

# Gender differences between parental pregnancy characteristics and nonalcoholic fatty liver disease in adolescents

Short Title: Gender difference in parent characteristics and adolescent NAFLD

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## Gender differences between parental pregnancy characteristics and nonalcoholic fatty liver disease in adolescents

Nonalcoholic fatty liver disease (NAFLD) is a complex chronic liver disorder. Examination of parental pregnancy-related characteristics may provide insights into the origins of risk of

NAFLD in offspring. We examined relationships between parental pregnancy-related characteristics and NAFLD in 1170 adolescent offspring aged 17-years participating in the Western Australian Pregnancy (Raine) Cohort Study. Fatty liver was diagnosed using liver ultrasound. NAFLD was diagnosed in 15.2% of the adolescents at age 17 years. In univariate analysis, maternal factors associated with NAFLD in female offspring were younger maternal age ( $p=0.02$ ), higher maternal pre-pregnancy BMI ( $p<0.001$ ), higher maternal weight gain by 18 weeks gestation ( $p<0.001$ ) and maternal smoking during pregnancy ( $p=0.04$ ). Paternal age or BMI were not associated with NAFLD in female offspring. In contrast, higher paternal BMI ( $p<0.001$ ), maternal pre-pregnancy BMI ( $p<0.001$ ) and lower family socio-economic status at time of birth ( $p=0.001$ ), but not parental age nor maternal gestational weight gain, were associated with NAFLD in male offspring. Using multivariate logistic regression, factors independently associated with NAFLD after adjusting for obesity in adolescent females included maternal obesity (OR 3.46, 95% CI 1.49-8.05,  $p=0.004$ ) and maternal weight gain  $\geq 6.0$ kg by the 18th week of gestation (OR 1.10, 95% CI 1.04-1.15,  $p < 0.001$ ). In adolescent males, family socio-economic status at the time of birth (OR 9.07, 95% CI 1.54-53.29,  $p=0.02$ ) remained significantly associated with NAFLD after multivariate modeling adjusted for adolescent obesity.

**Conclusion:** Early life contributors to NAFLD show considerable sexual dimorphism. Maternal obesity and higher early-mid gestational weight gain were associated with NAFLD in female offspring, while lower family socio-economic status at birth was associated with NAFLD in male offspring independent of adolescent obesity.

Nonalcoholic fatty liver disease (NAFLD) is a common, potentially progressive, complex chronic liver disorder. NAFLD associates with insulin resistance and features of the metabolic syndrome (1,2). Obesity is a dominant risk factor for NAFLD, with both subcutaneous and visceral adiposity being associated with NAFLD (3). Recent data describes considerable risk of liver related morbidity and mortality with progressive adiposity gains during the life course, from childhood through adulthood (4-9). This is concerning, given changing dietary patterns (10,11), and the rising prevalence of obesity (12-14) and of NAFLD (15). NAFLD is now common in children and adolescents (3) and is associated with similar metabolic risk factors as in adults despite varied histology (16). Various genetic polymorphisms associated with an increased risk of NAFLD have been reported (17-20), however obesity in an individual remains a major risk factor for NAFLD. Additionally, metabolic risk factors are prevalent in first-degree relatives of individuals with NAFLD (21-23). Maternal obesity has been highlighted as a risk factor for NAFLD (24-26), while early onset paternal obesity has been associated with elevated serum ALT levels in adult offspring (27). Though existing data support the heritability, or at least familial clustering of NAFLD and metabolic risk factors, the relative contribution of environmental, genetic and epigenetic factors on the NAFLD phenotype within families is less well ascertained (28). Heritability of NAFLD within families is estimated to be 20-70% but influences of genes versus environment are inadequately defined, with the PNPLA-3 genotype explaining only 10–12% of the variance in development of NAFLD (17,29). Twin studies have attempted to disentangle part of the intricacies of this complex disorder by assuming control for genetic and epigenetic factors. Consequently, studies of adult twins from the United States of America and the United Kingdom identified heritability of hepatic steatosis and fibrosis phenotypes as well as genotypes associated with these phenotypes (30-32). By contrast a Hungarian study could not confirm heritability of NAFLD in twins (21). Translation of existing data into knowledge about prevention or targeted early intervention for NAFLD in individuals and populations is lacking. In particular, there remains a paucity of population-based prospective studies examining prediction or heritability of NAFLD from pre-pregnancy and

pregnancy-related parent phenotypic and socio-demographic characteristics. Yet, such factors may be important regulators of inter-generational liver and metabolic disease through epigenetic and imprinting influences that potentially impact the health of offspring.

We have previously described gender-specific differences in the prevalence and phenotype of NAFLD in 17-year-old adolescents in the Western Australian Pregnancy (Raine) Cohort study (3). Adolescent males had a more adverse NAFLD phenotype, with a higher prevalence of the metabolic syndrome, greater visceral adipose thickness, higher fasting glucose, transaminase levels and systolic blood pressure but lower serum adiponectin and high-density lipoprotein levels than females (3). There was also a more pronounced association between body mass index (BMI) and skinfold thickness trajectories from early childhood through adolescence, and the NAFLD outcome in males than in females (4).

Further, adolescent females in the Raine Cohort who were diagnosed with NAFLD in addition to polycystic ovary syndrome (PCOS) had a similar phenotype to adolescent males with NAFLD that appeared mediated through obesity rather than androgen levels (33). A study from the same cohort has also found associations between higher maternal pre-pregnancy BMI and early pregnancy weight gain rate, and an adverse adolescent offspring cardio-metabolic profile (34). Given the recognized gender-different inter-relationship between NAFLD and cardio-metabolic risk factors (3), we sought to extend that observation by examining relationships between parental pregnancy-related and offspring birth characteristics and a subsequent diagnosis of NAFLD during adolescence.

The aims of this study were; (a) to examine inter-generational relationships between parental anthropometry, pregnancy-related factors and family socio-demographic characteristics coinciding with pregnancy, and a future diagnosis of NAFLD in adolescent offspring, and (b) to examine gender-specific relationships between parental pre-pregnancy obesity and NAFLD during adolescence in same-sex and opposite-sex offspring.

