FINAL MASTER THESIS

THE EFFECTS OF A MATERNAL DIET RICH IN FIBER AND POLYPHENOLS ON THE LIPID METABOLISM OF THE MOTHERS AND THEIR OFFSPRING

"MÁSTER INTERUNIVERISTARIO DE NUTRICIÓN Y METABOLISMO"

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1. Abstract

Maternal nutrition during gestation and early life is a key driver of fetal programming, with long-lasting effects on the offspring metabolic health. In the context of the growing obesity epidemic, increasing evidence highlights how maternal dietary habits during gestation and lactation can have long-term effects on lipid metabolism and disease susceptibility in the progeny. Diets rich in fiber and polyphenols have shown beneficial metabolic effects due to their anti-inflammatory, antioxidant, and lipid-regulating properties. Little is known about their influence during the perinatal period, especially in the absence of a high-fat challenge. The impact of a maternal diet enriched in fiber and polyphenols, administered during different stages of the perinatal period, on the lipid metabolism of mothers and their offspring, at weaning and adulthood, has been evaluated. Plasma lipid profiles have been characterized to identify diet-induced alterations. Morphological changes in white and brown adipose tissues have been assessed. Moreover, the expression of key genes involved in lipid metabolism has been quantified in the adipose tissues. Results showed no significant differences in total lipid content between dietary groups. However, specific changes were observed in certain lipid species and minor subclasses such as altered bile acid profiles in dams. In their offspring, an increased pattern of the hydroxylated fatty acids and a decreased ratio of omega-6/omega-3 fatty acid was detected. No histological differences were seen in the white adipose tissue. The brown adipose tissue morphology was only altered in the offspring at weaning. Gene expression analysis revealed some modifications primarily in the mothers, with some changes persisting in the adult offspring, suggesting a long-term effect of the transmission. These findings indicate that maternal intake of fiber and polyphenols can have some influence on the lipid metabolism of the mothers and their offspring, highlighting the potential of early nutritional strategies in shaping metabolic health.

Keywords: Maternal diet, polyphenols, fiber, lipid metabolism, lipidomics, white adipose tissue, brown adipose tissue, gene expression.

2. Table of contents

3. Introduction

3.1 Mother-offspring relationship

Events during intrauterine life can result in developmental adaptations that permanently affect tissues and organs, modulating the risk of metabolic, endocrine, and cardiovascular diseases in adulthood. This process, known as fetal programming [1], is influenced by maternal factors such as nutrition, environment, and health status. Both nutrient deficiencies and unhealthy diets during pregnancy can impair fetal growth or predispose the offspring to overweight and obesity in adult life, among other illnesses. Emerging evidence suggests that some of these effects may be mediated by diet-induced epigenetic alterations, such as DNA methylation, miRNA expression, and histone modification [2].

In addition to the gestation period, post-partum early-life nutrition plays a critical role in shaping long-term health outcomes [3], not only physiologically but also by influencing host-microbiota interactions. The type of infant feeding, breast milk or formula, can drive microbial colonization patterns, leading to distinct metabolic adaptations. Moreover, factors such as antibiotics, mode of delivery, and environmental pollutants impact microbiota development from preconception through the first 1000 days of life. A suboptimal microbial environment during this critical window may impair immune maturation and increase disease susceptibility. Therefore, early modulation of the microbiome offers a promising strategy to promote immune health and prevent alterations later in life [4].

Apart from microbiota-derived mechanisms, other factors including the transmission of maternal cells, cytokines, antibodies, as well as antigens and allergens also play a crucial role in programming the infant immune system towards tolerance development. After birth, the transfer continues through breast milk, whose composition is influenced by multiple factors. Maternal physiology, anthropometric traits, lifestyle, and particularly nutritional status and diet can alter the levels of macronutrients and bioactive compounds [5]. However, there is limited understanding of the factors driving short- and long-term health outcomes, the findings remain inconsistent, and the mechanisms involved are not yet fully elucidated.

3.2 Lipid metabolism

Obesity is a major global health issue in modern society, affecting both children and adults at increasing rates. It is linked to numerous chronic diseases, including type 2 diabetes, cardiovascular disease, and certain cancers; and places a significant burden on healthcare systems. Lifestyle is a key contributor, such as poor diet and reduced physical activity, making obesity a complex and pressing public health challenge [6].

Studying lipid metabolism is particularly relevant since obesity is associated with changes in the composition and function of various lipid species, known as dyslipidemia. This can disrupt cellular signaling, promote chronic inflammation, and contribute to insulin resistance and metabolic syndrome [7].

Maternal obesity and an intake of an obesogenic diet during the perinatal period have been related to adverse effects on the development of the neonates and may predispose the offspring to obesity and metabolic syndrome in adulthood [3]. Although the mechanisms are only partially understood, intervention studies in rodents have shown that the mammary gland function and structure is affected, leading to alterations in milk composition. Milk lipids, primarily triacylglycerols (TG), are the main energy source for the offspring and play a critical role in early tissue programming. Milk TGs are composed of fatty acids (FA) that originate from *de novo* synthesis in the mammary gland, dietary intake, and maternal fat stores. Therefore, both the quantity and type of fat consumed during lactation influence milk FA composition [8].

3.3 Mediterranean diet

The Mediterranean Diet, rich in dietary fiber (DF) and polyphenols, has been studied for its health benefits. Several clinical trials have shown that it lowers the risk of developing cardiovascular diseases, metabolic syndrome, and cancer [9].

3.3.1 Dietary fiber

According to the definition proposed in 2009 by the Codex Alimentarius, DF are carbohydrate polymers of plant origin with three or more monomeric units that resist digestion and are not absorbed in the small intestine [10]. It can be classified depending on different characteristics (Table 1).

Table 1. Dietary fiber classifications [10].

Characteristic	Classifications	Examples
Length	Polysaccharides	cellulose, inulin, pectin
	Oligosaccharides	fructoOS, galactoOS
Solubility	Soluble	inulin, FOS, GOS, pectin
	Insoluble	cellulose, hemicellulose, lignin
Viscosity	Viscous	β-glucans, pectins, mucilages
	Non-viscous	inulin, FOS, GOS
Fermentability	Fermentable	inulin, FOS, GOS, pectin
	Non-fermentable	cellulose, lignin

The biological effects of DF depend on its physicochemical properties. Viscous fibers, for example, delay nutrient digestion and absorption, contributing to glycemic regulation. Soluble fibers are not digested and reach the distal gut, where they can be fermented by the microbiota, generating new metabolites. Many DF act as prebiotics by modulating the composition and metabolic activity of the gut microbiota, particularly evident in soluble, fermentable fibers which promote the production of short-chain fatty acids and alter the local pH. Additionally, beyond microbiota-mediated effects, certain DF may exert microbiota-independent benefits, such as direct anti-infective actions [11].

Regarding health benefits, insoluble fibers improve insulin sensitivity and reduce the risk of developing type 2 diabetes, while soluble fibers support glycemic control, lipid profile, and weight regulation. Cardiovascular health is improved by lowering chronic inflammation and associated mortality risk. Moreover, DF contributes to gut health by promoting colonic integrity and motility, potentially lowering colorectal cancer risk [12].

3.3.2 Polyphenols

Polyphenols are bioactive compounds from the secondary metabolism of plants, derived from phenylalanine and characterized by the presence of aromatic rings with reactive hydroxyl groups. They play important protective roles in plants, such as defense against pathogens, protection from UV radiation, and mitigation of oxidative stress. Structurally, polyphenols are classified into four major groups: flavonoids, phenolic acids, stilbenes, and lignans [13]. Among them, flavonoids represent the largest class, and include flavonols, flavones, isoflavones, flavanones, flavanols and anthocyanidins (Table 2) [14].

Table 2. Flavonoid subclasses [15].

