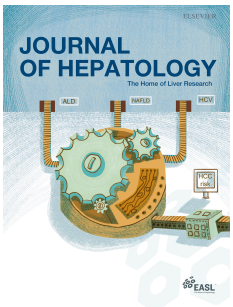
Journal Pre-proof



Maternal obesity increases the risk and severity of NAFLD in offspring

Hannes Hagström, Tracey G. Simon, Bjorn Roelstraete, Olof Stephansson, Jonas Söderling, Jonas F. Ludvigsson

PII: S0168-8278(21)01899-7

DOI: https://doi.org/10.1016/j.jhep.2021.06.045

Reference: JHEPAT 8354

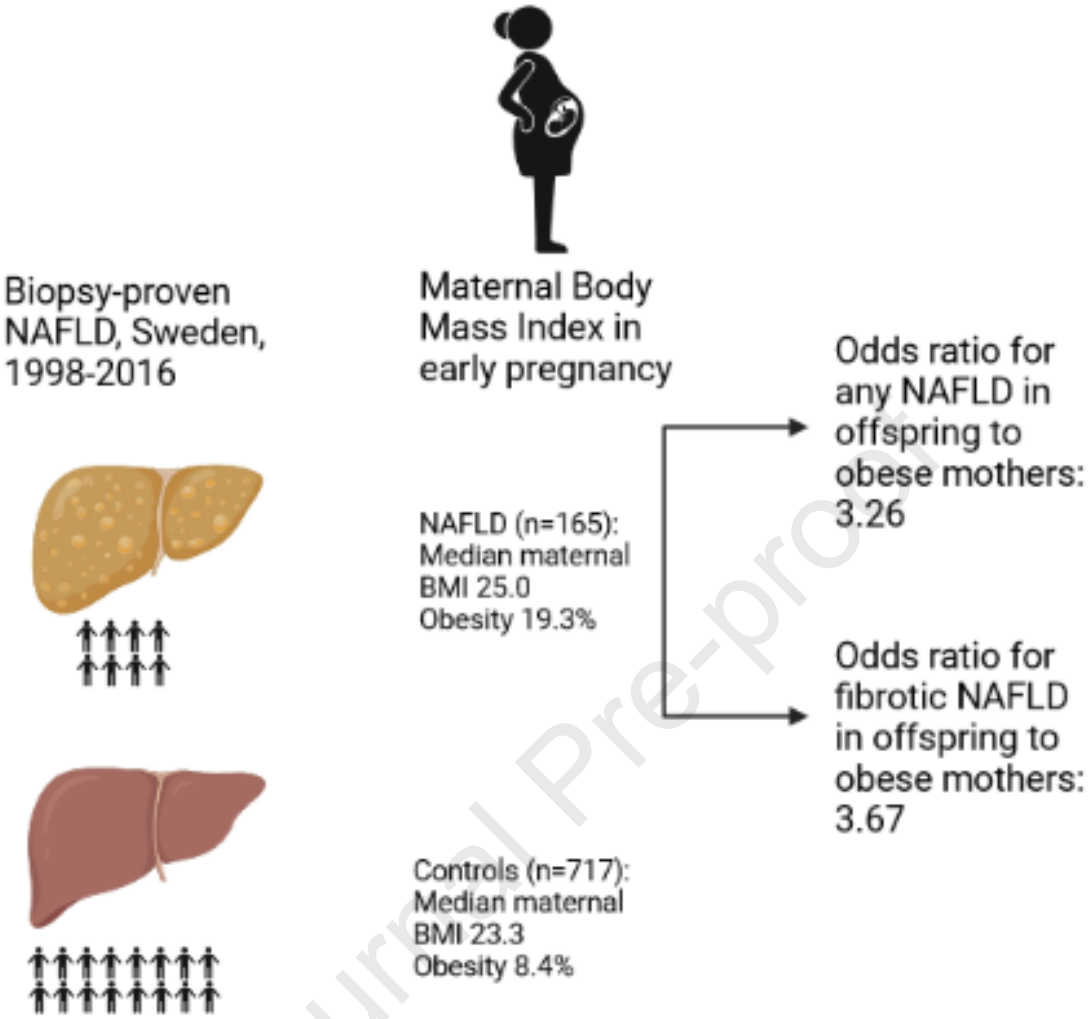
To appear in: *Journal of Hepatology*

Received Date: 22 March 2021

Revised Date: 22 June 2021

Accepted Date: 29 June 2021

Please cite this article as: Hagström H, Simon TG, Roelstraete B, Stephansson O, Söderling J, Ludvigsson JF, Maternal obesity increases the risk and severity of NAFLD in offspring, *Journal of Hepatology* (2021), doi: https://doi.org/10.1016/j.jhep.2021.06.045.



