Metabolic analysis of adipose tissues in a rodent model of pre-pregnancy maternal obesity combined with offsprings on high-carbohydrate diet

Andi Wang[a,1], Ting-Li Han[a,b,c,1], Zhu Chen[d], Xiaobo Zhou[a,b], Xinyang Yu[a,b], Hongbo Qi[a,b],Philip N. Baker[b,c,e], Hua Zhang[a,b]□

[a]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

[b]Canada - China -New Zealand Joint Laboratory of Maternal and Fetal Medicine, Chongqing Medical University, Chongqing 400016, People's Republic of China

[c]Liggins Institute, University of Auckland, Auckland, New Zealand

[d]Department of Obstetrics and Gynecology, Xin Qiao Hospital, The Second Medical College of Army Medical University, Chongqing, China

[e]College of Medicine, Biological Sciences and Psychology, University of Leicester, UK

[1]Authors contributed equally.

□Corresponding author at: Department of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China. E-mail address: zhanghua@hospital.cqmu.edu.cn (H. Zhang)

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Abstract

Maternal obesity is associated with adverse effects on the health of offsprings. Consumption of a high-carbohydrate (HC) diet has been found to promote abnormal fatty acid metabolism in adipose tissue. Therefore, we hypothesised that maternal obesity combined with an offspring HC diet would alter the fatty acid metabolism of adipose tissue and subsequently contribute to offspring obesity. Lepr$^{db/+}$ mice were used to model pre-pregnancy maternal obesity and the C57BL/6 wildtype were used as a control group. Offspring were fed either HC diet or a normal-carbohydrate (NC) diet after weaning. Brown adipose tissue (BAT) and white adipose tissue (WAT) were

collected from offspring at 20 weeks of age and their fatty acid metabolome was characterized using gas chromatography-mass spectrometry. We found that HC diet increased the body weight of offspring (males increased by 14.70% and females increased by 1.05%) compared to control mothers. However, maternal obesity alone caused a 7.9% body weight increase in female offspring. Maternal obesity combined with an offspring HC diet resulted in dynamic alterations of the fatty acid profiles of adipose tissue in male offspring. Under the impact of a HC diet, the fatty acid metabolome was solely elevated in female WAT, whereas, the fatty acid metabolites in BAT showed a similar trend in the male and female offsprings. 6,9-octadecadienoic acid and 12,15-cis-octadecatrienoic acid were significantly affected in female WAT, in response to offspring consumption of a HC diet. Our study demonstrated that maternal obesity and offspring HC diet have different metabolic effects on adipose tissue in male and female offsprings.

1. Introduction

Environmental and genetic factors such as diet and maternal obesity are important constituents of offspring health. Poor nutrition habits are a dominating contributor to obesity and chronic metabolic diseases worldwide [1, 2]. In the United States, approximately 27 % of women at reproductive age are overweight (body mass index (BMI) between 25-30 kg/m^2) and 37 % are obese (BMI ≥30 kg/m^2) [3-5]. Maternal overweight and obesity are also rising in the developing world, particularly in urban settings [6]. In addition, maternal obesity seems to have long-term effects on offspring health outcomes [7] and has been associated with medical complications in later life, such as hypertension, type-2 diabetes, and metabolic syndrome [8-10].

The Prospective Urban Rural Epidemiology study, published in 2017, investigated individuals from 18 countries across five continents (n=135 335, median follow up 7.4 years, 5796 deaths) and reported that a high-carbohydrate (HC) intake was associated with an increased risk of mortality [11]. Indeed, another prospective cohort study also showed that HC consumption was related to a significantly higher risk of all-cause mortality [12]. Previous observational studies have also shown that a HC diet with a high glycemic index and glycemic load increase the risk of type-2 diabetes mellitus in

Chinese women [13]. These study outcomes indicate that a HC diet may play a critical role in the development and progression of metabolic diseases. Franca *et al* (2014) demonstrated that following a HC diet promoted fatty acid biosynthetic pathways in the adipose tissue of rats. Although some rodent studies have previously examined the relationship between maternal obesity and diabesity in her offspring [14-16], few studies have investigated the combined impact of maternal obesity and an offspring HC diet on the metabolic health of the offspring [17]. Therefore, we hypothesised that the combined consequences of maternal obesity and an offspring HC diet would alter the fatty acid metabolism of adipose tissue and subsequently contribute to offspring obesity.

Our study aims to explore the interaction of both maternal obesity and offspring HC diet on adipose tissue of the offspring. Maternal obesity was modelled using a gene knockout-mouse model, and offspring were exposed to either a HC diet or a normal-carbohydrate (NC) diet. Here we report the results of the first metabolomic screen of metabolites detected from brown adipose tissue (BAT) and from white adipose tissue (WAT) in offspring fed with a HC diet, delivered by obese mothers.

2. Method

2.1. Animal experiment

$Lepr^{db/+}$ mice are heterozygous for a loss-of-function mutation in the leptin receptor gene, and unlike its homozygous counterpart, is fertile. In the nongravid state, $Lepr^{db/+}$ mice have a comparable body weight and fasting glucose as its parental strain C57BL/6 [18, 19]. Spontaneous mutations ($Lepr^{db/+}$) cause the mice to become obese at approximately three to four weeks of age. $Lepr^{db/+}$ mice exhibit hyperinsulinemia and compensatory hyperplasia of islet beta cells that persist throughout their lifespan [20].

We included thirteen $Lepr^{db/+}$ heterozygous mice in the study to represent the obese mother model and nine C57BL/6 mice were included as controls. The same batch of male C57BL/6 rodents from the same parental line was used to mate with the C57BL/6 and $Lepr^{db/+}$ mothers, thus preventing the father's biometric influence in this study (**Figure 1**). All animals in this study were purchased from the Model Animal Research

Center of Nanjing University. The animal care and practices in this study were consistent with guidelines and protocols approved by the Institutional Animal Use and Care Committee (IACUC). All efforts were made to minimize the number of animals sacrificed and their suffering. The mice received 100 grams of fresh food and 250 milliliters of fresh water (daily diet and water changes) per cage; the temperature was set at 22°C ± 2°C; the humidity was between 40 % and 60 %; and their light/dark cycle repeats every 12 hours.

The caudal arterial pressure was measured with "tail-cuff" method by a blood pressure recorder (BP-2000 Blood Pressure Analysis System, Series II, Visitech System, Apex NC, USA). The mice tails were occluded with the proper size tube-shaped tail cuff linked to the tail cuff device. Basal level blood pressure was recorded for 20 min, at 5 min intervals [21, 22].

Plasma concentrations of insulin and leptin were measured using a mouse insulin ELISA kit (Shanghai Jianglai Biotech, Shanghai, P.R. China, #KB11459), according to the manufacturer's instructions.

