RESEARCH REPORT

Breast feeding and blood lipid concentrations in male Brazilian adolescents

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Objective: To assess the association between breast feeding and blood lipid levels in adolescence.
Design: Population based prospective birth cohort study.
Setting: City of Pelotas, Brazil.
Subjects: All hospital births taking place in 1982; 79% of all males (n = 2250) were followed up for 18 years, and 2089 blood samples were available.
Interventions: None.
Main outcome measures: Total cholesterol and fractions (very low density lipoprotein cholesterol (VLDL), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL)), LDL/HDL ratio, serum triglycerides.
Results: Three breast feeding variables were studied: total duration of breast feeding, duration of exclusive or predominant breast feeding, and ever compared with never breast fed. Adjusted analyses were controlled for family income, household assets index, maternal education, maternal pre-pregnancy body mass index (BMI), skin colour, birth weight, gestational age, maternal smoking during pregnancy, and adolescent BMI, and behavioural variables (fat content of diet, physical activity, smoking, and alcohol drinking). Only one association reached borderline significance (p = 0.05): LDL cholesterol was slightly higher among never (mean 41.0 mg/dl; 95% CI 39.4 to 42.7) than among ever breast fed men (38.6 mg/dl; 95% CI 38.6 to 40.3), in the adjusted analyses. All other associations were not significant ($p \geq 0.09$). There was no evidence of effect modification according to preterm status, intrauterine growth retardation, socioeconomic level, growth velocity in the first two years of life, or nutritional status at 2 years of age.
Conclusions: There was no clear association between breast feeding duration and serum lipid concentrations at the age of 18 years in this sample of Brazilian men.

The availability of results from long term birth cohort studies has permitted the assessment of the long term health consequences of early life exposures. A recent publication by British authors reported on two parallel randomised controlled trials in which preterm newborns were allocated to receive either donated banked human milk or formula (study 1) or two different types of formula (study 2). At the ages of 13–16 years, adolescents who had been randomised to banked breast milk had lower low density lipoprotein (LDL) to high density lipoprotein (HDL) cholesterol ratio (mean difference 0.34 (14% lower), 95% CI −0.67 to −0.01; p = 0.04) than those given preterm formula. There was also a dose-response relation with the intake of breast milk in infancy, thus suggesting a long term impact of breast milk feeding on the risk of atherosclerosis.[1] The evidence from several other non-randomised studies carried out in developed countries[2–10] is not clear cut. Of 11 analyses presented in these articles (some included separate analyses by sex), three showed breast feeding to be protective, six did not find a significant effect, and two found increased risk. Few studies have assessed the effect of infant feeding on LDL cholesterol concentrations during adolescence. Fall[10] and Kolacek[6] found that breast feeding duration was inversely related to LDL concentrations, whereas two other studies failed to see a significant association.[4 5] Finally, Ravelli *et al*[9] found a lower LDL/HDL ratio among subjects who had been breast fed. Owen *et al*,[5] in addition to reporting original data, carried out a meta-analysis that failed to show a protective effect, with a pooled mean difference in total cholesterol of 0.00 mmol/l (95% confidence interval: −0.07 to 0.07 mmol/l). We are not aware of any meta-analyses of the LDL/HDL ratio.

In the literature reviewed, there were no studies from developing countries where malnutrition in childhood is prevalent. It is possible that the effect of breast feeding on cholesterol concentrations may be modified by childhood nutritional status. To fill this gap, we analysed data from a population based birth cohort from Pelotas, in southern Brazil, that we have been following up from 1982 to the present.

METHODS

The study was carried out in Pelotas (current urban population 320 000) in southern Brazil. The population is mostly white, of southern European descent. This is a comparatively developed part of Brazil, but there are wide inequalities in health and in socioeconomic status. The infant mortality rate in the birth cohort was 38 per thousand live births and at the age of 18 years 17.4% of all male subjects were overweight (body mass index, or BMI, above 25 kg/m^2).

Children born in 1982 in the city have been followed up on several occasions.[11] All 6011 infants born in three maternity hospitals (over 99% of all births in the city) were initially recruited. The 5914 live born infants were examined, 3037

Abbreviations: BMI, body mass index; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 1 Distribution of the sample according to blood lipid values

Indicator	Mean	Standard deviation	Number
Total cholesterol	143.1	29.0	2083
HDL cholesterol	40.1	9.7	2059
LDL cholesterol	88.2	25.1	2059
VLD cholesterol	14.8	9.6	2059
LDL/HDL ratio	2.31	0.81	2059
Triglycerides	76.1	48.0	2059

All values are expressed in mmol/l, except for the LDL/HDL ratio.

were boys, and their mothers interviewed on socioeconomic, demographic, and health related variables.

