Interactive effects of maternal and weaning high linoleic acid intake on hepatic lipid metabolism, oxylipins profile and hepatic steatosis in offspring

Justine Marchix, Daniel D. Catheline, Cécile Duby, Nathalie N. Boulier-Monthéan, Françoise Boissel, Frédérique F. Pédrono, Gaëlle Boudry, Philippe P. Legrand

Title page:

Interactive effects of maternal and weaning high linoleic acid intake on hepatic lipid metabolism, oxylipins profile and hepatic steatosis in offspring.

Author's names: Justine Marchix[1], Daniel Catheline[1], Cécile Duby[1], Nathalie Monthéan-Boulier[1], Francoise Boissel[1], Frédérique Pédrono[1], Gaëlle Boudry[2] and Philippe Legrand[1]

[1]Laboratoire de Biochimie et Nutrition Humaine, INRA USC 1378, Agrocampus Ouest, Rennes, France

[2] Institut NuMeCan INRA, INSERM, Univ Rennes, Rennes, France

J.M. : justine.marchix@gmail.com, D.C. : daniel.catheline@agrocampus-ouest.fr, C.D.: cecile.duby@agrocampus-ouest.fr, N.M-B. : nathalie.monthean@agrocampus-ouest.fr, F.B. : francoise.boissel@agrocampus-ouest.fr, F.P.: frederique.pedrono@agrocampus-ouest.fr, G.B.: gaelle.boudry@inra.fr, P.L.: philippe.legrand@agrocampus-ouest.fr

Pr. Legrand and Dr. Marchix will accept correspondence and proofs.

Corresponding author: Pr. Philippe Legrand, Laboratoire de Biochimie, Agrocampus Ouest, 65 rue de Saint Brieuc, 35000 Rennes, Tel: +33223485920, fax: +33223485550, e-mail: philippe.legrand@agrocampus-ouest.fr

Running title: High linoleic acid alters offspring lipid metabolism

Abstract:

Non-alcoholic fatty liver disease (NAFLD) has been described as a hepatic manifestation of the metabolic syndrome. When several studies correlated maternal linoleic acid (LA) intake with the development of obesity, only few links have been made between n-6 fatty acid (FA) and NAFLD. Herein, we investigated the influence of both maternal and weaning high LA intake on lipid metabolism and susceptibility to develop later metabolic diseases in offspring. Pregnant rats were fed a control-diet (2% LA) or a LA-rich diet (l2% LA) during gestation and lactation. At weaning, offspring was assigned to one of the two diets, i.e. either maintained on the same maternal diet or fed the other diet for six months. Physiological, biochemical parameters and hepatic FA metabolism were analyzed. We demonstrated that the interaction between the maternal and weaning LA intake altered metabolism in offspring and could lead to hepatic steatosis. This phenotype was associated with altered hepatic FA content and lipid metabolism. Interaction between maternal and weaning LA intake led to a specific pattern of n-6 and n-3 oxylipins that could participate to the development of hepatic steatosis in offspring. Our findings highlight the significant interaction between maternal and weaning high LA intake to predispose offspring to later metabolic disease and support the predictive adaptive response hypothesis.

Keywords**:** maternal diet, weaning diet, n-6 polyunsaturated fatty acid, fatty liver, OXLAMs, eicosanoids.

1. Introduction:

The importance of both maternal lifestyle and nutrition in the adequate development of the fetus has been increasingly described [1, 2]. During the pregnancy and/or lactation period, diet represents one of the essential environmental factors that can have beneficial or adverse consequences on phenotype and health of offspring [1]. Evidence that the quantity but also the type of dietary fatty acids (FA) in maternal diet during this period could influence the fetal programming has emerged [3-5]**.** Essential polyunsaturated FA (PUFA) and their long-chain derivatives (LC-PUFA) are central nutrients required for cell membrane structure and bioactive molecule synthesis. Because fetus and newborn have a limited capacity to synthesize LC-PUFA, their intake mainly depends on the maternal availability and hepatic conversion from precursors. Several reports suggested that maternal LC-PUFA precursor's intake during the early stages of fetal development can influence lipid metabolism and signaling pathways leading to the development of later disorders in offspring [3, 5, 6].

Linoleic acid (LA), essential n-6 PUFA, is one of the most consumed FA in the Western diet. Changes in agricultural and industrial practices have led to the widespread use of LA-rich products resulting in a dramatic overconsumption of n-6 PUFA [7]. When recommendations for doses of dietary LA intake varied controversially between 1 to 4% [7, 8] or between 5 to 10% of total energy [9], its consumption in Western countries may account for up to 7% in diet and had reached up to 12% of energy in the maternal milk [7]. Studies have emphasized that excessive dietary LA is correlated with the prevalence, the onset and the development of chronic diseases including obesity in animals as well as in human [7, 10-13]. Indeed, Massiera et *al.* demonstrated that a Western-like fat diet induces a gradual fat mass enhancement over four generations of mice revealing the transgenerational pathology of obesity [10]. Similarly, Moon et *al.* also suggested that maternal plasma n-6 PUFA status during pregnancy may influence offspring adiposity in childhood [11]. However, the role of n-6 PUFA in chronic diseases such as obesity is still controversial and the mechanisms are not fully determined.

The progression of obesity can further lead to secondary risk factors for the metabolic syndrome such as insulin resistance, type 2 diabetes or liver disorders. Non-alcoholic fatty liver disease (NAFLD) has been

described as a hepatic manifestation of the metabolic syndrome [14, 15]. This clinico-histopathological disease represents a large spectrum of diseases covering from the simple triglyceride (TG) accumulation within hepatocytes (hepatic steatosis, also described as the first "hit") to a progressive non-alcoholic steatohepatitis (NASH) associated with inflammation, fibrosis and cirrhosis (or second "hit") [16]. Several reports suggested that the maternal quantity of fat intake plays a major role in the offspring development by showing that offspring of obese or high fat diet (HFD) fed mothers are more susceptible to develop the features of NAFLD [2, 17-19]. However, the importance of maternal dietary type of fat has also been highlighted in the development of hepatic inflammation and steatosis independently from an excess of caloric consumption [3, 20]. The hepatic FA analysis of NAFLD patients have shown an imbalance of n-6/n-3 PUFA in patients but the link with their diet has not been reported and the mechanisms remain unclear. Accumulating evidence in animal and human studies recently stated the potential role for the n-6 PUFA derivatives in the development of liver injuries [3, 21, 22].

Taken altogether, the role of LA in the development of metabolic diseases has been mainly studied in association with high fat intake and only few data have considered the role of the maternal diet. The focus on high fat intake rather than on the diet composition have led to controversial conclusions on the n-6 PUFA effects. We hypothesized that an excess of LA in a moderate fat diet during the pregnancy and lactation period to adulthood could induce the development of obesity and further associated complications including hepatic alterations. Herein, we aimed to analyze the properties of LA across one generation with a limited calorie intake which could unmask specific effects of this essential FA. Therefore, we investigated the interaction of both maternal and weaning high LA intake on the adult offspring metabolic status and its susceptibility to later metabolic diseases. We further aimed to identify the metabolic pathways or lipid derivatives involved in the LA-induced alterations of offspring metabolic status.

2. Material and methods

2.1. Animal and diets

All experiments were performed in accordance with the European Union Guidelines for animal care and use (2003/35/CEE) and approved by the Ethics & Animal Experimentation Committee of Rennes (MENESR authorization number: 01375.02) governing the use of experimental animals.

2.1.1. Experimental diets. The control diet (CTL-diet, 2% of energy) and the LA-rich diet (LA-diet, 12.3% of energy) were isocaloric and isolipidic. Both diets contained 10.0% of fat (21.0% of energy), 22.0% of proteins, 70.3% of carbohydrates, 2.0% of fiber and 5.7% of minerals and vitamins. The percentage of fat was chosen in order to allow high proportions of LA intake without reaching high fat diet proportions (40-60 % of energy) [23]. The diets were made from a combination of commercial vegetable oils, C16:0 TG and ethyl LA (TCI Europe, Zwijndrecht, Belgium) at the Unité de Production d'Aliments Expérimentaux (INRA, Jouy en Josas, France). The use of ethyl FA has been commonly used to specifically enrich experimental diets and while it provides source of ethanol, the percentage does not appear sufficient to induce alcoholic liver disease (ethanol intake, 30-40% of energy) [21]. The FA composition of diets was confirmed by gas chromatography (Table 1). Experimental diets were stored at -20°C and provided fresh every day.

