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Impact of Protein–energy Malnutrition on Growth, Body Composition, and Gut Short-chain Fatty ACID Profiles in Formula-fed Infants Compared to Breastfed Controls With Congenital Heart Defects

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Article

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Abstract

Background/Objectives: This study examined whether PEM severity in formula-fed infants with CHD is associated with alterations in body composition and fecal short-chain fatty acid (SCFA) concentrations. The primary analytical comparison was conducted within the CHD cohort according to PEM severity (Grade I vs. Grade II). Comparisons with an exclusively breastfed CHD reference group without PEM were included to provide contextual reference values.

Methods: In this observational cross-sectional single-center study, we enrolled 46 infants aged 0–12 months with confirmed congenital heart defects (CHD). Twenty-six formula-fed infants had PEM (Grade I, n = 15; Grade II, n = 11), and 20 age-matched exclusively breastfed infants with CHD and normal nutritional status served as the reference group. Anthropometry was assessed using WHO standards, body composition was measured by bioelectrical impedance analysis, and fecal SCFAs (C2–C6 and isoforms) were quantified by gas chromatography. Group comparisons were performed using one-way ANOVA (with Tukey's post hoc test) for approximately symmetric variables and Kruskal–Wallis testing (with Dunn's post hoc comparisons and Benjamini–Hochberg FDR correction) for non-normally distributed variables. Multivariable regression models adjusted for age, sex, and CHD type.

Results: Compared with the reference group, infants with PEM had lower BMI and smaller central anthropometric measures (all $p < 0.001$). Within the CHD cohort, fat mass was 22% lower in Grade II than in Grade I PEM (2.65 vs. 3.40 kg; $p = 0.018$), and PEM severity independently predicted reduced fat mass ($\beta = -0.74$ kg; 95% CI: −1.32 to −0.16; $p = 0.018$). Total fecal SCFA concentrations decreased progressively with increasing PEM severity (Kruskal–Wallis, $p < 0.001$; $\varepsilon^2 \approx 0.51$). **Conclusions:** In infants with CHD, increasing PEM severity is associated with reduced fat mass and lower fecal SCFA concentrations. These findings suggest an association between nutritional status severity and microbial metabolic output in this high-risk population.

1. Introduction

Congenital heart defects (CHD) are among the most common congenital anomalies, affecting approximately 1% of live births worldwide. Infants with CHD are particularly vulnerable to protein–energy malnutrition (PEM) due to increased energy demands, frequent hospitalizations, and feeding difficulties. Early nutritional deficits can impair growth, alter body composition, and disrupt metabolic processes that are critical during the first year of life [1, 2, 3].

Globally, exclusive breastfeeding rates remain suboptimal. According to UNICEF and WHO, fewer than 50% of infants under six months of age are exclusively breastfed worldwide, and rates are often lower among medically fragile populations [4, 5]. Infants with severe CHD may struggle with breastfeeding because of fatigue, impaired coordination of sucking and swallowing, and reduced feeding efficiency, which can limit nutrient intake [4, 5]. Consequently, many infants rely on formula feeding, which, while essential, may not fully replicate the protective and nutritional benefits of human milk.

Formula-fed infants with CHD are therefore at increased risk of PEM, which may contribute to impaired growth and adverse changes in body composition. Disruptions in gut microbial metabolic activity—reflected by altered SCFA profiles—may also be associated with impaired energy metabolism and intestinal function [3, 6]. Quantification of fecal short-chain fatty acids represents a targeted metabolomic approach that provides functional insight into the biochemical output of the gut microbiota. Despite advances in cardiac care, nutritional status remains a key determinant of outcomes in infants with CHD [6]. Early recognition of PEM and targeted nutritional strategies are essential to support growth, body composition, and gut health, particularly in formula-fed infants with CHD who do not receive the full benefits of breast milk. Accordingly, this study aimed to evaluate whether PEM severity within formula-fed infants with CHD is associated with altered body composition and fecal SCFA concentrations. We further compared these findings with those of a reference group of clinically stable infants with CHD without PEM to provide contextual reference values [1, 7]. The primary outcome was total fecal SCFA concentration. Secondary outcomes included individual SCFA fractions (C2, C3, C4, and isoforms), anthropometric indices, and bioimpedance-derived body composition parameters (fat mass, active cell mass, skeletal muscle mass, and related measures).

2. Materials and Methods

2.1. Study Design and Participants

This observational cross-sectional single-center study was conducted at the Specialized Scientific and Practical Medical Center of Cardiology and Cardiac Surgery of the Aral Sea region. A total of 46 infants during the first year of life (20 girls and 26 boys; mean age 0.75 ± 0.25 years) were enrolled. All participants had confirmed congenital heart defects (CHD) in the preoperative period. Infants were stratified according to nutritional status. The PEM cohort consisted of 26 infants with secondary protein–energy malnutrition (PEM), including 15 infants classified as Grade I and 11 infants as Grade II PEM. The comparison group included 20 age-matched infants with CHD who demonstrated normal nutritional status, defined as weight-for-length and BMI-for-age Z-scores ≥ −2 SD according to WHO standards [11]. These infants had no anthropometric or body composition abnormalities and were exclusively breastfed at the time of assessment. Because feeding modality was intrinsically linked to nutritional status in the present cohort (formula feeding in PEM groups and exclusive breastfeeding in the reference group), feeding type could not be independently analyzed as an exposure variable, and comparisons involving the reference group should therefore be interpreted cautiously. The sample size reflects the total number of eligible patients during the recruitment period in this geographically limited population.

2.2. Inclusion and Exclusion Criteria

Inclusion criteria were: infants of both sexes aged 0–1 year with a confirmed diagnosis of CHD, receiving exclusive formula feeding (for PEM groups), without congenital anomalies of other organs or known genetic disorders. Eligible participants had no history of gastrointestinal disease and had not

received antibiotics, hormonal therapy, or other medications for at least 10 days prior to fecal sample collection [1,6,24].

Exclusion criteria included acute illness, use of antibiotics, statins, glucocorticoids, or cytostatic agents, mixed or exclusive breastfeeding (for PEM groups), genetic syndromes, congenital anomalies of other organ systems, and lack of parental consent.

2.3.Classification of CHD

Congenital heart defect (CHD) diagnoses were confirmed by pediatric cardiologists based on transthoracic echocardiography and clinical assessment. Defects were classified according to anatomical type and hemodynamic characteristics and categorized as acyanotic or cyanotic lesions.

In the overall cohort (n = 46), ventricular septal defect (VSD) was the most common diagnosis, identified in 30 patients (65.2%). Patent ductus arteriosus (PDA) was present in 5 children (10.9%), atrial septal defect (ASD) in 4 children (8.7%), pulmonary artery stenosis in 2 children (4.3%), and tetralogy of Fallot (TOF) in 5 children (10.9%).

When grouped by physiological classification, acyanotic CHD accounted for 41 of 46 cases (89.1%), whereas cyanotic lesions (tetralogy of Fallot) comprised 5 of 46 cases (10.9%).

The distribution of CHD types across study groups was as follows:

• PEM Grade I (n = 15): VSD (8, 53.3%), ASD (2, 13.3%), PDA (2, 13.3%), pulmonary artery stenosis (2, 13.3%), TOF (1, 6.7%).
• PEM Grade II (n = 11): VSD (7, 63.6%), TOF (3, 27.3%), PDA (1, 9.1%).
• Control group (n = 20): VSD (15, 75.0%), ASD (2, 10.0%), PDA (2, 10.0%), TOF (1, 5.0%).

