HEPATOLOGY

HEPATOLOGY, VOL. 00, NO. 00, 2017

Sex Differences Between Parental Pregnancy Characteristics and Nonalcoholic Fatty Liver Disease in Adolescents

Oyekoya T. Ayonrinde,[1–3] Leon A. Adams,[1,4] Trevor A. Mori,[1] Lawrence J. Beilin,[1] Nicholas de Klerk,[5] Craig E. Pennell,[6] Scott White,[6] and John K Olynyk[2,3,7]

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 1,170 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 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 prepregnancy 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 body mass index (BMI) were not associated with NAFLD in female offspring. In contrast, higher paternal BMI ($P < 0.001$), maternal prepregnancy BMI ($P < 0.001$), and lower family socioeconomic status (SES) 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 (odds ratio [OR], 3.46; 95% confidence interval [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 SES 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, whereas lower family SES at birth was associated with NAFLD in male offspring independent of adolescent obesity. (HEPATOLOGY 2017; 00:000-000).

Nonalcoholic fatty liver disease (NAFLD) is a common, potentially progressive, complex chronic liver disorder. NAFLD associates with insulin resistance (IR) and features of metabolic syndrome (MetS).[1,2] Obesity is a dominant risk factor for NAFLD, with both subcutaneous and visceral adiposity being associated with NAFLD.[3] Recent data describe considerable risk of liver-related morbidity and mortality with progressive adiposity gains during the life course, from childhood through adulthood.[4–9]

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GGT, gamma-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostasis model for insulin resistance; HS, hepatic steatosis; IQR, interquartile range; IR, insulin resistance; IRSAD, Index of Relative Socio-economic Advantage and Disadvantage; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; SES, socioeconomic status.

Received March 12, 2017; accepted June 26, 2017.

Supported by the National Health and Medical Research Council project grants (403968, 634445, 353514, 403981, and 634445), a postgraduate scholarship to Oyekoya T. Ayonrinde (404166), National Health and Medical Research Council Practitioner Fellowship to John K. Olynyk (1042370) and a research fellowship to Trevor A. Mori, the Gastroenterology Society of Australia (Astra Zeneca Career Development Award to Leon A. Adams), the Fremantle Hospital Medical Research Foundation (medical research grant), and a University of Western Australia Ada Bartholomew grant.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.29347

Potential conflict of interest: Nothing to report.

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] whereas early-onset paternal obesity has been associated with elevated serum alanine aminotransferase (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 patatin-like phospholipase-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 and UK identified heritability of hepatic steatosis (HS) 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 prepregnancy and pregnancy-related parent phenotypic and sociodemographic characteristics. Yet, such factors may be important regulators of intergenerational liver and metabolic disease through epigenetic and imprinting influences that potentially impact the health of offspring.

We have previously described sex-specific differences in 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 MetS, 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] Furthermore, adolescent females in the Raine Cohort who were diagnosed with NAFLD in addition to polycystic ovary syndrome had a similar phenotype to adolescent males with NAFLD that appeared to be mediated through obesity rather than androgen levels.[33] A study from the same cohort has also found associations between higher maternal prepregnancy BMI and early pregnancy weight gain rate, as well as an adverse adolescent offspring cardiometabolic profile.[34] Given the recognized sex-different inter-relationship between NAFLD and cardiometabolic 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 to: (1) examine intergenerational relationships between parental anthropometry, pregnancy-related factors, and family

ARTICLE INFORMATION:

From the [1]School of Medicine and Pharmacology, The University of Western Australia, Perth, WA, Australia; [2]Department of Gastroenterology and Hepatology, Fiona Stanley Hospital, Murdoch, WA, Australia; [3]Faculty of Health Sciences, Curtin University, Bentley, WA, Australia; [4]Department of Hepatology, Sir Charles Gairdner Hospital, Nedlands, WA, Australia; [5]Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia; [6]School of Women's and Infants Health, University of Western Australia, Crawley, WA, Australia; and [7]School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA, Australia.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Oyekoya T. Ayonrinde, M.B.B.S., F.R.A.C.P.
Department of Gastroenterology and Hepatology, Fiona Stanley Hospital
11 Robin Warren Drive
Murdoch 6150, Australia
E-mail: oyekoya.ayonrinde@health.wa.gov.au
Tel: +61861522827

sociodemographic characteristics coinciding with pregnancy, and a future diagnosis of NAFLD in adolescent offspring, and (2) examine sex-specific relationships between parental prepregnancy obesity and NAFLD during adolescence in same-sex and opposite-sex offspring.

