



Dietary fish oil supplementation during early pregnancy in rats on a cafeteria-diet prevents fatty liver in adult male offspring



Clara Sánchez-Blanco, Encarnación Amusquivar, Kenia Bispo, Emilio Herrera*

Department of Chemistry and Biochemistry, Universidad San Pablo CEU, Madrid, Spain

ARTICLE INFO

Keywords:

Cafeteria diet
Dietary fish oil supplement
Long-term effects
Adult rat offspring
Fatty liver disease
Gender effects

ABSTRACT

We studied in rats the effects of cafeteria diet (CD) supplemented (or not) with fish oil (FO) during just the first 12 days of pregnancy, or during the whole of pregnancy and lactation in 14-month old offspring. Female rats were given standard diet (STD) or CD and after mating some animals remained on STD or CD; for some CD rats the diet was supplemented with 8.78% FO. After 12 days, half of the CD-FO group returned to CD (CD-FO12) and the others remained on CD-FO. From weaning all offspring were given STD. The adiposity index of male offspring of CD dams increased but was normal in CD-FO males. Plasma triacylglycerols (TAG) and individual fatty acid concentrations were similar among the groups. Liver total lipids, TAG, fatty acid concentrations, $\Delta 9$ -desaturase indices and the mRNA expression of fatty acid synthase were higher in male offspring of CD than in those of STD; most of these differences disappeared in male offspring of CD-FO12 and CD-FO dams. Female offspring showed smaller changes. Thus, a moderate supplement with FO during just the first half of gestation or during pregnancy and lactation in rats on CD decreases the liver steatosis in male adult offspring.

1. Introduction

It is now well-known that maternal over-nutrition during pre-pregnancy, pregnancy and lactation influences offspring development and may cause permanent changes in adults, including the susceptibility for chronic diseases such as obesity, type-2 diabetes and cardiovascular disease; that is to say that the changes have been programmed (Hull et al., 2011; Boney et al., 2005; Schack-Nielsen et al., 2010; Godfrey and Barker, 2000; Lucas et al., 1999). In this sense, the composition of the diet may play an important role, as is the case for the polyunsaturated fatty acids (PUFA), which have a direct effect regulating the gene expression (Clarke, 2000) and major dietary implications during pregnancy (Herrera et al., 2012). To date there are limited data on the long-term influence of high-calorie processed foods during the perinatal stages. Recently we have studied the influence of a cafeteria diet, which is a highly palatable diet containing high fat and sugars, during pregnancy and lactation on pups' body weight and fatty acid profiles in rats, and also determined how a fish oil supplement rich in n-3 long-chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) modified the response (Sanchez-Blanco et al., 2016). Dietary depletion of DHA is known to cause adverse neurological outcomes in animals (Carlson and Neuringer, 1999) and it has been suggested that

increasing the consumption of fish oil could prevent certain diseases (Hodge et al., 1996; Black and Sharpe, 1997). These findings have prompted the issuing of advice to pregnant and lactating women to consume fish oil supplements (Escolano-Margarit et al., 2013; Ribeiro et al., 2012; Palmer et al., 2012).

It has also been shown, however, that supplementing diets with n-3 LCPUFA in adult subjects decreases arachidonic acid (ARA, 20:4n-6) levels (Buckley et al., 2004; Grimsgaard et al., 1997; Burns et al., 2007). It has been proposed that ARA has a growth-promoting effect during the perinatal stage (Koletzko and Braun, 1991; Clandinin and VanAerde, 2003). In fact, we found in rats that both dietary fish oil (Amusquivar et al., 2000) or cafeteria diet with or without fish oil supplement during pregnancy decreases pups body weight that could be related to decreased plasma ARA levels (Sanchez-Blanco et al., 2016; Amusquivar et al., 2000). On the other hand, it has been shown in rats that fish oil alleviated high-fat diet-induced non-alcoholic fatty acid disease (Yuan et al., 2016) and we have previously found that fish oil supplements during pregnancy and lactation in rats have a long-term effect reducing the age-dependent insulin resistance in male but not female offspring (Sardinha et al., 2013).

Stearoyl-CoA desaturase-1 (SCD-1) is a lipogenic $\Delta 9$ -desaturase (Ntambi et al., 2002) that is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. SCD-1 converts saturated fatty

* Corresponding author.

E-mail addresses: clarasanchezblanco@gmail.com (C. Sánchez-Blanco), eamusqui@ceu.es (E. Amusquivar), kenia.bispo@hotmail.com (K. Bispo), eherrera@ceu.es (E. Herrera).

<https://doi.org/10.1016/j.fct.2018.12.006>

Received 22 September 2018; Received in revised form 3 December 2018; Accepted 6 December 2018

Available online 10 December 2018

0278-6915/ © 2018 Elsevier Ltd. All rights reserved.

acids (16-carbon PAM, 16:0 and 18-carbon STA, 18:0) to mono-unsaturated fatty acids (palmitoleic, PAO, 16:1n-7, and OLA, 18:1n-9, respectively). The PAO/PAM and OLA/STA ratios have been widely used as desaturation indices for SCD-1 in both human (Sjogren et al., 2008; Warensjo et al., 2009; Vessby et al., 2013; Yee et al., 2014) and rat (Hofacer et al., 2012; Cedernaes et al., 2013) tissues. When female rats were on a high-fat diet prior to and throughout pregnancy and lactation, their 6-month old male pups had liver desaturation indices that were higher than in controls (Seet et al., 2015).

In the current study we followed the same protocol as before, where rats were given the cafeteria diet supplemented or not with fish oil during either the first 12 days of their 21.5-day pregnancy or throughout pregnancy and lactation (Sanchez-Blanco et al., 2016). The main objective on this occasion was to study the long-term effects for the offspring of changing dietary composition during perinatal life.