In 1983, children born from January to April 1982 were visited at home. In 1984 and 1986, a city census covered about 70 000 homes in search of children born in 1982; 87% and 84% of the original cohort were located, respectively, at the average ages of 20 months and 42 months.[11] Mothers were interviewed in every round, and detailed information on duration of breast feeding and age at introduction of other feeds (non-breast milk, other fluids, semi-solids, and solids) were obtained. Feeding information is available for 90% of the cohort.

In 2000, all men in the birth cohort who were still resident in Pelotas were legally obliged to undergo a medical examination at the local army base. They were then invited to answer a research questionnaire and to donate a blood sample. Typically, conscripts had continental style breakfast at home at around 5 30 am, because they had to arrive at 6 00 am at the army base where the examinations were carried out. Blood samples were collected by venopuncture between 10 30 am and 12 00 noon, after the whole round of medical, physical, and intellectual examinations had been completed.

The following outcome variables were studied: total serum cholesterol; very low density lipoprotein cholesterol (VLDL); LDL; HDL; LDL/HDL ratio, and serum triglycerides. Total and HDL cholesterol, and tryglicerides were measured using enzyme methods (Dimension clinical chemistry system; Dade Behring). VLDL cholesterol was estimated from triglyceride levels.[12] All values are expressed in mmol/l, except for the LDL/HDL ratio.

Two explanatory variables were studied. Total breast feeding duration (in months and days) was collected in the 1983, 1984, and 1986 follow up studies. Total breast feeding duration was classified as <1 month (because of evidence of misclassification between infants who were never breast fed or who were breast fed only for a few days); 1–2.9, 3–5.9, 6–8.9, 9–11.9, and ⩾12 months. The data were also reanalysed comparing ever and never breast fed subjects. The duration of predominant breast feeding recorded the age at regular introduction of foods other than breast milk or teas/water, according to the standard World Health Organisation definitions of breast feeding indicators.[13] Exclusive breast feeding was extremely short, as nearly all children also received teas or water from the first week of life, being classified in the predominant breast feeding category. This variable was recoded as <1 month, 1–1.9, 2–2.9, 3–3.9, and ⩾4 months. The earliest available information on stopping breast feeding or introduction of other foods was used to reduce recall bias.

Confounding variables collected in the early phases of the study included monthly family income, maternal education, household assets index (obtained through factor analysis and based on the ownership of household goods),[14] birth weight (g), maternal smoking in pregnancy (non-smokers, 0–14 or 15 or more cigarettes per day), gestational age, and maternal pre-pregnancy BMI. Information on ethnicity/skin colour was based on self classification during the 2000 interview (white, mixed, black, Asian, native Brazilian). Current behavioural variables (fat content of diet, physical activity, smoking, and alcohol drinking) and BMI were also included in the

Table 2 Blood lipid values of 18 year old men according to total duration of breast feeding

	Duration of total breast feeding (months)							
	<1	1–2.9	3–5.9	6–8.9	9–11.9	⩾12	p Value*	Number
HDL	40.4 (39.6, 41.3)	39.4 (38.6, 40.2)	40.1 (39.3, 41.0)	41.6 (40.1, 43.0)	39.9 (37.8, 42.0)	39.3 (38.2, 40.3)	0.083	1993
LDL	89.7 (87.4, 92.0)	87.0 (84.9, 89.1)	87.2 (84.9, 89.5)	91.1 (87.2, 95.0)	86.1 (81.1, 91.1)	89.5 (86.5, 92.5)	0.195	1993
Total cholesterol	144.5 (141.8, 147.2)	141.5 (139.1, 143.9)	142.3 (139.6, 145.0)	147.9 (143.4, 152.4)	140.4 (135.0, 145.8)	143.5 (140.1, 147.0)	0.116	2017
VLD	14.3 (13.4, 15.2)	15.1 (14.3, 15.9)	15.2 (14.3, 16.2)	15.4 (14.1, 16.8)	14.4 (12.9, 15.9)	14.6 (13.6, 15.6)	0.584	1993
LDL/HDL ratio	2.33 (2.25, 2.40)	2.32 (2.25, 2.39)	2.28 (2.20, 2.35)	2.31 (2.19, 2.44)	2.27 (2.09, 2.44)	2.38 (2.29, 2.47)	0.639	1993
Triglycerides	73.4 (69.0, 77.8)	77.5 (73.4, 81.6)	78.2 (73.3, 83.0)	78.8 (72.0, 85.6)	74.2 (66.8, 81.7)	74.8 (69.8, 79.9)	0.603	1993

*Analysis of variance. Values are means (95% confidence intervals). All values are expressed in mg/dl, except for the LDL/HDL ratio.