Materials and Methods

We used prospectively collected data from the longitudinal Raine Cohort study to determine associations between prepregnancy and antenatal socioanthropometric 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 asessment of offspring was conducted at ages 1, 2, 3, 5, 8, 10, 14, 17, and 20 years. Assessments involved questionnaires regarding sociodemographic 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 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 socioeconomic status (SES) 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 for the family's residential postcode.[36] Records of prepregnancy parental sociodemographic 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 breastmilk 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 adolescents was defined as a waist circumference $\geq$80 cm in females and $\geq$94 cm in males, consistent with age- and sex-specific criteria of the International Diabetes Federation.[37] 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, because we have previously found that waist circumference identified a higher proportion of obese adolescents than BMI. All references to parental, maternal, or paternal BMI or obesity are specific to prepregnancy assessments, whereas 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 prepregnancy weight. Maternal height was measured to the nearest 0.1 cm during the prerecruitment assessment and again at the subsequent assessment between 16 and 20 weeks' gestation. Maternal prepregnancy BMI was calculated. Maternal weight at approximately 18 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 prepregnancy weight, whereas gestational weight gain at 34 weeks was the

TABLE 1. Parental Pregnancy-Related Characteristics Reported as Mean (SD) or Proportions

Parental Characteristics	Value
Maternal characteristics	
Age at study recruitment (years) (n = 1,170)	28.7 (5.8)
Race (white), n (%)	1,036/1,149 (90.2)
Parity >2, n (%)	267/1,163 (23)
Singleton pregnancy, n (%)	1,128/1,170 (96.4)
Prepregnancy weight (kg) (n = 1,149)	59.6 (11.9)
Prepregnancy BMI (kg/m^2) (n = 1,121)	22.2 (4.2)
Prepregnancy obese, n (%)	64/1,121 (5.7)
Prepregnancy overweight/obese, n (%)	192/1,121 (17.1)
Prepregnancy diabetes, n (%)	46/1,169 (3.9)
Essential (chronic) hypertension n (%)	19/1,170 (1.6%)
Weight at 18 weeks of pregnancy (kg) (n = 1,097)	64.4 (12.4)
Weight gain by 18 weeks of pregnancy (kg) (n = 1,097)	4.8 (4.3)
Weight at 34 weeks of pregnancy (kg) (n = 1,085)	73.2 (12.6)
Weight gain by 34 weeks of pregnancy (kg) (n = 1,085)	13.6 (5.4)
Mean weekly gestational weight gain (kg) (n = 1,083)	0.40 (0.16)
Gestational diabetes mellitus, n (%)	19/1,170 (1.6)
Gestational hypertension, n (%)	297/1,170 (25.4)
Proteinuric pre-eclampsia, n (%)	24/1,170 (2.1)
Education ≥12 years	561/1,160 (48.4)
High household income (≥$36,000) during pregnancy, n (%)	436/1,111 (39.2)
Smoking during pregnancy, n (%)	251/1,166 (21.5)
Married/*de facto* relationship, n (%)	997/1,149 (86.8)
Paternal characteristics	
Age (years) (n = 1,134)	31.0 (6.8)
Race (white), n (%)	1,033/1,149 (90)
BMI (kg/m^2) (n = 943)	24.4 (3.4)
Paternal obesity, n (%)	57/943 (6.0)
Paternal overweight/obesity, n (%)	326/943 (34.6)

Differences in the denominator reflect missing data. Obesity = BMI ≥30 kg/m^2; n = number.

difference between the 34-week pregnancy weight and prepregnancy weight.

OFFSPRING BIRTH AND 17-YEAR ANTHROPOMETRIC ASSESSMENT

Birth weight was documented in kilograms. Ponderal index, a measure of leanness, was calculated from weight (kg)/height (m)^3 and percentage of expected birth weight defined as the percentage of expected weight adjusted for gestational age. Obesity in 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 et al. that provides 92% sensitivity and 100% specificity for the histological diagnosis of >10% HS.[38] Trained ultrasonographers performed liver ultrasound assessments as 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, which included an echotexture score of at least 1. Severity of HS 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 steatosis). Intraobserver reliability (j statistic) for fatty liver was 0.78 (95% confidence interval [CI], 0.73-0.88). A NAFLD diagnosis required sonographic fatty liver and exclusion of significant alcohol consumption.[3] Testing for hepatitis B (HBV) or C virus (HCV) infections was not performed because notification rates for HBV and HCV infections were, on

average, less than 24 per 100,000 and 23 per 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 sex and maternal prepregnancy 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 SD whereas nonparametric data are reported as the median and interquartile range (IQR). The main outcome variables were the presence or absence of NAFLD and the severity of sonographic HS. Differences in continuous anthropometric variables between adolescents with or without NAFLD were examined using the independent *t* test or analysis of variance for normally distributed variables and nonparametric 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; IBM Corp., Armonk, NY).

