Alterations in Plasma Short-Chain Fatty acids in Preadolescence Children: The Hokkaido Study

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Abstract

The purpose of this study is to explore the plasma short-chain fatty acid (SCFA) concentrations in 9–12-year-old Japanese children collected in the Hokkaido study, focusing on how factors such as age, sex, and body mass index (BMI) correlate with these levels. The Hokkaido Study on Children's Health is an ongoing longitudinal study since 2002, encompassing 20,926 pregnant women in Hokkaido Prefecture, Japan, between 2003 and 2012. We contacted 1881 children aged 9–12 born between April 2006 and January 2010, and 342 non-fasting plasma samples (boys = 181, girls = 161) were obtained from this cohort, alongside assessments of their height and weight. Plasma SCFA concentrations were determined using *N,N*-dimethylethylenediamine derivatization method coupled with liquid chromatography-mass spectrometry. Ethyl acetate was used to extract SCFAs from plasma, and the recovery ranged from 83% to 108%. Our findings indicate that acetic acid had the highest concentration across all age groups and sexes. The concentrations of butyric acid, valeric acid, and hexanoic acid increased with age, peaking in 12-year-old children. Conversely, the level of 4-hydroxy valeric acid showed a decreasing trend with increasing age groups. This study also explored the correlation between BMI and SCFA concentrations, comparatively higher level of propionic acid was observed in the overweight group. The results obtained in this study enhance our understanding of the role of SCFAs in the growth and development of children and provide a foundation for future nutritional intervention and health promotion strategies.

Keywords

Short-chain fatty acids, Metabolites, Plasma, Children, Liquid chromatography, Mass spectrometry

Abbreviations: 3HC3, 3-Hydroxypropionic acid; 3HC4, 3-Hydroxybutyric acid; 3HC6, 3-Hydroxyhexanoic acid; 4HC5, 4-Hydroxypentanoic acid; ACN, Acetonitrile; BMI, Body mass index; C2, Acetic acid; C3, Propionic acid; C4, Butyric acid; C5, Valeric acid; C6, Hexanoic acid; CMPI, 2-Chloro-1-methylpyridinium iodide; DMED, N,N-dimethylethylenediamine; H-ESI, Heated electrospray ionization; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; MRM, Multiple reaction monitoring; QC, Quality control; RSD, Relative standard deviation; SCFAs, Short-chain fatty acids; TEA, Triethylamine

Data availability

Data will be made available on request.

1. Introduction

Short-chain fatty acids (SCFAs), primarily composed of acetic acid, propionic acid, and butyric acid, are significant products of the fermentation of indigestible carbohydrates by the gut microbiota [1]. These small-molecule fatty acids play a crucial role in maintaining intestinal health and regulating systemic metabolism, particularly during

65 critical growth and developmental stages in children. The effects of SCFAs on health and
66 disease progression are important [2,3]. They provide the main energy source for the
67 intestinal epithelial cells and maintain the balance of the gut microbiota by regulating the
68 pH of the intestine, thereby inhibiting the growth of harmful bacteria [4]. Additionally,
69 SCFAs play an important role in regulating the immune system and help to establish early
70 immune tolerance in children by influencing the function and distribution of immune cells,
71 which are crucial for preventing allergies and autoimmune diseases [5,6].

72 SCFAs are also potentially important for the cognitive development of children.
73 Studies have indicated that SCFAs can cross the blood–brain barrier and directly affect
74 brain function and development. For example, butyrate has been found to promote the
75 growth and differentiation of neural cells, potentially positively impacting brain health
76 and cognitive function [7]. Plasma concentrations of SCFAs in children and adults with
77 attention deficit hyperactivity disorder are lower than those in healthy controls [8].
78 Furthermore, SCFAs play a significant role in regulating energy metabolism and
79 preventing obesity by modulating the metabolism of fats and sugars [9], and have been
80 increasingly recognized for their role in appetite regulation [10]. SCFAs contribute to
81 satiety signaling and appetite suppression by influencing the release of gut hormones,
82 such as peptide YY and glucagon-like peptide-1 [11–13]. Rapid physical growth, immune
83 system maturation, and significant cognitive and social skill development occur at an
84 early age [14]. These aspects of SCFA activity are critical for rapidly growing children,
85 in which a balance between energy intake and expenditure is essential for healthy

