments. The SFA sum was higher in FO relative to LSO ($p < 0.05$), although similar to FO-EE and DHA-SL groups ($p > 0.05$). Except for the 11:0 fatty acid which reached the highest percentage in the DHA-SL ($p < 0.001$) diet relative to the others, no additional variations were found for the remaining SFA ($p > 0.05$). Even if total MUFA was kept similar across dietary groups ($p > 0.05$), the 18:1*n*-9 percentage was higher in the LSO group in comparison to the DHA-SL ($p < 0.05$). The 18:1*n*-7 and the 20:1*n*-9 fatty acids reached the lowest percentages in the DHA-SL group ($p < 0.05$ and $p < 0.001$, respectively). Total PUFA, as well as the *n*-6 family, showed no variations across dietary groups ($p > 0.05$), including arachidonic acid (20:4*n*-6) ($p > 0.05$). In contrast, the LSO group had the highest percentage of *n*-3 PUFA sum in comparison to FO and FO-EE groups ($p < 0.01$). Not surprisingly, the DHA-SL group presented the highest percentage of DHA in relation to FO and FO-EE ($p < 0.001$) groups. EPA (20:5*n*-3) was undetermined in BAT.

3.6. PCA on Plasma Hormones and Adipose Tissues' Fatty Acids

The Figure 3A displays the projection of PC1 and PC2 in the plane using the levels of plasma hormones (insulin, leptin and adiponectin) and the percentage of fatty acids determined in all 3 fat depots (subcutaneous WAT, retroperitoneal WAT and interscapular BAT). Both PC combined accounted for 46.34% of the total variance. The PC1 was characterized by variables with positive loadings, such as 18:0 (0.698), 19:0 (0.697), 18:3*n*-4 (0.653), 20:1*n*-9 (0.619), 18:1*n*-9 (0.574) and 18:2*n*-6 (0.537), and by variables with negative loadings, like 15:0 (-0.846), 14:0 (-0.815), 16:1*n*-7 (-0.815), 16:0 (-0.741), 11:0 (-0.684) and 16:3*n*-4 (-0.596) (Figure 3A). In turn, the PC2 was positively defined by 17:0 (0.709), 18:0 (0.619), 17:0iso (0.614) and 16:0 (0.570), and negatively by 18:1*n*-7 (-0.688), 18:4*n*-3 (-0.540) and 18:1*n*-9 (-0.519) (Figure 3A). Interestingly, insulin, leptin and adiponectin were not related to any of the PC, and therefore, they were positioned close to the origin of the plot. This result is due to the absence of variations on plasma hormones across dietary treatments (Figure 2). The projection of scores in the PC1 × PC2 plane set apart 3 well defined clusters matching the 3 analyzed fat depots: the interscapular BAT was individualized in quadrant B; the retroperitoneal WAT was located in quadrants A and D; and the subcutaneous WAT was positioned in quadrants C and D (Figure 3B). Surprisingly, the cluster of subcutaneous WAT showed more dispersion than the ones delimited for interscapular BAT and retroperitoneal WAT, in this particular order (Figure 3B). The PCA on plasma

hormones and adipose tissues' fatty acids discriminated BAT from WAT. The cluster defined for interscapular BAT did not overlap the others in opposition to subcutaneous and retroperitoneal WATs which were relatively merged into each other (Figure 3B). It is worth notice that the discrimination of dietary treatments (LSO, FO, FO-EE and DHA-SL) within each adipose tissue was unattainable (Figure 3B).

