BREAST MILK CONSUMPTION IN PRETERM NEONATES AND CARDIAC SHAPE IN ADULTHOOD

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Short title: Breast milk consumption and cardiac shape

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Abbreviations: EHM = Exclusively Human Milk; EFF = Exclusively Fed Formula

What's Known on This Subject: Preterm-born young adults exhibit an adverse cardiac morphology and function. The preterm postnatal period is a key developmental window during which cardiac changes emerge, though it is not known whether postnatal nutrition is relevant to long-term cardiac structure and function.

What This Study Adds: This study provides the first evidence of a beneficial association between postnatal breast milk consumption and cardiac morphology in adulthood in preterm-born individuals. These findings support promotion of human milk for preterm infant care to reduce long-term cardiovascular risk.

Contributor's Statement: Professor Lucas conceptualized and designed the initial milk feeding trial. Dr Lewandowski, Professor Neubauer and Professor Leeson conceptualized and designed the follow-up study.

Dr Lewandowski drafted the initial manuscript, and approved the final manuscript as submitted.

Dr Lewandowski, Dr Lamata, Mrs Francis, Dr Piechnik, Dr Ferreira and Dr Boardman carried out data collection and analysis. The data was interpreted with input from all co-authors.

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ABSTRACT

Background and Objectives– Preterm birth relates to long-term alterations in cardiac morphology and function. Understanding whether preterm postnatal life is a tractable period of cardiovascular development that can be positively altered by nutrition is relevant to long term outcomes. We hypothesized that being fed human breastmilk during early postnatal life is beneficial to long-term cardiac structure and function in preterm-born individuals compared to infant formulas.

Methods– 926 preterm-born infants originally took part in a randomized controlled trial of postnatal milk feeding regimes between 1982-1985 across five different UK centres. Preterm-born individuals were randomly assigned to either breastmilk donated by unrelated lactating women or nutrient-enriched formulae. We followed up 102 individuals from this cohort; 30 of which had been randomized to being fed exclusively human milk and 16 to being fed exclusively formula. As a comparison group, we recruited an additional 102 individuals born term to uncomplicated pregnancies. Cardiac morphology and function were assessed by magnetic resonance imaging.

Results- Preterm-born individuals fed exclusively human milk as infants had increased left and right ventricular end-diastolic volume index (+9.73%, P=0.04 and +18.2%, P<0.001), as well as left and right ventricular stroke volume index (+9.79%, P=0.05 and +22.1%, P=0.01) compared to preterm-born individuals who were exclusively formula fed as infants.

Conclusions– This study provides the first evidence of a beneficial association between breast milk and cardiac morphology and function in adult life in those born preterm and supports promotion of human milk for the care of preterm infants to reduce long-term cardiovascular risk.

Keywords: Preterm birth, cardiac remodeling, infant nutrition, breastmilk, prematurity

INTRODUCTION

Increased survival of preterm babies has resulted in growing numbers now reaching adulthood. Recently we demonstrated these young adults exhibit a unique, adverse cardiac morphology and function, with reduced ventricular volumes and function and disproportionately increased mass.[1,2] Experimental models implicate the early postnatal period as a key developmental window during which cardiac changes emerge. Preterm birth and the subsequent ex utero environment result in abnormal myocardial maturation, hypertrophy and fibrosis.[3,4] We therefore hypothesized different nutritional exposures during this period might modify preterm cardiac development. In particular, human milk feeding has been shown to preferentially influence the cardiovascular phenotype, which may in part relate to bioactive factors in breastmilk.[5-7] Therefore, we reanalyzed our data on cardiac morphology and function to take into account exposure to different milk feeding regimes.

METHODS

Participants

The study participants were recruited from a cohort of 926 preterm-born infants who participated in a randomised controlled trial, initially investigating the effects of early diet on later cognitive function (Figure 1). Details of the trial have been published comprehensively elsewhere,[8] but briefly, babies born between 1982 and 1985 with no major congenital anomalies and of birthweight less than 1850g were recruited in five UK centres. They were randomly assigned to either breastmilk donated by unrelated lactating women, nutrient-enriched preterm formula (Farley's Osterprem) or standard term formula (Farley's Ostermilk). Within each trial the diets were randomly assigned in two strata: the trial diets alone (A) and, in mothers who elected to express their own milk, the trial diets were assigned as supplements to mother's milk (B; Figure 1). The composition of the assigned diets has

previously been described.[8,9] Follow-up studies were designed to test the hypothesis that early diet influences risk factors for cardiovascular disease.[10]

102 of these subjects, aged between 23 and 28 years, were able to attend an appointment in Oxford for detailed cardiovascular phenotyping, including cardiac magnetic resonance imaging (Figure 1).[1,2] Of these, 30 individuals had been randomized to exclusively human milk (EHM) in the initial feeding trial, while the remaining 62 were randomized to either term formula or preterm formula. As we wished to only study those individuals that received the assigned milk as sole diet, 16 of the 62 were included as being exclusively fed formula (EFF). We recruited 102 young adults born term to uncomplicated pregnancies with age and sex distributions similar to those of the preterm-born young adults via advertisement to undergo identical investigations. All data was coded with subject and study-specific IDs to ensure anonymity and blinded analysis. The follow-up study was registered with ClinicalTrials.gov (NCT01487824) and the protocol and recruitment strategy have previously been reported.[1,2,11-13] The study was approved by the relevant ethics committee (Oxfordshire Research Ethics Committee A: 06/Q1604/118) and all participants provided signed informed consent.