Maternal obesity increases the risk and severity of NAFLD in offspring

Hannes Hagström[1,2,3*], Tracey G Simon[4,5,6*], Bjorn Roelstraete[7], Olof Stephansson[2,8], Jonas Söderling[2], Jonas F Ludvigsson[7,9-11]

[1] Division of Hepatology, Department of Upper GI, Karolinska University Hospital, Stockholm, Sweden
[2] Clinical Epidemiology Division, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden
[3] Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden
[4] Division of Gastroenterology and Hepatology, Massachusetts General Hospital, Boston, MA, USA
[5] Harvard Medical School, Boston, MA, USA
[6] Clinical and Translational Epidemiology Unit (CTEU), Massachusetts General Hospital, Boston, MA, USA
[7] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[8] Division of Women's Health, department of Obstetrics, Karolinska University Hospital, Stockholm, Sweden
[9] Department of Pediatrics, Orebro University Hospital, Orebro, Sweden.
[10] Division of Epidemiology and Public Health, School of Medicine, University of Nottingham, UK.
[11] Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York, USA
*Equal contribution.

Correspondence and reprint requests:
Hannes Hagström, C1:77, Division of Hepatology, Karolinska University Hospital, 141 86 Stockholm, Sweden
Phone: +46 (0) 8 5858 2305, Fax: +46 (0) 8 5858 2335
E-mail: hannes.hagstrom@ki.se

Keywords: obesity; steatosis; fibrosis; cirrhosis; children
Short title: Maternal BMI and offspring NAFLD

Abbreviations: aOR, adjusted odds ratio. BMI, body mass index. CI, confidence interval. ESPRESSO, Epidemiology Strengthened by Histopathology Reports in Sweden. IQR, interquartile range. MBR, Swedish medical birth register. NAFLD, non-alcoholic fatty liver disease. NASH, non-alcoholic steatohepatitis. OR, odds ratio. PIN, personal identity number. SD, standard deviation.

Total word count: 3817 (introduction to references). **Abstract: 243**. **Tables: 4**. **Figures: 0**. **Character count for title: 114.**

Disclosure / Conflict of interest declaration: Conflict of interest: Dr Hagström's institution has received independent research grants from Intercept, Gilead, Astra Zeneca, EchoSens, MSD and Pfizer. Board advisory for Gilead and Bristol-Myers Squibb. These concern works unrelated to this project.
Dr. Simon has received grants to the institution from Amgen and has served as a consultant to Aetion for work unrelated to this project.
Dr Ludvigsson coordinates a study on behalf of the Swedish IBD quality register (SWIBREG). This study has received funding from Janssen corporation.

Grant Support (Funding): Dr Hagström is supported by grants from Region Stockholm (clinical postdoctoral appointment).
Dr. Simon is supported by grants from the National Institutes of Health (NIH) (K23 DK122104), the Dana-Farber/Harvard Cancer Center (DF/HCC) Gastrointestinal (GI) Specialized Program in Research Excellence (SPORE).

Author's contributions: Study conception and design: All
Acquisition of data: JFL
Statistical analysis: JS, BR
Analysis and interpretation of data: All
Drafting of manuscript: HH, TGS, JFL
Critical revision: All
Guarantor of article: JFL
All authors approved the final version of the article, including the authorship list.

Writing Assistance: None.

Abstract

Background and Aims: Maternal obesity has been linked to the development of cardiovascular disease and diabetes in offspring, but its relationship to non-alcoholic fatty liver disease (NAFLD) is unclear.

Methods: Through the nationwide ESPRESSO cohort study we identified all individuals in Sweden with biopsy-verified NAFLD ≤25 years of age diagnosed between 1992 and 2016 (n=165). These were matched on age, sex, and calendar year with up to 5 controls (n=717). Through linkage with the nationwide Swedish Medical Birth Register (MBR) we retrieved data on maternal early-pregnancy body mass index (BMI), and possible confounders, in order to calculate adjusted odds ratios (aORs) for future offspring NAFLD.

Results: Maternal BMI was associated with offspring NAFLD (underweight women (aOR=0.84; 95%CI=0.14-5.15); normal weight (reference, aOR=1), overweight (aOR=1.51; 0.95-2.40), and obesity (aOR=3.26; 1.72-6.19). Also, severe NAFLD (biopsy-proven fibrosis or cirrhosis) was more common in offspring of overweight (aOR=1.94; 95%CI=0.96-3.90) and obese mothers (aOR=3.67; 95%CI=1.61-8.38). Associations were similar after adjusting for maternal pre-eclampsia and gestational diabetes. Socio-economic parameters (smoking, mother born outside the Nordic countries and less than ten years of basic education) were also associated with offspring NAFLD but did not materially affect the effect size of maternal BMI in a multivariable model.

Conclusions: This nationwide study found a strong association between maternal overweight/obesity and future NAFLD in the offspring. Adjusting for socio-economic and metabolic parameters in the mother did not affect the finding. This suggests that maternal obesity may be an independent risk factor for offspring NAFLD.

Lay summary

In a study of all young persons in Sweden with a liver biopsy consistent with fatty liver, the authors found that compared to matched controls, the risk of fatty liver was much higher in those with obese mothers. This was independent of available confounders, and suggest that the high prevalence of obesity in younger persons might lead to a higher risk of fatty liver in their offspring.

