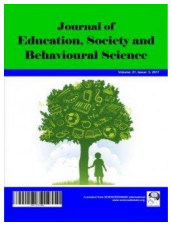

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Different Levels of Urinary Short-chain Fatty Acids in Overweight and Normal-weight Children

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Obesity may bring about various co-morbidities, which commonly not only include cardiometabolic disorders but also mood and cognitive disorders. Gut microbiota which attracts researchers' much attention recently plays an important role in maintaining human health. As the major metabolites of gut microbiota, short-chain fatty acids (SCFAs) detected in faces have been found to be associated with childhood obesity. But it has been unclear whether SCFAs in urines have the same association. This study focuses on the comparison of urinary SCFAs concentrations from overweight and normal children, trying to find certain interplay between urinary SCFAs and childhood obesity.
Methods: We analyzed the data from 23 overweight children and 23 normal weight children aged 5-6 years. The concentrations of eight urinary short-chain fatty acids -- acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, hexanoic acid, heptanoic acid, were measured using gas chromatography-mass spectrometer (GC-MS).
Results: Overweight children showed significantly higher levels of acetic acid (P<.001), propionic

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acid (*P*<.001), isobutyric acid (*P*=.003), isovaleric acid (*P*<.001), hexanoic acid (*P*<.001) and heptanoic acid (*P*<.001) than normal-weight children. Positive correlations were found between BMI and acetic acid (r=.460, *P*=.001), and propionic acid (r=.452, *P*=.002), and isovaleric acid (r=.366, *P*=.012), and hexanoic acid (r=.648, *P*<.001).

Conclusion: Urinary SCFAs are related to childhood obesity as well, compared to fecal ones. Overweight/obese children might have higher levels of SCFAs than normal ones. Our results suggest that detecting short-chain fatty acids in urine samples is a feasible method in studying multiple obesity-associated health consequences.

Keywords: SCFAs; urine; overweight; children.

1. INTRODUCTION

Prevalence of overweight and obesity in children has increased substantially around the world, including in China [1,2]. And the age when the prevalence dramatically increased became earlier [3,4]. Emerging evidence indicates that obesity may bring about various co-morbidities, which commonly not only include cardiometabolic disorders but also mood and cognitive disorders [5].

Overweight and obesity in childhood could have a particularly relevant impact since it is a critical period for neurodevelopment and neuronal plasticity [6], where negative experiences can alter brain functions, behaviors and mood states in adulthood [7]. Several studies with obese children expressed alterations in attention and attentional shifting [8,9] and visuospatial abilities [10,11] compared to normal children. Some other studies also found higher presence of anxiety and depression levels in obese children than in normal-weight children [12,13]. It was reported that obesity was evenly related to social disorders like autism spectrum disorder [14].

The associations established between obesity and mental disorders (cognitive impairment and mood and behavioral alterations) in epidemiological and experimental studies indicate shared contributing factors and pathophysiological mechanisms, which could be related to the alterations of gut microbiota [5]. Gut microbiota, which is an extremely complex ecosystem, comprising over 1000 different bacterial species with more than 7000 strains, plays an irreplaceable role in human health including immune, metabolic and neurobehavioral traits [15,16].

Underlying obesity and mental disorders, obesity-associated microbiota may contribute to dysregulation of the HPA-axis with overproduction of glucocorticoids, and alterations in levels of neuroactive metabolites such as short-chain fatty acids (SCFAs) [5]. SCFAs are among the most important and pleiotropic functional components of microbe-to-host signaling in the microbiota-gut-brain axis, as the major microbial metabolites produced during anaerobic fermentation in the gut [17,18]. Studies in adults have shown that variations in the relative abundance of two phyla Firmicutes and Bacteriodetes, and changes in SCFAs production, are related to the condition of accumulation of body fat [19]. But few studies pay attention on the correlation between gut microbiota with its metabolites SCFAs and overweight/obesity in children. Additional, our previous work has reported higher levels of cortisol which is one kind of glucocorticoids in overweight children [20], which might suggest the dysregulation of the HPA-axis in them. In this work, we determined eight SCFAs concentrations in overweight children compared with normal-weight children, trying to find whether certain relation exists between these neuroactive metabolites in urine and overweight/obesity in children.