Flavonoid subclass	Examples	Found
Flavonols	quercetin, kaempferol	onions, apples, berries
Flavones	glycosides of luteolin, apigenin	fruit skin, parsley, celery
Isoflavones	genistein, daidzein	leguminous plants, soy
Flavanones	naringenin, hesperidin	citrus fruits
Flavanols	epicatechin, catechin	cocoa, tea
Anthocyanidins	cyanidin, malvidin	red wine, berry fruits

Most polyphenols are poorly absorbed in the small intestine and reach the colon, where they are metabolized by the gut microbiota. In these cases, they may act as prebiotics, and some promote the production of beneficial short-chain fatty acids [16].

Flavonoids have been associated with a variety of beneficial health effects in humans, not only through their antioxidant properties but also via non-antioxidant mechanisms. They have been linked to protective effects against cancer [17], cardiovascular [18] and neurodegenerative diseases [19], among other pathologic conditions. Anti-inflammatory and immunomodulating mechanisms [20] have also been described. Higher dietary polyphenol intake has been associated with a reduced waist-to-hip ratio [21] and lower risk of chronic diseases.

Several studies have reported that maternal polyphenol intake during pregnancy may exert beneficial effects, such as reducing the risk of complications like gestational diabetes and preeclampsia. However, adverse effects have also been observed, particularly during early gestational stages [22]. On the other hand, the placenta acts as a barrier for xenobiotics, thereby limiting fetal exposure; but it has been reported in flavonoid-supplemented pregnant rats the presence of high concentrations of flavanols and their conjugated forms in the placenta [23]. Moreover, some polyphenols can interfere with DNA methylation enzymes, inducing epigenetic modifications that may influence cellular phenotype through dynamic regulation of gene expression [24]. Therefore, further research is required to better understand the safety and impact of polyphenol consumption during pregnancy.

4. Hypothesis

This work is based on the hypothesis that a maternal diet enriched in fiber and polyphenols will induce significant alterations in the lipid metabolism of mothers and that these effects will also be reflected in their offspring, both in early life but also in adulthood. Moreover, it is expected that the most pronounced effects will occur when the dietary intervention is applied throughout the entire perinatal period (pre-gestation, gestation, and lactation), followed in decreasing order by the interventions during lactation, gestation, or pre-gestation only.

5. Objectives

This work has been performed under the project “Impact of a maternal diet enriched in fiber and polyphenols on the immunedevelopment of the neonate and the long-term immuneprogamming effect (FyP-SI)”. The project’s main objective is to establish the impact of a fiber/polyphenol-enriched maternal diet inducing the best immunological/microbial maternal status and breast milk quality which in turn will promote both a better neonatal immune development and immune response later in life.

In this context, the main objective of this master thesis is to evaluate the impact of a maternal diet high in dietary fiber and polyphenols on the lipid metabolism of the mothers and their offspring at weaning and in adulthood.

In the above subjects, the specific objectives will be to:

1. Characterize plasma lipid profiles and identify diet-induced alterations in lipid species.
2. Evaluate the morphological changes in the white and brown adipose tissues.
3. Quantify the expression of key genes involved in lipid metabolism.

6. Materials and methods

6.1 Animals

Six-week-old female Wistar rats (n=40), were purchased from Janvier Labs (Saint-Berthevin, France) and housed at the animal facility located on the Diagonal Campus of the Faculty of Pharmacy and Food Sciences at the University of Barcelona. After a one-week acclimation period, the females entered a 3-week pre-gestation period.

Then, the females were paired with males (in a 2:1 ratio) for four days. After this mating period, the females were separated and housed individually.

Throughout the study, animals were maintained under standard laboratory conditions, including a controlled temperature of 21 ± 2 °C, relative humidity of 50–55%, and a 12-hour light/dark cycle. Food and water were provided *ad libitum*. All experimental procedures were reviewed and approved by the Ethics Committee for Animal Experimentation at the University of Barcelona (CEEA/UB ref. 240/19) and the "Generalitat de Catalunya" (DAAM 10933).

The number of animals required was calculated using the Appraising Project Office software developed by the "Universidad Miguel Hernández de Elche" (Alicante, Spain). The estimation was based on expected changes in intestinal immunoglobulin A levels and Toll-like receptor 4 gene expression, assuming a two-sided type I error rate of 0.05 and no dropout. The final sample size was also adjusted following the University ethical guidelines and the principles of the 3Rs in animal research [25].

6.2 Diets and experimental design

Two diets were administered during the study: a reference diet (REF diet) based on the AIN-93G formulation (Envigo, Indianapolis, IN, USA) and an experimental diet (HFP diet). The HFP diet was developed to reflect the DF and polyphenol intake characteristic of a healthy dietary pattern observed in pregnant and lactating women from a Spanish Mediterranean cohort [26].

This experimental diet used the AIN-93G as its base (Table 3A) but was enriched with 8% inulin (Fibruline™, derived from chicory roots, Cosucra, Warcoing, Belgium) and 1% pectin (PE21006, extracted from citrus fruits, Gojira Fine Chemicals, LLC, Mundelein, IL, USA) as sources of dietary fiber. Additionally, it included a 0.5% polyphenol blend, primarily composed of flavonoids (Table 3B). The flavonoid compounds, catechin, epicatechin, hesperidin, naringenin, and quercetin, were all obtained from Millipore Sigma (Madison, WI, USA). This diet was supplied by Envigo, vacuum-sealed to prevent oxidation and contamination, and stored at 4 °C until administration.

Table 3. Composition of experimental diets.

Table 3A. Common components

Components	REF Diet (g/kg)	HFP Diet (g/kg)
Casein	200	200
L-Cysteine	3	3
Maltodextrin	132	132
Sucrose	100	100
Soybean Oil	70	70
Cellulose	50	50
Mineral Mix (TD94049)	48	48
Ferric Citrate	0.3	0.3
Vitamin Mix (TD94047)	15	15
Choline Bitartrate	2.5	2.5
Butylhydroquinone	0.014	0.014

Table 3B. Nutritional differences

Components	REF Diet (g/kg)	HFP Diet (g/kg)
Cornstarch Flour	379.186	289.186
Inulin	**0**	**80**
Pectin	**0**	**10**
Polyphenols	**0**	**5**
Catechin	0	1
Epicatechin	0	1
Hesperidin	0	1.5
Naringenin	0	0.75
Quercetin	0	0.75

The females were randomly divided into five groups, each consisting of six pregnant rats (n = 6/group). Groups were based on the feeding period of the HFP diet (see Figure 1). The control group (REF group) received the REF diet for the entire dietary intervention (9 weeks). The four experimental groups were fed the HFP diet during different life stages. The pre-gestation group (P group) the three weeks prior to mating. The gestation group (G group) the three weeks of the gestation period. The suckling group (S group) the three weeks of the lactation period. Finally, the PGS group received it throughout all three stages, pre-gestation, gestation and lactation (9 weeks). After weaning, the offspring from both the REF and PGS groups were maintained on the REF diet for seven weeks, until they reached adulthood.

