The Lancet Regional Health - Europe 000 (2021) 100206

Contents lists available at ScienceDirect

The Lancet Regional Health - Europe

journal homepage: www.elsevier.com/lanepe

Research paper

Association of maternal pre-pregnancy BMI and breastfeeding with NAFLD in young adults: a parental negative control study

Kushala WM Abeysekera, MRCP[a,b,*], James G Orr, PhD[b], Paul Madley-Dowd, PhD[a], Gwen S Fernandes, PhD[a], Luisa Zuccolo, PhD[a,c], Fiona H Gordon, FRCP[b], Deborah A Lawlor, PhD[a,c,d], Jon Heron, PhD[a], Matthew Hickman, PhD[a,c]

[a] *Population Health Science, Bristol Medical School, University of Bristol, Bristol, UK*
[b] *Department of Liver Medicine, University Hospitals Bristol and Weston NHS Foundation Trust, UK*
[c] *MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK*
[d] *Bristol NIHR Biomedical Research Centre*

ARTICLE INFO

Article History:
Received 4 June 2021
Revised 5 August 2021
Accepted 13 August 2021
Available online xxx

Keywords:
ALSPAC
Non-alcoholic fatty liver disease
Young adults
Transient elastography
Controlled attenuation parameter
Breastfeeding
Maternal pre-pregnancy BMI

ABSTRACT

Background: The importance of the maternal-infant dyad in the genesis of nonalcoholic fatty liver disease (NAFLD) is of increasing interest. The Avon Longitudinal Study of Parents and Children (ALSPAC) showed that at age 24, 1 in 5 had NAFLD measured by transient elastography and controlled attenuation parameter (CAP). Our aim was to investigate the association between breastfeeding duration and maternal pre-pregnancy BMI on offspring NAFLD in young adulthood.

Methods: 4021 participants attended clinic for FibroScan and CAP measurement using Echosens 502 Touch®. 440 participants with Alcohol Use Disorders were excluded. Offspring of 100 non-singleton pregnancies were excluded. 2961 valid CAP measurements for NAFLD were analysed. Exposures of interest were breastfeeding of any duration, ≥6months exclusive breastfeeding, and maternal pre-pregnancy BMI. Multivariable regression models estimated the odds of NAFLD at 24 years. We performed a paternal negative control test to explore residual confounding in the analyses of pre-pregnancy BMI.

Findings: There was a modest inverse association of exclusive and non-exclusive breastfeeding ≥6 months having a protective effect on NAFLD in offspring (OR 0·92 [95%CI 0·66-1·27] and OR 0·90 [0·67-1·21] respectively).The odds of offspring NAFLD in overweight pre-pregnancy maternal BMI and paternal BMI was OR 2·09 [1·62-2·68] and OR 1·33 [95%CI 1·07-1·65] respectively, with the ratio of effect sizes OR 1·57 [1·11-2·22]. Similarly, odds of offspring NAFLD with obese pre-pregnancy maternal BMI and paternal BMI was OR 2·66 [1·71-4·14] and OR 1·35 [0·91-2·00] respectively, with the ratio of effect sizes OR 1·98 [1·05-3·74].

Interpretation: Higher maternal pre-pregnancy BMI was associated with offspring NAFLD, having accounted for shared parental confounding. We did not replicate previous work that found a strong association between breastfeeding and NAFLD.

Funding: Medical Research Council UK, Alcohol Research UK, David Telling Charitable Trust

1. Introduction

In the last decade there has been an increasing interest in the role of early life exposures, in utero and postnatally, in priming the livers of offspring to develop non-alcoholic fatty liver disease (NAFLD) within the complex maternal-infant dyad. Two areas of focus of research have been the exposures of breastfeeding duration and maternal pre-pregnancy weight.

The value of breastfeeding and its benefits to infants is widely recognised amongst the scientific community and general public, including a reduction in infections and sudden infant death syndrome [1-4]. Different infant feeding practices have been studied in terms of the developmental origins of health and disease, but the evidence remains uncertain for many outcomes [4,5]. Several studies have demonstrated a protective association of breastfeeding against NAFLD, in part related to generating a favourable gut microbiome [6-8].

Multiple possible physiological mechanisms have been suggested for how maternal obesity may influence NAFLD genesis in offspring. Murine models have demonstrated that maternal obesity exacerbates

* Corresponding Author: Oakfield House, Oakfield Grove, Bristol BS8 2BN
E-mail address: k.abeysekera@bristol.ac.uk (K.W. Abeysekera).

https://doi.org/10.1016/j.lanepe.2021.100206

Research in context panel

Evidence before this study

Non-alcoholic fatty liver disease (NAFLD) is the commonest liver condition in the western world, closely linked to the worldwide obesity health crisis. Previous studies have suggested breastfeeding for longer periods, particularly ≥6 months exclusively, was protective against children getting NAFLD as teenagers, whilst mothers having obesity increases the odds of their children going on to develop NAFLD.

Added value of this study

In one of the largest to date to examine these relationships, we were unable to demonstrate evidence longer breastfeeding duration was protective against offspring developing NAFLD. We did find an overweight or obese BMIs in mothers does contribute to children developing NAFLD in addition to a child's environment, despite accounting for the shared parental confounding using a parental negative control test.

Implications of all the available evidence

This study demonstrates an early life effect in offspring of individuals with an overweight or obese BMI pre-pregnancy in developing NAFLD. In doing so, it supports the role of the maternal infant dyad in NAFLD pathogenesis, thus illustrating the pervasive intergenerational consequences of obesity. This highlights the importance of public health strategies designed to make it easier for individuals to make healthier choices regarding diet and exercise, in tackling the obesity epidemic.

the development of NAFLD associated with innate immune dysfunction in offspring fed with a hypercalorific obesogenic diet [9]. Previously, McCurdy and colleagues demonstrated that high fat diet exposure affected offspring *in utero*, associated with hepatic oxidative stress in the third trimester, mediating NAFLD development in infant primates [10]. Maternal obesity may also influence infant NAFLD development via their microbiome. For example, germ-free mice colonised with stool from infants born to obese mothers versus normal weight mothers, demonstrated increased hepatic gene expression of endosplasmic reticulum stress, with hepatic periportal and lobular inflammation consistent with the histological features of paediatric NAFLD [11].

Importantly, the interplay between maternal pre-pregnancy body mass index (BMI) and breastfeeding is complex with breastfeeding duration has been repeatedly demonstrated to be lower in mothers who are overweight or have obesity [12-14]. In addition, breastmilk composition could differ between mothers with obesity compared to those with a normal BMI [15,16], although the role of breastmilk components in shaping future offspring health remains unclear.

One of the largest attempts to interrogate the association between breastfeeding duration and maternal pre-pregnancy BMI with offspring NAFLD used the Raine birth cohort in Western Australia. Ayonrinde and colleagues demonstrated ≥6 months exclusive breastfeeding was associated with a lower odds of NAFLD outcomes in late adolescents (17years) and pre-pregnancy obesity more than doubled odds of NAFLD outcomes in offspring in late adolescence [6]. Longer breastfeeding duration was also associated with lower serum gamma glutamyl transferase (GGT) and triglycerides by the age of 17 years, whilst early introduction of supplementary formula, but not complimentary feeding, was associated with offspring NAFLD [6]. The same cohort also identified sexual dimorphism in NAFLD development. For example, socioeconomic status at birth and paternal obesity were strongly associated with NAFLD specifically in male adolescent offspring [17].