Hemodynamic significance was determined using echocardiographic parameters, including shunt magnitude, chamber enlargement, and pulmonary arterial pressure. Lesions were categorized as hemodynamically significant or non-significant according to established pediatric cardiology criteria. Structural complexity was further classified as simple, moderate, or complex in accordance with the American College of Cardiology/American Heart Association (ACC/AHA) classification system [9].

2.4.Definition of Formula Feeding

In the present study, “formula-fed” was defined as exclusive artificial feeding from birth without exposure to breast milk. All infants in the PEM groups were exclusively fed commercial infant formulas during the preoperative period (from birth up to 12 months of age).Standard cow’s milk–based formulas were used, including NAN OptiPro®, Malyutka®, and Bellakt®. No infants received specialized hydrolyzed, high-calorie, or therapeutic formulas. The estimated caloric intake was assessed using the WHO CINDI dietary questionnaire. In the majority of infants with CHD and PEM, daily caloric intake was below age-adjusted recommendations, reflecting reduced feeding volume and limited energy

consumption (kcal/kg/day), primarily due to feeding intolerance, fatigue during feeding, and increased metabolic demands associated with CHD.

2.5. Anthropometric and Body Composition Assessment

Anthropometric assessment included body weight, length, and waist and hip circumferences. Body mass index (BMI) was calculated as weight/height² and expressed as BMI Z-scores (BMI SDS). Physical development was evaluated using WHO Anthro and AnthroPlus software according to age- and sex-specific WHO reference standards [9,10]. The waist-to-hip ratio (WHR) was calculated [11,12].

Body composition was assessed using a SECA mBCA 514/525 medical bioelectrical impedance analyzer. Measurements were performed in the supine position under standardized pediatric conditions at least 3 hours after feeding to minimize hydration-related variability. The device applies proprietary pediatric-adjusted prediction algorithms incorporating impedance, body weight, height, age, and sex; the specific equations are not publicly disclosed. Given the limited availability of universally validated prediction equations for infants under 12 months of age, BIA-derived parameters were interpreted primarily for within-cohort comparative analysis rather than absolute quantification [13,14]. Therefore, BIA-derived absolute values should be interpreted cautiously in infants under 12 months. The device was calibrated according to the manufacturer's recommendations. Each measurement was performed twice, and mean values were used for analysis (coefficient of variation <5%). Recorded parameters included fat mass (FM), fat-free mass (FFM), skeletal muscle mass (SMM), active cell mass (ACM), basal metabolic rate (BMR), total body water (TBW), and extracellular water (ECW).

Protein–energy malnutrition (PEM) severity was classified according to WHO weight-for-length Z-scores (WLZ) using WHO Anthro software. Grade I PEM corresponded to moderate acute malnutrition (−3 SD ≤ WLZ < −2 SD), and Grade II PEM corresponded to severe acute malnutrition (WLZ < −3 SD). Infants with WLZ ≥ −2 SD were classified as having normal nutritional status. As a sensitivity analysis, PEM classification was additionally evaluated using BMI-for-age Z-scores (BAZ), yielding consistent group allocation [11]. Cross-classification of WLZ and BAZ categories demonstrated high concordance (93.5% agreement; Supplementary Table S6). Bioelectrical impedance analysis (BIA) was performed in all enrolled infants (n = 46). Summary statistics are presented in Table 2, and individual participant-level BIA profiles are provided in Supplementary Table S2.Descriptive BIA parameters were obtained for both PEM and control groups. Inferential comparisons were primarily conducted between PEM Grade I and Grade II subgroups to evaluate differences according to malnutrition severity, whereas the control group served as a normative reference group.

2.6. Fecal Sample Collection and Short-Chain Fatty Acid Analysis

Fecal samples were collected immediately after spontaneous defecation into sterile containers. Samples were frozen at −22 °C during transport (≤12 h) [3,15,16], and subsequently stored at −80 °C until analysis to preserve metabolite stability.

Sample Preparation. Approximately 0.5 g of fecal material was homogenized in 5 mL of distilled water and centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was acidified with 50% sulfuric acid (final pH < 2.0) prior to chromatographic analysis.

Gas Chromatographic Analysis. SCFAs (C2–C6 and isoforms) were quantified using a GC-2010 system (Shimadzu, Japan) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 μm FFAP capillary column. Injector and detector temperatures were maintained at 200 °C and 250 °C, respectively. Nitrogen served as the carrier gas at a flow rate of 1.2 mL/min.Quantification was performed using the manufacturer's pre-configured external calibration integrated into the instrument software. No internal standard was applied. The limits of detection (LOD) were 0.5 μmol/g for acetic acid and 0.1 μmol/g for propionic and butyric acids. The intra-assay coefficient of variation ranged from 3.2% to 6.5%. Calibration curves were constructed over a concentration range of 0.1–50 μmol/g for each SCFA fraction. Linearity was considered acceptable at a coefficient of determination (R^2) ≥ 0.995. Detailed analytical validation parameters are provided in Supplementary Table S5. Quality control was ensured by duplicate sample analysis and periodic measurement of pooled QC samples throughout the analytical run. Absolute concentrations were expressed as μmol/g of wet fecal weight. Total SCFA content was calculated as the sum of all measured fractions. The anaerobic index (C2/C4 ratio) was calculated as an integrative marker of gut microbial metabolic activity [20,21]. Values below the detection limit were assigned the minimum detectable concentration. Dietary intake was assessed using the WHO CINDI standardized questionnaire [20,21,25].

2.7. Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan (protocol code: 6/15-5107; date of approval: 27 May 2025). Written informed consent was obtained from the parents or legal guardians of all participants prior to inclusion in the study.

No identifiable personal data or images are included in this study.

2.8. Statistical Analysis

The primary outcome was total fecal SCFA concentration. Secondary outcomes included individual SCFA fractions (C2–C6), anthropometric parameters, and body composition indices derived from bioelectrical impedance analysis. Statistical analyses were performed using IBM SPSS Statistics version 30.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro–Wilk test [26]. Continuous variables are reported as median (interquartile range, IQR) for non-normally distributed data and as mean ± SD for approximately symmetric data; categorical variables are reported as counts and percentages. No outliers were excluded from the analysis; interquartile ranges were calculated using the full dataset. For comparisons across three groups (PEM Grade I, PEM Grade II, and the reference group), one-way ANOVA with Tukey's post hoc testing was used for approximately symmetric variables. For non-normally distributed variables, the Kruskal–Wallis test was applied,

followed by Dunn's post hoc pairwise comparisons with Benjamini–Hochberg false discovery rate (FDR) correction. The Kruskal–Wallis effect size was quantified using epsilon-squared (ε^2), calculated as $\varepsilon^2 = (H - k + 1) / (n - k)$, where H is the Kruskal–Wallis statistic, k is the number of groups, and n is the total sample size. Pearson or Spearman correlation coefficients were calculated, depending on data distribution by Zar [27], to assess associations between fat mass, BMI, and PEM severity. The effect size for correlations was reported as r, with values of 0.1, 0.3, and 0.5 representing small, medium, and large effects, respectively. Correlation strength was interpreted as weak ($r < 0.3$), moderate (0.3–0.5), and strong (>0.5). A p-value < 0.05 was considered statistically significant.

Multivariable linear regression analysis was conducted to evaluate the independent association between PEM severity and body composition parameters. Fat mass was included as the dependent variable. Age, sex, and CHD type were entered as covariates based on clinical relevance. Multicollinearity was assessed using variance inflation factors (VIF < 5 was considered acceptable), and model assumptions were verified by inspection of standardized residual plots. A two-sided p-value < 0.05 was considered statistically significant.

