Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Effect on splanchnic oxygenation of breast milk, fortified breast milk, and formula milk in preterm infants

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BACKGROUND: Enteral feeding induces mesenteric hemodynamic changes in preterm infants, which may vary according to the milk used. Our aim in this study was to evaluate changes of splanchnic regional oxygenation (rSO_2S) measured by near-infrared spectroscopy (NIRS) in infants fed with mother's own milk (MOM), fortified human milk (FHM), or preterm formula (PTF).

METHODS: Infants born at 25–31 weeks of gestational age ($n = 54$) received a bolus of MOM, FHM, or PTF. rSO_2S and splanchnic fractional oxygen extraction ratio (FOES) were recorded 60 min before (T_0), and 30 min (T_1) and 120 min (T_2) after the beginning of bolus feeding.

RESULTS: In the MOM group, rSO_2S and FOES did not change during the study period. In the FBM group, rSO_2S decreased from T_0 to T_1 and increased from T_1 to T_2, while FOES changed in reverse. In the PTF group, rSO_2S decreased from T_0 to T_1 and from T_1 to T_2, while FOES changed in reverse.

CONCLUSIONS: Splanchnic oxygenation was not affected by MOM feeding, was transiently decreased by FBM feeding, and was persistently decreased by PTF. These results suggest that preterm infants who received PTF has higher splanchnic tissue oxygen extraction compared to those who received MOM or FBM.

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IMPACT:

- Human milk feeding is associated to a lower splanchnic energy expenditure than preterm formula feeding.
- Fortified human milk transiently increases splanchnic energy expenditure.
- Preterm formula should be used only in the absence of human milk.

INTRODUCTION

The food of choice for preterm infants is human milk (HM).[1] When mother's own milk (MOM) is insufficient, preterm infants should be supplemented with pasteurized donor human milk (DHM) and, in the absence of MOM and DHM, with preterm formula (PTF).[2] In fact, recent meta-analyses concluded that HM decreases the risk of necrotizing enterocolitis (NEC)[2,3] and, possibly, late-onset sepsis (LOS), and severe retinopathy of prematurity (ROP)[2] in comparison with PTF. Nonetheless, fortification of HM is required, both for MOM and DHM, to meet nutrient requirements of preterm infants, especially protein and minerals, allowing for adequate growth and development and decreasing the risk of extra-uterine growth restriction.[4] Consistently, fortification of HM has been found to improve in-hospital rates of growth without increasing the risk of NEC,[5] although it has been reported that fortification might impair feeding tolerance.[6,7]

The availability of noninvasive methods to measure feeding effects on splanchnic circulation, such as near-infrared spectroscopy (NIRS), makes it possible to evaluate the potential correlation between enteral feeding strategies and mesenteric hemodynamic changes. Noninvasive measurement of splanchnic regional oxygenation (rSO_2S) has become possible and it has been studied in preterm infants in different clinical conditions. We have recently demonstrated that continuous enteral feeding increases rSO_2S in HM-fed preterm infants in comparison with bolus feeding, and, therefore, could help limit the risk of hypoxic −ischemic gut damage in severely ill patients.[8] Moreover, Grometto et al.[9] found that MOM feeding is associated with lower rSO_2S and higher cerebral regional oxygenation (rSO_2C) than PTF feeding in preterm infants, suggesting that the use of MOM is associated to a better cerebro-splanchnic hemodynamic redistribution than PTF.[9] However, the effect on splanchnic oxygenation of fortified MOM feeding has never been investigated and its knowledge could add another piece to the understanding of mesenteric hemodynamic changes in the relationship to the type of milk used.

On this basis, we hypothesized that rSO_2S changes after MOM feeding were lower than that after FHM and PTF in very preterm infants. Thus, to assess this hypothesis, we prospectively studied three cohorts of preterm infants who were fed with MOM, FHM, or PTF in whom rSO_2S was measured by NIRS.

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METHODS

Patient population

This was a prospective observational study performed at the third-level NICUs of Careggi University Hospital of Florence, Italy, and C. Arrigo Children's Hospital of Alessandria, Italy, between September 2017 and September 2019. The study was approved by local ethics committees.