Follow-up visit in young adulthood

Anthropometry, blood pressure, blood samples and lifestyle questionnaire- Subjects attended the Oxford Cardiovascular Clinical Research Facility in the morning following a 12-hour overnight fast. Anthropometric data, blood pressure measures and blood sample collection were done as previously described.[1,2,11-13] Data on medical history, smoking, parental medical history and lifestyle were obtained using a validated questionnaire.[14]

Cardiovascular magnetic resonance- Cardiovascular magnetic resonance was performed on a 1.5T Siemens Sonata scanner.[1,2] Steady-state free precession (SSFP) cine sequences were used to acquire localization images followed by optimized left ventricular horizontal and vertical long-axis cines. From these, a left ventricular short-axis cine stack was obtained with standardized basal slice alignment with a 7mm slice thickness and 3mm inter-slice gap. The cine images were stored on a digital archive for post-processing, which was undertaken as detailed below. We also acquired ShMOLLI T1 maps using previously described methods[15] and assessed thoracic dimensions and pulmonary artery diameters[16] using our localizer images (see 'Online Supplement').

Quantification of cardiac geometry and mass

Image analysis for right and left ventricular volumes, mass and dimensions was performed offline using Argus (Siemens Medical Solutions, Germany) as previously described.[1,2] Briefly, left and right ventricular short-axis epicardial and endocardial borders were manually contoured for each slice at end-diastole and endocardial borders at end-systole to allow automated calculation of right and left ventricular mass and volumes, indexed to body surface area. Left ventricular internal cavity diameters were measured on the mid-ventricular short-axis slice at end-diastole while left ventricular lengths and right ventricular diameters were measured on the horizontal long-axis cines.

Assessment of left ventricular geometry

We have previously created left ventricular statistical meshes for this population using novel, in-house methods,[17,18] which have been made available to the scientific community at: amdb.isd.kcl.ac.uk.[19] Principal component analysis was undertaken to identify the key modes of variation of the shape between groups.

Statistical analysis

Statistical analysis was carried out using SPSS Version 22. Normality of variables was assessed by visual assessment of normality curves and Shapiro-Wilk test. Comparison between groups for continuous variables was performed using a two-sided, independent-samples Student's t-test. For categorical variables, comparison was done using a Chi-Square test. Pearson correlations (r) were used for bivariate associations and unstandardized regression coefficients (B) were used for bivariable and multivariable linear regression models. Results are presented as mean±standard deviation. *P*-values were adjusted using the Bonferroni method by multiplying the respective unadjusted *P*-values by three (the number of pairwise comparisons when there are three groups) where multiple comparisons were performed between groups. Comparisons between groups were also adjusted for age and sex. *P*-values <0.05 were considered statistically significant.

RESULTS

Characteristics of cohorts

There were no significant differences between preterm-born young adults who had been fed EHM as infants compared to those exclusively fed EFF in perinatal characteristics (Table S1) or anthropometrics, demographics, blood biochemistry, or blood pressure in young adulthood (Table 1). However, both groups had altered blood biochemistry profiles and increased blood pressures compared to young adults born term. There were no significant differences in number of smokers, personal and family medical history, or lifestyle factors such as socioeconomic status, physical activity or diet (*P*>0.05) between the two milk feeding preterm groups, nor were there any differences between these groups compared to the control groups of adults born at term to uncomplicated pregnancies (Table 1 and Table S2).

Left ventricular size and function

Preterm-born individuals fed EHM as infants have an increased mean left ventricular end-diastolic volume index (+9.73%; 73.3±7.6 vs 66.8±6.7mL/m^2, *P*=0.04) and left ventricular stroke volume index (+9.79%; 47.1±5.9 vs 42.9±6.3mL/m^2, *P*=0.05) compared to preterm-born individuals who were fed EFF as infants (Figure 2 and Table 2). Although both feeding groups still had significantly smaller left ventricular end-diastolic volume index and stroke volume index than adults born term, the reduction in the group fed EHM was only around 9% compared to 18% in the group fed EFF for both left ventricular end-diastolic volume index and stroke volume index. Both preterm-born groups had similar left ventricular mass index and ejection fraction (*P*>0.99), though the preterm group fed EFF had significantly shorter left ventricles (*P*=0.05) with reduced left ventricular luminal diameters (*P*=0.009). The range of values for the left ventricular parameters in the term-born controls are similar to normal ranges previously published using cardiovascular magnetic resonance.[20]

Left ventricular shape and native T1 imaging

Shape analysis confirmed the differences in size observed using standard metrics (Figure 3), which particularly highlighted the significant increase in left ventricular length (*P*<0.001) in the EHM group compared to the preterms fed EFF. Importantly, there were no other major geometric variations identified between preterm-born individuals fed EHM and those fed EFF (Figure 3) or term-born adults. We did not observe significant differences in myocardial tissue characterization by T1 mapping. Preterm-born young adults fed EFF had similar native T1 values to those fed EHM (961.0±43.1 vs 959.0±27.3ms, *P*>0.99), and term-born young adults (969.1±24.7ms, *P*=0.26 and *P*=0.51, respectively), in range of the normal population values.[15]

Right ventricular size and function

Interestingly, the percentage differences between preterm-born young adults who had been fed EHM as infants compared to those fed EFF were much greater for right ventricular end-diastolic volume index (+18.2%, $P<0.001$) and right ventricular stroke volume index (+22.1%, $P=0.01$). Furthermore, while there was only a 5.42% reduction in right ventricular end-diastolic volume index and 8.13% in right ventricular stroke volume index in preterm-born young adults fed EHM compared to term-born controls, preterm-born young adults fed EFF showed substantially greater reductions in these cardiac parameters when compared to term-born controls (25.0% and 24.8%, respectively).