## Methods

We used prospectively collected data from the longitudinal Raine Cohort study to determine associations between pre-pregnancy and antenatal socio-anthropometric characteristics of parents, and outcomes of NAFLD in offspring at age 17 years. The Raine Study was initiated as a pregnancy cohort comprising 2,868 live-born children from 2,900 pregnancies. Between 1989 and 1992, women between 16 and 18 weeks' gestation were recruited mainly from the antenatal clinics of King Edward Memorial Hospital for Women, located in Perth, Western Australia. Gestational age was ascertained by maternal recall of the date of the last menstrual period, if certain, or else determined by 18-week ultrasound studies. Cross-sectional assessment of offspring was conducted at ages 1, 2, 3, 5, 8, 10, 14, 17 and 20 years. Assessments involved questionnaires regarding socio-demographic and lifestyle-related characteristics, anthropometry, basic resting cardiovascular examination and fasting blood tests. Age at menarche was prospectively recorded and checked during the 14 and 16-year assessments. The 17-year assessment of the adolescent cohort, conducted between July 2006 and June 2009, involved detailed questionnaires, anthropometric and cardiovascular examination, liver ultrasound and fasted blood tests. Blood testing was performed and NAFLD was diagnosed by liver ultrasound as previously described (3). Alcohol consumption per week reflecting typical intake during the preceding year was documented by self-reporting in a questionnaire and by the completion of a validated semiquantitative food frequency questionnaire (4). Adolescents with sonographic fatty liver and weekly alcohol intake of less than 210 g and 140 g for males and females, respectively, were classified as having NAFLD, consistent with recent NAFLD diagnosis guidelines (35). Institutional ethics committee approval was obtained from the Princess Margaret Hospital for Children Human Research Ethics Committee. Signed informed consent from parents or legal guardians and adolescent assent at 17 years were obtained.

The socio-economic status of the family at the time of birth was determined according to the Index of Relative Socio-economic Advantage and Disadvantage (IRSAD) domain of the Australian Bureau of Statistics Socio-economic Indexes For Areas (SEIFA) for the family's

residential postcode (36). Records of pre-pregnancy parental socio-demographic characteristics and anthropometry, antenatal health and neonatal birth anthropometry were reviewed. Pregnancy-related complications such as gestational diabetes, pre-eclampsia and eclampsia were collected from the antenatal records. Psychosocial and pregnancy-associated stressful events, such as pregnancy-related health problems, marital or relationship difficulties, financial, employment or residential difficulties or difficulties with another child or other children were also recorded. Mothers recorded the age at which breast-milk feeding stopped in a diary and this was clarified by direct interview during the ages 1,2 and 3-year surveys (26).

Central obesity in the adolescents was defined as a waist circumference  $\geq 80$  cm in females and  $\geq 94$  cm in males, consistent with age and gender-specific criteria of the International Diabetes Federation (37). Body mass index (BMI) was derived from weight (kg)/ height ( $m^2$ ), with underweight, normal weight, overweight and obese defined by BMI  $< 18.5$ ,  $18.5-24.9$ ,  $25-29.9$  and  $\geq 30$   $kg/m^2$  respectively. We defined adolescent obesity by waist circumference, as we have previously found waist circumference identified a higher proportion of obese adolescents than BMI. All references to parental, maternal or paternal BMI or obesity are specific to pre-pregnancy assessments, while adolescent refers to adolescent offspring.

### **Maternal pregnancy assessments**

At the time of recruitment into the study, women completed baseline questionnaires and provided an estimate of their pre-pregnancy weight. Maternal height was measured to the nearest 0.1cm during the pre-recruitment assessment and again at the subsequent assessment between 16 and 20 weeks gestation. Maternal pre-pregnancy BMI was calculated. Maternal weight at approximately 18 weeks and 34 weeks of gestation were obtained from the routine medical records. Pregnancy-associated or gestational weight gain at 18 weeks was computed as the difference between the 18-week pregnancy weight and

the pre-pregnancy weight, while gestational weight gain at 34 weeks was the difference between the 34-week pregnancy weight and pre-pregnancy weight.

### **Offspring birth and 17 year anthropometric assessment**

Birth weight was documented in kg. Ponderal index, a measure of leanness, was calculated from weight (kg)/height (m)<sup>3</sup> and percentage of expected birth weight defined as the percentage of expected weight adjusted for gestational age. Obesity in the 17-year-old adolescents was determined by waist circumference measured as the average of two measurements taken at the level of the umbilicus to the nearest 0.1 cm.

### **Abdominal ultrasound assessment and diagnosis of NAFLD**

The diagnosis of fatty liver (steatosis) during the 17-year cross-sectional assessment relied on specified liver ultrasound characteristics. We used the ultrasound protocol described by Hamaguchi and colleagues that provides 92% sensitivity and 100% specificity for the histological diagnosis of > 10% hepatic steatosis (38). Trained ultrasonographers performed liver ultrasound assessments as previously described (3). A single specialist radiologist who was blinded to the clinical and laboratory characteristics of the subjects interpreted the ultrasound images. Scores of 0 to 3, 0 to 2, and 0 to 1 were determined from captured images for liver echotexture (bright liver and hepatorenal echo contrast), deep attenuation (diaphragm visibility), and vessel blurring (intrahepatic vessel visibility), respectively. The diagnosis of fatty liver required a total score of at least 2, that included an echotexture score of at least 1. The severity of hepatic steatosis was classified by the total fatty liver score, as 0 to 1 (no fatty liver), 2 to 3 (mild fatty liver), or 4 to 6 (moderate to severe fatty liver). The intraobserver reliability (j statistic) for fatty liver was 0.78 (95% CI 0.73-0.88). A NAFLD diagnosis required sonographic fatty liver and exclusion of significant alcohol consumption (3). Testing for hepatitis B or C virus infections was not performed because notification rates for hepatitis B and C virus infections were on average less than 24/100,000 and 23/100,000,

respectively, for Western Australian teenagers between the ages of 15 and 19 years over the study period (3).

### Statistical analysis

Case-control analysis was used to identify parent factors associated with adolescent NAFLD.

We detected a significant interaction between gender and maternal pre-pregnancy BMI ( $p < 0.001$ ) in relation to the NAFLD outcome. Consequently, data for males and females were analyzed separately. Parametric descriptive data are presented as the mean and standard deviation while non-parametric data are reported as the median and interquartile

range. The main outcome variables were the presence or absence of NAFLD and the severity of sonographic hepatic steatosis. Differences in continuous anthropometric variables between adolescents with or without NAFLD were examined using the independent t-test or

analysis of variance (ANOVA) for normally distributed variables and non-parametric analysis (Mann-Whitney U or Kruskal-Wallis test) for non-normally distributed data. All p values were

reported as two-sided and interpreted at the 5% level of significance. Multiple logistic regression analysis was used to calculate the odds of NAFLD in the offspring from parent

anthropometric characteristics. Data were analyzed using IBM SPSS statistics for Windows (version 20.0; Armonk, NY: IBM Corp.).

