**Maternal high fat diet in mice alters immune regulation and lung function in the offspring**

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**Abstract**

Polyunsaturated fatty acids (PUFA) modulate immune function and have been associated with risk of childhood atopy and asthma. We investigated the effect of maternal fat intake in mice on PUFA status, elongase anddesaturasegene expression*,* inflammatory markers and lung function in the offspring. C57BL/6J mice (n=32) were fed either standard chow (C, 21% kcal fat) or a high fat diet (HFD, 45% kcal fat) for 4 weeks prior to conception and during gestation and lactation. At 21 days of age, offspring were weaned onto either the HFD or C, generating four experimental groups: C/C, C/HF, HF/C and HF/HF. Plasma and liver fatty acid composition were measured by gas chromatography and gene expression by qPCR. Lung resistance to methacholine was assessed. Arachidonic acid concentrations in offspring plasma and liver phospholipids were increased by HFD; this effect was greater in the post-natal HFD group. Docosahexaenoic acid concentration in offspring liver phospholipids was increased in response to HFD and was higher in the post-natal HFD group. Post-natal HFD increased hepatic *FADS2* and *ELOVL5* expression in male offspring, whereas maternal HFD elevated expression of *FADS1* and *FADS2* in female offspring comparing to males. Post-natal HFD increased expression of *IL-6* and *CCL2* in perivascular adipose tissue. The HFD lowered lung resistance to methacholine. Excessive maternal fat intake during development modifies hepatic PUFA status in offspring through regulation of gene expression of enzymes that are involved in PUFA biosynthesis and modifies the development of the offspring lungs leading to respiratory dysfunction.

**Background**

The incidence of immune-mediated inflammatory diseases increased world-wide in recent decades and these have become a leading cause of chronic illness in children and young people([1](#_ENREF_1)). A range of evidence shows that early dietary exposures may influence the development of these diseases(1). A number of studies have also linked maternal obesity during pregnancy with an increased risk of obesity in the offspring, which persists across the lifespan([2](#_ENREF_2)). While epidemiological studies in humans are limited in their ability to separate the effects of maternal obesity from those of the maternal diet on fetal development, some evidence suggests that a maternal high fat diet (HFD) increases the likelihood of obesity in the offspring irrespective of maternal weight([3](#_ENREF_3)). Animal models using maternal HFD have shown a range of effects on the offspring such as altered lung development and function([4](#_ENREF_4)), metabolic disorders([5](#_ENREF_5)), and adipo-immunologic aging([6](#_ENREF_6)). We have previously shown that maternal obesity leads to long term changes in vascular function in mice([7](#_ENREF_7)) but whether maternal exposure to a HFD diet alters immune regulation in the offspring through fatty acid dysregulation remains unknown.

Polyunsaturated fatty acids (PUFA) can modulate the immune system in part through regulation of lipid mediator synthesis. In general, eicosanoids derived from the n-6 PUFA arachidonic acid (ARA) are proinflammatory and immunoactive, whereas eicosanoids and docosanoids derived from the n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory and inflammation resolving functions([8](#_ENREF_8)). ARA, EPA and DHA are formed from precursor PUFA through a pathway involving desaturation and elongation. The rate limiting step in the pathway is catalysed by delta-6 desaturase, encoded by the fatty acid desaturase 2 (*FADS2*) gene. Elongation of very long-chain fatty acids (ELOVL) 5 converts the products of delta-6 desaturase into fatty acids that are 2-carbons longer. This is followed by a further desaturation catalysed by delta-5-desaturase (encoded by fatty acid desaturase 1 (*FADS1*)) to generate ARA and EPA([9](#_ENREF_9)). In a rat model, maternal fat intake was demonstrated to inversely correlate with the proportions of ARA and DHA in offspring liver and with epigenetic regulation of *FADS2* transcription in offspring liver([10](#_ENREF_10)). A recent systematic review of diet during pregnancy and infancy and risk of allergic or autoimmune disease study concluded that that maternal fish oil supplementation may reduce risk of eczema and allergic sensitization to food in children([1](#_ENREF_1)). Fish oil is a source of EPA and DHA. In addition, a recent trial demonstrated that maternal fish oil supplementation altered the child's metabolome at age 6 months, with lower levels of n-6 PUFA pathway-related metabolites and decreased risk of the child developing asthma at age 5 years([11](#_ENREF_11),[12](#_ENREF_12)). We have previously observed that gestational oily fish intake epigenetically modifies genes encoding key enzymes of PUFA synthesis, and alters allergic risk in early childhood([13](#_ENREF_13)).

