# A protective effect of breastfeeding on the progression of non-alcoholic fatty liver disease

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## ABSTRACT

**Objective:** Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease characterised by accumulation of large-droplet fat in hepatocytes with possible progression to inflammation and fibrosis. Breastfeeding has benefits for child health, both during infancy and later in life, reducing the risk of manifestations of the metabolic syndrome. Here we investigated the association between early type of feeding (breastfed versus formula-fed and duration of breastfeeding) and later NAFLD development.

**Study design:** We investigated 191 young Caucasian children (3–18 years old) with NAFLD consecutively enrolled between January 2003 and September 2007 in our centre. 48% of these children (n = 91) had been breastfed for a median (interquartile range) time of 8 (7) months.

**Results:** After correction for age, waist circumference, gestational age and neonatal weight, the odds of non-alcoholic steatohepatitis (NASH) (OR 0.04, 95% CI 0.01 to 0.10) and fibrosis (OR 0.32, 95% CI 0.16 to 0.65) were lower in breastfed versus not breastfed infants. Moreover, the odds of NASH (OR 0.70, exact 95% CI 0.001 to 0.87) and fibrosis (OR 0.86, exact 95% CI 0.75 to 0.98) decreased for every month of breastfeeding.

**Conclusions:** This observational study suggests that earlier feeding habits might affect the clinical expression of NASH from 3 to 18 years later, with an apparent drug-like preventive effect of breastfeeding.

### What is already known on this topic

- In the last 30 years many studies have suggested that breastfeeding may be crucial to infant health.
- Importantly, breastfeeding may have protective effects against obesity, hypertension, hypercholesterolaemia and type 2 diabetes.
- However, no data on the possible association between early type of feeding and non-alcoholic fatty liver disease have been reported to date.

### What this study adds

- Earlier feeding habits might affect the clinical expression of non-alcoholic steatohepatitis (NASH) 3 to 18 years later, with an apparent drug-like preventive effect of breastfeeding.
- By demonstrating a possible protective effect on NASH, this study reinforces the notion that breastfeeding is important for later health.

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease characterised by the accumulation of large-droplet fat in hepatocytes with possible progression to inflammation and fibrosis.[1] This metabolic disorder occurs mainly in overweight and obese individuals.[2][3] The mechanism of disease primarily involves hyperinsulinaemia and hepatic insulin resistance but not alcohol abuse.[4] NAFLD may be the hepatic manifestation of the metabolic syndrome, traditionally associated with type 2 diabetes mellitus and cardiovascular disease.[5] NAFLD ranges from simple steatosis, which is usually non-progressive, to non-alcoholic steatohepatitis (NASH) to cirrhosis.[6] The high prevalence of NAFLD in children has been recognised only in recent years as rates of childhood obesity have soared.[5]

Early life metabolic programming is a well-known phenomenon in animals and is receiving increasing attention in humans.[7] Early environmental experiences, including the supply of nutrients during intrauterine and early extrauterine life, might have long term effects on the expression of chronic-degenerative disorders.[8–10] Besides supporting the growth and development of infants, breastfeeding has also benefits for child health,

both during infancy and later in life, reducing the risk of manifestations of the metabolic syndrome. Protective effects have been recognised against overweight and obesity (at least in childhood), hypertension, hypercholesterolaemia and type 2 diabetes.[11–14]

However, no data on the relationship between early feeding and later NAFLD have been reported to date. The aim of the present study was therefore to investigate the effects of the early type of feeding (breastfeeding versus formula feeding and breastfeeding duration) in children with NAFLD as diagnosed by liver biopsy.

## METHODS

### Patients

A total of 191 young Caucasian children (3–18 years old) with NAFLD were consecutively enrolled into the study at the Liver Unit of the ''Bambino Gesù'' Pediatric Hospital (Rome, Italy) between January 2003 and September 2007. The study is based on two clinical series previously reported by our group.[15][16] Criteria for suspecting NAFLD were: (i) serum aminotransferases either persistently elevated and/or fluctuating between normal and high levels (with at least two high levels during the 6 months prior to enrolment); (ii) a diffusely hyperechogenic liver at ultrasonography; (iii) absence of alcohol intake; and (iv) absence

of HBV and HCV infection, parenteral nutrition and any pharmacological treatment. The final diagnosis of NAFLD was reached by liver biopsy in all cases. The study protocol conformed to the Declaration of Helsinki and the recommendations of the Ethics Committee of the "Bambino Gesù" Hospital. The nature and purpose of the study were carefully explained to the parents or guardians of the children who gave their written consent.