2. Materials and methods

2.1. Animals

Female Sprague Dawley rats of 40–45 days of age were obtained from the animal quarter of University San Pablo CEU, Madrid, Spain. Rats were given a standard pellet diet (Harlan Global Diet, 2014; Madison, WI) and maintained for 5 days in shared cages (5 per cage) under controlled conditions ($22 \pm 2^\circ\text{C}$, $55 \pm 10\%$ relative humidity and 12-hourly cycling of light and dark). The experimental protocol was approved by the Animal Research Committee of the University San Pablo CEU (reference 220113).

Rats were then randomly assigned to one of two dietary treatment groups: the standard diet group (STD) and the cafeteria diet group (CD). After 21 days on their respective diet rats were mated with males that had been given the standard diet and the day that spermatozoa appeared in vaginal smears was considered to be day 0 of pregnancy. At this time pregnant rats were placed in individual cages and the CD group was subdivided into two, one maintained on the same diet and the other on a diet supplemented with fish oil (CD and CD-FO groups). On the 12th day of pregnancy half of the CD-FO group was returned to the cafeteria diet without the fish oil supplement (CD-FO12) and the other half remained on the fish oil supplemented cafeteria diet throughout the whole of pregnancy and lactation (CD-FO). Rats had free access to the assigned diet and tap water. Pups were maintained with their mothers until weaning and all litters were culled to 9 pups within 48 h of delivery by selecting the excess number of pups at random and removing them. At weaning (day 21 post-natal) female and male pups from each experimental group were separated from each other and housed in collective cages (3–4 per cage) and given the standard diet until the end of the experiment.

When the offspring were 14 months old, they were fasted for 12 h then decapitated under CO_2 sedation; trunk blood from the neck wound was collected into ice-chilled receptacles containing Na_2EDTA . Livers and the different fat depots were rapidly dissected and placed into liquid nitrogen for weighing and kept at -80°C until analysis. Plasma from fresh blood was separated by centrifugation at $1500 \times g$ for 30 min at 4°C and stored at -80°C until analysis.

2.2. Diets

Each diet was prepared as a homogeneous paste by mechanically blending the components shown in Table 1 and they were stored at -20°C until use. The amount of fish oil present in the diet of the CD-FO group (8.78g/100g) was based on previous studies by us (Sanchez-Blanco et al., 2016; Amusquivar et al., 2000; Sardinha et al., 2013), being lower than the amount used by others for high-fat diet in rats (Nakashima, 2008; Viggiano et al., 2016; Hernandez-Casner et al., 2017).

2.3. Analytical methods

Plasma triacylglycerols (TAG), cholesterol (Spinreact Reactives, Spain) and non-esterified fatty acids (NEFA) (Wako Chemicals, Germany) were determined by enzymatic methods using commercial kits. Liver TAG and cholesterol were determined in purified lipid extracts (Folch et al., 1957) as described elsewhere (Carr et al., 1993). For the analysis of the fatty acids profile, nonadecenoic acid (19:1) (Sigma Chemical Co.) was added as the internal standard to fresh aliquots of each diet, of frozen plasma and frozen livers, which were used for lipid extraction and purification (Folch et al., 1957). The final lipid extract was evaporated to dryness under vacuum and the residue resuspended in methanol/toluene and subjected to methanolysis in the presence of acetyl chloride at 80°C for 2.5 h as previously described (Amusquivar et al., 2011). Fatty acid methyl esters were separated and quantified on a Perkin-Elmer gas chromatograph (Autosystem) with a flame ionization detector and a 20 m Omegawax capillary column (internal diameter 0.25 mm). Nitrogen was used as carrier gas, and the fatty acid methyl esters were compared with purified standards (Sigma Chemical Co.). Quantification of the fatty acids in the sample was performed as a function of the corresponding peak areas compared to that of the internal standard.

To extract protein from the tissues, between 50 and 60 mg of frozen liver were homogenized in a lysis buffer using a Tissue-Lyzer (Qiagen, Hilden, Germany) and after three thermic shocks at 37°C . The homogenate was centrifuged at 4°C for 30 min at 12000 rpm. After removing the upper fat layer the supernatant was used for protein analysis using a commercial kit (Pierce BCA Protein Assay kit, Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20°C until use for Western blot analysis. Proteins were combined with molecular weight markers (Precision Blue, Bio-Rab Laboratories, Hercules, CA, USA) and subjected to electrophoresis in 10% polyacrylamide gel at 90 V. Proteins were transferred to a nitrocellulose membrane (Bio-Rab Laboratories, Hercules, CA, USA) for the expression of fatty acid synthase (FAS). The membranes were incubated with different antibodies and latterly with secondary antibody marked with horseradish peroxidase (HRP). Immunoreactive bands were visualized by chemiluminescence using a commercial kit (Clarity Western ECL Kit, Bio-Rab Laboratories, Hercules, CA, USA) and quantified by densitometry (Quantity One Analysis Software, Bio-Rab Laboratories, Hercules, CA, USA).

2.4. Desaturation index

After determining the amounts of the different fatty acids, the $\Delta 9$ desaturation index (SCD-1) was calculated as the ratio of the mono-unsaturated fatty acid to the corresponding saturated fatty acid: PAO (16:1n-7)/PAM (16:0) or OLA (18:1n-9)/STA (18:0).

2.5. Adiposity index

Adiposity index was determined by the sum of inguinal, mesenteric, perirenal, lumbar and epididymal pad weights divided by body weight $\times 100\%$, and expressed as adiposity percentage.

2.6. Statistical analysis

Results are expressed as mean \pm standard error. Statistical analysis was carried out using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to compare the changes due to the different diets. When treatment effects were significantly different ($P < 0.05$) values were analyzed by the Newman-Keuls post hoc test. For TAG concentrations, a log transformation was carried out before statistical testing. When two groups were compared the Student *t*-test was applied.

Table 1
Composition of the diets.