### Results

Parental characteristics at the time of pregnancy are summarized in Table 1 and offspring characteristics at birth and during late adolescence (age 17 years) are summarized in Table

2. At age 17 years, 1170 community-based 17-year-old adolescents (592 male) had liver ultrasound assessment for fatty liver. Adolescent males and females had similar alcohol intake patterns (Table 2). Three adolescents (2 female and 1 male) with ultrasound-

diagnosed fatty liver were excluded from a NAFLD diagnosis due to excessive alcohol consumption. All adolescent females were post-menarchal (mean age at menarche 12.8 [standard deviation 1.1] years. Oral corticosteroid use was reported by 0.03% of participants,

while oral contraceptive pill use was reported by 28.6% of females. NAFLD was diagnosed in 15.2% and central obesity in 21.1% of adolescents. Both NAFLD and central obesity were more common in females than in males, however NAFLD was more prevalent in centrally obese males compared with centrally obese females (Table 2). Age at menarche ( $p=0.53$ ), oral corticosteroid use ( $p=1.0$ ) and oral contraceptive use ( $p=0.8$ ) were not associated with NAFLD.

#### **Association between parental pre-pregnancy BMI category and metabolic risk factors**

Maternal BMI category was associated with increasing waist circumference, serum leptin and glucose ( $p<0.05$  for all; Figures 1a-1c) in adolescent female offspring, but with increasing serum leptin alone in adolescent male offspring. In contrast, paternal BMI category was associated with waist circumference in female and serum leptin in male offspring ( $p<0.05$  for both).

#### **Association between maternal pre-pregnancy BMI and NAFLD**

Amongst the mothers of the Raine cohort adolescents, 17.1% were overweight or obese prior to pregnancy. Further, the mothers of adolescent offspring with NAFLD had a higher pre-pregnancy BMI than mothers of adolescents without NAFLD (Table 3). The proportion of adolescents with NAFLD increased with increasing maternal pre-pregnancy BMI category from underweight/ normal weight to overweight to obese in females (18.2% vs. 23.1% vs. 41.2%,  $p=0.005$ ) and males (9.3% vs. 14.3% vs. 26.7%,  $p=0.007$ ).

#### **Maternal weight or weight gain during pregnancy and NAFLD in adolescents**

Mean pregnancy weight at 18 and 34 weeks of gestation, as well as pregnancy weight gain by 18 weeks of pregnancy were higher in mothers of female cohort adolescents diagnosed with NAFLD compared with those without NAFLD (Table 3). In contrast, gestational weight gain was not associated with adolescent male NAFLD. Maternal pregnancy weight gain by 34 weeks of gestation was not associated with NAFLD in adolescent males or females (both

$p > 0.05$ ). There was a significantly higher prevalence of NAFLD in female, but not in male, adolescents who had a mother with gestational weight gain  $\geq 6.0$  kg compared with gestational weight gain  $< 6.0$  kg by 18 weeks (Table 3).

#### **Paternal BMI and NAFLD in adolescents**

Amongst the fathers of the cohort adolescents, 34.6% were overweight or obese prior to their partner's pregnancy. NAFLD was more prevalent in male cohort adolescents if the father was obese at the time their partner was pregnant. Pre-pregnancy BMI was higher in fathers of adolescent males diagnosed with NAFLD than those without NAFLD but there was no association of paternal BMI with NAFLD in female offspring (Table 3). The prevalence of NAFLD increased with paternal BMI category from underweight/normal weight to overweight to obese in adolescent males (8.4% vs. 16.2% vs 23.8% respectively,  $p = 0.01$ ) but not in females (20% vs. 22.6% vs. 25% respectively,  $p = 0.69$ ).

#### **Association of parental BMI with the severity of hepatic steatosis in adolescent offspring**

Increasing maternal BMI was associated with increasing severity of hepatic steatosis in adolescent offspring of both genders but paternal BMI was associated with severity of hepatic steatosis only in adolescent male but not female offspring (Figures 2a and 2b). However, gestational weight gain rate was associated with severity of steatosis in female but not in male adolescents (Figure 2c).

#### **Association of parental pre-pregnancy obesity with offspring NAFLD prevalence, liver enzymes, adipokines and insulin resistance**

Daughters of obese mothers had a higher prevalence of NAFLD, higher serum leptin, glucose, insulin and HOMA levels, compared with daughters of non-obese mothers (Table 4). While NAFLD was most prevalent if both parents were obese than if neither or just one parent was obese, maternal obesity had a dominant association with NAFLD compared with

paternal obesity (Tables 3 and 5). Serum ALT and AST levels were higher in male offspring of obese fathers compared with non-obese fathers, while serum leptin and GGT were higher if the mother was obese (Table 4).

### **Parent Socio-demographic status and NAFLD in adolescents**

Maternal socio-demographic characteristics during pregnancy are summarized in Table 1.

There was no association between maternal marital status and NAFLD (married/ de facto relationship 15.2% vs. not in a relationship 15.1%,  $p=0.99$ ) nor maternal educational achievement and NAFLD (post-secondary 14.3% vs. secondary 15.9%,  $p=0.46$ ). Table 3

compares parental pregnancy-associated characteristics based on the presence or absence of NAFLD in female and male adolescents. The mean age of mothers of adolescent females with NAFLD was lower than of mothers of adolescent females without NAFLD, however

maternal age was not associated with NAFLD in adolescent males. Paternal age was not associated with NAFLD in offspring of either gender. Adolescent males born into families

with the lowest socio-economic status, as determined by the lowest quartile of index of social advantage and disadvantage (IRSAD) at the time of birth, were nearly half as likely to have

been breastfed for  $\geq 6$  months (33.3% vs. 61.2%,  $p<0.001$ ) and had 3 times the odds of

NAFLD compared with birth into a family within the highest IRSAD quartile (Table 5). By

contrast, the family socio-economic status at birth was not significantly associated with breastfeeding for  $\geq 6$  months (35.8% vs. 48.2%,  $p=0.08$ ), or with NAFLD in adolescent

females when comparing the lowest and highest quartiles of IRSAD at the time of birth (OR 1.34, 95% CI 0.72-2.63,  $p=0.34$ ). A similar pattern was observed comparing associations of

NAFLD with the lowest and highest quartiles of family IRSAD at age 17 years (males OR 2.52, 95% CI 1.12-5.69,  $p=0.03$ ; females OR 1.12, 95% CI 0.61-2.07,  $p=0.71$ ).

### **Mode of delivery and NAFLD in adolescents**

The modes of delivery were spontaneous vaginal delivery (698[59.9%]), assisted vaginal delivery (219 [18.8%]), elective Cesarean section (135[11.6%]) and non-elective Caesarian

section (113[9.7%]). There was no difference in NAFLD prevalence based on the mode of delivery ( $p=0.13$ ) between genders. Birth anthropometry was not associated with NAFLD (Table 2).