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a large prospective birth cohort, based in Bristol, United Kingdom (UK) [18]. ALSPAC assessed the cohort as young adults (n=4021; mean age 24 years) using transient elastography (FibroScan®) and controlled attenuated parameter (CAP) and found NAFLD prevalence to be over 20% [19].

Using existing data on maternal BMI and infant feeding practices at ALSPAC, we aim to replicate the previous Raine cohort study and test whether exposures of any breastfeeding, ≥6 months exclusive breastfeeding, and maternal pre-pregnancy BMI are associated with NAFLD in young adulthood. To strengthen a causal inference, we used a negative control approach by testing the association of paternal pre-pregnancy BMI and comparing this effect size to that of maternal pre-pregnancy BMI.

2. Materials and methods

2.1. Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective birth cohort study from southwest England [18,20]. The study website contains details of all available data through a fully searchable data dictionary and variable search tool (www.bristol.ac.uk/alspac/researchers/our-data). Briefly, ALSPAC invited pregnant women in Avon, UK with expected delivery dates between April 1, 1991 and December 31, 1992 into the cohort [21]. The initial number of pregnancies enrolled is 14,541. Of these initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age.

When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. Following 3 further phases of recruitment, this resulted in an additional 913 children being enrolled. The total sample size for analyses using any data collected after the age of seven is therefore 15,454 pregnancies, resulting in 15,589 foetuses. Of these 14,901 were alive at 1 year of age [21].

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Between June 2015 and October 2017, 10018 remaining "active" ALSPAC study participants were invited to the "Focus@24+" clinic (see Fig. 1). Of 10018 participants invited, 40·1% (n=4021/10018) attended; mean age 24.0 years (SD 0·8; IQR 23-25 years; 1507 males, 2510 females).

1060 participants were excluded from the final analysis. 293 non-core participants (i.e. not enrolled in the original recruitment phase of ALSPAC) did not have recorded maternal measures. 97 twins and triplets were excluded as non-singleton pregnancies are independently associated with lower breastfeeding duration [22-24]. 440 participants attending the F@24+ clinic had evidence of Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-V) criteria for alcohol use disorder (AUD) were also excluded from analysis [25]. 94 participants did not attend the FibroScan session. Of those who did 99 had insufficient information e.g. did not have 10 valid CAP measurements. Finally, 37 participants withdrew consent to continue being part of ALSPAC. Thus, 2961 participants were included in this study.

Study data were collected and managed using REDCap electronic data capture tools hosted at University of Bristol [26]. In line with ALSPAC confidentiality policy, any analysed groups with less than

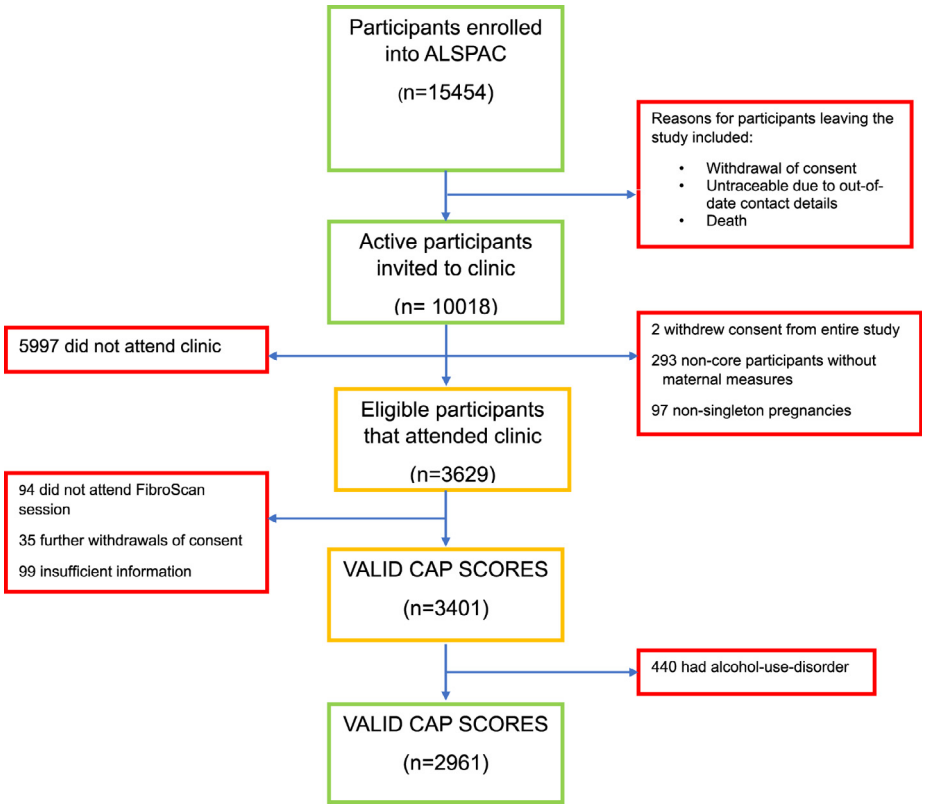

Fig. 1. Flow diagram of study

five participants are expressed as n = "<5". This figure when expressed can include zero.

In the F@24+ clinic no one was reported to have viral hepatitis or taking nucleoside analogues/direct-acting antivirals. In the F@24+ clinic, there were less than 5 participants identified with: autoimmune hepatitis on azathioprine, or autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome on prednisolone, mycophenolate mofetil, ursodeoxycholic acid, who were not removed as this was a general population study.

2.2. Outcomes

Prior to Focus@24+ clinic attendance, participants were fasted for a minimum of 6 hours or overnight before blood tests and subsequent transient elastography.

Imaging. In the F@24+ clinic participants were assessed with transient elastography (FibroScan®, Echosens 502 Touch®, Echosens, Paris) and controlled attenuation parameter (CAP). These are a standardised non-invasive measure of fibrosis and quantifying steatosis in NAFLD [27,28]. CAP score cut off values for different grades of steatosis for both groups were derived from a meta-analysis on CAP technology [28]. Ten valid readings within the range of 100-400dB/m were required to derive a CAP score. NAFLD was defined as ≥248dB/m (S1 equivalent steatosis).

Serology. Fasted blood tests analysed included liver function: alanine transaminase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT). Lipid profiles were taken including cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein (VLDL-C), and high-density lipoprotein (HDL). Glucose and insulin levels were also sent and used to calculate the homeostasis model assessment for insulin resistance (HOMA-IR), using the equation HOMA-IR score = (Fasting insulin [μU/ml] x Fasting glucose [mmol/l])/22·5 [29,30].

As a secondary analysis we sought to evaluate if there was a difference in liver enzyme levels and metabolic markers between participants with NAFLD who were breastfed for more or less than 6 months, as the previous Raine cohort study had noted some difference including lower serum GGT, triglycerides, and HOMA-IR in participants breastfed for ≥6months [6].