development.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a sensitive method that has been widely employed to conduct thorough analyses of human plasma SCFA levels and uncover their roles in health and development [15–17]. In fact, we previously determined the serum butyric acid levels in 547 adult Japanese volunteers from the Hokkaido region using LC-MS [18]. Furthermore, we previously developed a highly sensitive and selective method for SCFA analysis using *N,N*-dimethylethylenediamine (DMED) derivatization coupled with LC-MS/MS [19]. Derivatization assisted LC-MS analysis of SCFAs is widely used in the literature as for example, a study by Fristedt et al demonstrated the quantitative analysis of SCFAs with 3-nitrophenylhydrazine derivatization in small volume of blood samples from animals and humans [20]. They even included a quenching step in their analysis after derivatization to remove excess derivatizing agent, as it could eliminate the contamination peaks. To the best of our knowledge, there are no reports on the analysis of plasma SCFAs in healthy preadolescent children. Hence, in this study, we quantitatively determined the plasma SCFA concentrations in 9–12-year-old children and examined correlation with sexes, age, and BMI. This study focused on analyzing SCFAs in the plasma of preadolescents, a critical period for physiological and psychological development. Understanding the levels and composition of SCFAs in children during this critical period is essential to reveal their potential impact on child health and development.

2. Experimental section

2.1 Human samples

Plasma samples were collected from non-fasting children aged 9–12 years (n = 342, boys = 181, girls = 161) participating in the Hokkaido Study on Environment and Children's Health, the Hokkaido Cohort [21–24]. The detailed criteria for participant selection are described in previous studies [25,26]. The height and weight of participants were measured on the same day of the sample collection. Both the research objectives and methods were thoroughly explained to the participants, with written informed consent obtained from all parents, while informed assent was obtained from all children.

Ethical approval for this study was granted by the Ethics Review Board of the Faculty of Health Sciences, Hokkaido University (approval number: 22–33) and Center for Environmental and Health Sciences, Hokkaido University (approval number: 21–136).

2.2 Materials

The following analytical grade reagents such as triethylamine (TEA), DMED, 3-hydroxybutyric acid (3HC4), and aceticacid-d4 (C2-d4) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 2-chloro-1-methylpyridinium iodide (CMPI), propionic acid (C3), butyric acid (C4), valeric acid (C5), and hexanoic acid (C6) were purchased from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Acetic acid (C2) and 1 M ammonium acetate solution were obtained from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). LC–MS grade methanol and acetonitrile were purchased from the Kanto Chemical Co., Inc. (Tokyo, Japan). 3-yydroxypropanoic acid (3HC3), 4-hydroxypentanoic acid (4HC5), 3-hydroxyhexanoic acid (3HC6), and pentanoic acid-d9

(C5-d9) were purchased from Cayman Chemical (Ann Arbor, MI, USA). All three reaction reagents (TEA, DMED, and CMPI) were dissolved in LC-MS grade acetonitrile of 2 mM concentration and stored at −28°C.

2.3 Short-chain fatty acids extraction

Plasma samples were preserved at –80 °C after collection. Before the extraction, the samples were thawed in an ice bath. A volume of 50 µL of plasma was subjected to a series of extraction steps using ethyl acetate by the previously reported method [27]. Each step involves the addition of 200 µL of ethyl acetate and 10 µL of formic acid (0.5 %, v/v) to the plasma. The mixture was subjected to vortex at 3500 rpm for a duration of 1 min, followed by centrifugation at 20,630×g for 3 min. After centrifugation, supernatants were collected. The residue was reextracted with ethyl acetate and 10 µL of formic acid (0.5 %, v/v) twice, and then the combined extracts were evaporated under vacuum using a centrifuge evaporator. The concentrated extracts were redissolved in 100 µL of acetonitrile.

To the obtained 100 µL of SCFA extract in acetonitrile, 100 µL of aceticacid-d4 (20 µM) and 100 µL of pentanoic acid-d9 (20 µM) were added, followed by vortex mixing. The extracts were subjected to DMED-derivatization by adding 20 µL of TEA (2 mM) and 10 µL of CMPI (2 mM) and vortexed for 3 min. Subsequently, 20 µL of DMED (2 mM) was added, and the mixture was further vortexed for 30 min, followed by centrifugation (20,630×g, 20 min, 4°C) [19]. The centrifugate was collected and transferred to LC-MS vials. A 5 µL aliquot of the prepared samples was injected for LC-

MS analysis. A detailed extraction and derivatization work-flow for plasma SCFAs is shown in **Fig. 1**.

2.4 LC-MS/MS analysis and quantification of SCFAs

Chromatographic separation was achieved using a Hypersil GOLD column (100 mm × 2.1 mm, 1.9 μm). The column oven temperature was set at 40°C, and the autosampler temperature was maintained at 4°C. The mobile phase consisted of solvent A (Milli-Q water with 20 mM ammonium acetate) and solvent B (50/50 (v/v) mixture of methanol and acetonitrile), delivered at a flow rate of 0.3 mL/min. The gradient profile was programmed as follows: pump B at 90% (0–2 min), followed by 50% (3–5 min), reduce to 20% at 6 min and held until 7 min, increase to 90 % at 7.5 min, and maintain at 90 % (7.5–10 min). The process was concluded with a stop command at 10 min, and the injection volume for the analysis was 5 μL.