In humans, a recent study linked minor allele carriers of several *FADS* alleles associated with lower blood ARA, with reduced risk of atopic eczema at age of 13 years([14](#_ENREF_14)) while a higher proportion of n-6 PUFA was associated with higher risk of allergy in children([15](#_ENREF_15)). In addition, n-3 PUFA supplementation has been shown to decrease plasma levels of n-6 PUFA, increase plasma n-3 PUFA levels, and influence desaturase and elongase activities([16](#_ENREF_16)). In this study, the authors identified that minor alleles of *FADS* variants are associated with higher delta-6 desaturase activity and with lower delta-5-desaturase activity, while minor alleles of *ELOVL* variants were associated with higher elongase activity following n-3 PUFA supplementation. Also, genetic variants of the *FADS1* and *ELOVL5* modified the effect of breastfeeding on cognition, suggesting functional importance of such variants to early development([17](#_ENREF_17)).These findings support the hypothesis that gene-diet interactions modulate the activities of desaturases and elongases, and that n-3 and n-6 PUFA are competitive toward these enzymes resulting in altered levels of precursors of eicosanoids and docosanoids. Changes in the fatty acid composition of inflammatory cells affect production of both lipid and peptide mediators of inflammation, and as a result ARA, EPA and DHA may play an important role in the development of allergy([18](#_ENREF_18)).

Obesity increases the risk of common conditions including atherosclerosis, severe asthma and airway inflammation through neutrophil accumulation, generation of inflammatory cytokines (interleukin-1ß, tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6)), an altered gut microbiome, and vitamin D deficiency([19](#_ENREF_19)). Previous studies reported that maternal exposure to a HFD causes changes in lung morphology([20](#_ENREF_20)), leads to innate airway hyperresponsiveness([21](#_ENREF_21)), persistent metabolic abnormality and chronic airway inflammation in offspring([22](#_ENREF_22)). The perivascular adipose tissue (PVAT) is important for the regulation of vascular/endothelial function and dysfunction of PVAT is characterized by its inflammatory character, oxidative stress, and increased production of paracrine factors such as resistin, leptin, cytokines (TNF-α and IL-6) and chemokines (CCL5 and CCL2)([23](#_ENREF_23)).

The aim of this study was to investigate the effect of maternal and post-natal HFD on PUFA status, on the genes encoding enzymes of fatty acid biosynthesis,on inflammatory gene expression in PVAT and on lung function in the offspring.

**Methods**

*Ethics statement*

All animal procedures were performed under the regulations of the British Home Office Animals (Scientific Procedures) Act 1986 and were conducted under Home Office License number 70-6457. The study received Institutional approval from the University of Southampton Biomedical Research Facility Research Ethics Committee.

*Animals and diets*

The animal diets were from Special Diet Services UK as previously described([7](#_ENREF_7)). Weaned female C57BL/6J mice (n=32) housed in autoclaved cages with autoclaved bedding were fed either standard chow (C, RM1A, fat as 20.4 kcal%) or a high fat (fat as 39.9 kcal%) diet (HFD) for 4-6 weeks prior to conception and throughout gestation and lactation (Fig. 1). Dietary macronutrients and energy are shown in Table 1. At 21 days of age, offspring were weaned onto either the HFD or C, generating four experimental groups: C/C, C/HF, HF/C and HF/HF (N=7-10/group). Following weaning of the offspring, dams were killed by cervical dislocation. Two sets of dams were used to generate the 15 and 30 week old offspring to avoid maternal bias. At 15 weeks of age, a subgroup of offspring was killed by cervical dislocation and liver (left lobe) and perivascular fat from around mesenteric arteries were dissected and snap frozen in liquid nitrogen and stored at -80° C until further analyses. Blood was collected by cardiac puncture into tubes containing lithium heparin and the plasma separated, frozen in liquid nitrogen and stored at -80° C. At 30 weeks of age, offspring were killed by cervical dislocation and lungs dissected and frozen in liquid nitrogen. In a subgroup at 30 weeks, lung function was assessed.