Components (g/100g)	Diet		
	Standard (STD)	Cafeteria (CD)	Cafeteria plus Fish Oil (CD-FO)
Pellets ^a	100	25.2	25.2
Condensed milk (Nestlé, Barcelona)	–	11.0	11.0
Sucrose (Azucarera, Barcelona)	–	17.2	17.2
Muffins (Panrico SA, Barcelona)	–	6.58	6.58
Croissants (Bimbo, Barcelona)	–	6.58	6.58
Powdered milk (Central Lechera Asturiana, Asturias)	–	24.7	24.7
Lard (El Pozo, Murcia)	–	8.78	–
Fish oil (Fagron Iberica, Barcelona)	–	–	8.78

^a From Harlan, Global Diet 2014, Madison, MI.

Table 2
Fatty acid (FA) composition of the experimental diets.

Fatty acids (μmol/g)	Diet		
	Standard (STD)	Cafeteria (CD)	Cafeteria plus Fish Oil (CD-FO)
MA, 14:0	N.D. ^A	40.7 ± 1.23 ^B	49.4 ± 1.6 ^C
PAM, 16:0	28.2 ± 1.6 ^A	185 ± 26 ^B	151 ± 3 ^B
STA, 18:0	3.62 ± 0.13 ^A	73.5 ± 2.3 ^B	36.8 ± 1.0 ^C
PAO, 16:1n-7	0.611 ± 0.042 ^A	15.1 ± 0.4 ^B	31.8 ± 0.6 ^C
OLA, 18:1n-9	33.4 ± 1.3 ^A	232 ± 6 ^B	156 ± 2 ^C
LNA, 18:2n-6	83.3 ± 3.1 ^A	77.9 ± 0.7 ^A	63.4 ± 2.2 ^B
DGLA, 20:3n-6	N.D.	N.D.	N.D.
GLA, 18:3n-6	N.D. ^A	N.D. ^A	0.69 ± 0.03 ^B
ARA, 20:4n-6	N.D. ^A	0.835 ± 0.024 ^B	1.58 ± 0.06 ^C
DPAn-6, 22:5n-6	N.D. ^A	N.D. ^A	0.543 ± 0.008 ^B
ALA, 18:3n-3	5.67 ± 0.22 ^A	6.35 ± 0.07 ^B	7.49 ± 0.18 ^C
EPA, 20:5n-3	N.D. ^A	0.712 ± 0.040 ^B	20.2 ± 0.3 ^C
DPAn-3, 22:5n-3	N.D. ^A	N.D. ^A	3.32 ± 0.08 ^B
DHA, 22:6n-3	N.D. ^A	N.D. ^A	22.5 ± 0.2 ^B
Sum of saturated FA	32.8 ± 1.7 ^A	304 ± 26 ^B	243 ± 6 ^B
Sum of monounsaturated FA	34.5 ± 1.3 ^A	252 ± 6 ^B	244 ± 2 ^B
Sum of n-6 polyunsaturated FA	83.3 ± 3.1 ^A	78.2 ± 2.5 ^A	66.6 ± 3.7 ^B
Sum of n-3 polyunsaturated FA	5.67 ± 0.22 ^A	7.06 ± 0.06 ^B	53.5 ± 0.5 ^C

Values correspond to mean of triplicate samples ± SEM. Statistical comparisons between the diets are shown by upper-case superscript letters (different letters indicate $P < 0.05$). N.D. = not detected.

3. Results

The fatty acid composition of the diets is shown in Table 2 which shows that the CD diets have a higher content of fatty acids than the STD diet, the greatest difference being in the total amounts of saturated and monounsaturated fatty acids. The CD-FO diet has higher amounts of myristic (MA, 14:0), palmitoleic (PAO, 16:1n-7), gamma-linolenic (GLA, 18:3n-6), arachidonic (ARA, 20:4n-6), docosapentaenoic n-6 (DPAn-6, 22:5n-6), alpha-linolenic (ALA, 18:3n-3), docosapentaenoic n-3 (DPAn-3, 22:5n-3) acids, EPA, and DHA as well as total n-3 PUFA, but less linoleic acid (LNA, 18:2n-6) and total n-6 PUFA than the others. Body and tissue weights at different time points during pregnancy and lactation and of pups at birth and at weaning (21 days of age) have previously been reported (Sánchez-Blanco et al., 2016). When pups were studied at 14 months of age there were no statistical differences between the groups in terms of the weights of their body, liver and adipose tissues (data not shown). The adiposity index in males (Fig. 1A) was higher in pups whose mothers were given the cafeteria diet (CD) than in those with mothers fed the standard diet (STD) ($P < 0.05$ by *t*-

test). Pups from dams fed CD supplemented with fish oil during the first 12 days of pregnancy (CD-FO12) also had higher adiposity indices than STD pups ($P < 0.05$ by *t*-test) and that difference disappeared in pups from CD dams receiving the fish oil supplement for the whole pregnancy and lactation (CD-FO). In females similar tendencies were seen (Fig. 1B), although the difference was significant only between CD and CD-FO groups.

When studying the plasma concentrations of TAG, NEFA, glucose, cholesterol or any of the individual fatty acids, including the sum of saturated, monounsaturated, n-6 fatty acids or n-3 fatty acids, no differences between the groups were found, with the only exception of a higher concentration of gamma-linolenic acid (GLA, 18:3n-6) in those male pups of the CD, CD-FO12 or CD-FO groups vs. those of the STD group and a lower concentration of alpha-linolenic acid (ALA, 18:3n-3) also in male CD, CD-FO12 and CD-FO groups vs. STD with no differences in females (supplement Table 1). However, major differences between groups were found in liver. As shown in Fig. 2A, the total concentration of lipids in livers from males was higher in those pups from dams fed the CD during pregnancy and lactation than those of the STD group ($p < 0.001$). Pups of cafeteria diet dams that received dietary fish oil supplement for just the first 12 days of pregnancy (CD-FO12) had lower liver lipid concentrations than those of the CD group ($p < 0.001$) and no difference was found between CD-FO12 and those of the STD group. This decrease in the liver total lipid concentration was even more pronounced in those male pups from CD dams that were given the fish oil supplement during pregnancy and lactation (CD-FO) ($p < 0.001$). These differences between the groups in males were similar to those of liver TAG concentrations (Fig. 2B), with values in the CD group being much higher than in the STD group ($p < 0.001$). Again, these values decrease in the CD-FO12 group ($p < 0.01$) and even more so in the CD-FO group ($p < 0.001$) where the values did not differ from those in the STD group. As shown in Fig. 2C and D no significant statistical differences between the groups were found in the female pups.