2.1.2. Experimental design. 8-weeks old female Wistar rats from Janvier Labs (Le Genest-Saint-Isle, France) were fed with the CTL or LA diets during the pregnancy and lactation period constituting two groups ("c" for maternal CTL-diet or "l" for maternal LA-diet). At birth, pups were culled into 8 per litter to ensure a similar growth between the litters. At weaning, on *postnatal day 21*, offspring of CTL-fed and LA-fed dams were randomly separated into two groups of 6 rats each. Pups were maintained on the same maternal diet or fed other experimental diet for 6 months thereby generating 4 groups that differed from maternal and/or weaning diets (c/CTL, c/LA, l/CTL and l/LA) (supplementary Fig. 1). The experiment was performed once and included overall four groups of 6 rats. All animals were housed on a 12h light/dark cycle and maintained at 22± 2°C with free access to water and food *ad libitum*.

Body weight was measured every day. At the end of the 6-month period, fasted rats were anesthetized with an intraperitoneal injection of pentobarbital (140 mg/kg, Merial, Lyon, France) and blood samples were collected by cardiac puncture into EDTA K2-treated vacutainers (Dominique Dutscher, Brumath, France). Plasma was obtained by centrifugation (3000 g, 15 min) and stored at -20°C until further analysis. Liver and adipose tissues were removed, weighted, snap-frozen in liquid nitrogen and stored at -80°C. Some liver samples were washed and fixed in 4% paraformaldehyde for staining.

2.2. Plasma biochemistry and cytokines

Plasma concentrations of glucose, triglycerides, total cholesterol and HDL-cholesterol were measured by using enzymatic kits (BioMerieux, France) according to the manufacturer's instructions. Plasma insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA) using a rat/mouse insulin commercial kit (EZRMI-13K, Millipore, St. Charles, MO, USA).

Plasma proinflammatory cytokine concentrations were also measured using a commercially available rat IL-1β ELISA kit (ER2IL1B, ThermoScientific, Rockford, IL, USA), IL-6 ELISA kit (KRC0061, Invitrogen, Camarillo, CA, USA) and TNF-α ELISA kit (RTA00, R&D Systems, Abingdon, UK). The concentrations of the cytokines were determined using curve fitting software to generate the standard curve (five parameter logistic functions).

2.3. Histology

Liver were fixed in phosphate buffered saline (PBS) containing 4% paraformaldehyde. Liver segments were then dehydrated in graded xylene and ethanol series solutions and embedded in paraffin using a tissue processor (Excelsior ES, ThermoFischer Scientific, Waltham, MA, USA) at the Histopathology Facility H2P2 (Alain Fautrel, Biogeneouest, Université de Rennes I, France). Segments were cut into 4-µm-thick sections. Paraffin-embedded samples were deparaffinized and hydrated using routine techniques. Liver sections were stained with hematoxylin, eosin and safran using an automated slide trainer ST5020 Multistainer (LEICA Microsystems, Wetziar,German) and observed under a light microscope to confirm

the integrity of livers. Thereafter, the stained sections were scanned with the NanoZoomer 2.0 RS (Hamamatsu, Tokyo, Japan) and images were captured at 20x magnification.

2.4. Hepatic lipid extraction and triglyceride isolation in liver

2.4.1. Fatty acid analysis. Liver TG were extracted, separated and performed as previously described [24].

2.4.2. Quantification. Hepatic TG content was measured from liver extracted TG. The organic layer was dried under nitrogen gas and solubilized in absolute ethanol for the determination of TG content using enzymatic kit (BioMerieux, France). Triolein was used as the calibration standard to measure TG levels.

2.5. Stearoyl-CoA Desaturase-1 expression

The Stearoyl-CoA Desaturase (SCD)-1 expression was estimated by Western blot from liver post-mitochondrial fraction proteins resolved by a 12% SDS-PAGE electrophoresis, transferred onto nitrocellulose membrane and immunoblotted with appropriate primary antibodies. The expression of SCD-1 was evaluated using specific antibodies against the respective antigens as previously described [25].

2.6. Real time qPCR

Total RNA was extracted from frozen liver samples with TRIzol® (Life Technologies, Saint-Aubin, France) and reverse transcripted using the iScript kit (Biorad). mRNA were quantified by Real-Time PCR with SYBRGreen® mix (Biorad) or with Taqman® Mix (Applied Biosystem, Courtaboeuf, France) as previously described [25]. Primers and Taqman® probes used are presented in supplementary Table 1. The gene expression level was normalized relative to 18S expression level (RT-CKYD-18S Taqman®, Eurogenetec). The differential gene expression was determined from the cycle thresholds (Ct) using the ΔCt approach.

2.7. Plasma oxylipin concentrations

LA, arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) derivatives were quantified in plasma using LC-MS/MS at the **MetaToul lipidomic facility** as previously described [26]. The analytical platform used for the plasma PUFA metabolite analysis allowed the detection of 33 individual lipid derivatives. Of these, 6 metabolites were not included due to low levels under the limit of detection.

2.8. Statistical analysis

The values (n=6) were expressed as mean ± standard error of the mean (SEM). Data were compared using a two-way ANOVA (maternal diet x weaning diet). Multiple comparisons were analyzed by a Tukey's *post-hoc* test (GraphPad Prism 6.00 Software Inc., La Jolla, CA, USA). Normality of the residuals displayed a normal distribution according to the Shapiro test. Different letters indicate significant differences ($p<0.05$).

Heatmap, PCA and Biplot representations were performed using Metaboanalyst 3.0, an open source R-based program specifically designed for metabolomics[18]. Prior to analysis, metabolite concentrations were transformed (log transformation) and auto-scaled on MetaboAnalyst 3.0.

3. Results:

3.1. Interaction of maternal and weaning LA-diet on the prime features of metabolic syndrome in adult offspring

High dietary LA intake has been shown to induce an obese-like phenotype [10], therefore we examined the phenotypic and biochemical changes associated with obesity in adult offspring. After 6 months of experimental diets, body weight gain among the offspring groups was not significantly affected by the maternal diet nor the weaning diet (Table 2A). The fat accumulation was estimated by calculating the adiposity index as the sum of epididymal, retroperitoneal, inguinal, mesenteric fat pad weight divided by body weight x100. The adiposity index was mainly affected by the weaning diet ($p=0.026$). Offspring fed LA-diet after weaning exhibited a lower adiposity index compared to offspring fed CTL-diet regardless of

the maternal diet. The difference was mainly due to a lower abdominal fat deposition of epididymal white adipose tissue (epWAT) and retroperitoneal (rp) WAT. The percentage of epWAT was significantly affected by both maternal and weaning diets ($p<0.001$; Table 2A) with a lower epWAT percentage in offspring fed LA-diet after weaning compared to their respective CTL-diet fed groups. However, offspring of dams fed LA-diet also had a higher epWAT percentage compared to offspring of dams fed CTL-diet indicating opposite effects of maternal and weaning LA-diet in inducing adiposity. The percentage of rpWAT was only affected by the weaning diet ($p=0.005$) with a significant reduction of rpWAT deposition in c/LA offspring compared to c/CTL group. Liver relative weight was significantly affected by the weaning diet ($p=0.045$) with higher weight in offspring fed LA-diet compared to offspring fed CTL-diet.

We then analyzed fasting plasma biochemical parameters as a key component of the metabolic syndrome associated with obesity (Table 2B). Fasting TG concentrations were only affected by the weaning diet ($p<0.01$) with lower plasma TG levels in offspring fed LA-diet that reached significance in offspring of dams fed LA diet as well. Plasma concentrations of total-cholesterol and glucose were statistically affected by the maternal diet. Offspring of dams fed LA-diet had higher cholesterol levels compared to the offspring of CTL-fed dams with a significant increase in l/CTL offspring compared to c/CTL offspring. Fasting glucose concentration was significantly higher in offspring of dams fed LA-diet compared to the offspring of dams fed CTL-diet. HDL-cholesterol and insulin levels were not significantly affected by the maternal nor the weaning diets. These findings demonstrated that while the maternal LA-diet could promote features of the metabolic syndrome, the exposure to LA-diet for six months induced opposite effects in adult offspring. According to the phenotype observed in adult offspring, plasma IL-1β, TNF-α but not IL-6 levels were differently affected by the maternal diet or the weaning diet (supplementary Fig. 2). While the weaning LA-diet induced lower concentrations of IL-1β ($p=0.034$), the offspring of dams fed LA-diet exhibited higher plasma TNF-α levels ($p=0.045$) indicating the opposite effects of maternal and weaning LA-diet on the inflammatory status involved in metabolic disorders.