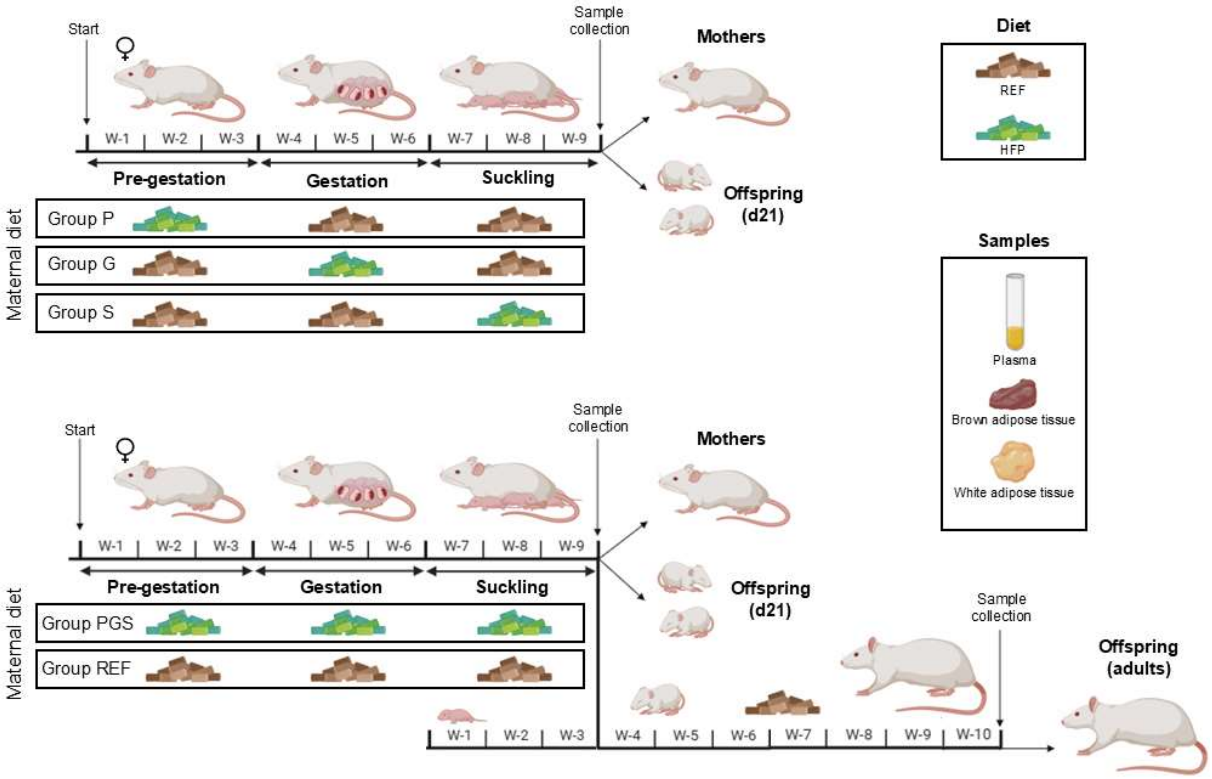

***Figure 1. Study design and dietary intervention timeline**. Created with BioRender.com [27].*
REF: reference diet; HFP: diet high in fiber and polyphenol; Groups receiving the maternal HFP during pre-gestation (P), gestation (G), suckling (S) and all periods (PGS); Group receiving the maternal REF diet during all periods (REF); d21: day 21

6.3 Sample collection and processing

Samples were obtained from the dams (n=6/group) and their offspring (n=12/group, 2/dam) at postnatal day 21 (d21) at the end of the lactation period, and from the adult offspring (n=12/group, 2/dam) at the end of the study.

The rats were anesthetized via intramuscular injection with ketamine (90 mg/kg; Merial Laboratorios, S.A., Barcelona, Spain) and xylazine (10 mg/kg; Bayer A.G., Leverkusen, Germany), followed by exsanguination through cardiac puncture. The collected blood was subjected to centrifugation at 10000 *g* for 5 min at room temperature and the resultant plasma was stored at -80 °C for the lipidomics assay. Gonadal white adipose tissue (WAT) and subscapular brown adipose tissue (BAT) samples were collected from all animals from the REF and PGS groups.

6.4 Lipid profiling

The targeted lipidomics assay was carried out by Eurecat (Centre Tecnològic de Catalunya) with the package “MetLC Lipidomics-I: analysis of lipids, including lysophospholipids, free fatty acids, bile acids, hormones and oxylipins using LC-MS” [28].

Briefly, plasma samples were thawed at 4 °C, and each sample was mixed with 100% methanol containing a predefined mixture of labeled internal standards. The mixture was vortexed and centrifuged at 21000 *g* for 10 min at 4 °C. The resulting supernatants were vacuum-filtered through Oasis HLB 96-well plates. An additional methanol was used to elute the analytes. An aliquot of the eluate was collected for analysis using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-qTOF). The remaining volume was evaporated to dryness using a SpeedVac concentrator, and the resulting residue was reconstituted in methanol for further analysis by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QQQ-MS).

The identification of lipid species was achieved by matching their accurate mass and tandem mass spectrum. Additionally, the chromatographic behavior of pure standards for each family, together with bibliographic information, was used to support their putative identification. Results were obtained as nM of lipid species.

6.5 Histology

Tissue samples of WAT and BAT were fixed in 4% buffered formaldehyde for 24 h at room temperature. Then, samples were rinsed in phosphate-buffered saline for 3 h, dehydrated in graded ethanol (70%, 90%, and 100%), and permeated with xylene (Panreac Química SLU, Barcelona, Spain). Finally, samples were embedded in melted paraffin (Merck, Madrid, Spain). Paraffin blocks were sectioned at 5 µm thickness using a microtome (RM2135, Leica, Wetzlar, Germany) and stained with hematoxylin and eosin as previously described [29].

Sample observation was performed using a light microscope (Olympus BX41, Olympus Corporation, Shinjuku Tokyo, Japan) equipped with a digital camera (Olympus XC50, Olympus Barcelona, Spain). Representative images were captured for each tissue type: WAT (200X) and BAT (400X). Image analysis was conducted using ImageJ software (Image Processing and Analysis in Java, National Institute of Mental Health, Bethesda, MD, USA). In WAT, the number of adipocytes per section and the adipocyte area and perimeter were quantified. In BAT, the number of nuclei per section and the area and perimeter of the lipid droplets larger than 50 µm² were determined.

6.6 Gene expression analysis

Tissue samples from WAT and BAT were preserved in RNAlater® (ThermoFisher Scientific) at 4 ºC overnight and subsequently stored at -20 ºC until the gene expression analysis.

RNA extraction was performed as previously described [29]. Approximately 100 mg of tissue was transferred into lysing matrix tubes (MP Biomedicals, Illkirch, France) containing an appropriate lysis buffer and homogenized using a FastPrep® instrument (MP Biomedicals) for 60 s. The lysates were centrifuged at 12000 *g* for 15 min to eliminate residual tissue debris. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Madrid, Spain) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoPhotometer (BioNova Scientific S.L., Fremont, CA, USA).

Complementary DNA was synthesized using TaqMan® Reverse Transcription Reagents (Applied Biosystems (AB), Weiterstadt, Germany). Quantitative real-time PCR was performed with the ABI Prism 7900 HT system (AB) to analyze gene expression using the specific TaqMan probes and PCR primers (AB) shown in Table 4. Gene expression levels were normalized to the expression of the housekeeping gene *Gusb* and calculated using the $2^{-\Delta\Delta Ct}$ method. Expression levels for each experimental group are presented as a percentage relative to the mean value of the REF group, which was set to 100%.

Table 4. Description of the specific TaqMan primers (Applied Biosystems)

Gene	Encoded protein	Related to	Reference
Cidea	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	Lipid droplet formation	Rn04181355_m1, I
Ucp-1	Uncoupling protein 1	Thermogenesis	Rn00562126_m1, I
Prmd16	PR domain containing 16	Adipogenesis	Rn01516224_m1, I
Pparγ	Peroxisome proliferator activated receptor γ	Adipogenesis	Rn00440945_m1, I
IL-1β	Interleukin-1β	Inflammation	Rn00580432_m1, I
Gusb	β-glucuronidase	“Housekeeping”	Rn00566655_m1, I

6.7 Statistical analysis

SPSS Statistics 30.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. To assess normal distribution and homogeneity of variance of the

data, Shapiro-Wilk and Levene test were used. Normal and homogeneous results were analyzed by independent samples T-test. U-Mann Whitney was performed when results did not follow a normal and equal distribution (REF vs PGS). Finally, Kruskal-Wallis test was performed when results did not follow a normal and equal distribution to assess significant differences among groups (P, G, S, PGS vs REF) followed by multiple comparisons between groups. Differences were considered statistically significant when p value < 0.05.

