Concentrations of very long-chain fatty acid in whole blood are associated with cardiovascular risk factors in children

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Concentrations of very long-chain fatty acid in whole blood are associated with cardiovascular risk factors in children

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ABSTRACT

Background: Fatty acid metabolism has a close relationship with metabolic syndrome. Saturated very long-chain fatty acid (hexacosanoic acid; C26:0) was recently reported to be associated with cardiovascular risk factors in adults.
Methods: Eighty-eight children (47 male, 41 female; average age, 10.9 y) participated in this study. Concentrations of C26:0 in whole blood were measured.
Results: Compared with reported concentrations in the whole blood of adults, children had lower C26:0 concentrations, which had a close relationship with abdominal obesity, increased concentrations of low-density lipoprotein cholesterol and high blood pressure. C26:0 concentrations increased with increasing number of risk factors.
Conclusions: Elevation of C26:0 concentrations may be one of the metabolic features of children with cardiovascular risk factors.

1. Introduction

Cardiovascular risk factors, including the components of metabolic syndrome, are common in children in recently urbanized areas [1]. Studies suggest that the composition of fatty acids in blood and tissue, which affect various important physiological functions related to the development of cardiovascular disease, change in abdominal obesity [2–4]. In children, abdominal adiposity has also been shown to have a close relationship with cardiovascular risk factors [5,6]. Children with abdominal obesity have a characteristic long-chain fatty acid metabolism; increased activity of stearoyl-CoA desaturase [7], and increased activity of delta-6 desaturase [8,9].

Saturated very long-chain fatty acid (hexacosanoic acid; C26:0), which is a minor component of fatty acids in human tissue, was recently reported to be associated with cardiovascular risk factors. The relative content of C26:0 in erythrocyte membranes is reported to have a close positive relationship with concentrations of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDLC), and an inverse relationship with concentrations of high-density lipoprotein cholesterol (HDLC), in healthy adults [10]. Absolute concentrations of C26:0 in whole blood appear to be associated with metabolic syndrome [11]. Information about the relationship between C26:0 concentrations and cardiovascular risk factors in children is not available. We measured the concentrations of C26:0 in whole blood in school-age children, and investigated its relationship to cardiovascular risk factors.

2. Materials and methods

2.1. Study population

Eighty-eight children (47 male, 41 female; average age, 10.9 y) in the fourth grade of an elementary school that volunteered to participate in school health checks in 2006 were the subject population. Standing height and body weight were measured. Relative weight (%) was calculated according to the standard weight obtained for sex, age and height according to data from the Ministry of Education, Science, Sports and Culture [12]. Waist circumference was measured at the concentration of the umbilicus, and the ratio of waist to height (WHtR) obtained. Blood sampling was done in the morning after overnight fasting. Concentrations of TC, HDLC and TG were measured by enzymatic methods (Autoanalizer 7600, Hitachi High Technologies Inc., Tokyo, Japan). LDLC concentrations were obtained using the Friedewald formula [13]. Concentrations of adiponectin in serum were measured by enzyme-linked immunosorbent assay (ELISA) as reported by Arita et al. [14]. Plasma glucose concentrations were measured enzymatically using a Glucose Analyzer 06 (T&A Inc., Yokohama, Japan), and insulin concentrations were determined by Elecsys insulin assay (Roche Diagnostics K.K. Tokyo, Japan). The homeostasis model of assessment ratio (HOMA-R) was obtained using the Matthews' formula as an index of insulin resistance [15].

2.2. C26:0 analysis with gas chromatography–mass spectrometry (GC–MS)

The concentrations of C26:0 were analyzed according to the procedures previously described [11]. C26:0 in whole blood was directly transmethylated with 14% boron

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Table 1
Characteristics of the study subjects