3.2. Maternal LA–diet induced triglyceride accumulation within hepatocytes in offspring

Because LA intake has also been associated with hepatic alterations [27], we then assessed histological tissue sections of the offspring livers (Fig. 1A). The c/CTL livers showed normal architecture and lipid accumulation (Fig. 1Aa). The c/LA group exhibited mainly microvesicular fatty livers (Fig. 1Ab). Both l/CTL and l/LA groups exhibited lipid droplets throughout the liver parenchyma including microvesicular and mainly macrovesicular steatosis (Fig. 1Ac, 1Ad). Steatosis was more severe in the l/LA livers with extensive fat accumulation (Fig. 1Ad). These histological analyses were corroborated by the hepatic TG quantification demonstrating that the maternal LA-diet significantly increased TG accumulation within hepatocytes ($p=0.027$; Fig. 1B). Our data demonstrated the effects of the maternal diet on later development of hepatic steatosis in offspring without major liver injury as described in steatohepatitis.

3.3. Maternal and weaning LA-diet differently altered lipid metabolism pathways involved in hepatic triglyceride accumulation

In NAFLD patients, hepatic TG accumulation is known to result from excessive TG diet intake, free FA (FFA) from adipose tissue or *de novo* lipogenesis (DNL) [28]. Therefore, we examined the hepatic expression of key genes involved in these different metabolic pathways. Fatty acid uptake was evaluated by the fatty acid translocase/cluster of differentiation 36 (*Fat/Cd36)* and fatty acid transport protein (*Fatp)* mRNA expression. None of these genes were affected by the maternal or the weaning diets (Fig. 2A). β-oxidation evaluated by hepatic mRNA expression of the transcriptional factor peroxisome proliferator-activated receptor α (*Ppar-α)* and carnitine palmitoyltransferase 1 *(Cpt-1)* was mainly affected by the maternal diet (Fig. 2B). The offspring of dams fed LA-diet exhibited lower relative *Ppar-α* mRNA expression ($p=0.018$) compared to the offspring of dams fed CTL-diet. Inversely, hepatic *Cpt-1* mRNA expression was higher in the offspring of dams fed LA-diet ($p=0.015$) with a trend to be exacerbated when offspring were also exposed to LA-diet after weaning ($p=0.051$). The lipogenic pathway was evaluated by mRNA expression of key enzymes involved in FA biosynthesis, acetyl-CoA carboxylase (*Acc)* and fatty acid synthase *(Fasn)* and key enzymes involved in MUFA synthesis, fatty acid elongase 6 (*Elovl6)* and

stearoyl-CoA desaturase 1 (*Scd-1)* in the offspring liver (Fig. 2C). The hepatic transcript levels of *Fasn*, involved in C16:0 synthesis, were significantly higher in the l/CTL livers than the c/CTL and l/LA groups indicating the combined and opposite effects of maternal (p=0.018) and weaning (p=0.002) LA-diets. The transcript levels of *Elovl6* were significantly lower in the c/LA livers compared to c/CTL group indicating the downregulated effects of the weaning LA-diet on the elongation of C16:0 (p=0.001). Hepatic transcript levels of *Scd-1*, involved in the conversion of SFA to MUFA was altered mainly by the weaning diet (p=0.008) with a reduced mRNA expression in offspring fed LA-diet compared to the CTL-diet (Fig. 2C). The transcription factor regulating *de novo* lipogenesis, sterol regulatory element-binding protein-1c (Srebp-1c) was also significantly reduced in offspring fed LA-diet compared to their control groups (p=0.045) (Fig. 2D). These findings demonstrated that both maternal and weaning LA-diet altered lipid metabolism pathways in offspring livers. While the maternal LA-diet affected mainly the β-oxidation pathway and to a lower extend the lipogenic pathway, the FA, MUFA, and TG synthesis were mainly altered by the weaning LA-diet.

3.4. Maternal and weaning LA-diet altered SCD-1 protein expression and desaturation index

To further explore the lipogenic pathway and especially the MUFA synthesis required for the TG synthesis, we assayed the SCD-1 protein level by Western Blot (Fig. 3A). Two major protein isoforms were observed for SCD-1. Relative quantification demonstrated a lower protein level in the c/LA, l/CTL and l/LA livers compared to the c/CTL group. The offspring fed LA-diet after weaning had a reduced SCD-1 expression compared to the offspring fed CTL-diet ($p<0.001$) which was only significant in offspring of dams fed CTL-diet. The l/CTL group showed a decreased protein level compared to the c/CTL group indicating the potential downregulated effects of the maternal LA-diet on SCD-1 protein expression in offspring. These data demonstrated the combined effects of maternal and weaning LA-diet in the alteration of hepatic SCD-1 protein levels in offspring.

The SCD-1 desaturation index was evaluated as both ratios of SCD-1 products to the precursors (C16:1 n-7/C16:0 and C18:1 n-9/C18:0) in liver (Supplementary Fig. 3B,C) [29]. The offspring fed LA-diet

exhibited a hepatic decrease of the SCD-1 ratio on C16:0 but only the l/CTL group had significant higher values compared to the c/LA group (Supplementary Fig. 3B). Hepatic SCD-1 ratio on C18:0 was inversely affected by the maternal ($p=0.014$) and the weaning ($p<0.001$) LA-diets with significant higher value in the l/CTL group compared to the others (Supplementary Fig. 3C). Similar profile of both SCD-1 ratios were also observed in plasma (data not shown) and epWAT indicating mainly the effects of the weaning diet ($p<0.0001$) on the SCD-1 regulation (Supplementary Fig. 5D-E). However, it is important to note that these differences of the SCD-1 ratios are likely to be explained by the diet FA composition. Taken together, these data suggested that both maternal and weaning LA-diet altered the regulation of enzymes involved in the lipogenic pathway including SCD-1.

3.5. Maternal and Weaning LA-diet altered liver TG composition in adult offspring

In NAFLD patients, changes in PUFA composition have also been described [30], therefore we analyzed the FA profile of hepatic TG in the liver of offspring. As shown in Table 3, the global FA composition of hepatic TG fraction was affected by both maternal and weaning diets. The hepatic TG composition of offspring fed LA diet revealed non-significant changes in the proportions of saturated FA (SFA) but lower proportions of monounsaturated FA (MUFA) and an enrichment of n-6 PUFA (especially LA) and n-3 PUFA compared to their respective CTL-diet fed offspring. This indicates the effectiveness of the dietary regimen in modulating the FA composition of organs such as liver or adipose tissue (supplementary Fig. 5A-C). Specifically, the proportions of C16:1 n-7 and C18:1 n-7 were lower in liver TG of offspring fed LA-diet compared to the CTL-diet ($p<0.001$) and likely depend on the relative smaller intake of these FA as well as their SFA precursors in the LA-diet. Among the n-6 PUFA, the proportions of LA and its derivatives such as C20:4 n-6 were significantly increased in the hepatic TG fraction of offspring fed LA-diet compared to their respective controls ($p<0.001$). The weaning LA-diet also induced higher proportions of some n-3 LC-PUFA ($p<0.001$).

Additionally, the type of maternal diet received during the pregnancy/lactation period also altered the FA profile of liver TG in offspring. More precisely, the offspring of dams fed LA-diet exhibited lower

proportions of C18:0 ($p=0.002$) and C18:1 n-9 ($p<0.001$) but relative higher proportions of PUFA including C20:4 n-6 ($p=0.034$), C18:3 n-3 ($p=0.022$) and C22:5 n-3 ($p<0.001$) compared to the respective offspring of dams fed CTL-diet. The proportions of C20:4 n-6, C22:5 n-3, C22:6 n-3 and the n-6/n-3 PUFA ratio were affected by significant synergic effects of both maternal and weaning diets. These data demonstrated that, although hepatic FA composition is mainly determined by the weaning diet, the maternal diet during gestation and lactation had still lasting effects on liver FA composition in adult offspring.