### **Association between maternal pregnancy complications, stressful events during pregnancy and NAFLD in adolescents**

None of the mothers was diagnosed with eclampsia, however 19 mothers had chronic or primary hypertension and 19 mothers experienced gestational diabetes. There was no difference in the prevalence of NAFLD based on the presence or absence of maternal chronic hypertension, diabetes or gestational diabetes (Table 3). Hypertension during any stage of the pregnancy was observed in 297 (25.4%) and proteinuric preeclampsia in 24 (2.1%) of mothers, however there was no significant difference in the prevalence of NAFLD in association with the presence or absence of hypertension (16.6% vs. 14.6%,  $p=0.41$ ) or proteinuric hypertension (4.2% vs. 15.3%,  $p=0.16$ ). Further, no significant differences were seen between genders. Stressful life events during pregnancy such as pregnancy-related problems, marital or relationship, financial, employment or residential difficulties or difficulties with another child or other children were described by 19.1% of mothers. There was no significant difference in NAFLD prevalence in association with maternal stressful life events during pregnancy (17.1% vs. 14.8%,  $p=0.40$ ). There was no significant difference between genders.

### **Prediction of adolescent NAFLD from parental pregnancy-related factors**

Using multiple binary logistic regression analysis, covariates that were independently associated with NAFLD in adolescent offspring were determined from parent pregnancy-related characteristics that were statistically significant in univariate analyses. In female adolescents, maternal pre-pregnancy obesity, gestational weight gain  $\geq 6.0$ kg by the 18th week of gestation, maternal age during pregnancy, and parity were independently associated with NAFLD (Table 5). Maternal obesity and gestational weight gain remained

significant after adjusting for adolescent obesity. Paternal BMI, paternal age, and IRSAD did not significantly contribute to NAFLD prediction in female adolescents. In male adolescents, family socio-economic status at the time of childbirth (i.e. lowest quartile compared with highest quartile of IRSAD) was independently associated with NAFLD after adjusting for paternal and maternal pre-pregnancy obesity (Table 5). Family socio-economic status at the time of childbirth remained significantly associated with NAFLD in male adolescents after adjusting for male adolescent obesity and parent pre-pregnancy obesity.

## Discussion

The Raine study provides an opportunity to examine inter-generational relationships between parental pregnancy-related anthropometric and socio-demographic characteristics and subsequent adolescent NAFLD. We identified significant associations between parental characteristics and a future diagnosis of NAFLD in their children at age 17. Notably, there was sexual dimorphism in the association of parental factors at the time of pregnancy on NAFLD, hepatic enzymes, leptin and insulin resistance in adolescents. Maternal pre-pregnancy obesity was associated with a higher prevalence of NAFLD, greater severity of hepatic steatosis, and higher serum leptin levels in adolescent male and female offspring but significantly greater serum glucose, insulin and HOMA in female offspring. Similarly, in a rodent model maternal adiposity independently induced hyperleptinemia and insulin resistance in offspring, as well as an increased body weight that persisted into adulthood (39). However, in our study paternal obesity at the start of pregnancy was associated with NAFLD and raised serum transaminases in male offspring only. The relationship of paternal obesity with raised ALT and AST is consistent with a previous study (27). As previously reported, serum adipokine levels show gender differences. Leptin levels are positively associated with subcutaneous adiposity and severity of hepatic steatosis, particularly in females while adiponectin levels are inversely associated with visceral adiposity and steatosis severity in males (3). Similarly, maternal BMI associated more with serum leptin in female offspring compared with males.

Gender-specific associations between parental BMI or gestational weight gain, and NAFLD in offspring have not previously been described. In multivariable logistic regression analysis, NAFLD in adolescent females was independently associated with higher maternal pre-pregnancy BMI and greater gestational weight gain up to the 18th week of pregnancy. However, NAFLD in male offspring was associated with lower parental socio-economic status at the time of birth, independent of parental obesity at the start of pregnancy and adolescent offspring obesity. In both genders NAFLD appeared to be largely mediated by adolescent obesity.

Earlier studies have linked maternal pre-pregnancy obesity and excessive gestational weight gain with obesity (40-42) and adverse cardio-metabolic health in offspring (43). Higher maternal early-pregnancy weight gain was associated with an increased metabolic risk in adolescent offspring (34). In the Raine study, adult offspring obesity associated more with maternal obesity than paternal obesity (42). Environmental factors have been suggested to have a dominant effect over genetic influences on gender-different associations of parent-child BMI (44,45). The finding that maternal pregnancy weight gain only up to the 18th week, was associated with NAFLD in adolescent females suggests a possible vulnerability of the female fetus to excessive gestational weight gain early in pregnancy, particularly the first trimester. The strength of the association of early gestational weight gain with female offspring NAFLD suggests that the intrauterine environment during that period may be critical to metabolic risk, as previously proposed by other authors (34,42,46,47).

Maternal pregnancy-related obesity, high fat diet and insulin resistance have been shown to increase the risk of hepatic steatosis and lipotoxicity in intrauterine and postnatal life in animal models (24,25) and in humans (48). This has been proposed as the “first hit” in developmental programming for NAFLD (25). It is plausible that shared environmental factors such as feeding patterns or epigenetic effects superimposing on genetic and environmental factors influence NAFLD in offspring. Maternal obesity associates with metabolic risk factors and NAFLD in females, while paternal obesity and low family socio-

economic status at the time of birth show a distinct association with NAFLD in male offspring. Nevertheless, maternal pre-pregnancy obesity is associated with NAFLD in both genders.

Low socio-economic status is a determinant of higher risk of metabolic syndrome later in life (49). We are unable to determine a precise cause of the gender selective association of low family socio-economic status at birth with NAFLD in male adolescent offspring or the association between maternal obesity and offspring hyperleptinaemia and insulin resistance in female adolescent offspring. However, we have found an association between low socio-economic status and shorter breastfeeding duration that is linked to NAFLD in male offspring.

We speculate that there is a contribution of gender-different gene-environment interactions influencing the NAFLD phenotype and modifiable by breastfeeding, as described with BMI increases (50). Family upbringing practices, including nutrition and sedentary lifestyle are additional factors that may have an effect on individual and family risk of NAFLD. We did not find an association between gestational diabetes and NAFLD in offspring. This may relate to the small number of mothers with diagnosed gestational diabetes at the time, as routine oral glucose tolerance testing in pregnancy was not the norm during the study period and consequent under-diagnosing and underreporting of gestational diabetes is possible.

Strengths of this study include the large sample size with detailed, frequent follow-up assessments over many years and prospective data collection. Limitations of the study include participant attrition, self-reporting of maternal pre-pregnancy weight that was used to calculate pre-pregnancy BMI, and missing data. It is likely that the relatively small number of adolescent males with NAFLD and missing paternal BMI data attenuated the statistical power to infer stronger associations between paternal BMI and NAFLD in male offspring in the multivariate analysis. A diagnosis of NAFLD or other metabolic risk factors apart from maternal type II diabetes mellitus pre-pregnancy in parents was not available for analysis.