The concentrations of individual fatty acids in liver were determined (supplement Table 2). In male pups, stearic acid (STA, 18:0), dihomogamma-linolenic acid (DGLA, 20:3n-6), docosapentaenoic acid n-6 (DPAn-6, 22:5n-6), EPA and DHA liver concentrations showed no differences between the 4 groups, whereas the concentration of ARA was lower in the CD-FO than in the rest of the groups ($p < 0.01$). However, the concentration of the remaining fatty acids was significantly higher in liver of CD than in the STD group, an effect which decreased or even disappeared in those of the CD-FO12 and CD-FO groups. In female pups these differences between the groups were once again much smaller for all the studied fatty acids in liver. In Fig. 3 the sums of the concentrations of all saturated, monounsaturated, n-3 PUFA and n-6 PUFA are summarized; it can be seen that in males (Fig. 3A) the pattern for all the groups of fatty acids again resembles that found for the liver TAG (see above): i.e., significant increases in CD vs. STD, a decline in CD-FO12 and completely normalized in pups from the CD-FO

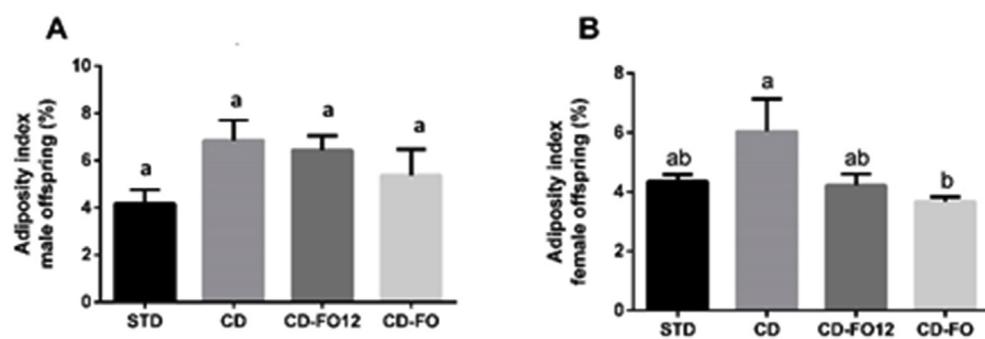


Fig. 1. Adiposity index of 14-month old male (A) and female (B) offspring of dams that were given either standard diet (STD) or cafeteria diet during pre-gestation, gestation and lactation (CD), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil during only the first 12 days of pregnancy and cafeteria diet thereafter until weaning (CD-FO12), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil throughout pregnancy and lactation (CD-FO). All pups were given standard diet from weaning. Statistical comparisons by ANOVA between the groups are shown by the lower case letters (different letters indicate $P < 0.05$). Number of 14-month old pups: 5/group.

group. In the case of females (Fig. 3B), the sum of all the n-6 PUFA was lower in those of the CD-FO group than in CD, but no significant differences appeared between the groups for the remaining fatty acid groups.

The SCD-1 indices were derived from the liver fatty acid concentration profiles and, as shown in Fig. 4A and B (corresponding to the PAO/PAM and OLA/STA ratios in males respectively), they were significantly higher in CD pups than in STD both for SCD-1 ($p < 0.01$). These effects were lower or had completely disappeared in both CD-FO12 and CD-FO pups. No differences between the groups were found in females (Fig. 4C and D). In order to relate these findings to lipogenesis, the expression of fatty acid synthase (FAS) was determined in the livers of males and as shown in Fig. 5, its amount changed between the groups in parallel to the SCD-1 values, being higher in CD pups than in STD ($p < 0.05$) and the effect was completely abolished in both CD-FO12 and CD-FO pups.

4. Discussion

We had previously found that, when 21-day-old pups of dams that were given a cafeteria diet during pre-gestation, gestation and lactation,

they showed hypertriaclyglycerolemia that completely disappeared when the cafeteria diet was supplemented with fish oil during just the first 12 days of pregnancy or throughout pregnancy and lactation (Sánchez-Blanco et al., 2016). Using the same protocol here, we have shown in 14-month old offspring that there were no differences between the studied groups either in plasma TAG concentrations or in their plasma fatty acid profiles. However, it was found that 14-month old male pups from dams given a cafeteria diet during pregnancy and lactation have increased both hepatic TAG and concentrations of all the groups of fatty acids in the liver. When the cafeteria diet dams also received a dietary supplement with fish oil during just the first half of pregnancy or throughout pregnancy and lactation these variables were completely normalized to the values found in STD group, the effect being gender-dependent since it did not appear in females. The 14 month-old male pups of dams given the cafeteria diet during pregnancy and lactation also showed higher liver SCD-1 indices and higher amounts of FAS protein, which indicated an increased lipogenic activity that was normalized in those of dams that received the fish oil supplement during just the first 12 days of pregnancy or throughout the whole pregnancy and lactation. Since both SCD-1 and FAS have been positively associated to lipogenesis (Ntambi et al., 2002; Seet et al.,

