Contents lists available at ScienceDirect

Clinical Nutrition Open Science

An open-label pilot single-subject study to monitor the impact of a Food-Based enteral formula on faecal short-chain fatty acid concentrations in children admitted to intensive care with sepsis

Graeme O'Connor [a,*], Yuxin Sun [b], Breeana Gardiner [a], Grace Audu [c], Mona Bajaj-Elliott [b], Simon Eaton [d]

[a] Great Ormond Street Hospital, Dietetics Department, London, United Kingdom
[b] Infection, Immunity & Inflammation Department, Institute of Child Health, University College London, United Kingdom
[c] Infection, Immunity & Inflammation Department, Great Ormond Street Institute of Child Health, University College London, United Kingdom
[d] Developmental Biology & Cancer Department, UCL Great Ormond Street Institute of Child Health, University College London, United Kingdom

ARTICLE INFO

SUMMARY

Background: Non-digestible dietary fiber undergoes fermentation by the intestinal microbiota to produce short-chain fatty acids (SCFAs). Intestinal SCFAs control the production of T-helper cells, antibodies and are involved in maintaining homeostasis of the mucosal system. Sepsis is the leading cause of mortality in hospitalised children and is treated with antibiotics which disrupts the normal maturation of the microbiome causing dysbiosis. This study assessed the impact of a high-fibre Food-Based formula on feed tolerance and faecal SCFA concentrations in children admitted to intensive care with sepsis.

Methods: An open-label single-subject study was based on repeated observations over 14 days in children admitted to intensive care with sepsis who commenced a high-fiber Food-Based enteral formula Compleat®Paediatric, (Nestle Health Science). Stool samples were collected to measure SCFA concentrations (acetate, butyrate and propionate). A Wilcoxon Signed-Rank test was used to measure change in SCFA concentrations. Other data collection included feed tolerance, anthropometrics, antibiotic administration and inflammatory markers.

* Corresponding author. Great Ormond Street Hospital, Dietetics Department, WC1N 3JH, London, United Kingdom.
  E-mail address: Graeme.oconnor@nhs.net (G. O'Connor).

Results: Twenty children with sepsis were recruited over six months. The mean age was 10.8 years ($\pm$5.6 years SD). The most common sepsis-related organ failure was the respiratory tract (50 %). The mean duration of mechanical ventilation was 9 days ($\pm$4 SD), 25 % of children were treated with more than two antibiotics during their time in intensive care. Faecal propionate and butyrate concentrations were maintained during the children's time in intensive care. Stool frequency reduced from 2.6 per day ($\pm$1.08 SD) at baseline to 1.2 per day ($\pm$0.45 SD) after one week in intensive care ($p < 0.004$).

Conclusion: In this pilot study children admitted to intensive care with sepsis tolerated a Food-Based formula. Faecal butyrate and propionate concentrations were maintained whilst feeding on a high fiber Food-Based formula. Further research is warranted to assess whether a Food-Based formula is superior to a standard enteral formula in preserving the intestinal microbiota, thereby mitigating gastrointestinal complications associated with antibiotic-related dysbiosis.

Background

The human intestinal microbiota consists of several hundred bacterial species [1]. The microbial community of the gut conveys significant benefits to human physiology at an intestinal epithelial and systematic inflammatory level [2,3,4]. The diversity and relative abundance of microbial metabolites are heavily dependent on specific dietary components [5]. Non-digestible dietary fiber such as oligosaccharides and inulin demonstrate resistance to digestion in the human small intestine [6]. In the large bowel dietary fiber undergoes fermentation by colonic microbiota to produce short-chain fatty acids (SCFAs); acetate, butyrate and propionate, which act as the primary carbon energy source for colonocytes [5].

The synergistic relationship between the host and intestinal SCFA concentrations includes the concomitant reduction of the luminal pH, which by itself inhibits pathogenic microorganisms and increases the absorption of some nutrients [7]. Furthermore, intestinal SCFAs control the production of T-helper cells, antibodies, and cytokines and are also involved in maintaining homeostasis of the mucosal system [8,9]. The effects of SCFA on lymphocytes appear to work together with those on epithelial cells and myeloid cells to strengthen intestinal barrier immunity, regulate microbes, and prevent harmful inflammatory responses. A significant portion of intestinal SCFAs are transported out of the gut affecting immune cells beyond the cells in the gut [10].