	Male	Female	
	$n=47$	$n=41$	p
Relative weight (%)	107.3±2.7	107.9±2.9	0.9833
Waist circumference (cm)	61.6±1.3	61.7±1.3	0.9900
Waist/height ratio	0.45±0.01	0.45±0.01	0.5940
Systolic blood pressure (mmHg)	112.1±1.6	114.8±1.9	0.3421
Diastolic blood pressure (mmHg)	65.4±1.2	65.7±1.3	0.9100
Total cholesterol(mg/dl)	180.9±4.1	182.7±4.1	0.6941
LDL-cholesterol (mg/dl)	90.4±3.2	93.6±3.3	0.4642
HDL-cholesterol (mg/dl)	65.6±1.7	63.7±1.9	0.3771
Triglyceride (mg/dl)	53.1±3.7	57.0±5.5	0.6665
Fasting insulin (μU/ml)	7.5±0.6	10.0±1.2	0.0533
Fasting glucose (mg/dl)	93.3±0.8	90.2±0.7	0.0059
HOMA-R	1.7±0.1	2.2±0.3	0.0952
Adiponectin (μg/ml)	5.7±0.5	5.0±0.5	0.4219
Hexacosanoic acid (μg/ml)	1.91±0.04	1.86±0.03	0.4718

Mean±SE Mann–Whitney *U*-test.

trifluoride methanol solution (Sigma-Aldrich Japan, Tokyo, Japan) at 90 °Cfor 90 min. The quantification of C26:0 was carried out on a GC–MS system (QP2010, Shimadzu Corporation, Kyoto, Japan) equipped with fused silica capillary column (Rtx-5MS, 30 m×0.25 mm i.d.; 0.25 μm film thickness, Restek, USA) using nonacosanoic acid (C29:0) methyl ester as an internal standard. In this measurement system, intraassay CV was 5.2% and intraclass correlation was 96.5%.

2.3. Cardiovascular risk factors

Informed consent was obtained from the children and their parents before participation in this study. The study protocol was approved by the University Ethics Committee of Nihon University, Itabashi Hospital, Tokyo, Japan. In the present study, metabolic syndrome in children was defined as having abdominal obesity (waist circumference≥75 cm and/or WHtR≥0.5) plus≥2 of the following: (i) dyslipidemia: TG≥120 mg/dl and/or HDLC<40 mg/dl; (ii) elevated systolic blood pressure (≥125 mmHg) and/or diastolic blood pressure (≥70 mmHg); or (iii) increased concentration of fasting plasma glucose (≥100 mg/dl) [16]. Elevated LDLC concentrations (≥110 mg/dl) were included as cardiovascular risk factors [17], as well as all components of metabolic syndrome. Children were disease-free except for dyslipidemia and obesity.

2.4. Statistical analyses

Data are expressed as mean±standard error. Differences in mean values were analyzed by Mann–Whitney *U*-test. Correlation coefficients between 2 variables were determined by simple regression analysis. Multiple regression analysis was carried out to determine variables explaining C26:0 concentrations in whole blood. The relationship between C26:0 concentrations in whole blood and the number of cardiovascular risk factors was analyzed by one-way ANOVA with *post hoc* test. A $p<0.05$ was considered statistically significant. Statistical analyses were conducted using the statistical package STATVIEW (ver. 4.5; Abacus Concepts, Berkeley, CA).

3. Results

3.1. Patient characteristics

Patient characteristics are shown in Table 1. Males had significantly higher fasting glucose concentrations than females, but no other difference based on sex was demonstrated. The concentration of C26:0 in whole blood was 1.91±0.04 μg/ml in males and 1.86±0.03 μg/ml in females. Two children had metabolic syndrome, and their C26:0 concentration was 1.96±0.04 μg/ml.

Table 2
Simple correlation coefficients between very long-chain fatty acid and variables

	Correlation coefficient	
	r	p
Relative weight (%)	0.212	0.0470
Waist circumference (cm)	0.223	0.0363
Waist/height ratio	0.217	0.0424
Systolic blood pressure (mmHg)	0.301	0.0044
Diastolic blood pressure (mmHg)	0.389	0.0002
Total cholesterol (mg/dl)	0.227	0.0335
LDL-cholesterol (mg/dl)	0.229	0.0316
HDL-cholesterol (mg/dl)	−0.018	0.8648
Triglyceride (mg/dl)	0.173	0.1071
Fasting insulin (μU/ml)	0.077	0.4784
Fasting glucose (mg/dl)	0.033	0.7593
HOMA-R	0.073	0.4995
Adiponectin (μg/ml)	0.165	0.1234