2.3. Exposures

The data used in this study were collected from ALSPAC mothers during pregnancy and over the index child's first year of life. Data on method of infant feeding, including introduction of supplementary milk formula and complimentary feeding, were obtained from questionnaires completed by mothers when the index child was 6 and 15 months of age [31]. Two categorical variables were created. "Any breastfeeding duration" was trichotomized into never breastfed (referent), non-exclusive breastfeeding <6 months, and non-exclusive breastfeeding ≥6months. "Exclusive breastfeeding ≥6 months" was compared to having never breastfed (referent), and mixed feeding (i.e. supplementary milk formula) or duration of breastfeeding <6 months combined. Exclusive breastfeeding ≥6 months was defined as no reported supplementary milk formulas (including cow's milk, soya, hypoallogenic and follow-on formula) in the first 6 months. A cut-off of ≥6 months breastfeeding was specified to facilitate direct comparison with the Raine cohort analysis.

In questionnaires at around 18 weeks of gestation women reported their pre-pregnancy weight and height and this was used to calculate maternal pre-pregnancy BMI (correlations between BMI based on weight and height reported by the women and BMI based on weight and height extracted from the first antenatal clinic visit were high; Pearson correlation coefficient: 0·9).

2.4. Confounders

Confounders were defined a priori based on prior evidence of them being known or plausible causes of the exposure and outcome

Please cite this article as: K.W. Abeysekera et al., Association of maternal pre-pregnancy BMI and breastfeeding with NAFLD in young adults: a parental negative control study, The Lancet Regional Health - Europe (2021), https://doi.org/10.1016/j.lanepe.2021.100206

(separately for each exposure). The following were considered confounders for maternal pre-pregnancy BMI: occupational social class (derived from maternal and partner questionnaires at 12- and 18-weeks' gestation respectively), maternal age, smoking in pregnancy. The following were considered confounders for breastfeeding: social class, maternal age, smoking in pregnancy, maternal pre-pregnancy BMI, birthweight.

3. Statistical analysis

Mean serology results for participants with and without NAFLD based on CAP value in the F@24+ clinic were compared using univariable regression for normally distributed data (cholesterol, LDL-C, HDL). Wilcoxon rank sum was used to compare differences between non-normally distributed data (ALT, AST, GGT, HOMA-IR, VLDL-C, triglycerides). Univariable regression models were used to examine the exposure of supplementary formula and complimentary feeding on outcomes of offspring NAFLD in the first 6 months. Confounder adjusted multivariable logistic regression models were used to examine the associations of pre-pregnancy BMI (in mothers and fathers), exclusive breastfeeding for more or less than 6 months, and any breastfeeding duration with NAFLD.

3.1. Paternal negative control

Negative control studies in epidemiology compare results from the hypothesized (real) association and a negative control association where either the exposure is changed to an exposure that does not plausibly cause the outcome, or vice versa [32-34]. In studies of the developmental origins of disease, paternal exposure has been increasingly used as a negative control for maternal exposure as data are often available on the same exposures in the father that are potentially causal intrauterine factors in mothers [35,36]. Here we compared confounder adjusted paternal BMI categories of normal, overweight and obesity associations with offspring NAFLD to the same associations of maternal BMI categories with offspring NAFLD.

For this parental negative control comparison, we also mutually adjusted each parents' exposure for the same exposure in the other parent as failing to do this can result in bias in the presence of assortative mating [37]. To simplify the paternal negative control test, maternal and paternal BMI were dichotomised in two models: (1) normal BMI vs overweight & obese BMI and (2) normal and overweight BMI vs obese BMI. In each model maternal and paternal BMI exposures were mutually adjusted for, following which effect size estimates were compared at the different thresholds (overweight and obese BMI). The null hypothesis was there is no difference between the maternal and paternal estimates.

3.2. Dealing with missing data

This study only included participants with complete CAP data and imputed missing exposure and confounder data, with this being the highest for maternal pre-pregnancy BMI (9·2% of participants did not have this measure). Statistical analysis was performed using Stata MP 15·1. (For further details on the imputation strategy see Supplementary Methods).

4. Results

2961 participant with valid CAP results were eligible for analysis (63·9% female). Overall, 20·7% (95%CI 19·2-22·1%) of participants attended the Focus@24+ clinic had NAFLD (≥S1 equivalent steatosis), with 10·1% having S3 equivalent steatosis CAP measurements (>66% steatosis).

Characteristics of the main exposures analysed are summarised in Table 1. Offspring NAFLD prevalence within male and female sex were interrogated (see Table 2). There did not appear to be any relationship between longer breastfeeding duration between male offspring participants with or without NAFLD (p=0·27). No relationship

Table 1
Characteristics of exposures amongst participants (n=2961).

Variable		S0 (n=2350; 79·4%)	S1 (n=215; 7·3%)	S2 (n=99; 3·3%)	S3 (n=297; 10·0%)	
Sex	Male (n=1070)	789 (73·7%)	93 (8·7%)	44 (4·1%)	144 (13·5%)	p<0·0001*
	Female (n=1891)	1561 (82·5%)	122 (6·4%)	55 (2·9%)	153 (8·1%)	
Maternal pre-pregnancy BMI	BMI <25kg/m^2 (n=2196)	1795 (81·7%)	144 (6·6%)	73 (3·3%)	184 (8·4%)	p<0·0001*
	Overweight (25 - <30kg/m^2) (n=371)	261 (70·3%)	39 (10·5%)	14 (3·8%)	57 (15·4%)	
	Obesity (≥30kg/m^2)	71	14	<5	32	
Paternal BMI	BMI <25kg/m^2 (n=2196)	972 (82·0%)	87 (7·3%)	36 (3·0%)	90 (7·6%)	p<0·0001*
	Overweight (25 - <30kg/m^2) (n=371)	638 (76·7%)	62 (7·4%)	35 (4·2%)	97 (11·7%)	
	Obesity (≥30kg/m^2)	106	11	<5	28	
Breastfeeding	Never breastfed (n=404)	307 (76·0%)	33 (8·2%)	15 (3·7%)	49 (12·1%)	p=0·15*
	Mixed feeding (n=1747)	1398 (80·0%)	130 (7·4%)	60 (3·4%)	159 (9·1%)	
	Exclusive breastfeeding for ≥6months (n=584)	471 (80·6%)	33 (5·6%)	14 (2·4%)	66 (11·3%)	
Maternal smoking during pregnancy	No (n=2446)	1955 (79·9%)	175 (7·1%)	81 (3·3%)	235 (9·6%)	p=0·40*
	Yes (n=467)	359 (76·9%)	35 (7·5%)	17 (3·4%)	56 (12·0%)	
Maternal age (years)	Mean (SD)	29.5 (4·6)	28.7 (4·5)	28.5 (4·4)	28.8 (4·8)	p=0·001¥
Maternal education	Lower than O-level (n=502)	374 (74·5%)	46 (9·2%)	19 (3·8%)	63 (12·5%)	p=0·03*
	O-level (n=987)	765 (77·5%)	78 (7·9%)	38 (3·8%)	106 (10·7%)	
	Higher education (n=1385)	1132 (81·7%)	90 (6·5%)	39 (2·8%)	124 (9·0%)	
Social class	IV-V (partly skilled and unskilled occupation)	65	9	<5	6	p=0·43*
	III (non-manual and manual occupation) (n=879)	686 (78·0%)	64 (7·3%)	33 (3·7%)	97 (11·0%)	
	II (managerial and technical occupation) (n=1264)	1000 (79·1%)	95 (7·5%)	35 (2·8%)	134 (10·6%)	
	I (professional occupation) (n=530)	435 (82·1%)	36 (6·8%)	18 (3·4%)	41 (7·7%)	
Birthweight (grams)	Mean (SD)	3413·7 (513·4)	3475 (554·8)	3434 (457·0)	3479 (509·5)	p=0·02¥

Steatosis grade based on CAP score ranges from S0-S3. S0 <248 dB/m (<10% steatosis); S1 248 to <268 dB/m (10%–<33% steatosis [mild]); S2 268 to <280 dB/m (33%–<66% steatosis [moderate]); and S3 ≥280 dB/m or more (≥66% steatosis [severe])

* Pearson's Chi^2 test

¥ Univariable logistic regression

Please cite this article as: K.W. Abeysekera et al., Association of maternal pre-pregnancy BMI and breastfeeding with NAFLD in young adults: a parental negative control study, The Lancet Regional Health - Europe (2021), https://doi.org/10.1016/j.lanepe.2021.100206

Table 2
Comparison of offspring characteristics by sex corresponding with presence or absence of NAFLD

		Male participants			Female participants		
		No NAFLD (n=789)	NAFLD (n=281)		No NAFLD (n=1561)	NAFLD (n=330)	
Breastfeeding pattern	Never breastfed	68·2%	31·8%	p=0·27*	79·8%	20·2%	p=0·28*
	Mixed feeding	75·0%	25·0%		83·1%	16·9%	
	Exclusive breastfeeding for ≥6months	73·7%	26.2%		84·4%	15·6%	
Maternal pre-pregnancy BMI (n=2688)		21·9 (20·5-24·0)	22·7 (20·9-25·3)	p=0·001‡	21·8 (20·3-23·6)	22·9 (21·0-26·0)	p<0·0001‡
Paternal BMI (n=2167)		24·6 (22·8-26·6)	25·0 (23·4-27·2)	p=0·01‡	24·6 (22·8-26·5)	25·4 (23·5-28·1)	p<0·0001‡
Social class (n=2757)	IV-V (partly skilled and unskilled occupation)	80·0%	20·0%	p=0·47*	75·9%	24·1%	p=0·13*
	III (non-manual and manual occupation)	70·7%	29·3%		81·8%	18·2%	
	II (managerial and technical occupation)	74·9%	25·1%		81·6%	18·4%	
	I (professional occupation)	75·0%	25·0%		82·4%	17·6%	

‡ Wilcoxon rank sum test
* Pearson's Chi^2 test

was observed in female offspring participants with or without NAFLD (p=0·28). Both in male and female offspring participants, maternal pre-pregnancy BMI was strongly associated with offspring NAFLD (p=0·001 and p<0·0001 respectively). Similarly paternal BMI was also strong associated with offspring in both male and female sexes (p=0·01 and p<0·0001 respectively). Finally, no difference in socio-economic status was identified between male and female offspring with and without NAFLD (p=0·47 and p=0·13 respectively).

4.1. Any Breastfeeding

Complete data on patterns of breastfeeding existed on 2409 participants when restricting to those with available CAP measurements. Participants were categorised ordinally into those that were not breastfed (n=342/2409), those that breastfed (with or without mixed feeding) for less than 6 months (n=1048/2409) and those that breastfed (with or without mixed feeding) for ≥6months (n=1019/2409) as a dose response exposure. (see Table 3.) There was a weak association of breastfeeding duration ≥6months being associated with lower odds of NAFLD, compared to <6 months or no breastfeeding (19·0% vs 20·6% vs 24·0% respectively; unadjusted OR 0·78 (0·58-1·04; p=0·09). However, this association attenuated when adjusted for confounders including social class, maternal age, smoking in pregnancy, birthweight and the pre-pregnancy BMI exposure variable (OR 0·92 [0·67-1·26]; p=0·60). Findings were similar following the use of multiple imputation (OR 0·90 [0·67-1·21]; p=0·50).

4.2. Exclusive breastfeeding ≥6 months

(see Table 3.) When interrogating ≥6months exclusive breastfeeding, participants were categorised into those who never breastfed (n=342/2409), those with mixed feeding or duration of breastfeeding <6 months combined (n=1544/2409), and those who exclusively breastfed for ≥6months (n=523/2409). Lower prevalence of NAFLD were seen amongst participants who were exclusively breastfed for ≥6months, compared to participants who received mixed feeding and those who never breastfed, but the confidence interval included the null (19·3% vs 20·0% vs 24·0% respectively; OR 0·78 [0·56-1·09]; p=0·14). Effect estimates were attenuated further after adjusting for confounders including social class, maternal age, smoking in pregnancy, birthweight, and the pre-pregnancy BMI exposure variable (0·89 [0·63-1·27]; p=0·53). This did not alter following imputation of a complete dataset (0·92 [0·66-1·27]; p=0·60).

4.3. Timing of introduction of supplementary milk formula and complementary feeding

(see Table 4.) In the first 6 months, no association was identified between the month supplementary milk formula was introduced and the presence of NAFLD in univariable analysis (OR 0·97 [0·93-1·01; p=0·13]). Whilst there was a stronger association between timing of complementary feeding being commenced in the first 6 months and lower odds of NAFLD, the confidence interval again included the null (OR 0·91 [0·82-1·01; p=0·07]).

4.4. Pre-pregnancy maternal BMI

(see Table 3.) 13·8% (n=371/2688) of mothers were overweight, whilst 4·5% had pre-pregnancy obesity (n=121/2688). Presence of NAFLD in offspring ranged from 18·3% amongst mother with a BMI<25 kg/m^2 to 41·3% amongst mother with pre-pregnancy obesity.

Complete data on maternal pre-pregnancy BMI was available for 2505 participants when interrogating NAFLD outcomes. Mothers' BMI was categorised into normoweight (<25kg/m^2; n=2051/2505), overweight (25≤ BMI <30kg/m^2; n=346/2505), and obese (≥30kg/m^2; n=108/=2505). Crude associations of overweight and obese maternal pre-pregnancy BMI with offspring NAFLD outcomes were OR 2·00 ([1·55-2·58]; p<0·0001) and OR 3·28 ([2·20-4·88]; p<0·0001) respectively. Following adjustment for confounders including social class, maternal age and smoking in pregnancy and imputation to a complete data set overweight and obese maternal pre-pregnancy BMI with offspring NAFLD outcomes were OR 1·84 ([1·44-2·37]; p<0·0001) and OR 3·06 ([2·11-4·42]; p<0·0001) respectively.

4.5. Paternal negative control

A negative control test entails the comparison of magnitude of the maternal and paternal association estimates within a mutually adjusted model. (see Table 5.) For completeness Table 5 contains crude estimates. 2316 participants had paternal BMI data.