### Anthropometry

Weight and height were measured using standard procedures.[17] The standard deviation score (SDS) of BMI was calculated using US reference data.[18] Waist circumference was measured at the highest point of the iliac crest.[19] A "large waist" was defined as a waist value ≥90th percentile for age and gender using US reference values.[20 21]

### Laboratory and clinical assessment

Fasting glucose, triglycerides and total, HDL and LDL cholesterol were measured using standard laboratory methods. Insulin was measured by radioimmunoassay (MYRIA Technogenetics, Milan, Italy) with a lower limit of sensitivity of 0.3 µU/ml and an inter-assay coefficient of variation from 2.9% to 4.8%. Impaired fasting glucose or diabetes mellitus was defined as fasting glucose ≥100 mg/dl, hypertriglyceridaemia as fasting triglycerides ≥150 mg/dl, and low HDL cholesterol as fasting HDL <40 mg/dl in males and <50 mg/dl in females.[21] The homeostasis model assessment index of insulin resistance (HOMA) and the insulin sensitivity index (ISI) were calculated as surrogate markers of insulin sensitivity.[22 23] After a 5 min rest, blood pressure was measured with an aneroid sphygmomanometer (Taylor Instruments, Asheville, NC) equipped with a cuff appropriate for arm size.[24] Three measurements were performed and the average of the last two was taken as the blood pressure value. Hypertension was defined as a systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg.[21] The metabolic syndrome was defined as three or more of the following: large waist, low HDL cholesterol, hypertriglyceridaemia, hypertension and impaired fasting glucose or diabetes.[21]

### Neonatal, breastfeeding and family history

Neonatal data (gestational age and birth weight) were collected from clinical charts. Breastfeeding and its duration were obtained from the same source. Weight for gestational age was classified as ≤5th percentile (low), between 6th and 94th percentile (normal) and ≥95th percentile (high) for age using US reference data.[25] The family history of cardio-metabolic disease (hypertension, chronic ischaemic disease, diabetes and dyslipidaemia) was ascertained by interview with parents. The job of the parents was used as a surrogate measure of socioeconomic status (1 = pensioner; 2 = working; 3 = white collar; 4 = professional).

### Liver histology

Biopsies were performed as described elsewhere.[26] Steatosis, inflammation, hepatocyte ballooning and fibrosis were scored using the NAFLD Clinical Research Network criteria.[27] Features of steatosis, lobular inflammation and hepatocyte ballooning were combined to obtain the NAFLD activity score (NAS) and patients with NAS ≥5 were considered to have NASH.[27]

### Statistical analysis

Continuous variables are given as median, interquartile range (IQR) and minimum and maximum values because of skewed distributions. IQR was calculated as the difference between the 75th and 25th percentile. Between-group comparisons of continuous variables were performed with the Wilcoxon–Mann–Whitney test and those of categorical variables with Fisher's exact test. The contribution of breastfeeding (1 = yes; 0 = no) to NASH (1 = yes; 0 = no) and liver fibrosis (any degree of liver fibrosis = 1; absence of liver fibrosis = 0) was evaluated using univariable and multivariable binary logistic regression. The multivariable models included age, waist circumference, gestational age and birth weight as predictors together with breastfeeding. Age and waist circumference were included on the basis of previous evidence that they are independent predictors of liver fibrosis in children with NAFLD.[15] Gestational age and birth weight were included as anthropometric and developmental markers of the neonatal period. All these potential confounders were modelled as continuous. In the subgroup of breastfed children, the contribution of the duration of breastfeeding (continuous) to NASH and fibrosis was modelled using binary logistic regression. Linearity of logits was ascertained using the Box–Tidwell test for univariable models and multivariable fractional polynomials for multivariable models. Exact binary logistic regression was used whenever asymptotic assumptions were not met.[28] Model fit of multivariable binary logistic models was checked using the Hosmer–Lemeshow statistic. The effect of breastfeeding (no = 0; yes = 1) on selected ordinal outcomes (NAS, steatosis, inflammation, ballooning, fibrosis, birth weight status and socioeconomic status) was evaluated using ordinal logistic regression with a continuation ratio model.[29] Proportionality of odds ratios across response categories was evaluated using a likelihood ratio test. Statistical significance was set at a p value <0.05. All statistical tests are two-tailed. Statistical analysis was performed using Stata 10.1 (StataCorp, College Station, TX) and StatXact 8.0 and LogXact 8.0 (Cytel Inc, Cambridge, MA).