An a priori power analysis was conducted using G*Power version 3.1.9.7 (Heinrich Heine University Düsseldorf, Germany) [28], to determine the required sample size. Because subgroup sizes were relatively small (particularly the Grade II PEM group, n = 11), multivariable regression analyses should be considered exploratory and interpreted with caution. The models were therefore limited to a small number of clinically relevant covariates to reduce the risk of overfitting. To achieve a power ($1 - \beta$) of 0.80 with a significance level (α) of 0.05 and a predicted medium effect size ($f = 0.25$) for group comparisons, a total sample size of 46 infants was required. Descriptive statistics are presented as mean ± standard deviation (SD) with 95% confidence intervals (CI) for normally distributed variables, or as median with interquartile range (IQR) for non-parametric data. Therefore, inclusion of 46 infants in the study was considered sufficient to detect clinically meaningful differences. Given the exploratory nature of subgroup analyses, effect sizes were emphasized alongside p-values to enhance interpretability.

3. Results

3.1 Anthropometric Characteristics

Between September and December 2025, a total of 46 infants with congenital heart defects (CHD) were enrolled in the study. Of these, 26 were formula-fed infants diagnosed with protein–energy malnutrition (PEM), and 20 were exclusively breastfed infants with normal nutritional status. All participants underwent standardized anthropometric and body composition assessment in the Khorezm region, Uzbekistan.

The distribution of CHD phenotypes within the cohort is presented in Figure 1. Ventricular septal defect (VSD) was the most common anomaly, accounting for 30 of 46 cases (65.2%).

Baseline demographic and clinical characteristics were comparable across study groups (Supplementary Table S10). There were no significant differences in age distribution (p = 0.842) or sex ratio (p = 0.985) between PEM Grade I, PEM Grade II, and control infants. Acyanotic CHD predominated in all groups, accounting for 93.3% of cases in Grade I, 72.7% in Grade II, and 95.0% in controls. Cyanotic lesions (tetralogy of Fallot) were more frequent in Grade II infants (27.3%) compared with Grade I (6.7%) and controls (5.0%), although this difference did not reach statistical significance (p = 0.214). CHD structural complexity did not differ significantly between groups (p = 0.912).

Infants with PEM grade I exhibited significantly lower anthropometric indices compared to controls (p < 0.001; Table 1).

.**Table 1.** Anthropometric characteristics of infants with congenital heart defects (CHD) stratified by protein–energy malnutrition (PEM) severity. WHR—waist-to-hip ratio.

Parameter	PEM Grade I (n=15)	PEM Grade II (n=11)	Control (n=20)	Overall p-value
BMI (kg/m²)	14.74 ± 0.73	13.52 ± 0.96	16.51 ± 1.32	<0.001
Post-hoc	I vs II: 0.004	I vs C: <0.001	II vs C: <0.001	
Waist (cm)	37.32 ± 1.92	34.52 ± 1.86	43.69 ± 2.77	<0.001
Post-hoc	I vs II: 0.006	I vs C: <0.001	II vs C: <0.001	
Hip (cm)	30.59 ± 2.63	27.39 ± 1.83	34.69 ± 3.39	<0.001
Post-hoc	I vs II: 0.011	I vs C: <0.001	II vs C: <0.001	
WHR	0.88 ± 0.02	0.82 ± 0.03	0.78 ± 0.03	<0.001
Post-hoc	I vs II: 0.002	I vs C: <0.001	II vs C: 0.003	

Data are presented as mean ± standard deviation (SD). Overall group differences were assessed using one-way ANOVA. Pairwise comparisons were performed using Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant

These results indicate progressive anthropometric impairment with increasing PEM severity, with the most pronounced deficits observed in Grade II

3.2. Body Composition Assessment

Bioelectrical impedance analysis (BIA) was performed in all enrolled infants (n = 46). Descriptive parameters were obtained for both PEM and control groups; however, inferential comparisons of body composition focused on PEM Grade I and Grade II subgroups to evaluate differences according to malnutrition severity, as control infants demonstrated values within age-appropriate reference ranges. Median BMI Z-scores were lower in Grade II (−3.66 [−3.99, −3.35]) than in Grade I (−2.56 [−2.97, −2.25]); however, this difference did not reach statistical significance (Mann–Whitney U test, p = 0.08). Fat mass

(FM) was significantly higher in Grade I (3.4 [3.1–3.6] kg) compared with Grade II (2.65 [2.2–2.95] kg; p = 0.018). Absolute basal metabolic rate (BMR) was also higher in Grade I (176.5 [170–183] kcal/day) than in Grade II (168.2 [162–175] kcal/day; p = 0.024). In contrast, when normalized per kilogram of body weight, specific BMR (kcal/kg/day) was significantly higher in Grade II (p = 0.028). Active cell mass (%) was modestly but significantly higher in Grade II (p = 0.045). These findings may reflect lower total body mass in more severe PEM, resulting in relatively greater proportional representation of metabolically active tissue. No statistically significant differences were observed for fat-free mass (FFM), skeletal muscle mass (SMM), total body water (TBW), or extracellular water (ECW) between the PEM groups (all p > 0.05) (Table 2).

Table 2. Body composition parameters in infants with CHD according to PEM severity. ACM—active cell mass; BMR—basal metabolic rate; FFM—fat-free mass; SMM—skeletal muscle mass; TBW—total body water; ECW—extracellular water.

Parameter	PEM Grade I (n=15)	PEM Grade II(n=11)	p-value
BMI Z-score	−2.56 [−2.97;−2.25]	−3.66 [−3.99; −3.35]	0.08
Fat Mass (FM, kg)	3.4 [3.1–3.6]	2.65 [2.2–2.95]	0.018
BMR (kcal/day)	176.5 [170–183]	168.2 [162–175]	0.024
ACM (%)	56 [53.1–57.6]	58 [55–60]	0.045
sBMR (kcal/kg/day)	68.5 [65–74]	72 [68–76]	0.028
FFM (kg)	10.2 [9.8–10.6]	10.5 [10.0–11.0]	>0.05
SMM (kg)	4.5 [4.2–4.8]	4.7 [4.4–5.0]	>0.05
TBW (L)	7.1 [6.8–7.5]	7.3 [7.0–7.7]	>0.05
ECW (L)	2.4 [2.2–2.5]	2.5 [2.3–2.6]	>0.05
Bone Mass Reduct., n (%)	33.3%	63.6%	0.039

*Data are presented as median [interquartile range].

Group comparisons were performed using the Mann–Whitney U test.

Data are presented as median [interquartile range] or percentage, as appropriate. Group comparisons were performed using the Mann–Whitney U test. Statistically significant differences are shown in bold (p < 0.05).

Multivariable linear regression models were constructed to examine the independent association between PEM severity and body composition parameters, adjusting for age, sex, and CHD type. In Model 1 (fat mass as the dependent variable), PEM severity (Grade II vs. Grade I) was independently associated with lower fat mass (β = −0.74 kg, 95% CI: −1.32 to −0.16; p = 0.018), whereas age, sex, and CHD type

were not significant predictors. In Model 2 (fat-free mass as the dependent variable), PEM severity ($p < 0.001$), age ($p < 0.001$), and sex ($p = 0.015$) were all independently associated with fat-free mass, indicating that lean tissue mass is influenced not only by malnutrition severity but also by developmental and sex-related factors. The strong age dependency of fat-free mass is consistent with expected physiological increases in lean tissue during infancy.