2.2. Founder (F0) and offspring generation

$Lepr^{db/+}$ female mice and C57BL/6 female mice were mated between ten and twelve-weeks-old, and only the offspring delivered at the 14 weeks were selected for our study. Dams were reduced to seven pups at birth to avoid food competition during the suckling period. All parental mice were fed with the standard diet (protein = 19.3% kcal, carbohydrate = 64% kcal, fat = 16.7% kcal). The HC diet contained high availability of calories from sucrose. Starting from three weeks of age, all offspring were separated from their mother, with up to two pups per litter. Mice from the same litter were divided into different cages and fed with a standard feed [23]. The offspring mice were converted to a high-carbohydrate (HC) (Diet D12102C, protein = 20% kcal, carbohydrate = 70% kcal, fat = 10% kcal) or to a normal-carbohydrate diet (NC) (Diet D12109C, protein = 20% kcal, carbohydrate = 40% kcal, fat = 40% kcal) eight to ten weeks later (**Table 1**).

The descriptions and nomenclature of the mice used in this study were as follows: F-OB-NC = Female offspring (n=6) from obese mothers were fed a normal-carbohydrate

diet; F-OB-HC = Female offspring (n=8) from obese mothers were fed a high-carbohydrate diet; F- N-NC: Female offspring (n=10) from normal mothers were fed a normal-carbohydrate diet; F-N-HC= Female offspring (n=9) from normal mothers were fed a high-carbohydrate diet. M-OB-NC = Male offspring (n=9) from obese mothers were fed a normal-carbohydrate diet; M-OB-HC = Male offspring (n=8) from obese mothers were fed a high-carbohydrate diet; M-N-NC: Male offspring (n=7) from normal mothers were fed a normal-carbohydrate diet; M-N-HC = Male offspring (n=6) from normal mothers were fed a high-carbohydrate diet.

2.3. Genotyping

Genotyping was performed at four weeks in the offspring from Leprdb/+ mother and C57BL/6 mother. The offspring containing a leptin gene was eliminated from the study. Mice were anesthetized with diethyl ether and 0.5 cm of their tail was cut and lysed in 25 mM NaOH and 0. 2mM disodium EDTA buffer (pH=12) for one hour. All tails were centrifuged at 4000 rpm for three minutes to purify DNA after neutralizing with 40 mM Tris–HCl (pH=5), and subsequently subjected to gene-specific primer PCR reactions (Forward: AGAACGGACACTCTTTGAAGTCTC, Reverse: CATTCAAACCATAGTTTAGGTTTGTGT, KK5702, KAPA, USA). The RasI enzyme (r0167s, New England Bio, USA) was added to the lysed DNA overnight. Genotyping was confirmed by gel electrophoresis of the reaction product [24].

2.4. Sample preparation for brown adipose tissue (BAT) and white adipose tissue (WAT) samples

The BAT was collected from scapular and perirenal fat, and the WAT was collected from inguinal, gonadal and subcutaneous fat.BAT and WAT samples were collected and frozen in -80 °C, prior to metabolite extraction. 20 mg of the BAT and WAT from each subject was weighed at 4 °C. Metabolites were extracted from the samples using 2 mL of methanol/toluene (4:1 v/v ratio) solution containing nonadecanoic acid (20 µg/mL, Nu-Chek Prep, Inc., USA) and tridecanoic acid (20 µg/mL, Nu-Chek Prep, Inc., USA) as internal standards (ISs). 20 mg of BAT sample and 20 mg of WAT sample were extracted and weighed at 4 °C. 200 µl of acetyl chloride (ADAMAS Reagents, China) was added to each sample and vortexed continuously for 1 min and incubated at

100 °C for an hour. After samples were cooled in tap water, 5 ml of 6 % potassium carbonate aqueous solution (ADAMAS Reagent, China) was added to each test tube. The test tube contents were then vortexed at room temperature for 10 s and centrifuged at room temperature at 2000 rpm for 10 minutes. The toluene phase was extracted and analyzed by gas chromatography-mass spectrometry (GC-MS).

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis and metabolite identification

Extracted metabolites were analyzed using an Agilent 5977 A MSD system linked to an Agilent 7890B GC system. Metabolites were separated using a ReSTEK RTX α-2330 column (90% biscyanopropyl/ 10% phenylcyanopropylpolysiloxane, 100 m, 0.25 MID, 0.2 µm DF). 1 µL of sample was injected into the inlet and it was operated in a split-less mode at 250 °C throughout the entire analysis. The helium pressure was set at a constant flow rate of 1 mL/min. The oven temperature program and mass spectrometer parameters were set according to Smart *et al*. GC-MS peaks were deconvoluted using the Automatic Mass Spectral Deconvolution Identification System (AMDIS) software. Metabolites were identified by comparing their fragmentation spectral pattern and retention times with the in-house lipid mass spectral library built using chemical standards [25].

2.6. Data normalization and statistical analysis

The relative quantification of the identified metabolites was extracted using XCMS R-scripts by selecting the most abundant reference ions within a one-minute retention time bin. Metabolite levels were normalized using two internal standards (non-nonanoic acid and tridecanoic acid), batch correction was applied using quality control (QC) samples, and the dilution effect was corrected for using the weight of the adipose tissue biomass [26]. Data were presented as mean ± SEM. Comparisons between two groups were evaluated using an unpaired *t*-test, while comparisons between more than two groups were analysed using two-way ANOVA and a Tukey *post hoc* test, with both diet and maternal obesity as sources of variation. A false discovery rate (FDR) was calculated using R, to account for multiple comparisons. The variables of importance for the projection (VIP) of partial least squared-discriminant analysis (PLS-

DA) was determined using the ropls R-package. Only fatty acids with a p-value < 0.05, q-value (FDR) < 0.05, and VIP > 1 were considered statistically significant [27]. Receiver operating characteristic (ROC) curves were constructed using pROC ver. 1.8 [28] to plot significant metabolites. Graphical illustrations of the significant differences were plotted on a heatmap using gplots and ggplot2 R packages [29].

3. Results

3.1. The maternal body weight and blood glucose of Lepr$^{db/+}$ mice and C57BL/6 mice at 13-15 weeks

The Lepr$^{db/+}$ mice (28.53 ± 0.36 g) were significantly heavier compared to C57BL/6 mice (21.31 ± 0.90 g) at 13 to 15-weeks-old, prior to conception (**Figure 2A**). The blood glucose levels measured were elevated in obese mothers (n=13) compared to normal mice (n=9), which indicated an impaired glucose tolerance before pregnancy in the obese mothers (**Figure 2B**).