The principal component analysis (PCA) was performed in Rstudio using the R package *stats* and the function *prcomp* [30] and was used to search clusters of similarities between samples in terms of lipidomic composition. Heatmaps were performed in Rstudio using the R package *pheatmap* [31].

7. Results

7.1 Lipid profiling

First, and independently of the nutritional interventions, the PCA of plasma lipidomic profiles revealed a clear separation between generations, indicating that the overall lipid composition differed among dams, offspring at d21, and adult offspring (Figure 2A). However, within each generation, no distinct clustering according to maternal dietary consumption was observed (Figure 2C-E). Moreover, total lipid levels did not show significant differences across dietary groups (Figure 2F-H).

During data exploration, one individual from the offspring at d21, encoded as T2.5 from the PGS group (Figure 2A), was identified as an outlier. This individual contributed 13% to the variance in dimensions 1-2, while the next individual with the highest contribution was only 5%. All the other samples showed similar and lower contributions. The individual was removed from further analyses to avoid biasing the results.

Sex was considered as a possible confounding factor [32]. All mothers were females, and all the adult offspring were males. However, the offspring at d21 included both sexes. The PCA of the offspring at d21 (Figure 2B) male and female individuals did not show distinct clustering, indicating no sex-dependent separation in lipid profiles at this stage. Therefore, the offspring at d21 were analyzed together, grouped only by diet.

The lipid species analyzed in plasma samples were present at varying concentrations, being free FA the most abundant, followed by lysophosphatidylcholines (LPC),

lysophosphatidylethanolamines (LPE), bile acids (BA), hydroxy fatty acids (FA-OH), oxylipins (Oxy), and hormones (Figure 3 and Figure 4).

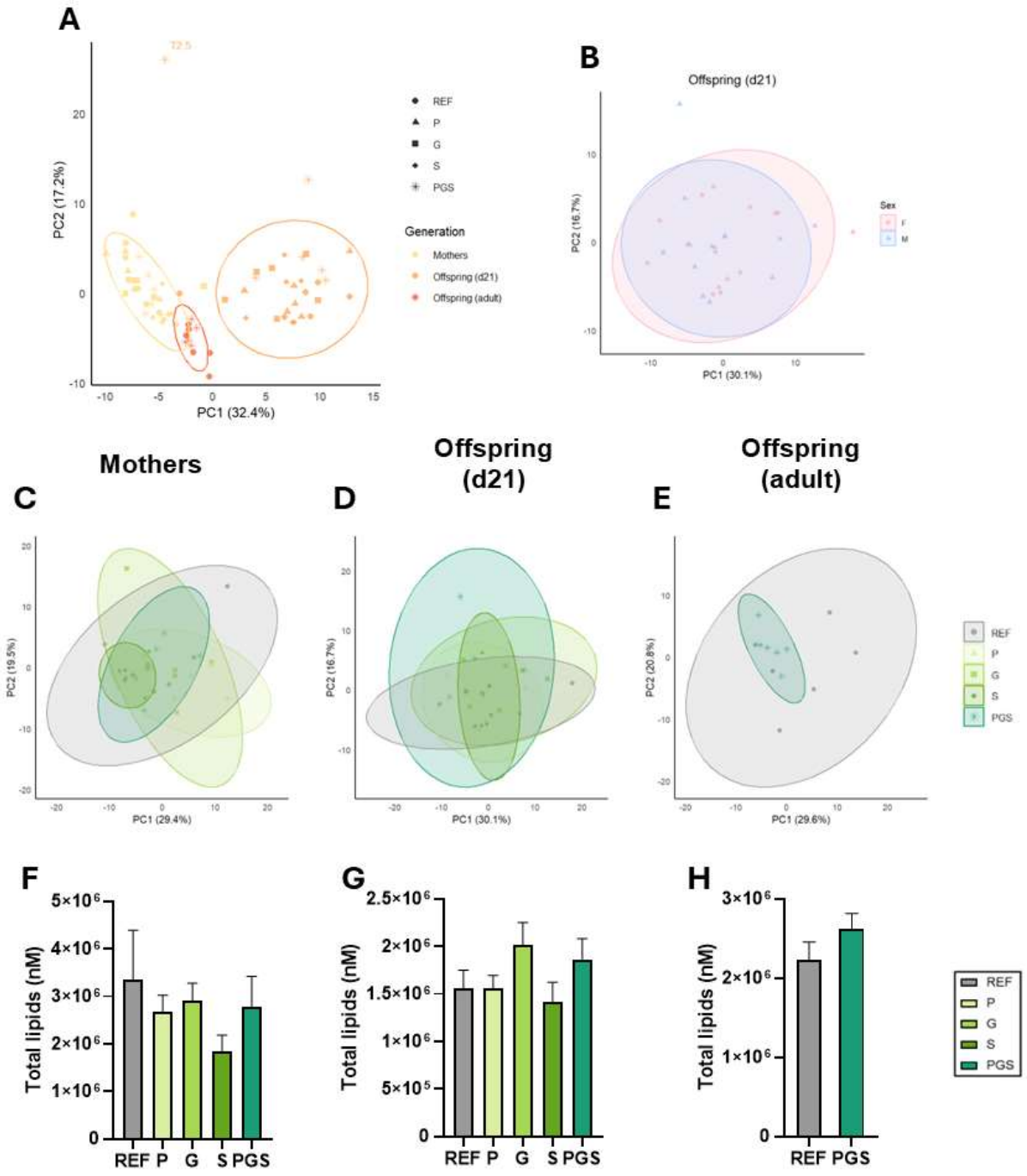

Figure 2. Lipidomic analysis of plasma samples from fiber and polyphenol enriched diet during pre-gestation (P), gestation (G), suckling (S) and all periods (PGS). (A) *PCA of all individuals colored by generation and shaped by diet.* ***(B)*** *PCA of offspring (d21) colored and shaped by sex.* ***(C-E)*** *PCA of mothers (C), offspring (d21) (D) and offspring (adults) (E) colored and shaped by diet.* ***(F-G)*** *Total concentration of plasma lipids (nM) across diets from mothers (F), offspring (d21) (G) and offspring (adults) (H). Results are expressed as mean ± S.E.M (n = 5-6). No statistical significances were found between diets. REF: reference group; PCA: Principal components analysis; d21: day 21.*

The lipid species present at the highest concentrations (FA, LPC, LPE) are shown in Figure 3.