Prior to mutual adjustment, parental pre-pregnancy BMI was dichotomised into (1) BMI<25kg/m^2 vs BMI ≥25kg/m^2(overweight and obese) and (2) BMI<30kg/m^2 vs BMI ≥30kg/m^2 (obese). In model (1) the odds of offspring NAFLD in overweight/obese pre-pregnancy maternal BMI and paternal BMI was OR 2·09 [95%CI 1·62-2·68; p<0·0001] and OR 1·33 [95%CI 1·07-1·65; p=0·01] respectively, with the ratio of effect sizes OR 1·57 [95%CI 1·11-2·22; p=0·01]. Similarly, in model (2) the odds of offspring NAFLD with obese pre-pregnancy

Please cite this article as: K.W. Abeysekera et al., Association of maternal pre-pregnancy BMI and breastfeeding with NAFLD in young adults: a parental negative control study, The Lancet Regional Health - Europe (2021), https://doi.org/10.1016/j.lanepe.2021.100206

Table 3
Exposures associated with presence of NAFLD (n=2961)

Model	Independent variable	Variable	Presence of NAFLD	Crude OR[95% CI]	Adjusted (1) OR[95% CI]	Adjusted (2) OR[95% CI]
					Socioeconomic status Maternal age Smoking in pregnancy	Socioeconomic status Maternal age Smoking in pregnancy Pre-pregnancy BMI Birthweight
1	Pre-pregnancy maternal BMI	Underweight or Normal	18·3%	Referent	Referent	
		Overweight	29·6%	2·00 [1·55-2·58; p<0·0001]	1·99 [1·54-2·57; p<0·0001]	
		[imputed result]			1·84 [1·44-2·37; p<0·0001]	
		Obese	41·3%	3·28 [2·20-4·88; p<0·0001]	3·19 [2·13-4·76; p<0·0001]	
		[imputed result]			3·06 [2·11-4·42; p<0·0001]	
		Number of participants with complete data available = 2505		p<0·0001†	p<0·0001†	
2	Paternal BMI	Underweight or Normal	18·0%	Referent		
		Overweight	23·3%	1·39 [1·12-1·73; p=0·003]		
		Obese	28·9%	1·85 [1·26-2·72; p=0·002]		
		Number of participants with complete data available = 2069		p=0·0007†		
3	≥6 months Exclusive breastfeeding	Never breastfed	24·0%	Referent	Referent	Referent
		Mixed feeding	20·0%	0·81 [0·62-1·07; p=0·14]	0·85 [0·64-1·13; p=0·26]	0·88 [0·66-1·18; p=0·40]
		[imputed result]				0·89 [0·68-1·16; p=0·38]
		≥ 6months exclusive breast-feeding	19·3%	0·78 [0·56-1·09; p=0·14]	0·83 [0·59-1·18; p=0·31]	0·89 [0·63-1·27; p=0·53]
		[imputed result]				0·92 [0·66-1·27; p=0·60]
		Number of participants with complete data available = 2409		p=0·16†	p=0·50†	p=0·70†
4	Any breastfeeding	Never breastfed	24·0%	Referent	Referent	Referent
		<6 months	20·6%	0·83 [0·62-1·11; p=0·21]	0·84 [0·62-1·13; p=0·24]	0·86 [0·64-1·17; p=0·33]
		[imputed result]				0·89 [0·68-1·17; p=0·40]
		≥6 months	19·.0%	0·78 [0·58-1·04; p=0·09]	0·85 [0·62-1·17; p=0·32]	0·92 [0·67-1·26; p=0·60]
		[imputed result]				0·90 [0.67-1·21; p=0·50]
		Number of participants with complete data available = 2409		p=0·25†	p=0·50†	p=0·63†

† Likelihood ratio test

maternal BMI and paternal BMI was OR 2·66 [95%CI 1·71-4·14; p<0·0001] OR 1·35 [95%CI 0·91-2·00; p=0·014] respectively, with the ratio of effect sizes OR 1·98 [95%CI 1·05-3·74; p=0·04].

4.6. Association of duration of breastfeeding with serum liver enzymes, cardiometabolic markers, and young adults with NAFLD

(see Table 6.) Median ALT, AST, and GGT levels were compared between participants identified with NAFLD who had been breastfed exclusively for ≥6 months vs < 6 months breast/mixed feeding, with no difference found (p=0·21, 0·12 and 0·66 respectively). Similarly, there was no difference in mean cholesterol, LDL-C, HDL, and median triglyceride levels between groups (p=0·86, 0·22, 0·30 and 0·40 respectively). Median HOMA-IR values amongst participants with NAFLD who received exclusive breastfeeding for ≥6 months were higher compared to participants with NAFLD who breastfed for <6 months (3·5 vs 2·8, p=0·07) with both groups in the insulin resistance range. This is despite similar levels of obesity (38·4% vs 38·7% respectively) and similar sex distribution (56·2% bs 50·6% respectively) amongst the 2 groups.

Further analyses including analysing liver fibrosis (liver stiffness measurement of ≥7.9kPa equivalent to F2≥ fibrosis) as the outcome with the same exposures can be found in Supplementary Results.

Table 4
Timing of introduction of milk formula feeding, complementary feeding, and the association with NAFLD

	Introduction of formula feeding		Introduction of complementary feeding	
Month	`N	% NAFLD	N	% NAFLD
0	936	22·0	50	30·0
1	212	19·3	41	19·5
2	291	21·3	230	23·0
3	292	21·2	1613	20·7
4	202	20·3	719	18·8
5	185	16·8	111	21·6
6	673	19·3	27	14·8
	OR 0·97 [0·93-1·01; p=0·13]¥		OR 0·91 [0·82-1·01; p=0·07] ¥	

¥ Univariable logistic regression

5. Discussion

5.1. Main findings

We found evidence of a specific early life effect of overweight and obese maternal pre-pregnancy BMI with higher odds of offspring NAFLD in young adulthood, that could not be explained by the shared parental confounding structure. Whilst we found an association with maternal pre-pregnancy BMI and NAFLD outcomes in offspring, this was also the case when modelling the association of paternal BMI as a negative control exposure. Therefore, whilst there may be an early life effect, this is in addition to environmental factors in the genesis NAFLD.

We did not find evidence that longer breastfeeding duration and ≥6 months exclusive breastfeeding duration were associated with a lower odds of NAFLD in offspring by young adulthood. We found insufficient evidence supporting the hypothesis that mixed feeding is

Table 5
Paternal negative control test. Crude and adjusted OR refer to outcomes of NAFLD.

Model	Independent variable		Crude OR [95% CI]	Mutually Adjusted OR [95% CI]	Ratio of effect sizes[95% CI]
1	Pre-pregnancy maternal BMI	Underweight or Normal (n=2333)	Referent	Referent	1·57 [1·11-2·22; p=0·011] †
		Overweight or obese (n=536)	2·15 [1·74-2·68; p<0·0001]	2·09 [1·62-2·68; p<0·0001]	
	Paternal BMI	Underweight or Normal (n=1267)	Referent	Referent	
		Overweight or obese (n=1049)	1·45 [1·18-1·79; p<0·0001]	1·33 [1·07-1·65; p=0·01]	
2	Pre-pregnancy maternal BMI	Not obese (n=2736)	Referent	Referent	1·98 [1·05-3·74; p=0·036] †
		Obese (n=133)	2·83 [1·95-4·12; p<0·0001]	2·66 [1·71-4·14; p<0·0001]	
	Paternal BMI	Not obese (n=2153)	Referent	Referent	
		Obese (n=163)	1·61 [1·11-2·33; p=0·012]	1·35 [0·91-2·00; p=0·14]	

† ORs are ratio of mutually adjusted ORs between maternal and paternal BMI exposure on NAFLD outcomes in offspring at 24 years.

more deleterious to offspring developing NAFLD in young adulthood compared to exclusive breastfeeding.