Additional multivariable models were constructed for selected SCFA fractions (total SCFA and butyrate). Model diagnostics demonstrated acceptable residual normality (Shapiro–Wilk $p > 0.05$), low multicollinearity (VIF < 2), and absence of influential observations (Cook's D < 0.5). Adjusted R^2 values ranged from 0.398 to 0.781, indicating moderate to substantial explanatory power (Supplementary Table S4).

An exploratory regression model including both PEM severity and feeding modality was constructed for total SCFA concentrations. However, because feeding modality and nutritional status were structurally linked in the study design, this model should be interpreted cautiously and cannot disentangle independent effects.

Bone mass reduction was observed in 33.3% of Grade I and 63.6% of Grade II infants. Male infants had higher skeletal muscle mass than females in both PEM groups ($p < 0.05$). No significant sex-related differences were observed for total or individual fecal SCFA concentrations ($p > 0.05$; Supplementary Table S7). A strong positive correlation was observed between fat mass and BMI ($r = 0.81$, $p < 0.001$), confirming internal consistency between anthropometric and bioimpedance-derived adiposity measures. In contrast, fat mass was inversely correlated with PEM severity ($r = -0.72$, $p < 0.05$), reflecting progressive depletion of adipose tissue with increasing malnutrition severity.

3.3. SCFA Profile

In infants under one year of age with Grade I and II PEM, we observed a pattern consistent with reduced saccharolytic fermentation activity. Total fecal SCFA concentrations differed significantly across groups (Kruskal–Wallis, $p < 0.001$), with the lowest values observed in Grade II PEM (18.10 [13.47–24.08] μmol/g), followed by Grade I PEM (29.36 [27.55–30.18] μmol/g), and the highest concentrations in the reference group (34.30 [30.45–40.36] μmol/g) (Table 3). Post hoc Dunn comparisons with Benjamini–Hochberg FDR correction demonstrated significant differences between all pairwise group comparisons. Detailed pairwise comparisons with effect size estimates are presented in Supplementary Table S3. The magnitude of the group effect was large ($\varepsilon^2 \approx 0.51$), indicating that approximately 51% of the variance in total SCFA concentrations was explained by group membership. Effect size estimates for all group comparisons and correlation analyses are summarized in Supplementary Table S8.

In contrast to the major saccharolytic SCFAs, branched-chain fatty acids (iso-C4, iso-C5, and iso-C6) were present at substantially lower absolute concentrations. Although statistically significant differences across groups were detected, the magnitude of these differences was modest and unlikely to be

clinically meaningful, suggesting relative stability of proteolytic fermentation activity despite reduced saccharolytic output.

Table 3. Fecal short-chain fatty acid (SCFA) concentrations across study groups

SCFA (μmol/g)	PEM Grade I (n = 15) median [IQR]	PEM Grade II (n = 11) median [IQR]	Control (n = 20) median [IQR]	p-value (Kruskal–Wallis)
Acetate (C2)	16.50 [15.50–16.95]	8.40 [6.00–11.15]	18.20 [15.80–21.30]	< 0.001
Propionate (C3)	6.08 [5.82–6.20]	4.50 [3.30–5.95]	8.40 [7.45–9.80]	< 0.001
Butyrate (C4)	4.35 [4.13–4.45]	3.10 [2.30–4.00]	5.90 [5.50–7.00]	< 0.001
Iso-butyric (iso-C4)	1.20 [1.13–1.23]	1.00 [0.88–1.20]	0.90 [0.84–1.03]	< 0.001
Iso-valeric (iso-C5)	0.80 [0.75–0.84]	0.70 [0.63–0.83]	0.65 [0.59–0.75]	0.011
Iso-caproic (iso-C6)	0.50 [0.45–0.54]	0.40 [0.37–0.46]	0.34 [0.30–0.39]	< 0.001
Total SCFA	29.36 [27.55–30.18]	18.10 [13.47–24.08]	34.30 [30.45–40.36]	< 0.001

Similarly, propionate (C3) and butyrate (C4) concentrations were significantly lower in infants with PEM compared with the reference group (Kruskal–Wallis, $p < 0.001$ for both comparisons). Median C3 concentrations were 6.08 [5.82–6.20] μmol/g in Grade I PEM and 4.50 [3.30–5.95] μmol/g in Grade II PEM, compared with 8.40 [7.45–9.80] μmol/g in the reference group. Median C4 concentrations were 4.35 [4.13–4.45] μmol/g in Grade I PEM and 3.10 [2.30–4.00] μmol/g in Grade II PEM, compared with 5.90 [5.50–7.00] μmol/g in the reference group (Table 3). These differences are illustrated in Figure 3.

The magnitude of the group effect was large ($\varepsilon^2 = 0.51$), indicating substantial between-group differences in total fecal SCFA concentrations. A strong negative correlation was observed between total fecal SCFA concentrations and fecal pH ($r = -0.58$, $p < 0.05$; Figure 4A). In contrast, fecal pH showed a moderate positive correlation with PEM severity ($r = 0.38$, $p < 0.05$; Figure 4B).

3.4. SCFAs and Nutritional Factors

The impact of dietary factors on fecal short-chain fatty acid (SCFA) levels in infants with congenital heart defects (CHD) was investigated. A negative correlation was observed between fecal propionic acid (C3) levels in formula-fed CHD patients and the frequency of early introduction of commercial cereals into their diet (Spearman's correlation coefficient = −0.276, $p = 0.017$).

In contrast, among breastfed infants in the control group, there was a positive correlation between the total fecal monocarboxylic acid content and the weekly intake of dietary fiber from fruits and vegetables (Spearman's correlation coefficient = 0.277, p = 0.012). r = −0.58, 95% CI: −0.74 to −0.39, p < 0.05.

No other dietary factors included in the analysis demonstrated a significant effect on fecal SCFA concentrations in infants with CHD.

3.5. SCFAs and Body Mass Index (BMI)

Comparison of SCFA profiles across study groups revealed significant differences in the relative abundance of propionic acid (C3), a key microbial metabolite implicated in glucose and lipid metabolism. Pairwise comparisons indicated that infants with underweight or protein–energy malnutrition exhibited lower relative C3 levels than their normal-weight counterparts (Mann–Whitney U test, p = 0.05 and p = 0.018, respectively).

These results suggest that impaired microbial fermentation in infants with CHD and PEM may contribute to suboptimal energy extraction from the diet, potentially exacerbating deficits in growth and body composition. Reduced propionate availability may hypothetically influence host energy metabolism, including gluconeogenesis and fatty acid pathways, potentially contributing to alterations in body composition. The observed associations between SCFA concentrations and BMI further support the role of gut microbiota in modulating host energy balance and highlight the potential for targeted dietary interventions to normalize microbial metabolite profiles and improve nutritional outcomes. Corresponding effect size estimates are summarized in Supplementary Table S8, and the full Spearman correlation matrix is presented in Supplementary Table S9.

4. Discussion

This exploratory, hypothesis-generating study comprehensively assessed anthropometry, body composition, and gut microbial metabolites in 46 infants with congenital heart defects (CHD) from the Khorezm region of Uzbekistan. The cohort included 26 formula-fed infants with protein–energy malnutrition (PEM) and 20 exclusively breastfed infants with CHD and normal nutritional status serving as a reference group. Importantly, the primary inference of this study is derived from stratified analyses conducted within the formula-fed CHD cohort according to PEM severity (Grade I vs. Grade II). The exclusively breastfed CHD group was included to provide contextual reference values rather than to serve as the principal comparator for causal interpretation. Accordingly, between-group differences involving the breastfed reference group should be interpreted cautiously, as feeding modality and baseline nutritional status may introduce additional confounding. The principal finding is that increasing PEM severity among formula-fed infants with CHD is associated with impaired body composition and reduced fecal SCFA concentrations.

Anthropometric measurements included weight, length, BMI Z-scores, waist and hip circumferences, and waist-to-hip ratio (WHR), while body composition parameters—including fat mass (FM), skeletal

muscle mass (SMM), active cell mass (ACM) [2,4], and bone mass—were assessed using bioelectrical impedance analysis (SECA mBCA analyzers) . While SECA mBCA analyzers are not a direct replacement for gold-standard methods such as air displacement plethysmography or DXA, they provide a reproducible non-invasive tool for within-cohort comparative body composition analysis. Previous studies comparing BIA devices with DXA have shown good relative agreement in pediatric populations, reinforcing the suitability of medical BIA when used under standardized conditions. In addition, the use of a standing-platform BIA device adapted for supine infant assessment may introduce measurement variability, although identical standardized conditions were maintained across groups. Fecal short-chain fatty acid (SCFA) concentrations (C2–C6, isoacids) were quantified via high-performance gas chromatography, and dietary intake was evaluated using the WHO CINDI questionnaire [21,22,30]. Normality testing confirmed the appropriateness of parametric or non-parametric methods for each variable. Due to the low prevalence of CHD combined with protein–energy malnutrition (PEM) in infancy, this single-center cohort represents the total number of eligible patients during the recruitment period [5,24,31]. While the limited sample size may restrict generalizability, the study provides detailed mechanistic insights into the relationships between malnutrition, body composition, and microbial metabolism in medically fragile infants.

Infants with CHD frequently exhibit early feeding difficulties due to reduced endurance, impaired sucking reflex, and rapid fatigue, resulting in insufficient breast milk intake and energy deficit [11,12,32]. Consequently, most infants transitioned to formula feeding, yet despite micronutrient-fortified adapted formulas, anthropometric deficits persisted (Table 1). The anthropometric and body composition analysis further characterizes the nutritional state of the study population. Specifically, infants with Grade I PEM exhibited BMI Z-scores of −2.56 [−2.97, −2.25], while those with Grade II PEM showed a further decline to −3.66 [−3.99, −3.35]. A critical finding was the significantly higher fat mass (FM) in the Grade I group (3.4 kg [3.1–3.6]) compared to the Grade II group (2.65 kg [2.2–2.95]; p = 0.018), reflecting the progressive depletion of energy reserves as malnutrition severity increases. Furthermore, the ACM proportions were slightly higher in Grade II, likely reflecting relative shifts in tissue composition rather than absolute preservation of metabolically active mass. These findings corroborate previous evidence that inadequate energy intake in infants with CHD, compounded by increased metabolic demands, impairs lean tissue accretion and somatic growth, while also contributing to the depletion of adipose reserves and compromised cellular development (Table 2) [5,31].

Infants with PEM exhibited lower median concentrations of acetate (16.50 [15.50–16.95] μmol/g in Grade I and 8.40 [6.00–11.15] μmol/g in Grade II) compared with the reference group (18.20 [15.80–21.30] μmol/g). Similar between-group differences were observed for propionate and butyrate (Table 3). Notably, the progressive decline in propionate (C3) and butyrate (C4) concentrations with increasing PEM severity may have specific pathophysiological implications. Butyrate serves as the primary energy substrate for colonocytes and plays a central role in maintaining epithelial barrier integrity through regulation of tight junction proteins and suppression of NF-κB–mediated inflammatory signaling [32,34,35]. Reduced butyrate availability may therefore compromise intestinal barrier function, increase mucosal permeability, and exacerbate systemic inflammatory activation. In medically fragile infants with

CHD, who may already experience hypoxia-related metabolic stress, such alterations could further impair nutrient absorption and growth [33]. Similarly, propionate contributes to hepatic gluconeogenesis and systemic energy homeostasis [3]. Severe protein–energy malnutrition is frequently associated with reduced availability of fermentable substrates and decreased abundance of saccharolytic bacterial taxa, leading to diminished propionate production. The observed decline in C3 concentrations in Grade II PEM may thus reflect impaired microbial carbohydrate fermentation capacity, potentially contributing to further deterioration of host energy balance [38]. Total fecal SCFA concentrations differed significantly across groups (Kruskal–Wallis, $p < 0.001$), with a large effect size ($\varepsilon^2 \approx 0.51$), indicating that approximately 51% of the variance in total SCFA concentrations was explained by group membership. Individual SCFA concentrations and corresponding participant characteristics are provided in Supplementary Table S1.

To better understand the clinical relevance of these findings, several biological mechanisms may be considered. Beyond serving as markers of microbial activity, short-chain fatty acids (SCFAs) exert critical metabolic, mitochondrial, and immunomodulatory functions that may be particularly relevant in infants with congenital heart defects (CHD) [20,34]. Butyrate represents the primary energy substrate for colonocytes and supports epithelial barrier integrity, whereas propionate contributes to hepatic gluconeogenesis and systemic energy balance, and acetate participates in lipid metabolism and peripheral energy signaling [20,35]. In the context of CHD, where infants exhibit elevated basal metabolic demands, increased resting energy expenditure, and limited nutritional reserves, reduced SCFA availability may further aggravate negative energy balance and impair nutrient utilization.

Importantly, SCFAs also influence mitochondrial function and cellular bioenergetics. Butyrate has been shown to enhance mitochondrial oxidative phosphorylation and stimulate mitochondrial biogenesis through activation of AMPK–PGC-1α–dependent pathways, thereby improving ATP production efficiency [36]. In states of protein–energy malnutrition, where mitochondrial dysfunction and reduced oxidative capacity may already be present, diminished SCFA availability could exacerbate cellular energy deficits and contribute to impaired tissue growth and altered body composition. This mechanism may be particularly relevant for metabolically active tissues, including skeletal muscle and myocardium, which rely heavily on mitochondrial ATP generation.

In addition to their metabolic roles, SCFAs regulate host inflammatory responses via activation of G-protein–coupled receptors (GPR41 and GPR43) and inhibition of NF-κB–mediated proinflammatory signaling [34,37]. Reduced SCFA production may therefore contribute to increased intestinal permeability and low-grade systemic inflammation. Infants with CHD frequently experience chronic inflammatory activation and oxidative stress, particularly in the presence of hypoxemia-related metabolic strain. Under these conditions, diminished microbial metabolite output may further exacerbate cardiometabolic vulnerability.

Emerging evidence suggests that chronic inflammation and impaired metabolic flexibility contribute to adverse cardiac remodeling in congenital and acquired heart disease [29]. By modulating inflammatory

tone and mitochondrial efficiency, SCFAs may indirectly influence myocardial substrate utilization and structural adaptation. Although causality cannot be inferred from the present cross-sectional data, reduced SCFA concentrations observed in infants with more severe malnutrition may represent a metabolic environment less favorable for optimal myocardial energy metabolism and adaptive remodeling.

Furthermore, adequate metabolic resilience is essential for post-surgical recovery in infants undergoing corrective cardiac procedures. Surgical stress induces catabolic activation, systemic inflammation, and increased energy requirements [39]. In this context, compromised microbial metabolite production and associated mitochondrial inefficiency could theoretically limit recovery capacity and prolong inflammatory responses. These considerations suggest that SCFA depletion in infants with CHD may represent a functionally relevant pathway linking malnutrition, altered body composition, mitochondrial energetics, inflammatory regulation, and potentially postoperative resilience.