3.6. Maternal and weaning LA-diet altered oxylipin profile in plasma of offspring

To further explore the potential causes of hepatic TG accumulation in offspring, we performed a targeted lipidomic analysis to measure plasmatic levels of bioactive derivatives generated from their PUFA precursors. Bioactive derivatives can be generated mainly via three enzymatic pathways: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome-P450 epoxygenases (CYPs). A non–enzymatic pathway is also possible. The principal component analysis of the plasma oxylipins revealed the clustering of two groups differentiated mainly by the weaning diet (supplementary Fig. 3A). Principal component 1 (PC1) explained 34.4% of the metabolite variance and principal component 2 (PC2) explained 22.6% of it. The first cluster was composed of c/CTL and l/CTL groups without any clear separation between the two groups. The second cluster was composed of c/LA and l/LA groups with a possible separation between the two groups suggesting an additional impact of the maternal diet on the metabolite profile in offspring of dams fed LA-diet. The Biplot representation showed the main metabolites contributing to the PC1 and PC2 variables (Supplementary Fig. 3B). The heatmap visualization confirmed the separation into two clusters according to the weaning diet (supplementary Fig. 3C). A detailed list of plasma oxylipins measured in offspring is shown in supplementary Table 2.

3.6.1. Oxidative derivatives of LA. LA is converted to ARA but part of it can also be oxidized into oxidized LA metabolites (OXLAMs). 9-HODE and 13-HODE, LA metabolites generated by LOX

enzyme were measured in the plasma of offspring at 6 months (Fig. 4A, B). The concentration of both metabolites were not affected by the maternal diet but, were increased in offspring fed LA-diet ($p<0.0001$) compared to their respective controls. Of note, plasma levels of 9-HODE were lower by half compared to 13-HODE concentrations. As shown in Fig. 4C, D, the linear regression between hepatic TG content and plasmatic OXLAM concentration was not significant for 13-HODE 9 ($p=0.338$) nor 9-HODE ($p=0.385$) suggesting that OXLAMS may not be directly involved in the development of steatosis in our model.

3.6.2. Oxidative derivatives of ARA. ARA is known as a precursor of numerous bioactive metabolites. Eicosanoids including prostaglandins (PGs) and thromboxanes (TXs) are the major ARA products of the COX pathway (Fig. 5A). Among them, the increased levels of TXB_2 and PGD_2 in plasma of offspring were respectively affected by the maternal LA-diet ($p=0.039$) and the weaning LA-diet ($p=0.037$). The levels of 6-keto $PGF_{1\alpha}$ were significantly higher in c/LA group compared to both c/CTL and l/LA group indicating the opposite interaction between maternal ($p=0.029$) and weaning diets ($p=0.022$). The levels of the other prostaglandins, PGE_2 and 11β-$PGF_{2\alpha}$, were not affected by the diet. Hydroxyeicosatetraenoic acids (HETEs), lipoxins (LXs) and leukotrienes (LTs) are the major ARA products of the LOX pathway (Fig. 5B). Of note, HETEs were the predominant metabolites with the highest plasma concentration of 5-HETE and 12-HETE. The levels of pro-inflammatory 5-HETE, 12-HETE and LTB_4 were significantly affected by both maternal and weaning diets with increased concentrations in offspring of dams fed LA-diet which was exacerbated when the offspring were also fed LA-diet. The levels of the downstream product of 5-HETE, 5-oxo-ETE and 8-HETE were increased by the weaning diet (respectively, $p=0.002$ and $p=0.016$; supplementary Table 2). Higher levels of the lipoxin A_4 (LXA_4), recently identified as a specialized pro-resolving mediator (SPM) [31], were also observed when offspring were fed LA-diet whereas the concentration of its precursor, 15-HETE, was not affected by the diet. (Supplementary Table 2).

Epoxyeicosatrienoics (EET) are the main ARA products of the CYP/sEH pathway (Fig. 5C). There was no difference in the levels of 5,6-EET while the levels of its downstream product, 5,6-DiHETE, were

significantly increased in offspring by both maternal and weaning LA-diets. Conversely, maternal ($p=0.002$) and weaning ($p<0.001$) LA-diets induced decreased levels of 8,9-EET in l/LA offspring compared to c/LA offspring. The levels of the non-enzymatically derived ARA-product, 8-iso-PGA_2, were affected by both maternal ($p=0.038$) and weaning ($p=0.008$) diets with a significant increase in the plasma of the l/LA group compared to c/CTL and l/CTL groups.

The linear regression between hepatic TG content and plasmatic ARA derivatives showed a significant correlation between TXB_2 ($r=0.481$, $p= 0.017$), 5-HETE ($r=0.487$, $p= 0.016$), 12-HETE ($r=0.608$, $p= 0.002$), LTB_4 ($r=0.435$, $p= 0.033$) and 8-iso-PGA_2 ($r=0.471$ $p= 0.020$) levels and hepatic TG in offspring (supplementary Fig. 4) suggesting a role of these bioactive derivatives in the development of steatosis in offspring.

3.6.3. Oxidative derivatives of n-3 EPA and DHA. n-6 PUFA, ARA, and n-3 PUFA, EPA/DHA, represent competitive substrates for the enzyme machinery including LOX, COX and CYP, therefore we also analyzed the n-3-derivatives in the plasma of offspring at 6 months (Fig. 6). The n-3 PUFA, EPA and DHA, serve as substrates for the conversion of a novel series of lipid mediators, the SPMs, involved in the resolution of inflammation which includes resolvins, lipoxins, protectins and maresins [31]. The EPA-derived metabolite, LTB_5, was significantly higher in the l/CTL group compared to c/CTL and l/LA groups indicating the opposite effects of maternal and weaning LA-diets (Fig. 6A). Similarly, the levels of 18-HEPE, precursor of E-series resolvins, were significantly affected by both maternal and weaning with a decrease in the c/LA group compared to c/CTL group. The LOX-generated derivatives of DHA, PDX, 7MaR1 and 17-HDHA were only affected by the weaning diet whereas 14-HDHA was affected by both maternal and weaning diets (respectively, $p<0.001$ and $p=0.001$; Fig. 6B). The levels of PDX and its precursor, 17-HDHA, were strongly reduced in the plasma of offspring fed LA-diet after weaning (respectively, $p=0.020$ and $p=0.015$) compared to the offspring fed CTL-diet. The levels of the maresin 7MaR1 were also significantly decreased in offspring fed LA diet during growth while the concentrations

of its precursor, 14-HDHA were higher in offspring of dams fed LA-diet with a significant difference between the l/CTL and c/CTL groups.

The linear regression between hepatic TG content and plasmatic EPA and DHA derivatives showed a significant correlation between LTB_5 (r=0.576, p= 0.003) and 14-HDHA (r=0.486, p= 0.016) levels and hepatic TG in offspring supporting an important role of both maternal and weaning LA-diet mediated by the levels of n-3 oxidative derivatives (supplementary Fig. 4).

4. Discussion:

In our study, we investigated the effects of both maternal and long-term weaning high LA intake within a moderate fat diet on lipid metabolism and the susceptibility for offspring to later develop metabolic diseases. Herein, we did not demonstrate that a high LA intake during pregnancy, lactation and growth induces obesity in offspring as previously suggested [1, 2, 7]. Although high maternal LA intake might induce primes features of the metabolic syndrome including hyperglycaemia, higher cholesterol levels and increased epWAT adiposity, the exposure to LA-diet after weaning led to an opposite phenotype in offspring with a reduced adiposity index and lower plasma TG. [32] Interestingly, we showed that maternal LA-rich diet altered the hepatic metabolism leading to TG accumulation within hepatocytes (hepatic steatosis or “first-hit”) with few signs of inflammation (plasma TNF-α levels) in offspring, as previously discussed [33, 34]. NAFLD is considered as the hepatic manifestation of the metabolic syndrome resulting from obesity and insulin-resistance [16]. The absence of major inflammation signs, increased adiposity and insulin level changes in our model did not lead here to the multiple (former “two-hit”) steps of NAFLD [35].