Despite the associations we have detected between parent and offspring phenotypes and characteristics, our study is unable to attribute causality or apportion relative contribution of genetic versus acquired familial factors in defining risk of NAFLD. While liver ultrasound has

limited sensitivity for fatty liver compared with liver histology and MRI we used a validated protocol with high sensitivity and specificity for fatty liver, acknowledging that liver biopsy of a community-based cohort of asymptomatic adolescents was not justifiable in this observational study. We are consequently unable to determine whether any of the adolescents had NASH or alcohol-related liver disease. Despite the limitations, the cohort was representative of the Western Australian population during the 17-year assessment (3).

In conclusion, heritability of anthropometric, metabolic and hepatic factors associated with NAFLD in offspring is complex and is influenced by parental gender differentially. The influence of the family socio-economic situation on NAFLD appears to associate with male offspring while pregnancy-related maternal obesity and gestational weight-gain associate more with female offspring. However, the magnitude of contribution of genetic, intrauterine, postnatal and later life influences on development of NAFLD is yet to be systematically unraveled. While genetic or epigenetic influences may be important, a potential contribution of the home environment and identification with the same-sex parent should not be overlooked. Strategies to protect against the risk of developing future NAFLD in offspring should ideally begin before pregnancy, striving for normal parental BMI pre-pregnancy, a healthy maternal diet during pregnancy and healthy first trimester gestational weight gain. Population health guidelines need to engage the community in risk reduction prior to conception rather than waiting for postnatal and childhood developmental stages when environmental overlay on epigenetic and genetic influences and the pathway to metabolic dysregulation, including NAFLD, may already be established.

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Accepted

**Table 1.** Parental pregnancy-related characteristics reported as mean (standard deviation) or proportions. Differences in the denominator reflect missing data. BMI = body mass index; Obesity = BMI  $\geq$  30 kg/m<sup>2</sup>, n = number.

<b>Parental characteristics</b>	<b>Value</b>
<b>Maternal characteristics</b>	
Age at study recruitment (years) (n=1170)	28.7 (5.8)
Race (Caucasian), n (%)	1036/1149 (90.2)
Parity >2, n (%)	267/1163 (23)
Singleton pregnancy, n (%)	1128/1170 (96.4)
Pre-pregnancy weight (kg) (n=1149)	59.6 (11.9)
Pre-pregnancy BMI (kg/m <sup>2</sup> ) (n=1121)	22.2 (4.2)
Pre-pregnancy obese, n (%)	64/1121 (5.7)
Pre-pregnancy overweight/ obese, n (%)	192/1121 (17.1)
Pre-pregnancy diabetes, n (%)	46/1169 (3.9)
Essential (chronic) hypertension n (%)	19/1170 (1.6%)
Weight at 18 weeks of pregnancy (kg) (n=1097)	64.4 (12.4)
Weight gain by 18 weeks of pregnancy (kg) (n=1097)	4.8 (4.3)
Weight at 34 weeks of pregnancy (kg) (n=1085)	73.2 (12.6)
Weight gain by 34 weeks of pregnancy (kg) (n=1085)	13.6 (5.4)
Mean weekly gestational weight gain (kg) (n=1083)	0.40 (0.16)
Gestational diabetes mellitus, n (%)	19/1170 (1.6)
Gestational hypertension, n (%)	297/1170 (25.4)
Proteinuric pre-eclampsia, n (%)	24/1170 (2.1%)
Education $\geq$ 12 years	561/1160 (48.4%)
High household income ( $\geq$ \$36,000) during pregnancy, n (%)	436/1111 (39.2)
Smoking during pregnancy, n (%)	251/1166 (21.5)
Married/ de facto relationship, n (%)	997/1149 (86.8)
<b>Paternal characteristics</b>	
Age (years) (n=1134)	31.0 (6.8)
Race (Caucasian), n (%)	1033/1149 (90)

BMI (kg/m <sup>2</sup> ) (n=943)	24.4 (3.4)
Paternal obesity, n (%)	57/943 (6.0)
Paternal overweight/ obesity, n (%)	326/943 (34.6)

**Table 2.** Comparison of offspring characteristics at birth and during late adolescence presented as mean (standard deviation), median (interquartile range) or proportions. N = number. Excessive alcohol is defined as >140 grams per week for females and >210 grams per week for males; Central obesity in adolescents is defined by waist circumference as  $\geq 80$  cm in females and  $\geq 94$  cm in males; NAFLD = non-alcoholic fatty liver disease. P values <0.05 are considered statistically significant.

Offspring characteristics	Female (N=578)	Male (N=592)	P value
<b>Birth characteristics</b>			
Caeserian delivery, N (%)	118/576 (20.5)	130/589 (22.1)	0.51
Weight (kg)	3.28 (0.58)	3.38 (0.57)	0.008
Ponderal index (kg/m <sup>3</sup> )	28.3 (3.2)	27.9 (2.9)	0.04
Percentage of expected birth weight	98.4 (12.7)	96.9 (12.9)	0.046
Duration of breastfeeding (months)	6(2-11)	7(2-12)	0.84
<b>Adolescent characteristics</b>			
Age (years)	17.1 (0.3)	17.0 (0.2)	0.03
Alcohol intake (g) per week	10 (0-75)	10 (0-100)	0.13
Excessive alcohol intake, N (%)	56/577 (9.7)	67/585 (11.5)	0.33
Weight (kg)	63.2 (12.7)	72.1 (14.5)	<0.001
Body mass index (kg/m <sup>2</sup> )	23.0 (4.3)	22.6 (4.2)	0.18
Waist circumference (cm)	77.4 (11.2)	80.3 (10.7)	<0.001
Central obesity, N (%)	186/570 (32.6)	58/588 (9.9)	<0.001
NAFLD, N (%)	113/576 (19.6)	63/528 (10.7)	<0.001
NAFLD in centrally obese adolescents N, (%)	67/185 (35)	36/58 (62.1)	<0.001
Systolic blood pressure (mm Hg)	109.6 (9.6)	119.9 (10.3)	<0.001
Diastolic blood pressure (mm Hg)	66.9 (9.8)	59.4 (6.6)	0.41
Total cholesterol (mg/dL)	166.5 (28.7)	152.1 (28.1)	<0.001
High density lipoprotein (HDL) cholesterol (mg/dL)	54.1 (12.0)	46.4 (9.4)	<0.001
Low density lipoprotein (LDL) cholesterol (mg/dL)	94.0 (24.9)	86.7 (25.6)	<0.001
Triglycerides (mg/dL)	91.5 (43.2)	93.9 (50.0)	0.44
Alanine aminotransferase (ALT) (U/L)	18.4 (10.9)	24.0 (13.4)	<0.001
Aspartate aminotransferase (AST) (U/L)	21.9 (5.2)	27.5 (9.3)	<0.001

