# Breastfeeding and late adolescent lipid sub-fraction: a Hong Kong birth cohort study (abridged secondary publication)

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**KEY MESSAGES**

1. Apolipoprotein (Apo) B is a key lipid in cardiovascular disease.
2. In a population-representative Chinese birth cohort (n=3462), a history of exclusive breastfeeding in the first 3 months of life was associated with lower ApoB at the age of 17.6 years.
3. Waist-hip ratio may partly mediate the association between breastfeeding and ApoB.
4. Whether some effects of breastfeeding are only actuated at the completion of growth (puberty) should be investigated.

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## Introduction

Associations of infant formula feeding with early life adiposity have not been confirmed in randomised trials or in observational studies from regions where breastfeeding is not associated with higher socioeconomic position.[1] The association of formula feeding with early life adiposity may be related to lipids.[2] In a randomised controlled trial of individuals born prematurely, apolipoprotein (Apo) B at a mean age of 15 years was higher in those fed pre-term formula as babies than those fed banked breast milk as babies; however, a history of formula feeding did not affect high-density lipoprotein-cholesterol (HDL-c), triglycerides, or ApoA-1.[3] Nonetheless, the trial was small and its follow-up rate was low (23%). ApoB (rather than low-density lipoprotein-cholesterol or triglycerides) is now thought to be a major lipid that contributes to ischaemic heart disease.[4]

To determine the long-term effects of breastfeeding on ApoB and hence its role in cardiovascular disease and mortality, we assessed the associations of breastfeeding with ApoB, triglycerides, and HDL-c in the Hong Kong 'Children of 1997' population-representative birth cohort in late adolescence. Considering differences in cardiovascular disease rates by sex, we also assessed whether associations varied by sex. Finally, we explored the possibility of mediation by adiposity, which could serve as a potential intervention target.

## Methods

The Hong Kong 'Children of 1997' birth cohort is a population-representative cohort (n=8327) comprising 88% of births in Hong Kong during April and May 1997. Participants were recruited at all the 49 Maternal and Child Health Centres; these provide universal routine checks and vaccinations for infants and toddlers in Hong Kong. A self-administered questionnaire in Chinese was used to collect baseline information on birth and family characteristics, second-hand smoking, and infant feeding at the first visit after birth. A similar questionnaire was administered at subsequent routine visits during 1997-98 (ages 3, 9, and 18 months). Additional information was collected via record linkage to routine health checks.[1] In 2007, contact was re-established and three telephone/postal surveys were administered; a Biobank Clinical follow-up (phase 1) was conducted in 2013-16 (mean age, 17.5 years), followed by a supplementary Biobank Clinical follow-up (phase 2) in the second half of 2017 (mean age, 19.5 years). Blood samples were collected after an overnight fast and stored at -80°C. After each phase of data collection, comparisons were made with previously collected data and anomalies resolved.

Breastfeeding status during the first 3 months of life was categorised as never breastfed, mixed feeding, or exclusively breastfed. Automated nuclear magnetic resonance spectroscopy was used to obtain ApoB, HDL-c, and triglycerides measurements from serum stored at -80°C; measurements that failed quality control criteria were excluded.

Multivariable linear regression was used to assess the associations of breastfeeding with ApoB and other lipids, after adjusting for potential confounders and age at follow-up. To obtain P-for-trend values regarding the association of

breastfeeding with ApoB, the three breastfeeding categories were presented as a continuous variable (0, 1, and 2). Sex-specific estimates were compared using z-tests. Pearl's mediation formula was used to assess mediation.

Multiple imputation and inverse probability weighting were used to minimise potential biases related to missing information and any differences between respondents and non-respondents. Missing values of breastfeeding and confounders were predicted 50 times with a flexible additive regression model. Multivariable logistic regression showed that breastfeeding, gestational age, maternal age, maternal birthplace, highest parental education, the interaction of highest parental education and maternal birthplace, and possibly sex were associated with non-response to the Biobank Clinical follow-up and thus were missing ApoB values. Logistic regression was used to calculate the probability that an observation would be present. Finally, we analysed the 50 complete datasets separately, considering the probability of inclusion, then summarised the results using single estimated beta coefficients with confidence intervals adjusted for missing-data uncertainty.

## Results

Of the 8327 individuals originally included in the 'Children of 1997' birth cohort, 28 had permanently withdrawn and 33 had died before the Biobank Clinical follow-up. Of the remaining 8266, 6850 were potentially contactable for the Biobank Clinical follow-up. After repeated attempts, 3460 and 158 participants attended phase 1 and phase 2 of the Biobank Clinical follow-up, respectively. Of these participants, 3462 had valid ApoB measurements (1688 girls and 1774 boys). Mean ApoB was higher in girls than in boys (0.75 vs 0.73 g/L). ApoB was strongly associated with age at follow-up in boys (0.03 g/L higher per 1 year older, 95% confidence interval=0.02-0.04, P=3.1e-12) but not in girls (0.007 g/L higher per 1 year older, 95% confidence interval= −0.001 to 0.015, P=0.13).

Participants did not differ from the original cohort with respect to socioeconomic position at birth (ie, parental income and highest parental education), parity, type of delivery, or birth weight. However, they differed in terms of maternal birthplace because some of the participants were born to migrants in Hong Kong who intended to return to China; accordingly, there were differences in breastfeeding status, maternal age, gestational age, highest parental occupation, and maternal second-hand smoking (SHS)/smoking.

During the first 3 months of life, 1811 participants were never breastfed, 1341 participants had mixed feeding, and 256 participants were exclusively breastfed; 54 participants had unknown breastfeeding status. A history of breastfeeding was not associated with birth weight, gestational age, maternal age, or age at follow-up; however, it was associated with female sex, higher parity, maternal birth outside Hong Kong, lower parental socioeconomic position, no maternal SHS/smoking, and vaginal delivery. Notably, ApoB was not associated with any other potential confounders apart from sex.

A history of exclusive breastfeeding in the first 3 months of life was associated with lower ApoB, with similar estimates by sex (Table 1). A history of breastfeeding was not associated with HDL-c or triglycerides, with similar estimates by sex (Table 2). In terms of potential mediators, a history of breastfeeding was associated with higher body mass index z-score at 3 months and a lower waist-hip ratio at 17.6 years but not with body mass index at 12 years or 17.6 years or with total fat percentage at 17.6 years. The association of breastfeeding with ApoB was slightly attenuated after adjusting for mediation by waist-hip ratio; the proportion of mediation was approximately 10%, according to causal mediation analysis.

## Discussion

Consistent with the previous finding that in premature babies a history of breastfeeding was associated with lower ApoB at the age of 15 years,[3] our study showed a similar association in older adolescents (~17.6 years) but no association of breastfeeding

TABLE 1. Associations of breastfeeding in first 3 months of life with nuclear magnetic resonance spectroscopy–assessed apolipoprotein B at 17.6 years in the Hong Kong 'Children of 1997' birth cohort after adjusting for potential confounders*

| Sex | Beta (95% confidence interval) | P value | P-for-trend | P value for sex difference |
|---|---|---|---|---|
| Overall | | | | |
| Never breastfed | Reference | | | |
| Mixed feeding | -0.005 (-0.016 to 0.005) | 0.33 | | |
| Exclusively breastfed | -0.027 (-0.046 to -0.007) | 0.007 | 0.016 | 0.63 |
| Boys | | | | |
| Never breastfed | Reference | | | |
| Mixed feeding | -0.005 (-0.020 to 0.009) | 0.48 | | |
| Exclusively breastfed | -0.021 (-0.051 to 0.008) | 0.15 | 0.17 | |
| Girls | | | | |
| Never breastfed | Reference | | | |
| Mixed feeding | -0.007 (-0.022 to 0.009) | 0.40 | | |
| Exclusively breastfed | -0.031 (-0.057 to -0.004) | 0.02 | 0.037 | |

* Sex, birth weight, gestational age, maternal age, maternal birthplace, highest parental education, parental income, parental occupation, maternal second-hand smoking/smoking, age at the biobank clinical follow-up, interaction of maternal birthplace and highest parental education, and interaction of age at follow-up and sex

TABLE 2. Associations of breastfeeding in first 3 months of life with nuclear magnetic resonance spectroscopy–assessed high-density lipoprotein-cholesterol (HDL-c) and triglycerides at 17.6 years in the Hong Kong 'Children of 1997' birth cohort after adjusting for potential confounders*

| Lipids | Beta (95% confidence interval) | P value | P-for-trend | P value for sex difference |
|---|---|---|---|---|
| HDL-c | | | | |
| Never breastfed | Reference | | | |
| Mixed feeding | -0.022 (-0.046 to 0.002) | 0.07 | | |
| Exclusively breastfed | -0.018 (-0.062 to 0.026) | 0.43 | 0.11 | 0.56 |
| Triglycerides | | | | |
| Never breastfed | Reference | | | |
| Mixed feeding | -0.016 (-0.045 to 0.012) | 0.26 | | |
| Exclusively breastfed | -0.037 (-0.090 to 0.016) | 0.17 | 0.11 | 0.53 |

* Sex, birth weight, gestational age, maternal age, maternal birthplace, highest parental education, parental income, parental occupation, maternal second-hand smoking/smoking, age at the biobank clinical follow-up, interaction of maternal birthplace and highest parental education, and interaction of age at follow-up and sex

with HDL-c or triglycerides in individuals who had largely been born at term. The associations were similar by sex, and some mediation by current waist-hip ratio cannot be excluded.

It is unclear how breastfeeding might affect ApoB. One possible explanation is that breastfeeding contributes to nutritional programming in early life, but the effects are only actuated at the completion of growth (ie, in late adolescence). Breastfeeding largely coincides with the mini-puberty of infancy, a developmental window with effects on fertility that only become evident during or after puberty; it is unclear whether breastfeeding modulates mini-puberty or its effects in later life.

The present study had some limitations. First, similar to most long-running cohort studies, attrition and missing data occurred. We used multiple imputation and inverse probability weighting to address these issues. Second, breastfeeding status was assessed using a questionnaire, so misclassification is possible. However, misclassification is unlikely to influence ApoB many years later; thus, we expect a bias towards the absence of an effect. Third, this study was not designed to study the effects of long-term (>3 months) breastfeeding, which was uncommon in Hong Kong in 1997.

The results of this study support breastfeeding, considering its short-term benefits and potential lifelong benefits. It would be useful to explore how breastfeeding duration affects ApoB (ie, whether >6 months of breastfeeding is more beneficial than 3 months of breastfeeding); this information could have major implications for mothers' lives and maternity policies. A follow-up study examining the associations of breastfeeding with hormones would be timely and relevant, particularly because there is increasing evidence that hormones affect cardiovascular disease[5] and hormone-related cancers.

## Conclusion

A history of breastfeeding was associated with lower ApoB, but not with triglycerides or HDL-c, in older adolescents; these associations were similar by sex. Breastfeeding may have important beneficial effects on the key lipid contributing to cardiovascular disease and longevity.

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## Disclosure

The results of this research have been previously published in:

1. Schooling CM, Au Yeung SL, Kwok MK, Leung GM. Breastfeeding and ApoB in late adolescence: a Hong Kong birth cohort study. Eur J Pediatr 2023 Jun 8.

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