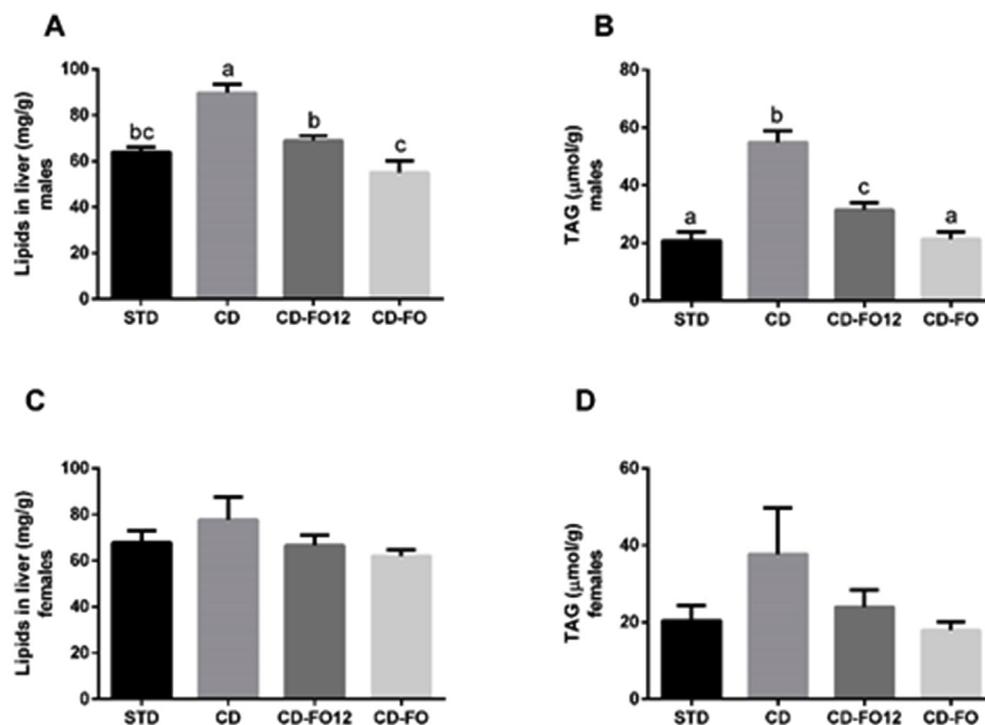


Fig. 2. Total lipids and TAG in liver of 14-month old male (A and B) and female (C and D) offspring of dams that were given either standard diet (STD) or cafeteria diet during pre-gestation, gestation and lactation (CD), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil during only the first 12 days of pregnancy and cafeteria diet thereafter until weaning (CD-FO12), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil throughout pregnancy and lactation (CD-FO). All pups were given standard diet from weaning. Statistical comparisons by ANOVA between the groups are shown by the lower case letters (different letters indicate $P < 0.05$. No letters in the graph indicate no statistical significance). Number of 14-month old pups: 5/group.

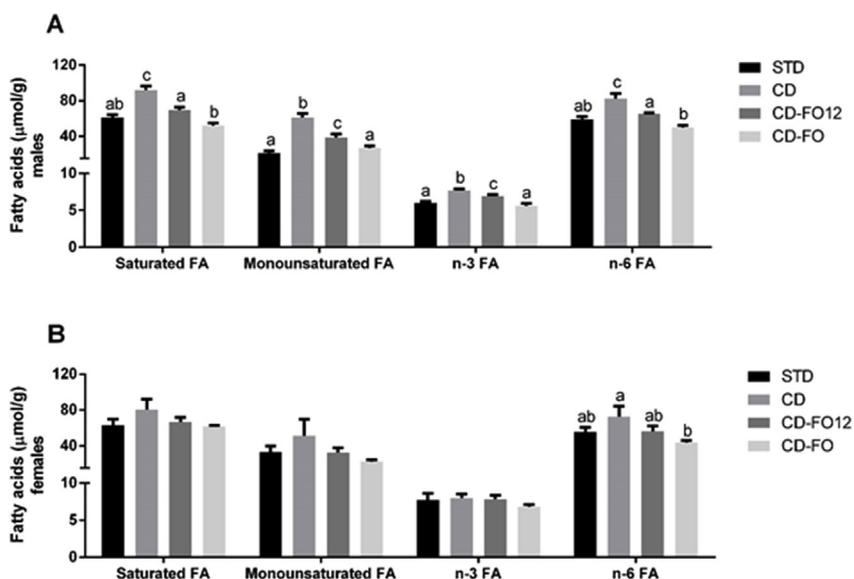


Fig. 3. Groups of fatty acid (FA) concentrations in liver of 14-month old male (A) and female (B) offspring of dams that were fed either standard diet (STD) or cafeteria diet during pre-gestation, gestation and lactation (CD), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil during the only the first 12 days of pregnancy and cafeteria diet thereafter until weaning (CD-FO12), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil throughout pregnancy and lactation (CD-FO). All pups were given standard diet from weaning. Statistical comparisons by ANOVA between the groups are shown by the lower case letters (different letters indicate $P < 0.05$). No letters in the graph indicate no statistical significance). Number of 14-month old pups: 5/group.

2015; Strable and Ntambi, 2010) current findings indicate that those FO supplements in dams on cafeteria diet during just the first 12 days of pregnancy or throughout pregnancy and lactation prevented the increased lipogenic effect in 14-month-old male pups. These findings extend those previously recorded by others in just 6-month old offspring of rats fed high fat diet during pregnancy and lactation showing increased liver SCD-1 indices (Seet et al., 2015) and in those of rats showing that 4 months of fish oil protects against the fatty liver developed by high-fat diet (Yuan et al., 2016). Although fish oil supplementation in rats during pregnancy has been shown to reduce the risks of adult disease in offspring (Joshi et al., 2003), this is the first time that the effect has been shown to be produced with a dietary fish oil supplement for just the first half of pregnancy.

The mechanism by which maternal fish oil supplements during just the first half of pregnancy in rats fed the cafeteria diet during pregnancy and lactation reverses the increased liver concentration of TAG and of most fatty acids as well as lipogenesis in male offspring of 14 months of

age is unknown. However there is already enough experimental support to show that fatty acids, in particular polyunsaturated fatty acids, can modify the epigenome (Burdge and Lillycrop, 2014), and we have previously shown that the type of fatty acid consumed by the rat mother during early pregnancy elicits epigenetic mechanisms through miRNAs modulation in male offspring (Casas-Agustench et al., 2015). The present findings also show that the response to fish oil during the perinatal stage reverses those long-term effects of cafeteria diet on fatty acid metabolism was sex-dependent, since it was clearly found in males but not in females. This finding agrees with previous findings in the sense that there are sex differences in response to high-fat diet feeding in rats (Amengual-Cladera et al., 2012) and that fish oil intake during early pregnancy in rats reduces the age-dependent insulin resistance also in a sex-dependent manner (Sardinha et al., 2013).