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, 1,170 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 females and 1 male) with ultrasound-diagnosed fatty liver were

TABLE 2. Comparison of Offspring Characteristics at Birth and During Late Adolescence Presented as Mean (SD), Median (IQR), or Proportions

Offspring characteristics	Female (N = 578)	Male (N = 592)	*P* Value
Birth characteristics			
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^3)	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
Adolescent characteristics			
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
BMI (kg/m^2)	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 cholesterol (mg/dL)	54.1 (12.0)	46.4 (9.4)	<0.001
Low-density lipoprotein 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
ALT (U/L)	18.4 (10.9)	24.0 (13.4)	<0.001
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 (μg/L)	26.0 (15.5-41.5)	2.7 (1.4-6.4)	<0.001
C-reactive protein (mg/L)	0.8 (0.3-2.1)	0.4 (0.2-1.0)	<0.001

N = number. Excessive alcohol is defined as >140 g/week for females and >210 g/week for males. Central obesity in adolescents is defined by waist circumference as ≥80 cm in females and ≥94 cm in males; *P* values <0.05 are considered statistically significant.
Abbreviation: HOMA-IR, homeostatic model assessment of insulin resistance.

excluded from a NAFLD diagnosis because of excessive alcohol consumption. All adolescent females were postmenarchal (mean age at menarche, 12.8 [SD, 1.1] years). Oral corticosteroid use was reported by 0.03% of participants, whereas 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 PREPREGNANCY BMI CATEGORY AND METABOLIC RISK FACTORS

Maternal BMI category was associated with increasing waist circumference, serum leptin and glucose ($P < 0.05$ for all; Fig. 1A-C) 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 PREPREGNANCY BMI AND NAFLD

Among the mothers of the Raine cohort adolescents, 17.1% were overweight or obese before pregnancy. Furthermore, mothers of adolescent offspring with NAFLD had a higher prepregnancy BMI than mothers of adolescents without NAFLD (Table 3). The proportion of adolescents with NAFLD increased with increasing maternal prepregnancy 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 to 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

Among the fathers of the cohort adolescents, 34.6% were overweight or obese before their partner's pregnancy. NAFLD was more prevalent in male cohort adolescents if the father was obese at the time their partner was pregnant. Prepregnancy 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). 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 HS IN ADOLESCENT OFFSPRING

Increasing maternal BMI was associated with increasing severity of HS in adolescent offspring of both sexes, but paternal BMI was associated with severity of HS only in adolescent male, but not female, offspring (Fig. 2A,B). However, gestational weight gain rate was associated with severity of steatosis in female but not in male adolescents (Figure 2c).

ASSOCIATION OF PARENTAL PREPREGNANCY 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