Right ventricular mass index and length were similar between preterm groups (Table 2). However, preterm-born young adults fed EHM had similar right ventricular ejection fractions to term-born young adults ($P=0.31$), while preterm-born young adults fed EFF showed reduced right ventricular ejection fractions compared to term-born controls ($P<0.001$). The range of values for the right ventricular parameters in the term-born controls are similar to normal ranges previously published using cardiovascular magnetic resonance.[20]

Predictors of cardiac changes

We have previously demonstrated that gestational age is the strongest predictor of left and right ventricular end-diastolic volumes and stroke volumes.[1,2] To test whether being fed EHM was also an independent predictor of these cardiac parameters in preterm-born individuals, we created separate multivariable regression models for each of our four major cardiac changes and included those fed EFF or EHM postnatally. In multivariable models including gestational age, sex, postnatal milk feeding group, and postnatal weight gain, gestational age remained an independent predictor for all four cardiac measures ($P<0.001$). However, being

fed EHM was also an independent predictor of left ventricular end-diastolic volume index (P=0.007), left ventricular stroke volume index (P=0.03), right ventricular end-diastolic volume index (P=0.002) and right ventricular stroke volume index (P=0.004). In line with the changes between preterm feeding groups being greater for the right ventricle, in a multivariable regression model across the entire preterm group (n=102) with gestational age, sex, percentage of human milk in the diet, and postnatal weight gain as the predictors, the percentage of human milk was positively related to both right ventricular end-diastolic volume index (P=0.009) and stroke volume index (P=0.04), but not left ventricular parameters. There were no correlations between percentage of expressed milk and cardiac measures within the group fed EHM.

Thoracic cavity size and pulmonary artery diameters

Preterm-born young adults fed EFF had reduced thoracic cavity dimensions compared to those fed EHM (Table S3). While there were no differences between preterm-born young adults fed EFF vs EHM in aortic diameters (2.35±0.30 vs 2.42±0.22cm, P=0.36), those in the EFF group had increased main pulmonary artery diameters (2.41±0.21 vs 2.16±0.23cm, P=0.002) and pulmonary artery to aortic diameter ratios (1.04±0.12 vs 0.89±0.07, P<0.001). Furthermore, pulmonary artery to aortic diameter ratios were inversely related to right ventricular end-diastolic volume index and stroke volume index (r=-0.41, P=-0.005 and r=-0.40, P=0.007, respectively) but not left ventricular parameters.

DISCUSSION

We provide the first evidence of an association between early postnatal nutrition in preterm-born infants and cardiac structure and function in later life. Left and right ventricular end-

diastolic and stroke volumes in the EHM group approached values seen in term-born controls with particularly striking findings for the right ventricle.

Breastmilk contains a greater bioactivity and bioavailability of a number of growth factors, enzymes and antibodies compared to even the best infant formulas.[21,22] These factors are relevant to normal growth and development, as well as improving preterm infant health. While the content of preterm formulas in particular have been modified to better meet the needs of the growing preterm infant since the 1980s, such as the inclusion of polyunsaturated fatty acids,[23] the American Academy of Pediatrics continues to recommend that preterm infants be given pasteurized donor milk rather than preterm formula if a mother is unable to provide adequate breastmilk volume.[24] The benefits of human breastmilk have also been demonstrated to extend to other parts of the cardiovascular system. Human milk intake in preterm infants shows a beneficial association with proximal cerebral arterial vessel tortuosity: a marker of cerebrovascular development that is reduced in preterm infants.[5] Breastmilk may act through similar pathways to protect vascular and cardiac development as it contains essential growth factors, such as vascular endothelial growth factor, which are of particular benefit to early stages of vasculogenesis and angiogenesis in preterm infants.[25] Altered vasculogenesis and angiogenesis in preterms has been shown as early as fetal life and extends into adulthood,[13,26] and is an important contributor to poor lung development in preterm infants as it plays a pivotal role in alveolarization.[27] It is therefore possible that, due to the interdependence of the lungs and the right ventricle, the reason we see greater benefits in right ventricular volumes and function in preterm-born young adults who were fed EHM postnatally as compared to the left ventricle is due to essential vascular growth factors contained in breastmilk that benefit lung function and development.[28] Though we did not collect lung function data in this cohort, the slight reduction in thoracic cavity dimensions

and the increase in pulmonary artery size in the group fed EFF supports this notion, as does the close relationship between pulmonary artery dimensions and right ventricular morphology. The data suggests that breastmilk may reduce the risk of pulmonary arterial hypertension and future cardiopulmonary disease in preterm-born individuals.[16]

As our study participants were still relatively young, continued follow-up will be necessary to assess clinical outcome as they reach later adulthood. Data on the relevance of cardiac morphology and function to disease development are sparse in young populations. Nevertheless, reductions in end-diastolic volume and stroke volume, which were much greater in the group fed EFF, lead to proportional changes in maximal exercise capacity;[29] an independent predictor of cardiovascular morbidity and mortality.[30,31] Furthermore, the reductions in right ventricular systolic function in the group fed EFF are of independent and additive prognostic value in chronic heart failure and are powerful predictors of mortality in left heart failure,[32] and may therefore directly contribute to the onset of clinical heart failure.[33] We have not performed LGE imaging in this cohort to assess for scar burden. However, native T1 values were within the normal range,[15] with no statistical differences between the groups studied. Given the lack of significantly elevated T1 values typically seen in areas of infarction[34,35] and the lack of regional wall motion abnormalities, it is unlikely that large areas of focal scarring are missed; although small areas of scarring cannot be ruled out with absolute certainty, this is unlikely to alter the main message of the manuscript.

Use of our computational atlas allowed us to confirm that cardiac differences between groups were proportional size changes, rather than alterations in geometric dimensions. These findings suggest the changes in cardiac morphology observed in preterm-born individuals relate primarily to size rather than geometric alterations, and that breastmilk leads to

proportional normalization of size. Interestingly, fetal growth restricted offspring, who have more spherical hearts in fetal and postnatal life, also showed benefits of human breastmilk, with longer breastfeeding durations relating to normalization of left ventricular sphericity.[7] While breastmilk appears to have differing effects in these offspring, the findings support the necessity of breastmilk as part of early nutrition for individuals born to pregnancy complications as a protective mechanism to cardiovascular development.