To the best of our knowledge, feces and blood are usually chosen as biological samples for the detection of SCFAs while urines are nearly not. Murugesan et al. found that altered short-chain fatty acid concentration in feces is associated to the overweight and obese conditions of Mexican children [21]. Although fecal samples are non-invasive compared with blood sampling, they contain much more interfering components than urines that are non-invasive as wellresulting a difficullty in acquiring reliable results. In addition, urine samples are more easily to be obtained from children than blood or feces samples. Hence, in this experiment, urines are selected as test specimens for the first time in SCFAs detection for a cohortstudy in childhood obesity.

2. MATERIALS AND METHODS

2.1 Subjects

All children participating in this study were recruited from one kindergarten in Suzhou of Jiangsu, China. According to Cole et al. [22], twenty three overweight and twenty three normal-weight children aged 5-6 years were chosen, and the sex ratio in the overweight group was 14/9 (males/females) while that in the normal-weight group was 13/10 (males/females). Selected subjects were healthy and had not received any antibiotics in the immediately previous 3-months period. Informed consent was signed by the legal guardians of children in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The research protocol was approved by the Ethics Committee of Zhongda Hospital Affiliated to Southeast China University.

2.2 Sample Collection

Participants' first morning urine samples were chosen for the determination of urinary SCFAs. The guardians of all children were asked to collect first-morning-void urine from children after they woke in the morning at about 8 am in the same normal school day, and to send the urine samples to researchers at about 9 am when taking children to the kindergarten. The samples were transported to the laboratory using frozen ice packs as soon as possible and immediately stored in a -80°C freezer until further processing and analysis.

2.3 Detection of SCFAs in Children's Urine

According to our previously reported method [23], a pre-treatment processing of the urine samples were conducted and the quantitation of eight SCFAs in urines including acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, hexanoic acid, heptanoic acid were analyzed by gas chromatography-mass spectrometer (GC-MS).

2.4 Statistical Analysis

Statistical analysis was performed using SPSS version 22 (IBM SPSS Statistics). Owing to the small number of subjects, non-parametric statistical evaluation was performed to analyze differences in SCFAs between overweight and normal weight groups. Correlations were analyzed using Pearson's coefficients rho. A *P* value lower than 0.05 was regarded as statistically significant.

3. RESULTS

The results of urinary SCFAs levels from both overweight and normal weight children are shown in Table 1. It can be seen from it that the concentrations of six detected SCFAs in the urines from overweight group were significantly higher than that from normal weight group, including acetic acid (*P*<.001), propionic acid (*P*<.001), isobutyric acid (*P*=.003), isovaleric acid (*P*<.001), hexanoic acid (*P*<.001) and heptanoic acid (*P*<.001). But no significant differences were found between these two groups in other two SCFAs, including butyric acid (*P*=.198) and valeric acid (*P*=.229).

Table 2 shows the association between BMI and SCFAs concentrations (n=46). Positive correlations were weak-to-moderate between BMI and acetic acid (r=.460, *P*=.001), propionic acid (r=.452, *P*=.002), and isovaleric acid (r=.366, *P*=.012), while the correlation was moderate between BMI and hexanoic acid (r=.648, *P*<.001).

Table 1. Concentrations of SCFAs in urines from overweight and normal weight children (mean ± SD)

Analytes (ng ml^{-1})	Overweight (n=23)	Normal weight (n=23)	*P* value
Acetic acid	19001.73±9614.10	9816.36±5148.59	<.001
Propionic acid	123.81±90.75	45.30±28.03	<.001
Isobutyric acid	88.42±84.89	34.50±21.11	.003
Butyric acid	39.48±47.14	30.28±27.61	.198
Isovaleric acid	17.38±17.08	3.04±2.70	<.001
Valeric acid	27.92±28.24	14.30±6.15	.229
Hexanoic acid	20.53±11.84	2.39±1.73	<.001
Heptanoic acid	47.71±28.05	4.71±1.86	<.001

Table 2. Pearson's correlation coefficients between BMI and SCFAs concentrations

Analytes		AA	PPA	IBA	BA	IVA	VA	HEA	HPA
BMI	r value	.460**	.452**	.288	.130	.366*	.249	.648**	.234
	P value	.001	.002	.052	.389	.012	.095	<.001	.118