Sepsis is the leading cause of mortality in hospitalised children [11]. A prospective point prevalence study involving over a thousand intensive care units across the world found that on any given day 75 % of patients admitted to PICU received antibiotics [12]. Antibiotics disrupt the normal maturation of the microbiome altering basic physiological equilibria and causing dysbiosis [13]. The composition and diversity of the intestinal microbiome in critical illness are impacted by poor intestinal perfusion, hypoxia, lack of enteral feeds, and antibiotic therapy. This creates opportunities for the proliferation of potentially pathogenic species associated with adverse outcomes, including secondary infection and mortality [14]. The gut microflora in critically ill patients can consist of ultra-low-diversity communities of multidrug-resistant pathogenic microbes [15]. A study by Rooney *et al.* (2020), reported that antibiotic exposure was associated with reduced microbiome diversity and richness, and with changes in bacterial abundance. Each additional day of antibiotics was associated with a lower richness of anaerobes and butyrate-producers within one week after therapy [16].

Paradoxically, antibiotic management of sepsis can increase susceptibility to opportunistic and nosocomial infections by affecting the resistance of the intestinal microbiota to colonization [17]. An

additional consequence of antibiotic-associated dysbiosis is diarrhoea [18]. Feeding intolerance is one of the main reasons that enteral nutrition is withheld in the paediatric intensive care unit [19]. Clinicians manage these symptoms by imposing gut rest and implementing an oral hydration solution or hydrolysed protein formula to mitigate symptoms are aid absorption [20].

Dietary and microbiome-based therapies are being explored for the potential to preserve and support recovery of healthy gut commensal populations during and after critical illness [21,22]. In the paediatric population, interest is growing in the use of a blended diet for optimising feed intolerances [23] Blended diets were well tolerated in gastrostomy-fed children and are associated with clinical improvement of upper GI symptoms [24]. Although there may be benefits to using blended diets, there are concerns around safety and practical issues remain [25]. A blended diet is not always possible to implement in an acute clinical setting due to post-pyloric feeding (perceived microbial contamination risk) and imposed fluid restrictions [26].

Industry has responded to this shift in feeding practices and developed a high-fiber Food-Based enteral formula with food-derived ingredients, which has been shown to improve enteral feed tolerance [26,27]. In the United Kingdom, we currently have access to one commercially available Food-Based formula. Therefore, we implemented an open-label, single-subject design to assess the feed tolerance of a Food-Based formula and monitor its impact on faecal SCFA concentrations in children admitted to intensive care with sepsis.

Materials and methods

Study population

In this pilot study children were recruited sequentially who were admitted to our tertiary level intensive care unit with sepsis between January to July 2022, requiring mechanical ventilation, enteral feeding, and had an expected length of stay within the hospital of at least seven days (aged 1 year to 16 years). We excluded children who were discharged from the hospital before the seven days of admission, who did not receive enteral nutrition, were under the age of 1 year old (Food-Based formula licenced for over one-year-old), started antibiotics within one month before admission to our PICU, required total or partial parenteral nutrition, had a dairy intolerance or vegetarian (Food-Based formula contains cow's milk protein and rehydrated chicken). Ethical approval was granted by the Health Research Authority and Health and Care Research Wales on 1st July 2021 — reference: 279901 21/PR/0809.

Study design

This single-subject study was based on repeated observations over 14 days, subjects served as their own control. The primary outcome measure was to observe the impact of a Food-Based formula on faecal SCFA in children admitted to intensive care with sepsis. Secondary outcome measures were to monitor feed tolerance, anthropometrics, antibiotic administration, and inflammatory markers. Children who met the inclusion criteria commenced on a Food-Based enteral formula Compleat®Paediatric (Nestlé Health Science), a nutritionally complete enteral tube feed 1.2 kcal/ml, containing 14 % food-derived ingredients in the form of rehydrated chicken, peas, green beans, and orange juice, providing 1 g fiber/100 ml.

Study procedures

Faecal short-chain fatty acid concentrations

Faecal samples were collected at baseline (admission to intensive care), day 7 and day 14 (or within 48 hours of spontaneous bowel movement) or until discharge from the hospital. Faecal samples were collected from nappies, placed in sterile plastic containers, and stored at -80 °C until aliquoted. We first calibrated our faecal SCFA quantification using a pool of five faecal samples. The frozen stool was aliquoted to an average 100 mg transferred into a separated plastic vial and weighed (precision scale). To reduce the degradation of the SCFA, the stool samples were kept on dry ice whilst homogenization

[in 1 ml buffer (0.1 M tris, 0.15 M NaCl, 1 M urea, 10 mM CaCl2, 0.1 M citric acid monohydrate, 5 g/L bovine serum albumin and 0.25 mM thimerosal, pH 8.0) in fresh glass vial] was performed [18].