Infants with gestational age between 25^{+0} and 31^{+6} weeks were enrolled in the study, after parental informed consent, if they were clinically stable and tolerating full bolus enteral feeding for 1 week with a total daily amount between 140 and 160 mL/kg, without intravenous support. Eligibility for the study also included tolerance of orogastric tube feeding by gravity during a period of 5−10 min given every 3 h, on the day of the study.

Exclusion criteria were the need for cardiovascular support, signs of abdominal distension, previous NEC, gastroschisis or congenital diaphragmatic hernia, and infection developing within 1 week before enrollment. These exclusion criteria were selected because catecholamine administration may reduce perfusion by pathological vasoconstriction and because of concerns over the integrity of splanchnic perfusion following pathological gastrointestinal disorders.

Study design

All infants followed the same enteral nutrition protocol: trophic feeding was initiated within 24 h after birth and was continued at 20–40 mL/kg/d as tolerated for up to 5 days. Subsequently, the amount was increased by 20 mL/kg every day if enteral nutrition was tolerated. Decisions about whether to increase feeding volume, halt feeding advancement, and fortification were made by the clinical team based on local protocols. The goals for enteral nutrition were 150 mL/kg/d and 120 kcal/kg/d. MOM was enriched with a fortifier (Prenidina FM85®, Nestlè, La Tour-de-Peilz, France; 1 g/25 mL of milk) when enteral feeding of 120 mL/kg/d was reached. PTF was administered only when HM was not available.

For the purpose of the study, each infant was given a bolus of fresh MOM, or fortified fresh MOM (FBM), or PTF for ~10 min through an orogastric tube. NIRS measurements were taken with each infant in the supine position. During data recording, infants were mostly quiet or sleeping and, to reduce NIRS artifacts, the handling of patients during the study period was minimized. Oxygen arterial saturation (SpO_2) and heart rate (HR) were continuously recorded.

The following data were also recorded for each studied infant: gestational age (GA), birth weight (BW), GA and weight at NIRS measurement, gender, duration of parenteral nutrition, age at full enteral feeding, occurrence of PDA requiring medical treatment, blood transfusion within 1 week before enrollment, bronchopulmonary dysplasia (BPD), NEC, sepsis, intraventricular hemorrhage (IVH), ROP, and duration of stay in hospital. BPD was defined as oxygen requirement at 36 weeks of postmenstrual age.[10] PDA was diagnosed by echocardiography; NEC was defined as Bell's stage 2 or higher;[11] sepsis was defined as positive blood culture. IVH was classified according to the Papile classification scheme;[12] and ROP was graded according to the international classification of ROP.[13]

NIRS monitoring

Enrolled patients were studied continuously for 150 min by NIRS (INVOS 5100®; Somanetics Corporation, Troy, MI, USA) for measurement of rSO_2S. A self-adhesive transducer that contains a light-emitting diode and two distant sensors was placed on the infra-umbilical abdomen region. The rSO_2S measurements obtained with the NIRS technique reflect a combination of intravascular oxygenated/deoxygenated venous, arterial, and capillary hemoglobin in a ratio of approximately 75:20:5.[14] However, this ratio has only been validated for the brain,[14] although it has been extrapolated to all human tissue.

On the basis of the measurements of rSO_2S and SpO_2, we calculated the splanchnic fractional oxygen extraction ratio (FOES),[15] which is the difference between arterial SpO_2 measured by pulse oximetry and rSO_2S measured by NIRS ($FOES = (SpO_2 - rSO_2S)/SpO_2$). This parameter reflects the balance between oxygen delivery and oxygen consumption. Therefore, an increase in FOES suggests an increase in the oxygen extraction by tissues, due to higher oxygen consumption in relation to oxygen delivery, while its decrease suggests less oxygen use in comparison with the supply.[16]

All of the NIRS data were recorded 30 min before (T_0), 30 min after the beginning of bolus feeding (T_1), and 120 min after feeding finished (T_2). All infants were studied just one time.