Our finding that gestational age remained a significant, independent predictor of cardiac parameters when milk-feeding diets were taken into account is in line with our previous results.[1,2] While our data suggest that is in not possible to reverse all cardiac changes related to the degree of prematurity through breastmilk feeding, the magnitude of the differences between preterm-born adults fed EHM and those fed EFF is substantial and occurs independent of gestational age. Our finding that percentage of human milk consumed was positively associated to right ventricular morphology and function suggests that even small amounts of human milk in the preterm postnatal diet may be beneficial. Understanding the benefits and optimum duration of breastmilk feeding on cardiac remodeling across a large range of gestational ages of preterm-born individuals will be of benefit to better understand feeding approaches for different gestational age categories of preterm birth.

Despite the magnitude of the cardiac differences between milk feeding groups, this study does not prove causality. Firstly, the number of participants available for comparison is relatively small compared to the size of the original cohort. Due to loss of follow-up and the long-term prospective nature of our study, the study was not fully randomized, and as such, there is risk of ascertainment bias. It is therefore possible that other associated factors in the perinatal period account for a proportion of the differences we have identified, such as

genetic risk, epigenetic changes, and environmental factors.[36,37] However, our preterm groups were similar in baseline demographics and demographic characteristics in young adulthood. Furthermore, unlike other studies that often rely on questionnaires and recall,[7] we can be certain of the exact dietary intake of our preterm-born individuals during early postnatal life. It is possible that mothers who elect to breastfeed may be fundamentally different from mothers who do not. However, we found no evidence of this in our group fed EHM, suggesting that donor milk provides a similar level of benefit as the expressed breastmilk from their mothers. The randomized preterm population was previously followed up at age 13-16 years,[6] and in that study breastmilk consumption had a beneficial impact on blood pressure in adolescents born preterm resulting in a 4mmHg lower mean arterial pressure. Our group was smaller and, as a result, our analysis was not powered to identify this size of difference in blood pressure, although was still able to identify the proportionally larger variation in cardiac morphology associated with breastmilk consumption. Interestingly, the similar blood pressures and metabolic profiles between groups does support the concept that the differences between EHM and EFF preterm-born young adults occurs independently of other cardiovascular risk factors. Longer-term follow-up will be required to understand the true impact of this variation on cardiovascular risk.

CONCLUSION

In summary, we provide the first evidence of a beneficial association between breastmilk and cardiac morphology in adult life in those born preterm. The findings implicate early preterm postnatal life as a potentially tractable period of cardiovascular development, relevant to long-term outcomes, and support promotion of human milk for the care of preterm infants to reduce long-term cardiovascular risk.

REFERENCES

1. Lewandowski AJ, Augustine D, Lamata P, et al. Preterm Heart in Adult Life: Cardiovascular Magnetic Resonance Reveals Distinct Differences in Left Ventricular Mass, Geometry and Function. *Circulation* 2013; **127**(2): 197-206.
2. Lewandowski AJ, Bradlow WM, Augustine D, et al. Right ventricular systolic dysfunction in young adults born preterm. *Circulation* 2013; **128**(7): 713-20.
3. Bertagnolli M, Huyard F, Cloutier A, et al. Transient neonatal high oxygen exposure leads to early adult cardiac dysfunction, remodeling, and activation of the renin-angiotensin system. *Hypertension* 2014; **63**(1): 143-50.
4. Bertagnolli M, Luu TM, Lewandowski AJ, Leeson P, Nuyt AM. Preterm Birth and Hypertension: Is There a Link? *Current Hypertension Reports* 2016; **18**(4): 1-8.
5. Vasu V, Durighel G, Thomas L, et al. Preterm nutritional intake and MRI phenotype at term age: a prospective observational study. *BMJ Open* 2014; **4**(5): e005390.
6. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *The Lancet* 2001; **357**(9254): 413-9.
7. Rodriguez-Lopez M, Osorio L, Acosta-Rojas R, et al. Influence of breastfeeding and postnatal nutrition on cardiovascular remodeling induced by fetal growth restriction. *Pediatric Research* 2015: [Epub ahead of print].
8. Lucas A, Gore SM, Cole TJ, et al. Multicentre trial on feeding low birthweight infants: effects of diet on early growth. *Archives of Disease in Childhood* 1984; **59**(8): 722-30.
9. Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. *British Medical Journal* 1998; **317**(7171): 1481-7.
10. Lucas A, Morley R. Does early nutrition in infants born before term programme later blood pressure? *British Medical Journal* 1994; **309**(6950): 304-9.
11. Kelly BA, Lewandowski AJ, Worton SA, et al. Antenatal Glucocorticoid Exposure and Long-Term Alterations in Aortic Function and Glucose Metabolism. *Pediatrics* 2012; **129**(5): e1282-e90.
12. Lewandowski AJ, Lazdam M, Davis E, et al. Short-Term Exposure to Exogenous Lipids in Premature Infants and Long-Term Changes in Aortic and Cardiac Function. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2011; **31**(9): 2125 - 35.
13. Lewandowski AJ, Davis EF, Yu G, et al. Elevated Blood Pressure in Preterm-Born Offspring Associates With a Distinct Antiangiogenic State and Microvascular Abnormalities in Adult Life. *Hypertension* 2015; **65**(3): 607-14.
14. Leeson CPM, Kattenhorn M, Morley R, Lucas A, Deanfield JE. Impact of Low Birth Weight and Cardiovascular Risk Factors on Endothelial Function in Early Adult Life. *Circulation* 2001; **103**(9): 1264-8.
15. Piechnik SK, Ferreira VM, Lewandowski AJ, et al. Normal variation of magnetic resonance T1 relaxation times in the human population at 1.5 T using ShMOLLI. *Journal of Cardiovascular Magnetic Resonance* 2013; **15**(1): 1-11.
16. Truong QA, Massaro JM, Rogers IS, et al. Reference Values for Normal Pulmonary Artery Dimensions by Noncontrast Cardiac Computed Tomography: The Framingham Heart Study. *Circulation: Cardiovascular Imaging* 2012; **5**(1): 147-54.
17. Lamata P, Sinclair M, Kerfoot E, et al. An automatic service for the personalization of ventricular cardiac meshes. *Journal of The Royal Society Interface* 2013; **11**(91).
18. Lamata P, Niederer S, Nordsletten D, et al. An accurate, fast and robust method to generate patient-specific cubic Hermite meshes. *Med Image Anal*; **15**(6): 801-13.

19. Kerfoot E, Lamata P, Niederer S, Hose R, Spaan J, Smith N. Share and enjoy: anatomical models database—generating and sharing cardiovascular model data using web services. *Medical & Biological Engineering & Computing* 2013; **51**(11): 1181-90.
20. Hudsmith LE, Petersen SE, Francis JM, Robson MD, Neubauer S. Normal human left and right ventricular and left atrial dimensions using steady state free precession magnetic resonance imaging. *J Cardiovasc Magn Reson* 2005; **7**(5): 775-82.
21. Ballard O, Morrow AL. Human Milk Composition: Nutrients and Bioactive Factors. *Pediatric clinics of North America* 2013; **60**(1): 49-74.
22. Schanler RJ, Hurst NM, Lau C. The use of human milk and breastfeeding in premature infants. *Clinical Perinatology* 1999; **26**(2): 379-98.
23. Fleith M, Clandinin MT. Dietary PUFA for preterm and term infants: review of clinical studies. *Critical reviews in food science and nutrition* 2005; **45**(3): 205-29.
24. American Academy of Pediatrics SoB. Breastfeeding and the use of human milk. *Pediatrics* 2012; **129**(3): e827-e41.
25. Siafakas CG, Anatolitou F, Fusunyan RD, Walker WA, Sanderson IR. Vascular Endothelial Growth Factor (VEGF) Is Present in Human Breast Milk and Its Receptor Is Present on Intestinal Epithelial Cells. *Pediatric Research* 1999; **45**(S5): 652-7.
26. Ligi I, Simoncini S, Tellier E, et al. A switch toward angiostatic gene expression impairs the angiogenic properties of endothelial progenitor cells in low birth weight preterm infants. *Blood* 2011; **118**(6): 1699-709.
27. Thébaud B, Ladha F, Michelakis ED, et al. Vascular Endothelial Growth Factor Gene Therapy Increases Survival, Promotes Lung Angiogenesis, and Prevents Alveolar Damage in Hyperoxia-Induced Lung Injury: Evidence That Angiogenesis Participates in Alveolarization. *Circulation* 2005; **112**(16): 2477-86.
28. Ogbuanu IU, Karmaus W, Arshad SH, Kurukulaaratchy RJ, Ewart S. Efect of breastfeeding duration on lung function at age 10 years: a prospective birth cohort study. *Thorax* 2009; **64**(1): 62-6.
29. Grewal J, McCully RB, Kane GC, Lam C, Pellikka PA. Left ventricular function and exercise capacity. *The Journal of the American Medical Association* 2009; **301**(3): 286-94.
30. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise Capacity and Mortality among Men Referred for Exercise Testing. *New England Journal of Medicine* 2002; **346**(11): 793-801.
31. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality: A prospective study of healthy men and women. *The Journal of the American Medical Association* 1989; **262**(17): 2395-401.
32. Haddad F, Doyle R, Murphy DJ, Hunt SA. Right Ventricular Function in Cardiovascular Disease, Part II: Pathophysiology, Clinical Importance, and Management of Right Ventricular Failure. *Circulation* 2008; **117**(13): 1717-31.
33. Kawut SM, Barr RG, Lima JAC, et al. Right Ventricular Structure Is Associated With the Risk of Heart Failure and Cardiovascular Death: The Multi-Ethnic Study of Atherosclerosis (MESA)-Right Ventricle Study. *Circulation* 2012; **126**(14): 1681-8.
34. Liu A, Wijesurendra RS, Francis JM, et al. Adenosine Stress and Rest T1 Mapping Can Differentiate Between Ischemic, Infarcted, Remote, and Normal Myocardium Without the Need for Gadolinium Contrast Agents. *JACC: Cardiovascular Imaging* 2016; **9**(1): 27-36.
35. Kali A, Choi E-Y, Sharif B, et al. Native T1 Mapping by 3-T CMR Imaging for Characterization of Chronic Myocardial Infarctions. *JACC: Cardiovascular Imaging* 2015; **8**(9): 1019-30.
36. Lewandowski AJ, Leeson P. Preeclampsia, prematurity and cardiovascular health in adult life. *Early Human Development* 2014; **90**(11): 725-9.

37. Davis EF, Lewandowski AJ, Aye C, et al. Clinical cardiovascular risk during young adulthood in offspring of hypertensive pregnancies: insights from a 20-year prospective follow-up birth cohort. *BMJ Open* 2015; **5**(6).

TABLES

Table 1: Characteristics of Cohorts

	Preterm-born EFF (n=16)	Preterm-born EHM (n=30)	P-Value ψ	Term-born Controls (n=102)	P-Value Δ	P-Value ¥
Demographics & Anthropometrics						
Gestational Age, weeks	29.7±2.5	30.8±2.3	0.22	39.6±0.9	**<0.001**	**<0.001**
Maternal preeclampsia, n (%)	4 (25)	8 (26.7)	>0.99	0 (0)	>0.99	>0.99
Age, years	24.8±1.5	25.4±1.4	0.42	25.0±2.6	>0.99	>0.99
Males, n (%)	7 (43.8)	14 (46.7)	>0.99	47 (46.1)	>0.99	>0.99
Smokers, n (%)	4 (25.0)	8 (26.7)	>0.99	20 (19.6)	>0.99	>0.99
Birthweight, grams	1250.5±309.2	1365.3±257.8	0.41	3460.0±417.0	**<0.001**	**<0.001**
BMI, kg/m^2	24.4±8.1	24.9±4.1	>0.99	22.9±3.1	0.26	0.08
Height, m	1.70±0.09	1.70±0.09	>0.99	1.74±0.09	0.18	0.12
Weight, kg	69.7±16.9	71.5±12.5	>0.99	69.3±12.5	>0.99	>0.99
BSA, m^2	1.81±0.23	1.83±0.18	>0.99	1.83±0.20	>0.99	>0.99
Waist:Hip	0.76±0.06	0.82±0.07	0.38	0.81±0.06	0.19	0.89
Biochemistry						
Total Cholesterol, mmol/L	5.23±0.98	4.69±0.89	0.44	4.23±0.86	**0.003**	**0.04**
HDL-C, mmol/L	1.72±1.5	1.50±0.25	0.65	1.47±0.41	>0.99	>0.99
LDL-C, mmol/L	2.87±1.14	2.76±0.77	>0.99	2.37±0.66	**0.05**	**0.04**
Triglycerides, mmol/L	0.91±0.35	0.93±0.44	>0.99	0.87±0.40	>0.99	>0.99
Glucose, mmol/L	4.81±0.43	5.07±0.41	0.32	4.61±0.30	**0.05**	**0.003**
Insulin, pmol/L	59.8±32.8	61.2±36.9	0.57	35.6±15.9	**<0.001**	**<0.001**
Blood Pressure, mmHg						
Systolic	119.6±11.3	121.0±9.3	>0.99	112.9±10.1	**0.03**	**<0.001**
Diastolic	72.3±4.5	72.8±8.2	>0.99	68.8±7.0	0.14	**0.02**
Mean arterial pressure	88.0±5.7	88.9±7.2	>0.99	83.5±7.1	**0.03**	**<0.001**
Pulse pressure	47.4±8.2	48.2±9.9	>0.99	44.1±8.5	**0.05**	**0.03**

EFF indicates exclusively fed formula postnatally; EHM indicates exclusively fed human breast milk postnatally. Values as Mean±Standard Deviation unless stated otherwise. *P*-values were adjusted using the Bonferroni method for multiple group comparisons (3).

ψ=Preterm-born EHM vs. Preterm-born EFF. Comparisons adjusted for age and sex.
Δ= Preterm-born EFF vs. Term-born Controls. Comparisons adjusted for sex.
¥=Preterm-born EHM vs. Term-born Controls. Comparisons adjusted for sex.

Table 2: Cardiac Parameters

	Preterm-born EFF (n=16)	Preterm-born EHM (n=30)	P-Value ψ	Term-born Controls (n=102)	P-Value Δ	P-Value ¥
Left Ventricle						
End-Diastolic Volume Index (mL/m^2)	66.8±6.7	73.3±7.6	**0.04**	80.2±11.7	**0.003**	**0.004**
End-Systolic Volume Index (mL/m^2)	23.9±5.7	26.3±5.6	>0.99	29.1±6.4	**0.03**	**0.03**
Stroke Volume Index (mL/m^2)	42.9±6.3	47.1±5.9	**0.05**	51.3±8.9	**0.02**	**0.03**
Ejection Fraction (%)	64.3±7.5	64.3±5.7	>0.99	64.1±4.9	>0.99	>0.99
Mass index (g/m^2)	65.9±11.1	66.4±10.7	>0.99	55.4±11.4	**<0.001**	**<0.001**
Mass/End-Diastolic Volume	0.95±0.16	0.91±0.15	0.70	0.70±0.12	**<0.001**	**<0.001**
Length (cm)	8.78±0.47	9.28±0.62	**0.05**	9.81±0.73	**<0.001**	**0.001**
Luminal Diameter (cm)	4.83±0.40	5.34±0.45	**0.009**	5.64±0.48	**<0.001**	**0.001**
Right Ventricle						
End-Diastolic Volume Index (mL/m^2)	70.8±8.5	83.7±9.7	**<0.001**	88.5±11.8	**<0.001**	**0.04**
End-Systolic Volume Index (mL/m^2)	32.0±4.8	35.2±7.1	0.15	35.6±7.7	0.54	>0.99
Stroke Volume Index (mL/m^2)	39.8±7.6	48.6±9.1	**0.01**	52.9±7.2	**<0.001**	**0.02**
Ejection Fraction (%)	54.5±7.1	57.8±7.9	0.15	60.0±5.3	**<0.001**	0.31
Mass index (g/m^2)	24.2±3.7	24.8±3.0	>0.99	20.4±3.4	**<0.001**	**<0.001**
Mass/End-Diastolic Volume	0.35±0.06	0.29±0.05	**0.002**	0.23±0.03	**<0.001**	**<0.001**
Length (cm)	8.06±0.75	8.55±0.69	0.10	8.97±0.76	**<0.001**	**0.001**
Luminal Diameter (cm)	3.92±0.42	4.34±0.41	0.11	4.63±0.55	**<0.001**	**0.003**

EFF indicates exclusively fed formula postnatally; EHM indicates exclusively fed human breast milk postnatally. Values as Mean±Standard Deviation unless stated otherwise. *P*-values were adjusted using the Bonferroni method for multiple group comparisons (3).

ψ=Preterm-born EHM vs. Preterm-born EFF. Comparisons adjusted for age and sex.
Δ= Preterm-born EFF vs. Term-born Controls. Comparisons adjusted for sex.
¥=Preterm-born EHM vs. Term-born Controls. Comparisons adjusted for sex.

FIGURE LEGENDS

Figure 1: Derivation of preterm-born study population followed up at age 23-28 years. A=infants receiving assigned milk as sole diet; B=infants receiving assigned milk as supplement to mother's expressed milk.