The crosstalk between adipose tissue and liver is known as a central player in the development of NAFLD but the mechanisms remain yet to be fully elucidated. Our nutritional model did not reproduce the LA-induced physiological alterations observed mainly in high fat diet studies [7, 10, 11]. However, our results are supported by previous evidence suggesting the ability for LA to limit the development of adipose

tissue [32, 36] and to reduce serum TG [37]. Indeed, Shimomura et al demonstrated that a safflower diet (rich in LA) induced less fat accumulation and reduced serum triacylglycerol due to a greater thermogenesis and fat oxidation in adipose tissue and muscle. Interestingly, the adipose tissue and liver changes observed in our model also mimics the main physiological alterations induced by methionine-choline deficient (MCD) diet [38, 39] or conjugated LA-diet [40]. In the MCD model, increased metabolism and thermogenesis are also evoked to explain the diet-induced alterations [39, 41]. Thus, the use of high LA intake without excessive calories intake might reveal specific properties of this essential n-6 PUFA leading to hepatic steatosis.

In NAFLD patients, Donnelly et *al.* demonstrated that liver TG content derived or originated from around 60% from free FA influx from adipose tissue, 25% from *DNL* and 15% from the diet [28]. Herein, we demonstrated that maternal LA intake altered FA oxidation pathway as previously observed [18, 42]. Additionally, we showed the combined and rather opposite effects of maternal and weaning LA-rich diet on the regulation of DNL in offspring liver. While maternal high LA intake rather promoted higher lipogenic gene expression, long-term exposure to high LA induced a significant downregulation of the expression of key genes involved in FA synthesis and particularly in MUFA synthesis. Our results concurred with previous studies demonstrating that dietary LA reduced the lipogenic pathway [43-46]. However, the mechanisms which led offspring to develop a hepatic steatosis despite a downregulation of *de novo* lipogenesis remain unclear. In patients with NAFLD, the lipogenic pathway is enhanced and higher SCD1 levels is thought to be protective of the progression of NAFLD to NASH by partitioning excess lipid into secreted TG.[35, 47, 48]. Our results are in line with previous studies showing that the inhibition of *Scd-1* expression could lead to increased hepatic TG levels in rodents as well as humans [21, 49-52]. The comparison with MCD diet give us some potential mechanism insights [38, 39, 41]. We can speculate that the hepatic steatosis could result from impaired TG synthesis and secretion due to the LA-induced inhibition of SCD-1 [38, 39, 53, 54] and lowered proportions of MUFA in the LA-diet. This hypothesis is supported by previous results showing that n-6 supplementation lowered VLDL and

triglycerides concentrations [55] in human and that the blockade in lipoprotein secretion is accompanied by hepatic steatosis [56]. Another mechanism could involve AMPK activation which coordinate the partitioning of fatty acids between biosynthetic and oxidative pathways [41, 57]. Further studies are required to reveal the mechanisms involved in LA-induced metabolic changes observed in offspring and to deeper analyze the adipose tissue and liver crosstalk.

The consequences of maternal HFD or obese mothers on later liver injuries have been well described [2, 17-19], but the link between n-6 PUFA intake and NAFLD is still controversial [30, 58-60]. In our study, we demonstrated the synergic effects of high LA intake in maternal and weaning diets to enrich n-6 PUFA proportions of several organs including the liver. The maternal LA-exposition during pregnancy and lactation induced lasting effect on the FA composition in offspring as previously found in different nutritional contexts [4]. In NAFLD patients, changes in the hepatic ratio of n-6: n-3 PUFA have been highlighted and a link between higher n-6 PUFA and the pathogenesis of the disease has emerged due to the pro-inflammatory functions of their derivatives [22, 60, 61]. Most of the pro-inflammatory effects have been attributed to the ARA derivatives but recent studies also demonstrated a potential role of LA derivatives. Ramsden et al. demonstrated that lowering LA intake in the diet might help to reduce NAFLD development *via* a reduction of OXLAMS concentration [62]. In line with previous studies in animal and humans [61, 63], we demonstrated that enriched LA intake after weaning, but not *in utero* and during the suckling period, increases the levels of 9-HODE and 13-HODE which were not directly correlated with the hepatic steatosis as recently described [46, 64]. In addition, we demonstrated the synergic effects of maternal and weaning high LA intake to increase concentrations of ARA derivatives in the plasma of offspring. The main correlations between the hepatic steatosis and bioactive metabolites were observed for the ARA derivatives generated through the LOX pathway (5-HETE, 12-HETE, LTB_4) and the non-enzymatically derived 8 iso-PGA_2, which has been previously shown to be up-regulated in a model of LA-induced hepatic gene expression alterations [27]. Most of ARA derivatives including eicosanoids and HETEs have been described for their pro-inflammatory properties and consequently, for their roles in the

assessment of NAFLD in humans [22, 64]. However, some ARA-derived metabolites have been recently shown to be anti-inflammatory and/or pro-resolving mediators [65, 66]. In our study, high LA intake induced lower levels of the anti-inflammatory 8,9-EET while increasing plasma concentrations of the anti-inflammatory and pro-resolving lipoxin LXA_4 as previously suggested [21, 65]. The simultaneous synthesis of both pro- and anti-inflammatory and pro-resolving mediators question the mechanism by which the bioactive derivatives act on the development of hepatic steatosis. In addition, we demonstrated the combined effects of both maternal and weaning LA-diets in inducing an increased production of n-6 derivatives at the expense of n-3 PUFA derivatives as described in high fat diet studies [21, 67]. We demonstrated that high LA intake decreased plasma levels of SPM precursors, 14-HDHA and 17-HDHA, and their downstream products MaR1 and PDX, known for their anti-inflammatory and pro-resolving properties. Our data are in lines with recent evidence showing that obesity was associated with a downregulation of EPA- and DHA-derived SPM [21, 67-69]. The authors have linked the n-3 derived SPMs with the development of adipose tissue inflammation [68], the resolution of inflammatory responses [67] and the rescue of obesity-induced insulin-resistance and fatty liver [69]. It is interesting to note that, despite an absence of excessive calorie intake, high LA intake during the weaning period can induce lower n-3 PUFA derivatives which might contribute to the metabolic alterations seen in offspring [68, 69]. The mechanisms by which increased LA- and ARA-derived and lowered EPA- and DHA- derived metabolites induce steatosis are not yet fully elucidated [46]. Further investigations are required to better define the weight and the pathway of each derivative as well as their interactions in the induction of metabolic alterations.

Our findings demonstrated that a high LA intake received *in utero* and during growth affect adult metabolism but mechanisms involved in later adaptations in offspring are not fully understood. We demonstrated that maternal high LA intake during pregnancy and lactation programmed liver FA metabolism in offspring, in accordance with previous reports [4, 27, 42, 70]. Our results also demonstrated some opposite effects between the maternal and weaning diets on lipid metabolism particularly seen when

the offspring of dams fed LA-diet received the CTL-diet during growth. The marked changes observed in the l/CTL group suggested a phenomenon contributing to early life programming events called "predictive adaptive responses (PAR)" [71]. The discrepancy between the gestational nutrition and predicted weaning nutritional environment might have led offspring to develop hepatic steatosis caused by metabolic adaptations as previously described [17, 27]. Further studies will be required to investigate *in utero* mechanisms leading to later metabolic diseases in offspring regarding the weaning diet.

In conclusion, our results demonstrated the complex interaction of maternal and weaning high LA intake within a moderate fat diet on the alteration of hepatic lipid metabolism and plasma oxylipin profile that likely predispose offspring to a later development of NAFLD. Our findings might give some clues on the role of LA and its different bioactive derivatives to further study the impact of maternal nutrition on the later fetal development and its susceptibility to metabolic diseases in adulthood.

List of Abbreviations: LC-PUFA, long-chain polyunsaturated fatty acid, LA, linoleic acid, NAFLD; non-alcoholic fatty liver disease, NASH, non-alcoholic steatohepatitis, HFD, high fat diet, IL, interleukin, SCD, stearoyl-CoA desaturase, WAT, white adipose tissue, SFA, saturated fatty acid, DNL, *de novo* lipogenesis

Acknowledgments: The authors thank X. Blanc (UPAE, Jouy-en-Josas, France) for the preparation of the diets, Dr. A. Fautrel (H2P2 Federation de Recherche, Biosit, University of Rennes 1) for histological analysis.