The homogenized samples were centrifuged at 15000 rpm for 5 min at 4 °C. 20 μL of supernatant was transferred in a sterile glass vial and mixed with 15 μL internal standard (400 μM stock of labelled 13C2-acetate, d5-propionate, and 13C4-butyrate 100 μL H2O, 500 μL of 0.1 M tetrabutylammonium and 500 μL of 2 % pentafluorobenzyl bromide in dichloromethane. Each sample was sonicated for 60 minutes and then extracted into 2 mL hexane and centrifuged at 1500 rpm for 5 min. The extract was eluted into a new glass vial and submitted to mass spectrometry. Briefly, all samples undergo vaporization in the hot inlet (280 °C) before the gaseous sample being carried by helium and column separation [28]. Faecal SCFA reflects both colonic SCFA production and absorption rates.

Feed tolerability assessment

Stool consistency is a central component in the description of normal or altered bowel habits. Stool form can be considered as a proxy measure for stool consistency and refers to the shape and apparent texture of the stool, which can be assessed visually. Stool form scales are a standardised and inexpensive method of classifying stool form into a finite number of categories that can be used by healthcare professionals and researchers. The Bristol stool form scale is an ordinal scale of stool types ranging from the hardest (Type 1) to the softest (Type 7) [29]. Types 1 and 2 are abnormally hard stools (and in conjunction with other symptoms indicative of constipation) while Types 6 and 7 are considered abnormally loose/liquid stools (and in conjunction with other symptoms indicative of diarrhoea). Type 3, 4 and 5 are therefore generally considered to be the most 'normal' stool form [30,31].

Tolerance and details of stooling patterns will be recorded each day at a ward level. Descriptions of feeding intolerance included: stool consistency and frequency (number of stools in 24 hours) constipation was defined as Rome IV Criteria, less than three defecations a week, and painful and hard stools [32]. Diarrhoea was defined as three or more loose stools a day lasting longer than 48 hours [33].

Other clinical data collection

Children's clinical information was collected from the hospital's electronic records (EPIC, Madison, WI, USA), including demographics (age and sex), anthropometric measurements, feeding information (ml/day), and admission diagnosis. Enteral feeding was delivered as a continuous infusion as per our PICU feeding protocol. Serial C-reactive protein (CRP) levels, at least two CRP levels, obtained 24 hours apart, with levels below or equal to 10 mg/L, are needed to identify infants unlikely to be infected [34]. CRP was measured daily as part of the patient's routine assessment to classify the degree of sepsis. CRP data were collected from the hospital's electronic system. The CRP reading that was recorded closest to stool sample collection was used for data analysis. Children's diagnosis that required intensive care admission was recorded and then categorized into single organ category. Days free of intensive care and days free of mechanical ventilation at 30 days were used as a measure of clinical outcome.

Statistical analysis

Normally distributed continuous variables are expressed as means and $\pm$ standard deviation (SD), while medians and interquartile ranges (IQR) are used to describe non-normal distributions. We defined the intensive care-free days as 30 minus the number of days in the PICU (range, 0–30 days). For patients who survived and were in the PICU for less than 30 days, the intensive care-free day's outcome measure was obtained by subtracting the length of the PICU stay from 30. Ventilation-free days are defined as 30 minus the number of days on conventional ventilation (excluding non-invasive ventilation). The nutrition status, weight-for-age and height-for-age, was assessed using z-scores [35]. Moderate undernutrition was identified if z-scores were between -2 and -3 standard deviation (SD) and severe undernutrition was identified if the z-scores were below -3 (SD) [36]. Conversely, moderate overnutrition was defined if z-scores were between $+2$ and $+3$ SD [25].

Descriptive statistics of between-group differences in subject characteristics were tested for significance. Individual SCFA concentrations of acetate, propionate, and butyrate (μmol per gram dry

faeces), distribution violated the assumption of normality, therefore the difference between timepoints was analysed using a Wilcoxon Signed-Rank tests, presented as Z statistics. Statistical tests were conducted in a two-sided manner and the significance level was set at 5 % ($p < 0.05$). The Statistical analysis was performed using Package for the Social Sciences Version 21 (SPSS Statistics 22, IBM Corp., Armonk, NY).

Results

This study sequentially recruited 20 critically ill children, the mean age was 10.8 years (±5.6 years SD), of which 30%, 6 of the 20 children were female. The most common sepsis-related organ failure was the respiratory tract (50 %). The general characteristics of the population are described in Table 1. The mean time to commence enteral feed after admission to PICU was 34 ± 11 hours. The mean daily feed volume during intensive care admission was 950 ± 230 ml, providing a mean fiber dose of 9.5 ± 3 g/day The mean duration on mechanical ventilation was 9 ± 4 days. The median 30-day free of intensive care and 30-day free from ventilation were 8 days (IQR: 1, 22) and 10 days (IQR: 10, 25), respectively (Table 1). All children recruited to the study survived to PICU discharge.