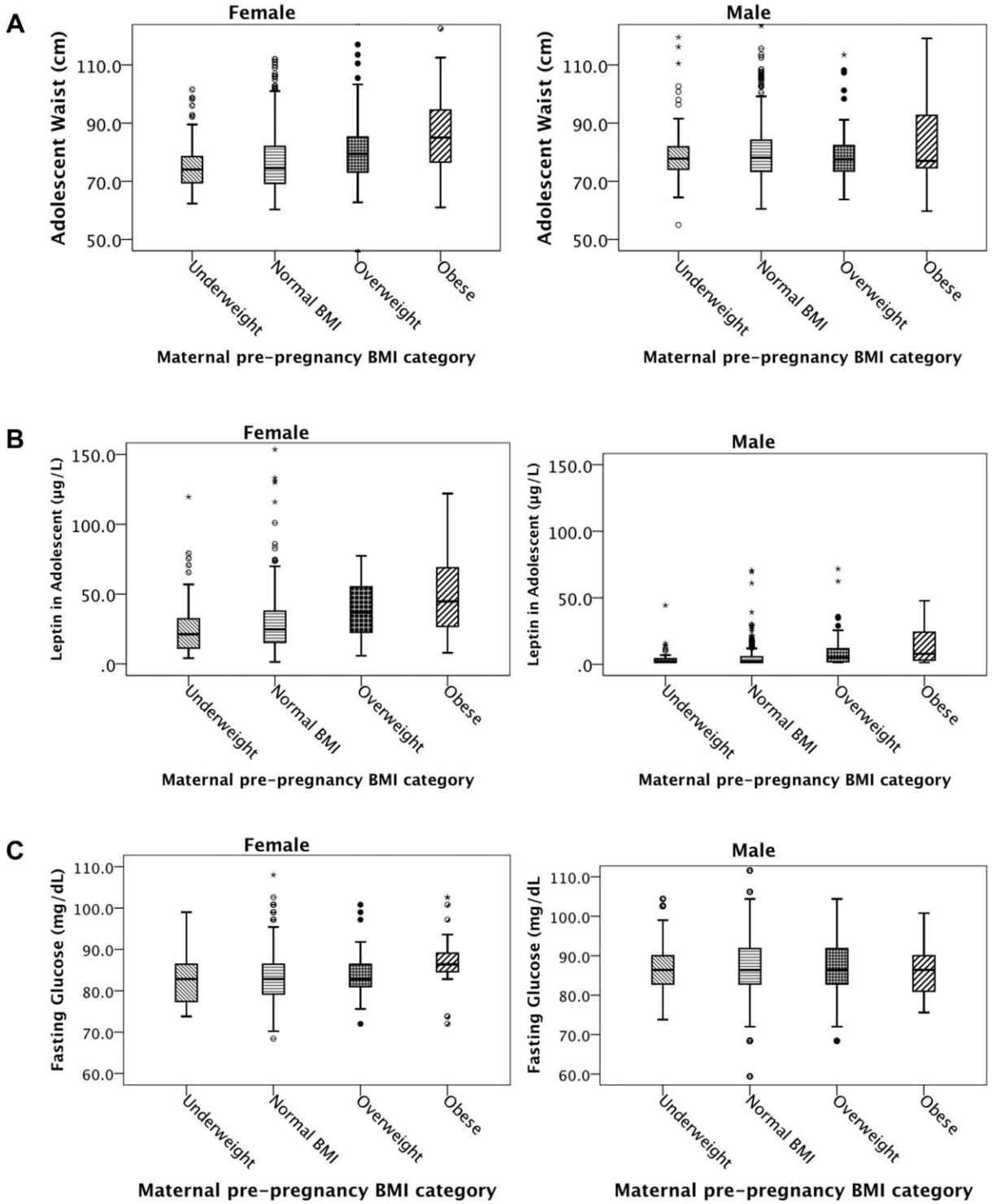

FIG. 1. Relationship between maternal prepregnancy BMI category and waist circumference, serum leptin and fasting glucose in adolescent offspring.

homeostasis model assessment (HOMA) levels, compared to daughters of nonobese mothers (Table 4). Whereas 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 to paternal obesity (Tables 3 and

TABLE 3. Comparison of Parent Pregnancy-Related Characteristics in Relation to the Presence or Absence of NAFLD in Adolescent Males and Females

	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 prepregnancy BMI (kg/m^2)	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 ≥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 prepregnancy BMI (kg/m^2)	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

Results are presented as mean (SD) 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. *P* values <0.05 are considered statistically significant.

5). Serum ALT and aspartate aminotransferase (AST) levels were higher in male offspring of obese fathers compared with nonobese fathers, whereas serum leptin and gamma-glutamyl transpeptidase (GGT) were higher if the mother was obese (Table 4).

PARENT SOCIODEMOGRAPHIC STATUS AND NAFLD IN ADOLESCENTS

Maternal sociodemographic 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 (postsecondary 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. 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 sex. Adolescent males born into families with the lowest SES, as determined by the lowest quartile of IRSAD at the time of birth, were nearly half as likely to have been breastfed for ≥6 months (33.3% vs. 61.2%; $P < 0.001$) and had 3 times