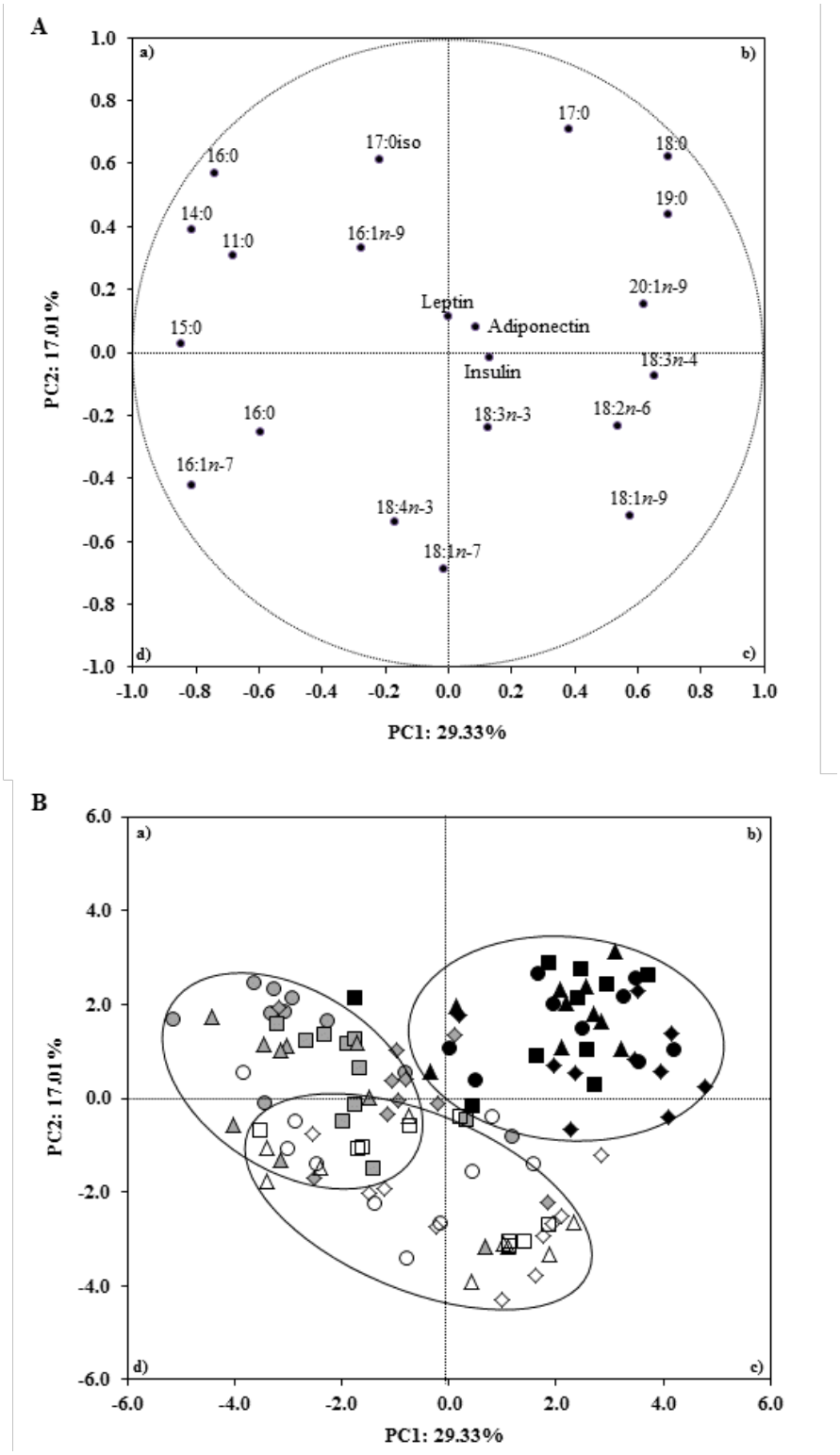

Figure 3. Loadings plot of the first and second principal components of the pooled FAME and plasma hormones (A) and component's score vectors (B) of subcutaneous white adipose tissue (white markers), retroperitoneal white adipose tissue (gray markers) and interscapular brown adipose tissue (black markers) from hamsters fed on LSO (◇), FO (Δ), FO-EE (□) and DHA-SL (○) diets

4. Discussion

Despite the current obesity epidemic and a growing body of evidence indicating that the regular consumption of the *n*-3 LCPUFA, notably docosahexaenoic acid (DHA, 22:6*n*-3) and eicosapentaenoic acid (EPA, 20:5*n*-3) can influence fat metabolism [36], little attention has been paid to the stereospecific structure of dietary lipids, in particular the potential of DHA placed at the *sn*-2 position

of TAG to affect its bioavailability and to modulate body fat composition.

In this study, similar adipose tissue weights from subcutaneous WAT, retroperitoneal WAT and BAT were found in hamsters across dietary treatments along with body weight gain and final body weight at the end of the experiment [35]. Contrarily to our study, Parrish and co-workers [37] used Wistar rats fed on high fat diets containing 20% TAG from fish oil or 20% TAG from lard for 3 weeks and found significantly less perirenal and epididymal fat in the fish oil fed group but no differences in body weight, like us. The fat lowering effect previously reported was the result of adipocyte size (i.e. adipocytes filling) reduction with no difference in adipocyte number [37,38]. Hainault et al. [39] reported in rats fed on *n*-3 fatty acids 20 to 30% less subcutaneous and visceral fat, respectively. Ruzicvkova et al. [40] fed obesity-prone mice a range of high fat diets (20-25% fat w/w) with varying levels of EPA plus DHA (1-12% w/w) for 5 weeks and reported that while DHA and EPA attenuated epididymal fat accumulation, there was a limited effect on the subcutaneous fat. Later on, Pérez-Matute et al. [41] showed that feeding rats EPA (1 g/kg/d) while consuming a high fat diet (62% w/w) for 5 weeks attenuated retroperitoneal adipose tissue accumulation, but associated this fact with increase in plasma concentrations of the appetite-suppressing hormone leptin and reduced food intake [42], suggesting that some of the effects of these fatty acids on body fat may be mediated by an appetite-suppressing action. Conversely, our data showed neither variation on leptin concentrations or on daily feed intake [35]. In view of what was above described, data on these parameters are consistent with each other. According to the literature, it appears that the protective benefits of these *n*-3 LCPUFA may be achieved through a lesser accumulation of fat in existing adipocytes, with these effects being primarily evident in visceral fat depots [37,40,43] exhibiting a dose-response relationship, and secondly in subcutaneous fat. The preferential reduction in visceral fat deposition may be mediated by a low ratio of EPA/DHA [40], suggesting that DHA, rather than EPA, is the main responsible for promoting the anti-obesity effect of increased *n*-3 LCPUFA intake. The arguments provided by Buckley and Howe [36] for the loss of body weight and improved body fat composition in mice switched to an enriched *n*-3 LCPUFA diet, focused essentially on reduced metabolic efficiency, through suppression of appetite, improvement of circulation (whereby nutrient delivery to skeletal muscle might be facilitated) and variation in gene expression which shift metabolism towards increased accretion of lean tissue, enhanced fat oxidation, increased energy expenditure [5], and ultimately reduced fat deposition [36,44,45]. In opposition, Todoric et al. [46] using genetically diabetic mice described that the incorporation of *n*-3 LCPUFA (EPA + DHA) into a high fat diet (30% of energy) exacerbated weight gain with no differences in energy intake. Unfortunately, these authors failed to assess changes in body fat content, making it difficult to determine whether the increased weight gain was actually due to greater fat deposition. This suggests that *n*-3 LCPUFA may exert contrasting effects on body fat accumulation supporting the lack of reduction on adipose tissue deposits weight, WAT or BAT, observed by us.

