Research Article

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Infant nutrition and maternal obesity influence the risk of non-alcoholic fatty liver disease in adolescents

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Background & Aims: The pathway to non-alcoholic fatty liver disease (NAFLD) in adolescents may have its origins in adiposity gains, nutrition and sedentary lifestyle established during childhood. There is inadequate knowledge regarding the associations between infant nutrition and subsequent NAFLD. We examined the association of maternal factors and infant nutrition, with the subsequent diagnosis of NAFLD in adolescents.

Methods: Adolescents aged 17 years in the Western Australian Pregnancy (Raine) Cohort study had fatty liver assessment using liver ultrasound. Prospectively recorded data on maternal pregnancy and infant feeding were examined against a NAFLD outcome during late adolescence.

Results: NAFLD was diagnosed in 15.2% of the 1,170 adolescents examined. Ninety-four percent had been breastfed as infants. The duration of breastfeeding before starting supplementary milk was $\geqslant$4 months in 54.4% and $\geqslant$6 months in 40.6%. Breastfeeding without supplementary milk $\geqslant$6 months (adjusted odds ratio [OR]: 0.64; 95% confidence interval [CI]: 0.43–0.94, $p = 0.02$), maternal pre-pregnancy obesity (adjusted OR: 2.29; 95% CI: 1.21–4.32, $p = 0.01$) and adolescent obesity (adjusted OR: 9.08; 95% CI: 6.26–13.17, $p < 0.001$) were associated with NAFLD independent of a Western dietary pattern at 17 years of age. Adolescents with NAFLD who had been breastfed for $\geqslant$6 months had a less adverse metabolic profile compared with adolescents breastfed for <6 months. Supplementary milk intake starting before 6 months was associated with a higher prevalence and ultrasound severity of NAFLD compared with intake starting after 6 months (17.7% *vs.* 11.2%, $p = 0.003$ and 7.8% *vs.* 3.4%, $p = 0.005$ respectively).

Conclusion: Though NAFLD is generally mediated through adiposity gains, breastfeeding for at least 6 months, avoidance of early supplementary formula milk feeding, and normal maternal pre-pregnancy BMI may reduce the odds of a NAFLD diagnosis during adolescence.

Lay summary: Non-alcoholic fatty liver disease (NAFLD) is a common liver disorder in which there is too much fat in the liver of people who do not consume excessive amounts of alcohol. In this large study, we found that infants who consumed breast milk for less than 6 months before starting infant formula milk, infants who were obese as teenagers or had mothers who were obese at the start of pregnancy, were much more likely to have NAFLD at 17 years of age. Based on our findings we consider that reducing the risk of NAFLD in teenagers needs to start before birth, by encouraging normal body mass index before pregnancy, as well as breastfeeding without infant formula milk consumption for the first 6 months of life.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a complex disorder in which there is an excessive fat deposition in the liver that is commonly associated with obesity and insulin resistance in the absence of excessive alcohol intake. NAFLD is now the most common liver disorder in humans,[1] with a general population prevalence of 2.6% in children,[2] 15.2% in adolescents[3] and 19–25% in adults.[4–6] Population data from the National Health and Nutrition Examination Survey in the USA showed a doubling of the prevalence of suspected NAFLD in adolescents over a 20-year period, up to 2010.[7] Severe hepatic steatosis, diagnosed using ultrasound, has been shown to be independently associated with increased liver disease morbidity and mortality.[8] Furthermore, the histologic spectrum of NAFLD, comprising plain steatosis, non-alcoholic steatohepatitis (NASH) and NASH-associated cirrhosis can occur from childhood through to adulthood.[9] However,

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despite increasing evidence that adiposity gain during childhood and adolescence is a significant risk factor for NAFLD in adolescence[10,11] and in adulthood,[12] the role of early life nutrition, including breastfeeding has not been adequately elucidated.

Prior to 2001, the World Health Organization (WHO) recommended that infants should be exclusively breastfed for four to six months before introducing complementary foods, however, recommendations now suggest exclusive breastfeeding for the first six months of life.[13] Benefits of breastfeeding extend beyond nutritional value to include potential reduction in rates of childhood infection, obesity and allergies later in life.[14] There are, however, inconsistent reports about the influence of breastfeeding on later obesity,[15] with some studies describing a possible protective effect on obesity[16,17] considered more likely with a threshold of six months of breastfeeding,[18–21] and other studies unable to demonstrate this.[22–24] The contradictions may reflect varied breastfeeding definitions,[22] mixed feeding patterns including infant formula milk or complementary feeding, recall bias, maternal and individual factors including prevalent dietary habits and sedentary lifestyle. Maternal obesity has been associated with shorter durations of breastfeeding, early introduction of supplementary formula milk and complementary food, possibly unhealthy food preferences in childhood and later obesity.[25] There are, however, few studies examining whether maternal obesity, infant nutrition and early feeding habits prospectively influence the development of NAFLD. In particular, there is no current evidence that breastfeeding causally reduces NAFLD risk in humans. In the only published observational study examining the effect of breastfeeding on the development of NASH in humans, Nobili *et al.* found longer duration of breastfeeding was associated with a reduction in the risk of NASH in children and adolescents.[26]

The aim of this study was to examine associations between the duration of breastfeeding and age at introduction of complementary milk or solid food, maternal pre-pregnancy obesity and adolescent obesity, on the diagnosis of NAFLD in adolescents from the Western Australian Pregnancy Cohort (Raine Cohort) at 17 years of age.

Materials and methods

The Raine study is a longitudinal cohort study with prospectively collected maternal, birth, child and adolescent data, including detailed nutritional data in the early years of life and serial follow-up every 2–3 years. The Raine study was initiated as a pregnancy and birth cohort comprising 2,868 live-born children from 2,900 pregnancies recruited mainly from the antenatal clinics of King Edward Memorial Hospital for Women in Perth, Western Australia between 1989 and 1992. The background and serial assessments of the Raine cohort has been detailed previously.[21] The following terms are explained: antenatal refers to the period during pregnancy, neonate refers to the newborn and infant is the child under 1 year. Antenatal data on mothers was prospectively collected, incorporating socio-demographic characteristics, history of gestational diabetes, hypertension during pregnancy, weight and height and calculated body mass index (BMI). Neonatal data included mode of delivery, birth anthropometry, early feeding pattern and age when discharged home. Each subsequent child assessment involved detailed questionnaires on lifestyle, health, medications, and physical assessments including anthropometry and cardiovascular assessments. Lists of medications given to the infant, including antibiotics, were documented by the mother or care-giver at the 1-year assessment. Aspects of infant nutrition examined were the duration of breast milk feeding, age at introduction of non-breast milk and solid feeding and types of milk consumed, as reported by the parents or primary care-giver of the child during the first 3 years of life. 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. Exclusive breastfeeding is defined as per the WHO, as breastfeeding with no supplementary milk or complementary food intake.[13] For this study, the terms breastfeeding and breast milk feeding are used interchangeably while consumption of supplementary milk or infant formula milk are considered the same. The age at which individuals stop breastfeeding and age of starting infant formula milk may have different metabolic effects. For example, in the Raine study, infants breastfed for >4 months but introduced to other milk at ⩽4 months (mixed feeding), had the highest increase in BMI at age 14 years.[17] Therefore, we considered the duration of breastfeeding and age at introduction of supplementary milk and complementary food separately, given the potential mixed patterns of feeding. Breastfeeding with supplementary milk intake and breastfeeding with no supplementary milk are used to describe feeding patterns regardless of any other complementary food intake.

The 17-year cross-sectional assessment of the cohort was conducted between July 2006 and June 2009, at which time the participating cohort was representative of the broader Western Australian population.[3] At age 17, liver ultrasound was performed to assess for fatty liver. Other data collected at the time were derived from detailed questionnaires, anthropometric, clinical and biochemical measurements as previously described.[3] Laboratory assessments were performed with venous blood samples taken from an antecubital vein after an overnight fast. Serum glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), ferritin, transferrin saturation, high sensitivity C-reactive protein (hsCRP), adiponectin, and leptin levels were assayed. We did not test for hepatitis B or C virus infections 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] Anthropometric measurements (weight, height, waist circumference, hip circumference, and skinfold thickness [SFT]) and cardiovascular assessments (resting pulse rate, systolic blood pressure, and diastolic blood pressure) were conducted by trained examiners. BMI was derived from weight (kg)/height^2 (m^2). Central obesity in the adolescents was defined by waist circumference ⩾80 cm in females and ⩾94 cm in males, consistent with age and gender-specific metabolic syndrome criteria of the International Diabetes Federation.[27] We defined adolescent obesity by waist circumference since we previously identified a higher proportion of adolescents with central obesity using waist circumference than using BMI.[3] The homeostasis model assessment for insulin resistance (HOMA-IR) score was calculated as follows: HOMA-IR score = (Fasting insulin [μU/ml] × Fasting glucose [mmol/L])/22.5.

Previously published reports describe the liver ultrasound methodology[3] and protocol.[28] The diagnosis of hepatic steatosis (fatty liver) by ultrasound required a total fatty liver score of at least 2, including a liver echotexture score of at least 1. The ultrasound score was computed from liver echotexture (bright liver and hepato-renal echo contrast) 0–3, deep attenuation (diaphragm visibility) 0–2, and vessel blurring (intrahepatic vessel visibility) 0–1. NAFLD steatosis severity was derived from 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). We used an alcohol intake threshold of <140 grams per week for females and <210 grams per week in males, consistent with recent NAFLD diagnosis and management guidelines,[29] to refine the ultrasound diagnosis of fatty liver to a clinical diagnosis of NAFLD. At 14 years of age, the adolescent, the parent or care-giver completed a semi-quantitative food frequency questionnaire (FFQ) developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO).[30,31] From the FFQ data, two dietary patterns, described as the "healthy" pattern or "Western" pattern were defined and the extent of intake of these during the preceding 12 months was estimated. A z-score was assigned for each dietary pattern, indicating how closely the reported intake corresponded with the two patterns.[31] We have previously described associations between dietary patterns and NAFLD in adolescents in the Raine cohort.[31] Institutional ethics committee approval was obtained from the Princess Margaret Hospital for Children Human Research Ethics Committee. Signed informed parental consent and adolescent assent at 17 years were obtained.

Statistical analysis

Variables were summarized by the mean and standard deviation for symmetrical distributions and median and interquartile range (IQR) for asymmetric distributions. Differences in normally distributed data were analysed using Student's *t* test or analysis of variance, while non-normally distributed data were analysed using the Mann-Whitney *U* test. Chi-square or Fisher's exact test, as appropriate, were used to compare proportions. Multivariable logistic regression analysis was used to identify predictors of NAFLD from maternal data, adolescent obesity, adolescent dietary patterns and infant feeding data. All statistical tests were

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