Fasting glucose (mg/dL)	83.6 (7.3)	87.4 (11.8)	<0.001
Fasting insulin (mU/L)	7.8 (5.3-11.2)	7.1 (4.6-10.5)	0.01
HOMA-IR	1.6 (1.1-2.3)	1.0 (1.5-2.3)	0.15
Adiponectin (mg/L)	10.0 (7.3-14)	7.4 (5.2-10.6)	<0.001
Leptin ( $\mu$ g/L)	26.0 (15.5-41.5)	2.7 (1.4-6.4)	<0.001
C-reactive protein (CRP) (mg/L)	0.8 (0.3-2.1)	0.4 (0.2-1.0)	<0.001

**Table 3.** Comparison of parent pregnancy-related characteristics in relation to the presence or absence of NAFLD in adolescent males and females. Results are presented as mean (standard deviation) or percentages. P values <0.05 are considered statistically significant. The table compares parent characteristics at the time of pregnancy based on whether adolescent offspring were diagnosed with NAFLD or not. N = number; IRSAD = index of relative socio-economic advantage or disadvantage; P values < 0.05 are considered statistically significant.

	Adolescent females (N=576)			Adolescent males (n=591)		
	NAFLD	No NAFLD	P value	NAFLD	No NAFLD	P value
Maternal age (years)	27.5 (6.1)	29.0 (5.9)	0.02	28.7(5.9)	28.8 (5.6)	0.80
Parity ≥2						
• Yes, N (%)	20/144 (13.9)		0.04	15/122 (12.3)		0.54
• No, N (%)	93/430 (21.6)			48/464 (10.3)		
Diabetes						
• Yes, N (%)	4/21 (19)		1.00	1/25 (4.0)		0.35
• No, N (%)	109/555 (19.6)			62/565 (11.0)		
Gestational diabetes						
• Yes, N (%)	2/8 (25.0)		1.00	0/11 (0)		0.39
• No, N (%)	111/ 568 (19.5)			63/580 (10.9)		
Essential (chronic) hypertension						
• Yes, N (%)	2/9 (22.0)		1.00	2/10 (20.0)		0.61
• No, N (%)	111/567 (19.6)			61/581 (10.5)		
Maternal pre-pregnancy body mass index (BMI) (kg/ m <sup>2</sup> )	23.8 (6.0)	22.0 (3.7)	<0.001	23.9 (6.0)	21.9 (3.8)	<0.001

Maternal weight at 18 weeks gestation (kg)	71.1 (18.2)	63.3 (10.4)	<0.001	68.6 (17.6)	63.5 (11.1)	0.002
Maternal weight at 34 weeks gestation (kg)	79.2 (18.0)	72.4(11.0)	<0.001	76.5 (16.8)	72.3(11.4)	0.01
Gestational weight gain $\geq$ 6.0 kg by 18 weeks (%)	29.1%	15.5%	<0.001	10.3%	9.1%	0.66
Maternal weight gain by 18 weeks gestation (kg)	6.2 (5.1)	4.4 (4.3)	<0.001	5.0 (6.3)	4.8 (3.9)	0.68
Maternal weight gain by 34 weeks gestation (kg)	14.3 (6.4)	13.4 (5.3)	0.14	12.6 (6.8)	13.7 (5.0)	0.13
Mean weekly pregnancy weight gain (kg)	0.42 (0.19)	0.39 (0.16)	0.16	0.37 (0.20)	0.40 (0.14)	0.12
Paternal age (years)	30.2 (7.1)	31.4 (6.9)	0.11	29.9 (6.2)	31.1 (6.8)	0.20
Paternal pre-pregnancy body mass index (BMI) (kg/ m <sup>2</sup> )	24.6 (4.0)	24.5 (3.2)	0.84	25.8 (3.8)	24.0 (3.2)	<0.001
Parent obesity						
• Neither parent obese	19.9%			10.0%		
• Maternal obesity only	37.5%		0.008	28.6%		0.05
• Paternal obesity only	16.7%			17.6%		
• Both parents obese	66.7%			33.3%		
Family IRSAD at birth						
Lowest quartile	22.7%		0.41	13.8%		0.02
Highest quartile	17.6%			4.0%		
Maternal smoking during pregnancy (%)	30.1%	21.1%	0.04	23.8%	19.9%	0.46

**Table 4.** Comparison of hepatic and metabolic characteristics of adolescents relative to the presence or absence of parental obesity during pregnancy. The table examines differences in liver and metabolic variables in adolescent females and males based on whether each of their parents was obese or not at the start of pregnancy. BMI = body mass index; Obesity = BMI  $\geq$  30 kg/m<sup>2</sup>; NAFLD = nonalcoholic fatty liver disease; LSM = liver stiffness measurement by transient elastography; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase; HOMA-IR = homeostasis model for insulin resistance. P values < 0.05 are considered statistically significant.