Targeted analyte quantification was conducted using a prominence ultra-fast liquid chromatograph (Shimadzu, Kyoto, Japan), interfaced with a TSQ quantum mass spectrometer (Thermo Fisher, San Jose, CA, USA). The mass spectrometer analysis was conducted using a heated electrospray ionization (H-ESI) probe in positive mode. Data were acquired in the multiple reaction monitoring (MRM) mode [19]. The spray voltage was set at 3.5 kV, and the vaporizer temperature was maintained at 150 °C.The sheath and auxiliary gas pressures were held at 40 and 25 arbitrary units, respectively. The capillary temperature was maintained at 250°C. The tube lens voltage and collision energy were set to compound-specific parameters in the ranges of 45–54 V and 9–14 eV,

respectively [19]. Data acquisition was conducted using Xcalibur 2.2 software.

Standards and internal standards were introduced into the plasma samples using two distinct preparation procedures to assess the recovery efficiency. For post-extraction samples, the mixture was spiked into plasma after extraction. For pre-extraction samples, the same mixture was added to the plasma before extraction commenced. Each approach was replicated five times (n = 5) to ensure consistency. The recovery rate was subsequently determined as the ratio of the post-extraction to pre-extraction concentration, with values ranging from 83% to 108%. The precision of this analysis was determined using data from quality control (QC) samples composed of SCFA extracts from a pooled mixture of several plasma specimens. When injecting 342 individual samples, QC samples were introduced between every 30–40 runs. The overall precision of the QC samples, as indicated by the relative standard deviation, predominantly fell within the range of 10–20%. Quantification was performed by constructing the linear curves for each SCFA.

2.5 Data visualization and statistics

Data visualization and statistical analyses were performed using GraphPad Prism version 9.5.0 software (CA, USA). The concentrations of SCFAs were assessed for normality using the Shapiro-Wilk test, which demonstrated a non-normal distribution ($p < 0.05$). A subsequent Kruskal-Wallis test indicated the presence of statistically significant differences among the groups ($p < 0.05$). The Dunn's multiple comparisons test was then employed to further analyze the Kruskal-Wallis findings, with a $p < 0.05$

was considered as statistically significant. For the comparative analysis involving two variables, two-way ANOVA followed by Šídák's multiple comparisons test was conducted, with a significance threshold set at $p < 0.05$.

3. Results and Discussion

In the quantitative analysis of SCFAs in the plasma samples of preadolescent children, we utilized LC-MS/MS to achieve the separation and precise detection of each SCFA. The extracted ion chromatograms (EICs) of authentic SCFA standards and the endogenous SCFAs detected in the plasma samples were provided in the supporting information **Fig. S1**. The elution of SCFAs was accomplished within a period of 2 min, demonstrating the robustness of this methodology. Furthermore, DMED derivatization enhanced the detection sensitivity of SCFAs to low femtomolar levels [19].

The characteristics, including height, weight, BMI, and number of children of 9–12-year-old are summarized in **Table 1**. The BMI of 342 children (181 boys; 161 girls) ranged from 16.2 ± 2.2 to 19.4 ± 3.3, and very few children (2.3%) were classified as overweight. Plasma SCFA concentrations in boys and girls according to age groups are reported as median (interquartile range) in **Table 2**. The detailed concentrations of SCFAs in the individual sample are provided in the supporting information **Table S1**. The results showed that C2 was predominant in both sexes at all ages, followed by 3HC3 and C5. Whereas 4HC5 was present at the lowest levels across all age groups in both boys and girls. All the analytes were almost universally detected in the children, with C3 being the exception, found in 160 individuals (46.8%). There are no significant differences in SCFA

concentrations between boys and girls, as shown in **Fig. 2**. Quantitative variations in SCFAs by age group and sex are shown in **Fig. 3**. In both boys and girls, the concentrations of C4, C5, and C6 increased with age, peaking at 12 years. Conversely, in both boys and girls, the levels of 4HC5 showed a downward trend as age increased. This suggests that in girls entering puberty, specific metabolic or microbial adjustments may lead to changes in the production and consumption of SCFAs.