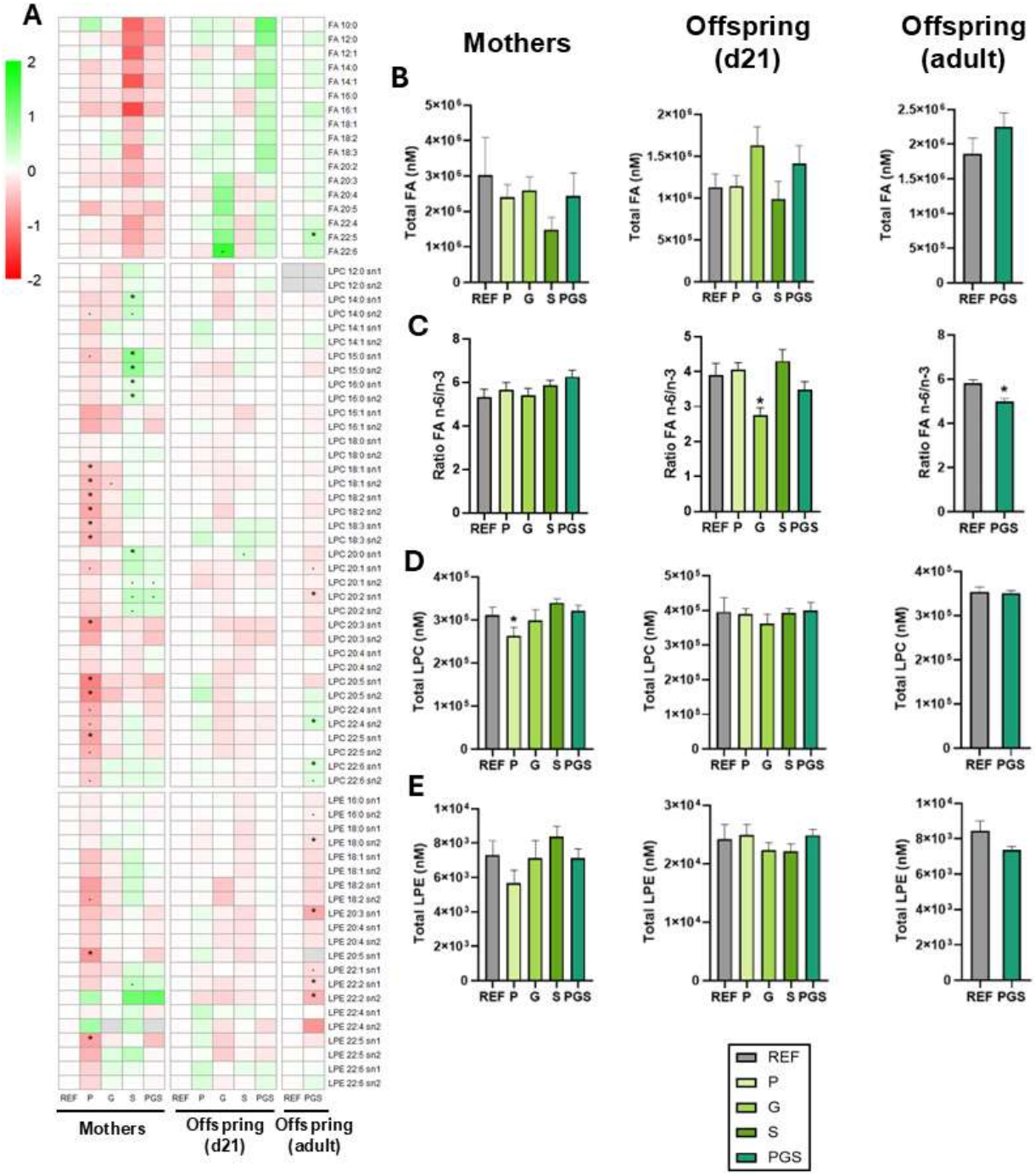

Figure 3. Analysis of lysophosphatidylcholines (LPC), lysophosphatidilethanolamines (LPE) and free fatty acids (FA) from plasma samples in response to a diet high in fiber and polyphenols during pre-gestation (P), gestation (G), suckling (S) and all periods (PGS). *(A) Heatmap of the lipid species from mothers, offspring (d21) and offspring (adults). Results are expressed as log2(FC), increased levels are indicated in green, decreased levels in red and gray indicates no presence. Statistical differences: * p< 0.05, · p<0.1, vs REF.* ***(B-E)*** *Analysis of total concentration of lipidic species: FA (nM) (B), ratio FA n-6/n-3 (C), LPC (nM) (D) and LPE (nM) (E), of mothers, offspring (d21) and offspring (adult). Results are expressed as mean ± S.E.M (n = 5-6). Statistical differences: * p<0.05, vs REF. REF: reference diet; FA n-6/n-3: fatty acids omega6/omega3; d21: day 2; FC: fold change.*

Regarding the FA pattern shown in the heatmap (Figure 3A), an apparent reduction was observed in the S and PGS groups in dams, and an apparent increase in the PGS group in the offspring, both at d21 and adult age. However, none of these differences

were statistically significant. Likewise, there were no differences between total FA levels in any generation due to the diet (Figure 3B). As for unsaturated FA, the omega-6 to omega-3 ratio was not altered in mothers. However, it significantly decreased in the G group of the offspring at d21 and in the PGS group of the adult offspring (Figure 3C). These differences were driven by an increase in omega-3 fatty acids.

Regarding LPCs, the dams showed no consistent pattern between groups (Figure 3A). While the P group generally showed reduced levels, the S group showed increased levels. In the offspring at d21, no significant differences were detected between dietary groups, and similarly, no differences were observed in the adult offspring. Regarding total LPC levels (Figure 3D), only the P group in dams showed a significant reduction. The offspring did not show differences either at d21 or adulthood.

LPEs followed a similar pattern (Figure 3A) to LPC in mothers and the offspring at d21, although with lower statistical significance in the latter. In contrast, the adult offspring from the PGS group showed a consistent decrease in nearly all LPE species, with some statistical differences. However, there were no differences across groups in total LPE levels in any generation (Figure 3E).

Among the less abundant lipid families analyzed (BA, FA-OH, Oxy, Hormones, Figure 4) the most pronounced effect of the HPF diet was observed in the BAs. A generalized decreasing pattern (Figure 4A) in relative BA levels was found in mothers and the offspring at d21, particularly for DCA, TDCA, and LCA. However, an increase in TCA and TCDCA was detected in PGS group of the offspring at d21. The effects did not persist in adulthood. Total BA levels (Figure 4B) showed a significant decrease in the P and G groups of mothers, but not in the offspring either at d21 or adulthood.

As for FA-OH (Figure 4A), only a decreasing pattern in the S group was observed in dams, and the specific lipid FA 12:3-3OH showed a significant decrease in most dietary groups. In the offspring, FA-OH relative levels were consistently increased in the PGS groups, both at d21 and in adulthood. Total FA-OH concentrations (Figure 4C) showed no differences in the dams but were significantly higher in the PGS group of the offspring at d21. An increasing trend ($p = 0.055$) in the PGS group of adult offspring was also observed.

No clear pattern was identified for oxylipins (Figure 4A). However, Oxy 9,10,13-triHOME, showed a reduction across all maternal groups, reaching statistical

significance in the P and G groups. No significant differences were observed in total oxylipin levels (Figure 4D) in any group across any generation.

Finally, hormone profiles showed no consistent pattern in any group and generation (Figure 4A). Moreover, in adult offspring, only epiandrosterone sulfate and T4 were detected. No significant differences in total hormone concentrations (Figure 4E) were found in any group across any generation.