*Measurement of phosphatidylcholine (PC) fatty acid composition*

Fatty acid compositions of liver and plasma PC were measured by gas chromatography as described elsewhere([24](#_ENREF_24),[25](#_ENREF_25)). Each sample (100 mg and 100 µl, respectively) was powdered under liquid nitrogen and lipids extracted using chloroform:methanol (2:1 v/v containing 50 mg/L BHT). PC was isolated by solid phase extraction. Fatty acids within PC were converted to fatty acid methyl esters (FAMEs) by an incubation with methanolic sulphuric acid (2% H2SO4 v/v). FAMEs were recovered by extraction with hexane. FAMEs were separated by gas chromatography using a BXP70 fused silica capillary column (30 m x 0.25 mm x 0.25 µm) on an Agilent 6890 GC system (Agilent, Stockport, Cheshire, UK) equipped with flame ionization detection. Running conditions were as described previously(22). FAMEs were identified by their retention times relative to standards and area under the peak was quantified using ChemStation software (Agilent).

*Gene expression analysis*

Total RNA was isolated from livers of 15 week old offspring, PVAT of 15 week old offspring and lung tissue of 30 week old offspring using Tri Reagent (Sigma, UK) according to the manufacturer’s instructions. RNA concentration and purity were assessed using a Nanodrop Fluorospectrometer (Nanodrop 3300, NanoDrop Technologies, USA) after extraction and purification. Fluorescence ratios (260/280nm and 260/230nm) were observed as an indication of purity. All samples underwent purification using an ethanol precipitation protocol before being used in reverse-transcription PCR. Complementary DNA was synthesised from total RNA by poly-A reverse transcription (Primerdesign, UK). cDNA template was amplified by a real-time PCR (Roche LightCycler® 480) under following conditions: 95° C for 10 min, then 15 sec at 5° C and 1 min at 60° C for 35 cycles. The primers and probes (Eurogentec, UK) are shown in Table 2. The expressionof *FADS1*, *FADS2* and *ELOVL5* were asssessed using Sybr Green (QIAGEN, UK). Each sample was assayed in triplicate and expression of the individual transcripts was normalized to the relevant housekeeper in a single 96-well plate, and a mean copy number was calculated. The housekeeper used for liver was YWHAS and for PVAT and lung it was GAPDH.

*Pulmonary function test*

A change in respiratory system resistance in response to increasing concentrations of inhaled methacholine was assessed *in vivo*. The trachea of 30 week-old offspring under deep anesthesia was exposed through a neck incision with an 18-gauge catheter inserted in a small cut below the cricoid cartilage([26](#_ENREF_26)). The catheter was connected via a nebulizer to a Flexivent system (SCIREQ, Montreal, Canada). Resistance was measured at baseline after nebulization of phosphate-buffered saline and then after nebulization of methacholine at cumulative doses of 0, 25, 50, and 100 mg/ml. On completion of the lung function assessment, mice were killed by cervical dislocation and lungs dissected and frozen in liquid nitrogen.

*Statistical methods*

The effects of maternal diet upon offspring plasma and liver PC fatty acid composition was assessed by 2-way-ANOVA with multiple comparison and sex adjustment (SPSS v26, Chicago, Illinois, USA). Relative mRNA expression levels among dietary groups were evaluated by 2-way-ANOVA and the comparison between two groups was tested by 1-way-ANOVA. Proportion of ARA and DHA in the offspring liver and plasma PC were tested among study groups by independent T test to evaluate the effect of pre- and post-natal HFD. Maximum resistance to methacholine was analyzed by 2-way-ANOVA among groups (GraphPad Prism v8, Sain Diego, California, USA). Results are expressed as mean and standard error of the mean (SEM) and sample size in each group was n≥5. Differences were considered to be statistically significant if the associated p value was less than 0.05 at two-sided significance levels.