Statistical analysis

A sample size of at least 15 infants for each group was calculated in order to detect a statistically significant change of 10% of rSO_2S 30 min after the bolus feeding (T_1) in infants fed with MOM vs. infants fed with PTF, with 80% power at 0.05 level.

Patients' clinical characteristics were described as mean ± SD or rate and percentage and were compared using the Student's *t* test for parametric data and the Fisher's exact test for categorical data.

For each NIRS variable (rSO_2S, FOES), we calculated the mean ± SD from the selected 5-min periods (12 data points per minute), which were chosen at the end of T_0, T_1, and T_2.[17] We made this choice to obtain the highest stability of NIRS signal, even if selecting a 5-min period could induce a selection bias. Sometimes this selection was not possible due to the occurrence of unwanted artifacts (generally infant movements); in this case the 5-min period without artifacts closest to the end of T_0, T_1, and T_2 was selected.

Median values of rSO_2S and FOES were compared by Kruskal−Wallis one-way ANOVA and Dunn's method, whereas difference vs. baseline ($T_1 - T_0$, $T_2 - T_1$) and between the rSO_2S and FOES of infants in the MOM, FBM, and PTF groups were compared using the Student's *t* test for parametric data and two-sided Mann−Whitney *U* test when parameters were not normally distributed.

Data analysis was performed using IBM SPSS Statistics version 20 (SPSS INC, Chicago, Illinois, USA).

Serial measurements of rSO_2C and FOES in the groups were compared by repeated-measures analysis of variance (ANOVA), while difference vs. baseline ($T_1 - T_0$, $T_2 - T_0$, $T_3 - T_0$) were compared between the groups using the Student's *t* test for parametric data, as our data have a normal distribution.

RESULTS

Fifty-four infants were enrolled in the study, 18 fed with MOM, 18 with FBM, and 18 with PTF. Clinical characteristics were similar between the groups with the exception of weight at NIRS measurement which was higher in the PTF than in the FBM group (Table 1). None of infants had PDA at NIRS measurement or received blood transfusion in the week before. SpO_2 and heart rate did not change during the study period and were similar between the groups (unreported data).

In the MOM group, rSO_2S and FOES did not change from T_0 to T_1, and from T_1 to T_2. (Table 2).

In the FBM group, rSO_2S decreased ($P < 0.001$) from T_0 to T_1, and increased ($P < 0.001$) from T_1 to T_2 returning to baseline. On the contrary, FOES increased ($P < 0.001$) from T_0 to T_1 and decreased ($P < 0.001$) from T_1 to T_2 returning to baseline (Table 2, Fig. 1).

In the PTF group, rSO_2S decreased from T_0 to T_1 and ($P < 0.001$) from T_1 to T_2. FOES increased ($P < 0.001$) from T_0 to T_1 and ($P < 0.05$) from T_1 to T_2 (Table 2, Fig. 2).

Table 1. Clinical characteristics of infants who were fed with MOM, FBM, or PTF. Mean ± SD, or rate and (%).

	MOM ($n = 18$)	FBM ($n = 18$)	PTF ($n = 18$)	*P*
GA (wks)	29 ± 2	27 ± 2	27 ± 2	Ns
Birth weight (g)	1200 ± 288	981 ± 205	884 ± 391	Ns
<10° percentile	1 (6)	2 (10)	2 (10)	
Male	5 (27)	10 (52)	12 (60)	NS
GA at NIRS recording (wks)	35 ± 2	36 ± 2	38 ± 3	NS
Weight at NIRS measurement (g)	1875 ± 348	1711 ± 346	1967 ± 359	<0.05
Parental nutrition duration (d)	24 ± 15	22 ± 11	30 ± 21	NS
Age at full enteral feeding (d)	25 ± 13	24 ± 12	26 ± 14	NS
PDA	8 (44)	14 (73)	13 (70)	NS
PDA at NIRS measurement	0 (0)	0 (0)	0 (0)	
BPD	0 (0)	3 (16)	7 (35)	NS
NEC	0 (0)	1 (5)	1 (5)	NS
Sepsis	6 (33)	5 (26)	8 (40)	NS
Sepsis within 1 week of NIRS	0 (0)	0 (0)	0 (0)	
IVH	2 (11)	8 (42)	2 (10)	NS
ROP	0 (0)	3 (16)	6 (30)	NS
Duration of hospital stay (d)	81 ± 29	83 ± 15	94 ± 45	NS

PDA patent ductus arteriosus, *BPD* bronchopulmonary dysplasia, *NEC* necrotizing enterocolitis, *IVH* intraventricular hemorrhage, *ROP* retinopathy of prematurity, *NS* not significant.