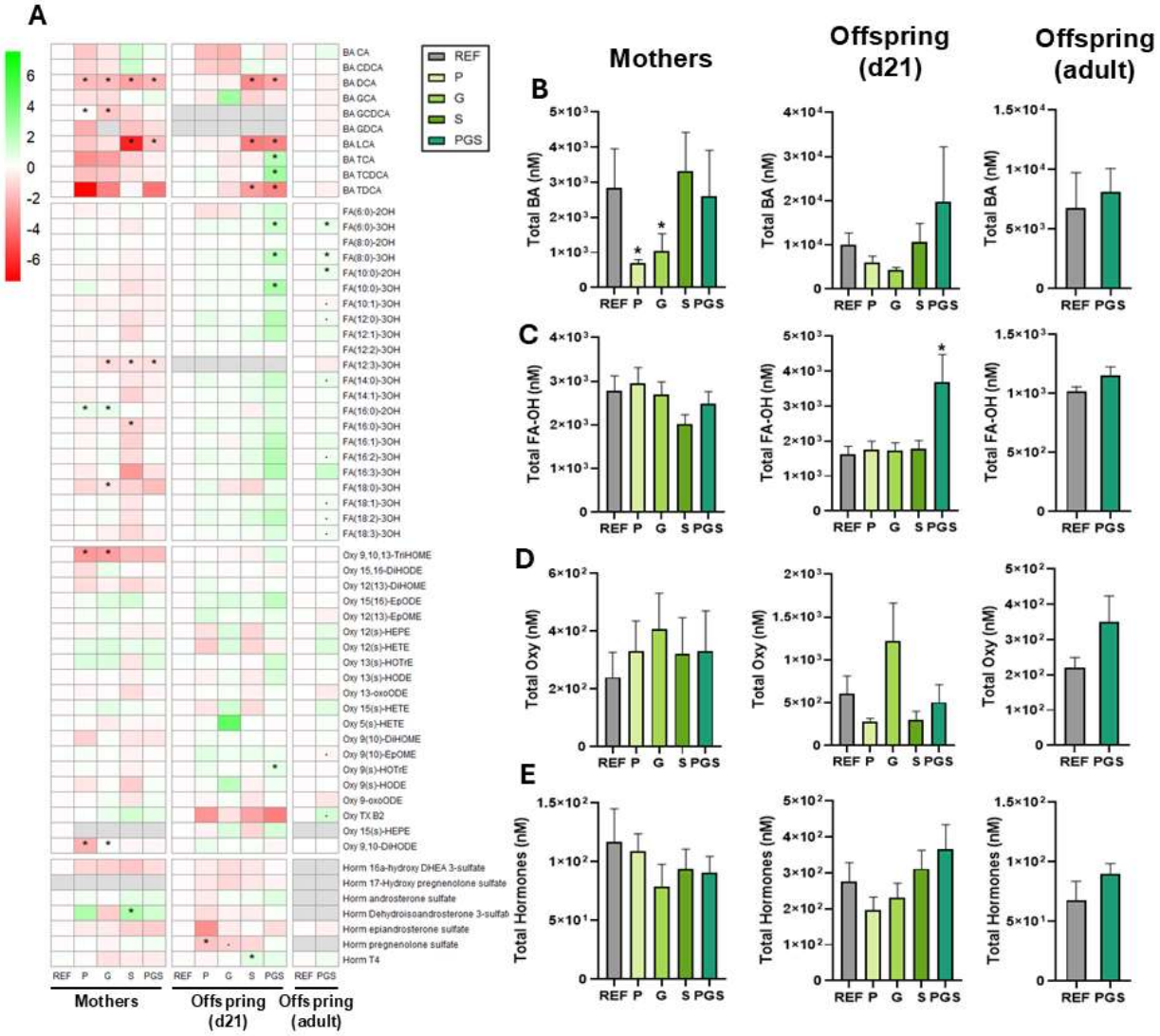

Figure 4. Analysis of bile acids (BA), hydroxylated fatty acids (FA-OH), oxylipins (Oxy) and hormones (Horm) from plasma samples in response to a diet high in fiber and polyphenols during pre-gestation (P), gestation (G), suckling (S) and all periods (PGS). ***(A)*** *Heatmap of the lipid species from mothers, offspring (d21) and offspring adults. Results are expressed as log2(FC), increased levels are indicated in green, decreased levels in red and gray indicates no presence. Statistical differences: * p< 0.05, · p <0.1, vs REF.* ***(B-E)*** *Analysis of total concentration of lipidic species: BA (nM) (B), FA-OH (nM) (C), Oxy (nM) (D) and Hormones (nM) (E) of mothers, offspring (d21) and offspring (adult). Results are expressed as mean ± S.E.M (n = 5-6). Statistical differences: * p< 0.05, vs REF. REF: reference diet; d21: day 21; DF: dietary fiber; FC: fold change*

7.2 Histology

Sex has been seen to have an impact on adipose tissue morphology [33]. In our study, all mothers were females. The offspring, both at d21 and adulthood, was formed by males and females, however, no significant changes were seen. Overall, the results were separated only by the maternal diet administered.

The HFP diet administered to the mothers throughout the entire perinatal period (PGS) did not alter the morphology of the WAT. Representative images of the histologic sections of WAT from mothers, the offspring at d21 and the adult offspring are shown in Figure 5A-C. No significant differences were found in the number of adipocytes, the adipocyte area, or the adipocyte perimeter across any generation (Figure 5D-F). Regarding BAT, representative images of the histological sections are shown in Figure **5**G-I. The HFP diet did not lead to changes in the BAT morphology of the adults, neither the mothers nor the adult offspring (Figure 5J,L). However, it significantly affected the BAT of the offspring at d21. An increased number of adipocytes ($p = 0.009$) was observed, accompanied by a reduction of the lipid droplet area ($p = 0.004$) and perimeter ($p = 0.002$) (Figure 5K).

7.1 Gene expression analysis

The maternal HFP diet during pre-gestation, gestation and suckling (PGS) altered the gene expression of WAT. In dams (Figure 6A), *PPARγ*, a marker for adipogenesis, showed a tendency to increase. No significant changes were detected in the offspring at d21 (Figure 6B). In contrast, the adult offspring (Figure 6C) showed a decrease in *Il-1β*, a marker of inflammation. In adults, *UCP-1* expression remained undetectable, both in mothers and adult offspring.

Regarding BAT, several changes were observed in the maternal group (Figure 6D). An increase in *UCP-1* expression, involved in thermogenesis, along with a reduction in *PPARγ*, and a trend towards increased *PRDM16,* involved in adipogenesis, and *Il-1β* expression. However, no differences were detected in the BAT of the offspring at d21 (Figure 6E), while in the adult offspring (Figure 6F), a reduction trend in *PPARγ* was observed.