5.2. Strength and limitations

This study can only comment on the relationships of maternal pre-pregnancy BMI and breastfeeding duration with NAFLD outcomes in the context of singleton pregnancies as we removed twin and multiple pregnancies to limit bias, as those pregnancies are associated with lower breastfeeding duration [22-24]. By treating BMI as a categorical variable to replicate the previous Raine cohort analysis, we accepted a loss of power for not treating BMI as a continuous variable.

Our diagnosis of NAFLD was based on controlled attenuation parameter diagnosis in the absence of harmful alcohol use. We acknowledge that cut-off values for CAP are validated in adults with histologically proven liver disease and these results should be interpreted in that context. However, in our large population study, gold standard liver biopsy was not ethically viable.

Whilst individuals with alcohol use disorder were removed from this analysis, ALSPAC did not collect data on grams of alcohol consumed by participants. Therefore, it is possible that participants with excess daily alcohol consumption (>30g/day in men and >20g/day in women) were included in the analysis.

We accepted that increased maternal pre-pregnancy BMI and breastfeeding cannot be considered entirely independent of each other. In addition to breastfeeding duration being lower in mothers with obesity, murine models showed in offspring of mice control-diets, when breastfed by obese mice on high-fat diets, had evidence of increased hepatic tumour necrosis factor, interleukin-6 expression, and triglyceride levels– highlighting a difference in breastmilk composition between mothers with obesity and those with normal BMI [12, 38, 39]. Maternal pre-pregnancy BMI was included as a confounder in regression models evaluating breastfeeding as an exposure to replicate and compare with the previous Raine cohort analysis. Despite utilising a large birth cohort, our study remained underpowered to assess the relationship between breastfeeding duration and outcomes of NAFLD in offspring (see Supplementary Methods for power calculation). Therefore, we cannot definitively comment on a lack of protective effect of longer breastfeeding duration.

The World Health Organisation advised exclusive breastfeeding duration for 4 to 6 months during the 1990s and changed their stance in 2001 to 6 months exclusive breastfeeding [40,41]. Overall, 21·2% (n=620/2928) of our participants were exclusively breastfed for ≥6 months in the early 1990s, which is substantially higher than 1% figure quoted in the 2010 UK Infant Feeding Survey, and the Raine cohort 7·4% [6,42]. Despite this relatively high number of exclusively breastfed participants, we were unable to elucidate a strong protective effect of longer exclusive breastfeeding duration for NAFLD development.

The ALSPAC Focus@24 clinic had a large clinic non-attendance, with only 40·1% of participants attending. Therefore, the ALSPAC birth cohort may not be entirely representative of the wider population [18]. The demographic profile of the catchment area in south-west England, and the differential attrition, has created an over-representation of affluent groups and an under-representation of ethnic minorities, with ALSPAC over 97% Caucasian [18]. Thus, it is unclear how our results apply to patients of other ethnicities with NAFLD. Higher maternal educational attainment is strongly associated with longer breastfeeding duration [43,44]. Despite this we could not demonstrate a strong association for the protective benefits of longer breastfeeding duration.

5.3. Other evidence

To the best of our knowledge, this is one of the largest studies to interrogate the relationship between breastfeeding, maternal pre-pregnancy BMI and NAFLD development in the offspring. It remains

Table 6
Relationship between exclusively breastfeeding ≥6 months vs breastfeeding < 6months, liver function and metabolic markers in young adult participants with or without NAFLD.

Variable		NAFLD F24			Normal F24		
		Exclusive Breastfeeding≥6 months (n=113)	Breastfeeding <6 months (n=446)	P value	Exclusive Breastfeeding ≥6 months (n=471)	Breastfeeding <6 months (n=1705)	P value
ALT (10-35U/l)	Median (IQR)	15·8 (12·4-20·2)	27·4 (18·9-49·8)	0·21‡	18·9 (14·7-26·2)	19.1 (14·1-27·6)	0·92‡
AST (10-35U/l)	Median (IQR)	24·8 (21·3-30·2)	26·3 (20·9-36·0)	0.12‡	23·9 (20·9-28·2)	23·7 (20·5-28·5)	0·76‡
GGT (IU/l) (<40 U/l)	Median (IQR)	19 (14-30)	20.5 (15-31)	0·66‡	15 (12-20)	16 (12-20)	0·94‡
HOMA-IR (<1·68)	Median (IQR)	3·5 (1·8-5·2)	2·8 (1·8-3·8)	0·07‡	1·6 (1·1-2·2)	1·6 (1·1-2·3)	0·61‡
Cholesterol (<5·2mmmol/l)	Mean (SD)	4·6 (0·9)	4·5 (0·8)	0·86†	4·4 (0·8)	4·4 (0·8)	0·82†
Triglycerides (<1.7mmol/l)	Median (IQR)	1·1 (0·7-1·7)	1·1 (0·7-1·6)	0·40‡	0·8 (0·6-1·1)	0·8 (0·6-1·1)	0·92‡
LDL-C (mmol/l)	Mean (SD)	2·6 (0·8)	2·8 (0·8)	0·22†	2·3 (0·7)	2·4 (0·7)	0·47†
VLDL-C (mmol/l)	Median (IQR)	0·5 (0·3-0·8)	0·5 (0·3-0·7)	0·10‡	0·4 (0·3-0·5)	0·4 (0·3-0·5)	0·81‡
HDL (>1·45mmol/l)	Mean (SD)	1·3 (0·4)	1·3 (0·3)	0·30†	1·7 (0·4)	1·6 (0·4)	0·03†

† T-test
‡ Wilcoxon rank sum test

the first to use a paternal negative control test to elucidate causality for maternal pre-pregnancy BMI leading to NAFLD in offspring. Whilst there was an association between increased paternal BMI and offspring NAFLD, when we mutually adjusted for maternal and paternal BMI and compared effect size estimates, there still appeared to be an increased association between overweight and obese maternal BMI and offspring NAFLD. This lends support to an early life effect increasing odds of NAFLD in offspring amongst mothers with elevated pre-pregnancy BMI, independent of environmental factors.

In a similar birth cohort, the Raine cohort (n=1170) based in Western Australia, 15·2% had NAFLD based on ultrasound assessment. Our findings evaluating the early life effects of maternal pre-pregnancy were consistent with the Raine Cohort in relation to maternal pre-pregnancy obesity and NAFLD outcomes (OR 2·29 [1·21-4·32]; p=0·01). Our paternal negative control test supported this association. In addition, a meta-analysis of current literature of maternal risk factors for paediatric NAFLD risk supports our findings regarding the relationship of elevated maternal pre-pregnancy BMI with offspring NAFLD [45]. The Raine cohort found ≥6months exclusive breastfeeding to be protective vs <6 months breastfeeding without supplementary milk (OR 0·64 [0·43-0·94], p=0·02); whereas after adjustment we did not find evidence of a similar protective effect with a larger sample size. However, our assessment of a protective breastfeeding effect is limited by being underpowered.