Introduction

Changes in food quality and a more sedentary lifestyle have led to a high prevalence of obesity globally (1). Trailing this epidemic is the rapid increase in the prevalence of non-alcoholic fatty liver disease (NAFLD), now the most common liver disease worldwide (2), and affecting an estimated 25% of the global population. Obesity has also become increasingly common even early in life, including in women of reproductive age (3). This does not only have consequences for affected women, but maternal obesity is also a risk factor for obesity, type 1 diabetes and cardiometabolic disease in the offspring (4-7).

Additional human studies have shown that adolescents with ultrasound-diagnosed NAFLD often have had obese mothers (8). A recently formulated hypothesis based on preclinical data suggests that adaptions to maternal obesity in early life environment impact the risk of offspring metabolic disorders, such as NAFLD both in rodent models and in humans (9-13). It is unclear if also the risk of severe NAFLD is increased, and importantly if the effect of maternal obesity on offspring liver-related disease can be confounded by other factors. An increase in risk could be explained partly by intrinsic maternal factors such as obesity, but possibly also by socio-economic determinants. Such a distinction might be important, since societal and individual changes including reduced food availability and intake, or increased education might improve obesity-associated diseases such as NAFLD if there are no "programmed" behaviours which are generally less susceptible to intervention.

Here, we hypothesised that maternal body mass index (BMI) in early pregnancy is a risk factor for biopsy-proven NAFLD in the offspring, and especially severe NAFLD.

Material and methods

Study population

We performed a population-based case-control study using the ESPRESSO (Epidemiology Strengthened by Histopathology Reports in Sweden) cohort (14). The ESPRESSO cohort holds detailed data on liver histopathology from all 28 Swedish pathology departments (1965-2017), defining histopathology findings using SNOMED coding, such as steatosis, non-cirrhotic fibrosis and cirrhosis (15). Reports also include the unique Swedish personal identity number (PIN) (16), which we used to link ESPRESSO data to several national registers containing validated prospectively-recorded data on demographics including socio-economic data and development of diseases with diagnoses made at hospital level (available since 1964 but nationwide from 1987), and since 2001 also on outpatient visits in specialized healthcare (17). Finally, data were linked to the Medical Birth Register (MBR) with data on BMI since 1992. The MBR contains data from the first antenatal visit until delivery and discharge from the delivery hospital (18).

For this study, we identified all liver biopsy specimens with a SNOMED diagnosis of hepatic steatosis. The process to identify NAFLD has been validated (positive predictive value 92%) and has been described elsewhere (19). We excluded participants aged >25, since they per definition were born prior to 1992 (with no data on maternal BMI in the MBR).
We then excluded individuals with a competing diagnosis that could potentially cause steatosis, such as alcohol-related liver disease, viral hepatitis or rare pediatric liver diseases that could cause steatosis (**eTable 1**). We further identified the NAFLD population with a more severe disease, defined as presence of liver fibrosis or cirrhosis based on SNOMED coding (M4900x or M4950x). As we expected, the number of individuals with cirrhosis to be low, cases with fibrosis or cirrhosis were merged into one subgroup, defined as "severe NAFLD".

We then matched each NAFLD case with up to five controls from the general population. Controls were systematically sampled by the central authority "Statistics Sweden" that hold detailed census-level data on all Swedish citizens through the Total Population Register (20). Controls were matched at the time of first liver biopsy in the index individual. Matching criteria were sex, exact age, county and calendar year. Exclusion and inclusion criteria were identical for cases and controls (**eTable 1**).

Next, we identified mothers to cases and controls (21). From the MBR, we obtained data on maternal and pregnancy-related parameters that *a priori* were considered important for the development of NAFLD in the offspring **(Table 1)**. Parameters obtained from the MBR included maternal age, calendar year, maternal country of

birth, smoking status, and level of education. Maternal country of birth was categorized as born in a Nordic country (yes/no). Smoking status was divided into three categories (non-smoker, smoking 1- 9, or 10 or more cigarettes per day, or missing data), and level of education into four categories (≤9 years, 10- 12 years; ≥13 years, missing). Information on height and weight was collected at the first antenatal visit. Height was self- reported, while weight was measured by a midwife. BMI was calculated and categorized as underweight (<18.5 kg/m^2), normal weight (18.5- 24.9 kg/m^2, used as the reference category), overweight (25.0- 29.9 kg/m^2) and obesity (≥30 kg/m^2).

We also retrieved data on diagnoses of commonly occurring comorbidities of NAFLD in the offspring from the National Patient Register (17). These included cardiovascular disease, diabetes type 1 or 2, hypertension and hyperlipidemia (definitions in **eTable 2**).

For a subsequent sensitivity analysis, we used the Total Population Register (20) to identify all full siblings to patients with a NAFLD biopsy, who then served as the control population, consistent with our prior work (19, 22). Sibling analyses have the advantage of addressing potential intrafamilial confounding due to shared genetics and early environmental factors.

Statistical analysis

We estimated adjusted ORs for the risk of NAFLD in offspring based on BMI categories using conditional logistic regression. As the causal pathway between maternal factors such as a high BMI and offspring NAFLD is unknown, we considered two statistical models *a priori*. First, we constructed a model adjusted only for the matching factors (age at NAFLD diagnosis, sex and municipality). Next, we used a model adjusted for the matching factors plus the following maternal factors: age, country of birth, education, parity and smoking at the time of first entry in the MBR.