3.2. Body weight, BAT and WAT weight, blood pressure, and blood glucose of the offspring at 20 weeks.

The body weight of both male and female offspring from the normal mothers were higher in the HC diet group (Male: 27.3 ± 2.5 g; Female: 20.3 ± 1.3 g) when compared to the NC diet group (Male: 23.8 ± 1.4 g; Female: 17.0 ± 1.2 g) (**Figure 3A & 3B**). The difference in percentage of body weight between NC diet and HC diet for the males and females was 14.70% and 1.05%, respectively (**Table 1**). Maternal obesity resulted in an increased body weight of the female offspring fed with NC diet (19.6 ± 1.7 g), compared to female offspring from normal mothers (17.0 ± 1.2 g). On the contrary, maternal obesity resulted in a lower body weight of male offspring fed with a HC diet (25.3 ± 1.6 g), compared to male offspring from normal mothers (27.3 ± 2.5 g). In addition, we observed lower WAT weight (from 0.78 ± 0.29 g to 0.53 ± 0.21 g) and BAT weight (from 1.03 ± 0.41 g to 0.61 ± 0.19 g) of male offspring fed with a HC diet, from obese mothers, compared to offspring fed with NC diet. Consequently, the lowered adipose tissue weight contributed to the reduced body weight of the male offspring.

The body weight measured at 8 weeks, 14 weeks, and 20 weeks is depicted as the offspring growth trajectory in **Supplementary Figure 1.**

We only observed differences in blood pressure between the treatment groups among female offspring (**Figure 3 C & D**). The blood pressure of female offspring from normal mothers was higher in the offspring group that followed a HC diet (120.5 ± 5.6 mmHg) compared to the offspring group that followed a NC diet (113.2 ± 4.7 mmHg). Meanwhile, female offspring born to obese mothers, and that were following a HC diet had a lower blood pressure (104.8 ± 5.4 mmHg) than female offspring following a HC diet, born to normal mothers (120.5 ± 5.6 mmHg). The BAT and WAT weight of both male and female offspring fed with a HC diet, from normal mothers, were elevated compared to the offspring fed with a NC diet. Meanwhile, only the BAT weight of both male and female offspring fed with HC diet, from obese mothers, was elevated compared to the offspring fed with a NC diet (**Figure 3E-3H**). Lastly, the female offspring following a HC diet had a higher glucose intolerance than those receiving the NC diet when comparing offspring from both the obese mothers or normal mothers (**Figure 3I**), but no trend was observed in the male offspring groups (**Figure 3J**). In addition, the plasma insulin level only significantly increased in female offspring from normal mothers fed with a HC diet compared to a NC diet (**Supplementary figure 3**).

3.3 Metabolite profiles of BAT and WAT in offspring

To investigate how maternal obesity and an offspring HC diet influence the offspring adipose tissue metabolome, we performed unbiased metabolic profiling of over 200 chromatographic entities with specific masses, from the offspring's adipose tissue extracts. A total of 105 compounds were identified in BAT and WAT using our in-house lipid mass spectral library. The principal component analysis (PCA) of metabolites showed that HC diet groups (OB-HC and N-HC) and NC diet groups (OB-NC and N-NC) were clustered within the same diet and separated according to gender, BAT, and WAT. The first two major components, PC1 and PC2, explained 44.1% and 10.5% of the metabolite variation in the female BAT (**Figure 4A**); 61.5% and 12.1% of the variation in the female WAT (**Figure 4B**); 55.8% and 9.1% of the variation in the male BAT (**Figure 4C**); and 70.5% and 10% of the variation in the male WAT (**Figure D**).

To examine the effect of maternal obesity on the offspring adipose tissue metabolome, the offspring groups from the obese and normal mothers following the same offspring diet were pair-wise compared (**Figure 5**). Some metabolite differences were observed in male offspring adipose tissues including one cholesterol, two long-chain saturated fatty acids, eleven long-chain unsaturated fatty acids, and one medium-chain saturated fatty acid, while only two long-chain unsaturated fatty acids were significantly altered in the female adipose tissue metabolome.

On the other hand, to investigate the effect of the different carbohydrate diets on offspring adipose tissues, offspring groups from the same type of mothers, consuming either the HC diet or NC diet were compared (**Figure 6**). We found substantial differences in the lipidomic profile of the offspring adipose tissues. In particular, the majority of fatty acid concentrations in BAT were significantly different between offspring following a HC diet and NC diet, and both genders shared a similar trend. These differences included one reduced cholesterol, seven long-chain saturated fatty acids (four elevated and three reduced levels); seventeen long-chain unsaturated fatty acids (twelve elevated and five reduced levels), and two elevated medium-chain saturated fatty acids. Interestingly, most of the noticeable fatty acid changes in WAT were only detected in female long-chain unsaturated fatty acids (sixteen increased and one reduced), whereas only three fatty acids were significantly different in the male WAT.

Since the lipidomic profiles demonstrated that male adipose tissue was prone to the influence of maternal obesity and female WAT tissue was most effected by the offspring carbohydrate diet, we performed a ROC analysis of these comparisons. The analysis showed that there was no significant AUC above 0.95 when comparing the effect of maternal obesity on adipose tissue of male offspring. In contrast, four fatty acids in female offspring WAT exhibited high sensitivity and specificity (AUC > 0.95) to discriminate between NC diet and HC diet, including 6,9-octadecadienoic acid, cis-5,8,11-eicosatrienoic acid, 11,14-cis-eicosadienoic acid, and 9,12,15-cis-octadecatrienoic acid (**Figure 7**). Lastly, there were three fatty acids in BAT that not only had AUC above 0.95, but also expressed the same metabolic changes in response

to diet intervention for both male and female offspring. These fatty acids included 10-cis-heptadecenoic acid, 6-trans-octadecenoic acid, and 9-cis-tetradecenoic acid (**Figure 8**). Interestingly, we have detected that 11,14-Eicosadienoic acid was elevated in both male and female offspring's serum and WAT (**S2**).

4. Discussion

In our study, we investigated the body weight, BAT and WAT weight, blood pressure, blood glucose, and fatty acid profiles of BAT and WAT in offspring affected by maternal obesity and following a HC diet. Our data provides evidence that a HC diet has a greater effect on the metabolism of adipose tissue in offspring than maternal obesity. Moreover, the adipose tissue lipid metabolome in male offspring was more sensitive to the consequences of maternal obesity (**Figure 5**), meanwhile, the adipose tissue lipid metabolome in female offspring was more likely to be altered by following a HC diet, particularly in WAT.

We also observed that the $Lepr^{db/+}$ mice model of maternal obesity during pregnancy and suckling partially protected offspring against obesity when exposed to a HC diet in later life. One particularly interesting facet of our study findings was that maternal obesity reduced the body weight of only male offspring fed with a HC diet (**Figure 3**). This is in an agreement with Monk *et al* (2018) that showed that breast milk from an obese dam may partially protect their offspring from the detrimental effects of an unhealthy diet [30]. The challenge here is to dissect out the relative interactions of intrauterine milieu, genetic constitutions, and the postnatal environment. This intergenerational interweaving may be explained by a predictive adaptive response model proposed by Gluckman *et al.* (2004) [30]. If plasticity developed during the prenatal environment matches the postnatal environment, this appropriate prediction will assist survival. Hence, offspring experiencing maternal obesity during pregnancy and lactation, and subsequently being fed with a NC diet may cause a disadvantageous mismatch between the pre- and postnatal environments, which in turn, increases the risk of obesity.