### RESULTS

A total of 191 Caucasian children with NAFLD (131 males and 60 females) aged 3.3–18.0 years were consecutively studied. Some 48% of them (n = 91) had been breastfed for a median (IQR) time of 8 (7) months (range 0.5–17 months).

Table 1 reports the anthropometric, laboratory and neonatal measurements of breastfed versus not breastfed children. Breastfed children had lower HDL levels (p = 0.012) and slightly higher values of diastolic blood pressure (p = 0.037), while no differences were seen among the other variables of interest.

Table 2 reports the frequency of the metabolic syndrome in the two study groups. Low HDL was more common in breastfed children (OR 2.28, p = 0.013), but there was no difference in the other components of the metabolic syndrome. Table 2 also reports the distribution of cardio-metabolic disease and the socioeconomic status of parents, which was similar in breastfed compared to not breastfed children.

Table 3 reports the findings of liver biopsy for breastfed versus not breastfed children. The NAS score (OR 0.12), steatosis (OR 0.15), lobular inflammation (OR 0.24), ballooning (OR 0.15) and fibrosis (OR 0.28) showed a clear trend of decreasing severity in breastfed as compared to not breastfed children (p<0.001 for all; ordinal logistic regression with continuation ratio model).

At univariable analysis, breastfeeding was protective against NASH (OR 0.04, 95% CI 0.01 to 0.10, p<0.001), and both the

**Table 1** Anthropometric, laboratory and neonatal measurements of breastfed versus not breastfed children

| | Breastfed (n = 91) | | | | Not breastfed (n = 100) | | Kruskal–Wallis test | | |
|---|---|---|---|---|---|---|---|---|---|
| | Median | IQR | Min | Max | Median | IQR | Min | Max | p Value |
| Age (years) | 12.1 | 3.7 | 3.3 | 18.0 | 12.1 | 3.4 | 4.7 | 17.5 | 0.930 |
| Weight (kg) | 62.0 | 31.5 | 14.0 | 128.0 | 63.0 | 18.2 | 26.0 | 115.0 | 0.578 |
| Weight (SDS) | 1.80 | 1.30 | −2.68 | 3.75 | 1.86 | 1.02 | −0.01 | 3.09 | 0.734 |
| Height (m) | 1.53 | 0.18 | 0.99 | 1.86 | 1.52 | 0.21 | 0.86 | 1.87 | 0.922 |
| Height (SDS) | 0.44 | 1.57 | −2.53 | 3.69 | 0.16 | 1.38 | −3.06 | 3.69 | 0.196 |
| BMI (kg/m²) | 25.9 | 6.8 | 15.2 | 38.9 | 26.0 | 4.5 | 20.1 | 40.7 | 0.307 |
| BMI (SDS) | 1.89 | 0.78 | −1.79 | 3.05 | 1.90 | 0.63 | 1.05 | 4.14 | 0.482 |
| Waist circumference (cm) | 90 | 14 | 60 | 110 | 93 | 11 | 61 | 109 | 0.297 |
| ALT (U/l) | 66 | 35 | 14 | 388 | 67 | 82 | 10 | 407 | 0.320 |
| AST (U/l) | 43 | 19 | 19 | 151 | 45 | 30 | 16 | 187 | 0.150 |
| GGT (U/l) | 21 | 9 | 11 | 130 | 21 | 25 | 10 | 130 | 0.493 |
| Cholesterol (mg/dl) | 156 | 50 | 75 | 243 | 164 | 43 | 75 | 250 | 0.130 |
| HDL cholesterol (mg/dl) | 48 | 23 | 20 | 101 | 54 | 17 | 21 | 101 | 0.012 |
| Triglycerides (mg/dl) | 80 | 61 | 31 | 351 | 90 | 64.5 | 24 | 424 | 0.059 |
| Glucose (mg/dl) | 79 | 13 | 52 | 138 | 80 | 12 | 56 | 138 | 0.718 |
| Insulin (μU/ml) | 11 | 9 | 4 | 78 | 13 | 10 | 3 | 79 | 0.403 |
| HOMA | 2.2 | 2.1 | 0.7 | 16.0 | 2.6 | 2.1 | 0.5 | 15.4 | 0.388 |
| ISI | 3.9 | 3.1 | 0.6 | 9.2 | 3.2 | 2.4 | 0.8 | 14.7 | 0.068 |
| Systolic blood pressure (mm Hg) | 110 | 20 | 94 | 161 | 110 | 22 | 90 | 160 | 0.543 |
| Diastolic blood pressure (mm Hg) | 70 | 10 | 50 | 84 | 69 | 10 | 50 | 81 | 0.037 |
| Gestational age (weeks) | 38.0 | 1.0 | 31.0 | 42.0 | 39.0 | 2.0 | 37.0 | 42.0 | 0.145 |
| Birth weight (kg) | 3.2 | 0.8 | 2.1 | 4.3 | 3.2 | 0.8 | 2.0 | 4.7 | 0.560 |