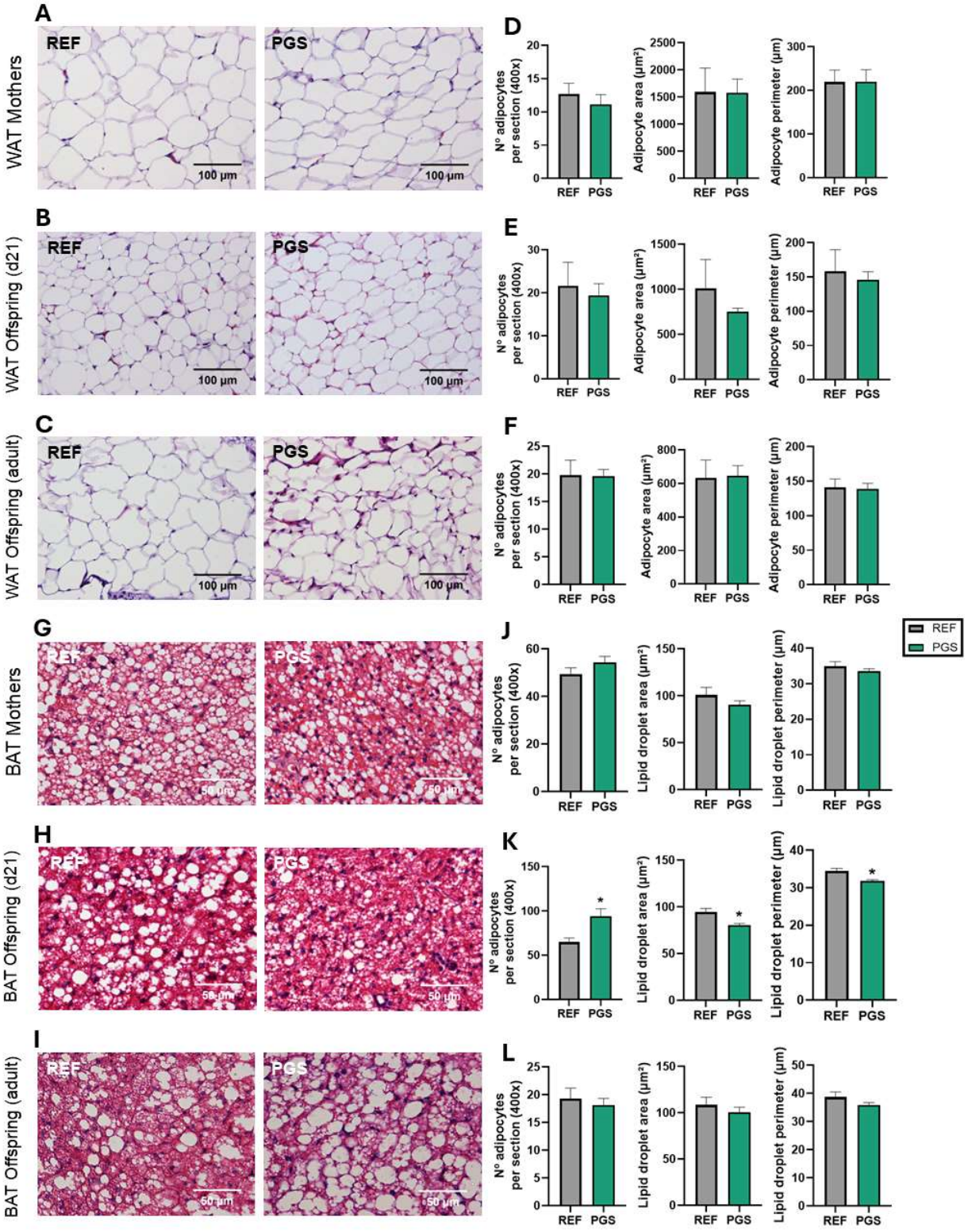

Figure 5. Impact of the PGS diet on the adipose tissues. (A-C) *Representative histological sections of WAT stained with hematoxylin and eosin at 200X of the REF and PGS groups, of mothers (A), offspring (d21) (B) and offspring (adult) (C).* ***(D-F)*** *Analysis of WAT: number of adipocytes per section (400X), adipocyte area and adipocyte perimeter, of mothers (D), offspring (d21) (E) and offspring (adult) (F).* ***(G-I)*** *Representative histological sections of BAT stained with hematoxylin and eosin at 400X of the REF and PGS groups, of mothers (G), offspring (d21) (H) and offspring (adult) (I).* ***(J-K)*** *Analysis of BAT: number of adipocytes per section (400X), lipid droplet area (area ≥ 50µm²)) and lipid droplet perimeter, of mothers (J), offspring (d21) (K) and offspring (adult) (L). Results are expressed as mean ± standard error of means (S.E.M) (n = 3-13). Statistical differences: * p< 0.05 REF vs PGS. WAT: White adipose tissue; BAT: Brown adipose tissue; REF: reference diet; PGS: diet high in fiber and polyphenol during pregestation, gestation and suckling; d21: day 21.*

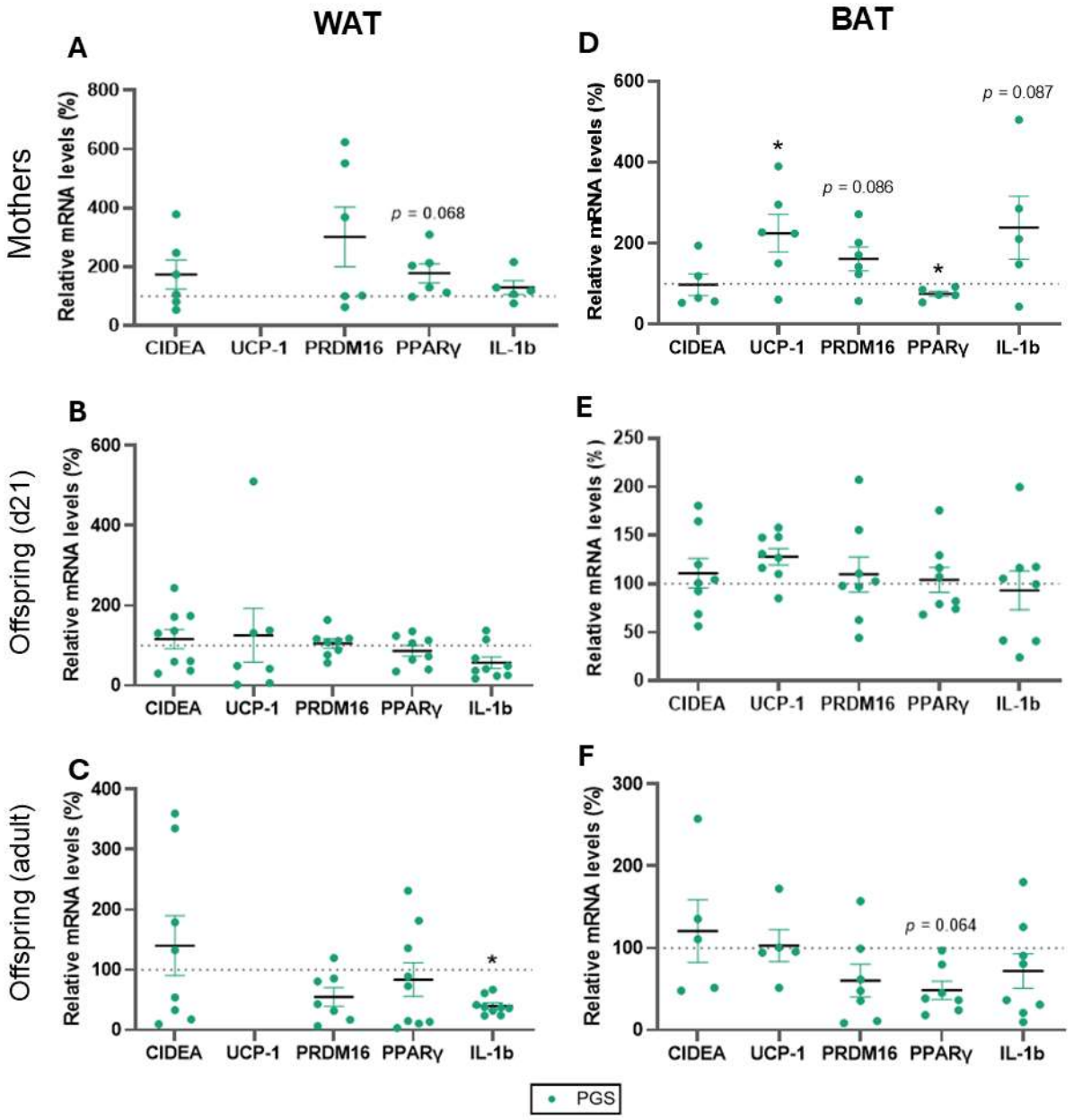

Figure 6. Impact of the PGS diet on the gene expression of the adipose tissues. (A-F) *Relative mRNA expression of the genes CIDEA, UCP-1, PRDM16, PPARγ, IL-1b, by real-time PCR, calculated relative to REF, with correspond to 100% of transcription (dashed line), in WAT (A-C) and BAT (D-F), of mothers (A, D), offspring (d21) (B, E) and offspring (adult) (C, F). Results are expressed as mean ± S.E.M. (n = 5-9). Statistical differences: * p< 0.05 REF vs PGS. WAT: White adipose tissue; BAT: Brown adipose tissue; REF: reference diet; PGS: diet high in fiber and polyphenol during pre-gestation, gestation and suckling; d21: day 21.*

8. Discussion

In our study, we evaluated the impact of a maternal diet enriched in DF and polyphenols on the lipid metabolism of mothers, and their offspring, both at weaning and adulthood. The diet was administered during different stages of the perinatal period: pre-gestation (group P), gestation (group G), lactation (group S), or covering

the full period (group PGS). However, most existing studies evaluate whether a similar diet improves lipid profiles under pathological conditions, such as obesity or atherosclerosis [7,8,34–38]. Furthermore, a study showed that supplementation with resveratrol, a polyphenol widely found in fruits, reduced the adipocyte area and plasma cholesterol in rats fed a high-fat diet, whereas animals on a low-fat diet, the same supplementation paradoxically led to an increase in adipocyte size and cholesterol plasma content [33]. So, there is limited literature comparing a standard diet with one enriched both with fiber and polyphenols in the absence of metabolic disturbances, making direct comparisons with our study challenging.

Most lipidomic studies tend to focus on the major lipid classes, such as TG, free FA, and cholesterol, while minor lipid species often remain unexplored [34,38–40] As a result, there is limited information available regarding the biological relevance and metabolic regulation of these less abundant lipid molecules, which may still play significant roles in physiological and pathological processes.