On admission, all children were prescribed antibiotics. The type of antibiotic class administered at the time of the first faecal sampling is indicated by the number of children receiving each antibiotic class (Table 2). Five of the 20 (25 %) children were treated with more than two classes of antibiotics, Aminoglycoside being the most frequently prescribed. The mean CRP on admission to PICU was 67 mg/l (±10 SD), which remained raised after one week 58 mg/l (±10 SD) (Table 3).

The total faecal SCFA concentration reduced from 290 μmol/g (±90 SD) at baseline to 140 μmol/g (±0.8 SD) (Z-value-1.3018, *P*-value = 0.09) after two weeks in hospital (Table 6). The SCFA concentrations of propionate and butyrate levels were maintained during the two weeks children were in intensive care. However, due to a reduction in acetate concentration, the total SCFA concentrations lowered although this was not clinically significant (Table 4). In this small sample size, we were unable to find an association between SCFA concentration with 30 days free from intensive care or 30 days free from ventilation.

Both stool frequency and consistency improved within one week after admission to intensive care. Stool frequency reduced from 2.6 per day (±1.1 SD) to 1.2 per day (±0.45 SD), $p < 0.004$ (95 % confidence interval: 0.65; 2) and stool consistency (Bristol Stool Chart Scale) improved from 6.6 (±0.4 SD) to 3.6 (±0.4 SD), $p < 0.001$ (95 % confidence interval: 2.72, 3.72) (Table 5). At baseline, the mean weight-Z-score and height-Z-score were -1.79 (±1.7 SD); -0.9 (±0.9 SD), respectively, suggesting children admitted to our intensive care unit were in an undernourished state. After one week in hospital, the weight-Z-scores continued to decrease to -2.14 (±1.8 SD); *P*-value 0.05 (95% confidence interval:-3.69, -0.59) but normalised to baseline after two weeks (Table 6).

Table 1
Demographic characteristics and clinical details of study participants

Gender, n, (%)	
Male	14 (70)
Female	6 (30)
Age, decimal years (SD)	10.8 (5.6)
Primary Organ Failure, n (%)	
Respiratory Tract	10 (50)
Cardiovascular	4 (20)
Neurological/Traumatic Head Injury	3 (15)
Gastrointestinal Tract	3 (15)
Mean duration on ventilation, days ($\pm$SD)	9 (4)
Mean feed volume per day, ml/day ($\pm$SD)	950 (230)
Mean fiber dose, grams/day ($\pm$SD)	9.5 (3)
30 Days Free intensive care, median, (Inter Quartile Range)	8 (1, 22)
30 Days free conventional ventilation, median (Inter Quartile Range)	10 (10, 25)

Table 2
Antibiotic class prescribed on admission to intensive care

Antibiotic administered	n (%)
Aminoglycoside	10 (34)
Meropem (Carbapenems)	8 (27)
Penicillin	5 (17)
Glycopeptides Fluoroquinolones	4 (13)
Clindamycin	2 (9)

Table 3
Children's C-Reactive Protein concentration during stay in intensive care

Inflammatory marker	Admission	Week 1	Week 2
C-Reaction protein, mean (±SD)	67.17 (10)	58.75 (10)	15.00 (3)
Mean difference (±SD); *P*-value		13 (10); 0.246	35 (21); 0.001

Table 4
Comparative analysis of longitudinal faecal short chain fatty acids concentrations (μmol/g) in study participants

Short chain fatty acid	Baseline N=20	Week 1 N=20	Baseline and FLA week 1 Z value (*P*-value)	Week 2 N=12	Baseline and week 2 Z value (*P*-value)
Acetate, mean (±SD)	210 (100)	90 (70)	−1.0142 (0.3)	70 (50)	−1.7529 (0.2)
Propionate, mean (±SD)	50 (40)	80 (40)	−1.9548 (0.2)	50 (30)	−0.2962 (0.4)
Butyrate, mean (±SD)	30 (20)	30 (20)	−0.3381 (0.8)	20 (10)	−0.2011 (0.2)
Total	290 (90)	200 (100)	−0.4258 (0.7)	140 (80)	−1.3018 (0.09)

Table 5
Stool consistency and frequency of study participants after one week receiving an enteral formula with food derived ingredients

	Baseline	One week	*P*-value, (95% confidence Interval)
Stool Frequency, mean (±SD)	2.6 (1.08)	1.2 (0.45)	p<0.004, (0.65; 2)
Stool consistency, mean (±SD)	6.6 (0.4)	3.6 (0.4)	p<0.001, (2.72; 3.72)

Table 6
Anthropometric measurements of study participants who commenced high fibre Food Based

	Baseline	One week on high fibre formula	*P*-value (95% confidence interval	Two weeks on high fibre formula	*P*-value (95% confidence interval
Weight, kg Mean (±SD)	31.3 (9)	27.3 (6)	0.01 (13, 42)	30.1 (7)	0.4 (32, −12)
Weight-Z-score Mean (±SD)	−1.8 (1.7)	−2.1 (1.8)	0.05 (−3.7, −0.6)	−1.87 (0.9)	0.1 (−2.77, 0.96)

Discussion

Short-chain fatty acids are the end products of fermentation of non-digestible dietary fibers by the anaerobic intestinal microbiota, which have been shown to exert multiple beneficial effects on mammalian energy metabolism and immune function [37]. This is the first study in the United Kingdom to report on SCFA concentrations in septic critically ill children who are receiving a Food-Based formula. Our study reports that the high fibre Food-Based formula was well tolerated during children's time in intensive care; both stool frequency and consistency improved despite children being on multiple antibiotics. Additionally, our pilot study found that children maintained faecal butyrate and propionate concentrations during their admission to PICU.

Our study found that children admitted to intensive care had similar SCFA concentrations to that of healthy children (200—600 μmol/g) [38,39]. Hayakawa *et al.*, (2011) reported that critically ill patients who had all been in good health just before their admission demonstrated a significant decrease in SCFA concentration even at 6 hours after admission, indicating that faecal SCFA concentrations change immediately after severe insults [40]. The dramatic decline in the concentrations of the SCFAs, especially butyrate, apparently resulted from the reduction of obligate anaerobes and Lactobacillus in critically ill patients just after the insults. Concluding, that such an effect may further impair the recovery of these patients [40]. The complications associated with a rapid deterioration in intestinal SCFA concentration is outlined in a prospective multicentre cohort study by Wijeyesekera *et al.* (2019), who state that, the disruption to the functional activity of the intestinal microbiome may result in worsening organ failure in the critically ill child. The team identified reduced faecal excretion of SCFAs (including butyrate, propionate, and acetate), demonstrating that these metabolites also distinguished between critical illness and health. Concluding, profiling of bacterial metabolites in faecal samples may support identification and treatment of intestinal dysbiosis in critical illness [41].

Of note, in our study children on Food-Based formula maintained faecal butyrate and propionate concentrations during their time in intensive care. Our findings support Majid HA *et al.* (2014), who delivered a randomised controlled trial and found that when fiber was added to enteral formulas and administered to critically ill patients SCFA levels were maintained [42]. The delirious impact of reduced SCFAs in critical illness are described by Valdes-Duque *et al.* (2020), who performed a descriptive, multicentre, observational study to determine the concentration of faecal SCFA in critically ill patients with sepsis when compared with the control group (healthy non hospitalised adults). The results reported significantly lower concentrations of faecal SCFA in critically ill patients with sepsis than in control subjects. Concluding that due to SCFA's role in intestinal integrity, barrier function, and anti-inflammatory effect, maintaining the concentration of SCFAs may be important in the intensive care patient [43].

Our study found that a quarter of children were on at least two types of antibiotics within the first week of admission to PICU. Despite children receiving multiple antibiotics, both stool consistency and frequency improved within the first week of intensive care. Of note, the degree of sepsis as measured by the CRP concentration in children remained raised after one week, which has clinical relevance in relation to the statistically significant improvements reported in stool frequency and consistency when compared during the same time, suggesting that feed tolerance may be related to Food-Based formula as opposed to an improvement in sepsis. Our findings support those of Kamerun *et al.* (2015), who performed a meta-analysis to investigate the impact of added fiber to enteral nutrition in relation to the incidence of diarrhoea. The team reported, that overall, fiber reduces diarrhoea in patients receiving enteral nutrition (OR = 0.47; 95%CI: 0.29—0.77; P = 0.02). However, unlike our study, subgroup analysis could not attribute the beneficial impact of fibre in critically ill patients (OR = 0.89; 95% CI: 0.41—1.92; P = 0.77) [44].

The mechanisms as to why Food-Based enteral formulas are better tolerated than standard enteral formula is unclear [45]. However, it stands to reason that 'food-derived ingredients' within the enteral formula aid normal gut functioning providing a superior clinical performance to standard commercially availability enteral formula [25,46]. Similar improvements in enteral feed tolerance have been described by Samela *et al.* (2017), who reported children with intestinal failure who were experiencing diarrhoea and inconsistent stooling improved in 90% of the children who transitioned to a Food-Based formula [47]. Numerous questions remain to be answered, including to what extent microbial disturbance influences dietary needs, metabolic status, intestinal permeability, and immunity in critically ill patients. More detailed knowledge of the short- and long-term health consequences of these major shifts in intestinal bacterial communities is needed [48]. The discontinuation of inappropriate antibiotic therapy is an important target for stewardship intervention [38].