n-3 LCPUFA are considered a valuable nutritional tool for preventing or diminishing insulin resistance in human obesity [47]. Insulin stimulates fatty acid synthesis as well as formation and storage of TAG in the adipose tissue and liver [48]. Herein, similar to the non-variation of insulin concentrations across dietary groups, the insulin resistance markers (HOMA-IR and QUICKI) were unchanged [35], without affecting insulin homeostasis. Noteworthy is the fact that structured DHA at the *sn*-2 position of TAG fed to hamsters showed the lowest HOMA-IR index among all dietary groups (<2.3) which characterizes high insulin sensitivity [6].

Adiponectin and leptin are adipokines responsible for normal adipocyte phenotype and function [49,50]. Leptin is almost exclusively produced by adipocytes in proportion to their TAG storage [51] and it is known to regulate lipolysis by controlling the level of hormone sensitive-lipase (HSL) [52], which is one of the enzymes responsible for fatty acids breakdown [52]. Using an *in vitro* setting, Ahn et al. [53] observed that fat accumulation in 3T3-L1 adipocytes and leptin secretion to the extracellular medium were reduced by a recognized anti-adipogenic fatty acid compound, the *trans*10,*cis*12 conjugated linoleic acid (CLA) isomer, without affecting adiponectin. Hence, it is our belief that the non-variation of leptin and adiponectin agrees with similar fat depots weight found across dietary treatments.

The fatty acid composition of adipose tissue has been considered as a gold standard for dietary fatty acids representation [54,55,56,57,58]. The fatty acid composition of adipose tissue is typically measured because approximately 99% of adipose tissue is comprised of TAG with 0.3% of cholesterol and less than 0.1% of PL [59].

Common to all fat depots studied (subcutaneous WAT, retroperitoneal WAT and BAT), palmitic acid was the main SFA while oleic acid was the main representative of MUFA, according to previous reports [55,60]. Oleic and linoleic acids combined made up the greatest proportion of fatty acids whereas palmitoleic and stearic acids were relatively least abundant, in agreement with Reidinger et al. [55]. In a study about time-course incorporation of dietary *n*-3 PUFA, fish-oil induced rapid and large replacements of *n*-6 fatty acids by *n*-3 fatty acids into phospholipids of liver, WAT and BAT in rats [61]. As a general trend, the ratio 22:6*n*-3/20:5*n*-3 was higher in liver than in WAT and BAT. Herein, DHA and EPA were not detected in subcutaneous and retroperitoneal WAT. The same trend was verified in BAT for EPA, but not for DHA, reaching the highest percentage in DHA-SL fed hamsters. In accordance to Leray et al. [61], the incorporation of these *n*-3 LCPUFA is commonly attainable in the liver, as previously reported [35] but considering the divergent findings with the literature [62], one can speculate about the small dosages of DHA and EPA used. Besides that, the strength of the association between diet and biomarker depends highly on the fatty acid analyzed. For instance, fatty acids not synthesized endogenously, such as *trans* or odd-chain fatty acids, 18:2*n*-6, 18:3*n*-3 and fish oil-derived *n*-3 LCPUFA in adipose tissue tend to correlate well with diet [56]. In turn, correlations are weaker for other SFA and MUFA. A possible explanation might be that it is difficult to modify the proportion of a fatty acid that is already present in a relatively high percentage in the diet or in the body [56].