Table 3 Blood lipid values of 18 year old men according to duration of predominant breast feeding

	Duration of predominant breast feeding (months)						
	<1	1–1.9	2–2.9	3–3.9	⩾4	p Value*	Number
HDL	40.5 (39.6, 41.3)	39.6 (38.5, 40.8)	39.5 (38.5, 40.5)	40.4 (39.6, 41.2)	39.9 (38.6, 41.3)	0.52	1907
LDL	89.4 (87.2, 91.5)	88.3 (85.7, 91.0)	89.5 (86.9, 92.2)	87.9 (85.7, 90.0)	86.5 (82.9, 90.1)	0.58	1907
Total cholesterol	144.8 (142.4, 147.3)	141.8 (138.8, 144.8)	144.0 (140.9, 147.0)	143.0 (140.5, 145.4)	142.2 (138.0, 146.4)	0.58	1907
VLD	14.9 (14.1, 15.8)	14.0 (13.0, 15.0)	14.9 (14.0, 15.9)	14.9 (14.0, 15.7)	15.6 (14.1, 17.0)	0.45	1907
LDL/HDL ratio	2.32 (2.25, 2.39)	2.34 (2.25, 2.43)	2.38 (2.30, 2.47)	2.26 (2.20, 2.33)	2.29 (2.17, 2.41)	0.28	1907
Triglycerides	76.7 (72.4, 81.1)	71.8 (66.9, 76.8)	76.7 (72.0, 81.3)	76.2 (72.1, 80.3)	79.9 (72.8, 86.9)	0.43	1907

*Analysis of variance. Values are means (95% confidence intervals). All values are expressed in mg/dl, except for the LDL/HDL ratio.

Table 4 Blood lipid values of 18 year old men according to total duration of breast feeding: adjusted analyses

	Breast feeding duration (months)						
	<1	1–2.9	3–5.9	6–8.9	9–11.9	⩾12	p Value*
HDL	40.1 (39.0, 41.2)	39.0 (37.9, 40.1)	39.5 (38.42, 40.7)	40.9 (39.3, 42.4)	39.6 (37.3, 41.8)	38.9 (37.5, 40.2)	0.15
LDL	91.0 (88.0, 93.9)	88.3 (85.4, 91.2)	88.2 (85.3, 91.1)	91.2 (87.1, 95.2)	84.8 (78.9, 90.7)	90.8 (87.2, 94.3)	0.15
Total cholesterol	146.4 (143.1, 149.8)	143.2 (139.9, 146.5)	143.8(140.5, 147.1)	147.6 (143.0, 152.2)	138.3 (131.6, 145.0)	145.5 (141.5, 149.6)	0.09
VLD	15.3 (14.2, 16.4)	15.8 (14.7, 16.8)	16.2 (15.1, 17.3)	15.6 (14.1, 17.1)	13.8 (11.6, 15.9)	15.5 (14.2, 16.8)	0.34
LDL/HDL ratio	2.38 (2.28, 2.47)	2.38 (2.29, 2.47)	2.34 (2.24, 2.43)	2.35 (2.22, 2.48)	2.25 (2.06, 2.44)	2.43 (2.32, 2.55)	0.50
Triglycerides	78.3 (72.9, 83.7)	80.8 (75.6, 86.1)	83.0 (77.7, 88.3)	79.7 (72.3, 87.1)	71.2 (60.4, 81.9)	79.8 (73.3, 86.2)	0.35

*General linear model (general factorial); estimates adjusted for family income, household asset index, maternal education and pre–pregnancy BMI, skin colour, birth weight, gestational age, maternal smoking during pregnancy, current BMI, and behavioural variables (fat content of diet, physical activity, smoking, and alcohol drinking). Values are means (95% confidence intervals). All values are expressed in mg/dl, except for the LDL/HDL ratio.