**Results**

*PC fatty acid composition of mice fed chow and high-fat diet*

The proportions of ARA (20:4n-6) and DHA (22:6n-3) in offspring liver and plasma PC were affected by diet (Tables 3 and 4). Specifically, the proportion of ARA in offspring was significantly higher in the HF/HF group compared to the other (C/C, HF/C, C/HF) dietary groups in plasma PC, and compared to the C/C and HF/C groups in liver PC (*P*<0.05; Table 3 and 4, respectively). The proportion of plasma PC DHA tended to be higher in the HF/HF group, but this did not reach statistical significance. However, DHA in liver PC in offspring in the C/HF and HF/HF groups was significantly higher than in the C/C and HF/C groups (*P*<0.05, respectively; Table 4).

To determine the effects of pre- and post- weaning HFD on composition of fatty acids, the study groups were combined as follows: C/C and HF/C considered as prenatal diet group; C/HF and HF/HF considered as post-natal diet group. The proportion of ARA was significantly higher in offspring plasma and liver PC in post-natal HF diet group (*P*=0.039 and *P*=0.001, respectively; Fig. 2a and c). Also, the proportion of DHA was significantly higher in offspring liver PC in the post-natal HF diet group (*P*<0.001, Fig. 2d). Post-natal diet did not change the proportion of plasma PC DHA in offspring (Fig. 2b).

*Gene expression of PUFA biosynthetic enzymes and pro-inflammatory cytokines and chemokines*

Next, hepatic expression of genes encoding enzymes of PUFA metabolism was examined to identify the potential effect of high fat feeding. *FADS1* mRNA expression in the liver of the offspring was higher in females than males, significantly so in HF/C and HF/HF groups (*P*=0.001 and *P*=0.003, respectively; Fig. 3a), but there was no effect of diet. There were significant effects of offspring sex and maternal diet on *FADS2* mRNA expression (Fig. 3b). *FADS2* expression was significantly higher in female than male offspring in the HF/C group (*P*=0.001) and tended to be higher in HF/HF group (*P*=0.056), and it was expressed more highly in HF/HF group than in the HF/C group (*P*<0.05, respectively; Fig. 3b). *ELOVL5* mRNA expression was higher in HF/HF males than in HF/C and C/HF males (*P*=0.038 and *P*=0.009, respectively; Fig. 3c).

Post-natal HFD (C/HF, HF/HF) was associated with higher expression of inflammatory markers *IL6* and *CCL2* in PVAT than seen in C/C and HF/C groups (*P*<0.001 and *P*=0.01, respectively; Fig. 4a and b). In addition, post-natal HFD (C/HF) significantly increased expression of pro-inflammatory cytokine *IL6* in offspring lung comparing to C/C offspring (*P*=0.042, Supplementary figure 1).

*Lung function of offspring*

In both male and female mice from pre-natal HFD groups (HF/C and HF/HF) methacholine generated significantly less airway resistance compared to mice in the pre-natal chow groups (C/C and C/HF, *P*<0.01, Fig. 5a and b). In male offspring, there was a significant interaction between the pre and post-natal diets but no such interaction was seen in the female offspring (HF/C vs CC and HF/HF vs C/HF, *P*<0.05, respectively, Fig. 5c and d).

**Discussion**

In this study, we have demonstrated a clear effect of a maternal HFD on ARA and DHA status in offspring plasma and liver phospholipids. Maternal HFD exposure also led to altered offspring responses to postnatal HFD with higher expression of genes encoding hepatic long chain PUFA synthesizing enzymes, *FADS2* and *ELOVL5* (in male offspring only), and impaired lung function. Postnatal HFD was also associated with an increased expression of genes encoding pro-inflammatory (*IL6*) and vascular inflammatory (*CCL2*) markers in offspring PVAT independent of maternal HFD. These observations suggest that exposure to a HFD during development programs the lipidomic, metabolic and immunologic profile of the offspring linked to respiratory dysfunction in early life.