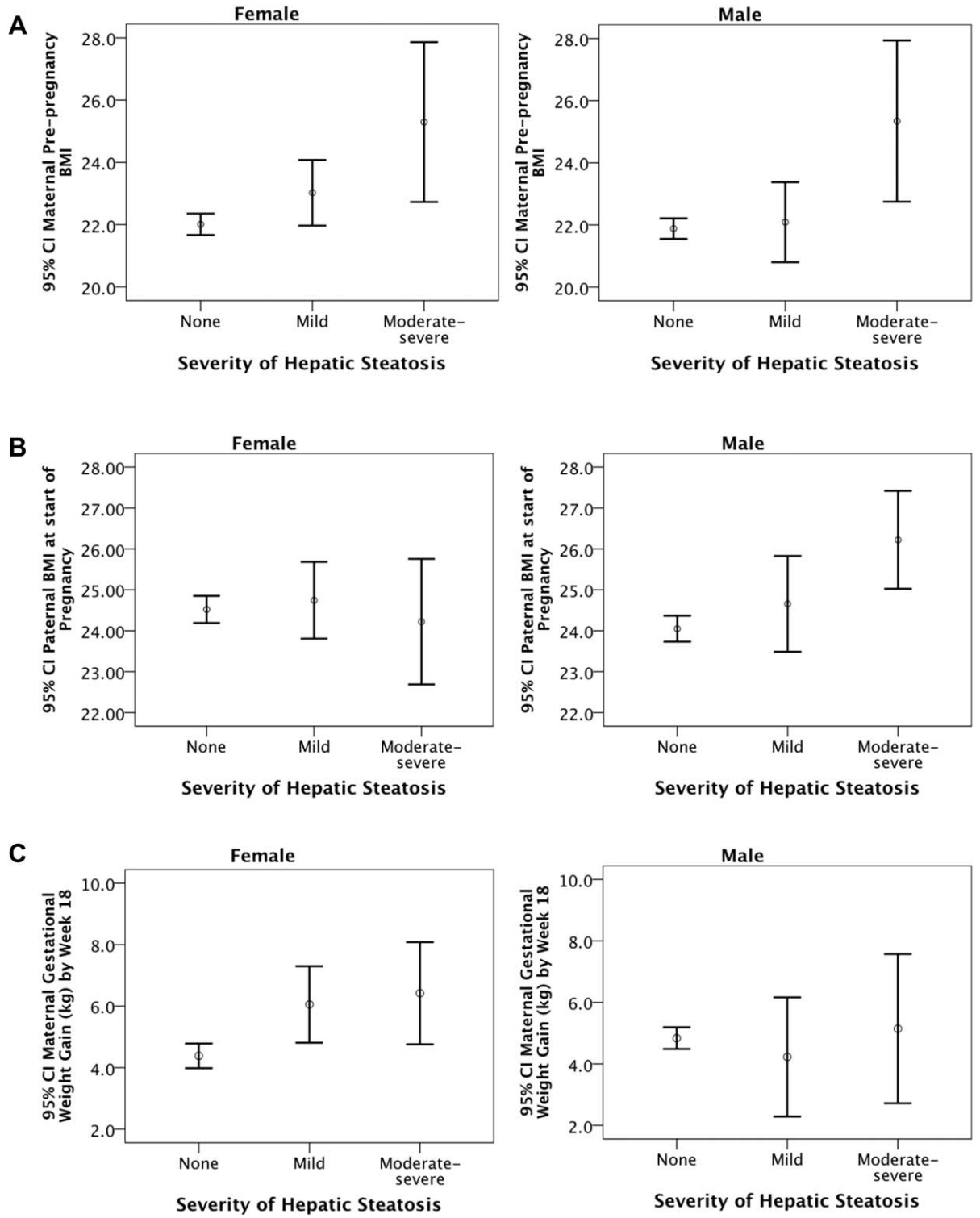

FIG. 2. Relationship between parent prepregnancy BMI, maternal gestational weight gain, and the severity of hepatic steatosis in adolescent offspring.

TABLE 4. Comparison of Hepatic and Metabolic Characteristics of Adolescents Relative to the Presence or Absence of Parental Obesity During Pregnancy

		Maternal Prepregnancy Obesity (N = 64)	Maternal Prepregnancy Nonobesity (N = 1,057)	*P* Value	Paternal Prepregnancy Obesity (N = 57)	Paternal Prepregnancy Nonobesity (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 (μg/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 (μg/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

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. Obesity = BMI $\geq$30 kg/m^2. *P* values <0.05 are considered statistically significant.
Abbreviation: LSM = liver stiffness measurement by transient elastography.

the odds of NAFLD compared with birth into a family within the highest IRSAD quartile (Table 5). By contrast, the family SES 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 (odds ratio [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

Modes of delivery were spontaneous vaginal delivery (698 [59.9%]), assisted vaginal delivery (219 [18.8%], elective Cesarean section (135 [11.6%]) and nonelective Caesarian section (113 [9.7%]). There was no difference in NAFLD prevalence based on mode of delivery ($P = 0.13$) between sexes. 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 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 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%; $P = 0.16$). Furthermore, no significant differences were observed between sexes. Stressful life events during pregnancy, such as pregnancy-related problems, marital or relationship, financial, employment or residential difficulties, or

TABLE 5. Univariate and Multivariate Associations Between Parent Pregnancy-Related Characteristics and Adolescent Obesity Associated With NAFLD in Adolescent Females and Males