The relative abundance of SCFA species in the plasma of children, irrespective of age or sex, are shown in **Fig. 4a**. C2 exhibited the highest relative abundance (54.8%), indicating its dominance in plasma SCFA profiles. 3HC3 (28.9%) and C5 (11.2%), which exhibited the next two greatest abundances, also represented significant proportions of the total SCFA levels in the plasma. Other SCFAs, including C3, C4, C6, 3HC4, 4HC5, and 3HC6, were present in minor quantities, ranging from 0.2 to 1.3%. During the critical growth period of 9–12 years, dietary diversity increases and affects the diversity of the gut microbiota, and gradually transitions to a composition that is more similar to that of adults [28]. In this phase, new metabolic pathways begin to form, which are crucial for the breakdown of complex nutrients and potentially activate interactions between the immune system and the gut microbiota, influencing disease resistance in children [29]. In particular, C4 is a vital energy source for intestinal epithelial cells, increasing its concentration, aiding in the maintenance and strengthening of the gut mucosal barrier [30] . This barrier further prevents pathogens and harmful substances from entering the bloodstream through the gut [31]. For example, inflammatory bowel disease is a hallmark

example of impaired gut barrier function [32], an increase in C4 can be indicative of a healthy gut environment to some extent. Moreover, changes in the living environment during this developmental stage along with increased physical activity and changes in sleep patterns can significantly affect the gut microbiome of children [33]. Our study cross sectional and obtained the data at one point in time. To better understand the role of SCFAs in children's growth and development long-term follow-up studies are required.

The information of height and weight of the children in this study was categorized the extent of childhood obesity. Obesity was defined according to the internationally accepted BMI criteria, categorizing individuals as: underweight (BMI < 18.5; n = 239), normal weight (BMI 18.5–25; n = 95), and overweight (BMI > 25; n = 8). The results of the correlation analysis between the BMI and the SCFA concentrations is depicted in **Fig. 4b**. The comparative analysis across BMI categories revealed no statistically significant differences in SCFA concentrations among the groups for either sex. Notably, while not reaching statistical significance, C3 concentrations were observed to be comparatively higher in the overweight group relative to the other groups. Some studies have reported the role of C3 in human adipose tissue, indicating its influence on obesity and suggesting potential roles in preventing obesity-related inflammation and associated diseases [34,35] . However, our study found the highest concentration of C3 in the overweight group, which was a seemingly contradictory outcome. This may be due to a compensatory increase as a mechanism to counteract the status of being overweight, however, this mechanism may not be sufficiently effective for weight reduction. Alternatively, the

254 increase in C3 level could be the result of alterations in the gut microbiota due to obesity.
255 As evidenced by a study where the transplantation of fecal microbiota from obese mice
256 into germ-free mice induced obesity in the recipients, highlighting the pivotal role of gut
257 microbiota in the pathogenesis of obesity [36].

258 Previous research found higher concentrations of SCFAs in the feces of obese
259 individuals than in leaner counterparts, with a greater abundance of related bacteria, such
260 as *Firmicutes*, *Lactobacillus spp*., and *Staphylococcus spp* [37]. Additionally, this
261 discrepancy could be attributed to the smaller dataset for the overweight group. In our
262 study, the proportion of overweight individuals among Japanese children was found to be
263 relatively small, which may be related to their dietary habits. Japanese cuisine is typically
264 characterized by its lightness, with foods often boiled rather than fried, emphasis on the
265 natural flavors of the ingredients; and the preference for unsweetened green tea, which
266 may also contribute to health and weight management benefits [38].

267 A Swedish study involving 148 infant-mother pairs compared the SCFA levels in
268 infant plasma, maternal plasma, and breast milk samples using LC-MS [39]. They found
269 a high amount of total SCFAs in infant plasma compared to maternal samples. However,
270 C4, C5, and C6 SCFAs are significantly higher in breast milk compared to plasma
271 samples. They also found a strong correlation between the SCFA concentration in infant
272 plasma with SCFAs in maternal plasma. Among SCFAs formic acid was found to be most
273 abundant in infant plasma followed by C2 whereas in our study we have not determined
274 formic acid levels but C2 is still abundant in Hokkaido children's plasma. The selection