Missing data for BMI (18.2% in cases and 18.3% in controls) and smoking (7.3% in cases and 5.7% in controls) were imputed using a multiple imputation regression model by fully conditional specification methods (FCS) with five iterations (23). Regression estimates from each of the five sets of data were combined using the MIANALYZE procedure in SAS (v9.4).

In sensitivity analyses, we added preeclampsia and gestational diabetes to the second model. We did so to explore whether any association between maternal obesity and offspring NAFLD might be mediated through pregnancy-related metabolic factors.

We chose not to adjust for parameters in the offspring such as diabetes, as such parameters were considered likely to be part of the causal pathway, which could introduce bias (24).

A second sensitivity analysis was restricted to complete-case data (no imputations), replicating the above analyses. In a third sensitivity analysis we compared NAFLD cases with full sibling comparators. A fourth analysis stratified the results on offspring sex.

Finally, we explored univariate associations in the regression analyses with offspring NAFLD.

Ethical considerations

The study was approved by the Stockholm Ethics Review Board on August 27, 2014 (No.2014/1287-31/4). Informed consent was waived as the study was register-based (25).

Results

We identified 718 cases with a liver biopsy-based diagnosis of NAFLD below the age of 25 years and without any differential diagnosis. From these, we excluded 530 cases with a birthdate prior to 1992, and therefore no data on maternal BMI. Further, we excluded 20 cases who were not born in Sweden, and three cases who were not singleton births. Thus, our sample consisted of 165 cases of NAFLD. These were matched with 717 controls, subject to the same exclusion criteria. A flowchart for study inclusion is presented in **eTable 1**.
Most NAFLD cases were diagnosed after 2010. They had a median age of 12.0 years (IQR=4.4-16.9), and 60.6% were male (**Table 1**). Interestingly, offspring with later NAFLD had a lower birth weight compared to matched controls (median 3.35 kg vs 3.59, p<0.001).

General population analyses
Maternal BMI was higher in cases with NAFLD compared with controls (**Table 1**). Logistic regression revealed a higher prevalence of maternal obesity in offspring with NAFLD (19.3%) compared with controls (8.4%), with evidence of a dose-response effect of maternal BMI (p_{trend}=0.006). Compared to mothers with a normal BMI, the risk for offspring NAFLD in mothers with a BMI ≥30 kg/m^2 was 3-fold higher (aOR=3.26, 95%CI=1.72-6.19). This risk was not statistically significant and numerically lower in mothers with overweight (aOR=1.51, 95%CI=0.95-2.40), and was also not seen in underweight mothers (aOR=0.84, 95%CI=0.14-5.15).

Further, 76 cases (46%) with NAFLD fulfilled our criteria for severe NAFLD (presence of fibrosis: n=71 or cirrhosis: n=5). The risk for severe offspring NAFLD was increased in both obese (aOR=3.67, 95%CI=1.61-8.38) and overweight mothers (aOR=1.94, 95%CI=0.96-3.90). The estimates for offspring NAFLD are presented in **Table 2**, and for severe NAFLD in **Table 3**.

Adjusting for maternal pre-eclampsia and gestational diabetes yielded similar results (**eTable 3**), while slightly higher risk estimates were seen in our complete case analysis (**eTable 4**) than in our main analysis.

Besides maternal BMI, especially socio-economic factors were significantly linked to offspring NAFLD (**Table 4**). Compared to women born outside the Nordic countries, women born in the Nordic countries had a significantly *lower* risk for offspring NAFLD (aOR=0.35, 95%CI=0.22-0.57). Smoking ≥10 cigarettes per day was associated with increased risk of offspring NAFLD (aOR=2.13, 95%CI=1.07-4.25), as was less than 10 years of completed education, albeit this association was not statistically significant (aOR=2.22, 95%CI=0.94-5.26).

Sibling analyses

In the sibling analysis, we compared 108 cases with NAFLD with their 156 siblings. Maternal BMI was similar between cases with NAFLD and their sibling controls (median 25.2 vs 25.3) (**eTable 5**). After multivariable adjustment, we found no association between maternal obesity and offspring NAFLD (aOR=1.38, 95%CI=0.35-5.39) (**eTable 6**).

Stratification on sex

There were 65 females and 100 males among the offspring. The odds for NAFLD in obese mothers were comparable for male (aOR=4.22, 95%CI=1.68-10.59) and female offspring (aOR=2.87, 95%CI=1.22-6.79) (**eTable 7a+b**).

Discussion

In this national, population-based case-control study, we demonstrate an increased risk of biopsy-proven NAFLD in offspring born to mothers with a high early-pregnancy BMI. This excess risk seems to be independent of several important socio-economic factors, as well as of smoking and gestational diabetes, that were otherwise linked to future risk of NAFLD. In fact, adjusting for the available socio-economic parameters increased the ORs for maternal BMI somewhat (from 3.07 to 3.26), suggesting that the association between maternal BMI and offspring NAFLD is unlikely to be fully explained by such factors.