Covariates	Adolescent Females		
Univariate Analysis	OR	95% CI	*P* Value
Pregnancy weight gain >6 kg by 18 weeks' gestation	2.24	1.45-3.46	<0.001
Maternal prepregnancy 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 ≥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
Multivariable regression analysis			
Model 1			
Pregnancy weight gain >6 kg by 18 weeks' gestation	3.34	1.47-3.84	<0.001
Maternal prepregnancy obesity	3.82	1.54-9.45	0.004
Maternal age	0.97	0.93-1.01	0.11
Parity ≥2	0.70	0.39-1.26	0.17
Model 2			
Pregnancy weight gain >6 kg by 18 weeks' gestation	1.10	1.04-1.15	<0.001
Maternal prepregnancy obesity	3.46	1.49-8.05	0.004
Maternal age	—	—	—
Parity ≥2			
Adolescent central obesity	4.27	2.68-6.79	<0.001
	Adolescent Males		
Univariate Analysis	OR	95% CI	*P* Value
Maternal prepregnancy obesity	3.33	1.41-7.84	0.006
Pregnancy weight gain >6 kg 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
Multivariable regression analysis			
Model 1			
Maternal prepregnancy 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
Model 2			
Maternal prepregnancy 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

Maternal prepregnancy obesity is defined as BMI ≥30 kg/m^2; paternal obesity is defined as BMI ≥30 kg/m^2; adolescent central obesity is defined by waist circumference as ≥80 cm in females and ≥94 cm in males. *P* values <0.05 are considered statistically significant.

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 sexes.

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 prepregnancy 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 SES 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 prepregnancy obesity (Table 5). Family SES at the time of childbirth remained significantly associated with NAFLD in male adolescents after adjusting for male adolescent obesity and parent prepregnancy obesity.

Discussion

The Raine study provides an opportunity to examine intergenerational relationships between parental pregnancy-related anthropometric and sociodemographicic 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 (IR) in adolescents. Maternal prepregnancy obesity was associated with a higher prevalence of NAFLD, greater severity of HS, 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 IR 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 reported, serum adipokine levels show sex differences. Leptin levels are positively associated with subcutaneous adiposity and severity of HS, particularly in females whereas 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 to males.

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

Earlier studies have linked maternal prepregnancy obesity and excessive gestational weight gain with obesity[40-42] and adverse cardiometabolic 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 was associated more with maternal obesity than paternal obesity.[42] Environmental factors have been suggested to have a dominant effect over genetic influences on sex-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 proposed by other investigators.[34,42,46,47]

Maternal pregnancy-related obesity, high-fat diet, and IR have been shown to increase the risk of HS 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, whereas paternal obesity and low family SES at the time of birth show a distinct association with NAFLD in male offspring. Nevertheless, maternal prepregnancy obesity is associated with NAFLD in both sexes. Low SES is a determinant of higher risk of MetS later in life.[49] We are unable to determine a precise cause of the sex-selective association of low family SES at birth with NAFLD in male adolescent offspring or the association between maternal obesity and offspring hyperleptinemia and IR in female adolescent offspring. However, we have found an association between low SES and shorter breastfeeding duration that is linked to NAFLD in male offspring. We speculate that there is a contribution of sex-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 be related to the small number of mothers with diagnosed gestational diabetes at the time, given that routine oral glucose tolerance testing in pregnancy was not the norm during the study period and consequent underdiagnosing and under-reporting 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 prepregnancy weight that was used to calculate prepregnancy 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 2 diabetes mellitus prepregnancy 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. Whereas liver ultrasound has limited sensitivity for fatty liver compared with liver histology and magnetic resonance imaging, 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 sex differentially. The influence of the family socioeconomic situation on NAFLD appears to associate with male offspring whereas 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. Whereas 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 prepregnancy, a healthy maternal diet during pregnancy, and healthy first trimester gestational weight gain. Population health guidelines need to engage the community in risk reduction before 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.

Acknowledgments: We thank the Raine Study participants and their families. We also thank the Raine Study team for cohort coordination and data collection. The National Health and Medical Research Council, the University of Western Australia, the Raine Medical Research Foundation, the Faculty of Medicine, Dentistry, and Health Sciences of the University of Western Australia, Telethon Kids Institute, the Women's and Infant's Research Foundation, Curtin University, and Edith Cowan University are

acknowledged for their support and funding of the Raine Study.