Dietary patterns also influenced microbial metabolic output. In formula-fed infants, early introduction of commercial cereals was negatively associated with propionic acid levels ($\rho = -0.276$, $p = 0.017$), whereas breastfeeding and higher dietary fiber intake were positively associated with total SCFA output ($\rho = +0.620$ and $+0.277$, $p < 0.05$), highlighting the importance of substrate availability for microbial fermentation during early life. In exploratory regression analyses including feeding modality, breastfed status was associated with higher SCFA concentrations. However, because feeding mode and nutritional status were structurally confounded in the present design, these findings cannot be interpreted causally and should be considered hypothesis-generating only.

The observed links between SCFA levels and anthropometric markers underscore the functional relevance of microbial metabolism to growth. Specifically, C3 and C4 concentrations positively correlated with BMI Z-score ($r = 0.41–0.37$) and ACM ($r = 0.43–0.48$), while inversely correlating with FM ($r = -0.59$ to -0.72). These findings suggest that reduced SCFA availability may be associated with alterations in somatic growth and body composition; however, causality cannot be inferred from the present cross-sectional design. [12,40,41,42]. Emerging evidence indicates that early-life gut microbial composition plays a critical role in regulating host energy metabolism and growth trajectories. Several recent studies have demonstrated that malnutrition during infancy is frequently associated with delayed maturation of the gut microbiota, reduced abundance of saccharolytic taxa, and decreased production of short-chain fatty acids, which collectively may impair nutrient utilization and metabolic efficiency. Reduced microbial diversity and diminished SCFA production have been reported in infants with undernutrition and chronic disease states, suggesting that alterations in microbial metabolic activity may contribute to impaired growth and altered body composition during early development [40–43].

Our findings are consistent with literature reporting early-life dysbiosis, reduced SCFA production, and impaired microbial diversity in malnourished infants or those with chronic disease [12,40-42,48]. Reduced acetate, propionate, and butyrate production likely suggest reduced activity of saccharolytic taxa (e.g., Bifidobacterium spp.), which play a critical role in carbohydrate fermentation and colonocyte

energy supply. The concomitant reduction in SCFAs may impair intestinal barrier function, nutrient absorption, and growth, emphasizing the importance of early microbial support [34].

Strengths of this study include integration of anthropometric, BIA, SCFA, and dietary data within a well-characterized cohort, providing a multidimensional perspective on PEM and intestinal metabolism in infants with CHD. The integration of anthropometric, bioimpedance, metabolomic, and dietary data represents a key strength of the present work, enabling a comprehensive assessment of host–microbe metabolic interactions in a clinically vulnerable population. This study has several limitations. The cross-sectional design, single-center recruitment, and measurement of SCFA concentrations at a single time point preclude causal inference [44]. In addition, feeding modality and nutritional status were structurally linked in the present study design, the independent contributions of feeding type and PEM severity to SCFA variation cannot be fully disentangled. Therefore, comparisons involving the breastfed reference group should be interpreted as contextual rather than causal. Infants with PEM were exclusively formula-fed due to clinical feeding difficulties, whereas the reference group comprised exclusively breastfed infants with preserved nutritional status. Consequently, between-group differences may reflect the combined effects of feeding modality, nutritional status, and illness severity rather than PEM alone. Because feeding mode was not independently distributed across nutritional strata, the independent contributions of PEM and feeding modality to gut microbial metabolism and body composition cannot be fully disentangled. Accordingly, multivariable models including both feeding modality and PEM severity are subject to structural confounding and should be interpreted as hypothesis-generating rather than confirmatory.

Furthermore, inflammatory biomarkers were not assessed, as the primary focus was on nutritional status and microbial metabolic output. Although SCFA profiling provides functional metabolic information, it does not identify specific microbial taxa or pathways. The absence of microbiome sequencing limits taxonomic resolution and mechanistic interpretation of the observed alterations [40–47]. While short-chain fatty acids represent end products of microbial fermentation and may reflect integrated metabolic activity more directly than compositional data alone, future longitudinal multi-omics studies integrating sequencing-based microbiome profiling with metabolomics are warranted to comprehensively characterize microbial compositional and functional alterations in infants with CHD.

Although the cohort represents the total number of eligible infants during the recruitment period in this geographically limited population, larger multicenter studies are required to confirm these findings and enhance generalizability. Given the relatively small subgroup sizes, particularly in the Grade II cohort (n = 11), multivariable regression analyses should be interpreted as exploratory and hypothesis-generating rather than confirmatory.Accordingly, multivariable analyses should be interpreted with caution.

In conclusion, our data indicate that early nutritional interventions and feeding strategies supporting beneficial microbial metabolism may represent potential targets for future nutritional strategies aimed at supporting microbial metabolic function and growth outcomes in infants with CHD [42]. Optimizing formula composition, introducing fiber-rich complementary foods, and promoting breastfeeding where

feasible may enhance SCFA production, nutrient absorption, and somatic growth during this critical developmental window. From a clinical perspective, these findings suggest that early nutritional strategies targeting microbial metabolic activity may complement conventional nutritional rehabilitation in infants with CHD.

5. Conclusions

Among formula-fed infants with congenital heart defects, increasing PEM severity was associated with impaired body composition and reduced fecal SCFA concentrations. Infants with more severe PEM demonstrated substantially lower total SCFA levels (18.10 vs 34.30 μmol/g in controls), corresponding to an approximate 47% reduction. These alterations were significantly correlated with anthropometric and bioimpedance parameters, including fat mass depletion ($r = -0.72$, $p < 0.05$) and reduced active cell mass, supporting an association between microbial metabolite availability and somatic growth.

Higher SCFA concentrations were observed in the breastfed reference group; however, given the non-matched study design, differences related to feeding modality should be interpreted as associative rather than causal.

Overall, these findings emphasize the relationship between nutritional status severity and gut microbial metabolic output in infants with CHD and highlight the potential relevance of individualized nutritional strategies. Future longitudinal and multi-omics studies are required to clarify underlying mechanisms and causal pathways.

Abbreviations

The following abbreviations are used in this manuscript:

CHD – congenital heart defects;
PEM – protein–energy malnutrition;
SCFA – short-chain fatty acids;
BIA – bioelectrical impedance analysis;
BMI – body mass index;
ACM – active cell mass;
FFM – fat-free mass;
SMM – skeletal muscle mass;
TBW – total body water;
ECW – extracellular water;
BMR – basal metabolic rate;

Declarations

7.Author Contributions

Conceptualization, B.F.R. and A.S.A.; methodology, B.F.R.; formal analysis, B.F.R.; investigation, B.F.R.; data curation, B.F.R.; writing—original draft preparation, B.F.R.; writing—review and editing, B.F.R. and S.A.A.; visualization, B.F.R.; supervision, A.S.A.; project administration, A.S.A. All authors have read and agreed to the published version of the manuscript.

8. Funding

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9. Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Human Research Ethics Committee of the Ministry of Health of the Republic of Uzbekistan (approval No. 6/15-5107, 27 May 2025).

10. Informed Consent Statement

Informed consent was obtained from all subjects involved in this study.

11. Data Availability Statement

All data generated or analysed during this study are included in this published article and its supplementary information files. Further inquiries can be directed to the corresponding author.

12. Acknowledgments

The authors would like to thank all study participants. During the preparation of this manuscript, the authors used ChatGPT for language editing and text structuring. The authors critically reviewed and edited all generated content and take full responsibility for the final manuscript.

13. Conflicts of Interest

The authors declare no conflicts of interest.