The main strength of this study is its ability to compare and combine comprehensive clinical characterisation of feed tolerance with faecal SCFA in critically ill children. As a pilot investigation, this study has limitations, the most crucial being its small sample size with no comparative group. The trial design has several limitations and despite the design's simplicity, the interpretation of the trial results must be approached pragmatically due to the inability to distinguish between the effect of the treatment, a placebo effect, and the effect of natural history. Responses could theoretically be due to the

efficacy of the treatment, a placebo effect of an inefficacious therapy, or a spontaneous or natural history improvement. For a subject that has responded, it could be argued that the subject would have responded even without treatment or that the subject responded because they thought that they were receiving efficacious therapy. Furthermore, it is also difficult to interpret the response without a frame of reference for comparison. Additionally, stool collection was difficult to obtain when diarrhoea was severe, which is a barrier associated with the clinical and pathological condition of these critically ill patients. However, a consideration for our future comparative study is the ability to assess the intestinal microbiome using urine metabolic profiling [41].

Conclusion

In this pilot study, children admitted to intensive care with sepsis tolerated a Food-Based formula. Faecal butyrate and propionate concentrations were maintained whilst feeding on a high fiber Food-Based formula. Further research is warranted to assess whether a Food-Based formula is superior to a standard enteral formula in preserving the intestinal microbiota, thereby mitigating gastrointestinal complications associated with antibiotic-related dysbiosis. A comparative validation study is required to substantiate our preliminary findings and confirm the potential impact of dietary fiber to support the recovery of healthy gut commensal populations during and after critical illness.

Funding

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Ethical approval was sought through the Health Research Authority and Health and Care Research Wales: 279901-21/PR/0809 on the 1st July 2021, and was conducted in accordance with the Declaration of Helsinki and STROBE statement.

Consent for publication

Not applicable.

Author contributions

GOC conceived and designed the study. BG and GA collected clinical data at a ward level. YS, SE and MBE analysed faecal samples and short-chain fatty acids. GOC and SE performed the statistical analyses. GOC wrote the manuscript. All the authors have read and approved the final version of the manuscript.

Conflicts interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nestle Health Science covered the costs for the mass spectrometry analysis of faecal short-chain fatty acids, which were performed independently at the

Institute of Child Health. This was a clinician-led study where all results are owned by Great Ormond Street Hospital, University College London. The authors have no other conflicts of interest.

Acknowledgements

References

[1] Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut 2016;65(2):330—910. 1136/gutjnl-2015-309990.

[2] Kindon S, Pain R, Kesby M. Participatory action research approaches and methods: connecting people, participation and place. Routledge Routledge studies in human geography London; 2007. December.

[3] McDonnell L, Gilkes A, Ashworth M, Rowland V, Harries TH, Armstrong D, et al. Association between antibiotics and gut microbiome dysbiosis in children: systematic review and meta-analysis. Gut Microbes 2021;13(1):1—1810. 1080/19490976.2020.1870402.

[4] Feng W, Liu J, Cheng H, Zhang D, Tan Y, Peng C. Dietary compounds in modulation of gut microbiota-derived metabolites. Frontiers in Nutrition 2022;910:3389. fnut.2022.939571.

[5] Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 2016;7(3):189—20010. 1080/19490976.2015.1134082.

[6] Lattimer JM, Haub MD. Effects of Dietary Fiber and Its Components on Metabolic Health. Nutrients 2010;2(12):1266—89.

[7] Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. Journal of AOAC International 2012;95(1):50—6010. 5740/jaoacint.sge_macfarlane.

[8] Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de Los Reyes-Gavilán CG, Salazar N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. Front Microbiol 2016;7:18510. 3389/fmicb.2016.00185.

[9] Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. Clinical & Translational Immunology 2016;5(4):e7310. 1038/cti.2016.17.

[10] Kim CH. Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. Cellular & Molecular Immunology 2021;18(5):1161—7110. 1038/s41423-020-00625-0.

[11] Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet 2020;395(10219):200—1110. 1016/s0140-6736(19)32989-7.

[12] Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. Jama 2009;302(21):2323—910. 1001/jama.2009.1754.

[13] Bhalodi AA, van Engelen TSR, Virk HS, Wiersinga WJ. Impact of antimicrobial therapy on the gut microbiome. Journal of Antimicrobial Chemotherapy 2019;74(Supplement_1):i6—1510. 1093/jac/dky530.