of an appropriate biological specimen is crucial for studying SCFA levels. Fecal samples are commonly used to assess the gut microbiome and its metabolic outputs; however, SCFA concentrations in feces are influenced by a multitude of factors, including the composition of the gut microbiota, type of dietary residues, and intestinal motility [40]. Variations in these factors may lead to fluctuations in fecal SCFA levels. In contrast, plasma SCFA concentrations are comparatively stable and less susceptible to external influences [41], thus providing more reliable data. Furthermore, plasma SCFA levels reflect the metabolic state across the entire body and not just the gut environment, indicating that plasma SCFAs can serve as markers of systemic metabolic activity and offer a comprehensive overview of health status [42]. In contrast, fecal samples primarily reflect the local conditions of the gut and may not comprehensively represent the metabolic state of the entire body [43]. Additionally, the collection and processing of plasma samples are relatively standardized, which helps minimize variability during sample handling. This standardization is particularly important in multicenter or longitudinal studies, as it ensures the comparability of samples collected at different times and locations. Therefore, the use of plasma samples for SCFA analysis can provide more stable and representative data and contribute to a more accurate assessment of the role of SCFAs in human health and development. However, the study as following limitations: (i) The sample size is relatively small compared to larger coherent studies, this could affect the obtained results (ii) Plasma samples were from non-fasting children's which could influence the variations in results (iii) The factors that influence the change in SCFA

296 levels such as dietary habits, exercise levels, and intestinal flora are not controlled. (iv)
297 The SCFAs determined in children plasma were at one point in time, to reveal their role
298 in children's growth and development a follow-up studies are essential. (v) The study
299 lacks the mechanistic investigation behand the increase of C3 levels in overweight
300 children plasma. Despite these limitations, as for our knowledge this is the first study
301 focusing on the determination of SCFAs level in Japanese children of different age groups.

302 **4. Conclusion**

303 This study substantially enhances our understanding of SCFAs and their distribution
304 in the plasma of non-fasting preadolescent children during their critical developmental
305 stages. By employing a derivatization-coupled LC-MS/MS technique, this study offers a
306 valuable perspective on the concentration profiles of various SCFAs in the plasma of
307 children, emphasizing age-, weight-, and sex-related variations. Acetic acid was the most
308 abundant SCFA found in the children plasma of both sexes, followed by 3-hydroxy
309 propanoic acid. This study also explored the relationship between BMI and SCFA
310 concentrations, highlighting comparatively higher levels of propionic acid in overweight
311 children. This research lays the groundwork for potential dietary and health interventions
312 aimed at optimizing child development and health outcomes.

313 **CRediT authorship contributions**

314 Yonghan Li: Writing – original draft, Visualization, Methodology, Formal analysis,
315 Data curation. Siddabasave Gowda B. Gowda: Writing – review & editing, Visualization,
316 Supervision, Resources, Methodology, Conceptualization. Divyavani Gowda: Writing –

review & editing, Visualization, Resources, Investigation, Formal analysis. Atsuko Ikeda: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Yu Ait Bamai: Writing – review & editing, Supervision, Resources, Investigation. Rahel Mesfin Ketema: Writing – review & editing, Resources, Project administration. Reiko Kishi: Writing – review & editing, Resources, Project administration. Hitoshi Chiba: Writing – review & editing, Supervision, Resources, Project administration. Shu-Ping Hui: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table. 1. Characteristics of non-fasting preadolescent children of 9–12-year-old. The values of height, weight, and BMI are shown as mean ± SD.

	Age (years old)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Under Weight (n)	Normal Weight (n)	Over Weight (n)
Boys	9 (n = 68)	134.9 ± 5.0	31.8 ± 6.1	17.4 ± 2.5	49	19	0
(n = 181)	10 (n = 63)	139.0 ± 6.4	35.0 ± 8.3	17.9 ± 3.3	45	15	3
	11 (n = 20)	148.3 ± 6.5	41.8 ± 8.1	19.0 ± 3.3	11	8	1
	12 (n = 30)	150.6 ± 9.4	44.2 ± 9.8	19.4 ± 3.3	14	14	2
Girls	9 (n = 46)	136.1 ± 5.7	30.2 ± 5.9	16.2 ± 2.2	41	5	0
(n = 161)	10 (n = 70)	140.4 ± 7.0	34.6 ± 8.1	17.4 ± 2.8	52	17	1
	11 (n = 16)	151.0 ± 5.0	39.5 ± 5.8	17.3 ± 2.0	12	4	0
	12 (n =29)	151.5 ± 6.5	42.6 ± 8.4	18.4 ± 2.6	15	13	1

Table. 2. Plasma SCFAs concentrations in boys and girls based on their age groups. The values are shown as median (interquartile range).