REFERENCES

1) Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2006;91:4753-4761.
2) Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006 Feb;43(2 Suppl 1):S99-S112.
3) Ayonrinde OT, Olynyk JK, Beilin LJ, Mori TA, Pennell CE, de Klerk N, et al. Gender-specific differences in adipose distribution and adipocytokines influence adolescent nonalcoholic fatty liver disease. Hepatology 2011;53:800-809.
4) Ayonrinde OT, Olynyk JK, Marsh JA, Beilin LJ, Mori TA, Oddy WH, et al. Childhood adiposity trajectories and risk of nonalcoholic fatty liver disease in adolescents. J Gastroenterol Hepatol 2015;30:163-171.
5) Anderson EL, Howe LD, Fraser A, Callaway MP, Sattar N, Day C, et al. Weight trajectories through infancy and childhood and risk of non-alcoholic fatty liver disease in adolescence: The ALSPAC study. J Hepatol 2014;61:626-632.
6) Zimmermann E, Gamborg M, Holst C, Baker JL, Sørensen TI, Berentzen TL. Body mass index in school-aged children and the risk of routinely diagnosed non-alcoholic fatty liver disease in adulthood: a prospective study based on the Copenhagen School Health Records Register. BMJ Open 2015;5:e006998.
7) Berentzen TL, Gamborg M, Holst C, Sørensen TI, Baker JL. Body mass index in childhood and adult risk of primary liver cancer. J Hepatol 2014;60:325-330.
8) Zimmermann E, Berentzen TL, Gamborg M, Sørensen TI, Baker JL. Sex-specific associations between birth weight and adult primary liver cancer in a large cohort of Danish children. Int J Cancer 2016;138:1410-1415.
9) Hagström H, Stål P, Hultcrantz R, Hemmingsson T, Andreasson A. Overweight in late adolescence predicts development of severe liver disease later in life: a 39 years follow-up study. J Hepatol 2016;65:363-368.
10) Popkin BM, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. Int J Obes Relat Metab Disord 2004;28(Suppl 3):S2-S9.
11) Nielsen SJ, Siega-Riz AM, Popkin BM. Trends in energy intake in U.S. between 1977 and 1996: similar shifts seen across age groups. Obes Res 2002;10:370-378.
12) Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. JAMA 2012;307:483-490.
13) Booth ML, Chey T, Wake M, Norton K, Hesketh K, Dollman J, et al. Change in the prevalence of overweight and obesity among young Australians, 1969-1997. Am J Clin Nutr 2003;77:29-36.
14) Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766-781.
15) Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988-1994 to 2007-2010. J Pediatr 2013;162:496-500.
16) Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42:641-649.
17) Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461-1465.
18) Adams LA, Marsh JA, Ayonrinde OT, Olynyk JK, Ang WQ, Beilin LJ, et al. Cholesteryl ester transfer protein gene polymorphisms increase the risk of fatty liver in females independent of adiposity. J Gastroenterol Hepatol 2012;27:1520-1527.
19) Adams LA, White SW, Marsh JA, Lye SJ, Connor KL, Maganga R, et al. Association between liver-specific gene polymorphisms and their expression levels with nonalcoholic fatty liver disease. Hepatology 2013;57:590-600.
20) Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011;7:e1001324.
21) Tarnoki AD, Tarnoki DL, Bata P, Littvay L, Osztovits J, Jermendy G, et al. Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. Liver Int 2012;32:1287-1293.
22) Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology 2009;136:1585-1592.
23) Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2006;4:1162-1169.
24) Oben JA, Mouralidarane A, Samuelsson AM, Matthews PJ, Morgan ML, McKee C, et al. Maternal obesity during pregnancy and lactation programs the development of offspring nonalcoholic fatty liver disease in mice. J Hepatol 2010;52:913-920.
25) Stewart MS, Heerwagen MJ, Friedman JE. Developmental programming of pediatric nonalcoholic fatty liver disease: redefining the "first hit". Clin Obstet Gynecol 2013;56:577-590.
26) Ayonrinde OT, Oddy WH, Adams LA, Mori TA, Beilin LJ, de Klerk N, Olynyk JK. Infant nutrition and maternal obesity influence the risk of nonalcoholic fatty liver disease in adolescents. J Hepatol 2017 May 22. pii: S0168-8278(17)30200-3. doi: 10.1016/j.jhep.2017.03.029. [Epub ahead of print]
27) Loomba R, Hwang SJ, O'Donnell CJ, Ellison RC, Vasan RS, D'Agostino RB, Sr., et al. Parental obesity and offspring serum alanine and aspartate aminotransferase levels: the Framingham heart study. Gastroenterology 2008;134:953-959.
28) Sookoian S, Pirola CJ. Genetic predisposition in nonalcoholic fatty liver disease. Clin Mol Hepatol 2017;230:1-12.
29) Dongiovanni P, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. Curr Pharm Des 2013;19:5219-5238.
30) Loomba R, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, et al.; Genetics of NAFLD in Twins Consortium. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. Gastroenterology 2015;149:1784-1793.
31) Cui J, Chen CH, Lo MT, Schork N, Bettencourt R, Gonzalez MP, et al.; for The Genetics Of NAFLD In Twins Consortium. Shared genetic effects between hepatic steatosis and fibrosis: A prospective twin study. Hepatology 2016;64:1547-1558.
32) Zarrinpar A, Gupta S, Maurya MR, Subramaniam S, Loomba R. Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study. Gut 2016;65:1546-1554.