As a result of studies in both humans and rats it is well known that diets with high fat contents, especially saturated fatty acids, promote the development of non-alcoholic fatty liver disease and consequent

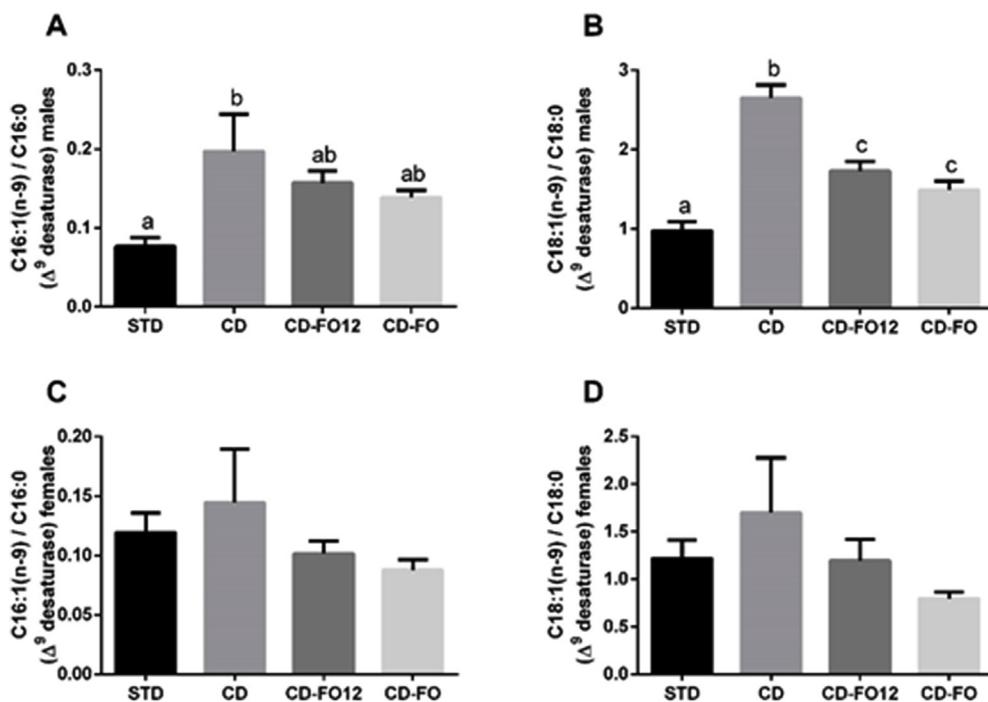


Fig. 4. Liver stearoyl-CoA desaturase-1 (SCD-1) indices of 14-month old male (A and B) and female (C and D) offspring of dams that were fed either standard diet (STD) or cafeteria diet during pre-gestation, gestation and lactation (CD), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil during only the first 12 days of pregnancy and cafeteria diet thereafter until weaning (CD-FO12), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil throughout pregnancy and lactation (CD-FO). All pups were given standard diet from weaning. Statistical comparisons by ANOVA between the groups are shown by the lower case letters (different letters indicate $P < 0.05$). No letters in the graph indicate no statistical significance). Number of 14-month old pups: 5/group.

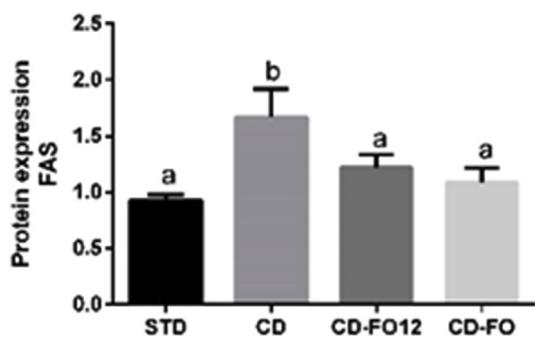


Fig. 5. Expression of fatty acid synthase in liver of 14-month old male offspring of dams that were fed either standard diet (STD) or cafeteria diet during pre-gestation, gestation and lactation (CD), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil during only the first 12 days of pregnancy and cafeteria diet thereafter until weaning (CD-FO12), or the cafeteria diet during pre-gestation followed by cafeteria diet supplemented with fish oil throughout pregnancy and lactation (CD-FO). All pups were given standard diet from weaning. Statistical comparisons by ANOVA between the groups are shown by the lower case letters (different letters indicate $P < 0.05$). Number of 14-month old pups: 5/group.

increased hepatic TAG concentration (Yuan et al., 2016; Musso et al., 2003; Gentile and Pagliassotti, 2008). This effect has been mainly attributed to a promotion of lipogenesis *de novo* associated with a high-fat diet-induced overexpression of the liver regulatory element binding protein-1 (SREBP1) encoded by the gene sterol regulatory element binding factor 1 (*Srebf1*), which is inhibited by fish oil consumption (Yuan et al., 2016; Ou et al., 2001; Levy et al., 2004). The current findings in 14-month old offspring are also consistent with reported findings in adult rat offspring of dams given a high-fat diet during pregnancy and lactation and then given a control diet from weaning; they developed a metabolic syndrome-like phenotype (Srinivasan et al., 2006; Desai et al., 2014) as well as increased liver SCD-1 (Seet et al., 2015), which has been positively correlated with lipogenesis and adiposity (Ntambi et al., 2002; Yee et al., 2014; Jeyakumar et al., 2009; Kotronen et al., 2011).