	Boys (μmol/L)				Girls (μmol/L)			
Analytes	9y	10y	11y	12y	9y	10y	11y	12y
C2	195.8 (132.4–282.0)	201.8 (100.6–380.1)	158.6 (72.6–297.7)	246.1 (169.5–378.6)	220.4 (121.3–312.7)	157.2 (97.0–311.0)	159.4 (114.5–227.5)	222.3 (163.4–281.7)
C3	1.7 (1.3–3.9)	2.1 (1.4–2.7)	2.0 (1.5–2.3)	1.8 (1.0–4.0)	1.6 (0.8–3.1)	2.4 (1.4–3.3)	2.0 (1.7–3.0)	1.8 (1.2–2.4)
C4	3.6 (2.5–5.0)	3.9 (1.6–5.3)	3.9 (1.6–5.6)	5.5 (4.5–6.4)	4.3 (2.5–5.4)	3.7 (1.6–4.7)	4.1 (2.7–5.1)	4.9 (4.3–6.0)
C5	47.8 (36.6–55.4)	49.7 (23.9–58.3)	49.9 (16.7–58.4)	61.7 (54.4–67.1)	47.8 (31.2–58.4)	43.4 (19.7–54.8)	48.3 (25.3–56.0)	57.4 (53.7–63.2)
C6	5.3 (3.9–6.3)	4.8 (3.4–5.7)	5.0 (2.9–5.8)	6.4 (5.4–6.8)	5.2 (3.8–6.2)	4.7 (3.0–5.9)	5.2 (3.5–6.7)	6.3 (5.3–6.9)
3HC3	96.7 (67.5–203.7)	102.0 (42.5–183.9)	64.1 (27.5–140.9)	102.5 (65.9–150.7)	102.1 (58.3–169.9)	83.3 (35.9–141.0)	76.8 (53.5–99.3)	112.7 (67.5–173.0)
3HC4	4.6 (2.2–6.9)	5.1 (2.6–7.6)	3.6 (2.5–5.1)	5.6 (3.8–8.6)	4.9 (3.5–6.8)	4.1 (2.6–6.3)	3.5 (2.7–6.0)	5.4 (4.4–8.0)
4HC5	1.0 (0.7–1.3)	1.0 (0.7–1.2)	0.7 (0.5–0.9)	0.6 (0.5–0.8)	0.8 (0.5–1.1)	0.9 (0.5–1.1)	0.6 (0.5–0.8)	0.6 (0.4–0.8)
3HC6	4.6 (3.9–5.3)	4.6 (3.8–6.2)	4.2 (3.3–5.0)	4.5 (3.6–5.3)	4.6 (3.8–5.5)	4.0 (3.4–5.4)	3.8 (3.4–4.5)	4.0 (3.5–5.1)

Figures

Fig. 1.

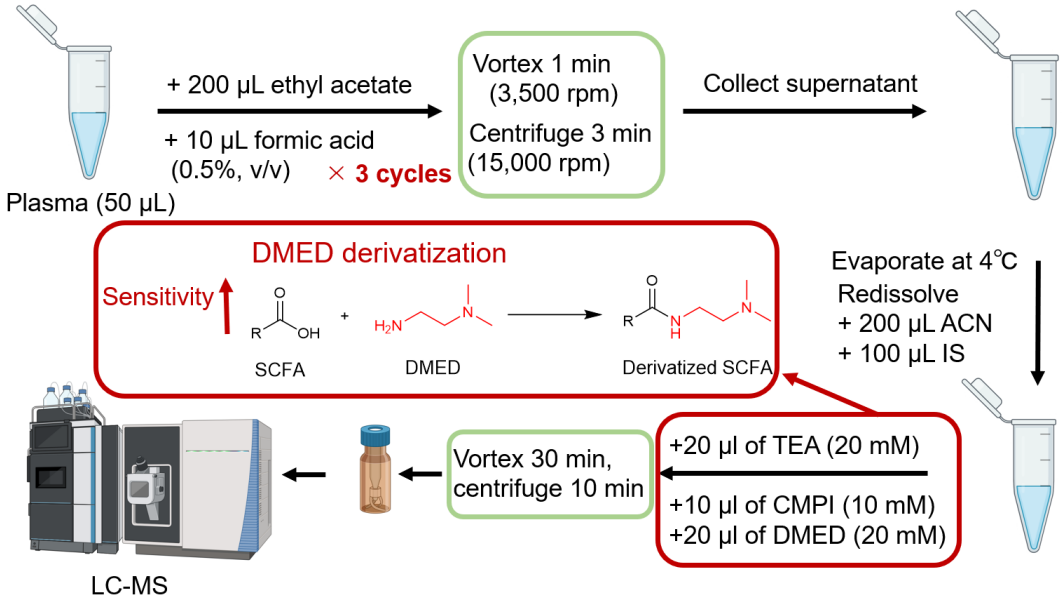


Fig. 1. Extraction and derivatization work-flow for plasma SCFAs determination.

Fig. 2.