In this model, C57BL/6J offspring were exposed to a HF maternal diet for 21 days *in utero* and via suckling until 3 weeks of life, before being weaned to either a control diet or a continued HFD. Physiological properties of individual PCs are heavily dependent on their FA composition. In general, PCs are crucial for maintaining membrane fluidity and for cellular differentiation and proliferation([27](#_ENREF_27)). PCs can also have an anti-inflammatory effect([28](#_ENREF_28)). In previous studies in mice, dietary DHA/EPA administration as phospholipids prevented weight gain and reduced plasma lipid concentrations([29](#_ENREF_29)), and n-3 PUFA supplementation in addition to a HFD reduced inflammation and size of adipocytes([30](#_ENREF_30)). In the current study, increased ARA and DHA levels in PC were observed in offspring following maternal HFD. Further, post-natal HFD additionally affected the proportions of hepatic and plasma ARA and hepatic DHA, shifting the profile to one with more ARA. Plasma samples from offspring fed the HFD showed no difference in DHA containing PC, in line with previous studies that showed no difference in DHA containing PC in the plasma following high n-3 PUFA diet and/or dietary supplements of DHA([31](#_ENREF_31),[32](#_ENREF_32)). Conceivably, DHA containing PC might rapidly convert to lysophosphatidylcholine (LPC) for delivery to extrahepatic tissues, with clearance from the circulation.

It has been shown that liver lipid accumulation, FA composition and inflammation are all modulated by the dietary composition of lipids, and that elongase and desaturase enzymes are also regulated by diet composition and via their activities can lead to alterations in cell lipid composition([33](#_ENREF_33)). Although Su et al. identified low expression of both *FADS1* and *FADS2* associated with human minor haplotypes, in mice dietary PUFA showed stronger effects than genotype on PUFA composition in liver([34](#_ENREF_34)). Also, PUFA composition in red blood cells was similar to that in liver phospholipids[34](#_ENREF_34). In our study, maternal HFD groups had lowered *FADS1* and *FADS2* expression in male offspring, while *FADS2* expression was elevated by post-natal HFD. We also observed increased *ELOVL5* expression by pre- and post-natal HFD among male offspring suggesting that male mice are more sensitive to the effects of a HFD in comparison to females.

DHA and EPA have both metabolic and anti-inflammatory actions, for example lowering hepatic lipogenesis, increasing adipose tissue insulin sensitivity and decreasing pro-inflammatory cytokines such as TNF-α and IL-6 in adipose tissue([35](#_ENREF_35)). Several investigators have reported decreased expression of genes encoding proteins involved in pro-inflammatory pathways in human, animal and cellular models in response to EPA and/or DHA. For example, a recent study revealed beneficial effects of n-3 PUFA in IL-17A-related inflammatory pathologies through downregulation of intercellular adhesion molecule-1 expression in monocytes and adipose-derived stem cells([36](#_ENREF_36)). In contrast, adipocyte-derived prostaglandin E2, a derivative of ARA, mediates recruitment of adipose tissue macrophages contributing to inflammation([37](#_ENREF_37)). Therefore, the imbalance between n-6 and n-3 PUFA has effects on inflammatory processes and overconsumption of one results in a significant decrease in products of the other([38](#_ENREF_38)). In line with these observations, we observed that a postnatal obesogenic diet (but not maternal HFD), upregulated proinflammatory gene expression in PVAT and lung tissue. This upregulation of *IL6* and *CCL2* in the post-natal HF groups agrees with previous findings that obesity leads to a pro-inflammatory phenotype([23](#_ENREF_23)). Taken together, the coordination of diet-induced DHA and ARA synthesis seems to be important for the regulation of inflammatory markers in adipose tissue as they share a common synthetic pathway.

Our observations of the effect of maternal HFD on offspring lung function are supported by the results of McDonald et al.([21](#_ENREF_21)) who reported increased airway hyperresponsiveness to methacholine and levels of inflammatory cytokines in bronchial alveolar lavage fluid following maternal HFD, suggesting that HFD causes lung dysfunction and increased susceptibility to inflammation at an early age. Although we assessed fatty acid and inflammatory markers at week 15, which occur prior to functional changes in the lung measured at week 30, further detailed investigation will be required to determine the longevity of these effects of maternal HFD during pregnancy of offspring development and whether they are associated with functional outcomes of clinical significance.

 In conclusion, a maternal high fat diet during pregnancy leads to altered hepatic PUFA status in offspring through regulation of gene expression of enzymes that are involved in fatty acid metabolism, increased inflammation, and respiratory dysfunction in early life (Fig. 6). This suggests that composition of maternal diet rather than maternal obesity *per se* may be important in increasing risk of asthma in offspring. This hypothesis needs to be examined in suitably designed human studies.

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**Conflict of interests**

The authors declare that they have no conflict of interest.

**Authors contributions**

P.L., J.W.H. and C.T. designed the study; P.L., L.P.M. and H.L.F. performed research and analyzed data; P.L., P.C.C., J.W.H. and C.T. wrote the manuscript; all authors reviewed and approved the final version of the manuscript.

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FIGURES

**Fig. 1.** Dietary model and study design. C-chow; HF-high fat diet.

**Fig. 2.** Proportion of ARA and DHA in the offspring plasma (a, b) and liver (c, d). Values are mean±SEM. Open bar-C/C+HF/C, closed bar-C/HF+HF/HF. \**P*<0.05, \*\**P*≤ 0.001.

**Fig. 3.** mRNA levels relative to YWHAS of *FADS1* (a), *FADS2* (b) and *ELOVL5* (c) in offspring liver. Values are mean±SEM. Open bar-male, closed bar-female. \**P*≤0.05, \*\**P*≤ 0.001.

**Fig. 4**. mRNA levels relative to GAPDH of *IL6* (a), *CCL2* (b) in perivascular adipose tissue from around mesenteric arteries. Values are mean±SEM.

\*Indicates p<0.05 pre- vs. post-natal fat feeding, \*\*\*Indicates *P*<0.001 pre- vs. post-natal fat feeding.

**Fig. 5.** Change in lung resistance in response to increasing doses of methacholine in 30-week male (a) and female (b) offspring fed either a CD or HFD. Lung resistance in maximum response to methacholine in male (c) and female (d) offspring. Open bar-C/C and HF/C, closed bar-C/HF and HF/HF. \*\*Indicates *P*<0.01 pre- vs. post-natal fat feeding, $ indicates *p*<0.05 interaction.

**Fig. 6.** This diagram provides a schematic representation of the effects that maternal high-fat rich diet alters plasma and hepatic FA composition in offspring through regulation of gene expression of FA enzymes. This leads to induced secretion of inflammatory markers and modifies lung development which may further increase risk of allergy in offspring.

**Table 1**. Macronutrient composition and energy content of the standard laboratory chow and high fat diet.

|  |  |  |
| --- | --- | --- |
|  | Chow | High Fat Diet |
| Percentage weight |  |  |
| Carbohydrate | 70.0 | 49.5 |
| Protein | 18.0 | 26.5 |
| Fat | 10.0 | 22.5 |
|  |  |  |
| Percentage energy  |  |  |
| Carbohydrate | 63.3 | 39.1 |
| Protein | 16.2 | 20.9 |
| Fat | 20.4 | 39.9 |
|  |  |  |
| Total energy (MJ/kg) | 18.49 | 21.17 |
|  |  |  |

**Table 2**. Primer and probe sequences used

|  |  |  |
| --- | --- | --- |
| Gene |  | Sequence 5’ – 3’ |
|  | forward primer | CCAGCTTTGAACCCACCAA |
| *FADS1* | reverse primer | CATGAGGCCCATTCGCTCTA |
|  | forward primer | TCAAAACCAACCACCTGTTCTTC |
| *FADS2* | reverse primer | GATGAACCAGGCAAGGCTTTC |
|  | forward primer | ATGGACACCTTTTTCTTCATCCTT |
| *ELOVL5* | reverse primer | ATGGTAGCGTGGTGGTAGACATG |
|  | forward primer | GCTACCAAACTGGATATAATCAGGA |
| *IL6* | reverse primer | CCAGGTAGCTATGGTACTCCAGAA |
|  | Probe | UPL Probe #6 |
|  | forward primer | Catccacgtgttggctca |
| *CCL2*  | reverse primer | Gatcatcttgctggtgaatgagt |
|  | Probe | UPL Probe #62 |
|  | forward primer | GGTGAACATGAGTCCCATCA |
| *IL33* | reverse primer | CGTCACCCCTTTGAAGCTC |
|  | Probe | UPL Probe #71 |
|  | forward primer | CTGTAGCCCACGTCGTAGC |
| *TNFα* | reverse primer | TTGAGATCCATGCCGTTG |
|  | Probe | UPL Probe #25 |

**Table 3**. Polyunsaturated fatty acid composition of plasma PC among dietary groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Fatty acid  | C/C, % | HF/C, % | C/HF, % | HF/HF, % |
|  n=6 | n=8 | n=5 | n=9 |
| 18:2n-6 | 23.20±1.62a | 22.68±1.15a | 26.20±1.21b | 22.92±0.55a |
| 18:3n-6 | 0.23±0.02a | 0.20±0.02a | 0.21±0.01ab | 0.14±0.01b |
| 18:3n-3 | 0.40±0.05a | 0.42±0.05a | 0.34±0.07ab | 0.26±0.02b |
| 20:2n-6 | 0.30±0.01 | 0.28±0.04 | 0.29±0.02 | 0.29±0.01 |
| 20:3n-6 | 2.14±0.14ab | 2.14±0.11ab | 1.79±0.11a | 2.30±0.23b |
| 20:4n-6 | 9.64±0.97a | 9.61±1.10a | 10.29±0.89a | 12.19±0.39b |
| 20:4n-3 | 0.07±0.04 | 0.04±0.02 | NA | 0.06±0.01 |
| 20:5n-3 | 0.35±0.02a | 0.29±0.02ab | 0.31±0.01ab | 0.28±0.01b |
| 22:5n-3 | 0.44±0.06abc | 0.50±0.04a | 0.33±0.02bc | 0.33±0.01c |
| 22:6n-3 | 5.62±0.49 | 5.72±0.45 | 5.85±0.26 | 6.10±0.32 |

Data are shown as mean±SEM. Values not sharing a superscript letter are different (*P*<0.05).

**Table 4**. Polyunsaturated fatty acid composition of liver PC among dietary groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Fatty acid | C/C, % | HF/C, % | C/HF, % | HF/HF, % |
| n=7 | n=8 | n=7 | n=10 |
| 18:2n-6 | 13.11±0.49abc | 12.33±0.45ac | 14.10±0.81b | 11.75±0.29c |
| 18:3n-6 | 0.29±0.03ac | 0.26±0.01a | 0.31±0.02c | 0.21±0.01b |
| 18:3n-3 | 0.12±0.01ab | 0.10±0.00ab | 0.13±0.01a | 0.10±0.00b |
| 20:2n-6 | 0.30±0.03 | 0.24±0.01 | 0.28±0.02 | 0.30±0.02 |
| 20:3n-6 | 2.31±0.17ab | 2.16±0.16a | 1.94±0.06a | 2.56±0.25b |
| 20:4n-6 | 15.20±0.63ac | 14.99±0.98a | 16.64±0.62bc | 17.93±0.30b |
| 20:4n-3 | 0.13±0.02 | 0.08±0.00 | 0.11±0.01 | 0.08±0.01 |
| 20:5n-3 | 0.39±0.04 | 0.41±0.03 | 0.43±0.01 | 0.35±0.02 |
| 22:5n-3 | 0.46±0.04ab | 0.52±0.04a | 0.41±0.02b | 0.39±0.03b |
| 22:6n-3 | 9.34±0.42a  | 9.68±0.55a | 12.01±0.24b | 11.65±0.48b |

Data are shown as mean±SEM. Values not sharing a superscript letter are different (*P*<0.05).













**Supplementary**

**Figure 1. Relative expression of *IL6*, *IL33* and *TNFα* in offspring lung.**

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Relative gene expression levels of inflammatory markers for all offspring. Phenotype gene expression compared by Two-way-ANOVA. Significant post-natal difference was identified in *IL6* expression when C/HF compared to C/C group (a, *P*=0.042). Relative expression of *IL33* (b) and *TNFα* (c) did not show any significant difference among study groups.