Author contributions: J.M. wrote the paper and F.P., G. B. and P.L. contributed to the manuscript, J.M. and P.L. designed the study. J.M., D.C., C.D., N.M-B., F.B., G.B contributed to the research, F.B. and N.M-B. contributed to animal cares. All authors read and approved the final manuscript.

Financial support: This work was supported by grants from Valorex. The funders had no role in data collection and analysis, decision to publish or the preparation of the manuscript. This work has been supported by the Société Française de Nutrition.

Conflict of interest: The authors declare no conflict of interest.

References

[1] Mennitti LV, Oliveira JL, Morais CA, Estadella D, Oyama LM, Oller do Nascimento CM, et al. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. J Nutr Biochem. 2015;26:99-111.
[2] Elahi MM, Cagampang FR, Mukhtar D, Anthony FW, Ohri SK, Hanson MA. Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. Br J Nutr. 2009;102:514-9.
[3] Mennitti LV, Oyama LM, Santamarina AB, do Nascimento C, Pisani LP. Early exposure to distinct sources of lipids affects differently the development and hepatic inflammatory profiles of 21-day-old rat offspring. J Inflamm Res. 2018;11:11-24.
[4] Hoile SP, Irvine NA, Kelsall CJ, Sibbons C, Feunteun A, Collister A, et al. Maternal fat intake in rats alters 20:4n-6 and 22:6n-3 status and the epigenetic regulation of Fads2 in offspring liver. J Nutr Biochem. 2013;24:1213-20.
[5] Kelsall CJ, Hoile SP, Irvine NA, Masoodi M, Torrens C, Lillycrop KA, et al. Vascular dysfunction induced in offspring by maternal dietary fat involves altered arterial polyunsaturated fatty acid biosynthesis. PLoS One. 2012;7:e34492.
[6] Ducheix S, Montagner A, Polizzi A, Lasserre F, Marmugi A, Bertrand-Michel J, et al. Essential fatty acids deficiency promotes lipogenic gene expression and hepatic steatosis through the liver X receptor. J Hepatol. 2013;58:984-92.
[7] Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog Lipid Res. 2006;45:203-36.
[8] Sioen I, van Lieshout L, Eilander A, Fleith M, Lohner S, Szommer A, et al. Systematic Review on N-3 and N-6 Polyunsaturated Fatty Acid Intake in European Countries in Light of the Current Recommendations - Focus on Specific Population Groups. Ann Nutr Metab. 2017;70:39-50.
[9] Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation. 2009;119:902-7.
[10] Massiera F, Barbry P, Guesnet P, Joly A, Luquet S, Moreilhon-Brest C, et al. A Western-like fat diet is sufficient to induce a gradual enhancement in fat mass over generations. J Lipid Res. 2010;51:2352-61.
[11] Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, et al. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. J Clin Endocrinol Metab. 2013;98:299-307.
[12] Rudolph MC, Young BE, Lemas DJ, Palmer CE, Hernandez TL, Barbour LA, et al. Early infant adipose deposition is positively associated with the n-6 to n-3 fatty acid ratio in human milk independent of maternal BMI. Int J Obes (Lond). 2017;41:510-7.
[13] Vidakovic AJ, Gishti O, Voortman T, Felix JF, Williams MA, Hofman A, et al. Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the Generation R Study. Am J Clin Nutr. 2016;103:1017-25.

[14] Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Prog Lipid Res. 2009;48:1-26.
[15] Boppidi H, Daram SR. Nonalcoholic fatty liver disease: hepatic manifestation of obesity and the metabolic syndrome. Postgrad Med. 2008;120:E01-7.
[16] Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998;114:842-5.
[17] Gregorio BM, Souza-Mello V, Carvalho JJ, Mandarim-de-Lacerda CA, Aguila MB. Maternal high-fat intake predisposes nonalcoholic fatty liver disease in C57BL/6 offspring. Am J Obstet Gynecol. 2010;203:495 e1-8.
[18] Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC, et al. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology. 2009;50:1796-808.
[19] Hughes AN, Oxford JT. A lipid-rich gestational diet predisposes offspring to nonalcoholic fatty liver disease: a potential sequence of events. Hepat Med. 2014;6:15-23.
[20] de Meijer VE, Le HD, Meisel JA, Akhavan Sharif MR, Pan A, Nose V, et al. Dietary fat intake promotes the development of hepatic steatosis independently from excess caloric consumption in a murine model. Metabolism. 2010;59:1092-105.
[21] Warner DR, Liu H, Ghosh Dastidar S, Warner JB, Prodhan MAI, Yin X, et al. Ethanol and unsaturated dietary fat induce unique patterns of hepatic omega-6 and omega-3 PUFA oxylipins in a mouse model of alcoholic liver disease. PLoS One. 2018;13:e0204119.
[22] Santoro N, Caprio S, Feldstein AE. Oxidized metabolites of linoleic acid as biomarkers of liver injury in nonalcoholic steatohepatitis. Clin Lipidol. 2013;8:411-8.
[23] Speakman JR. Use of high-fat diets to study rodent obesity as a model of human obesity. Int J Obes (Lond). 2019.
[24] Rioux V, Catheline D, Bouriel M, Legrand P. Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5 n-3 and C20:3 n-6 in the rat. Reprod Nutr Dev. 2005;45:599-612.
[25] Pedrono F, Boulier-Monthean N, Catheline D, Legrand P. Impact of a Standard Rodent Chow Diet on Tissue n-6 Fatty Acids, Delta9-Desaturation Index, and Plasmalogen Mass in Rats Fed for One Year. Lipids. 2015;50:1069-82.
[26] Le Faouder P, Baillif V, Spreadbury I, Motta JP, Rousset P, Chene G, et al. LC-MS/MS method for rapid and concomitant quantification of pro-inflammatory and pro-resolving polyunsaturated fatty acid metabolites. J Chromatogr B Analyt Technol Biomed Life Sci. 2013;932:123-33.
[27] Chechi K, McGuire JJ, Cheema SK. An interaction of the pre- and post-weaning diets rich in omega-6 polyunsaturated fats alters plasma lipids, hepatic gene expression and aortic vascular reactivity in adult C57BL/6 mice. Nutr Metab Insights. 2010;3:69-78.
[28] Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343-51.
[29] Flowers MT. The delta9 fatty acid desaturation index as a predictor of metabolic disease. Clin Chem. 2009;55:2071-3.
[30] Arendt BM, Comelli EM, Ma DW, Lou W, Teterina A, Kim T, et al. Altered hepatic gene expression in nonalcoholic fatty liver disease is associated with lower hepatic n-3 and n-6 polyunsaturated fatty acids. Hepatology. 2015;61:1565-78.
[31] Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest. 2018;128:2657-69.

[32] Shimomura Y, Tamura T, Suzuki M. Less body fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. J Nutr. 1990;120:1291-6.
[33] Torres Dde O, Dos Santos AC, Silva AK, Leite JI, De Souza JR, Beltrao EI, et al. Effect of maternal diet rich in omega-6 and omega-9 fatty acids on the liver of LDL receptor-deficient mouse offspring. Birth Defects Res B Dev Reprod Toxicol. 2010;89:164-70.
[34] Vaughan RA, Garrison RL, Stamatikos AD, Kang M, Cooper JA, Paton CM. A High Linoleic Acid Diet does not Induce Inflammation in Mouse Liver or Adipose Tissue. Lipids. 2015;50:1115-22.
[35] Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest. 2004;114:147-52.
[36] Matsuo T, Takeuchi H, Suzuki H, Suzuki M. Body fat accumulation is greater in rats fed a beef tallow diet than in rats fed a safflower or soybean oil diet. Asia Pac J Clin Nutr. 2002;11:302-8.
[37] Poudyal H, Kumar SA, Iyer A, Waanders J, Ward LC, Brown L. Responses to oleic, linoleic and alpha-linolenic acids in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. J Nutr Biochem. 2013;24:1381-92.
[38] Rizki G, Arnaboldi L, Gabrielli B, Yan J, Lee GS, Ng RK, et al. Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. J Lipid Res. 2006;47:2280-90.
[39] Rinella ME, Elias MS, Smolak RR, Fu T, Borensztajn J, Green RM. Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. J Lipid Res. 2008;49:1068-76.
[40] Vyas D, Kadegowda AK, Erdman RA. Dietary conjugated linoleic Acid and hepatic steatosis: species-specific effects on liver and adipose lipid metabolism and gene expression. J Nutr Metab. 2012;2012:932928.
[41] Dobrzyn A, Ntambi JM. The role of stearoyl-CoA desaturase in the control of metabolism. Prostaglandins Leukot Essent Fatty Acids. 2005;73:35-41.
[42] Jacometo CB, Schmitt E, Pfeifer LF, Schneider A, Bado F, da Rosa FT, et al. Linoleic and alpha-linolenic fatty acid consumption over three generations exert cumulative regulation of hepatic expression of genes related to lipid metabolism. Genes Nutr. 2014;9:405.
[43] Jeffcoat R, James AT. The control of stearoyl-CoA desaturase by dietary linoleic acid. FEBS Lett. 1978;85:114-8.
[44] Flick PK, Chen J, Vagelos PR. Effect of dietary linoleate on synthesis and degradation of fatty acid synthetase from rat liver. J Biol Chem. 1977;252:4242-9.
[45] Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res. 1999;40:1549-58.
[46] Schuster S, Johnson CD, Hennebelle M, Holtmann T, Taha AY, Kirpich IA, et al. Oxidized linoleic acid metabolites induce liver mitochondrial dysfunction, apoptosis, and NLRP3 activation in mice. J Lipid Res. 2018;59:1597-609.
[47] Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab. 2008;34:643-8.
[48] Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. J Gastroenterol. 2013;48:434-41.
[49] Gutierrez-Juarez R, Pocai A, Mulas C, Ono H, Bhanot S, Monia BP, et al. Critical role of stearoyl-CoA desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance. J Clin Invest. 2006;116:1686-95.