FA concentrations are typically elevated in metabolic disorders such as obesity and type 2 diabetes [41]. Dietary FA composition plays a critical role in modulating these levels. FAs omega-3 have been associated with metabolic benefits and FAs omega-6, common in the Western diet, have raised concerns. Evidence suggests that an elevated omega-6/omega-3 ratio is detrimental [42]. In our study, we did not see significant changes regarding individual and total FA profiles. However, the ratio of FA omega-6/omega-3 was significantly decreased in the G group of the offspring at weaning and in the PGS group in the adult offspring. Previous animal studies have demonstrated that healthy dietary interventions are capable of reversing lipidomic alterations induced by a high-fat diet in both mothers and their offspring [38].

LPC is the most abundant lysoglycerophospholipid found in the blood. LPCs have been proposed to function as a carrier of polyunsaturated fatty acids and choline to peripheral tissues. Although the physiological roles are not fully elucidated, LPCs have been reported to exert diverse biological effects, including inflammatory, anti-hemostatic, and cytotoxic activities. However, some LPCs can have either a pro-inflammatory or anti-inflammatory effect [43]. On the other hand, the findings regarding LPC levels in obesity are inconsistent. Some studies showed correlation of obesity with reduced LPC concentrations [44], while others report elevated levels [45]. This inconsistency is also reflected in our data. In mothers, the P group exhibited a reducing

LPC pattern, whereas the group S showed an increasing pattern. Further investigation is needed to elucidate these patterns. In the adult offspring, the LPC pattern seemed to be reduced, however, the LPC 22:4 and LPC 22:6 were significantly increased. Interestingly, polyunsaturated LPC species, such as LPC22:4 and LPC22:6, have been reported to exhibit anti-inflammatory properties [43].

LPE is the second most abundant lysoglycerophospholipid. Several studies have highlighted the involvement of LPE in various cellular functions, particularly in promoting cell differentiation and migration [46]. In our results, the only significant change was a reducing pattern in the adult offspring, however, the biological relevance of this reduction remains to be elucidated.

BAs have been reported to be elevated in individuals with obesity [47]. Additionally, diets rich in flavonoids have been shown to enhance biliary cholesterol excretion [48]. In our study, BA predominantly displayed a decreasing trend, potentially by modulating their synthesis, transformation, or excretion pathways.

Limited information is available regarding the biological functions of FA-OH [49]. However, specific molecules such as 2-hydroxyoleic acid have been shown to suppress the growth and induce autophagy in certain cancer cells [50]. Additionally, FA-OHs serve as precursors to branched fatty acid esters of hydroxy fatty acids (FAHFAs). FAHFAs have been associated with anti-inflammatory and antioxidant properties and have shown potential in improving insulin sensitivity and in the treatment of type 2 diabetes [51]. In our study, we observed an increasing trend in FA-OH levels in the PGS group among the offspring, both at weaning and in adulthood. Notably, the total content of FA-OH was significantly elevated in the offspring at weaning. These findings suggest that maternal intake of a diet rich in polyphenols and fiber can influence the levels of these bioactive lipids in offspring, with effects that may persist in adulthood.

Pregnancy is characterized by dynamic alterations in maternal adiposity, with an initial increase in adipose tissue mass during early gestation (lipogenic activities) followed by a reduction in fat mass in the later stages (lipolytic activities) [52]. Moreover, during lactation, maternal WAT stores are depleted, due to the requirements of milk synthesis [53]. On the other hand, a maternal high-fat diet has been linked to the reprogramming of WAT and BAT, characterized by elevated adipogenic and lipogenic markers [29,37]. In recent years the stimulation of BAT and the induction of browning in WAT have been

seen as potential strategies to combat metabolic disorders, including obesity. They enhance energy expenditure, primarily via increased fatty acid oxidation and thermogenesis [54]. It has been seen that an intake of polyphenols [35] and DF [36] can improve thermogenesis and browning.

In our study, we observed no significant morphological changes in WAT or BAT of the mothers at the end of suckling. However, there were changes in the gene expression in these tissues. In obesity, *PPARγ* activity is typically reduced, leading to impaired maintenance of adipocyte cellular identity [55]. In our study WAT *PPARγ* showed an increasing tendency, suggesting preserved adipogenic capacity. However, BAT *PPARγ* was significantly reduced. It has been described that a reduction in *PPARγ* is related with a decrease in the thermogenic activity of BAT [56], but we observed a significant increase in the uncoupling protein 1 (*UCP1*), a key protein involved in thermogenesis by dissipating excess energy as heat. This last observation aligns with findings from other studies, where polyphenols and dietary fiber enhance the expression of thermogenic genes in BAT [35,36]. *Prdm16* showed a tendency to increase, and it has been seen to regulate thermogenic gene programming [57]. Regarding *Il-1b*, its tendency to increase should be explored, since it is a marker regarding inflammation and it is usually increased in obesity [58], but due to high data variability, further analysis is needed to clarify this finding.

In the offspring, morphological differences were only observed in BAT at weaning. However, these differences were not reflected in gene expression, since there were no differences in any case. In adulthood, no morphological changes were observed either at WAT or BAT, but a significant decrease in *Il-1b*, in WAT was observed, indicating an improved inflammatory status [58]. However, a tendency towards a reduction in *PPARγ* in BAT was also observed, highlighting the need to take a close look at it in the future.

This supports the hypothesis that maternal intake of polyphenols and DF can influence offspring metabolism through the placenta [23] or breast milk [38]. However, these effects were not as strong as in mothers, who received the diet directly, and the effects were different at weaning and adulthood. This suggests that while early-life exposure may have transiently modulated the tissue morphology, it may not have been sufficient to induce long-term effects. However, differences were seen regarding gene expression, so it could be involved in epi-genetic programming [2].

This study presents several limitations that must be acknowledged. First, the lipidomics targeted analysis was limited to specific lipid subclasses, excluding key components such as TG and cholesterol, which could have provided a more comprehensive view of lipid metabolism. Further analysis will be performed to elucidate it. Subsequently, to establish a stronger link in the mother-milk-infant axis, the lipid and polyphenol composition of breast milk is currently being analyzed. This could clarify the direct transmission of dietary components and their impact on the offspring. Moreover, the observed changes in BA levels could not be fully interpreted, as fecal BA measurements were not conducted. This would have helped determine whether the alterations were due to increased excretion or decreased synthesis. The samples are stored, and the measurements could be performed in the future. Additionally, further gene expression analyses in the liver such as *CYP7A1* or *FAS* could have clarified BA [59] and lipid [60] synthesis pathways. Moreover, epigenetic studies, among other analyses, are being conducted to elucidate the relationship between diet and gene expression.

Other limitations of the experimental design include that the offspring begin consuming small amounts of solid food at day 16-18, so the effects observed on d21 cannot be attributed solely to maternal influence. Additionally, samples were collected only at the end of lactation, so the impact of the maternal diet during pre-gestation and gestation is reflected only at this point. It would be valuable to assess these effects after each period to better understand the temporal dynamics of dietary influence.

9. Conclusion

A maternal diet high in fiber and polyphenols administered during pre-gestation, gestation, suckling or the whole perinatal period induced the following effects:

1. The nutritional intervention induced modifications in individual lipid species, although the lipidomic profile of total lipid content and main subclasses was not affected. The main changes due to the diet were in the PGS and S group. Supplemented mothers had a reduction in biliary acids. In their offspring, hydroxylated fatty acids increased and the ratio of omega6/omega3 fatty acids was reduced.

2. The high polyphenol and fiber diet did not affect white adipose tissue morphology at any age but significantly modified the brown adipose tissue morphology in the offspring at weaning, suggesting a short-term effect.
3. Gene expression was predominantly altered in mothers following the enriched diet, although the results showed some inconsistencies. Some changes were also observed in their adult offspring, suggesting a potential long-term impact on the adipose tissues.

These findings suggest that maternal intake of dietary fiber and polyphenols had certain shaping effect on the lipid metabolism of mothers and their offspring, both at weaning and in adulthood.

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