Table 2. Changes of rSO_2S and FOES before (T_0), during (T_1) and after feeding (T_2) in MOM-, FBM-, and PTF-fed infants. Median and interquartile range (IQR).

	T_0	T_1	T_2
MOM group ($n = 18$)			
rSO_2S (%)	57 (51–62)	60 (55–68)	62 (59–65)
Difference vs. baseline		3[a]	5[b]
FOES	0.36 (0.24–0.42)	0.28 (0.21–0.39)	0.27 (0.24–0.30)
Difference vs. baseline		−0.08	−0.09[a]
FBM group ($n = 18$)			
rSO_2S (%)	57 (50–67)	55 (49–66)[c]	60 (50–67)[d]
Difference vs. baseline		−2	3
FOES	0.43 (0.31–0.51)	0.42 (0.29–0.51)[c]	0.36 (0.28–0.48)[d]
Difference vs. baseline		−0.01	−0.07
PTF ($n = 18$)			
rSO_2S (%)	59 (45–68)	54 (41–64)[c]	37 (35–68)[e]
Difference vs. baseline		−5	−22
FOES	0.39 (0.31–0.67)	0.44 (0.36–0.58)[c]	0.61 (0.31–0.64)[d,e]
Difference vs. baseline		0.05	0.22

[a] $P < 0.005$ vs. PTF.
[b] $P < 0.001$ vs. PTF.
[c] $P < 0.001$ vs. T_0.
[d] $P < 0.001$ vs. T_1.
[e] $P < 0.05$ vs. T_0.

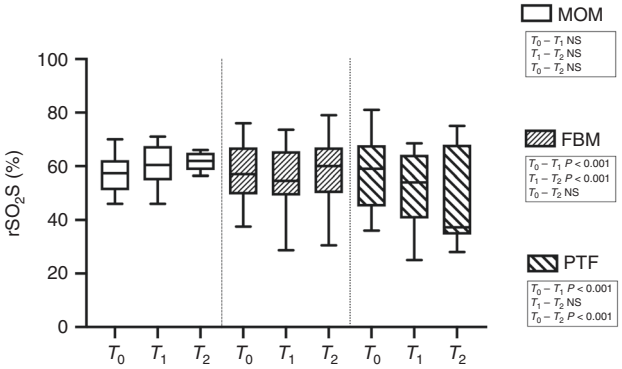

Fig. 1 Changes of rSO_2S before (T_0), during (T_1) and after feeding (T_2) in infants receiving MOM, FBM and PTF. Median and interquartile range (IQR).

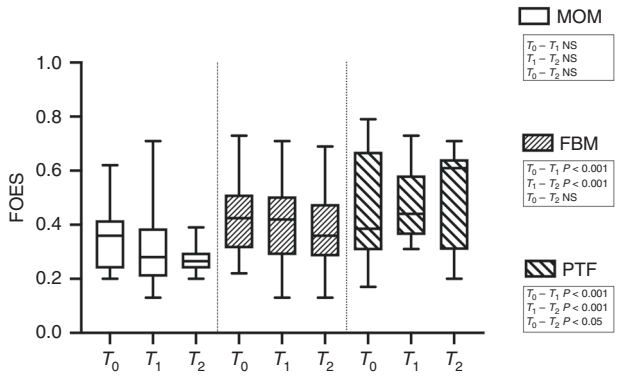

Fig. 2 Changes of FOES before (T_0), during (T_1) and after feeding (T_2) in infants receiving MOM, FBM and PTF. Median and interquartile range (IQR).