[50] Fernandez Gianotti T, Burgueno A, Gonzales Mansilla N, Pirola CJ, Sookoian S. Fatty liver is associated with transcriptional downregulation of stearoyl-CoA desaturase and impaired protein dimerization. PLoS One. 2013;8:e76912.
[51] Stefan N, Peter A, Cegan A, Staiger H, Machann J, Schick F, et al. Low hepatic stearoyl-CoA desaturase 1 activity is associated with fatty liver and insulin resistance in obese humans. Diabetologia. 2008;51:648-56.
[52] Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? Prog Lipid Res. 2013;52:15-42.
[53] Miyazaki M, Kim YC, Ntambi JM. A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. J Lipid Res. 2001;42:1018-24.
[54] Legrand P, Catheline D, Hannetel JM, Lemarchal P. Stearoyl-CoA desaturase activity in primary culture of chicken hepatocytes. Influence of insulin, glucocorticoid, fatty acids and cordycepin. Int J Biochem. 1994;26:777-85.
[55] Ooi EM, Watts GF, Ng TW, Barrett PH. Effect of dietary Fatty acids on human lipoprotein metabolism: a comprehensive update. Nutrients. 2015;7:4416-25.
[56] Bjorkegren J, Beigneux A, Bergo MO, Maher JJ, Young SG. Blocking the secretion of hepatic very low density lipoproteins renders the liver more susceptible to toxin-induced injury. J Biol Chem. 2002;277:5476-83.
[57] Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci U S A. 2002;99:11482-6.
[58] Cortez-Pinto H, Jesus L, Barros H, Lopes C, Moura MC, Camilo ME. How different is the dietary pattern in non-alcoholic steatohepatitis patients? Clin Nutr. 2006;25:816-23.
[59] El-Badry AM, Graf R, Clavien PA. Omega 3 - Omega 6: What is right for the liver? J Hepatol. 2007;47:718-25.
[60] Allard JP, Aghdassi E, Mohammed S, Raman M, Avand G, Arendt BM, et al. Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. J Hepatol. 2008;48:300-7.
[61] Warner DR, Liu H, Miller ME, Ramsden CE, Gao B, Feldstein AE, et al. Dietary Linoleic Acid and Its Oxidized Metabolites Exacerbate Liver Injury Caused by Ethanol via Induction of Hepatic Proinflammatory Response in Mice. Am J Pathol. 2017;187:2232-45.
[62] Ramsden CE, Ringel A, Feldstein AE, Taha AY, MacIntosh BA, Hibbeln JR, et al. Lowering dietary linoleic acid reduces bioactive oxidized linoleic acid metabolites in humans. Prostaglandins Leukot Essent Fatty Acids. 2012;87:135-41.
[63] Maciejewska D, Ossowski P, Drozd A, Ryterska K, Jamiol-Milc D, Banaszczak M, et al. Metabolites of arachidonic acid and linoleic acid in early stages of non-alcoholic fatty liver disease--A pilot study. Prostaglandins Other Lipid Mediat. 2015;121:184-9.
[64] Feldstein AE, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, et al. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J Lipid Res. 2010;51:3046-54.
[65] Schuck RN, Zha W, Edin ML, Gruzdev A, Vendrov KC, Miller TM, et al. The cytochrome P450 epoxygenase pathway regulates the hepatic inflammatory response in fatty liver disease. PLoS One. 2014;9:e110162.
[66] Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. Chem Rev. 2011;111:5922-43.

[67] Crouch MJ, Kosaraju R, Guesdon W, Armstrong M, Reisdorph N, Jain R, et al. Frontline Science: A reduction in DHA-derived mediators in male obesity contributes toward defects in select B cell subsets and circulating antibody. J Leukoc Biol. 2018.
[68] Neuhofer A, Zeyda M, Mascher D, Itariu BK, Murano I, Leitner L, et al. Impaired local production of proresolving lipid mediators in obesity and 17-HDHA as a potential treatment for obesity-associated inflammation. Diabetes. 2013;62:1945-56.
[69] Gonzalez-Periz A, Horrillo R, Ferre N, Gronert K, Dong B, Moran-Salvador E, et al. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J. 2009;23:1946-57.
[70] Seet EL, Yee JK, Jellyman JK, Han G, Ross MG, Desai M. Maternal high-fat-diet programs rat offspring liver fatty acid metabolism. Lipids. 2015;50:565-73.
[71] Gluckman PD, Hanson MA, Spencer HG. Predictive adaptive responses and human evolution. Trends Ecol Evol. 2005;20:527-33.

Figure legends

Fig. 1. Effects of dietary LA on hepatic triglyceride accumulation in adult offspring.

(A) Representative histological images of hematoxylin-eosin-stained liver sections showing normal TG accumulation in c/CTL group (a), microvesicular steatosis in c/LA group (b), micro- and macrovesicular steatosis in l/CTL (c) and in l/LA groups (d). Thick represents 300µm. (B) Triglyceride content in liver of adult offspring. Results (n=6) are presented as mean ± SEM.

two-way ANOVA followed by the Tukey's post-hoc test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.

Fig. 2. Hepatic gene expression of enzymes involved in fatty acid uptake, β-oxidation and lipogenesis pathway in adult offspring.

mRNA expression levels of genes involved in fatty acid (FA) uptake (*cd36, fatp*) (A) and β-oxidation (*ppar-α, cpt-1*) (B). mRNA expression levels of genes involved in *de novo* lipogenesis (*acc, fas, elovl6, scd-1*) (C) positively mediated by the transcriptional factor srebp-1c (D). Relative gene expression was determined by real-time PCR in liver with SYBRGreen® or Taqman® techonology. Results (n=6) are presented as mean ± SEM. Different letters indicate significant differences according to according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.

Fat/cd36: fatty acid translocase/cluster of differenciation 36; fatp: fatty acid transport protein; pparα: peroxisome proliferator-activated receptor α; cpt-1:carnitine palmitoyltransferase-1, acc:acetyl-CoA carboxylase; fas: fatty acid synthase: elovl6: elongation of very long chain fatty acids protein 6; scd-1: stearoyl-CoA desaturase-1; srebp-1c: stearoyl regulatory element-binding protein-1c

Fig. 3. Hepatic Stearoyl-CoA Desaturase-1 protein expression and desaturation index in adult offspring. A) Representative hepatic expression of SCD-1 expression vizualised by Western blot and

quantified relative expression. Stearoyl-CoA desaturase-1 (SCD-1) desaturation index was evaluated respectively as the ratio of C16:1 n-7/C16:0 (B) and the ratio of C18:1 n-9/C18:0 (C) in liver of adult offspring. Results (n=6) are presented as mean ± SEM. Different letters indicate significant differences according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.

Fig. 4. Plasma oxidized LA metabolites, OXLAMS, in adult offspring.