[14] Moron R, Galvez J, Colmenero M, Anderson P, Cabeza J, Rodriguez-Cabezas ME. The Importance of the Microbiome in Critically Ill Patients: Role of Nutrition. Nutrients 2019;11(12). https://doi.org/10.3390/nu11123002.

[15] Zaborin A, Smith D, Garfield K, Quensen J, Shakhsheer B, Kade M, et al. Membership and behavior of ultra-low-diversity pathogen communities present in the gut of humans during prolonged critical illness. mBio 2014;5(5). e01361-1410.1128/mBio.01361-14.

[16] Rooney AM, Timberlake K, Brown KA, Bansal S, Tomlinson C, Lee KS, et al. Each Additional Day of Antibiotics Is Associated With Lower Gut Anaerobes in Neonatal Intensive Care Unit Patients. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America 2020;70(12):2553—6010. 1093/cid/ciz698.

[17] Shah T, Baloch Z, Shah Z, Cui X, Xia X. The Intestinal Microbiota: Impacts of Antibiotics Therapy, Colonization Resistance, and Diseases. International Journal of Molecular Sciences 2021;22(12):6597.

[18] Kesavelu D, Jog P. Current understanding of antibiotic-associated dysbiosis and approaches for its management. Therapeutic Advances in Infectious Disease 2023;10. 2049936123115444310.1177/20499361231154443.

[19] Tume L, Carter B, Latten L. A UK and Irish survey of enteral nutrition practices in paediatric intensive care units. The British Journal of Nutrition 2013;109(7):1304—2210. 1017/s0007114512003042.

[20] Chouraqui JP, Michard-Lenoir AP. [Feeding infants and young children with acute diarrhea]. Archives de pediatrie: organe officiel de la Societe francaise de pediatrie 2007;14(Suppl 3):S176—8010. 1016/s0929-693x(07)80024-8.

[21] Oami T, Chihade DB, Coopersmith CM. The microbiome and nutrition in critical illness. Current Opinion in Critical Care 2019;25(2):145—910. 1097/mcc.0000000000000582.

[22] Choy A, Freedberg DE. Impact of microbiome-based interventions on gastrointestinal pathogen colonization in the intensive care unit. Therapeutic Advances in Gastroenterology 2020;13. 17562848209394471010.1177/1756284820939447.

[23] Schmitz É PCR, Silva ECD, Lins Filho OL, Antunes MMC, Brandt KG. Blenderized tube feeding for children: an integrative review, 40. Revista paulista de pediatria : orgao oficial da Sociedade de Pediatria de Sao Paulo; 2021. p. e202041910. 1590/1984-0462/2022/40/2020419.

[24] Batsis ID, Davis L, Prichett L, Wu L, Shores D, Au Yeung K, et al. Efficacy and Tolerance of Blended Diets in Children Receiving Gastrostomy Feeds. Nutrition in Clinical Practice 2020;35(2):282—8. https://doi.org/10.1002/ncp.10406.

[25] Coad J, Toft A, Lapwood S, Manning J, Hunter M, Jenkins H, et al. Blended foods for tube-fed children: a safe and realistic option? A rapid review of the evidence. Archives of Disease in Childhood 2017;102(3):27410. 1136/archdischild-2016-311030.

[26] O'Connor G, Watson M, Van Der Linde M, Bonner RS, Hopkins J, Saduera S. Monitor gastrointestinal tolerance in children who have switched to an "enteral formula with food-derived ingredients": A national, multicenter retrospective chart review (RICIMIX study). Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition 2022;37(4):929—3410. 1002/ncp.10812.

[27] Dipasquale V, Diamanti A, Trovato CM, Elia D, Romano C. Real food in enteral nutrition for chronically ill children: overview and practical clinical cases. Current Medical Research and Opinion 2022;38(5). 831-510.1080/03007995.2022.2052514.

[28] De la Cuesta-Zuluaga J, Mueller NT, Álvarez-Quintero R, Velásquez-Mejía EP, Sierra JA, Corrales-Agudelo V, et al. Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. Nutrients 2019;11(1):51.

[29] Huysentruyt K, Koppen I, Benninga M, Cattaert T, Cheng J, De Geyter C, et al. The Brussels Infant and Toddler Stool Scale: A Study on Interobserver Reliability. Journal of Pediatric Gastroenterology and Nutrition 2019;68(2):207—1310. 1097/mpg.0000000000002153.

[30] Bernard AC, Magnuson B, Tsuei BJ, Swintosky M, Barnes S, Kearney PA. Defining and Assessing Tolerance in Enteral Nutrition. Nutrition in Clinical Practice 2004;19(5):481—610. 1177/0115426504019005481.