TAG from white adipose tissue, subcutaneous and retroperitoneal, contained predominantly 18:1*n*-9 fatty acid. The next most abundant fatty acids were 18:2*n*-6 and 16:0. SFA, as 15:0 and 17:0 fatty acids were found in low concentrations, just like proportions described by Biong et al. [63]. The proportion of 16:1*n*-7 (palmitoleic acid) was more than two times higher than 18:0 (stearic acid). PUFA in TAG adipose tissue consist primarily of those provided in the diet, that is, 18:2*n*-6 and 18:3*n*-3 [57]. Of note are the very low proportions of HUFA and very long chain (20 or more carbons) PUFA found, in resemblance to Hodson et al. [56] findings in subcutaneous WAT, as the fatty acids in this tissue are primarily less than 18 carbons in length [58]. Although *n*-3 fatty acids are acceptable as low in abundance in adipose tissue, Leaf et al. [64] demonstrated the ability of adipose tissue to sequester *n*-3 fatty acids, reflecting dietary intake that together with higher oxidative and lipolytic capacity of TAG rich in long-chain fatty acids might explain the reduction in fat mass found with diets rich in long-chain fatty acids [4], but disappointingly not in our study, even after using a novel structure form of DHA, that is, DHA placed at the *sn*-2 position of TAG. DHA and also EPA have positive effects, lowering lipogenesis, increasing lipolysis and decreasing inflammation, all of which would be beneficial for adipose tissue biology [4]. In humans, comparisons between subcutaneous and intra-abdominal adipose tissue have been made more often [65,66,67]. Higher proportions of MUFA are found in subcutaneous adipose depots when compared to intra-abdominal sites, which are in line with our results even if the difference is lesser relevant. This increase in MUFA appears to be at the expenses of SFA. Interestingly, there is no difference in the distribution of linoleic acid (18:2*n*-6) between the two sites, as previously observed by others [65,66,67]. The abundance of DHA and EPA in humans' adipose tissue does not increase notably even after supplementation. This suggests that DHA and EPA are not preferentially long-term stored in adipose tissue TAG, rather they may be partitioned to oxidation pathways or storage in other lipid fractions, such as plasma phospholipids, cell phospholipids and red blood cells; all of them having a notably higher abundance of both DHA and EPA than adipose tissue [4,35].

The subcutaneous WAT in comparison to the retroperitoneal WAT was not very sensitive to changes in dietary fatty acid composition, probably because it is a tissue with a lower rate of metabolic interchange or the experimental period applied was too short to show the expected changes in this tissue [62]. Once again, this was particular the case of DHA, contrarily to Soriguer et al. [62] findings.

BAT is primarily distributed in interscapular and also, in axillary, paravertebral and perirenal area in mammals [68]. BAT thermogenesis is influenced by the fatty acid composition of diet [69], as fatty acids provide the major fuel of respiration in brown fat [70]. Essential fatty acids (mixed *n*-6 and *n*-3 PUFA) have been shown to have a stimulatory effect on BAT thermogenesis concomitant with improve insulin sensitivity and glucose metabolism by decreasing fat deposition [45] and weight gain in rats [71], and counter-acting obesity [72,73]. As previously debated, interscapular BAT fatty acid differences do not completely correspond to differences in the fatty acid composition of diets, but rather follow specific fatty acid profiles [74]. The ability to select, degrade, elongate, and desaturate the newly arrived fatty acids could be more important in determining the interscapular BAT fatty acid composition, as compared to what is accepted for other fat depots, in particular white adipose tissue [60], and could also be related to a more active metabolism of fatty acids in brown adipocytes specialized in thermogenesis. Although the capacity of BAT for *de novo* synthesis of fatty acids is very high [75], it is also possible that these fatty acids were synthesized in other tissues and taken up by BAT, given the high lipoprotein lipase (LPL) activity of this tissue to import fatty acids - the fuel for non-shivering thermogenesis - from circulating TAG [76,77,78]. In opposition to WAT, structured DHA at the *sn*-2 position of TAG, incorporated better than other DHA molecular structures (fish oil and fish oil fatty acid ethyl esters) into BAT [61] providing a role in the thermogenic function in this tissue [79], and validating for BAT the research hypothesis previously formulated. Besides DHA, differences on the majority of fatty acids in BAT were modest, and it is not clear if their levels would effectively translate into physiological differences.

According to Soriguer et al. [62], the tissue of origin is a more important source of variation than diet in the fatty acid content of fat. Moreover, adipose tissue TAG turnover may be influenced by the size of fat depot. This study corroborates the complexity of the interrelations between different families of fatty acids and of the specificity of each tissue to changes in view of dietary fatty acids composition. Taken together, these arguments justify the contrasting responsiveness on fatty acids deposition across subcutaneous and visceral WAT and BAT, as clearly proven by the discriminant statistical analysis.

5. Conclusions

In summary, our results do not document augmentation of the anti-adipogenic effect of DHA structured at the *sn*-2 position of TAG nor suggest that more DHA relative to EPA could reduce accumulation of hamsters' body fat. This might be the direct consequence of using normal-weight hamsters, which stands out as an important limitation of this study as the majority of evidence in both growing and adult rodents stresses out that dietary intake of *n*-3 LCPUFA may reduce body fat accumulation, particularly visceral fat, when growing animals are exposed to high fat diets, or reduce final body weight in adults that are already obese [5,80]. Moreover, this study underlines the existence of a clear individual signature of fatty acid deposition, which overall determines the discrimination of BAT from WAT. It also points out toward a possible conservative role of BAT which stands out as less permeable to dietary lipids, in opposition to both subcutaneous and visceral WAT. Even so, it is worth notice that BAT, in contrast to WAT, deposited DHA in hamsters fed on different molecular structures of DHA, reaching the highest percentage of accumulation when engineered at the *sn*-2 position of TAG, reflecting most probably cellular specific requirements associated with divergent anatomical location and metabolic function.

Acknowledgments

This study was supported by the Spanish Ministry of Science and Innovation through "Structured lipids: novel dietary strategies for improving human health" grant (AGL/25807/2011) and by Fundação para a Ciência e a Tecnologia (FCT, Lisbon, Portugal) through CIISA project (UID/CVT/00276/2013). PAL is a researcher from the FCT "Ciência 2008" program and Incentivo 2014 project (AGR/UI0276/2014). SVM is a recipient of an individual fellowship from FCT (SFRH/BPD/63019/2009).

Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

BAT; brown adipose tissue
DHA; docosahexaenoic acid
DHA-SL; structured docosahexaenoic acid at the *sn*-2 position of triacylglycerols
EPA; eicosapentaenoic acid
FAME; fatty acid methyl esters
FFA; free fatty acids
FO; fish oil
FO-EE; fish oil ethyl esters
LCPUFA; long-chain polyunsaturated fatty acid
LSO; linseed oil
MUFA; monounsaturated fatty acid
PCA; principal components analysis
SL; structured lipids
SFA; saturated fatty acid
TAG; triacylglycerols
WAT; white adipose tissue

References

[1] Simopoulos, A., "The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases", *Exp. Biol. Med.*, 233 (6), 674-688. June 2008.

[2] Calder, P., "*n*-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases", *Am. J. Clin. Nutr*., 83 (6), 1505S-1519S. June 2006.

[3] Calder, P., "Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?", *Br. J. Clin. Pharmacol.*, 75 (3), 645-662. March 2013.

[4] Todorčević, M. and Hodson, L., "The effect of marine derived *n*-3 fatty acids on adipose tissue metabolism and function", *J. Clin. Med*., 5 (1), 3.

[5] Belchior, T., Paschoal, V.A., Magdalon, J., Chimin, P., Farias, T.M., Chaves-Filho, A.B., Gorjão, R., St-Pierre, P., Miyamoto, S., Kang, J.X., Deshaies, Y., Marette, A. and Festuccia, W., "Omega-3 fatty acids protect from diet-induced obesity, glucose intolerance, and adipose tissue inflammation through PPARγ-dependent and PPARγ-independent actions", *Mol. Nutr. Food Res.*, 59 (5), 957-967. May 2015.

[6] Guilherme, A., Virbasius, J.V., Puri, V. and Czech, M.P., "Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes", *Nat. Rev. Mol. Cell Biol*., 9 (5), 367-377. May 2008.

[7] Miller, W.H. and Faust, I.M., "Alterations in rat adipose tissue morphology induced by a low temperature environment", *Am. J. Physiol. Endocrinol. Metabol*., 242 (2), E93-E96. February1982.

[8] Vitali, A., Murano, I., Zingaretti, M.C., Frontini, A., Ricquier, D. and Cinti, S., "The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes", *J. Lipid Res.*, 53 (4), 619-629. April 2012.

[9] Barbatelli, G., Murano, I., Madsen, L., Hao, Q., Jimenez, M., Kristiansen, K., Giacobino, J.P., De Matteis, R. and Cinti, S., "The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation", *Am. J. Physiol. Endocrinol. Metab*., 298 (6), E1244-E1253. June 2010.

[10] Capurso, C. and Capurso, A., "From excess adiposity to insulin resistance: the role of free fatty acids", *Vascul. Pharmacol.*, 57 (2-4), 91-97. September-October 2012.

[11] Park, H.T., Lee, E.S., Cheon, Y.P., Lee, D.R., Yang, K.-S., Kim, Y.T., Lee, K.W. and Kim, T., "The relationship between fat depot-specific preadipocyte differentiation and metabolic syndrome in obese women", *Clin. Endocrinol*., 76 (1), 59-66. January 2012.

[12] Birerdinc, A., Jarrar, M., Stotish, T., Randhawa. M. and Baranova, A., "Manipulating molecular switches in brown adipocytes and their precursors: a therapeutic potential", *Prog. Lipid Res.*, 52 (1): 51-61. January 2013.

[13] Smorlesi, A., Frontini, A., Giordano, A. and Cinti, S., "The adipose organ: white-brown adipocyte plasticity and metabolic inflammation", *Obesity Reviews*, 13(Suppl. 2), 83-96. December 2012.

[14] Rosell, M., Kaforou, M., Frontini, A., Okolo, A., Chan, Y.W., Nikolopoulou, E., Millership, S., Fenech, M.E., MacIntyre, D., Turner, J.O., Moore, J.D., Blackburn, E., Gullick, W.J., Cinti, S., Montana, G., Parker, M.G. andChristian, M., "Brown and white adipose tissues: intrinsic differences in gene expression and response to cold exposure in mice", *Am. J. Physiol. Endocrinol. Metab*., 306 (8), E945-E964. April 2014.

[15] Hunter, J.E., "Studies on effects of dietary fatty acids as related to their position on triglycerides", *Lipids*, 36 (7), 655-668. July 2001.

[16] Hong, D.D., Takahashi, Y., Kushiro, M. and Ide, T., "Divergent effects of eicosapentaenoic and docosahexaenoic acid ethyl esters, and fish oil on hepatic fatty acid oxidation in the rat", *Biochim. Biophys. Acta*, 1635 (1), 29-36. November 2003.

[17] Hoshi, T., Wissuwa, B., Tian, Y., Tajima, N., Xu, R., Bauer, M., Heinemann, S.H. and Hou, S., "Reply to Harris et al.: Differential impacts of omega-3 fatty acids and their derivatives on blood pressure", *Proc. Natl. Acad. Sci. USA*, 110 (25), E2260. June 2013.

[18] Lawson, L.D. and Hughes, B.G., "Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters", *Biochem. Biophys. Res. Commun*., 152 (1), 328-335. April 1988.