Table 5 Blood lipid values of 18 year old men according to duration of predominant breast feeding: adjusted analyses

	Breast feeding duration (months)					
	<1	1–1.9	2–2.9	3–3.9	⩾4	p Value*
HDL	40.3 (39.2, 41.4)	39.2 (37.9, 40.5)	39.0 (37.7, 40.2)	39.9 (38.8, 41.0)	39.5 (38.1, 41.0)	0.31
LDL	90.6 (87.7, 93.5)	89.6 (86.1, 93.0)	90.7 (87.4, 94.1)	88.9 (86.0, 91.7)	87.0 (83.1, 90.9)	0.38
Total cholesterol	146.8 (143.5, 150.1)	143.5 (139.6, 147.4)	145.9 (142.1, 149.7)	144.3 (141.1, 147.6)	143.1 (138.7, 147.5)	0.35
VLD	15.8 (14.8, 16.9)	14.8 (13.6, 16.1)	16.1 (14.8, 17.2)	15.5 (14.5, 16.6)	16.4 (14.9, 17.7)	0.37
LDL/HDL ratio	2.37 (2.27, 2.46)	2.40 (2.29, 2.51)	2.45 (2.34, 2.56)	2.32 (2.22, 2.41)	2.33 (2.20, 2.45)	0.15
Triglycerides	81.0 (75.8, 86.3)	76.1 (69.8, 82.3)	82.0 (75.9, 88.0)	79.7 (74.5, 84.9)	83.8 (76.8, 90.8)	0.35

*General linear model (general factorial); estimates adjusted for family income, household asset index, maternal education and pre-pregnancy BMI, skin colour, birth weight, gestational age, maternal smoking during pregnancy, current BMI, and behavioural variables (fat content of diet, physical activity, smoking, and alcohol drinking). Values are means (95% confidence intervals). All values are expressed in mg/dl, except for the LDL/HDL ratio.

Table 6 Blood lipid values of 18 year old men according to ever breast feeding: crude and adjusted analyses

	Breast feeding history					
	Crude analyses			Adjusted analyses		
	Never	Ever	p Value	Never	Ever	p Value*
HDL	41.1 (39.7, 42.4)	39.9 (39.5, 40.4)	0.14	41.0 (39.4, 42.7)	39.5 (38.6, 40.3)	0.05
LDL	88.9 (85.0, 92.8)	88.3 (87.2, 89.5)	0.79	91.1 (86.8, 95.5)	89.1 (86.9, 91.3)	0.34
Total cholesterol	143.6 (139.1, 148.2)	143.2 (141.9, 144.5)	0.85	147.1 (142.2, 152.0)	144.4 (141.9, 146.8)	0.24
VLD	13.8 (12.3, 15.3)	15.0 (14.5, 15.40)	0.14	15.1 (13.5, 16.6)	15.7 (14.9, 16.5)	0.44
LDL/HDL ratio	2.25 (2.14, 2.37)	2.32 (2.29, 2.36)	0.28	2.31 (2.17, 2.45)	2.37 (2.30, 2.44)	0.37
Triglycerides	71.2 (63.7, 78.7)	76.8 (74.6, 79.0)	0.15	77.4 (69.5, 85.3)	80.3 (76.3, 84.3)	0.45

*General linear model (general factorial); estimates adjusted for family income, household asset index, maternal education and pre-pregnancy BMI, skin colour, birth weight, gestational age, maternal smoking during pregnancy, current BMI, and behavioural variables (fat content of diet, physical activity, smoking, and alcohol drinking). Values are means (95% confidence intervals). All values are expressed in mg/dl, except for the LDL/HDL ratio.

multivariable analyses. Means of the outcome variables were compared using analysis of variance. General linear models were used to adjust for confounders. Stratified analyses were carried out in search of effect modification by preterm status (<37 weeks' gestation), intrauterine growth retardation (<10th centile of the Williamscurve),[15] presence of stunting at 2 years of age (height for age below −2 Z scores of the WHO/NCHS reference or above),[16] rapid growth velocity (gain of more than 0.66 Z score of weight for age in the first two years of life, NCHS/WHO reference), or low family income (<3 minimum wages a month).

The confidentiality of all information was ensured and informed consent was obtained in all phases of the study (verbal consent in the 1980s and written consent in 2000). The medical ethics committee of the University of Pelotas, affiliated with the Brazilian Medical Research Council, approved the study protocol.

What is already known on topic

There is no consensus in the literature about whether or not breast feeding contributes to lower blood lipid levels in adults, with some studies reporting an association while others do not.

RESULTS

A total of 2250 subjects were interviewed at the age of 18 years. Added to the 143 cohort boys known to have died, they represent 79% of all live born boys. Losses to follow up were more frequent among boys born to the poorest (27%) and the wealthiest families (23%), compared with 16% in the middle income group. There were no clear trends in follow up rates

What this paper adds

This is one of the few studies on this subject from low and middle income countries. Among over 2000 18 year old male Brazilians followed up prospectively from birth, total breast feeding duration was not associated to cholesterol levels. Predominant breast feeding duration was also not related to cholesterol levels.