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; GGT, gamma-glutamyl-transferase; HOMA, homeostatis model assessment of insulin resistance; ISI, insulin sensitivity index; IQR, interquartile range; Max, maximum value; Min, minimum value; SDS, standard deviation score.

size and variability of the effect did not change after correction for age, waist circumference, gestational age and neonatal weight at multivariable analysis (OR 0.04, 95% CI 0.01 to 0.10, p<0.001). At univariable analysis, breastfeeding was protective also against fibrosis (OR 0.31, 95% CI 0.16 to 0.58, p<0.001), but the variability of this effect increased slightly at multivariable analysis (OR 0.32, 95% CI 0.16 to 0.65, p<0.001) because of the already reported effect of age and waist circumference on liver fibrosis in children with NAFLD.[15]

Among breastfed children (n = 91), the odds of NASH decreased for increasing duration of breastfeeding (OR 0.07 for every month of breastfeeding, exact 95% CI 0.0005 to 0.55, exact p<0.001). Although substantially reduced, this protective effect persisted at multivariable analysis (OR 0.70 for every month of breastfeeding, exact 95% CI 0.001 to 0.87, exact

p = 0.001). Likewise, the odds of fibrosis decreased with the duration of breastfeeding at univariable analysis (OR 0.83 for every month of breastfeeding, exact 95% CI 0.73 to 0.93, exact p<0.001); this effect was confirmed at multivariable analysis (OR 0.86 for every month of breastfeeding, exact 95% CI 0.75 to 0.98, exact p = 0.025).

## DISCUSSION
This study is the first to investigate the association between breastfeeding and NAFLD in children. Our data suggest that earlier feeding habits might affect the later expression of NAFLD, with a protective effect of breastfeeding on NASH and liver fibrosis which is independent of the present or neonatal characteristics of the children.

**Table 2** Frequency of the metabolic syndrome in breastfed versus not breastfed children

| | Breastfed (n = 91) | Not breastfed (n = 100) | Breastfed versus not breastfed OR (95% CI) |
|---|---|---|---|
| Birth weight (⩽5th/6–94th/⩾95th percentile)† | 10/78/3 | 17/79/4 | 1.38 (0.67 to 2.87) |
| Large waist (Y/N) | 73/18 | 87/13 | 0.61 (0.28 to 1.32) |
| Impaired fasting glucose (Y/N) | 5/86 | 7/93 | 0.77 (0.24 to 2.53) |
| Hypertriglyceridaemia (Y/N) | 9/82 | 19/81 | 0.47 (0.20 to 1.10) |
| Low HDL (Y/N) | 33/58 | 20/80 | 2.28 (1.19 to 4.36)* |
| Hypertension (Y/N) | 9/82 | 19/81 | 0.47 (0.20 to 1.10) |
| Metabolic syndrome (⩾3 of the above) | 7/84 | 15/85 | 0.47 (0.18 to 1.22) |
| At least one parent with hypertension (Y/N) | 54/37 | 66/34 | 0.75 (0.42 to 1.35) |
| At least one parent with CHD (Y/N) | 32/59 | 39/61 | 0.85 (0.47 to 1.53) |
| At least one parent with diabetes (Y/N) | 14/77 | 21/79 | 0.68 (0.32 to 1.44) |
| At least one parent with dyslipidaemia (Y/N) | 65/26 | 69/31 | 1.12 (0.60 to 2.10) |
| Socioeconomic status - father's job (1/2/3/4) †‡ | 10/21/29/31 | 8/31/30/31 | 1.11 (0.72 to 1.71) |
| Socioeconomic status - mother's job (1/2/3/4) †‡ | 10/23/26/32 | 7/34/35/24 | 1.33 (0.86 to 2.05) |

CHD, coronary heart disease; N, no; Y, yes.
*p = 0.013 (likelihood-ratio test).
†Obtained from ordinal logistic regression using a continuation ratio model.
‡1 = Pensioner, 2 = working, 3 = white collar, 4 = professional.