Baseline values of rSO_2S and FOES were similar between the groups, but rSO_2S and FOES difference vs. baseline at T_1 and T_2 were lower in the MOM group than in the FBM (not statistically significant) and PTF (statistically significant) groups (Table 2).

DISCUSSION

In this study we measured changes of rSO_2S and FOES in preterm infants who were fed with MOM, FBM, or PTF. We found that they did not vary in the MOM group, while infants fed with FBM had a transient decrease of splanchnic oxygenation, balanced by an increase of oxygen blood extraction, which was followed by a return to the baseline values at the end of the study period. Moreover, we observed that infants fed with PTF had a progressive decrease of splanchnic oxygenation, balanced by an increase of oxygen blood extraction lasting the entire study period (120 min after the starting of milk bolus). Accordingly, changes of rSO_2S and FOES at T_1 and T_2 vs. baseline were higher in FBM and PTF than in MOM-fed infants.

Our results are in agreement with Grometto et al.[9] who demonstrated that MOM feeding does not increase the splanchnic oxygen requirement and induces a lower cerebro-splanchnic haemodynamic redistribution than PTF. For the remaining results, it is difficult to compare our results with previous studies because they investigated the effect of milk feeding on splanchnic oxygenation without selecting infants on the basis of milk fed and reported contradictory results: in 2014 we observed an increase of rSO_2S in infants who received a bolus of pasteurized MOM or human donor' milk;[8] Bozzetti et al.[18] found that rSO_2S was unchanged in infants fed with a bolus of fortified or not

fortified human milk (MOM or human donor' milk) or PFT; Corvaglia et al.[19] observed an increase of rSO_2S in infants after a bolus of fortified HM or PTF. Thus, the results achieved cannot be attributed to a specific type of milk.[8,18,19]

However, our findings suggest that MOM feeding does not impair the gut tissue oxygen delivery extraction balance and this might represent a protective mechanism against the development of hypoxic−ischemic injuries in high-risk preterm infants. In fact, although there is a lack of trials comparing the protective effect of MOM vs. donor's milk or PTF,[20] the better tolerance of HM and its effect in decreasing the risk of NEC in comparison with PTF has been previously reported.[2,3,21]

We observed for the first time that FBM increase oxygen extraction but this effect was transient and oxygen consumption shortly returned to baseline value. This evidence is important because it may suggest that the increased density and osmolarity of fortified HM[6] increase gut oxygen consumption and energy requirement. However, the transience of this effect is in agreement with previous studies that excluded an interference of HM fortification on gastric emptying, intestinal fortification, and, ultimately, feeding tolerance.[6,22]

In our population PTF feeding was associated with a persisting (2 h) decrease of splanchnic oxygenation. This result is in disagreement with Grometto et al. who found that gut oxygenation did not change during PTF feeding and increased after it. These differences could be due to different population gestational age (our infants were significantly more immature at birth) and different PTF used. However, since intestinal ischemia and secondary hypoxia have been found to be risk factors for NEC,[23] a prolonged increase of gut oxygen requirement and extraction is consistent with the higher risk of NEC reported in PTM-fed than in HM-fed preterm infants.[2,3]

A limitation of our study was that we did not measure cerebral rSO_2 in order to minimize patients' discomfort and, therefore, we could not evaluate possible cerebro-splanchnic haemodynamic redistribution. Moreover, we did not study infants fed with pasteurized HM that, theoretically, could induce different change of rSO_2S in comparison with MOM, FBM, and PTF.

CONCLUSIONS

We found that splanchnic oxygenation was not affected by MOM feeding, was transiently decreased by FBM feeding, and was persistently decreased by PTF feeding. These results suggest that HM feeding is associated to a lower oxygen extraction than PTF feeding and are consistent with previous studies demonstrating its better tolerance and protective effect against NEC.

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AUTHOR CONTRIBUTIONS

C.D. conceived the study and wrote the manuscript. D.G. conceived the study. C.C., S.M., G.R., C.P., C.S., E.M., M.S. collected data. All authors analyzed and interpreted patients' data, read and approved the final manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Consent to the study: Patients were enrolled after informed parental consent.

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