[31] Heaton KW, O'Donnell LJ. An office guide to whole-gut transit time. Patients' recollection of their stool form. Journal of Clinical Gastroenterology 1994;19(1):28—3010. 1097/00004836-199407000-00008.

[32] Russo M, Strisciuglio C, Scarpato E, Bruzzese D, Casertano M, Staiano A. Functional Chronic Constipation: Rome III Criteria Versus Rome IV Criteria. Journal of Neurogastroenterology and Motility 2019;25(1):123—810. 5056/jnm18035.

[33] McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Digestive Diseases (Basel, Switzerland) 1998;16(5):292—30710. 1159/000016879.

[34] Hengst JM. The role of C-reactive protein in the evaluation and management of infants with suspected sepsis. Advances in Neonatal Care : Official Journal of the National Association of Neonatal Nurses 2003;3(1):3—1310. 1053/adnc.2003.50010.

[35] Cole TJ. The development of growth references and growth charts. Annals of Human Biology 2012;39(5):382—9410. 3109/03014460.2012.694475.

[36] WHO. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatrica (Oslo, Norway : 1992) Supplement 2006;450:76—8510. 1111/j.1651-2227.2006.tb02378.x.

[37] den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. Journal of Lipid Research 2013;54(9):2325—4010. 1194/jlr.R036012.

[38] Łoniewska B, Fraszczyk-Tousty M, Tousty P, Skonieczna-Żydecka K, Maciejewska-Markiewicz D, Łoniewski I. Analysis of Fecal Short-Chain Fatty Acids (SCFAs) in Healthy Children during the First Two Years of Life: An Observational Prospective Cohort Study. Nutrients 2023;15(2). https://doi.org/10.3390/nu15020367.

[39] Gio-Batta M, Sjöberg F, Jonsson K, Barman M, Lundell AC, Adlerberth I, et al. Fecal short chain fatty acids in children living on farms and a link between valeric acid and protection from eczema. Scientific Reports 2020;10(1). 2244910.1038/s41598-020-79737-6.

[40] Hayakawa M, Asahara T, Henzan N, Murakami H, Yamamoto H, Mukai N, et al. Dramatic changes of the gut flora immediately after severe and sudden insults. Digestive Diseases and Sciences 2011;56(8):2361—510. 1007/s10620-011-1649-3.

[41] Wijeyesekera A, Wagner J, De Goffau M, Thurston S, Rodrigues Sabino A, Zaher S, et al. Multi-Compartment Profiling of Bacterial and Host Metabolites Identifies Intestinal Dysbiosis and Its Functional Consequences in the Critically Ill Child. Crit Care Med 2019;47(9):e727—3410. 1097/ccm.0000000000003841.

[42] Majid HA, Cole J, Emery PW, Whelan K. Additional oligofructose/inulin does not increase faecal bifidobacteria in critically ill patients receiving enteral nutrition: a randomised controlled trial. Clinical Nutrition (Edinburgh, Scotland) 2014;33(6):966—7210. 1016/j.clnu.2013.11.008.

[43] Valdés-Duque BE, Giraldo-Giraldo NA, Jaillier-Ramírez AM, Giraldo-Villa A, Acevedo-Castaño I, Yepes-Molina MA, et al. Stool Short-Chain Fatty Acids in Critically Ill Patients with Sepsis. Journal of the American College of Nutrition 2020;39(8):706—1210. 1080/07315724.2020.1727379.

[44] Kamarul Zaman M, Chin KF, Rai V, Majid HA. Fiber and prebiotic supplementation in enteral nutrition: A systematic review and meta-analysis. World Journal of Gastroenterology 2015;21(17):5372—8110. 3748/wjg.v21.i17.5372.

[45] Chandrasekar N, Dehlsen K, Leach ST, Krishnan U. Exploring Clinical Outcomes and Feasibility of Blended Tube Feeds in Children. JPEN Journal of Parenteral and Enteral Nutrition 2021;45(4):685—9810. 1002/jpen.2062.

[46] Klosterbuer A, Roughead ZF, Slavin J. Benefits of dietary fiber in clinical nutrition. Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition 2011;26(5):625—3510. 1177/0884533611416126.

[47] Samela K, Mokha J, Emerick K, Davidovics ZH. Transition to a Tube Feeding Formula With Real Food Ingredients in Pediatric Patients With Intestinal Failure. Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition 2017;32(2):277—8110. 1177/0884533616661011.

[48] Lankelma JM, van Vught LA, Belzer C, Schultz MJ, van der Poll T, de Vos WM, et al. Critically ill patients demonstrate large interpersonal variation in intestinal microbiota dysregulation: a pilot study. Intensive Care Medicine 2017;43(1):59—6810. 1007/s00134-016-4613-z.