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**Table 3** Distribution of histological findings after liver biopsy in breastfed and not breastfed children

| Score | Breastfed | | | | | | | | Not breastfed | | | | | | | | Breastfed versus not breastfed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | OR (95% CI)*† |
| NAS | – | 8 | 31 | 34 | 13 | 2 | 3 | 0 | – | 0 | 11 | 8 | 22 | 20 | 30 | 9 | 0.12 (0.07 to 0.20) |
| Steatosis | – | 46 | 37 | 8 | – | – | – | – | – | 14 | 32 | 54 | – | – | – | – | 0.15 (0.08 to 0.25) |
| Inflammation | 18 | 67 | 6 | 0 | – | – | – | – | 5 | 73 | 20 | 2 | – | – | – | – | 0.24 (0.12 to 0.50) |
| Ballooning | 66 | 21 | 4 | – | – | – | – | – | 34 | 18 | 48 | – | – | – | – | – | 0.15 (0.09 to 0.26) |
| Fibrosis | 42 | 44 | 5 | 0 | – | – | – | – | 21 | 62 | 3 | 14 | – | – | – | – | 0.28 (0.17 to 0.49) |

*p<0.001 for all values (likelihood-ratio test).
†Obtained from ordinal logistic regression using a continuation ratio model.

The fact that HDL was lower and diastolic blood pressure was slightly higher in breastfed children is somewhat surprising but may be just a matter of chance because of the high number of between-group comparisons performed. Our analysis was explorative so we were not able to calculate a sample size consistent with a given study hypothesis. It is possible that the size and the variability of the effect might be higher in larger samples, as shown for the association between breastfeeding and some of its later effects.[12]

Nevertheless, our study which is based on the gold standard method of liver biopsy, suggests the possibility of an early imprinting effect of breastfeeding and/or human milk that becomes more evident if the infant is later exposed to unfavourable metabolic conditions such as obesity and fatty liver. A similar preventive effect of early breastfeeding has been recently reported for lung compliance in 5- to 17-year-old children with cystic fibrosis, where breastfeeding longer than 4 months resulted in better lung function tests.[30]

Some nutritional components of human milk may interact with many regulatory systems, such as peroxisome proliferator-activated receptors (PPAR, alpha and gamma) that are implicated in protection against fibrosis.[31 32] Long-chain polyunsaturated fatty acids of the n-3 series, especially docosahexaenoic acid, delivered by human milk after prolonged lactation, could act as PPAR-agonists either directly or indirectly.[33 34] PPAR-gamma polymorphism variants are associated with differences in insulin sensitivity and long-chain polyunsaturated fatty acid (LCPUFA) levels.[35] PPAR-gamma expression is critical in reducing experimental liver fibrogenesis and a possible therapeutic role of its inhibition on NAFLD has been proposed.[36–38]

However, to what extent the early effects of breastfeeding could translate into later protection is a matter of speculation. Trophic factors present in human milk could interact with hepatocytes within a neurohormonal milieu that is quite different in breastfed as compared to formula-fed infants.[39] Since the protective effect of human milk on obesity is more evident during childhood, we suggest that the preventive effect of breastfeeding on liver fibrosis may be more evident in younger populations exposed to unfavourable situations, such as those encountered in NAFLD.[11] From this perspective, human milk should be recognised not just as a preventive substance but also as a drug-like modulator of growth and differentiation, whose role on the biological outcome of the individual should be further explored at the cellular and sub-cellular levels.

Only in recent years have some milk formula brands added long-chain polyunsaturated fatty acids (LCPUFA) to their starting formula products in Italy and, in any case, the biochemical mechanism through which they are provided in human milk is quite unusual.[40] Breastfeeding is associated with healthier practices and with more favourable environmental conditions in Italy as well in other western countries.[41 42] Even though some environmental confounders were taken into account by our analysis, we cannot exclude that the early type of feeding and prolonged breastfeeding are just surrogate indicators of other risk factors. This consideration applies indeed to all the available observational studies of breastfeeding which, especially when prolonged, is associated with the development of different eating patterns which may be partly responsible for the observed effects.[43–45] Clearly, more studies are needed to understand why breastfeeding may protect against NASH and liver fibrosis in children. However, our observational data point to another beneficial late effect of human milk and reinforce the notion that breastfeeding is important for later health.

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