Ayonrinde and colleagues also identified sexual dimorphism in the association of parental factors on NAFLD in offspring [17]. They found paternal obesity to be associated with male offspring NAFLD only. Female offspring NAFLD with independently associated with higher maternal pre-pregnancy BMI. Furthermore, lower parental socioeconomic status was associated with NAFLD in male offspring. We did not demonstrate such sexual dimorphism in our cohort analysis at 24 years or when our participants were 17.8 years during the first assessment for NAFLD using ultrasound [46]. This could be related to an overrepresentation of female participants in our cohort, who account for 63.9% of our cohort compared to 49.4% of the Raine cohort. In addition, NAFLD is far more prevalent in ALSPAC male participants compared to female participants, a reversal of the relationship seen in the previous Raine cohort analysis. This could be related to ALSPAC examining young adults as opposed to adolescents. Suzuki et al analysed paediatric biopsy proven NAFLD identifying a relationship between increasing pubertal stage and possibly worse histological features of NAFLD, although the study was underpowered to identify sex differences [47].

Our results contrast existing epidemiological literature which largely negates the association of pre-pregnancy BMI, breastfeeding duration, and outcomes of the related outcomes of childhood adiposity and obesity. The Promotion of Breastfeeding Intervention Trial (PROBIT) randomised controlled trial involving over 17,000 offspring did not demonstrate a difference in adiposity between infants with substantially greater durations and exclusivity of breastfeeding than the control arm at aged 6·5 years or 11·5 years [48,49]. One of the largest systematic reviews and meta-analyses (including 105 studies) to interrogate the association between breastfeeding and offspring being overweight or obese found only a modest pooled OR 0·87 [0·76-0·99], involving ≥1500 participants [50].

Whilst we found no evidence that longer breastfeeding durations were protective for offspring developing NAFLD, other studies have suggested it may have a protective effect for mothers within a maternal-infant dyad. Using an older American cohort study, Coronary Artery Risk Development in Young Adults, Ajmera and colleagues demonstrated that mothers who breastfed for longer durations (>6months) had >50% reduction in NAFLD outcomes compared to those who had breastfed for 0-1months, after adjusting for age, ethnicity education level and baseline BMI [51]. The mechanism postulated for this finding was of increased maternal basal metabolic rate associated with lactation, increasing insulin sensitivity [51,52].

5.4. Implications

The wide-ranging early life benefits of breastfeeding are indisputable. Whilst our findings are insufficient to conclusively rule out an effect, we found little evidence that longer breastfeeding durations or exclusive breastfeeding ≥ 6 months is protective for offspring developing NAFLD. With higher-income countries such as the UK having shorter breastfeeding duration, further studies are needed to clarify if a true protective benefit exists.

Similarly, we have demonstrated evidence for an early life effect associated with elevated maternal pre-pregnancy BMI, in addition to environmental factors, that contributes to offspring developing NAFLD as young adults. We must collectively address the obesogenic environment our patients live in and support them in behavioural change interventions to improve their diet and increase exercise whilst promoting policies that minimise perpetuation of unhealthy eating patterns. Ultimately this will reduce the risk of patients and their children developing advanced NAFLD fibrosis and cirrhosis.

Funding

The UK Medical Research Council and Wellcome (Grant ref: 217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. This work was undertaken with the support of the MRC and Alcohol Research UK (MR/L022206/1), and the David Telling Charitable Trust. We also acknowledge support from The Centre for the Development and Evaluation of Complex Interventions for Public Health Improvement (DECIPHer), a UKCRC Public Health Research Centre of Excellence (joint funding (MR/KO232331/1) from the British Heart Foundation, Cancer Research UK, Economic and Social Research Council, Medical Research Council, the Welsh Government and the Wellcome Trust, under the auspices of the UK Clinical Research Collaboration, the NIHR School of Public Health Research, NIHR Health Protection Research Unit in Behavioural Science and Evaluation and NIHR Biomedical Research Centre at the University Hospitals Bristol NHS Foundation Trust. LZ, DAL and HM work in a unit that receive funds from the University of Bristol and Medical Research Council (MC_UU_00011/6) and DAL's contribution to this paper is also supported by the Bristol NIHR Biomedical Research Centre. DAL (NF-0616-10102) and MH (G123511-101) are NIHR Senior Investigators. None of the funders influenced the research question, analysis plan or interpretation of results. The views expressed in this paper are those of the authors and not necessarily any listed funders.

Authors Contributions

KWMA (study design, data analysis, manuscript preparation); JGO (study design, critical review); PMD (advice on negative control study and critical review); (GSF (data analysis, critical review) LZ (critical review); FHG (critical review); DAL (advice on negative control study and critical review); JH (study design, data acquisition, data analysis, critical review); MH (study design, critical review, supervised entire project).

Declaration of Interest

DAL has received support from Medtronic Ltd and Roche Diagnostics for research unrelated to that presented here. MH has received lecturer honoraria from MSD and Gilead. Other authors declared no conflicts of interest.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory

technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. This publication is the work of the authors who will serve as guarantors for the contents of this paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lanepe.2021.100206.