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Figures

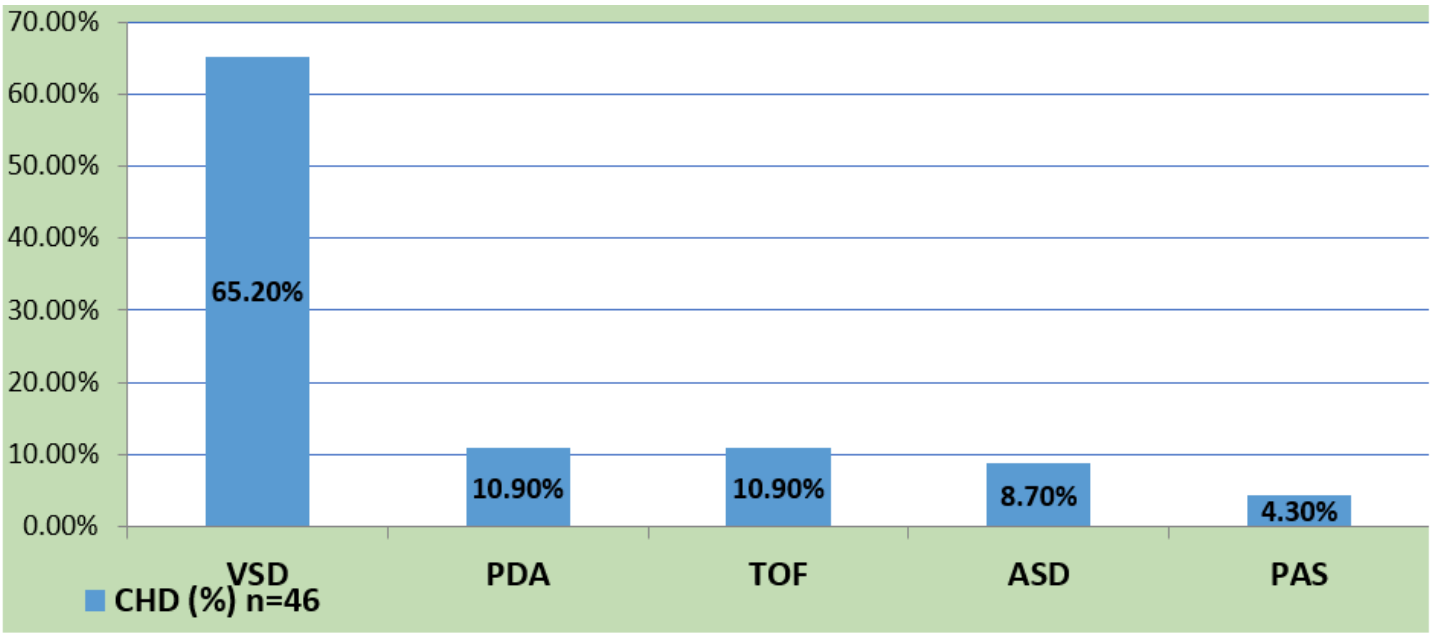


Figure 1

Distribution of congenital heart defect phenotypes among infants (n = 46).

Data are presented as n (%). Ventricular septal defect (VSD) was the most common diagnosis (30/46, 65.2%), followed by patent ductus arteriosus (PDA; 5/46, 10.9%), tetralogy of Fallot (TOF; 5/46, 10.9%), atrial septal defect (ASD; 4/46, 8.7%), and pulmonary artery stenosis (2/46, 4.3%).

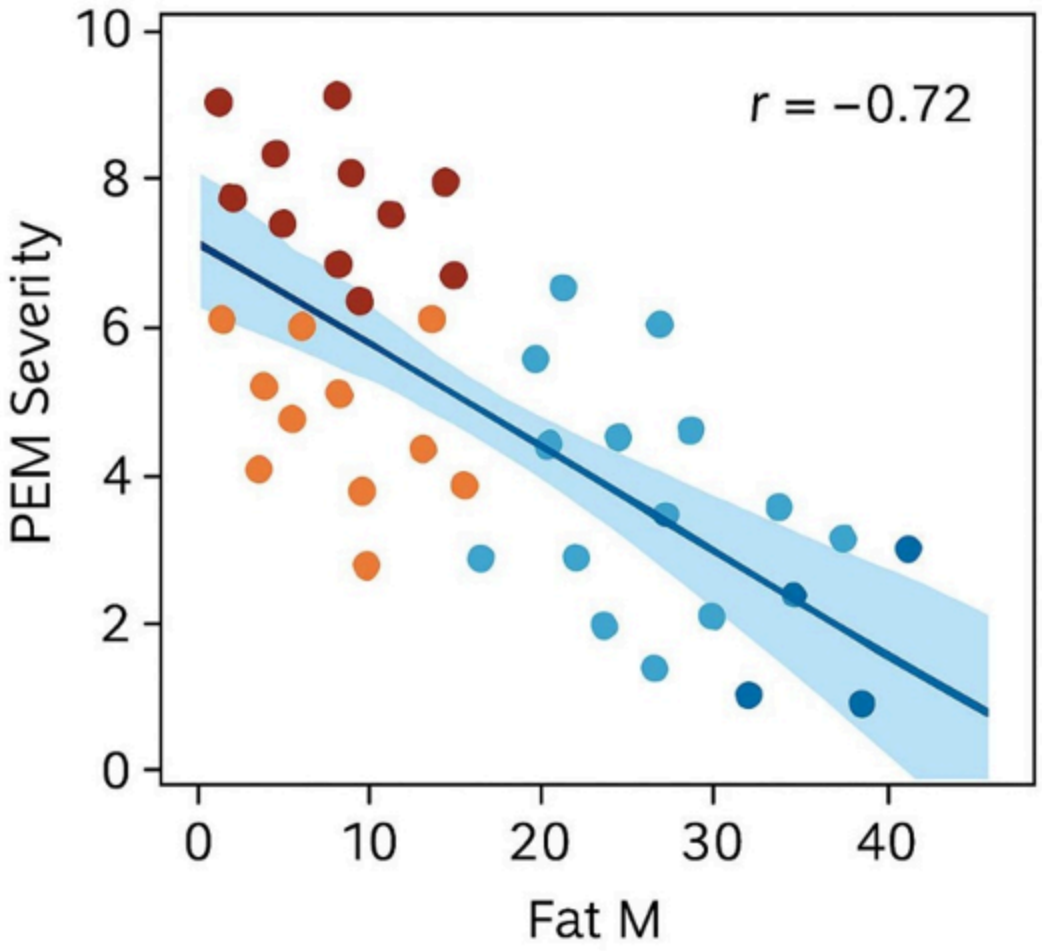


Figure 2

Correlation between fat mass and protein–energy malnutrition (PEM) severity. The scatter plot demonstrates a significant inverse correlation between fat mass (Fat M) and the severity of PEM ($r = -0.72$, $p < 0.05$). The solid line represents the linear regression, and the shaded area indicates the 95% confidence interval. Individual data points are color-coded by clinical subgroups.