The OXLAMs 9-HODE and 13-HODE represent the main LA derivatives generated by the lipoxygenase (LOX) pathway. (A) 9-HODE concentrations in the plasma of offspring. (B) 13-HODE concentrations in the plasma of offspring. Pearson correlation analysis between 9-HODE (C) and 13-HODE (D) and hepatic TG levels in offspring. Results (n=6) are presented as mean ± SEM. Different letters indicate significant differences according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.

Fig. 5. Main plasma ARA derivatives in adult offspring.

ARA derivatives can be generated by the cyclooxygenase (COX) pathway (A), the lipoxygenase (LOX) pathway (B), the cytochrome P450 (CYP) pathway (C) or non enzymatically (D). Results (n=6) are presented as mean ± SEM. Different letters indicate significant differences according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.

Fig. 6. Plasma EPA/DHA derivatives in adult offspring.

(A) EPA derivatives can be generated by the lipoxygenase (LOX) pathway or the cyclooxygenase (COX) pathway. (B) DHA derivatives can be generated by the LOX pathway. Results (n=6) are presented as

mean ± SEM. Different letters indicate significant differences according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.

Table 1. **Major fatty acid composition of experimental diets.**

Fatty acid	**Control-diet**	**LA-diet**
	% of total fatty acids	
C 16:0	16.1	14.6
C 18:0	2.8	3.0
∑ SFA	**19.5**	**17.9**
C 16:1 n-7	0.5	0.1
C 18:1 n-7	1.6	0.9
C 18:1 n-9	65.4	19.7
∑ MUFA	**68.4**	**20.9**
C 18:2 n-6	9.6	58.4
∑ n-6 PUFA	**9.6**	**58.4**
C 18:3 n-3	2.5	2.8
∑ n-3 PUFA	**2.5**	**2.8**
Total n-6/n-3 ratio	*3.9*	*20.9*
	% of energy	
SFA	4.1	3.8
MUFA	14.4	4.4
C 18:2 n-6	2.0	**12.3**
C 18:3 n-3	0.5	0.6

Both diets were isocaloric and isolipidic (21% kcal as fat). The enrichment of LA was compensated by lowering oleic acid. Both diets contained a similar amount of n-3 PUFA. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 2. Phenotypic characterisation of adult offspring.

	c/CTL	c/LA	l/CTL	l/LA	*MD* *p-value*	*WD* *p-value*	*Interaction* *p-value*
A. Physiological characteristics							
Body weight gain (%)	926.0 ± 28.9	988.5 ± 68.9	851.2 ± 13.7	937.9 ± 51.6	*p=0.188*	*p=0.120*	*p=0.793*
Adiposity index	18.6 ± 1.5	13.7 ± 0.8	18.1 ± 1.1	15.3 ± 1.7	*p=0.361*	*p=0.026*	*p=0.203*
epWAT (% body weight)	3.1 ± 0.1	2.6 ± 0.1	3.6 ± 0.1	3.1 ± 0.1	*p<0.001*	*p<0.001*	*p=0.751*
rpWAT (% body weight)	5.9 ± 0.6	3.8 ± 0.4	5.7 ± 0.4	4.9 ± 0.4	*p=0.305*	*p=0.005*	*p=0.161*
Liver (% body weight)	2.1 ± 0.1	2.3 ± 0.1	2.2 ± 0.0	2.3 ± 0.1	*p=0.819*	*p=0.045*	*p=0.191*
B. Plasma biochemistry							
Triglycerides (mg/dl)	182.2 ± 16.6	162.1 ± 18.4	245.7 ± 29.3	149.9 ± 3.7	*p=0.203*	*p=0.008*	*p=0.067*
Total-cholesterol (mg/dl)	58.4 ± 2.8	63.1 ± 3.6	75.9 ± 4.7	66.3 ± 4.0	*p=0.015*	*p=0.545*	*p=0.079*
HDL-cholesterol (mg/dl)	26.6 ± 5.5	27.6 ± 5.3	37.4 ± 3.6	41.1 ± 11.4	*p=0.152*	*p=0.775*	*p=0.871*
Glucose (mg/dl)	155.9 ± 13.1	158.9 ± 11.9	193.5 ± 18.7	184.1 ± 13.6	*p=0.043*	*p=0.830*	*p=0.674*
Insulin (pg/ml)	13.6 ± 1.3	14.7 ± 2.7	16.3 ± 2.6	18.6 ± 3.6	*p=0.229*	*p=0.531*	*p=0.817*

Results (n=6) are expressed in mean ± SEM. Different letters indicate significant differences according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). Adiposity index was calculated as the sum of

epididymal (ep), retroperitoneal (RP), perirenal, inguinal and mesenteric fat pad weight divided by body weight x100. “c, l” define maternal control or LA-rich diet; “CTL, LA” define weaning control or LA-rich diet. WAT: white adipose tissue; HDL: high density lipoprotein.

Table 3. Main fatty acid composition (in percent of total fatty acid) of liver triglycerides of adult offspring.

	c/CTL	c/LA	l/CTL	l/LA	*MD p-value*	*WD p-value*	*Interaction p-value*
			% of total FA				
C16:0	27.1 ± 0.8	25.4 ± 0.9	27.2 ± 1.1	25.2 ± 1.2	*p=0.9553*	*p=0.0899*	*p=0.8813*
C18:0	1.1 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	*p=0.0024*	*p=0.0936*	*p=0.6093*
∑ SFA	**28.9** ± 0.9	**27.5** ± 1.0	**29.2** ± 1.3	**27.3** ± 1.4	*p=0.9719*	*p=0.1374*	*p=0.8377*
C16:1 n-7	4.5 ± 0.6	2.7 ± 0.5	5.1 ± 0.3	2.9 ± 0.3	*p=0.8856*	*p<0.0001*	*p=0.2215*
C18:1 n-7	3.4 ± 0.1	3.0 ± 0.1	3.7 ± 0.2	2.3 ± 0.3	*p=0.8297*	*p=0.0007*	*p=0.1196*
C18:1 n-9	55.7 ± 0.7	20.3 ± 0.7	52.6 ± 0.6	18.0 ± 0.5	*p=0.0004*	*p<0.0001*	*p=0.5711*
∑ MUFA	**64.6** ± 1.5	**26.6** ± 1.1	**62.7** ± 1.1	**23.8** ± 1.4	*p=0.0159*	*p<0.0001*	*p=0.6116*
C18:2 n-6	4.7 ± 0.4	38.7 ± 1.7	6.2 ± 1.0	41.5 ± 1.1	*p=0.0650*	*p<0.0001*	*p=0.5613*
C20:4 n-6	0.3 ± 0.0	3.1 ± 0.3	0.3 ± 0.0	4.2 ± 0.4	*p=0.0343*	*p<0.0001*	*p=0.0228*
C22:4 n-6	0.1 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	1.0 ± 0.1	*p=0.0846*	*p<0.0001*	*p=0.0732*
C22:5 n-6	0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	*p=0.2927*	*p<0.0001*	*p=0.3882*
∑ n-6 PUFA	**5.3** ± 0.4	**44.1** ± 1.9	**6.7** ± 1.0	**48.8** ± 1.4	*p=0.0217*	*p<0.0001*	*p=0.1969*
C18:3 n-3	0.3 ± 0.0	0.9 ± 0.1	0.5 ± 0.1	1.0 ± 0.0	*p=0.0209*	*p<0.0001*	*p=0.7750*
C22:5 n-3	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	*p=0.0003*	*p<0.0001*	*p=0.0190*
C22:6 n-3	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	*p=0.0327*	*p=0.0045*	*p=0.0256*
∑ n-3 PUFA	**0.9** ± 0.1	**1.6** ± 0.1	**1.1** ± 0.2	**2.2** ± 0.1	*p=0.0047*	*p<0.0001*	*p=0.1178*
Total ratio n-6/n-3	6.0	27.7	6.4	22.1	*p=0.0064*	*p<0.0001*	*p=0.0027*

Results (n=6) are expressed in mean ± SEM. Different letters indicate significant differences according to two-way ANOVA (maternal diet (MD) x weaning diet (WD)) followed by the Tukey's *post-hoc* test ($p<0.05$). "c, l" define maternal control or LA-rich diet; "CTL, LA" define weaning control or LA-rich diet.