References

[1] Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics 2012;129(3):e827–41.
[2] Chen A, Rogan WJ. Breastfeeding and the risk of postneonatal death in the United States. Pediatrics 2004;113(5):e435–9.
[3] Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics 2005;115(5):1367–77.
[4] Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. The Lancet 2016;387(10017):475–90.
[5] Rollins NC, Bhandari N, Hajeebhoy N, Horton S, Lutter CK, Martines JC, et al. Why invest, and what it will take to improve breastfeeding practices? The Lancet 2016;387(10017):491–504.
[6] 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. J Hepatol 2017;67(3):568–76.
[7] 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.
[8] Ho NT, Li F, Lee-Sarwar KA, Tun HM, Brown BP, Pannaraj PS, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun 2018;9(1):4169.
[9] 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 2013;58(1):128–38.
[10] McCurdy CE, Bishop JM, Williams SM, Grayson BE, Smith MS, Friedman JE, et al. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. J Clin Invest 2009;119(2):323–35.
[11] Soderborg TK, Clark SE, Mulligan CE, Janssen RC, Babcock L, Ir D, et al. The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD. Nat Commun 2018;9(1):4462.
[12] Oddy WH, Li J, Landsborough L, Kendall GE, Henderson S, Downie J. The association of maternal overweight and obesity with breastfeeding duration. J Pediatr 2006;149(2):185–91.
[13] Donath SM, Amir LH. Does maternal obesity adversely affect breastfeeding initiation and duration? J Paediatr Child Health 2000;36(5):482–6.
[14] Amir LH, Donath S. A systematic review of maternal obesity and breastfeeding intention, initiation and duration. BMC Pregnancy Childbirth 2007;7:9.
[15] Enstad S, Cheema S, Thomas R, Fichorova RN, Martin CR, O'Tierney-Ginn P, et al. The impact of maternal obesity and breast milk inflammation on developmental programming of infant growth. Eur J Clin Nutr 2020.
[16] Leghi GE, Netting MJ, Middleton PF, Wlodek ME, Geddes DT, Muhlhausler ABS. The impact of maternal obesity on human milk macronutrient composition: A systematic review and meta-analysis. Nutrients 2020;12(4).
[17] Ayonrinde OT, Adams LA, Mori TA, Beilin LJ, de Klerk N, Pennell CE, et al. Sex differences between parental pregnancy characteristics and nonalcoholic fatty liver disease in adolescents. Hepatology 2018;67(1):108–22.
[18] Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, et al. Cohort Profile: the 'children of the 90s'–the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol 2013;42(1):111–27.
[19] Abeysekera KWM, Fernandes GS, Hammerton G, Portal AJ, Gordon FH, Heron J, et al. Prevalence of steatosis and fibrosis in young adults in the UK: a population-based study. The Lancet Gastroenterology & Hepatology; 2020.
[20] Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, et al. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol 2013;42(1):97–110.
[21] Northstone K, Lewcock M, Groom A, Boyd A, Macleod J, Timpson N, et al. The Avon Longitudinal Study of Parents and Children (ALSPAC): an update on the enrolled sample of index children in 2019. Wellcome Open Res 2019;4:51.
[22] Yokoyama Y, Wada S, Sugimoto M, Katayama M, Saito M, Sono J. Breastfeeding Rates Among Singletons, Twins and Triplets in Japan: A Population-Based Study. Twin Research and Human Genetics 2012;9(2):298–302.
[23] Damato EG, Dowling DA, Standing TS, Schuster SD. Explanation for cessation of breastfeeding in mothers of twins. J Hum Lact 2005;21(3):296–304.
[24] Ostlund A, Nordstrom M, Dykes F, Flacking R. Breastfeeding in preterm and term twins–maternal factors associated with early cessation: a population-based study. J Hum Lact 2010;26(3):235–41 quiz 327-9.
[25] American Psychiatry Association. Diagnostic and statistical manual of mental disorders. 5th edition. APA; 2013.
[26] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42(2):377–81.
[27] Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51(2):454–62.
[28] Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Ledinghen V, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol 2017;66(5):1022–30.
[29] Sarafidis PA, Lasaridis AN, Nilsson PM, Pikilidou MI, Stafilas PC, Kanaki A, et al. Validity and reproducibility of HOMA-IR, 1/HOMA-IR, QUICKI and McAuley's indices in patients with hypertension and type II diabetes. J Hum Hypertens 2007;21(9):709–16.
[30] Shashaj B, Luciano R, Contoli B, Morino GS, Spreghini MR, Rustico C, et al. Reference ranges of HOMA-IR in normal-weight and obese young Caucasians. Acta Diabetol 2016;53(2):251–60.
[31] Martin RM, Ness AR, Gunnell D, Emmett P, Davey Smith G, Team AS. Does breastfeeding in infancy lower blood pressure in childhood? The Avon Longitudinal Study of Parents and Children (ALSPAC). Circulation 2004;109(10):1259–66.
[32] Sanderson E, Macdonald-Wallis C, Davey, Smith G. Negative control exposure studies in the presence of measurement error: implications for attempted effect estimate calibration. Int J Epidemiol 2018;47(2):587–96.
[33] Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. Int J Epidemiol 2016;45(6):1866–86.
[34] Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. Epidemiology 2010;21(3):383–8.
[35] Brand JS, Gaillard R, West J, McEachan RRC, Wright J, Voerman E, et al. Associations of maternal quitting, reducing, and continuing smoking during pregnancy with longitudinal fetal growth: Findings from Mendelian randomization and parental negative control studies. PLoS Med 2019;16(11):e1002972.
[36] Santos Ferreira DL, Williams DM, Kangas AJ, Soininen P, Ala-Korpela M, Smith GD, et al. Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. PLoS Med 2017;14(8):e1002376.
[37] Madley-Dowd P, Rai D, Zammit S, Heron J. Simulations and directed acyclic graphs explained why assortative mating biases the prenatal negative control design. J Clin Epidemiol 2020;118:9–17.
[38] 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. J Hepatol 2010;52(6):913–20.
[39] Wesolowski SR, Kasmi KC, Jonscher KR, Friedman JE. Developmental origins of NAFLD: a womb with a clue. Nat Rev Gastroenterol Hepatol 2017;14(2):81–96.
[40] WHO. World Health Organisation's infant feeding recommendation. Weekly Epidemiological Record 1995;70:119–20.
[41] WHO. Infant and young child feeding. A tool for assessing national practices, policies and programmes. World Health Organization; 2003.
[42] McAndrew FT, J.; Fellows, L.; Large, A.; Speed, M.; Renfrew, M.J. . Infant Feeding Survery 2010 Health and Social Care Information Centre. 2012.
[43] Bertini G, Perugi S, Dani C, Pezzati M, Tronchin M, Rubaltelli FF. Maternal education and the incidence and duration of breast feeding: A prospective study. Journal of Pediatric Gastroenterology and Nutrition 2003;37(4):447–52.
[44] Flacking R, Nyqvist KH, Ewald U. Effects of socioeconomic status on breastfeeding duration in mothers of preterm and term infants. Eur J Public Health 2007;17(6):579–84.
[45] Querter I, Pauwels NS, De Bruyne R, Dupont E, Verhelst X, Devisscher L, et al. Maternal and perinatal risk factors for pediatric non-alcoholic fatty liver disease: A systematic review. Clin Gastroenterol Hepatol 2021.
[46] Lawlor DA, Callaway M, Macdonald-Wallis C, Anderson E, Fraser A, Howe LD, et al. Nonalcoholic fatty liver disease, liver fibrosis, and cardiometabolic risk factors in adolescence: a cross-sectional study of 1874 general population adolescents. J Clin Endocrinol Metab 2014;99(3):E410–7.
[47] Suzuki A, Abdelmalek MF, Schwimmer JB, Lavine JE, Scheimann AO, Unalp-Arida A, et al. Association between puberty and features of nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2012;10(7):786–94.
[48] Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, et al. Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y: evidence from a large randomized trial. Am J Clin Nutr 2007;86(6):1717–21.
[49] Martin RM, Patel R, Kramer MS, Guthrie L, Vilchuck K, Bogdanovich N, et al. Effects of promoting longer-term and exclusive breastfeeding on adiposity and insulin-like growth factor-I at age 11.5 years: a randomized trial. JAMA 2013;309(10):1005–13.
[50] Horta BL, Loret de Mola C, Victora CG. Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: a systematic review and meta-analysis. Acta Paediatr 2015;104(467):30–7.
[51] 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. J Hepatol 2019;70(1):126–32.
[52] Gunderson EP, Lewis CE, Wei GS, Whitmer RA, Quesenberry CP, Sidney S. Lactation and changes in maternal metabolic risk factors. Obstet Gynecol 2007;109(3):729–38.

Please cite this article as: K.W. Abeysekera et al., Association of maternal pre-pregnancy BMI and breastfeeding with NAFLD in young adults: a parental negative control study, The Lancet Regional Health - Europe (2021), https://doi.org/10.1016/j.lanepe.2021.100206