***Correlation is significant at the 0.01 level and *correlation is significant at the 0.05 level. AA - Acetic acid; PPA - Propionic acid; IBA - Isobutyric acid; BA - Butyric acid; IVA - Isovaleric acid; VA - Valeric acid; HEA - Hexanoic acid; HPA - Heptanoic acid; BMI – Body mass index*

4. DISCUSSION

Short-chain fatty acids (SCFAs) are the principal metabolites of gut microbiota, which have several important physiological roles in maintaining both physical and psychological health of the host. They act as intermediators between gut microbiota and host to regulate intestinal permeability, inflammation control, and bile acid metabolism, immunological functions, and disease control [24]. For instance, the colonic epithelium receives about 70% of its energy from SCFAs, mainly from butyric acid [25]; acetic acid is a substrate for cholesterol synthesis [26], as well as a suppressor of appetite through a central hypothalamic mechanism [27]; propionic acid was found to induce lipogenesis and adipogenesis [28].

It has been reported that obese humans and rodents seem to have an increased amount of faecal SCFA content compared to lean individuals [29]. This is consistent with the results found in this study that concentrations of six urinary SCFAs, including acetic acid, propionic acid, isobutyric acid, isovaleric acid, hexanoic acid and heptanoic acid, are significantly higher in overweight children than normal-weight children. The results that BMI and four SCFAs included in those six acids above has positives correlations also suggest the same trends of SCFAs changing in overweight/obese individuals. It might be explained that the rising level of propionic acid in overweight children leads to the increase of lipogenesis which can be induced by propionic acid, as mentioned above. Similarly, cholesterol synthesis might increase for higher levels of one of its substrate – acetic acid. The consistency indicates that the method of detecting urinary SCFAs concentrations is feasible in studying obesity.

Few studies on direct detection of SCFAs in urine samples of overweight or obese children have been reported as yet. Murugesan et al. [21] found lower concentrations of both propionic acid and butyric acid in obese children's fecal samples than in normal weight children's. The result is different from that in this study that overweight children display significantly higher levels of propionic acid in urine samples compared with normal weight counterparts, but no significantly higher or lower levels of butyric acid. It might be dietary differences which contributes to the inconsistent results. Different foods can affect the distribution and quantity of intestinal microorganisms and then influence the content of SCFAs, the main fermentation product of them [30]. Acetic acid, propionic acid and butyric acid are mostly produced by bacterial fermenting sugars, while isobutyric acid and isovaleric acid are mainly produced by bacterial fermenting proteins. Furthermore, besides diet, SCFAs concentration are also related to ages and living environments [31]. Thus, it is possible and reasonable that different or even opposite results will be obtained when subjects come from different regions.

Short-chain fatty acids are demonstrated not only to reach the bloodstream but also to cross the blood–brain barrier (BBB) and alter the hypothalamic leptin and adiponectin gene expression, affecting impulsivity [32]. SCFAs are thought to benefit the host by improving glucose homeostasis and stimulating enterocyte differentiation, but it should be noted that they also serve as an energy source for the host [33]. The significant alterations of six kinds of SCFAs levels in overweight children compared to normal-weight children suggest the overweight and obesity in children is associated with increased levels of some SCFAs and it can be speculated that differences exist between overweight children's neural activity and normal children's. It is unclear whether increased SCFAs concentrations positively or negatively affect cognitive impairment and mood and behavioral alterations. Therefore, more studies are needed to prove the interplay between urinary short-chain fatty acids, childhood obesity and cognitive function.

5. CONCLUSION

The results suggest that six urinary SCFAs concentrations in urines are associated with overweight in children and overweight/obese

children may have higher levels of SCFAs. Among all measured SCFAs, the results that significant differences for propionic acid and butyric acid between overweight and normal control groups are inconsistent with existing research results, which may be affected by diet and living environment. It is uncertain how SCFAs levels are related to mental disorders. Our findings emphasize the need for further research on the interplay between SCFAs, overweight/obesity and cognitive function in children and that it is a feasible method to study multiple obesity-associated health consequences by detecting short-chain fatty acids in urine samples.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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