Conversely, offspring following a HC diet, born to normal mothers, had a substantially elevated body weight. The HC diet contained high availability of calories from sucrose

and previous studies have revealed that certain dietary carbohydrates, like sugar-sweetened beverages, have been shown to be positively associated with weight gain [31-33]. In addition, we found a significantly elevated blood pressure, blood glucose level and plasma insulin level of female offspring fed with a HC diet. Indeed, consumption of a HC diet has repeatedly been shown to acutely impair conduit artery vasodilator function assessed by brachial artery flow-mediated dilation in females only [34]. Ranee Singh *et al.* (2014) also revealed that following a HC diet increased blood glucose concentration, which led to adverse consequences such as prediabetes and diabetes.

We found that the $Lepr^{db/+}$ mice model of maternal obesity resulted in a greater effect in the adipose tissue of male offspring. Studies investigating the effects of maternal over-nutrition on both male and female offspring have frequently shown that male offspring had a more pronounced detrimental phenotype [35-40]. Similar to our findings, a previous study utilised a maternal obesity-induced rodent model and followed offspring outcomes, and revealed that male offspring from obese mothers developed insulin resistance, hyperleptinemia, hyperuricemia, and hepatic steatosis; all these features were not observed in the female offspring [41]. We suggested that obesogenic stress derived from maternal obesity, through transmission of biological compounds via placenta and breast milk, during the earliest developmental phases seem to be sufficient to induce a sex-specific response in the adipose tissue metabolism of male offspring.

We detected that following a HC diet had a more profound impact on the body weight of male offspring (14.70% increase compared to the NC diet) than on female offspring (1.05% increase compared to the NC diet) (**Figure 3**). This gender-specific observation of offspring body weight in response to diet might be mediated by the hormonal milieu. This phenomenon aligned with previous reports that estrogens decrease food intake and favor energy expenditure [42]. We also documented that the increase of numerous unsaturated fatty acids in WAT in female offspring fed with HC diet to NC diet, which was not observed in male offspring (**Figure 6**). Estrogens are known to be important regulators of WAT in female rodents [43, 44], especially relative to 17 β-

estradiol's anti-inflammatory properties, through suppression of IL-6 and TNFα secretion by macrophage cells [45]. Furthermore, numerous clinical and preclinical evidence has demonstrated that fertile female mammals were less prone to chronic inflammatory diseases (e.g. diabetes) than males [46, 47]. Since obesity is considered a chronic inflammatory disease in which inflammatory chemokines and transcription factors are upregulated [48], and estrogens exhibit anti-inflammatory properties, it is plausible that estrogens may have a paracrine role in female adipose tissue [48] to alleviate inflammation and attenuate the development of obesity.

Lastly, we detected that 6,9-octadecadienoic acid and 9,12,15-cis-octadecatrienoic acid were elevated in female offspring WAT with an AUC＞0.95, in response to the influence of a HC diet (compared to a NC diet) (**Figure 8**). These two compounds are derived from linoleic acid, a polyunsaturated omega-6 long-chain fatty acid which is the most commonly represented n-6 polyunsaturated fatty acid (PUFA) [49]. Derivatives of linoleic acid include hepoxilins, which can be insulin sensitizers, known to increase the expression of anti-inflammatory high-density lipoproteins, and promote insulin release from pancreatic islet cells [50]. In contrary, linoleic acid is also a precursor for the biosynthesis of arachidonic acid, a fatty acid involved in the production and release of pro-inflammatory eicosanoids, such as PGE_2 and leukotriene B_4 (LTB_4) [51]. Recent studies have suggested that the increased consumption of n-6 PUFAs could increase the risk of chronic diseases, including obesity and type 2 diabetes [52-55]. An *in vivo* study has unraveled that increasing dietary linoleic acid in mice significantly elevated macrophage infiltration in WAT via LPS-TLR4-mediated signaling [56]. According to these findings, we propose that the increased n-6 PUFA metabolites in WAT of female offspring from following a HC diet may manifest as chronic inflammation and subsequently lead to adverse outcomes such as obesity in later life. Future studies should consider investigating the impact of 6,9-octadecadienoic acid and 12,15-cis-octadecatrienoic acid on the regulation of adipose tissue metabolism.

The differences in the PUFAs observed in the adipose tissues may be explained by expoxygenase gene expression or enzymatic activities. Expoxygenases are a set of

membrane-bound and heme-containing cytochrome P450 enzymes that metabolise PUFAs to produce eicosanoids. The most well-known substrate of the epoxygenases is the omega-6 fatty acids including arachidonic acid and linoleic acid, which were significantly altered in BAT and WAT in our study. Additionally, previous studies found that expoxygenase gene knockdown mice exhibited a prominent increase in WAT deposition relative to control mice [57]. Therefore, the expoxygenase enzyme might play a crucial role of PUFAs metabolism in adipose tissue. In addition, *Entringer's lab* has demonstrated that PUFAs 20:4 contained in alkyl-linked phosphatidylcholines (PCae) may protect against adiposity in the newborn, which may have implications for obesity risk. Future studies should consider investigating the PCae containing PUFAs 20:4 in adipose tissue.

There are some limitations of the current study that should be taken into consideration when designing future studies. Firstly, future studies should consider taking into account of the differences in the daily activity, basal metabolic rate, and 24h caloric intake of the offspring that could influence body fat deposition and fat utilisation. In addition, in humans, offspring normally follow the diet of their parents from weaning, but our study introduced the food intervention to offspring between 3-8 weeks post-weaning. The timing of initiation of the offspring dietary intervention was not ideal to model the human situation, but we believe that this feeding duration is sufficient to study the impact of diet on offspring outcomes. Secondly, plasma insulin levels should be measured in offspring, as they could influence the fatty acid profile in adipose tissue. Thirdly, the levels of estradiol and testosterone in both offspring genders should be analysed in order to investigate any interaction effects of sex hormones on the effect of maternal obesity and offspring diet on offspring outcomes. Lastly, future investigations should consider using high-fat diets to induce maternal obesity, as this would be more similar to how maternal obesity is achieved in humans, and the different ratios of the carbohydrate substitutes (protein and fat) in the offspring diet should be explored further.

5. Conclusion

In summary, this is the first metabolomics study to demonstrate that maternal obesity and an offspring HC diet contribute to the fatty acid metabolism of WAT and BAT in offspring. The synergistic interactions of maternal obesity and offspring HC diet on the lipid metabolism of WAT and BAT seem to occur in a sexually dimorphic manner. Our results imply that obesity in pregnancy and lactation as well as offspring HC diet may affect the fetal lipid metabolism, leading to persistent alterations in fatty acid metabolism of WAT and BAT. This study adds to our understanding of the effect of maternal obesity and offspring diet on the development of offspring adiposity and altered lipid metabolism.

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Table 1. Macronutrient composition of experimental diets.

Parameter	High-carbohydrate diet	Energy provided (KJ/g)	Normal-carbohydrate diet	Energy provided (KJ/g)
Protein (%)	20	3.31	20	3.31
Fat (%)	10	1.66	40	6.62
Soybean oil	5.55	0.92	5.55	0.92
Cocoa butter	4.44	0.74	34.44	5.7
Carbohydrate (%)	70	11.59	40	6.62
Sucrose	70	11.59	40	6.62

Table 2. Birth information of offspring from Lepr$^{db/+}$ and C57BL /6 mice.

Maternal mice	Lepr$^{db/+}$	C57BL /6
Pups factors		
Pups weight (g)	1.13±0.01	1.11±0.02
Litter size	7.2±0.4	7.0±0.4
Sex ratio (percentage of male)	46.32±3.67	49.23±3.67

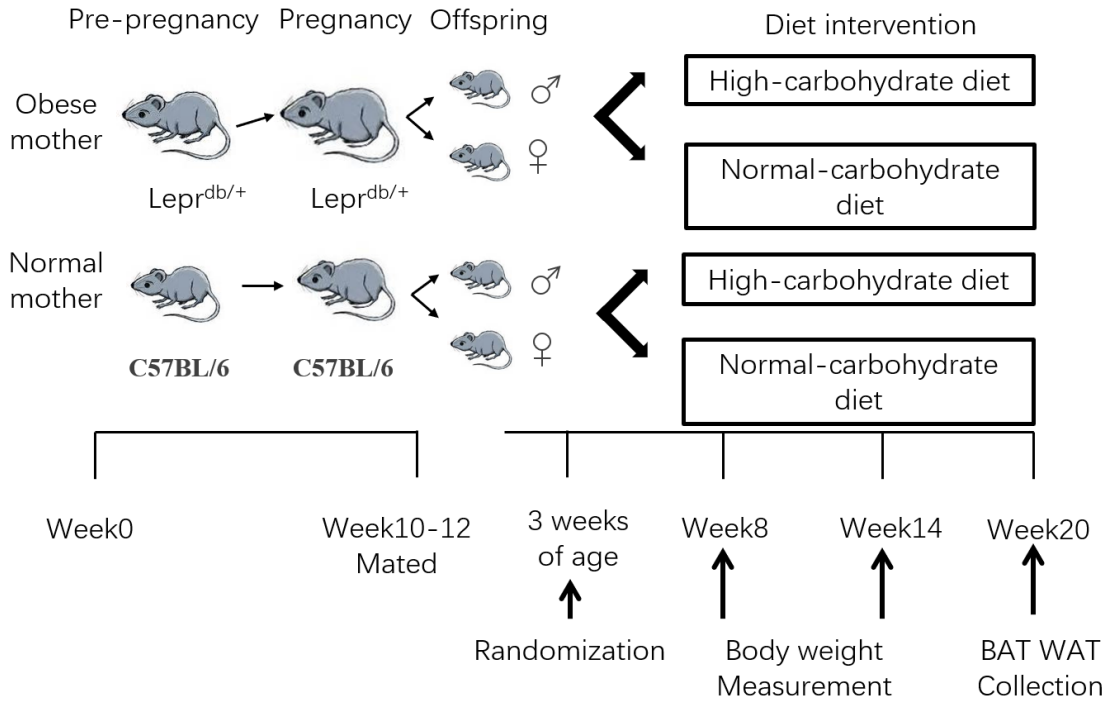

Figure 1. The overall experimental design. Both Lepr$^{db/+}$ mice (obese mothers) and the C57BL/6 mice (normal mothers) were mated at 10 to 12-weeks. The standard diet was fed continuously throughout pregnancy and lactation. Three-week old offspring from each dam were randomly assigned to either a high-carbohydrate (HC) diet or normal-carbohydrate (NC) diet for a total of 17 weeks until they reached 20 weeks of age. Brown adipose tissue (BAT) and white adipose tissue (WAT) were collected from the offspring at 20 weeks.

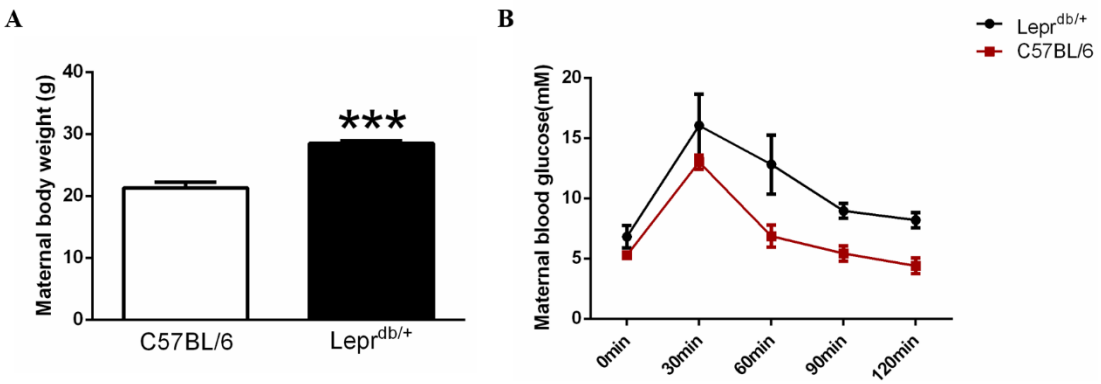

Figure 2. Maternal characteristics. **(A)** The body weight of control mothers (n=9) and Lepr$^{db/+}$ mothers (n=13) in grams at 14 weeks. **(B)** Oral glucose tolerance test of normal control mothers (red line and square; n=9) and Lepr$^{db/+}$ mothers (black line; n=13) at 14 weeks. Statistical differences for body weight and blood glucose levels were determined using a student t-test (***$p < 0.001$) and two-way ANOVA (**$p < 0.01$), respectively.

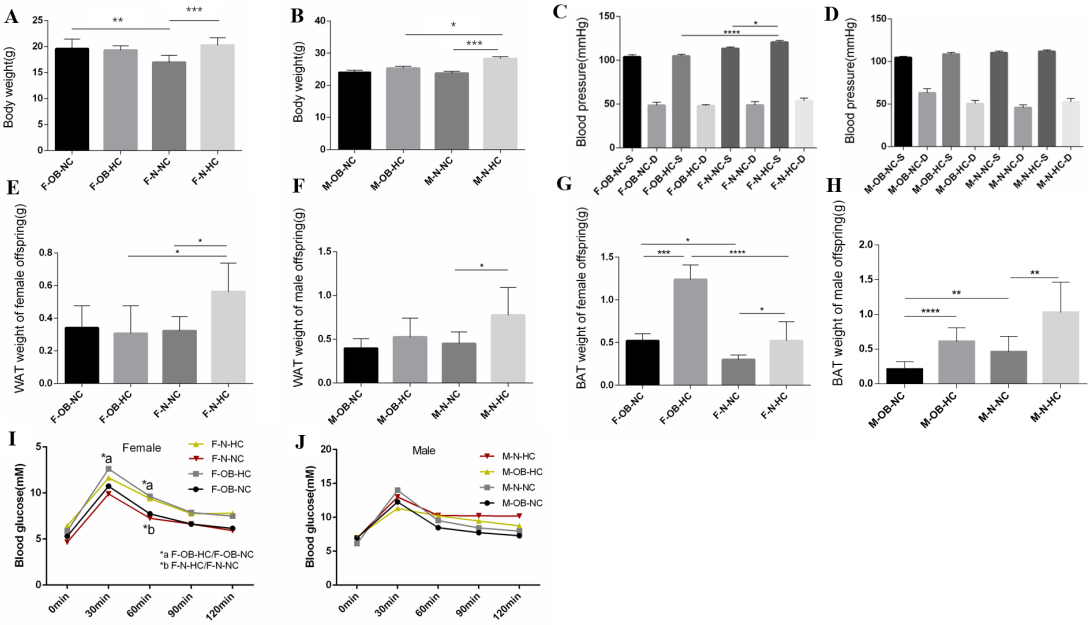

Figure 3. Offspring characteristics. (A) The body weight of female offspring. **(B)** The body weight of male offspring. **(C)** The systolic and diastolic blood pressure of female offspring. **(D)** The systolic and diastolic blood pressure of male offspring. **(E)** The brown adipose tissue (WAT) weight from female offspring. **(F)** The brown adipose tissue (WAT) weight from male offspring. **(G)** The brown adipose tissue (BAT) weight from female offspring. **(H)** The brown adipose tissue (BAT) weight from male offspring. **(I)** The blood glucose of female offspring. **(J)** The blood glucose levels of male offspring. Statistical differences for body weight, blood pressure, BAT weight, WAT weight, and blood glucose levels were determined using either a Tukey's multiple comparison test (****$p < 0.0001$) or a two-way ANOVA (*$p < 0.05$).

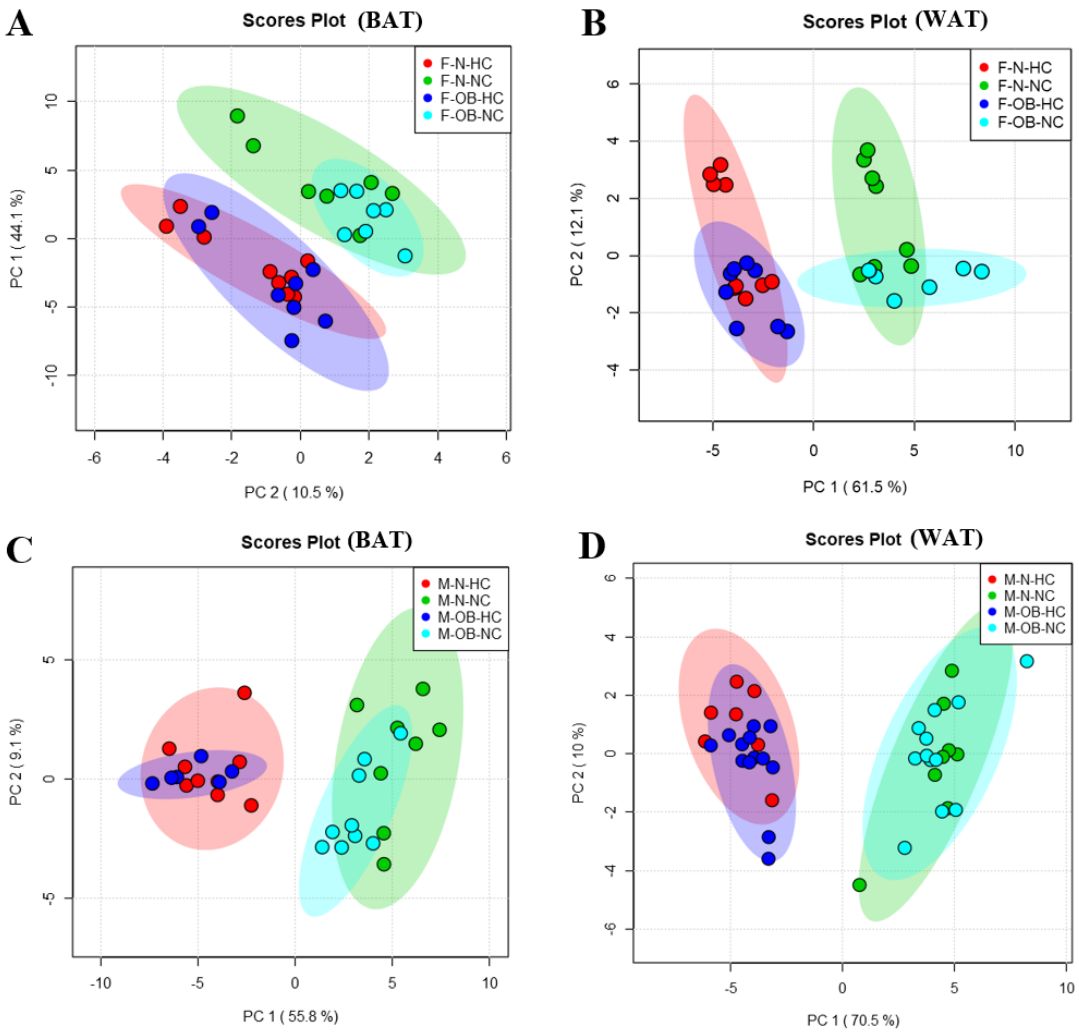

Figure 4. Principal component analysis (PCA). (A) The brown adipose tissue (BAT) collected from female offspring. **(B)** The white adipose tissue (WAT) collected from female offspring. **(C)** The BAT collected from male offspring. **(D)** The WAT collected from male offspring. The color codes of balls are listed as follows; blue color represents offspring gender (M= male; F= female) -obese mother-normal carbohydrate diet (M/F-OB-NC); Red color represents offspring gender-normal mother-high carbohydrate diet (M/F-N-HC); Purple color represents offspring gedner-obese mother-high carbohydrate diet (M/F-OB-HC); Green balls represent offspring gender-normal mother-normal carbohydrate diet (M/F-N-NC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

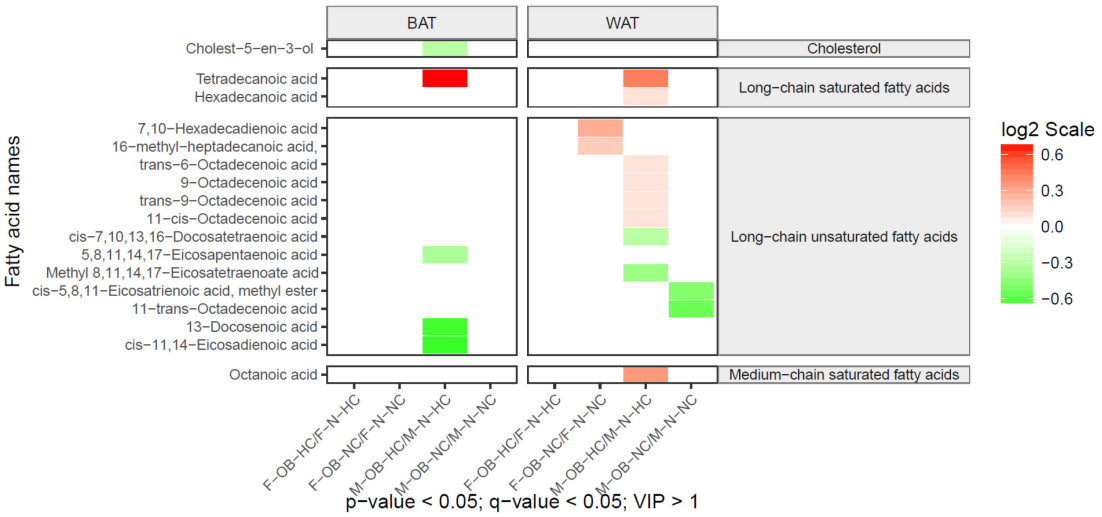

Figure 5. The heatmap demonstrates the offspring's metabolome profiles of brown adipose tissue (BAT) and white adipose tissue (WAT) affected by maternal obesity. The relative abundances of metabolites were plotted by using $\log_2$ scale. Fold changes of metabolite concentrations related to their corresponding controls are illustrated by red color (increasing levels when born to an obese mother) and green color (decreasing levels when born to an obese mother). Only the metabolites with a significant p-value (Tukey's HSD: $p < 0.05$), q-value (FDR: $q<0.05$), and VIP <1 are shown.

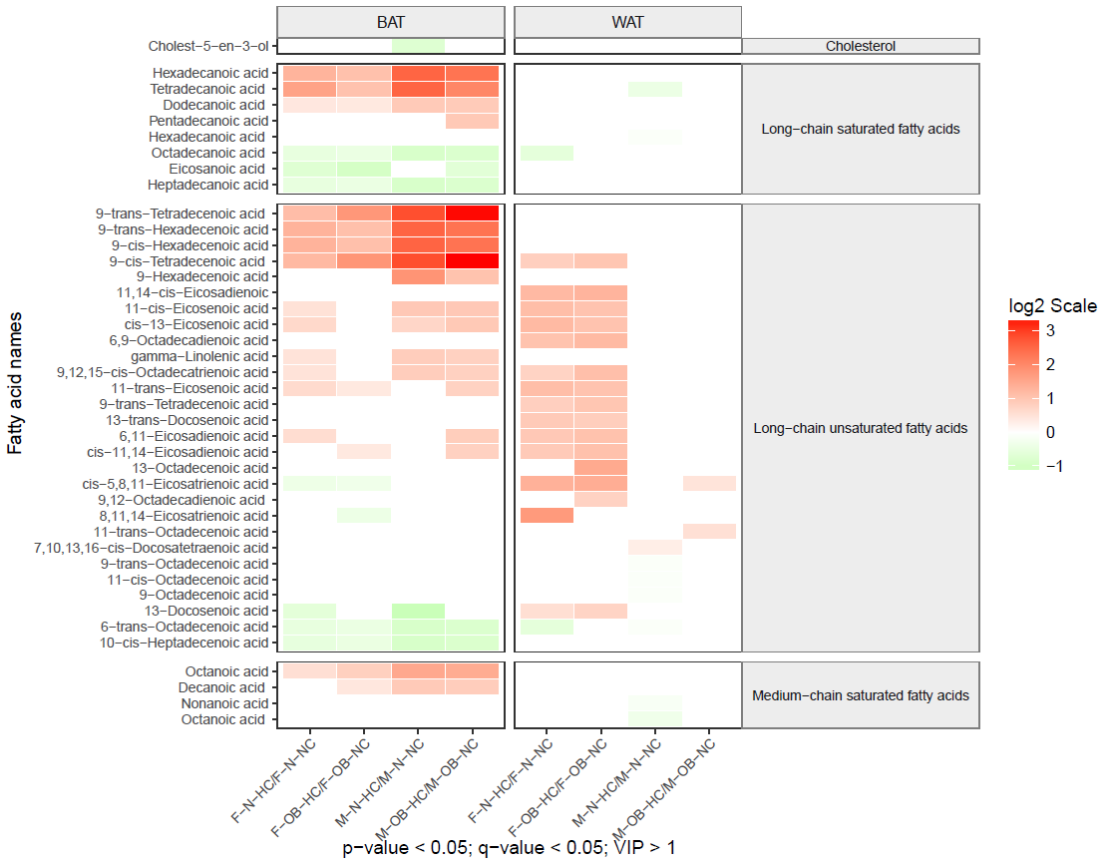

Figure 6. The heatmap shows the effect of a high-carbohydrate (HC) diet on the offspring's brown adipose tissue (BAT) and white adipose tissue (WAT) metabolomes. The relative concentration of metabolites was illustrated by using $\log_2$ scale. Fold changes of metabolite concentrations related to their normal-carbohydrate (NC) diet controls are shown with the red color (increasing levels in the HC diet group) and green color (decreasing levels in the HC diet group). Only the metabolites with a significant p-value (Tukey's HSD: $p < 0.05$), q-value (FDR: $q<0.05$), and VIP <1 are shown.

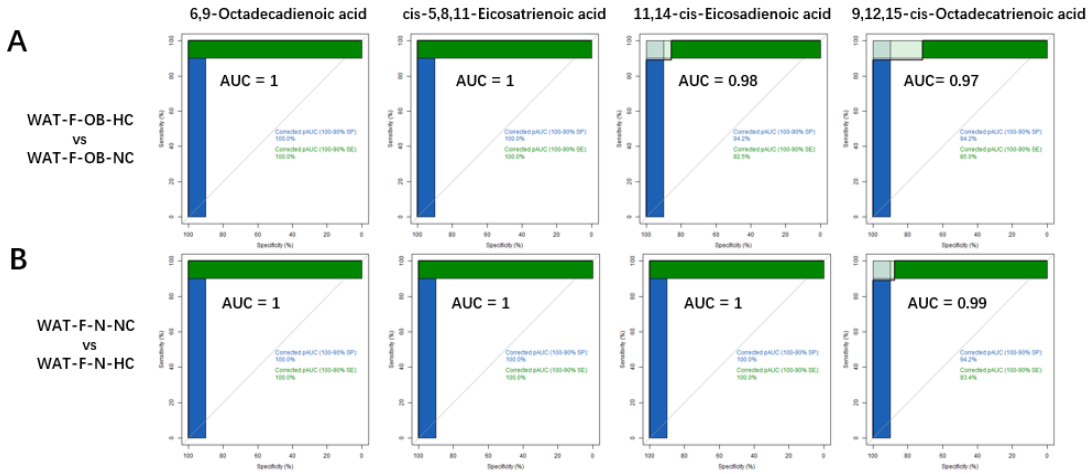

Figure 7. Receiver operating characteristic (ROC) curves of four fatty acids detected from the white adipose tissue (WAT) of female mice offspring under the influence of a high-carbohydrate (HC) diet. All fatty acids exhibited an area under the ROC curve greater than 0.95. (A) Comparison between F-OB-HC and F-OB-NC for 6,9-Octadecadienoic acid, cis-5,8,11-Eicosatrienoic acid, 11,14-cis-Eicosadienoic, and 9,12,15-cis-Octadecatrienoic acid. (B) Comparison between F-N-HC and F-N-NC for 6,9-Octadecadienoic acid, cis-5,8,11-Eicosatrienoic acid, 11,14-cis-Eicosadienoic and 9,12,15-cis-Octadecatrienoic acid. Abbreviations are listed as follows: F=female; OB=obese mother; N= normal mother; HC = high carbohydrate; NC= normal carbohydrate.

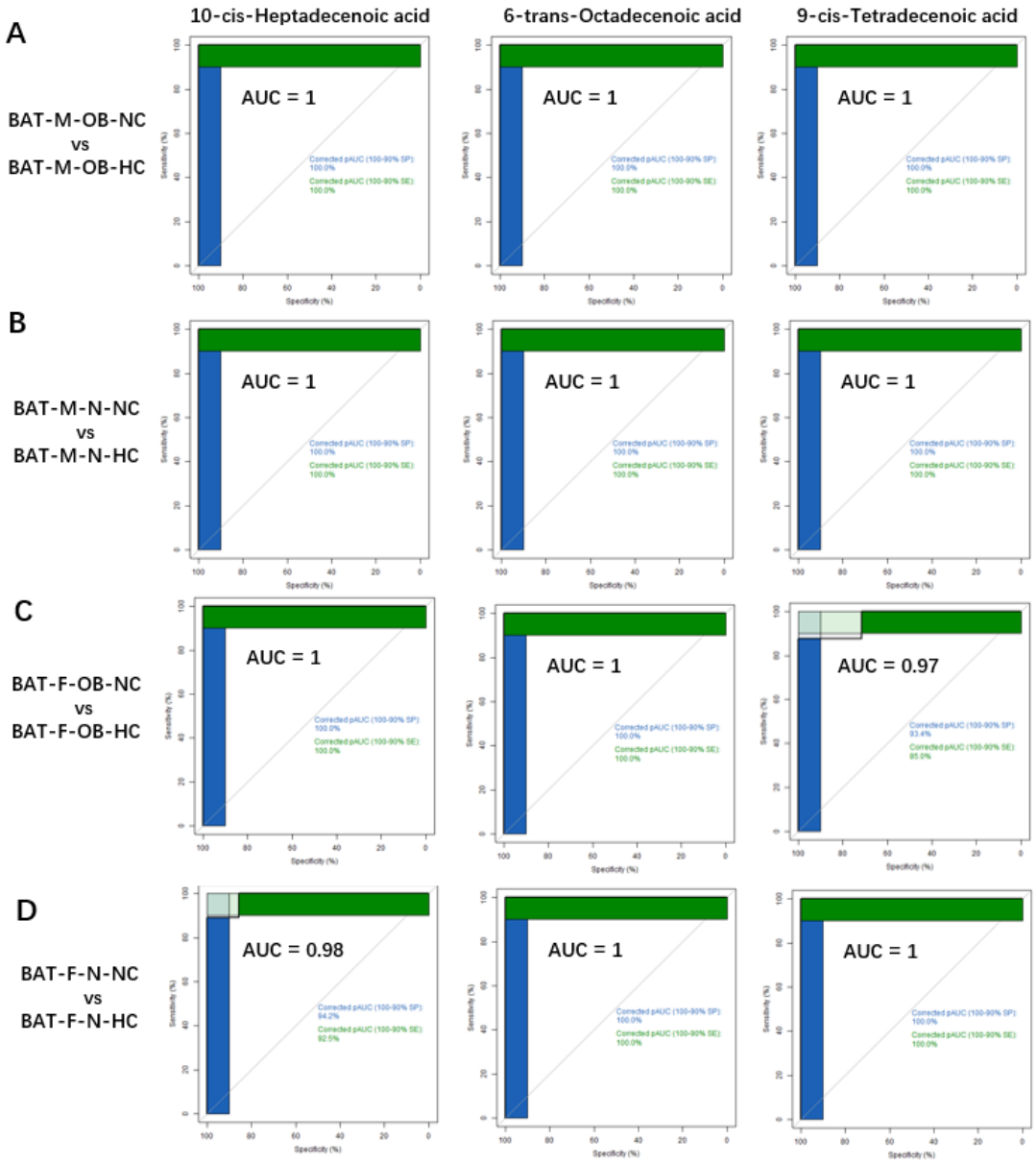

Figure 8. Receiver operating characteristic (ROC) curve of three fatty acids detected from the brown adipose tissue (BAT) of female mice offspring under the influence of a high-carbohydrate diet. All fatty acids exhibited an area under the ROC curve greater than 0.95. **(A)** Comparison between M-OB-HC and M-OB-NC for 10-cis-Heptadecenoic acid, 6-trans-Octadecenoic acid, and 9-cis-Tetradecenoic acid. **(B)** Comparison between M-N-HC and M-N-NC for 10-cis-Heptadecenoic acid, 6-trans-Octadecenoic acid, and 9-cis-Tetradecenoic acid. **(C)** Comparison between F-OB-HC and F-OB-NC for 10-cis-Heptadecenoic acid, 6-trans-Octadecenoic acid, and 9-cis-Tetradecenoic acid. **(D)** Comparison between F-N-HC and F-N-NC for 10-cis-Heptadecenoic acid, 6-trans-

Octadecenoic acid, and 9-cis-Tetradecenoic acid. Abbreviations are listed as follows: F=female; OB=obese mother; N= normal mother; HC = high carbohydrate; NC= normal carbohydrate.