		Maternal pre-pregnancy obesity (N= 64)	Maternal pre-pregnancy non-obesity (N= 1057)	P-value	Paternal pre-pregnancy obesity (N= 57)	Paternal pre-pregnancy non-obesity (N= 886)	P-value
Adolescent female	NAFLD %	41.2	18.8	0.002	25.8	20.8	0.67
	LSM (kPa)	5.3 (1.5)	4.6 (1.3)	0.03	5.0 (1.7)	4.6 (1.3)	0.26
	Serum ALT (U/L)	18.8 (8.3)	18.2 (9.6)	0.76	19.6 (8.2)	18.0 (9.7)	0.41
	Serum AST (U/L)	20.2 (4.4)	21.8 (5.0)	0.09	21.0 (4.1)	21.9 (5.2)	0.41
	Serum GGT (U/L)	13.5 (9.2)	13.2 (6.7)	0.80	14.1 (9.1)	12.9 (5.9)	0.35
	Serum Leptin ( $\mu$ g/L)	44.7 (26.3-69.3)	26.0 (15.5-40.6)	<0.001	29.8 (16.7-40.0)	26.0 (15.1-43.0)	0.32
	Serum adiponectin (mg/L)	10 (7.3-14.0)	10 (7.4-14.0)	0.63	9.2 (6.3-14.0)	10.1 (7.4-14.1)	0.31
	Serum Glucose (mg/dL)	87.2 (7.5)	83.3 (6.3)	0.002	84.6 (8.1)	83.3 (6.3)	0.33
	Serum Insulin (mU/L)	9.5 (7.4-17.2)	7.7 (5.2-11.3)	0.03	7.4 (5.8-12.8)	7.9 (5.2-11.2)	0.76
	HOMA	1.95 (1.37-3.93)	1.57 (1.05-2.33)	0.02	1.6 (1.1-2.8)	1.6 (1.0-2.3)	0.66
Adolescent male	NAFLD %	26.7	9.9	0.01	23.8	10.6	0.07
	LSM (kPa)	6.0 (1.5)	5.5 (1.4)	0.18	5.0 (1.1)	5.6 (1.4)	0.10
	Serum ALT (U/L)	28.7 (22.3)	23.8 (12.8)	0.07	32.1 (25.8)	24.1 (13.3)	0.03
	Serum AST (U/L)	27.6 (11.7)	27.5 (9.2)	0.95	37.8 (21.4)	27.4 (8.8)	<0.001
	Serum GGT (U/L)	20.1 (13.8)	16.0 (8.2)	0.02	15.8 (8.4)	16.3 (9.1)	0.84
	Serum Leptin ( $\mu$ g/L)	7.9 (3.0-25.0)	2.6 (1.4-6.2)	<0.001	2.3 (1.4-5.1)	2.6 (1.4-6.5)	0.55
	Serum adiponectin (mg/L)	7.5 (5.0-10.6)	7.4 (5.2-10.6)	0.80	7.3 (6.1-8.7)	7.4 (5.1-10.6)	0.87
	Serum Glucose (mg/dL)	87.4 (10.2)	87.4 (12.1)	1.00	85.1 (5.5)	87.3 (12.9)	0.48
	Serum Insulin (mU/L)	10.2 (5.1-15.5)	7.0 (4.6-10.3)	0.05	7.2 (4.4-9.3)	7.2 (4.8-10.4)	0.95
HOMA	2.10 (1.04-3.42)	1.48 (0.96-2.24)	0.05	1.6 (0.9-2.0)	1.5 (1.0-2.3)	0.98	

**Table 5.** Univariate and multivariate associations between parent pregnancy-related characteristics and adolescent obesity associated with NAFLD in adolescent females and males. Maternal pre-pregnancy obesity is defined as BMI  $\geq 30$  kg/m<sup>2</sup>; paternal obesity is defined as BMI  $\geq 30$  kg/m<sup>2</sup>; adolescent central obesity is defined by waist circumference as  $\geq 80$  cm in females and  $\geq 94$  cm in males; IRSAD = index of relative socio-economic advantage or disadvantage. P values < 0.05 are considered statistically significant.

Covariates	Adolescent Females		
	Odds ratio	95% confidence interval	P value
<b>Univariate analysis</b>			
Pregnancy weight gain > 6kg by 18 weeks gestation	2.24	1.45-3.46	<0.001
Maternal pre-pregnancy obesity	3.02	1.48-6.20	0.003
Paternal obesity during pregnancy	1.27	0.58-2.79	0.56
Maternal age	0.96	0.93-0.99	0.02
Parity $\geq 2$	0.58	0.35-0.99	0.045
Adolescent central obesity	4.61	2.97-7.15	<0.001
Parent obesity			
• Neither parent obese (reference)	-	-	-
• Maternal obesity only	2.42	1.02-5.73	0.04
• Paternal obesity only	0.81	0.30-2.17	0.67
• Both parents obese	8.07	1.45-44.85	0.02
Family IRSAD at birth (lowest vs. highest quartile)	1.36	0.71-2.6	0.36
<b>Multivariable regression analysis</b>			
<b>Model 1</b>			
Pregnancy weight gain > 6kg by 18 weeks gestation	3.34	1.47-3.84	<0.001
Maternal pre-pregnancy obesity	3.82	1.54-9.45	0.004
Maternal age	0.97	0.93-1.01	0.11
Parity $\geq 2$	0.70	0.39-1.26	0.17

<b>Model 2</b>			
Pregnancy weight gain > 6kg by 18 weeks gestation	1.10	1.04-1.15	<0.001
Maternal pre-pregnancy obesity	3.46	1.49-8.05	0.004
Maternal age	-	-	-
Parity ≥2			
Adolescent central obesity	4.27	2.68-6.79	<0.001
	<b>Adolescent Males</b>		
<b>Univariate analysis</b>	<b>Odds ratio</b>	<b>95% confidence interval</b>	<b>P value</b>
Maternal pre-pregnancy obesity	3.33	1.41-7.84	0.006
Pregnancy weight gain > 6kg by 18 weeks gestation	1.01	0.95-1.08	0.68
Paternal obesity during pregnancy	2.62	0.92-7.48	0.07
Maternal age	1.00	0.95-1.04	0.87
Parity ≥2	0.46	0.06-3.08	0.45
Family IRSAD at birth (lowest vs. highest quartile)	3.09	1.08-8.87	0.03
Adolescent central obesity	32.99	16.96-64.17	<0.001
Parent obesity			
• Neither parent obese (reference)	-	-	-
• Maternal obesity only	3.62	1.33-9.82	0.01
• Paternal obesity only	1.94	0.54-7.02	0.31
• Both parents obese	4.52	0.40-50.92	0.22
<b>Multivariable regression analysis</b>			
<b>Model 1</b>			
Maternal pre-pregnancy obesity	1.21	0.23-6.33	0.83
Paternal obesity during pregnancy	2.39	0.42-13.49	0.33

Family IRSAD at birth (lowest vs. highest quartile)	3.78	1.15-12.42	0.03
<b>Model 2</b>			
Maternal pre-pregnancy obesity	1.83	0.23-14.76	0.57
Paternal obesity during pregnancy	0.99	0.06-17.78	0.99
Family IRSAD at birth (lowest vs. highest quartile)	9.07	1.54-53.29	0.02
Adolescent central obesity	61.83	11.46-333.74	<0.001

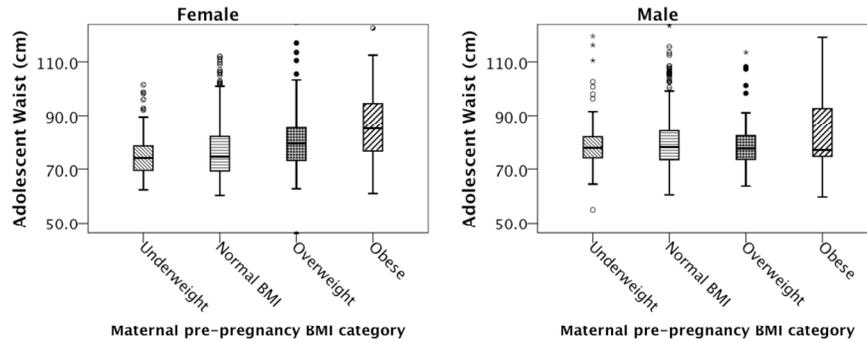


Figure 1a. Relationship between maternal pre-pregnancy BMI category and waist circumference in adolescent offspring. The box plots display median values and 25th and 75th percentiles; the whiskers represent the 5th and 95th percentiles.