33) Ayonrinde OT, Adams LA, Doherty DA, Mori TA, Beilin LJ, Oddy WH, et al. Adverse metabolic phenotype of adolescent girls with non-alcoholic fatty liver disease plus polycystic ovary syndrome compared with other girls and boys. J Gastroenterol Hepatol 2016;31:980-987.
34) **Gaillard R**, **Welten M**, Oddy WH, Beilin LJ, Mori TA, Jaddoe V, Huang RC. Associations of maternal prepregnancy body mass index and gestational weight gain with cardio-metabolic risk factors in adolescent offspring: a prospective cohort study. BJOG 2016;123:207-216.
35) Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012;142:1592-1609.
36) Pink B. Australian Bureau of Statistics: Socio-Economic Indexes for Areas (SEIFA)—Technical Paper, Canberra. Report no.: ABS Catalogue no. 2039.0.55.001. Canberra: Australian Bureau of Statistics; 2006.
37) Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet 2007;369:2059-2061.
38) Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol 2007;102:2708-2715.
39) Li M, Sloboda DM, Vickers MH. Maternal obesity and developmental programming of metabolic disorders in offspring: evidence from animal models. Exp Diabetes Res 2011;2011: 592408.
40) Kaar JL, Crume T, Brinton JT, Bischoff KJ, McDuffie R, Dabelea D. Maternal obesity, gestational weight gain, and offspring adiposity: the exploring perinatal outcomes among children study. J Pediatr 2014;165:509-515.
41) Oken E, Rifas-Shiman SL, Field AE, Frazier AL, Gillman MW. Maternal gestational weight gain and offspring weight in adolescence. Obstet Gynecol 2008;112:999-1006.
42) Rath SR, Marsh JA, Newnham JP, Zhu K, Atkinson HC, Mountain J, et al. Parental pre-pregnancy BMI is a dominant early-life risk factor influencing BMI of offspring in adulthood. Obes Sci Pract 2016;2:48-57.
43) Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol 2015; 30:1141-1152.
44) Perez-Pastor EM, Metcalf BS, Hosking J, Jeffery AN, Voss LD, Wilkin TJ. Assortative weight gain in mother-daughter and father-son pairs: an emerging source of childhood obesity. Longitudinal study of trios (EarlyBird 43). Int J Obes (Lond) 2009;33: 727-735.
45) Han TS, Hart CL, Haig C, Logue J, Upton MN, Watt GC, et al. Contributions of maternal and paternal adiposity and smoking to adult offspring adiposity and cardiovascular risk: the Midspan Family Study. BMJ Open 2015;5:e007682.
46) Laitinen J, Jääskeläinen A, Hartikainen AL, Sovio U, Vääräsmäki M, Pouta A, et al. Maternal weight gain during the first half of pregnancy and offspring obesity at 16 years: a prospective cohort study. BJOG 2012;119:716-723.
47) Andersen CS, Gamborg M, Sørensen TI, Nohr EA. Weight gain in different periods of pregnancy and offspring's body mass index at 7 years of age. Int J Pediatr Obes 2011;6:e179-e186.
48) Modi N, Murgasova D, Ruager-Martin R, Thomas EL, Hyde MJ, Gale C, et al. The influence of maternal body mass index on infant adiposity and hepatic lipid content. Pediatr Res 2011; 702:87-90.
49) Moore T, McDonald M, McHugh-Dillon H. Early childhood development and the social determinants of health inequities: a review of the evidence. Parkville, Victoria: Centre for Community Child Health at the Murdoch Childrens Research Institute and the Royal Children's Hospital; 2014. http://www.rch.org.au/uploadedFiles/Main/Content/ccch/151014_Evidence-review-early-childhood-development-and-the-social-determinants-of-health-inequities_Sept2015.pdf. Accessed April 28, 2017.
50) Abarin T, Yan Wu Y, Warrington N, Lye S, Pennell C, Briollais L. The impact of breastfeeding on FTO-related BMI growth trajectories: an application to the Raine pregnancy cohort study. Int J Epidemiol 2012;41:1650-1660.

Author names in bold designate shared co-first authorship.