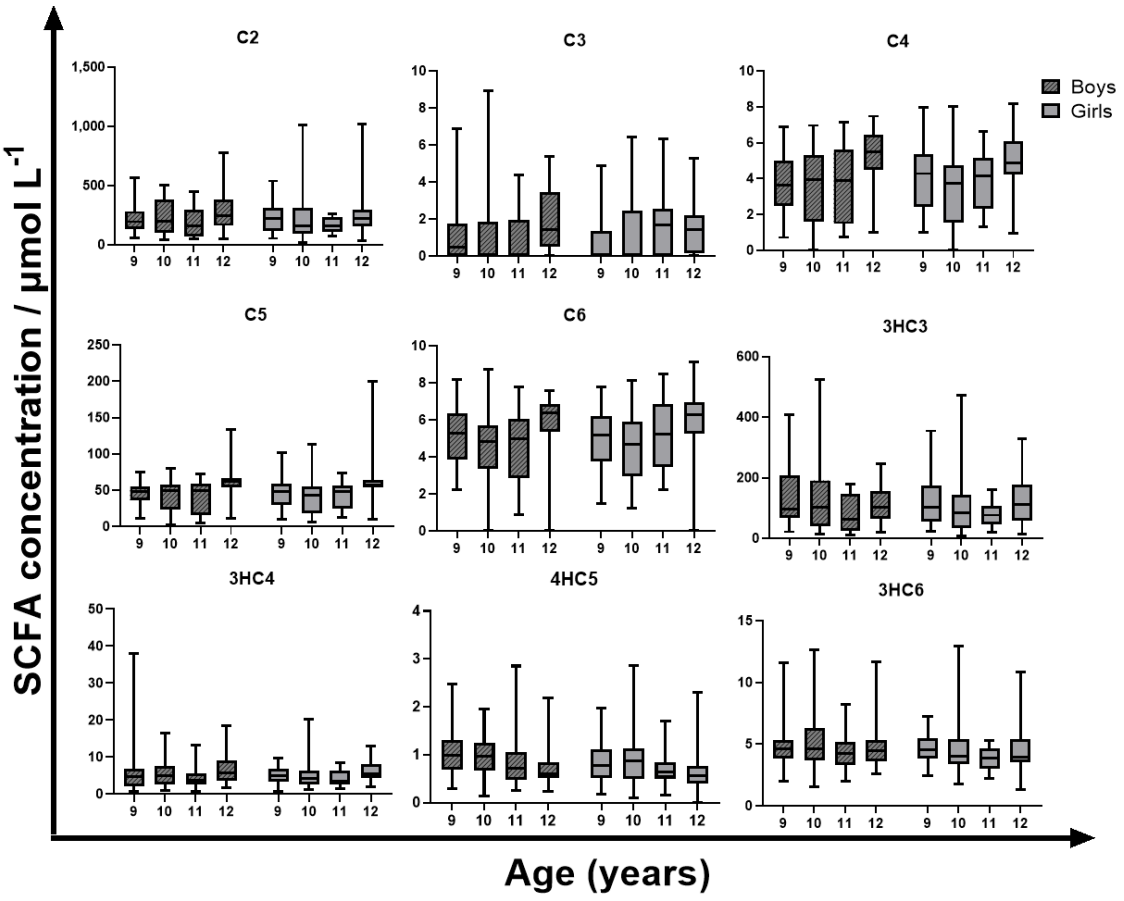
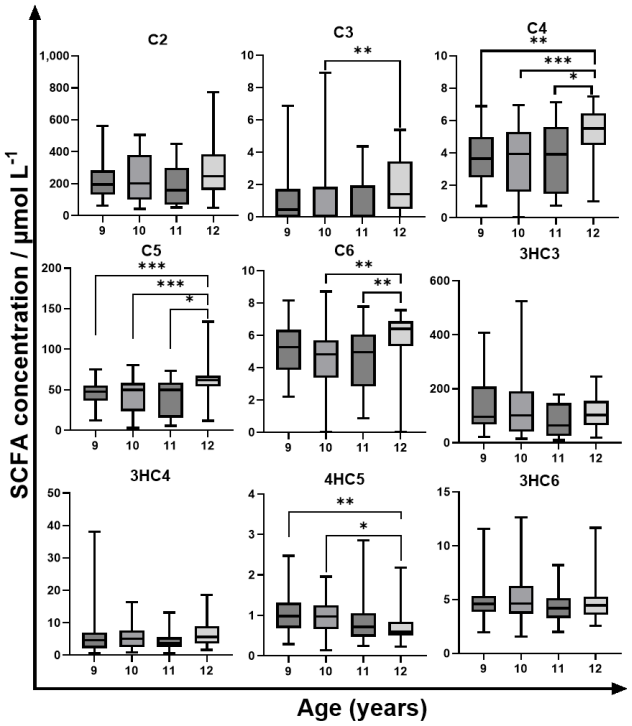


Fig. 2. Quantitative amount of SCFA concentrations in boys and girls. (Comparative analysis of the values was conducted via two-way ANOVA followed by Šídák's multiple comparisons test, which showed no significant differences between boys and girls across all SCFAs).

