Maternal-fetal hepatic and placental metabolome profiles are associated with reduced fetal growth in a rat model of maternal obesity

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Abstract

Maternal obesity is associated with a range of pregnancy complications, including fetal growth restriction (FGR), whereby a fetus fails to reach its genetically determined growth. Placental insufficiency and reduced nutrient transport play a role in the onset of FGR. Metabolomic profiling has the potential to reveal altered maternal and fetal metabolic pathways in a model of diet induced obesity during pregnancy, leading to reduced fetal growth. We examined the metabolome of maternal and fetal livers, and placenta following a high fat and salt intake. Sprague Dawley rats were assigned to a) control diet (CD;1% salt, 10% kcal from fat), b) high salt diet (SD; 4% salt, 10% kcal from fat), c) high fat diet (HF; 1% salt, 45% kcal from fat) or d) high-fat high-salt diet (HFSD; 4% salt, 45% kcal from fat) 21 days prior to pregnancy and during gestation. Metabolites from maternal and fetal livers, and placenta were identified using gas and liquid chromatography combined with mass spectrometry. Maternal HF intake resulted in reduced fetal weight. Altered metabolite profiles were observed in the HF maternal and fetal liver, and placenta. Polyunsaturated fatty acid metabolism was significantly altered in maternal and fetal liver by maternal fat intake. Results presented here show that an excess of essential fatty acids may be detrimental during placentation and associated with a reduction in fetal weight. Additionally, maternal, placental and fetal response to increased fat consumption seems likely to involve palmitoleic acid utilization as an adaptive response during maternal obesity.

Keywords: Maternal obesity; Fetal growth restriction; Metabolomics; Palmitoleic acid; Fat.

Introduction

Increased rates of obesity and related metabolic disorders worldwide are largely attributed to high fat and sugar intake and lack of exercise. Obesogenic lifestyle choices prior to and during pregnancy have been associated with a range of maternal complications such as gestational diabetes, preterm birth and fetal growth restriction (FGR). FGR is defined as the inability of a fetus to reach its genetically determined growth potential (Kusinski, Stanley, Dilworth, Hirt, Andersson, Renshall, Baker et al. 2012). Characteristics of FGR include reduced birth weight, abnormal placentation, fetal hypoglycaemia, hypoxia and increased fetal cortisol concentrations (Economides, Nicolaides, Campbell 1991; Kovo, Schreiber, Ben-Haroush, Cohen, Weiner, Golan, Bar 2013; Kyriakakou, Malamitsi-Puchner, Mastorakos, Boutsikou, Hassiakos, Papassotiriou, Kanaka-Gantenbein 2009; Mayhew, Ohadike, Baker, Crocker, Mitchell, Ong 2003). FGR is associated with 43-50 % of stillbirths (Gardosi, Kady, McGeown, Francis, Tonks 2005; McCowan, George-Haddad, Stacey, Thompson 2007; McCowan, Thompson, Taylor, North, Poston, Baker, Myers et al. 2013) and 42 % of early neonatal deaths (Roex, Nikpoor, van Eerd, Hodyl, Dekker 2012) in the Western world. The metabolic changes associated with maternal obesity such as insulin resistance, hypertension, elevated triglycerides, increased systemic inflammatory profiles and oxidative stress can lead to placental insufficiency (Hajer, van Haeften, Visseren 2008; Huang, Yu, Keim, Li, Zhang, Zhang 2014) and mitochondrial abnormalities in the placenta (Mele, Muralimanoharan, Maloyan, Myatt 2014). Similarly, excessive fat and/or salt intake during pregnancy may not provide the optimal balance of nutrients conducive to fetal growth and placental nutrient transfer, thereby resulting in a state of malnutrition (Ay, Kruithof, Bakker, Steegers, Witteman, Moll, Hofman et al. 2009).

To date, the use of metabolomics in pregnancy studies has largely been utilised to identify unique metabolic profiles prior to the onset of disease with an aim to develop effective

biomarkers and screening tests (Horgan, Broadhurst, Walsh, Dunn, Brown, Roberts, North et al. 2011; Kenny, Broadhurst, Dunn, Brown, North, McCowan, Roberts et al. 2010). In a comprehensive study of human plasma metabolic profiles in pregnancies complicated by reduced fetal weight, a phenotypic signature of reduced fetal growth was identified; this was validated in an animal model of placental insufficiency (Horgan, Broadhurst, Walsh, Dunn, Brown, Roberts, North et al. 2011). However, the influence of maternal fat and salt intake alone or in combination on maternal-fetal and placental metabolic profiles has not been examined previously. The current study, using state of the art metabolomics techniques, provides evidence that obesogenic maternal diet negatively impacts on the developing fetus and placental transfer which may potentiate risk of maternal metabolic disruption and subsequent reductions in fetal weight. Our animal model offers a robust nutritional paradigm in which the mechanistic aspects of maternal metabolism, placental transfer and subsequent fetal growth disruptions may be better understood.

Materials and Methods

Animal experiments

80 female Sprague Dawley rats were fed a standard control chow diet *ad-libitum* from weaning until day 90 of age and maintained at 25°C and a 12h light: 12h dark cycle. Rats were then randomly assigned to one of four dietary groups and fed *ad-libitum* for 21 days prior to pregnancy and throughout gestation. The experimental groups were fed either (1) Control (CD, n=20) purified standard chow diet (1% NaCl, 10% kcal from fat); (2) 4% Salt diet (SD; 4% NaCl, 10% kcal from fat, n=20); (3) High-fat diet (HF; 1% NaCl, 45% kcal from fat, n=20) or (4) High-fat 4% Salt (HFSD; 4% NaCl, 45% kcal from fat). Female rats (115 days of age ± 2, (n=20/group)) were time-mated using an estrous cycle monitor (Fine

Science Tools, USA). Day 0 of pregnancy was determined by the presence of spermatozoa after a vaginal lavage and females individually housed thereafter. Food and water intake and body weight of dams were recorded every two days. Pregnant dams were weighed, anaesthetised using isofluorane and killed by decapitation at gestational day 20. Fetuses were excised from the mother, separated from the placenta and sexed by ano-genital distance; fetal body weight and placental weight were recorded. All fetuses were then killed by decapitation. Maternal and fetal livers were excised and weighed. Tissues were snap frozen in liquid nitrogen and stored at -80^{o}C until further analysis. All animal procedures were approved by the Animal Ethics Committee of the University of Auckland (approval R1069).

Statistical analysis of maternal and fetal data

Statistical analysis was performed using SigmaPlot for Windows version 12.0 (Systat Software Inc., San Jose, CA). All the other data were analyzed by two-way factorial ANOVA, with maternal high fat and maternal high salt as factors. Holm–Sidak post hoc tests were performed where indicated to detect further differences between groups. Differences between groups were considered significant at $p<0.05$. All data are presented as means ± SEM unless otherwise stated.

Chemicals and reagents

L-Alanine-2,3,3,3-d_4, Indole-d_5, sodium hydroxide, pyridine and methylchloroformate (MCF) were purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol and chloroform were obtained from Merck (Darmstadt, Germany). Sodium bicarbonate and anhydrous sodium sulphate were purchased from Ajax Finechem (NSW, Australia).

Metabolite extraction

100 mg of frozen tissue was ground using an autolyser (TissueLyser II, Qiagen). Sample and methanol/water solutions were kept on dry ice prior to extraction. 1000µL of 50%

methanol/water solution was added along with internal standards – 40µL of 10mM d_4 alanine and 40µL of 0.5mM d_5 tryptophan, and vortexed. Samples were centrifuged at -5°C, 3500 rpm for 5 minutes. Following supernatant collection, the same procedure was performed using 80% and 100% methanol. Combined methanol extracts were vortexed and divided into two aliquots: 1000µL for liquid chromatography-mass spectrometry (LC-MS) and 1500µL for gas chromatography-mass spectrometry (GC-MS) analysis. 100µL from each sample was taken and pooled. This was then divided into QC samples respective to each tissue group and diet. Samples were dried by SpeedVac (Thermo Scientific Savant SC250EXP SpeedVac Concentrator with a Savant RVT4104 Refrigerated Vapor Trap) for 5h (no heat, 0.8 vacuum pressure) and subsequently stored at -80°C for chromatography analysis.

GC-MS and LC-MS analysis

GC-MS dedicated metabolic extracts were processed using a methyl chloroformate (MCF) method and analyzed in accordance with the protocol published by Smart *et al.* (Smart, Aggio, Van Houtte, Villas-Boas 2010).

Samples dedicated for LC-MS analysis were kept on ice and processed in the same random order as GC-MS samples. The dried samples were re-suspended in 300µL of chilled MQ water and centrifuged for 15 min at 12,000rpm at 4ºC. 200µL was transferred into a LC-MS vial and loaded for analysis. The samples were analysed by UHPLC-MS; Accela 1250 coupled to a Q-Exactive Orbitrap mass spectrometry system (Thermo Fisher Scientific Inc., MA, USA) operating in heated electrospray ionisation mode. Samples were analysed consecutively in positive ion mode. Chromatographic separations were performed using a Syncronis C18 column (100 x 2.1mm, 1.7µm; Thermo Fisher Scientific, Auckland, New Zealand). Solvent A and B were 0.1 % formic acid in water and 0.1% formic acid in acetonitrile, respectively. A flow rate of 0.40 mL/min was applied with a gradient elution profile (95% A for 30 s and subsequently ramped to 99% B at 13 min, followed by a 2 min

hold at 99% B before a rapid return to 95% A over 1 min and re-equilibrated for 4 min). The column and samples were maintained at temperatures of 30^{o}C and 4^{o}C, respectively. A 5µL tissue sample volume was injected. Centroid MS scans were acquired in the mass range of 80 –1200m/z using the Orbitrap mass spectrometer with mass resolution of 35,000 (FWHM as defined at m/z 400), AGC 1 e6, IT 100 ms, Sheath gas 40, Aux gas 10, Sweep gas 5, Spray voltage 4.00kV, Capillary temperature 320^{o}C, S-lens RF 50.0, and heater temperature 0^{o}C.

Data analysis

GC-MS data

Raw data from the GC-MS platform were directly processed with ChemStation Agilent software (MSD ChemStation E.01.00.237, 1989-2008 Agilent Technologies Inc., CA, USA) and converted to a NetCDF format. Data was deconvoluted and an ion report was produced using AMDIS v2.71 (NIST, Boulder, CO, USA) (Stein 1999). Metabolites were identified using in-house MS library and Metab3, an upgrade of an in-house R-script Metab (Aggio, Villas-Boas, Ruggiero 2011), compatible with R v3.0.2 (R Development Core Team 2008).

LC-MS data

Raw data from LC-MS was processed using SIEVE (version 2.1.377, Thermo Fisher). Frame settings were to include molecules where; mass to charge ratio was between 85 and 1200, and a retention time between 0 and 15 min with alignment was bypassed. Unaligned data were framed with parameters of 1000m/z width ppm, ICIS peak integration, retention time width of 1 min and abundance greater than 5 x 10^{7}. As some of the resulting frames, based on a width of 1 min, contained more than one peak, the raw data was processed a second time with a reduced width of 12s. SIEVE data processing resulted in a data matrix of retention time, ion mass and a given frame intensity.

Metabolite identification

All GC-MS metabolites were identified by mass to charge ratio (m/z) and retention time. Unidentified metabolites with m/z 55 are fatty acids with one double bond and were classified as a monounsaturated fatty acid (MUFA). Unknowns with m/z 67 and 79 have two and three or more double bonds and have been classified as polyunsaturated fatty acids (PUFA) (Zhang, Li, Sun, Hu, Wang, Zhang, Ding 2014).

LC-MS data was analysed using PutMedID software (Brown, Wedge, Goodacre, Kell, Baker, Kenny, Mamas et al. 2011) and metabolites with an H+ adduct were used for identification. Only statistically significant frames from LC-MS were assessed for validity *i.e.* one peak in each frame and correct integration. PutMedID, publicly available software (http://www.mcisb.org), was used to help with putative identification. Identification was based on four proposed levels of metabolite identification (Sumner, Amberg, Barrett, Beale, Beger, Daykin, Fan et al. 2007).

Metabolic pathway analysis

Significant differences in metabolites identified following univariate analysis led to pathway analysis in MetPA (MetabolAnalyst 2.0) (Xia, Wishart 2011) to identify potential pathways likely to be involved in the occurrence of reduced fetal weight. Differences between groups were considered significant at $p<0.05$

Statistical analysis of the metabolomics data

GC-MS and LC-MS data were normalised by the abundance of the internal standard (d_4-alanine for GC-MS and d_5-tryptophan for LC-MS) and the weight of the sample (~100mg). Normalised data were log-transformed and statistical analysis was performed using R software. A mixed-effects three-way ANOVA was performed, with fat, salt and fetal sex as

main factors (Gueorguieva, Krystal 2004). A student *t-test* was performed to calculate metabolite differences between purified experimental diets. To minimise the risk of increasing Type 1 errors through multiple comparisons, the false discovery rate (FDR) was controlled using a Benjamini Hochberg (BH) adjustment (Benjamini, Hochberg 1995). Differences between groups were considered significant at $p<0.05$ after the FDR adjustment. PCA analyses were performed using Pareto scaled values. Linear regression was performed on selected metabolites for significant correlations.

Results

Effect of diet on maternal weight

A significant effect of fat ($p=0.04$) was observed; dams in both HF and HFSD groups were heavier than CD and SD group dams. An overall effect of fat ($p=0.049$) was observed in HF-fed dam liver weights after adjusting for body weight (Table 1).

Table 1

Effect of maternal diet on fetal and placental weights

An overall effect of fat ($p=0.044$) was observed on male fetal and liver weight ($p=0.049$); both HF and HFSD groups had reduced body weight and liver weight (Table 2). An interaction effect ($p=0.002$) was observed in male placentas due to maternal HF reducing placental weight and weights in HFSD, being partially restored with the inclusion of salt in the maternal diet (HFSD). Following *post hoc* analysis, weight of HF male placentas were significantly less than all other groups ($p<0.05$) and HFSD placental weights significantly lower than CD and SD only ($p<0.05$) (Table 2.).

Table 2

In females, an interaction effect ($p=0.010$) was observed on body weight, with maternal HF fetuses having a reduced body weights when compared to all other groups. However, with the

introduction of salt into the diet (HFSD), this was reversed. Following *post hoc* analysis, female HF fetuses had a significantly lower body weight when compared to all other groups (Table 2).

Effect of maternal diet on placental metabolism

Following isotope removal, a total of 262 placental metabolic features underwent statistical analysis. Six metabolic peaks from GC-MS analysis were identified as significantly different in placental tissue in response to a HF maternal diet compared to control diet after the FDR adjustment ($p<0.001$; Figure 1). Significantly identified metabolic peaks belonged to the lipid class. Five out of six peaks were increased in the placenta with levels of palmitoleic acid reduced in response to a maternal HF diet. Principal Component Analysis (PCA) also revealed differences between HF and CD placenta (Figure 2). The principal metabolites influencing this separation were again from the lipids class. A pathway analysis identified the biosynthesis of unsaturated fatty acids ($p<0.001$), linoleic acid metabolism ($p=0.01$) and alpha linolenic acid metabolism ($p=0.03$) as being significantly altered in maternal HF placentas.

None of the metabolic peaks from GC- or LC-MS were classified as significantly different in response to a maternal SD with respect to salt following FDR adjustment.

Figure 1
Figure 2

Effects of maternal diet on the maternal and fetal hepatic metabolome

A total of 220 liver metabolic features underwent statistical analysis from GC- and LC-MS data. Thirteen metabolic peaks from GC- and LC-MS were identified to significantly differ in response to a maternal HF diet (Figure 3). Metabolic profiles did not change in response to a maternal SD and no interactions were observed. Common significant alterations in response to maternal HF diet for both maternal and fetal liver were observed only for 2 metabolites. Palmitoleic acid was significantly reduced by the maternal HF diet in both the maternal

($p<0.0001$, after FDR adjustment) and fetal livers ($p=0.01$, after FDR adjustment). The other metabolite down regulated by the high-fat maternal diet belonged to the polyunsaturated fatty acids group (PUFA), but could not be further identified. Fetal liver linoleic acid and its derivatives increased in response to the maternal HF diet, but no differences were observed in the maternal liver. Pathway analysis identified the biosynthesis of unsaturated fatty acids ($p<0.001$) and linoleic acid metabolism ($p=0.02$) as a significantly altered pathway in the fetal liver.

Figure 3

Fetal sex differences in placenta and fetal liver unrelated to maternal diet

Statistical analysis revealed that fetal sex had no effect on the differences observed in relation to HF and/or salt intake.

Metabolic composition of the control and high-fat purified diets used in the study

Following FDR adjustment, 30 metabolic peaks from 149 were identified as significantly different between CD and HF purified diets. 11 metabolites, primarily lipids, were increased and 19 metabolites, primarily amino acids, were decreased in the HF diet compared to the CD diet (Figure 4).

Figure 4

Discussion

Results presented here emphasize the importance of a nutritionally balanced diet during pregnancy and those considering pregnancy. Changes in maternal metabolites, particularly lipid moieties, following exposure to HF consumption can have detrimental consequences on fetal growth and development. There is extensive evidence that maternal obesity is associated with obstetric complications (Anderson, Sadler, Stewart, Fyfe, McCowan 2013; McDonald, Han, Mulla, Beyene, Grp 2010; Salihu, Mbah, Alio, Kornosky, Bruder, Belogolovkin 2009).

Reduced fetal weight and increased numbers of stillbirths are observed in experimental models of maternal obesity (Hayes, Lechowicz, Petrik, Storozhuk, Paez-Parent, Dai, Samjoo et al. 2012; Howie, Sloboda, Kamal, Vickers 2009). Changes in rodent placental blood vessel maturation, reduced growth in the labyrinth, reduced placental weights related to HF diet have also been reported (Akyol, Langley-Evans, McMullen 2009; Hayes, Lechowicz, Petrik, Storozhuk, Paez-Parent, Dai, Samjoo et al. 2012; Mark, Sisala, Connor, Patel, Lewis, Vickers, Waddell et al. 2011). However, metabolomic analysis has rarely been utilised to investigate biochemical pathways that may lead to disrupted placentation and fetal growth. We therefore utilised a well-established model of maternal diet-induced obesity to examine potential mechanisms involved in the metabolic disturbances leading to reduced fetal weight.

In the current study, alterations in metabolic profiles following a maternal HF diet were observed in the placenta and fetal liver. This was evident by significant increases in essential fatty acids (EFA) and long-chain PUFA (LCPUFA). EFAs can include linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3), which are both converted to the LCPUFA such as docosahexaenoic acid (C22:6n-3) and eicosapentaenoic acid (C20:5n-3by desaturation (Δ5- and Δ6-desaturases) and elongation enzymes. EFAs require an external dietary source as humans and rats cannot synthesise EFAs endogenously (Innis 2008). Therefore, the fetus relies solely on the maternal supply of EFAs. As LCPUFAs are essential to brain and retina development previous study has shown that an excess of these lipids may have a negative effect on fetal and placental development (Uauy, Hoffman, Peirano, Birch, Birch 2001). Our study shows that placenta and fetal liver from HF-fed mothers had higher concentrations of linoleic, α-linolenic and 11,14-eicosadeienoic acids. Interestingly, the HF purified diet contained higher levels of n-6, thereby increasing the n-6:n-3 PUFA ratio. It has been suggested that an increase in this ratio is detrimental to the placenta *via* promotion of inflammatory processes (Simopoulos 2011). Cetin *et al*. (Cetin, Giovannini, Alvino,

Agostoni, Riva, Giovannini, Pardi 2002) also reported changes in EFAs and LCPUFA in fetal plasma in FGR compared to normal human fetuses, however, the dietary fat intake in these subjects was not determined.

The pathway involving the biosynthesis of unsaturated fatty acids was affected by maternal HF intake in the placenta and the fetal liver. Pathways converting the saturated palmitic (C16:0) and stearic acid (C18:0) into monounsaturated palmitoleic (C16:1) and oleic acid (C18:1) by the rate-limiting enzymes stearoyl-CoA desaturase in the placenta and fetal liver were primarily altered by a maternal HF diet. Palmitoleic acid is an omega-7 MUFA, which is commonly found in the liver and is a prominent constituent of adipose tissue and primarily sourced from food or desaturated from palmitic acid (C16:0). In the current study, decreased palmitoleic acid (C16:1n-7c) was observed in all three tissues, in response to the maternal HF diet. This was unexpected as both palmitic and palmitoleic acids were significant components of the HF purified diet. However, palmitoleic acid increases insulin sensitivity, suppresses pro-inflammatory gene expression and improves hepatic lipid metabolism in mice (Yang, Miyahara, Hatanaka 2011). Fatty acids with different degrees of saturation have different effects on insulin sensitivity and lipid metabolism. Diets rich in palmitoleic acid improve circulating lipid profiles in animals (Matthan, Dillard, Lecker, Ip, Lichtenstein 2009) and humans (Garg, Blake, Wills, Clayton 2007). Palmitoleic acid functions as an adipose tissue-derived lipid hormone, stimulating muscle insulin action and suppressing hepatosteatosis (Cao, Gerhold, Mayers, Wiest, Watkins, Hotamisligil 2008). Given the effects on palmitoleic acid, we speculate that palmitoleic acid was metabolised by the maternal, placental and fetal tissues to counteract the seemingly negative effects of saturated fatty acids on maternal metabolism, which could, in part, explain the decrease in concentrations compared to the control diet. However, further research is necessary to investigate the exact pathways involved in the palmitoleic acid utilization and its direct effects on placentation and the fetal

growth. As palmitic acid concentrations were not different in any tissues, we speculate that the surplus C16:0 in HF diets was stored in adipose tissue or utilised as energy as suggested by PPARα and GLUT2 expression in the same cohort of animals (Reynolds, Vickers, Harrison, Segovia, Gray 2014).

Analysis of purified HF and CD diets showed that the abundance of most dietary amino acids was significantly lower in the maternal HF diet. However, this did not correspond to altered metabolism in the placenta or maternal and fetal liver. Previous studies of FGR suggest that in a hypoxic placenta, amino acid transport capacity is reduced (Regnault, de Vrijer, Galan, Wilkening, Battaglia, Meschia 2013). Down regulation of the fetal oxidative metabolism and growth rate results in normal or even higher concentrations of amino acids in the FGR fetuses compared to the healthy controls. In the current study, this may explain why no observable differences in the hepatic or placental amino acids abundance were seen between the dietary groups. In the same cohort of animals, previously published data from our group showed that amino acid transporters were also altered (Reynolds, Vickers, Harrison, Segovia, Gray 2014).

Concluding Remarks

In conclusion, EFAs, are necessary for fetal development. However, we showed that excessive EFAs may disrupt placentation and subsequent fetal growth, contributing to FGR. We speculate that maternal, placental and fetal response to saturated fatty acid excess involves palmitoleic acid utilization as a potential adaptive mechanism during maternal obesity, which may play an important role in the development or predisposition to FGR.

Author contributions: KS, PNB, CG and MHV designed research; KM and CJH conducted the research with support from KR in the biostatistical analysis; KS, CG and KM wrote manuscript; MHV, PNB, JLS, CMR, KR and SGV-B critically evaluated the manuscript.

Compliance with Ethical Standards: All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Animal procedures were approved by the Animal Ethics Committee of the University of Auckland (approval R1069). This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest: KM, CG, CMR, MHV, CJH, JLS, KR, SGV-B, PNB, KS declare that they have no conflict of interest.

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Figures legends

Figure 1.

Maternal high-fat feeding affects the metabolic profile within the placenta. Data were analysed by ANOVA, with fat, salt and sex as factors. High:control fat ratio with 95 % confidence intervals. Maternal control diet is constant at 1. Identification (ID) of metabolites: 1= identified compounds, 2= putatively annotated compounds, 3= putatively characterised compound classes, 4= unknown compound. Platform: GC= gas chromatography mass-spectrometry; LC= liquid chromatography mass-spectrometry. Significances are indicated by *p<0.05, **p<0.01, ***p<0.001.

Figure 2.

Score plot of significant metabolic peaks in the placenta in response to a maternal HF diet. Principal Component Analysis were performed using Pareto scaled values.

Figure 3.

Maternal high-fat feeding affects the maternal and fetal hepatic metabolic profile. Data were analysed by ANOVA, with fat, salt and sex as factors. A Benjamini Hochberg adjustment was made for multiple comparisons. High:normal fat ratio with 95 % confidence intervals. Maternal control diet fat is constant at 1. Identification (ID) of metabolites: 1= identified compounds, 2= putatively annotated compounds, 3= putatively characterised compound classes, 4= unknown compound. Platform: GC = gas chromatography mass-spectrometry; LC = liquid chromatography mass-spectrometry. Significances are indicated by *p<0.05, **p<0.01, ***p<0.001

Figure 4.

Metabolic composition of control and high-fat experimental diets. High fat:control diet ratio with 95 % confidence intervals. Control diet is constant at 1. Identification (ID) of metabolites: 1= identified compounds, 2= putatively annotated compounds, 3= putatively characterised compound classes, 4= unknown compound. Platform: GC = gas chromatography mass-spectrometry; LC = liquid chromatography mass-spectrometry. Logged data were analysed by t-test. Significances are indicated by *$p<0.05$, **$p<0.01$, ***$p<0.001$

Tables and Figures

Table 1. Effects of maternal diet on dam body and liver weights.

Pregnant Dam	Groups				P-value		
	CD	HF	SD	HFSD	Fat	Salt	F*S
Weight (g)	418±17	469±10	450±9	462±19	0.04	NS	NS
Liver ($mg.g^{-1}$)	37.8±1.1	37.3±1.2	37.8±1.1	36.75±1.1	0.049	NS	NS

Dams were fed one of four diets: control (CD, n=8); high salt (SD, n=8); high fat (HF, n=8) and high fat and salt (HFSD, n=8). NS=non-significant. Holm-Sidak post-hoc tests were performed to detect any differences between groups, with * indicating significantly different from CD, and # indicating significantly different from HFSD ($p<0.05$). Data presented as mean ± SEM.

Table 2. Effects of maternal diet on fetal weights.

Fetal	Groups				P-value		
	CD	HF	SD	HFSD	Fat	Salt	F*S
Male							
Birth weight (g)	3.92±0.12	3.80±0.05	4.08±0.04	3.79±0.09	0.044	NS	NS
Liver (mg)	297±14	275±11	316±16	280±16	0.049	NS	NS
Placenta (g)	0.67±0.02	0.51±0.01*	0.60±0.01	0.56±0.02*	NS	NS	0.002
Female							
Birth weight (g)	3.81±0.14	3.55±0.06*	3.75±0.04	3.81±0.04	NS	NS	0.010
Liver (mg)	283±19	282±13	310±7	284±18	NS	NS	NS
Placenta (g)	0.60±0.02	0.50±0.01*#	0.56±0.01	0.57±0.01	NS	NS	<0.001

Maternal diet: control (CD); high salt (SD); high fat (HFD) and high fat and salt (HFSD) n=34-57/group. Data were analysed by ANOVA with fat and salt as factors. NS=non-significant. Holm-Sidak post-hoc tests were performed to detect any differences between groups, with * indicating significantly different from CD, and # indicating significantly different from HFSD ($p<0.05$).

Figure 1. Maternal high-fat feeding affects the metabolic profile within the placenta.

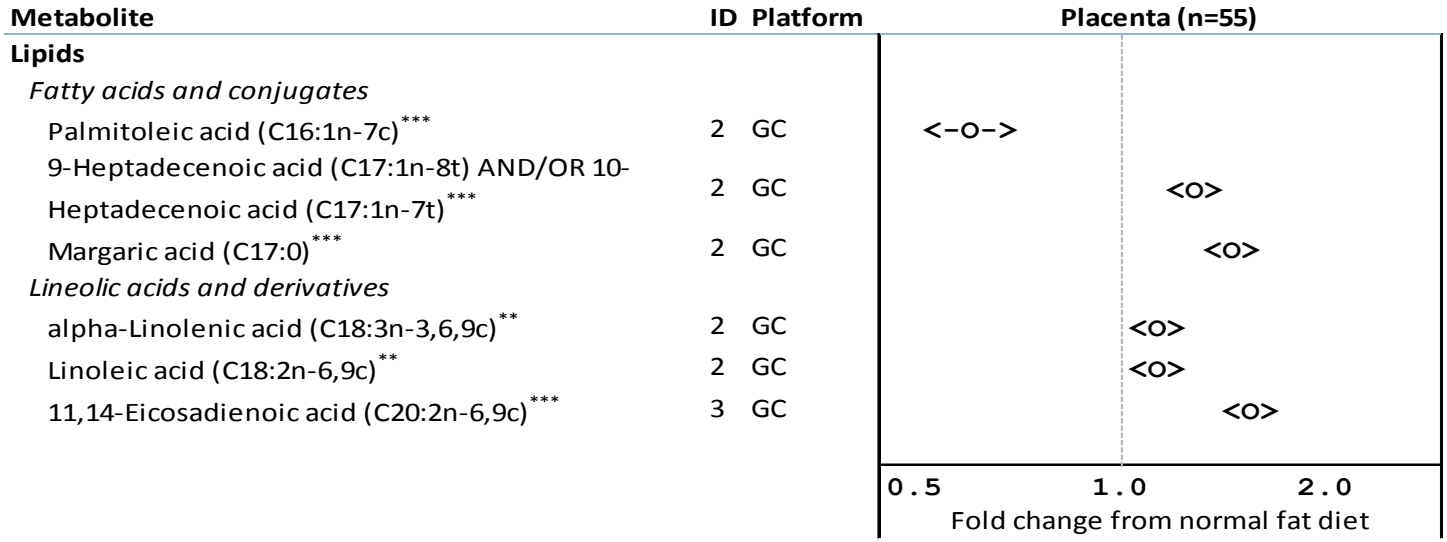

Figure 2. Score plot of significant metabolic peaks in the placenta in response to a maternal HF diet.

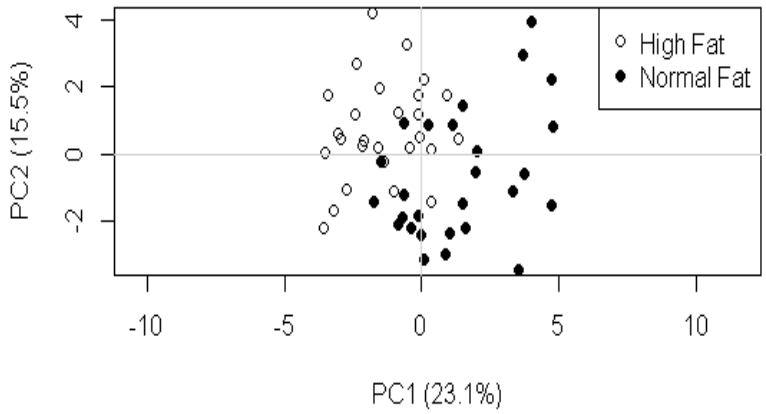

Figure 3. Maternal high-fat feeding affects the maternal and fetal hepatic metabolic profile.

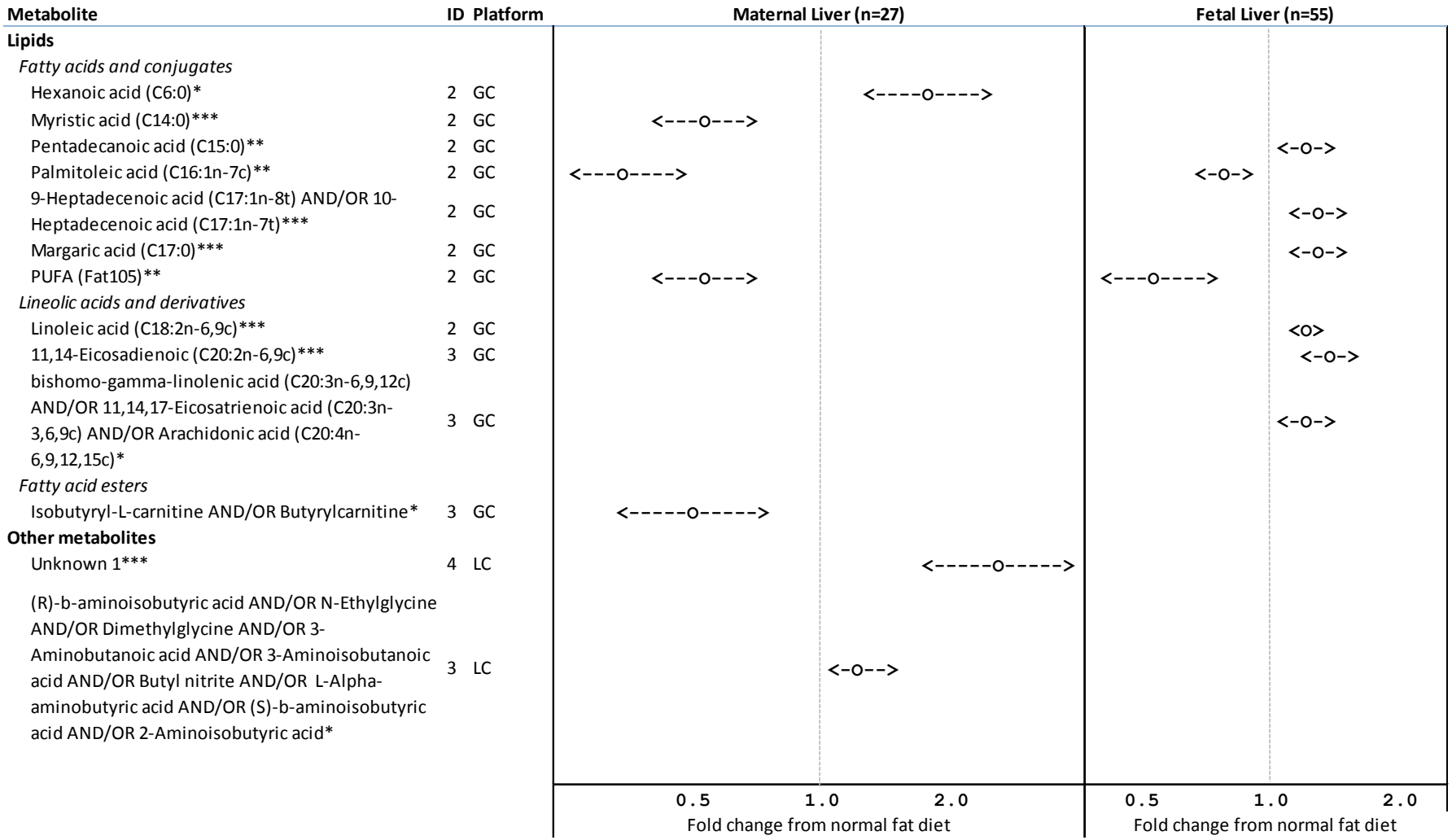

Figure 4. Metabolic composition of control and high-fat experimental diets.