Our results are largely consistent with those from preclinical rodent models (9-11). The prevalence of paediatric NAFLD in the US doubled between the end of the 1980s and 2010, when it was estimated at around 10% (26). Moreover, a secondary analysis of the Western Australian Pregnancy Cohort study found that out of 1170 17-year-old adolescents, 15.2% had ultrasound-defined NAFLD, and maternal obesity was a risk factor for offspring NAFLD, with an OR of 3.16, which is very similar to our point estimate (8). Our results supplement these findings by confirming the presence and severity of NAFLD by means of liver biopsy data, and further demonstrating that maternal BMI is linked to disease severity, even after adjustment for important maternal clinical factors and socio-economic parameters. Further, we show that adjusting for gestational diabetes and pre-eclampsia, as proxies for more severe metabolic disease, did not affect the risk for offspring NAFLD.

In the sibling-comparison, maternal obesity was not associated with offspring NAFLD. We cannot rule out that the observed association between maternal BMI and offspring NAFLD risk is mediated by other factors like foetal growth, comorbidities, diet or exercise that could differ between siblings, or that our sibling analysis was underpowered (precluding any sub-analyses). Another explanation could be that there is undiagnosed NAFLD in the siblings. In Sweden and elsewhere, siblings to individuals with biopsy-verified NAFLD do not routinely undergo liver biopsy for screening purposes, and this is not recommended in international guidelines (27).

The NAFLD diagnosis was most often made in older as compared to younger children, suggesting that NAFLD is less common in younger children. Metabolic comorbidities such as diabetes and hypertension were more common in the NAFLD patients as compared to controls, suggesting a shared dysmetabolic milieu. Moreover, we found that key socio-economic parameters such as being born to immigrant mothers, smoking and lower education were risk factors for offspring NAFLD, suggesting that groups with such characteristics are at a heightened risk for NAFLD and could be considered for focused public health interventions. We speculate that such factors are likely to be markers of a more sedentary lifestyle and a less healthy

diet, which to a large extent then is adopted by the offspring, leading to a higher risk for NAFLD. Indeed, previous studies have shown that individuals with lower education have higher BMI and more type 2 diabetes than the general population (28, 29). Given the known association between obesity and NAFLD, it is likely safe to assume that such factors also affect the prevalence of NAFLD and could help identify at-risk populations for public health interventions.

With the rising increase in prevalence of overweight and obesity in the population (1), including in pregnant women (3), our results suggest that the future prevalence of NAFLD in the paediatric and adolescent populations will increase, most likely continuing into later life. It has previously been shown that a high BMI early in life is associated with development of severe liver disease (30-32), and these results suggest that being exposed to obesity while at a reproductive age might also have cross-generational consequences. This further highlight the importance of obtaining a healthy lifestyle and a normal BMI prior to pregnancy, as part of family planning.

Women in reproductive age with an elevated BMI should receive active advice and education on how to reduce the risk for obesity-related conditions in themselves and their offspring, such as improvement in diet and physical exercise.

The current study has several strengths. First, it was nationwide and population-based, allowing us to identify all individuals with a biopsy-based diagnosis of NAFLD in Sweden during the study period. Second, maternal BMI and data on confounders were derived from national registers with prospectively collected and validated data, which reduces the risk for recall bias (often a threat to the internal validity of case-control studies). Third, we had data on liver biopsy which is the gold standard for both diagnosing and staging NAFLD and we have recently shown that the PPV for NAFLD in this cohort is 92% (19). Finally, we were able to exclude cases and controls with competing liver diseases.

Limitations include a risk for selection bias in that by nature of the study design, biopsy was mandated. Thus, we might have only captured the most severe cases of NAFLD, supported by the high prevalence of fibrosis in our study. Our results should however be generalizable to countries similar to Sweden. Additionally, we lack information on the indication for the liver biopsy, but the high prevalence of fibrosis and cirrhosis could suggest that one reason was suspicion of advanced NAFLD was a prominent reason for biopsy, which is in accordance with guidelines for when to perform a biopsy in paediatric NAFLD (27). The disease severity staging was derived from administrative coding that did not allow for more granular staging of fibrosis or presence of non-alcoholic steatohepatitis (NASH), such as defined by the NASH clinical research network system (33). We did not have data on ethnicity but used country of birth as a proxy. We did not have systematic ascertainment of NAFLD

status in the siblings, why those results should be interpreted with caution. We lacked detailed data on breastfeeding, which has been suggested to protect against offspring NAFLD (34, 35), but we also lacked data on diet, moderate alcohol consumption and exercise habits in the mother.

Even if we had access to prospective data on potential socio-economic and medical confounders, we cannot rule out residual confounding, especially in diet and physical activity patterns in mothers. As such, maternal obesity might not be a causal factor in that it induces specific changes in the foetal metabolism leading to a higher tendency for the offspring to develop NAFLD or other metabolic disease. An alternative hypothesis, partly supported by these data, is that mothers with NAFLD are more exposed to socio-economic determinants of poor health. Nevertheless, these data certainly suggest that maternal obesity is a marker and risk factor for offspring NAFLD. Finally, we did we not have granular data on offspring lifestyle factors. While such factors cannot impact on maternal BMI and are hence not confounders, they might have helped to explain the association with offspring NAFLD seen in this paper.

Conclusions

In this population-based case-control study, we show that maternal BMI early in pregnancy is an independent risk factor for the diagnosis and severity of NAFLD in their offspring. As obesity is increasing, this has implications for the future prevalence of NAFLD. Mothers with an elevated BMI should receive active counselling on how to reduce the risk of offspring NAFLD.

Tables

Table 1. Characteristics of patients with NAFLD and matched population comparators (birth year in 1992-2016)

Characteristic	NAFLD (N = 165)	Controls (N = 717)	P-value*
Sex, n (%)			
Women	65 (39.4%)	285 (39.7%)	0.93
Men	100 (60.6%)	432 (60.3%)	
Age at index date (years)			
Median (IQR)	12.0 (4.4-16.9)	11.7 (3.4-16.3)	
Categories, n (%)			
<11y	70 (42.4%)	324 (45.2%)	
11-<18y	70 (42.4%)	297 (41.4%)	0.75
18-≤25	25 (15.2%)	96 (13.4%)	
Birth year, n (%)			
1992-1999	102 (61.8%)	435 (60.7%)	
2000-2009	60 (36.4%)	268 (37.4%)	0.96
2010-2016	3 (1.8%)	14 (2.0%)	
Year of index date, n (%)			
1992-2000	20 (12.1%)	93 (13.0%)	
2001-2010	56 (33.9%)	255 (35.6%)	0.85
2011-2016	89 (53.9%)	369 (51.5%)	
Comorbidities ever before index date, n (%)			
Cardiovascular Disease	13 (7.9%)	13 (1.8%)	<0.001
Diabetes	9 (5.5%)	3 (0.4%)	<0.001
Hypertension	4 (2.4%)	0	<0.001
Dyslipidemia	0	0	-
NAFLD severity, n (%)			
Fibrosis	71 (43.0%)		-
Cirrhosis	5 (3.0%)		-
Cirrhosis or fibrosis	76 (46.1%)		-
Maternal and delivery characteristics			
Maternal BMI at first visit (kg/m^2)			
Median (IQR)	25.0 (22.0-29.0)	23.3 (21.3-26.6)	
Categories, n (%)			
<18.5	5 (2.8%)	27 (3.8%)	
18.5 - <25	77 (46.8%)	436 (60.8%)	<0.001
25 - <30	51 (31.2%)	194 (27.0%)	
≥30	32 (19.3%)	60 (8.4%)	
Gestational age (days)			
Median (IQR)	279 (272-285)	281 (273-287)	
Categories (weeks), n (%)			
<37	15 (9.1%)	34 (4.7%)	
37-41	142 (86.1%)	630 (87.9%)	0.05
≥42	8 (4.8%)	53 (7.4%)	
Birth weight (grams)			
Median (IQR)	3350 (3090-3773)	3590 (3265-3945)	<0.001
Maternal smoking in early pregnancy			
Non-smoking	130 (78.7%)	606 (84.5%)	
1-9 cig/day	16 (9.7%)	69 (9.7%)	0.04
≥10 cig/day	19 (11.6%)	42 (5.8%)	
Birth order - Parity			

1	65 (39.4%)	312 (43.5%)	
2	57 (34.5%)	255 (35.6%)	0.33
≥3	43 (26.1%)	150 (20.9%)	
Cesarean section, n (%)	22 (13.3%)	102 (14.2%)	0.77
Gestational diabetes, n (%)	4 (2.4%)	7 (1.0%)	0.13
Pre-eclampsia, n (%)	20 (12.1%)	48 (6.7%)	0.02
Maternal diabetes, n (%)	1 (0.6%)	2 (0.3%)	0.52
Maternal age at birth (years)			
Median (IQR)	28.7 (25.0-33.5)	29.7 (26.0-33.0)	
Highest level of education in parents			
≤9 years	11 (6.7%)	15 (2.1%)	
10 - 12 years	77 (46.7%)	306 (42.7%)	0.003
≥13 years	77 (46.7%)	396 (55.2%)	
Country of birth in mother, n (%)			
Nordic	120 (72.7%)	618 (86.2%)	<0.001
Other	45 (27.3%)	99 (13.8%)	
Living with partner			
Yes	140 (84.8%)	641 (89.4%)	0.24
No/missing	25 (15.2%)	76 (10.6%)	

*Student's t-test, Chi-squared test, or Wilcoxon-Mann-Whitney test were used as appropriate

Abbreviations: NAFLD, non-alcoholic fatty liver disease. BMI, body mass index. IQR, interquartile range.

Table 2 Odds ratio of biopsy-proven NAFLD of offspring by maternal BMI category using conditional logistic regression.

Maternal BMI	NAFLD (N=165)	Controls (N = 717)	OR (95% CI)*	OR (95% CI)**
<18.5	5 (2.8%)	27 (3.8%)	0.86 (0.16-4.81)	0.84 (0.14-5.15)
18.5 - <25 (reference)	77 (46.8%)	436 (60.8%)	1.00	1.00
25 - <30	51 (31.2%)	194 (27.0%)	1.50 (0.97-2.30)	1.51 (0.95-2.40)
≥30	32 (19.3%)	60 (8.4%)	3.07 (1.72-5.50)	3.26 (1.72-6.19)

*Conditioned on matching set (age, sex, county and calendar year);

**Conditioned on matching set and further adjusted for maternal age, maternal country of birth, parity, highest level education in parents, and smoking in early pregnancy.

Abbreviations: NAFLD, non-alcoholic fatty liver disease. BMI, body mass index. OR, odds ratio. CI, confidence interval.

Table 3 Odds ratio of biopsy-proven severe NAFLD (cirrhosis or fibrosis) of offspring by maternal BMI category using conditional logistic regression.

Maternal BMI	NAFLD (N=76)	Controls (N = 334)	OR (95% CI)*	OR (95% CI)**
<18.5	1 (1.8%)	12 (3.6%)	0.71 (0.08-5.98)	0.72 (0.08-6.32)
18.5 - <25 (reference)	31 (40.3%)	198 (59.3%)	1.00	1.00
25 - <30	27 (35.8%)	92 (27.5%)	1.89 (0.94-3.77)	1.94 (0.96-3.90)
≥30	17 (22.1%)	32 (9.6%)	3.48 (1.61-7.52)	3.67 (1.61-8.38)

*Conditioned on matching set (age, sex, county and calendar year);

**Conditioned on matching set and further adjusted for maternal age, maternal country of birth, parity, highest level education in parents, and smoking in early pregnancy

Abbreviations: NAFLD, non-alcoholic fatty liver disease. BMI, body mass index. OR, odds ratio. CI, confidence interval.

Table 4 Univariable and multivariable conditional* logistic regression for biopsy-proven NAFLD for parameters other than BMI. Model 1 presents univariable associations, that is the association between the parameter and NAFLD in the offspring. Model 2 presents the multivariable-adjusted associations for parameters other than BMI, which is presented in Table 3.

Parameter	Model 1*		Model 2**	
	OR (95%CI)	p-value	OR (95% CI)	p-value
Maternal age (continuous)	0.99 (0.96-1.02)	0.47	0.99 (0.95-1.02)	0.46
Nordic country of birth	0.39 (0.25-0.60)	<0.001	0.35 (0.22-0.57)	<0.001
Nulliparous (yes/no)	0.84 (0.60-1.19)	0.34	0.96 (0.65-1.43)	0.85
Maternal smoking in early pregnancy				
Non-smoking (reference)	1.09 (0.59-2.02)	0.77	0.89 (0.48-1.66)	0.71
1-9 cig/day	1.00	-	1.00	-
≥10 cig/day	2.16 (1.20-3.91)	0.01	2.13 (1.07-4.25)	0.03
Highest level of education in parents				
≤9 years	2.74 (1.23-6.09)	0.01	2.22 (0.94-5.26)	0.07
10 - 12 years (reference)	1.00	-	1.00	-
≥13 years	0.76 (0.53-1.09)	0.14	1.02 (0.69-1.52)	0.92

*Conditioned on matching set (age, sex, county and calendar year);

** Conditioned on matching variables, and further adjusted maternal age, maternal country of birth, parity, highest level education in parents, and smoking in early pregnancy.

Abbreviations: NAFLD, non-alcoholic fatty liver disease. BMI, body mass index. OR, odds ratio. CI, confidence interval.

References

1. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet. 2017;390(10113):2627-42.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology (Baltimore, Md). 2016;64(1):73-84.
3. Chen C, Xu X, Yan Y. Estimated global overweight and obesity burden in pregnant women based on panel data model. PloS one. 2018;13(8):e0202183.
4. Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. PLoS medicine. 2019;16(2):e1002744.
5. Persson M, Razaz N, Edstedt Bonamy AK, Villamor E, Cnattingius S. Maternal Overweight and Obesity and Risk of Congenital Heart Defects. Journal of the American College of Cardiology. 2019;73(1):44-53.
6. Lindell N, Carlsson A, Josefsson A, Samuelsson U. Maternal obesity as a risk factor for early childhood type 1 diabetes: a nationwide, prospective, population-based case-control study. Diabetologia. 2018;61(1):130-7.
7. Kahn S, Wainstock T, Sheiner E. Maternal obesity and offspring's cardiovascular morbidity - Results from a population based cohort study. Early Hum Dev. 2020;151:105221.
8. Ayonrinde OT, Oddy WH, Adams LA, Mori TA, Beilin LJ, de Klerk N, et al. Infant nutrition and maternal obesity influence the risk of non-alcoholic fatty liver disease in adolescents. Journal of hepatology. 2017;67(3):568-76.
9. 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 non-alcoholic fatty liver disease in mice. Journal of hepatology. 2010;52(6):913-20.
10. Mouralidarane A, Soeda J, Visconti-Pugmire C, Samuelsson AM, Pombo J, Maragkoudaki X, et al. Maternal obesity programs offspring nonalcoholic fatty liver disease by innate immune dysfunction in mice. Hepatology (Baltimore, Md). 2013;58(1):128-38.
11. Shrestha N, Ezechukwu HC, Holland OJ, Hryciw DH. Developmental programming of peripheral diseases in offspring exposed to maternal obesity during pregnancy. Am J Physiol Regul Integr Comp Physiol. 2020;319(5):R507-r16.
12. Dearden L, Ozanne SE. Early life origins of metabolic disease: Developmental programming of hypothalamic pathways controlling energy homeostasis. Front Neuroendocrinol. 2015;39:3-16.
13. Sun Y, Wang Q, Zhang Y, Geng M, Wei Y, Liu Y, et al. Multigenerational maternal obesity increases the incidence of HCC in offspring via miR-27a-3p. Journal of hepatology. 2020;73(3):603-15.

14. Ludvigsson JF, Lashkariani M. Cohort profile: ESPRESSO (Epidemiology Strengthened by histoPathology Reports in Sweden). Clinical epidemiology. 2019;11:101-14.
15. Cote RA, Robboy S. Progress in medical information management. Systematized nomenclature of medicine (SNOMED). Jama. 1980;243(8):756-62.
16. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. European journal of epidemiology. 2009;24(11):659-67.
17. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC public health. 2011;11:450.
18. Cnattingius S, Ericson A, Gunnarskog J, Kallen B. A quality study of a medical birth registry. Scandinavian journal of social medicine. 1990;18(2):143-8.
19. Simon TG, Roelstraete B, Khalili H, Hagström H, Ludvigsson JF. Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. Gut. 2020.
20. Ludvigsson JF, Almqvist C, Bonamy AK, Ljung R, Michaelsson K, Neovius M, et al. Registers of the Swedish total population and their use in medical research. European journal of epidemiology. 2016;31(2):125-36.
21. Stephansson O, Petersson K, Björk C, Conner P, Wikström AK. The Swedish Pregnancy Register - for quality of care improvement and research. Acta Obstet Gynecol Scand. 2018;97(4):466-76.
22. Lebwohl B, Green PHR, Söderling J, Roelstraete B, Ludvigsson JF. Association Between Celiac Disease and Mortality Risk in a Swedish Population. Jama. 2020;323(13):1277-85.
23. Liu Y, De A. Multiple Imputation by Fully Conditional Specification for Dealing with Missing Data in a Large Epidemiologic Study. Int J Stat Med Res. 2015;4(3):287-95.
24. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology (Cambridge, Mass). 2009;20(4):488-95.
25. Ludvigsson JF, Haberg SE, Knudsen GP, Lafolie P, Zoega H, Sarkkola C, et al. Ethical aspects of registry-based research in the Nordic countries. Clinical epidemiology. 2015;7:491-508.
26. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988-1994 to 2007-2010. The Journal of pediatrics. 2013;162(3):496-500.e1.
27. Dezsőfi A, Baumann U, Dhawan A, Durmaz O, Fischler B, Hadzic N, et al. Liver biopsy in children: position paper of the ESPGHAN Hepatology Committee. Journal of pediatric gastroenterology and nutrition. 2015;60(3):408-20.
28. Elinder LS, Hakimi S, Lager A, Patterson E. Global region of birth is an independent risk factor for type 2 diabetes in Stockholm, Sweden. European journal of public health. 2017;27(3):447-53.
29. Borrell LN, Dallo FJ, White K. Education and diabetes in a racially and ethnically diverse population. American journal of public health. 2006;96(9):1637-42.

30. Hagstrom H, Tynelius P, Rasmussen F. High BMI in late adolescence predicts future severe liver disease and hepatocellular carcinoma: a national, population-based cohort study in 1.2 million men. Gut. 2018;67(8):1536-42.
31. Hagstrom H, Stal P, Hultcrantz R, Hemmingsson T, Andreasson A. Overweight in late adolescence predicts development of severe liver disease later in life: A 39years follow-up study. Journal of hepatology. 2016;65(2):363-8.
32. Hagström H, Höijer J, Andreasson A, Bottai M, Johansson K, Ludvigsson JF, et al. Body mass index in early pregnancy and future risk of severe liver disease: a population-based cohort study. Alimentary pharmacology & therapeutics. 2019;49(6):789-96.
33. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313-21.
34. Ajmera VH, Terrault NA, VanWagner LB, Sarkar M, Lewis CE, Carr JJ, et al. Longer lactation duration is associated with decreased prevalence of non-alcoholic fatty liver disease in women. Journal of hepatology. 2019;70(1):126-32.
35. Nobili V, Bedogni G, Alisi A, Pietrobattista A, Alterio A, Tiribelli C, et al. A protective effect of breastfeeding on the progression of non-alcoholic fatty liver disease. Arch Dis Child. 2009;94(10):801-5.

Highlights

- Maternal obesity has been linked to offspring NAFLD
- All biopsy-proven NAFLD cases in Sweden aged 25 or younger were matched to controls
- Data on maternal body mass index and socio-economic confounders recorded
- Maternal obesity was a risk factor for offspring NAFLD
- Obesity might have inter-generational consequences