Policy implications

Breast feeding is essential for child health and development, as well as maternal health, particularly in low and middle income populations. If breast feeding can also be shown to protect against chronic diseases, this would be an additional argument for its strong promotion. The fact that we were not able to show an effect on blood lipid levels does not mean that breast feeding promotion should be neglected.

relative to maternal education, maternal skin colour, birth weight, or breast feeding duration.

Altogether 2083 subjects consented to provide a blood sample; total cholesterol was measured for all, but 24 subjects did not provide the amount of blood needed for the remaining tests, which were thus restricted to 2059 men (table 1).

Tables 2 (total breast feeding) and 3 (predominant breast feeding) are based on the unadjusted analyses. There was no association between breast feeding patterns and lipid levels. The lack of association remains when 12 potential confounding variables were added to the model (tables 4 and 5).

We also analysed the data according to whether or not the child was ever breast fed. Table 6 shows that there were no differences between the groups. Data were also reanalysed using the classifications of breast feeding duration used in the ALSPAC study[17] and those used by Singhal *et al*[1] but the lack of association persisted (data available upon request).

In addition, the possibility of effect modification was investigated, by repeating the above analyses after stratification by preterm status, by socioeconomic level, by growth velocity in the first two years of life, and by the presence stunting (low height for age) at 2 years. There was no evidence that any of these variables modified the effect of breast feeding (data available upon request).

DISCUSSION

Our study has the advantage of a prospective design relating blood breast feeding patterns measured in early childhood to lipid levels in late adolescence. The comparatively high rate of follow up (79%) is also worth noting. Its limitations include the restriction to men—because women are not required to undergo medical examination at the army—and the fact that blood lipids were not collected after a 12 hour fast. Typically our samples were collected after a six hour fast after a continental type breakfast; nevertheless, serum lipid levels were similar to results from other Latin American settings.[18 19]

Results that are not shown here confirm strong associations between blood lipids and their known determinants such as BMI, reported physical activity levels, activity levels, per cent body fat assessed through bioimpedance, and skin folds, thus suggesting that despite the short fasting periods it was possible to detect several significant associations in the expected direction.

Despite having examined our data in several different ways, we were unable to find an association between the duration of total or of predominant breast feeding and levels of blood lipids in Brazilian adolescents. The lack of association was consistent in the crude analyses, in those adjusted for true confounding factors (that are not part of the proposed causal chain between breast feeding and blood lipids), and even when potential mediating factors (for example, current BMI) were controlled. In our sample, there was no association between breast feeding patterns and BMI.[20]

The lack of association seems to be in agreement with most of the available literature.[1–10] An exception is the recent study by Singhal and colleagues,[1] which has the advantage of being a randomised trial, but also has its limitations. In the randomised analyses presented in their paper, 253 eligible preterm infants were allocated to receive banked breast milk for 40 days; of these, 66 (26%) were traced at ages 13–16 years. Of the 249 infants allocated to the formula group, 64 (26%) were followed up. Therefore, three quarters of the original subjects were not included in the blood lipid analyses. In addition, of the 66 infants who received banked breast milk, 50 were complemented with other types of milk; and of 64 randomised to formula, 47 also received other types of milk (including breast milk). This resulted in two groups that were not noticeably different in terms of early feeding. Given such low follow up and compliance rates, as well as the short duration of exposure (only 40 days) it is quite surprising that the authors were able to find a significant reduction ($p = 0.04$) in the LDL/HDL ratio for infants allocated to banked breast milk. Positive, but non-significant changes were also seen for some of the other outcomes. Their results are supported by additional analyses that ignored the randomisation and treated the data as if derived from an observational study. There were inverse associations between the amount of breast milk received in the first 40 days of life and LDL/HDL ratio in adolescence. There were no significant associations with either total, LDL, or HDL cholesterol levels. Therefore, despite the fact that the study was designed as a randomised trial, there are methodological issues that may affect the interpretation of their results.

Our cohort subjects grew up in a developing country; 9.1% presented a low birth weight, and 12.2% at about 2 years of age. Two other studies, from England and Holland, reported on cohorts that had faced food shortages during wartime. Ravelli *et al*[9] found a lower LDL/HDL ratio among subjects who had been breast fed, while Fall *et al*[10] found that breast feeding duration was inversely related to LDL. We searched for interactions between breast feeding and several other variables related to growth (preterm status, intrauterine growth restriction, growth velocity in the first two years of life, stunting at the age of 2 years), and to socioeconomic status, but there was no evidence that these variables modified the effect of breast feeding (or lack thereof) on lipid levels.

Our results suggest that breast feeding does not contribute to lower blood lipid levels in this sample of Brazilian adolescents.

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Competing interests: none.

Ethical approval: the Brazilian Medical Research Council approved the study protocol.

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