243x123mm (300 x 300 DPI)

Accepted

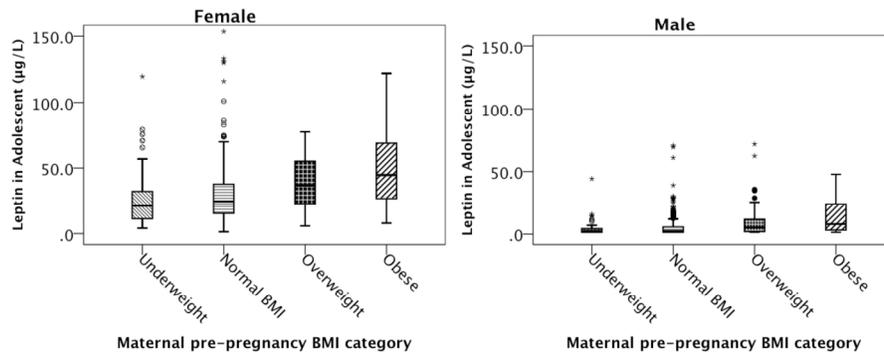


Figure 1b. Relationship between maternal pre-pregnancy BMI category and serum leptin in adolescent offspring. The box plots display median values and 25th and 75th percentiles; the whiskers represent the 5th and 95th percentiles.

243x132mm (300 x 300 DPI)

Accepted

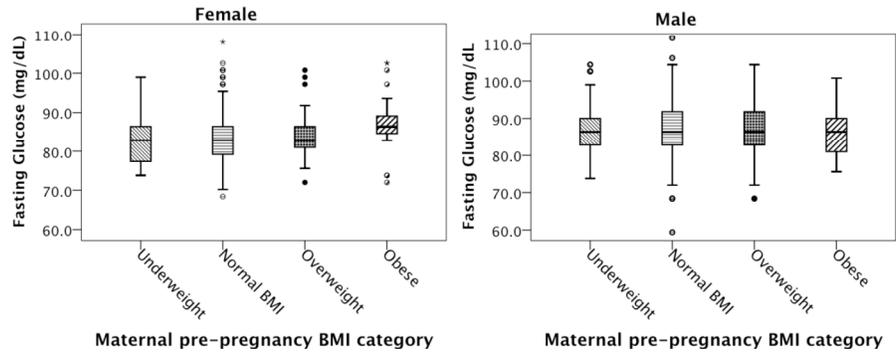


Figure 1c. Relationship between maternal pre-pregnancy BMI category and fasting serum glucose in adolescent offspring. The box plots display median values and 25th and 75th percentiles; the whiskers represent the 5th and 95th percentiles.

243x127mm (300 x 300 DPI)

Accepted

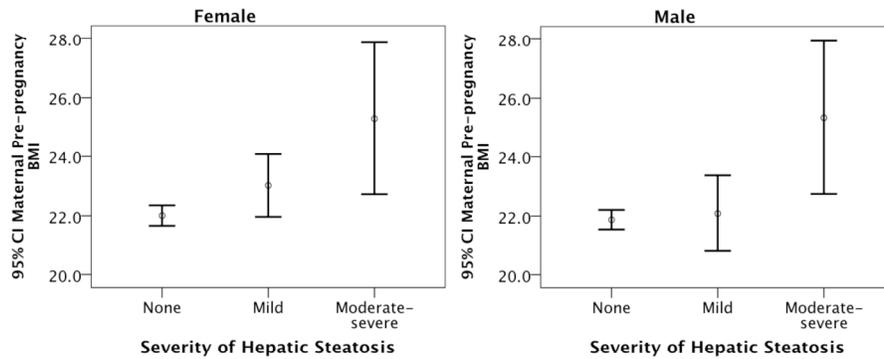


Figure 2a. Relationship between maternal pre-pregnancy BMI and the severity of hepatic steatosis in offspring. The figures display means and 95% confidence interval (CI) for maternal body mass index (BMI).

243x127mm (300 x 300 DPI)

Accepted

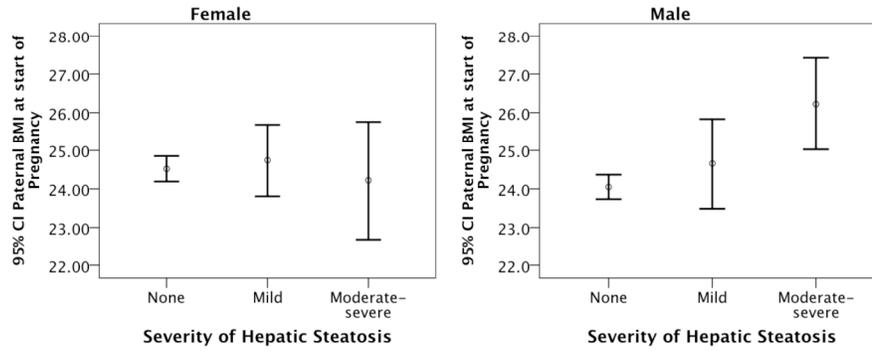


Figure 2b. Relationship between paternal pre-pregnancy BMI and the severity of hepatic steatosis in offspring. The figures display means and 95% confidence interval (CI) for paternal body mass index (BMI).

243x129mm (300 x 300 DPI)

Accepted

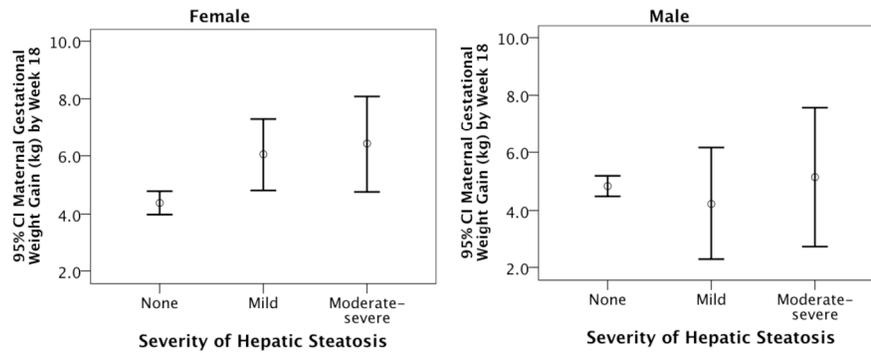


Figure 2c. Relationship between maternal gestational weight gain by week 18 and the severity of hepatic steatosis in offspring. The figures display means and 95% confidence interval (CI) for maternal gestational weight gain.

243x120mm (300 x 300 DPI)

Accepted