Fig. 3.

a



b

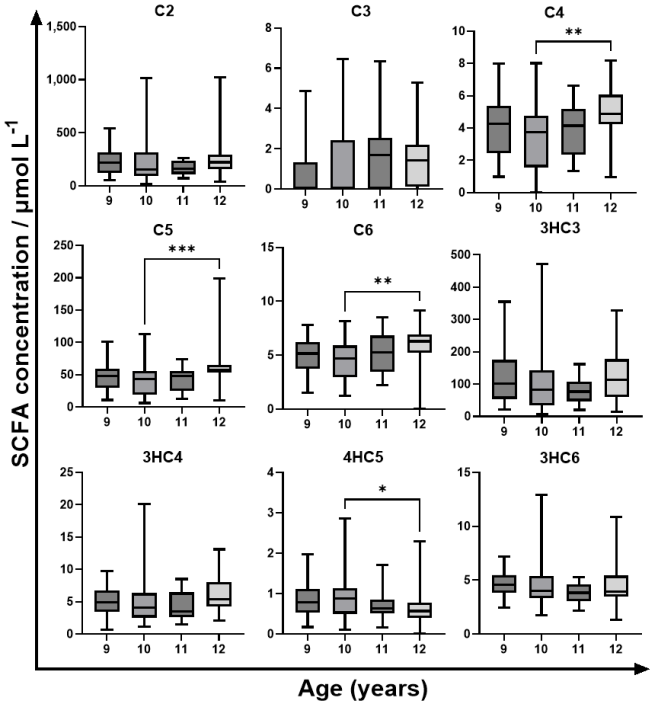
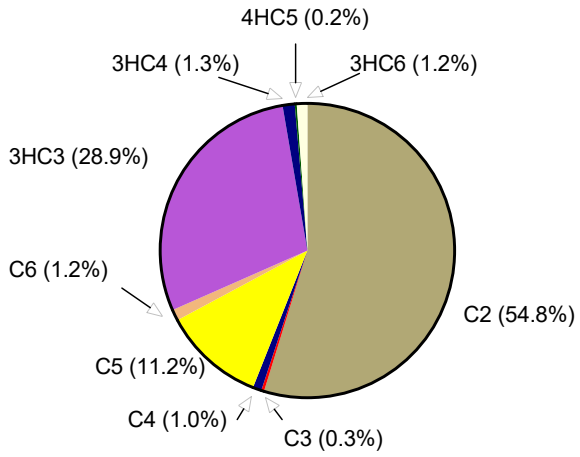


Fig. 3. Quantitative amount of SCFAs in boys (a) and girls (b) of different age groups. (Comparative analysis of the values was conducted via the Kruskal-Wallis test followed by Dunn's multiple comparisons test, significance levels indicated as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Fig. 4.

a



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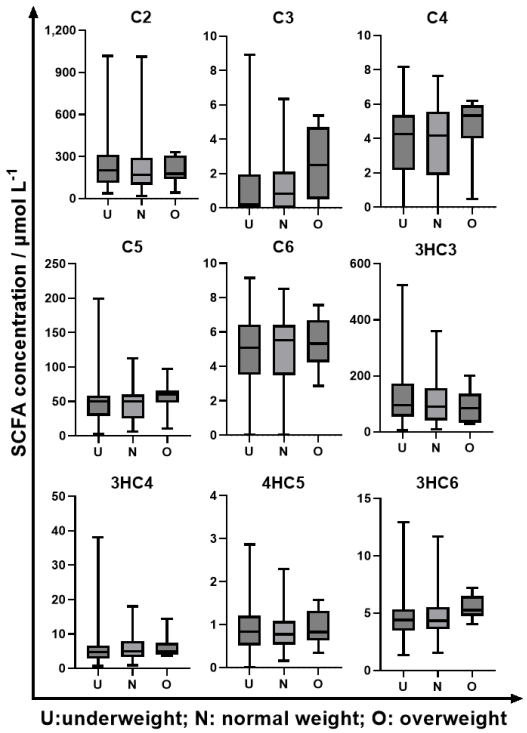

Fig. 4. (a) Relative abundance of quantitative representation of SCFA species in children's plasma (b) Plasma SCFA concentrations in underweight, normal weight, and overweight children visualized by bar graphs. (Comparisons conducted via the Kruskal-Wallis test followed by Dunn's multiple comparisons test show no statistically significant differences).

Graphical abstract: