einstein
Official Publication of the Instituto Israelita de Ensino e Pesquisa Albert Einstein

ISSN: 1679-4508 | e-ISSN: 2317-6385

ORIGINAL ARTICLE

Male sex is an independent risk factor for poor neurodevelopmental outcome at 20 months' corrected age, in human milk-fed very preterm infants: a cohort study

O sexo masculino é fator de risco independente para pior desenvolvimento neurológico na idade corrigida de 20 meses, em lactentes muito prematuros e alimentados com leite humano: estudo de coorte

Israel Macedo[1,2], Luis Pereira-da-Silva[2,3], Lília Brito[1], Manuela Cardoso[1]

[1] Maternidade Dr. Alfredo da Costa, Centro Hospitalar Universitário de Lisboa Central, Lisboa, Portugal.
[2] NOVA Medical School, Universidade NOVA de Lisboa, Lisboa, Portugal.
[3] Hospital Dona Estefânia, Centro Hospitalar Universitário de Lisboa Central, Lisboa, Portugal.

DOI: 10.31744/einstein_journal/2019AO4607

ABSTRACT

Objective: To determine associations between sex and neurodevelopmental outcomes in human milk-fed very preterm infants, adjusted to early measured nutrient intakes and other neonatal cofactors. **Methods:** Consecutive inborn human milk-fed infants, with gestational age <33 weeks, were eligible. In-hospital energy and protein intakes have relied on measured human milk composition. The *Bayley Scales of Infant Development II* mental and psychomotor developmental indexes were used to assess neurodevelopment at 20 months' corrected age. After univariate analysis, some covariables were used for linear multiple regression. **Results:** Thirty-two infants were included, with a mean (standard deviation) gestational age of 29.8 (1.8) weeks, and a median birth weight of 1168g (interquartile range 990-1419g). Minimum recommended intakes were achieved in 63.6% and 15.2% of infants for protein and energy, respectively. The mental and psychomotor developmental indexes were within normal limits in 93.8% of infants. The mean mental and psychomotor developmental indexes were significantly lower in males. Only male sex negatively and significantly affected the mental and psychomotor developmental indexes (B=-9.44; 95%CI: -17.64- -1.23; adjusted r^2=0.17; p=0.026), adjusted to gestational age and measured energy intake. **Conclusion:** In human milk-fed very preterm infants, males had a significantly lower mental and psychomotor developmental indexes score at 20 months' corrected age, adjusted for gestational age and measured energy intake.

Keywords: Human, milk; Neurodevelopment disorders; Nutrients; Infant, premature; Sex
ISRCTN ID: 27916681

RESUMO

Objetivo: Determinar a associação entre sexo e desfechos relativos ao neurodesenvolvimento em lactentes muito prematuros e alimentados com leite humano, ajustada para a ingestão de nutrientes medida nos primeiros dias de vida e outros cofatores neonatais. **Métodos:** Consideramos, para este estudo, lactentes alimentados com leite humano, consecutivamente nascidos em um centro especializado, com idade gestacional <33 semanas. A ingestão intra-hospitalar de energia e proteínas baseou-se na composição medida do leite humano. Os índices de desenvolvimento mental e psicomotor das *Bayley Scales of Infant Development II* foram usados para avaliar o neurodesenvolvimento na idade corrigida de 20 meses. Após a análise univariada, algumas

How to cite this article:
Macedo I, Pereira-da-Silva L, Brito L, Cardoso M. Male sex is an independent risk factor for poor neurodevelopmental outcome at 20 months' corrected age, in human milk-fed very preterm infants: a cohort study. einstein (São Paulo). 2019;17(3):eAO4607. http://dx.doi.org/10.31744/einstein_journal/2019AO4607

Corresponding author:
Israel Macedo
Maternidade Dr. Alfredo da Costa
Rua Viriato, 1
Zip code: 1050-170 – Lisboa, Portugal
Phone: +351 91983-9777
E-mail: israeljmacedo@gmail.com

Received on:
May 30, 2018

Accepted on:
Dec 20, 2018

Conflict of interest:
none.

covariáveis foram usadas para a regressão múltipla linear. **Resultados:** Incluímos 32 lactentes, com idade gestacional média (desvio padrão) de 29,8 (1,8) semanas e mediana de peso ao nascimento de 1168g (intervalo interquartil: 990-1419g). A ingestão mínima recomendada foi atingida em 63,6% e 15,2% dos lactentes, para proteínas e energia, respectivamente. Os índices de desenvolvimento mental e psicomotor ficaram dentro dos limites normais em 93,8% dos lactentes. A pontuação média nos índices de desenvolvimento mental e psicomotor foi significativamente menor no bebês do sexo masculino. Somente o sexo masculino afetou negativa e significativamente os índices de desenvolvimento mental e psicomotor (B=-9,44; IC95%: -17,64- -1,23; r^2 ajustado=0,17; p=0,026), ajustados para idade gestacional e ingestão de energia medida. **Conclusão:** Em lactentes muito prematuros e alimentados com leite humano, o sexo masculino teve pontuação significativamente mais baixa nos índices de desenvolvimento mental e psicomotor na idade corrigida de 20 meses, ajustada para idade gestacional e ingestão de energia medida.

Descritores: Leite humano; Transtornos do neurodesenvolvimento; Nutrientes; Recém-nascido prematuro; Sexo
ISRCTN ID: 27916681

INTRODUCTION

Male sex has been described as an independent risk factor for adverse neurodevelopmental outcomes in preterm infants.(1,2) Some reasons have been proposed to explain this disadvantage in male preterm infants, such as impaired adaptive response to prenatal stress,(3) a pro-oxidant state in the placentae,(4) and specific morphological characteristics of the brain.(5,6)

The type of feeding is an important independent factor for neurocognitive development. In very preterm infants, early human milk (HM) intake appears to be independently associated with better neurodevelopment.(7,8)

It is controversial whether nutritional requirements in the neonatal period differ between male and female preterm infants, and whether neurodevelopment is affected. A study involving preterm infants did not find an interaction between diet and sex on neurodevelopment at 9 months' corrected age.(9) In contrast, other authors have suggested that suboptimal in-hospital nutrition in preterm infants can alter brain organization and neurocognitive outcome, with particular sensitivity in males.(10,11) In addition, improved neurodevelopmental outcome in female preterm infants receiving higher intakes of protein(10) and docosahexaenoic acid(12) has been reported.

Published studies assessing the association between early diet and neurodevelopmental outcome adjusted to sex, in HM-fed preterm infants, have not measured the composition of HM. Instead, nutrient intakes provided by HM have relied on its assumed composition, or on growth as surrogate of nutritional support.(10,11,13-15)

In this study, we hypothesized a better outcome in females, adjusted for in-hospital measured energy, protein and protein-to-energy ratio intakes, and other relevant neonatal covariables.

OBJECTIVE

To determine the association between sex and neurodevelopmental outcome in human-milk fed very preterm infants.

METHODS

Study design and participants

This is a secondary analysis based on a birth cohort study primarily designed to evaluate the associations of in-hospital nutrient intakes, with body composition and neurodevelopmental outcome. In the present study, the association between sex and neurodevelopmental outcome at 20 months' corrected age was investigated. The study was performed in the neonatal intensive care unit of *Maternidade Dr. Alfredo da Costa, Centro Hospitalar de Lisboa Central*, Lisbon, Portugal. The study was approved by the Hospital Ethics Committee and is registered at ISRCTN (ID: 27916681). Informed written consent was obtained from the parents or legal representative of each infant.

The study protocol has been described elsewhere.(16) Briefly, consecutive inborn neonates at <33 weeks' gestation and who were HM-fed at least 80mL/kg/day (convenience criterion for tolerance to enteral feeding) were eligible for the study. Infants with major congenital malformations and triplets or more were not included. Infants with diagnosed inborn errors of metabolism, and those who were subsequently formula-fed >12.5% of the enteral volume intake, transferred, deceased, or unavailable for neurodevelopment assessment were excluded. In our unit, enteral feedings are administered every 3 hours (eight times per day); as a convenience criterion, the infants were considered to be predominantly HM-fed if no more than one of eight meals (12.5%) was replaced with formula.

The demographic and clinical independent variables recorded were sex, single or twin pregnancy, gestational age (GA), birth weight, small-for-GA (birth weight <10th percentile),(17) measured protein, energy, and protein-to-energy ratio daily intakes, Score for Neonatal Acute Physiology – Perinatal Extension II (SNAP-PE II) score,(18) use of prenatal and postnatal corticosteroids, diagnosis of late-onset sepsis,(19) severe necrotizing enterocolitis (grade ≥3B),(20) severe periventricular/intraventricular hemorrhage (grade ≥3),(21) multicystic periventricular leukomalacia,(22) days on invasive ventilation and on supplemental oxygen, retinopathy of prematurity (stage 3 or plus disease),[23] and chronic lung disease.[24,25] Gestational age was determined by an early prenatal ultrasonography or by the first day of the last menstrual period and, in the case of assisted reproductive technology, by adding two weeks to the conception age.[26]

Nutrition protocol

Infants were managed according to the neonatal intensive care unit nutrition protocol, based on international[27-29] and national[30,31] recommendations for neonatal parenteral nutrition (PN) and enteral nutrition. Briefly, PN was initiated within the first 2 postnatal hours with 2.5g/kg/day of amino acids and was increased up to 3.8-4.0g/kg/day; parenteral lipids were initiated within the first 24 postnatal hours with 1g/kg/day and increased up to 3g/kg/day. Early enteral trophic feeding (10 to 20mL/kg/day) was initiated within the first 2 to 4 postnatal days using HM; subsequently, enteral nutrition was increased as the PN was proportionally reduced. Until 35 weeks' corrected age exclusive HM (own mother's milk – OMM, or donor HM – DHM) was used. If the OMM was not sufficient after 35 weeks' corrected age, preterm formula was used owing to limited DHM stock. Nutrition was prescribed by physicians in collaboration with a nutritionist.

Donor human milk and OMM were stored frozen in the maternity milk bank.[16] For each infant, a 3mL homogenized sample of daily pools of OMM was analyzed using a mid-infrared HM analyzer (Miris AB, Uppsala, Sweden). The composition of DHM was always analyzed. The physicians, nutritionist, and psychologist were blinded to HM composition during the entire study period. When breastfeeding predominated (unknown volume intake and composition), the OMM composition analysis was suspended. An HM fortifier (Aptamil FMS®, Milupa/Danone GmbH, Friedrichsdorf, Germany) was used when the HM intake was at least 100mL/kg/day. The standard fortification method was supplemented with modular protein[32] (Aptamil Protein Supplement powder®, Milupa/Danone GmbH, Friedrichsdorf, Germany) and/or modular medium-chain triglycerides (MCT OIL, SHS Nutricia/Danone®, GmbH, Friedrichsdorf, Germany), considering the lowest reported HM protein content.[33] The administered volumes of OMM and DHM were used to calculate the energy, protein, and per intakes.[33,34] The volumes and powder weights of PN solutions and commercial products were also accounted for in these calculations. Following the nutrition protocol used in our unit, the minimum targeted daily intakes according to body weight, once the daily fluid intake of 140 to 150mL/kg was reached, were as follows: energy 110kcal/kg; protein (g/kg) 4.0 if <1000g, 3.7 if <1200g, 3.6 if <1800g, and 3.4 if >1800g; and protein-to-energy ratio of 3.6 if <1000g, 3.2 if <1800g, and 2.6 if >1800g.[27,29] The minimum recommended daily nutrient intakes for body weight, achieved in at least 75% of the days, after reaching a steady fluid intake, was assessed in each infant up to 35 weeks' corrected age.

Neurodevelopmental assessment

The mental developmental indexes (MDI) and psychomotor developmental indexes (PDI) of the Bayley Scales of Infant Development II (BSID-II)[35] were used to assess functional development in the study sample. This is a validated tool for infants up to 42 months of age.[36,37] The MDI measures cognitive, language, and personal-social functioning, and the motor scale measures control of gross and fine motor functions.[35] Reliability of scores of both scales was demonstrated in the normative sample of children, with internal consistency coefficients ranging from 0.78 to 0.93 for MDI and from 0.75 to 0.91 for PDI.[35] Test-retest score reliability in the normative sample is moderate to high for the approximate age range examined in the present study (r=0.91, for MDI, and r=0.79, for PDI, at 24 months of age).[35] The mean MDI and PDI scores were classified as within normal limits (≥85), mildly delayed (70 to 84), or severely delayed (<70).[38]

The neurodevelopmental assessment was initially scheduled at 18 months' corrected age. However, at this age other assessments have been scheduled, and it was decided to delay the assessment 2 months (20 months' corrected age) to assess the neurodevelopment in better conditions.

Statistical analysis

Statistical analysis was performed using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria) and Statistical Package for the Social Sciences (SPSS) version 13 (SPSS Inc., Chicago, IL, USA). To determine the association between in-hospital nutrient intakes and neurodevelopment at 20 months' corrected age, a sample of 75 infants was estimated to detect a difference ≥11 in MDI or PDI and a standard deviation (SD) ≥8 points[39] in a normally distributed variable, with a significance level of 0.05 and 80% power. The normality of continuous numerical variables was tested using the Shapiro-Wilk test, and the data are expressed according to adequate central and dispersion measures. Univariate analysis was performed using the Student's *t* test, the Mann-Whitney U test, Pearson's r, or Kendall's tau-b, as appropriate. Categorical variables were described by their absolute values, and relative frequencies compared using the χ^2 or Fisher's exact test. When OMM composition analysis was not possible, a *post-hoc* analysis for imputation of missing values was performed, as previously described.[16,22,24-26] The effect of covariables was first explored using univariate analysis, selecting associations with $p<0.15$ and fulfilled all the assumptions, for linear multiple regression. The backward method was then used, with MDI and PDI as the dependent variables and sex as independent variable.

RESULTS

The study was interrupted before the calculated sample size was reached, owing to logistical constraints. The period of enrollment was from February 1st, 2014, to February 28, 2015 (13 months), during which 156 eligible infants were identified (Figure 1).

Neurodevelopment was assessed in 32 infants, with 26 very preterm (≥28 weeks) and 6 extremely preterm (<28 weeks). Their characteristics and clinical outcomes, and comparison between sexes are summarized in table 1.

All infants received a complete cycle of antenatal betamethasone. No cases of small-for-GA, postnatal steroids, severe necrotizing enterocolitis, multicystic periventricular leukomalacia, retinopathy of prematurity, and transferred or deceased infants were recorded. No significant differences between sexes were found in relation to protein, energy and protein-to-energy ratio intakes (Table 2), twins, SNAP-PE II, late onset sepsis, days on supplemental oxygen, chronic lung disease, postnatal steroids, severe necrotizing enterocolitis, and severe intra-periventricular hemorrhage. Compared with the 56-excluded formula-fed infants, the female-to-male ratio did not differ significantly ($p=0.82$); however, the 33 infants who were enrolled had a lower GA (mean [SD] of 29.8 [1.8] *versus* median 31.7; interquartile range (IQR) 29.9-32.1 weeks of gestation; $p=0.002$); a lower prevalence of twins (12% *versus* 70%; $p<0.0001$) and longer hospital stay (median 51 [IQR: 35-62] *versus* 39 [IQR: 29-51] days; $p=0.016$).

During the hospital stay, infants were exclusively or predominantly HM-fed or breastfed. Fortified HM was started on postnatal day 7 (without differences between sexes) and generalized to all infants by postnatal day 28. The minimum recommended daily nutrient intakes was attained in 63.6% of infants for protein, 15.2% for energy, and 93.9% for protein-to-energy ratio. No differences between sexes were found either in initiation of fortified HM or in minimum recommended daily nutrient intakes. The median daily protein, energy, and protein-to-energy ratio intakes ranged from 2.7 to 4.2g/kg, 53.7 to 109.2kcal/kg, and 3.4 to 5.6, respectively.

In the entire sample, the mean (SD) score for MDI was 100.2 (11.5) and for PDI was 97.4 (8.0). The mean MDI score was within normal limits in 30 (93.8%) of infants, and mildly and severely delayed in two males, respectively. The mean PDI score was within normal limits in 30 (93.8%) of infants, and mildly delayed in one female and one male, respectively. No significant differences in MDI and PDI scores were found between extremely preterm and very preterm infants.

In the univariate analysis, the MDI distribution in females was skewed toward the upper scores. Mental developmental index and PDI in males, and PDI in both sexes, remained in a normal distribution.

The mean MDI score was significantly lower in males, and no significant differences were found in the mean PDI score between sexes (Table 3).

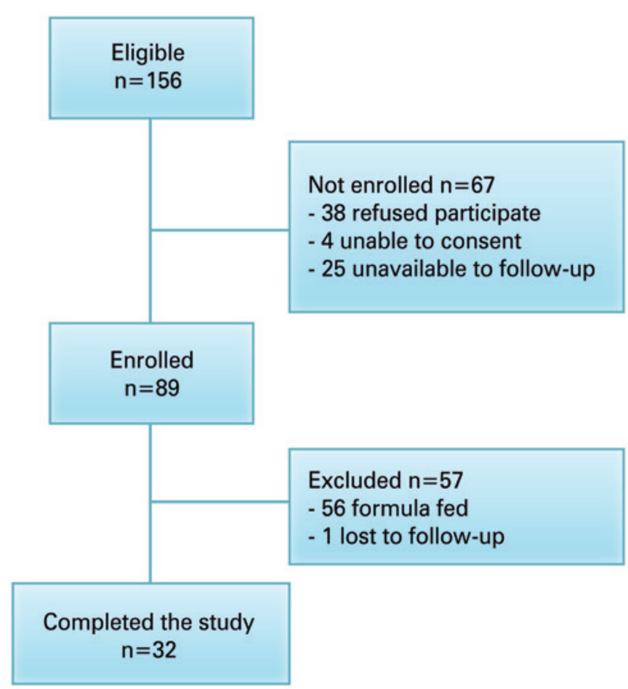

Figure 1. Flowchart of the cohort of very preterm infants with neurodevelopment assessment at 20 months' corrected age

Table 1. Characteristics of the infants included, and comparison between sexes

Characteristics	Total n=32	Female n=10	Male n=22	p value
Gestational age, weeks	29.8 (1.8)	30.1 (1.6)	29.6 (1.9)	0.27*
Extremely preterm	8	2	6	
Gestational age, weeks	28.4 (1.4)	28.7 (0.7)	28.3 (1.6)	0.79*
Very preterm	24	8	16	
Gestational age, weeks	30.2 (1.7)	30.4 (1.6)	30.1 (1.8)	0.65*
Birth weight, g	1168 [990-1419]	1279 [1073-1760]	1140 [960-1393]	0.35†
Twins	5 (15.6)	0 (0)	4 (19)	0.27‡
Antenatal steroids	32 (100)	11 (100)	21 (100)	NA
SNAP-PE II	13 [0-22]	8 [5-27.8]	14 [3.8-20]	0.88†
Late onset sepsis	6 (18.8)	1 (9.1)	3 (14.3)	>0.9‡
Days on invasive ventilation	0 [0-6]	2 [0-5]	0 [0-6]	0.57†
Chronic lung disease	3 (9.4)	1 (10.0)	2 (9.1)	>0.9‡
Steroids for chronic lung disease	1 (3.1)	0 (0)	1 (4.8)	NA
Severe periventricular/intraventricular hemorrhage	2 (6.3)	1 (10.0)	1 (4.5)	>0.9‡
Days on supplemental oxygen	21 [5-42]	2 [0-5]	0 [0-6]	0.57†

Results expressed as mean (standard deviation), n, median [interquartile range], or n (%). * Student *t* test; † Mann-Whitney test; ‡ χ^2.
SNAP-PE II: Score for Neonatal Acute Physiology – Perinatal Extension II; NA: not applicable.

Table 2. Initiation of fortified human milk and proportion of infants attaining the minimum recommended daily nutrient intakes. Comparison between sexes

	Total n=32	Female n=10	Male n=22	p value
Initiation of human milk fortification, days	7	6.4	6.5	0.98*
Infants attaining the minimum protein intake	63.6	40.0	68.2	0.24†
Infants attaining the minimum energy intake	15.2	20.0	13.6	>0.9†
Infants attaining the minimum protein-to-energy ratio	93.9	90.0	95.4	>0.9†

Results expressed as mean or %. * Mann-Whitney test; † χ^2.

Table 3. Comparison of mental development index and psychomotor developmental scores at 20 months' corrected age (n=32) between sexes

Index	Female	Male	p value
MDI	100.0 [98.0-115.5]	97.0 [91.8-104.0]	0.036*
PDI	99.6 (10.8)	96.4 (6.5)	0.299†

Results expressed as median [interquartile range] or mean (stadard deviation). * Mann-Whitney U test; † Student's *t* test.
MDI: mental development index; PDI: psychomotor developmental index.

Table 4. Adjusted effect of sex on mental development index score at 20 months' corrected age, with gestational age as covariate (n=32)*

Variable	B	95%CI for B	Adjusted r^2	p value
Sex, adjusted for gestational age, weeks	-9.44	-17.64; -1.23	0.17	0.026†

* Energy was removed in the first stage of iterations; † statistically significant.
95%CI: 95% of confidence interval.

Thus, only covariables for MDI were analyzed. The MDI score was weakly and negatively correlated with GA (r=-0.274; p=0.129), and weakly and positively correlated with daily energy intake (r=0.289; p=0.109).

Only sex, GA, and daily energy intake were selected for linear multiple regression, because other covariables did not meet criteria to enter in the multivariable models. Neither daily energy intake nor GA significantly affected the MDI score; only male sex negatively and significantly affected (B=-9.44; 95%CI: -17.64- -1.23; adjusted r^2 =0.17; p=0.026) the MDI score, adjusted to GA (Table 4). Specifically, the MDI score was 9.44 points lower (95%CI: -17.64- -1.23) in males than in females.

DISCUSSION

In this study, it was found that among infants born very prematurely, males had a significantly lower MDI score (-9.44) than females at 20 months' corrected age, adjusted for GA and daily energy intake. This model explains 17% of the variation in MDI. These results were found in a context of a suboptimal nutrition, with only 63.6% and 15.2% infants attaining the minimum recommended protein and energy intakes, respectively.

This reflects poor effectiveness of the fortification method used.[16] Despite early suboptimal nutrition, MDI and PDI were within normal limits in 93.8% of infants at 20 months' corrected age.

Several studies have reported that male sex is independently associated with poor neurodevelopmental outcomes in preterm infants at a wide range of GA. In late preterm and very preterm infants, significantly lower MDI scores at 24 months' corrected age have been reported in males.[40] In a large sample of very preterm infants from the National Institute of Child Health and Human Development Neonatal Research Network, male sex was found to be an independent risk factor for MDI <70 at 18 to 22 months' corrected age.[41] In another study including 797 infants born at 23 to 28 weeks gestation, male sex was significantly associated with cognitive delay at 24 months' corrected age.[42] Male sex is also a predictor of poor neurological outcome at extremely low GA, as demonstrated at 30 months' corrected age in surviving males from the EPICure cohort born at ≤25 weeks' gestation.[2]

Neurological outcome in male preterm infants appears to be more sensitive to suboptimal nutrition than in females.[10,11] In our sample, a weak-to-moderate correlation had been previously reported between suboptimal nutrient intakes and weight gain velocity.[16] A large cohort study of very preterm infants, a loss of weight z-score during neonatal hospitalization was associated with a poor neurological outcome at 24 months' corrected age in males.[10,11] In another cohort of very low birth weight infants, it was found that PDI assessed at 24 months' corrected age increased with greater protein and energy intake during the first postnatal week, especially in males.[10,11] In our study, the MDI score was weakly and positively correlated with in-hospital energy intake in male preterm infants, although this effect was lost after adjustment to GA.

The particular reason for male vulnerability is largely unclear, but may be related to impaired adaptive response to prenatal stress, with potential influence on early brain development.[3] A pro-oxidant state was observed in the placentae of preterm male infants born within 72 hours of antenatal betamethasone exposure, compared with females, conferring a physiological postnatal disadvantage for males.[4] In our sample, all infants received antenatal steroids. In three-dimensional magnetic resonance images, a smaller interface between the cortical gray and white matter was revealed in very preterm males compared with females.[5] In a study assessing sex differences in brain volumes at 8 years of age, only males born preterm exhibited significantly reduced white matter compared with term males, whereas white matter volumes were equivalent in preterm and term females.[6]

The present study has strengths that should be acknowledged. A homogeneous cohort of exclusively or predominantly in-hospital HM-fed preterm infants was studied. This is important because the early HM feeding is reported to be independently associated with better neurodevelopment.[7,8] In this context, this is the first study evaluating the association between sex and neurodevelopmental outcome, adjusted to measured nutrient intakes provided by HM, instead of estimated intakes or relying on growth as a surrogate of nutritional support.[10,11]

However, this study also had limitations. Owing to premature termination of the study, the sample became undersized and, in turn, probably underpowered to detect significant associations between nutrient intakes and neurodevelopmental outcomes. Nevertheless, in multivariable analysis the studied sample size was powered enough to find a significant association between sex and neurodevelopment, adjusted for nutritional intake. The unexplained variation in MDI might be due to underestimated variables owing to the small sample size, or by non-controlled confounders, including socioeconomic background, maternal education, and environmental stimuli.[11,43] In our cohort, sex was unevenly distributed; however, this factor was adjusted for in the multivariable models. Finally, a bias for withdrawal exists because enrolled infants completing the study were significantly more immature, were more frequently singletons, and stayed longer in the hospital than those who were excluded. With this bias, more vulnerable infants to neurodevelopment impairment could be included. Nevertheless, the main independent variable was not affected because the female-to-male ratio did not differ significantly between the included and excluded infants.

CONCLUSION

Male sex was found to be an independent risk factor for lower mental developmental index in human milk-fed very preterm infants, in a context of a suboptimal nutrition. This study reinforces that, in human milk fed preterm infants, an accurate evaluation of the effect of nutrition on neurodevelopmental outcome should consider sex and rely on measured human milk composition instead of estimated intakes or growth as a surrogate of nutritional support.

AUTHORS' INFORMATION

Macedo I: http://orcid.org/0000-0002-3355-0426
Pereira-da-Silva L: http://orcid.org/0000-0001-7069-6451
Brito L: http://orcid.org/0000-0003-3061-1841
Cardoso M: http://orcid.org/0000-0002-2397-7426

REFERENCES

1. Vohr BR, Wright LL, Dusick AM, Mele L, Verter J, Steichen JJ, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993-1994. Pediatrics. 2000;105(6):1216-26.
2. Wood NS, Costeloe K, Gibson AT, Hennessy EM, Marlow N, Wilkinson AR; EPICure Study Group. The EPICure study: associations and antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth. Arch Dis Child Fetal Neonatal Ed. 2005;90(2):F134-40.
3. Wainstock T, Shoham-Vardi I, Glasser S, Anteby E, Lerner-Geva L. Fetal sex modifies effects of prenatal stress exposure and adverse birth outcomes. Stress. 2015;18(1):49-56.
4. Stark MJ, Hodyl NA, Wright IM, Clifton VL. Influence of sex and glucocorticoid exposure on preterm placental pro-oxidant-antioxidant balance. Placenta. 2011;32(11):865-70.
5. Vasileiadis GT, Thompson RT, Han VK, Gelman N. Females follow a more "compact" early human brain development model than males. A case-control study of preterm neonates. Pediatr Res. 2009;66(5):551-5.
6. Reiss AL, Kesler SR, Vohr B, Duncan CC, Katz KH, Pajot S, et al. Sex differences in cerebral volumes of 8-year-olds born preterm. J Pediatr. 2004;145(2):242-9.
7. Isaacs EB, Gadian DG, Sabatini S, Chong WK, Quinn BT, Fischl BR, et al. The effect of early human diet on caudate volumes and IQ. Pediatr Res. 2008; 63(3):308-14.
8. Schanler RJ. Outcomes of human milk-fed premature infants. Semin Perinatol. 2011;35(1):29-33.
9. Lucas A, Fewtrell MS, Morley R, Singhal A, Abbott RA, Isaacs E, et al. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics. 2001;108(3):703-11.
10. Christmann V, Roeleveld N, Visser R, Janssen AJ, Reuser JJ, van Goudoever JB, et al. The early postnatal nutritional intake of preterm infants affected neurodevelopmental outcomes differently in boys and girls at 24 months. Acta Paediatr. 2017;106(2):242-9.
11. Frondas-Chauty A, Simon L, Branger B, Gascoin G, Flamant C, Ancel PY, et al. Early growth and neurodevelopmental outcome in very preterm infants: impact of gender. Arch Dis Child Fetal Neonatal Ed. 2014;99(5):F366-72.
12. Makrides M. DHA supplementation during the perinatal period and neurodevelopment: do some babies benefit more than others? Prostaglandins Leukot Essent Fatty Acids. 2013;88(1):87-90. Review.
13. Biasini A, Neri C, China MC, Monti F, Di Nicola P, Bertino E. Higher protein intake strategies in human milk fortification for preterms infants feeding. Auxological and neurodevelopmental outcome. J Biol Regul Homeost Agents. 2012;26(3 Suppl):43-7.
14. Ergenekon E, Soysal Ş, Hirfanoğlu İ, Baş V, Gücüyener K, Turan Ö, et al. Short- and long-term effects of individualized enteral protein supplementation in preterm newborns. Turk J Pediatr. 2013;55(4):365-70.
15. Rochow N, Fusch G, Mühlinghaus A, Niesytto C, Straube S, Utzig N, et al. A nutritional program to improve outcome of very low birth weight infants. Clin Nutr. 2012;31(1):124-31.
16. Macedo I, Pereira-da-Silva L, Cardoso M. Associations of measured protein and energy intakes with growth and adiposity in human milk-fed preterm infants at term postmenstrual age: a cohort study. Am J Perinatol. 2018; 35(9):882-91.
17. Fomon SJ, Nelson SE. Body composition of the male and female reference infants. Annu Rev Nutr. 2002;22(1):1-17. Review.
18. Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: simplified newborn illness severity and mortality risk scores. J Pediatr. 2001; 138(1):92-100.
19. Modi N, Doré CJ, Saraswatula A, Richards M, Bamford KB, Coello R, et al. A case definition for national and international neonatal bloodstream infection surveillance. Arch Dis Child Fetal Neonatal Ed. 2009;94(1):F8-12.
20. Bell RS, Graham CB, Stevenson JK. Roentgenologic and clinical manifestations of neonatal necrotizing enterocolitis. Experience with 43 cases. Am J Roentgenol Radium Ther Nucl Med. 1971;112(1):123-34.
21. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92(4):529-34.
22. de Vries LS, Eken P, Pierrat V, Daniels H, Casaer P. Prediction of neurodevelopmental outcome in the preterm infant: short latency cortical somatosensory evoked potentials compared with cranial ultrasound. Arch Dis Child. 1992;67(10 Spec No):1177-81.
23. Jefferies A. Retinopathy of prematurity: recommendations for screening. Paediatr Child Health. 2010;15(10):667-74.
24. Becker B, Reinhardt D, Risselmann K, Morgenroth K, Kemperdick H, Lemburg P. [Pulmonary dysplasia in infancy. Pathogenesis, pneumologic course studies and therapy possibilities]. Monatsschr Kinderheilkd. 1984;132(7):525-33. German.
25. Lovisatti L, Padovani EM, Osti G, Fanos V, Bergamo Andreis IA, Riggio S. [The lung of the severely premature infant: radiologic aspects]. Radiol Med. 1984;70(9):603-6. Italian.
26. Engle WA; American Academy of Pediatrics Committee on Fetus and Newborn. Age terminology during the perinatal period. Pediatrics. 2004; 114(5):1362-4.
27. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, Domellöf M, Embleton ND, Fusch C, Genzel-Boroviczeny O, Goulet O, Kalhan SC, Kolacek S, Koletzko B, Lapillonne A, Mihatsch W, Moreno L, Neu J, Poindexter B, Puntis J, Putet G, Rigo J, Riskin A, Salle B, Sauer P, Shamir R, Szajewska H, Thureen P, Turck D, van Goudoever JB, Ziegler EE; ESPGHAN Committee on Nutrition. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50(1):85-91.
28. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R; Parenteral Nutrition Guidelines Working Group; European Society for Clinical Nutrition and Metabolism; European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN); European Society of Paediatric Research (ESPR). 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr. 2005;41 Suppl 2:S1-87.
29. Ziegler EE. Meeting the nutritional needs of the low-birth-weight infant. Ann Nutr Metab. 2011;58(Suppl 1):8-18. Review.
30. Pereira-da-Silva L, Castela J, Malheiro L, Nona M, Macedo I, Rocha G, Rosa ML, Paulino E, Alexandrino AM; on behalf of the Portuguese Neonatal Society. [Parenteral Nutrition in the newborn: first update of the national consensus, 2008]. Acta Pediatr Port. 2008;39(3):125-34. Portuguese.
31. Pereira-da-Silva L, Gomes A, Macedo I, Alexandrino AM, Pissarra S, Cardoso M; on behalf of the Portuguese Neonatal Society. [Enteral feeding in infants born preterm: update of the National Consensus]. Acta Pediatr Port. 2014;45(4):326-39. Portuguese.
32. Alan S, Atasay B, Cakir U, Yildiz D, Kilic A, Kahvecioglu D, et al. An intention to achieve better postnatal in-hospital-growth for preterm infants: adjustable protein fortification of human milk. Early Hum Dev. 2013;89(12):1017-23.
33. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BMC Pediatr. 2014; 14(1):216. Review.

34. Wojcik KY, Rechtman DJ, Lee ML, Montoya A, Medo ET. Macronutrient analysis of a nationwide sample of donor breast milk. J Am Diet Assoc. 2009;109(1):137-40.
35. Bayley N. Bayley Scales of Infant and Toddler Development. 2a ed. San Antonio: Psychological Corporation; 1993. 374 p.
36. Cirelli I, Bickle Graz M, Tolsa JF. Comparison of Griffiths-II and Bayley-II tests for the developmental assessment of high-risk infants. Infant Behav Dev. 2015;41:17-25.
37. Connolly BH, Dalton L, Smith JB, Lamberth NG, McCay B, Murphy W. Concurrent validity of the Bayley Scales of Infant Development II (BSID-II) Motor Scale and the Peabody Developmental Motor Scale II (PDMS-2) in 12-month-old infants. Pediatr Phys Ther. 2006;18(3):190-6.
38. Bos AF. Bayley-II or Bayley-III: what do the scores tell us? Dev Med Child Neurol. 2013;55(11):978-9.
39. Ramel SE, Gray HL, Christiansen E, Boys C, Georgieff MK, Demerath EW. Greater early gains in fat-free mass, but not fat mass, are associated with improved neurodevelopment at 1 year corrected age for prematurity in very low birth weight preterm infants. J Pediatr. 2016;173:108-15.
40. Romeo DM, Brogna C, Sini F, Romeo MG, Cota F, Ricci D. Early psychomotor development of low-risk preterm infants: influence of gestational age and gender. Eur J Paediatr Neurol. 2016;20(4):518-23.
41. Hintz SR, Kendrick DE, Vohr BR, Kenneth Poole W, Higgins RD, The Nichd Neonatal Research Network F; Nichd Neonatal Research Network. Gender differences in neurodevelopmental outcomes among extremely preterm, extremely-low-birthweight infants. Acta Paediatr. 2006;95(10):1239-48.
42. Peacock JL, Marston L, Marlow N, Calvert SA, Greenough A. Neonatal and infant outcome in boys and girls born very prematurely. Pediatr Res. 2012;71(3):305-10.
43. Leijon I. Factors of importance for neurodevelopment in preterm infants. Acta Paediatr. 2010;99(5):642-4.