In conclusion, although current findings cannot be extrapolated to the human condition by obvious reasons, we demonstrate for the first time that a moderate supplement of dietary fish oil during just the first 12 days of pregnancy is able to decrease the development of liver steatosis in male adult offspring of rats given a cafeteria diet during pregnancy and lactation; the effect was even greater when the fish oil supplement was maintained throughout gestation and lactation and can be related to a reduction in lipogenic activity.

Conflicts of interest

None of the authors had any financial or personal conflicts of interest.

Acknowledgements

The authors thank Milagros Morante for excellent technical help and Dr. Peter Dodds for editing and linguistic revision of the manuscript. The study has been carried out with the financial support of Fundación Ramón Areces (CIVP16A1835) and University CEU San Pablo (PPC03-2014), Madrid, Spain. This manuscript does not contain clinical studies or patient data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2018.12.006>.

References

- Amengual-Cladera, E., Llado, I., Gianotti, M., Proenza, A.M., 2012. Sex differences in the effect of high-fat diet feeding on rat white adipose tissue mitochondrial function and insulin sensitivity. *Metabolism* 1108–1117.
- Amusquivar, E., Ruperez, F.J., Barbas, C., Herrera, E., 2000. Low arachidonic acid rather than alpha-tocopherol is responsible for the delayed postnatal development in offspring of rats fed fish oil instead of olive oil during pregnancy and lactation. *J. Nutr.* 130, 2855–2865.
- Amusquivar, E., Schiffner, S., Herrera, E., 2011. Evaluation of two methods for plasma fatty acid analysis by GC. *Eur. J. Lipid Sci. Technol.* 113, 711–716.
- Black, P.N., Sharpe, S., 1997. Dietary fat and asthma: is there a connection? *Eur. Respir. J.* 10, 6–12.
- Boney, C.M., Verma, A., Tucker, R., Vohr, B.R., 2005. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 115, e290–e296.
- Buckley, R., Shewring, B., Turner, R., Yaqoob, P., Minihane, A.M., 2004. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. *Br. J. Nutr.* 92, 477–483.
- Burdge, G.C., Lillycrop, K.A., 2014. Fatty acids and epigenetics. *Curr. Opin. Clin. Nutr. Metab. Care* 17, 156–161.
- Burns, T., Maciejewski, S.R., Hamilton, W.R., Zheng, M., Mooss, A.N., Hilleman, D.E., 2007. Effect of omega-3 fatty acid supplementation on the arachidonic acid:eicosapentaenoic acid ratio. *Pharmacotherapy* 27, 633–638.
- Carlson, S.E., Neuringer, M., 1999. Polyunsaturated fatty acid status and neurodevelopment: a summary and critical analysis of the literature. *Lipids* 34, 171–178.
- Carr, T.P., Andresen, C.J., Rudel, L.L., 1993. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clin. Biochem.* 26, 39–42.
- Casas-Agustench, P., Fernandes, F.S., Tavares do Carmo, M.G., Visioli, F., Herrera, E., Davalos, A., 2015. Consumption of distinct dietary lipids during early pregnancy differentially modulates the expression of microRNAs in mothers and offspring. *PLoS One* 10, e0117858.
- Cedernaes, J., Alsio, J., Vastermark, A., Riserus, U., Schioth, H.B., 2013. Adipose tissue stearoyl-CoA desaturase 1 index is increased and linoleic acid is decreased in obesity-prone rats fed a high-fat diet. *Lipids Health Dis.* 12, 2.
- Clandinin, M.T., VanAerde, J., 2003. Formula supplementation and growth. *Pediatrics* 112, 1456–1458.
- Clarke, S.D., 2000. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *Br. J. Nutr.* 83 (Suppl. 1), S59–S66.
- Desai, M., Jellyman, J.K., Han, G., Beall, M., Lane, R.H., Ross, M.G., 2014. Maternal obesity and high-fat diet program offspring metabolic syndrome. *Am. J. Obstet. Gynecol.* 211, 237 e231–237 e213.
- Escolano-Margarit, M.V., Campoy, C., Ramirez-Tortosa, M.C., Demmelmair, H., Miranda, M.T., Gil, A., et al., 2013. Effects of fish oil supplementation on the fatty acid profile in erythrocyte membrane and plasma phospholipids of pregnant women and their offspring: a randomised controlled trial. *Br. J. Nutr.* 109, 1647–1656.
- Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509.
- Gentile, C.L., Pagliassotti, M.J., 2008. The role of fatty acids in the development and progression of nonalcoholic fatty liver disease. *J. Nutr. Biochem.* 19, 567–576.
- Godfrey, K.M., Barker, D.J., 2000. Fetal nutrition and adult disease. *Am. J. Clin. Nutr.* 71, 1344S–52S.
- Grimsgaard, S., Bona, K.H., Hansen, J.B., Nordoy, A., 1997. Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *Am. J. Clin. Nutr.* 66, 649–659.
- Hernandez-Casner, C., Ramos, J., Serafine, K.M., 2017. Dietary supplementation with fish oil prevents high fat diet-induced enhancement of sensitivity to the behavioral effects of quinpirole. *Behav. Pharmacol.* 28, 477–484.
- Herrera, E., Amusquivar, E., 2012. Polyunsaturated fatty acids and their dietary implications during pregnancy. In: Van Dijk, M., Vitek, J. (Eds.), *Fish Oil Production, Consumption and Health Benefits*. Nova Science Publishers Inc, New York, pp. 73–106.
- Hodge, L., Salome, C.M., Peat, J.K., Haby, M.M., Xuan, W., Woolcock, A.J., 1996. Consumption of oily fish and childhood asthma risk. *Med. J. Aust.* 164, 137–140.
- Hofacer, R., Magrisso, I.J., Jandacek, R., Rider, T., Tso, P., Benoit, S.C., et al., 2012. Omega-3 fatty acid deficiency increases stearoyl-CoA desaturase expression and activity indices in rat liver: positive association with non-fasting plasma triglyceride levels. *Prostaglandins Leukot. Essent. Fatty Acids* 86, 71–77.
- Hull, H.R., Thornton, J.C., Ji, Y., Paley, C., Rosenn, B., Mathews, P., et al., 2011. Higher infant body fat with excessive gestational weight gain in overweight women. *Am. J. Obstet. Gynecol.* 205, 211–217.
- Jeyakumar, S.M., Lopamudra, P., Padmini, S., Balakrishna, N., Giridharan, N.V., Vajreswari, A., 2009. Fatty acid desaturation index correlates with body mass and adiposity indices of obesity in Wistar NIN obese mutant rat strains WNIN/Ob and WNIN/GR-Ob. *Nutr. Metab.* 6, 27.
- Joshi, S., Rao, S., Golwilkar, A., Patwardhan, M., Bhonde, R., 2003. Fish oil supplementation of rats during pregnancy reduces adult disease risks in their offspring. *J. Nutr.* 133, 3170–3174.
- Koletzko, B., Braun, M., 1991. Arachidonic acid and early human growth: is there a relation? *Ann. Nutr. Metab.* 35, 128–131.
- Kotronen, A., Yki-Jarvinen, H., Sevastianova, K., Bergholm, R., Hakkarainen, A., Pietilainen, K.H., et al., 2011. Comparison of the relative contributions of intra-abdominal and liver fat to components of the metabolic syndrome. *Obesity* 19, 23–28.