Figure 2: Preterm-born young adults fed exclusively human milk as infants (EHM; orange) had increased left and right ventricular end-diastolic volume index (Panels A and C), and left and right ventricular stroke volume index (Panels B and D) compared to preterm-born young adults exclusively fed formula as infants (EFF; blue). Term-born young adults (YAT) are shown in green.

Figure 3: The statistical average shapes of the left ventricle are shown across each group derived from computational atlas formation. Statistical comparisons across the first 10 modes for the preterm-born young adults exclusively fed formula (EFF; blue) and fed exclusively human milk (EHM; orange) are shown, with mode 1 being the key differentiating mode between groups ($P<0.01$). Term-born young adults (YAT) are shown in green.

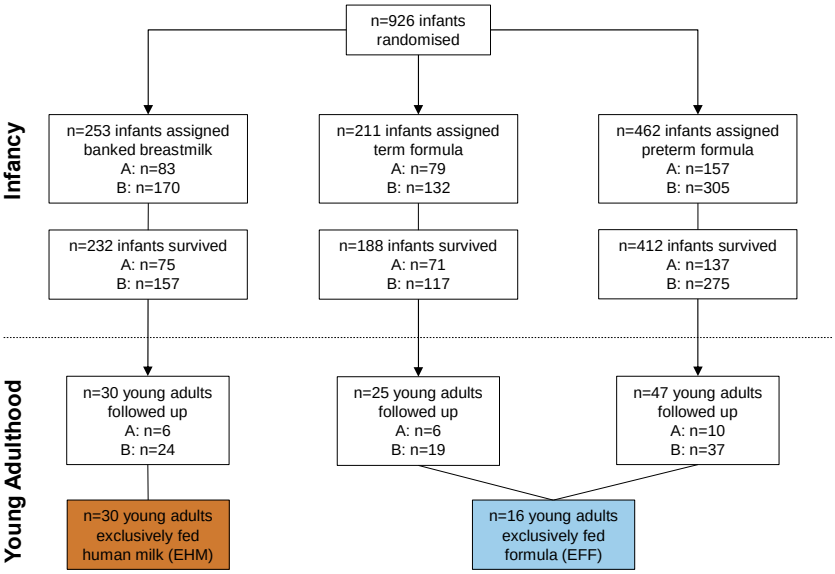

Figure 1

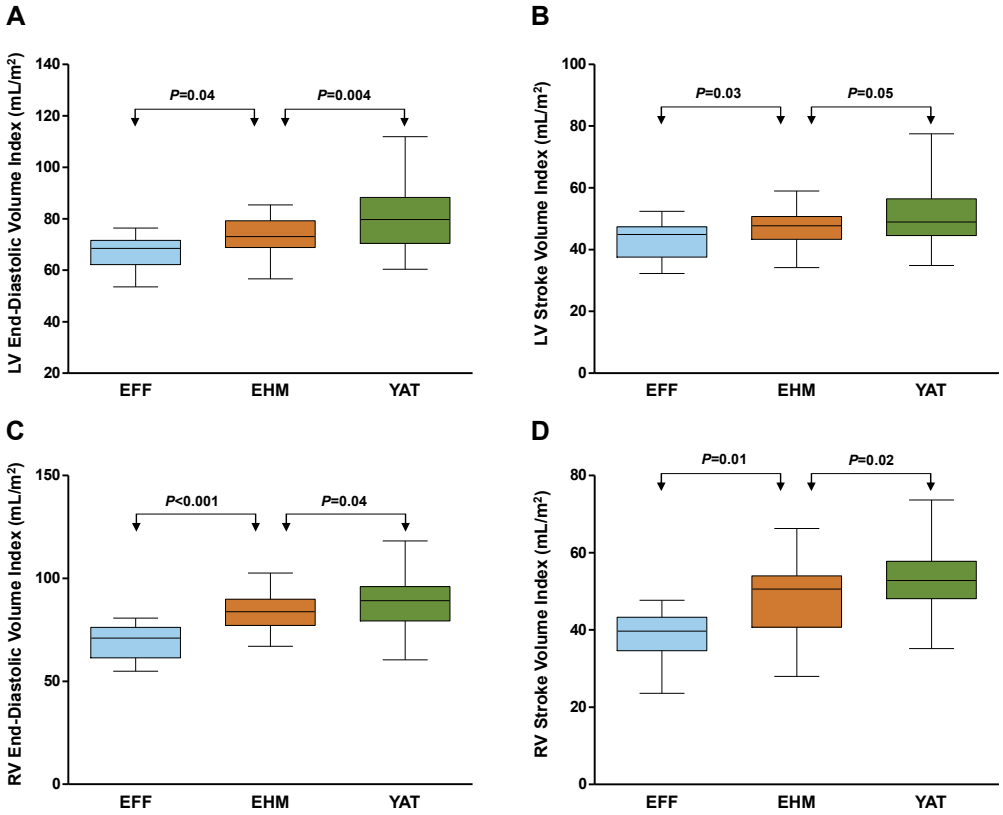

Figure 2!

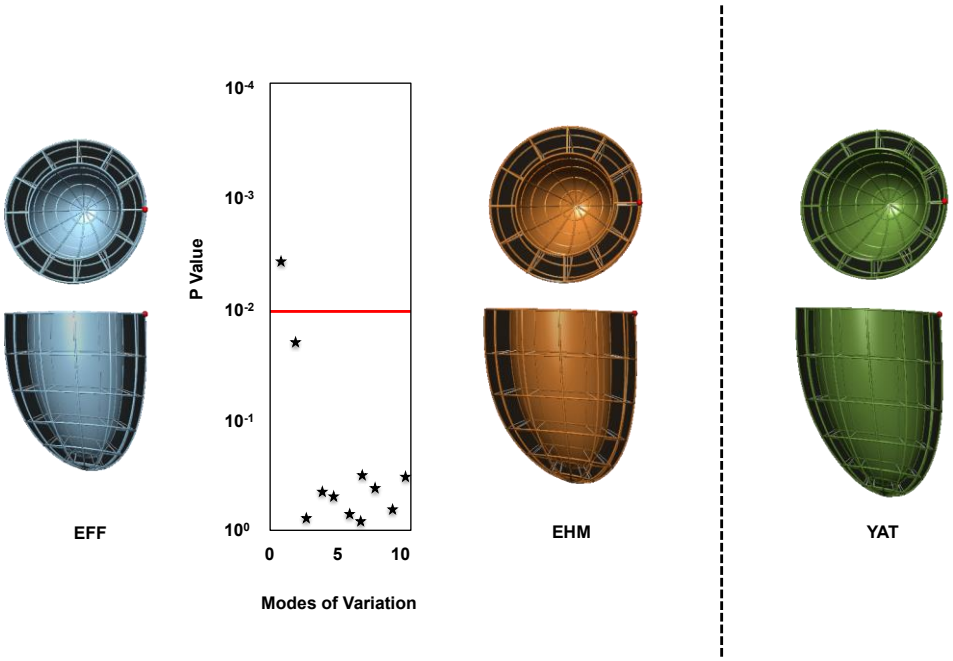
EFF
P Value
10-4
10-3
10-2
10-1
100
0
5
10
Modes of Variation
EHM
YAT

Figure 3

ONLINE SUPPLEMENT

TABLES

Table S1: Birth characteristics of preterm-born feeding groups

	EFF (n=16)	EHM (n=30)	P-value
Birth weight z score	-0.70±1.33	-0.92±1.19	0.56
Small for gestational age, n (%)	4 (25.0)	7 (23.3)	>0.99
Ventilation (days)	4.60±3.99	4.87±3.80	0.32
Maternal smoking during pregnancy, n (%)	2 (12.5)	5 (16.7)	0.93
Paternal smoking during pregnancy, n (%)	4 (25.0)	9 (30.0)	0.88
Antenatal glucocorticoids, n (%)	4 (25.0)	6 (20.0)	0.70
5-minute Apgar Score			0.76
0-3, n (%)	0 (0)	1 (3.33)	
4-6, n (%)	4 (25.0)	7 (23.3)	
7-10, n (%)	12 (75.0)	22 (73.3)	

EFF indicates exclusively fed formula postnatally; EHM indicates exclusively fed human breast milk postnatally. Values as Mean±Standard Deviation unless stated otherwise.

Table S2: Sociodemographic characteristics of preterm-born feeding groups

	EFF (n=16)	EHM (n=30)	P-value
Relationship status			0.91
Single, n (%)	9 (56.3)	16 (53.3)	
Long-term relationship, n (%)	4 (25.0)	8 (26.7)	
Married, n (%)	3 (18.9)	6 (20.0)	
Divorced, n (%)	0 (0)	0 (0)	
Educational level			0.94
None, n (%)	0 (0)	0 (0)	
GCSE or equivalent, n (%)	0 (0)	2 (6.67)	
A-levels or equivalent, n (%)	8 (50.0)	15 (50.0)	
Degree or higher, n (%)	8 (50.0)	13 (43.3)	
Employment Status			0.88
Full-time paid, n (%)	9 (56.3)	15 (50.0)	
Part-time paid, n (%)	3 (18.9)	8 (26.7)	
Caring for the home or children full time, n (%)	1 (6.25)	0 (0)	
Student/apprentice, n (%)	3 (18.9)	7 (23.3)	

EFF indicates exclusively fed formula postnatally; EHM indicates exclusively fed human breast milk postnatally.

Table S3: Thoracic cavity dimensions

	EFF (n=16)	EHM (n=30)	P-value
Left thoracic dimensions			
Height	16.6±2.0	17.9±2.4	0.09
Diameter	9.12±0.73	9.72±1.40	0.14
AP distance	10.7±1.4	12.2±1.5	**0.004**
Right thoracic dimensions			
Height	16.2±1.8	17.4±2.5	0.13
Diameter	9.66±1.05	10.2±1.1	0.08
AP distance	10.9±1.3	12.4±1.4	**0.002**

METHODS

Native T1 Cardiac ShMOLLI - We also acquired ShMOLLI T1 maps without the administration of any invasive agents over the basal, mid and apical parts of the ventricle to help detect any possible major myocardial pathophysiology. Imaging was performed with SSFP using flip 35° angle. Each image readout was preceded by 5 ramp up LISA pulses, and followed by a single 17.5° pulse at a TR/2 distance. Inversions were performed using a hyperbolic secant pulse.[1] All images were analysed using in-house software as previously described (Interactive Data Language, Ver. 6.1, Boulder, USA).[1] Myocardial contours were drawn directly on the T1 maps with full control over image windowing in order to separate consistent myocardial tissue with minimal partial volume of the neighbouring tissues.

Thoracic Dimensions and Pulmonary Artery Diameters - On the coronal localizer, we measured left and right thoracic height from the base of the diaphragm to the top of the thorax and measured diameters from the costophrenic angle to the spine on both sides using Siemens analytical software (Argus, Siemens Medical Solutions, Germany). On the transaxial localizer at the level of the bifurcation of the right pulmonary artery, we measured the anterior to posterior distance on the left and right sides of the thorax. Main pulmonary artery

and aortic diameters were measured on ECG-gated transaxial localizer images at the level of the right pulmonary artery bifurcation.[2]

REFERENCES

1. Piechnik SK, Ferreira VM, Lewandowski AJ, et al. Normal variation of magnetic resonance T1 relaxation times in the human population at 1.5 T using ShMOLLI. *Journal of Cardiovascular Magnetic Resonance* 2013; **15**(1): 1-11.
2. Truong QA, Massaro JM, Rogers IS, et al. Reference Values for Normal Pulmonary Artery Dimensions by Noncontrast Cardiac Computed Tomography: The Framingham Heart Study. *Circulation: Cardiovascular Imaging* 2012; **5**(1): 147-54.