[19] Beckermann, B., Beneke, M. and Seitz, I., "Comparative bioavailability of eicosapentaenoic acid and docosahexaenoic acid from triglycerides, free fatty acids and ethyl esters in volunteers", *Arzneimittelforschung*, 40 (6), 700-704. June 1990.

[20] Davidson, M.H., Johnson, J., Rooney, M.W., Kyle, M.L. and Kling, D.F., "A novel omega-3 free fatty acid formulation has dramatically improved bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: the ECLIPSE (EPANOVA® compared to Lovaza® in a pharmacokinetic single-dose evaluation) study", *J. Clin. Lipidol.*, 6 (6), 573-584. November-December 2012.

[21] Ghasemifard, S., Turchini, G.M. and Sinclair, A.J., "Omega-3 long chain fatty acid "bioavailability": a review of evidence and methodological considerations", *Prog. Lipid Res*., 56, 92-108. October 2014.

[22] Kew, S., Gibbons, E.S., Thies, F., McNeill, G.P., Quinlan, P.T. and Calder, P.C., "The effect of feeding structured triacylglycerols enriched in eicosapentaenoic or docosahexaenoic acids on murine splenocyte fatty acid composition and leucocyte phagocytosis", *Br. J. Nutr*., 90 (6), 1071-1080. December 2003.

[23] Iwasaki, Y. and Yamane, T., "Enzymatic synthesis of structured lipids", *Adv. Biochem. Eng. Biotechnol*., 90, 151-171. February 2004.

[24] Mu, H. and Porsgaard, T., "The metabolism of structured triacylglycerols", *Prog. Lipid Res*., 44 (6), 430-448. November 2005.

[25] Yin, W., Carballo-Jane, E., McLaren, D.G., Mendoza, V.H., Gagen, K., Geoghagen, N.S., McNamara, L.A., Gorski, J.N., Eiermann, G.J., Petrov, A., Wolff, M., Tong, X., Wilsie, L.C., Akiyama, T.E., Chen, J., Thankappan, A., Xue, J., Ping, X., Andrews, G., Wickham, L.A., Gai, C.L., Trinh, T., Kulick, A.A., Donnelly, M.J., Voronin, G.O., Rosa, R., Cumiskey, A.M., Bekkari, K., Mitnaul, L.J., Puig, O., Chen, F., Raubertas, R.,

Wong, P.H., Hansen, B.C, Koblan, K.S., Roddy, T.P., Hubbard, B.K. and Strack, A.M., "Plasma lipid profiling across species for the identification of optimal animal models of human dyslipidemia", *J. Lipid Res*., 53 (1), 51-65. January 2012.

[26] Dalbøge, L.S., Pedersen, P.J., Hansen, G., Fabricius, K., Hansen, H.B., Jelsing, J. and Vrang, N., "A hamster model of diet-induced obesity for preclinical evaluation of anti-obesity, anti-diabetic and lipid modulating agents", *PLoS One*, 10 (8), e0135634. August 2015.

[27] AOAC, *AOAC Official Methods of Analysis*, AOACS, Washington DC, USA, 1995.

[28] Bandarra, N.M., Batista, I., Nunes, M.L. and Empis, J.M., "Seasonal variation in the chemical composition of horse-mackerel (*Trachurus trachurus*)", *Eur. Food Res. Technol*., 212 (5), 535-539. May 2001.

[29] Hamam, F. and Shahidi, F., "Synthesis of structured lipids via acidolysis of docosahexaenoic acid single cell oil (DHASCO) with capric acid", *J. Agric. Food Chem*., 52 (10), 2900-2906. May 2004.

[30] Itah, R., Gitelman, I. and Davis, C., "A replacement for methoxyflurane (Metofane) in open-circuit anaesthesia", *Lab. Anim.*, 38 (3), 280-285. July 2004.

[31] Bandarra, N.M., Batista, I., Nunes, M.L., Empis, J.M. and Christie, W.W., "Seasonal changes in lipid composition of sardine *Sardina pilchardus*". *J. Food Sci*., 62 (1), 40-42. January 1997.

[32] Christie, W.W., "Preparation of methyl ester and other derivatives", In: Christie WW, editor, *Gas Chromatography and Lipids - A Practical Guide*, Pergamon Press, New York, USA, p. 64-84. 1989.

[33] Lee, K.T., Akoh, C.C. and Dawe, D.L., "Effects of structured lipid containing omega 3 and medium chain fatty acids on serum lipids and immunological variables in mice", *J. Food Biochem*., 23 (2), 197-208. June 1999.

[34] Kadegowda, A.K., Pipierova, L.S. and Erdman, R.A., "Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression", *J. Dairy Sci*., 91 (2), 749-759. February 2008.

[35] Bandarra, N.M., Lopes, P.A., Martins, S.V., Ferreira, J., Alfaia, C.M., Rolo, E.A., Correia, J.J., Pinto, R.M.A., Ramos-Bueno, R.P., Batista, I., Prates, J. A.M. and Guil-Guerrero, J.L., "Docosahexaenoic acid at the *sn*-2 position of structured triacylglycerols improved *n*-3 polyunsaturated fatty acid assimilation in tissues of hamsters", *Nutr. Res*., 36 (5), 452-463. May 2016.

[36] Buckley, J.D. and Howe, P.R.C., "Long-chain omega-3 polyunsaturated fatty acids may be beneficial for reducing obesity – a review", *Nutrients*, 2 (12), 1212-1230. December 2010.

[37] Parrish, C., Pathy, D., Parkes, J. and Angel, A., "Dietary fish oils limit adipose tissue hypertrophy in rats", *Metabolism*, 39 (3), 217-219. March 1990.

[38] Belzung, F., Raclot, T. and Groscolas, R., "Fish oil *n*-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets", *Am. J. Physiol*., 264 (6 Pt 2), R1111-R1118. June 1993.

[39] Hainault, I., Carolotti, M., Hajduch, E., Guichard, C. and Lavau, M., "Fish oil in a high lard diet prevents obesity, hyperlipemia, and adipocyte insulin resistance in rats", *Ann. NY Acad. Sci*., 683, 98-101. June 1993.

[40] Ruzickova, J., Rossmeisl, M., Prazak, T., Flachs, P., Sponarova, J., Veck, M., Tvrzicka, E., Bryhn, M. and Kopecky, J., "Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue", *Lipids*, 39 (12), 1177-1185. December 2004.

[41] Pérez-Matute, P., Pérez-Echarri, N., Martínez, J.A., Marti, A. and Moreno-Aliaga, M.J., "Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-α", *Br. J. Nutr*., 97 (2), 389-398. February 2007.

[42] Takahashi, Y. and Ide, T., "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 (2), 175-184. August 2000.

[43] Baillie, R., Takada, R., Nakamura, M. and Clarke, S., "Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fishoil: a mechanism for decreased body fat deposition", *Prostaglandins Leukot. Essent. Fatty Acids*, 60 (5-6), 351-356. May-June 1999.

[44] Cunnane, S.C., McAdoo, K.R. and Horrobin, D.F., "*n*-3 essential fatty acids decrease weight gain in genetically obese mice", *Br. J. Nutr*., 56 (1), 87-95. July 1986.

[45] Clarke, S., "Polyunsaturated fatty acid regulation of gene transcription: A mechanism to improve energy balance and insulin resistance", *Br. J. Nutr*., 83 (Suppl 1), S59-S66. March 2000.

[46] Todoric, J., Löffler, M., Huber, J., Bilban, M., Reimers, M., Kadl, A., Zeyda, M., Waldhäusl, W. and Stulnig, T.M., "Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by *n*-3 polyunsaturated fatty acids", *Diabetologia*, 49 (9), 2109-2119. September 2006.

[47] Holness, M.J., Greenwood, G., Smith, N. and Sugden, M.C., "Diabetogenic impact of long-chain omega-3 fatty acids on pancreatic β-cell function and the regulation of endogenous glucose production", *Endocrinology*, 144 (9), 3958-3968. September 2003.

[48] Wilcox, G., "Insulin and Insulin Resistance", *Clin. Biochem. Rev*., 26 (2), 19-39. May 2005.

[49] Cowherd, R.M., Lyle, R.E. and McGehee, R.E., "Molecular regulation of adipocyte differentiation", *Semin. Cell Dev. Biol*., 10 (1), 3-10. February 1999.

[50] Cao, H., "Adipocytokines in obesity and metabolic disease", *J. Endocrinol*., 220 (2), T47-59. January 2014.

[51] Savage, D.B. and O'Rahilly, S., "Leptin: a novel therapeutic role in lipodystrophy", *J. Clin. Invest*., 109 (10), 1285-1286. May 2002.

[52] Duncan, R.E., Ahmadian, M., Jaworski, K., Sarkadi-Nagy, E. and Sul, H.S., "Regulation of lipolysis in adipocytes", *Annu. Rev. Nutr*., 27, 79-101. August 2007.

[53] Ahn, I.S., Choi, B.H., Ha, J.H., Byun, J.M., Shin, H.G., Park, K.Y. and Do, M.S., "Isomer-specific effect of conjugated linoleic acid on inflammatory adipokines associated with fat accumulation in 3T3-L1 adipocytes", *J. Med. Food*, 9 (3), 307-312. Fall 2006.

[54] Beynen, A.C., Hermus, R.J. and Hautvast, J.G., "A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man", *Am. J. Clin. Nutr*., 33 (1), 81-85. January 1980.

[55] Reidinger, R.F.Jr., Labows, J.N., Fellows, D.P. and Mason, J.R., "Fatty acid composition of adipose tissue as an indicator of diet: a preliminary assessment", *The Journal of Wildlife Management*, 49 (1), 170-177. January1985.

[56] Hodson, L., Skeaff, C.M. and Fielding, B.A., "Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake", *Prog. Lipid Res*., 47 (5), 348-380. September 2008.

[57] Abbott, S.K., Else, P.L., Atkins, T.A. and Hulbert, A.J., "Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance", *Biochim. Biophys. Acta*, 1818 (5), 1309-1317. May 2012.

[58] Zhou, A.L., Hintze, K.J., Jimenez-Flores, R. and Ward, R.E., "Dietary fat composition influences tissue lipid profile and gene expression in Fischer-344 rats", *Lipids*, 47 (12), 1119-1130. December 2012.

[59] Hirsch, J., Farquhar, J.W., Ahrens, Jr.E.H., Peterson, M.L. and Stoffel, W., "Studies of adipose tissue in man. A microtechnic for sampling and analysis", *Am. J. Clin. Nutr*., 8 (4), 499-511. July 1960.

[60] Body, D.R., "The lipid composition of adipose tissue", *Prog. Lipid Res.*, 27 (1), 39-60. 1988.

[61] Leray, C., Andriamampandry, M., Gutbier, G., Raclot, T. and Groscolas, R., "Incorporation of *n*-3 fatty acids into phospholipids of white and brown adipose tissues: A time-course study during fish-oil feeding", *J. Nutr. Biochem*., 6 (12), 673-680. December 1995.

[62] Soriguer, F.J., Tinahones, F.J., Monzón, A., Pareja, A., Rojo-Martínez, G., Moreno, F., Esteva, I. and Gómez-Zumaquero, J.M., "Varying incorporation of fatty acids into phospholipids from muscle, adipose and pancreatic exocrine tissues and thymocytes in adult rats fed with diets rich in different fatty acids", *Eur. J. Epidemiol*., 16 (6), 585-594. June 2000.

[63] Biong, A., Berstad, P. and Pedersen, J.I., "Biomarkers for intake of dairy fat and dairy products", *Eur. J. Lipid Sci. Technol*., 108 (10), 827-834. October 2006.

[64] Leaf, D.A., Connor, W.E., Barstad, L. and Sexton, G., "Incorporation of dietary n_3 fatty acids into the fatty acids of human adipose tissue and plasma lipid classes", *Am. J. Clin. Nutr*., 62 (1), 68-73. July 1995.

[65] Calder, P.C., Harvey, D.J., Pond, C.M. and Newsholme, E.A., "Site-specific differences in the fatty acid composition of human adipose tissue", *Lipids*, 27 (9), 716-720. September 1992.

[66] Schoen, R.E., Evans, R.W., Sankey, S.S., Weissfeld, J.L. and Kuller, L., "Does visceral adipose tissue differ from subcutaneous adipose tissue in fatty acid content?" *Int. J. Obes. Relat. Metab. Disord*., 20 (4), 346-352. April 1996.

[67] Garaulet, M., Perez-Llamas, F., Perez-Ayala, M., Martinez, P., Medina, Fd., Tebar, F. and Zamora, S.., "Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity", *Am. J. Clin. Nutr*., 74 (5), 585-591. November 2001.

[68] Ravussin, E. and Kozak, L.P., "Have we entered the brown adipose tissue renaissance?", *Obes. Rev*., 10 (3), 265-268. May 2009.

[69] Mercer, S.W. and Trayhurn, P., "Effect of high fat diets on energy balance and thermogenesis in brown adipose tissue of lean and genetically obese ob/ob mice", *J. Nutr*., 117 (12), 2147-2153. December 1987.

[70] Williamson, J.R., "Control of energy metabolism in hamster – brown adipose tissue", *J. Biol. Chem*., 245 (8), 2043-2050. April 1970.

[71] Nedergaard, J., Becker, W. and Cannon, B., "Effects of dietary essential fatty acids on active thermogenin content in rat brown adipose tissue", *J. Nutr*., 113 (9), 1717-1724. September 1983.

[72] Cannon, B. and Nedergaard, J., "Brown adipose tissue: function and physiological significance", *Physiol. Rev*., 84 (1), 277-359. January 2004.

[73] Nedergaard, J. and Cannon, B., "The changed metabolic world with human brown adipose tissue: therapeutic visions", *Cell Metab*., 11 (4), 268-272. April 2010.

[74] Rafecas, I., Esteve, M., Fernández-López, J.A., Remesar, X. and Alemany, M., "Deposition of dietary fatty acids in young Zucker rats fed a cafeteria diet", *Int. J. Obes. Relat. Metab. Disord*., 16 (10), 775-787. October 1992.

[75] Trayhurn, P., "Fuel selection in brown adipose tissue", P*roc. Nutr. Soc*., 54 (1), 39-47. March 1995.

[76] Carneheim, C., Nedergaard, J. and Cannon, B., "Beta-adrenergic stimulation of lipoprotein lipase in rat brown adipose tissue during acclimation to cold", *Am. J. Physiol.*, 246 (4 Pt 1), E327-E333. April 1984.

[77] Carneheim, C., Cannon, B. and Nedergaard, J., "Rare fatty acids in brown fat are substrates for thermogenesis during arousal from hibernation", *Am. J. Physiol*., 256 (1 Pt 2), R146-R154. January 1989.

[78] Algire, C., Medrikova, D. and Herzig, S., "White and brown adipose stem cells: from signaling to clinical implications", *Biochim. Biophys. Acta*, 1831 (5), 896-904. May 2013.

[79] Ohno, T., Ohinata, H., Ogawa, K. and Kuroshima, A., "Fatty acid profile of phospholipids in brown adipose tissue during cold acclimation and repetitive intermittent immobilization: with special reference to docosahexaenoic acid", *Jpn J.Physiol*., 46 (3), 265-270. June1996.

[80] Buckley, J.D. and Howe, P.R.C., "Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids", *Obes. Rev*., 10 (6), 648-659. November 2009.