- Levy, J.R., Clore, J.N., Stevens, W., 2004. Dietary n-3 polyunsaturated fatty acids decrease hepatic triglycerides in Fischer 344 rats. *Hepatology* 39, 608–616.
- Lucas, A., Fewtrell, M.S., Cole, T.J., 1999. Fetal origins of adult disease—the hypothesis revisited. *BMJ* 319, 245–249.
- Musso, G., Gambino, R., De Michieli, F., Cassader, M., Rizzetto, M., Durazzo, M., et al., 2003. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 37, 909–916.
- Nakashima, Y., 2008. Fish-oil high-fat diet intake of dams after day 5 of pregnancy and during lactation guards against excessive fat consumption of their weaning pups. *J. Nutr. Sci. Vitaminol.* 54, 46–53.
- Ntambi, J.M., Miyazaki, M., Stoehr, J.P., Lan, H., Kendziorski, C.M., Yandell, B.S., et al., 2002. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11482–11486.
- Ou, J., Tu, H., Shan, B., Luk, A., DeBose-Boyd, R.A., Bashmakov, Y., et al., 2001. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6027–6032.
- Palmer, D.J., Sullivan, T., Gold, M.S., Prescott, S.L., Heddle, R., Gibson, R.A., et al., 2012. Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. *BMJ* 344, e184.
- Ribeiro, P., Carvalho, F.D., Abreu Ade, A., Sant'anna Mde, T., Lima, R.J., Carvalho Pde, O., 2012. Effect of fish oil supplementation in pregnancy on the fatty acid composition of erythrocyte phospholipids and breast milk lipids. *Int. J. Food Sci. Nutr.* 63, 36–40.
- Sánchez-Blanco, C., Amusquivar, E., Bispo, K., Herrera, E., 2016. Influence of cafeteria diet and fish oil in pregnancy and lactation on pups' body weight and fatty acid profiles in rats. *Eur. J. Nutr.* 55, 1741–1753.
- Sardinha, F.L., Fernandes, F.S., Tavares do Carmo, M.G., Herrera, E., 2013. Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R313–R320.
- Schack-Nielsen, L., Michaelsen, K.F., Gamborg, M., Mortensen, E.L., Sorensen, T.I., 2010. Gestational weight gain in relation to offspring body mass index and obesity from infancy through adulthood. *Int. J. Obes.* 34, 67–74.
- Seet, E.L., Yee, J.K., Jellyman, J.K., Han, G., Ross, M.G., Desai, M., 2015. Maternal high-fat-diet programs rat offspring liver fatty acid metabolism. *Lipids* 50, 565–573.
- Sjogren, P., Sierra-Johnson, J., Gertow, K., Rosell, M., Vessby, B., de Faire, U., et al., 2008. Fatty acid desaturases in human adipose tissue: relationships between gene expression, desaturation indexes and insulin resistance. *Diabetologia* 51, 328–335.
- Srinivasan, M., Katewa, S.D., Palaniyappan, A., Pandya, J.D., Patel, M.S., 2006. Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. *Am. J. Physiol. Endocrinol. Metabol.* 291, E792–E799.
- Strable, M.S., Ntambi, J.M., 2010. Genetic control of de novo lipogenesis: role in diet-induced obesity. *Crit. Rev. Biochem. Mol. Biol.* 45, 199–214.
- Vessby, B., Gustafsson, I.B., Tengblad, S., Berglund, L., 2013. Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. *Br. J. Nutr.* 110, 871–879.
- Viggiano, E., Mollica, M.P., Lionetti, L., Cavaliere, G., Trinchese, G., De Filippo, C., et al., 2016. Effects of an high-fat diet enriched in lard or in fish oil on the hypothalamic amp-activated protein kinase and inflammatory mediators. *Front. Cell. Neurosci.* 10, 150.
- Warensjo, E., Rosell, M., Hellenius, M.L., Vessby, B., De Faire, U., Riserus, U., 2009. Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: links to obesity and insulin resistance. *Lipids Health Dis.* 8, 37.
- Yee, J.K., Mao, C.S., Ross, M.G., Lee, W.N., Desai, M., Toda, A., et al., 2014. High oleic/stearic fatty-acid desaturation index in cord plasma from infants of mothers with gestational diabetes. *J. Perinatol.* 34, 357–363.
- Yuan, F., Wang, H., Tian, Y., Li, Q., He, L., Li, N., et al., 2016. Fish oil alleviated high-fat diet-induced non-alcoholic fatty liver disease via regulating hepatic lipids metabolism and metaflammation: a transcriptomic study. *Lipids Health Dis.* 15, 20.