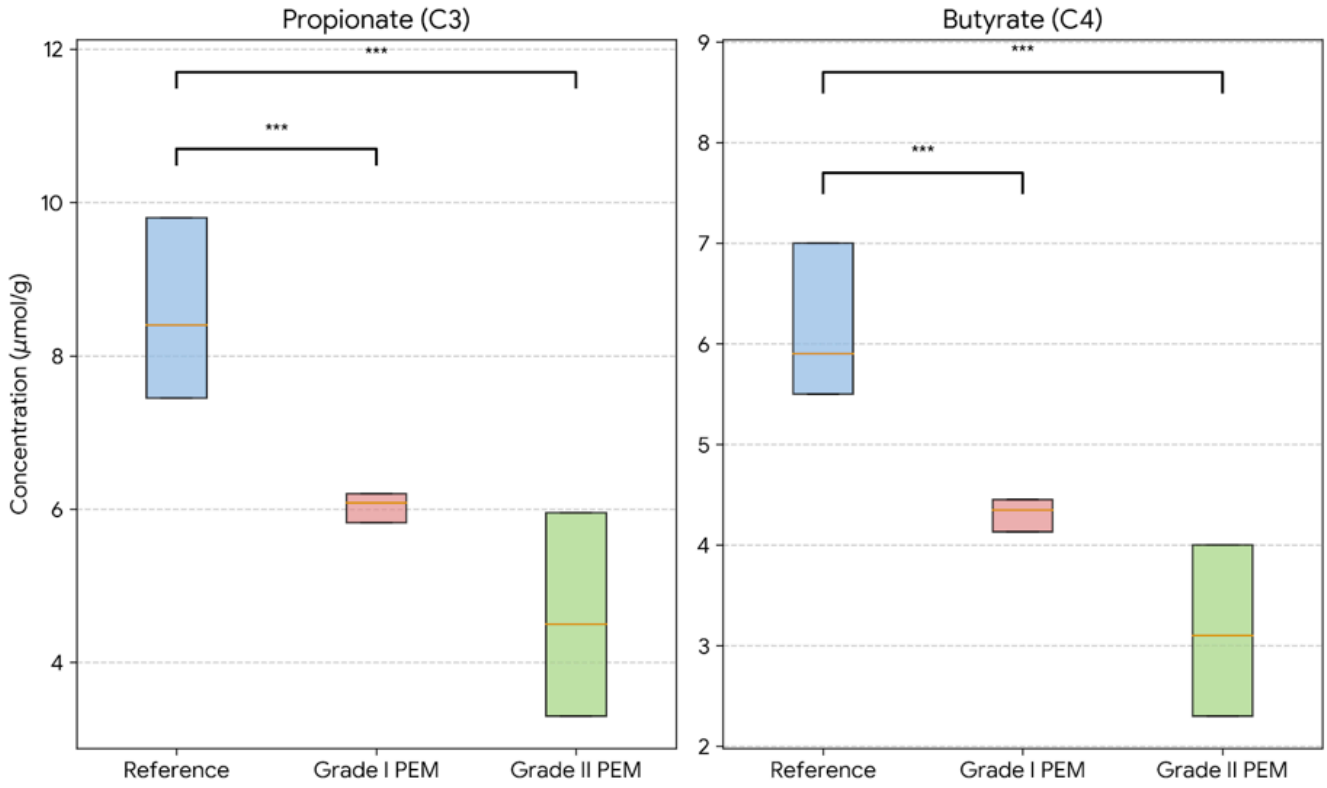


Figure 3

Box-and-whisker plots of fecal propionate (C3) and butyrate (C4) concentrations across study groups. Boxes represent the interquartile range (IQR), horizontal lines indicate medians, and whiskers denote minimum and maximum values. Group differences were assessed using the Kruskal–Wallis test followed by Dunn's post hoc comparisons with Benjamini–Hochberg false discovery rate (FDR) correction. Statistical significance was indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Reference (n = 20), Grade I PEM (n = 15), Grade II PEM (n = 11).

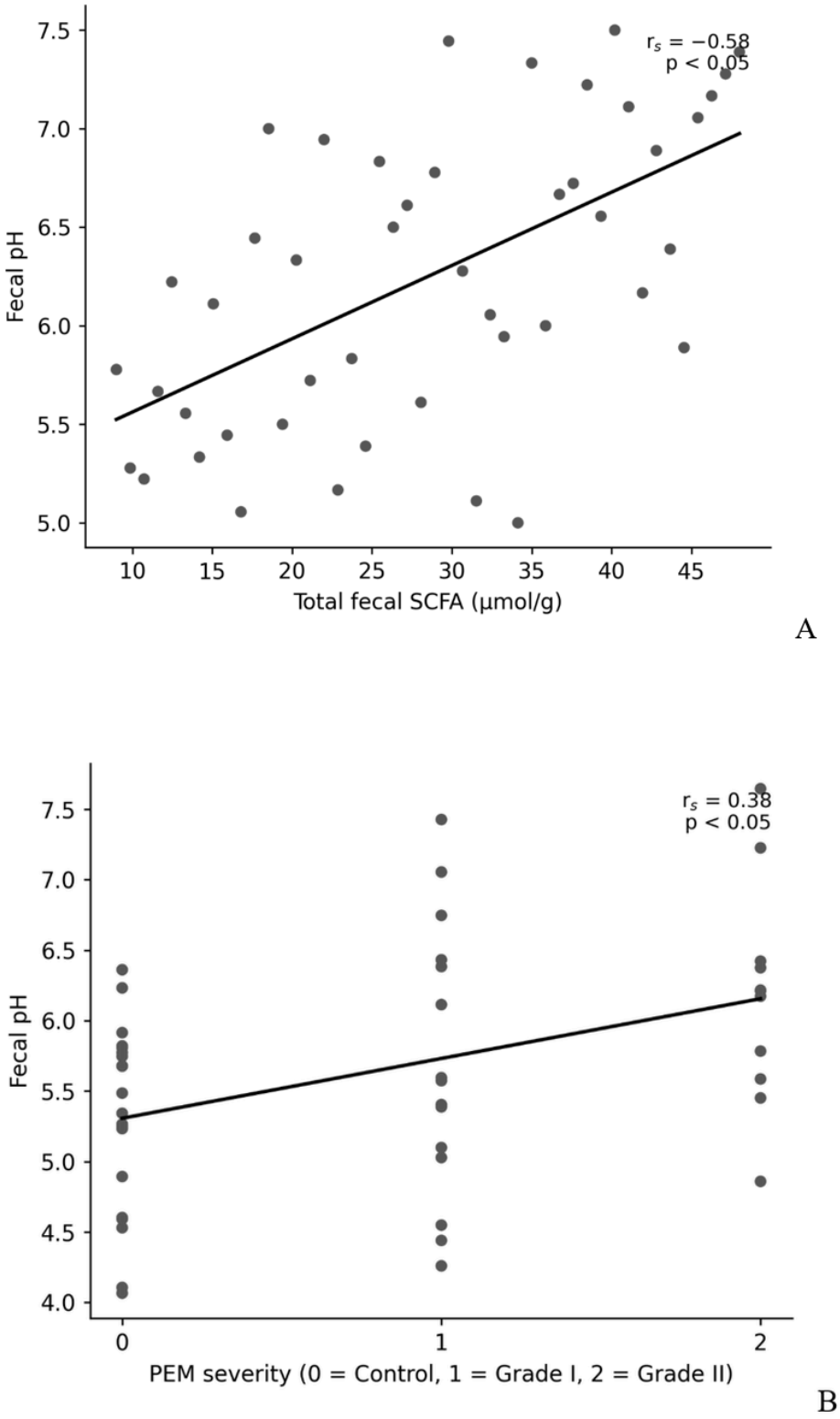


Figure 4

Correlation analysis between gut microbial metabolites and fecal pH in infants with CHD and PEM from the Aral Sea region (n = 46).

(A) Scatter plot demonstrating a moderate-to-strong inverse association between total fecal short-chain fatty acid (SCFA) concentrations and fecal pH (Spearman's r = −0.58, p < 0.05). Lower SCFA levels were

associated with higher fecal pH values.

(B) Scatter plot illustrating a moderate positive correlation between fecal pH and PEM severity (Spearman's r = 0.38, p < 0.05). These findings suggest that reduced microbial acid production may contribute to alterations in the intestinal luminal environment in infants with more severe malnutrition.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialsBabadjanova.zip