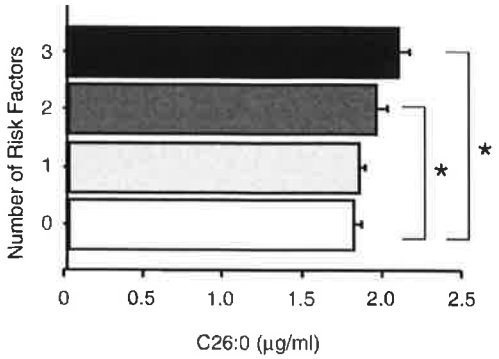

Fig. 1. Relationship between C26:0 concentrations in whole blood and the number of cardiovascular risk factors. *Statistical significance analyzed by ANOVA with Fisher's *post hoc* test.

3.2. C26:0 concentrations in whole blood and cardiovascular risk factors

The prevalences of abdominal obesity, dyslipidemia, elevated blood pressure and increased blood glucose concentrations were 19.3%, 19.3%, 38.6% and 10.2%, respectively. We found 29 children with 1 risk factor (33.0%), 17 children with 2 risk factors (8.0%) and 5 children with 3 risk factors (5.7%).

Concentrations of C26:0 in whole blood correlated positively with relative weight ($r=0.212$, $p=0.0470$), waist circumference ($r=0.223$, $p=0.0363$), WHtR($r=0.217$, $p=0.0424$), systolic blood pressure ($r=0.301$, $p=0.0044$), diastolic blood pressure ($r=0.389$, $p=0.0002$), TC concentration ($r=0.227$, $p=0.0355$) and LDLC concentration ($r=0.229$, 0.0316) (Table 2). In a multiple regression analysis that included waist circumference, diastolic blood pressure and LDLC concentration as independent determinants, waist circumference ($r=0.227$, $p=0.0214$) and diastolic blood pressure ($r=0.391$, $p=0.0001$) were the significant determinants of concentrations of C26:0 in whole blood, explaining 20.3% variability ($p<0.0001$).

The number of cardiovascular risk factors showed a significant association with C26:0 concentrations in whole blood (Fig. 1). Compared with children with no risk factors (1.83±0.04 μg/ml), C26:0 concentrations in whole blood of children with 2 or 3 risk factors were significantly higher (1.97±0.06 μg/ml, 2.10±0.07 μg/ml, respectively).

4. Discussion

We demonstrated C26:0 concentrations in whole blood in school-age children. C26:0 concentrations in whole blood were lower in children compared with those reported in adults [11]. Even in children, C26:0 concentrations in whole blood were closely related with cardiovascular risk factors. C26:0 concentrations increased with increasing number of risk factors in children.

C26:0 has been well investigated in peroxisomal disorders such as Zellweger syndrome, Refsum's disease and X-linked adrenoleukodystrophy [18,19] because peroxisome is essential in the β-oxidation of very long-chain saturated and unsaturated fatty acids. In these diseases, very long-chain fatty acid increases in plasma and is useful not only as a diagnostic marker, but also as a marker of clinical severity. Apart from genetic diseases, peroxisomal β-oxidation is also affected in alcoholic patients and crush syndrome. In these patients, active oxygen species or free radicals generated by chronic consumption of alcohol or ischemia—reperfusion may interrupt the beta-oxidation of fatty acids by peroxisome [20,21]. A mechanism linking high

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concentrations of C26:0 and cardiovascular risk factors has not been elucidated.

The activity of β-oxidation in peroxisomes is reported to decrease with age, leading to an accumulation of very long-chain fatty acids in mice [22]. In humans, C26:0 concentrations in whole blood increase with age [11]. In our study, children had lower concentrations of C26:0 than adults. Peroxisomal β-oxidation activity in humans may therefore also be affected by aging. Our results suggest that peroxisomal β-oxidation activity may be attenuated in children with cardiovascular risk factors, and that the degree of the attenuation depends on the severity of risk factors. Like the long-chain fatty acid metabolism by delta-6 desaturase or stearoyl-CoA desaturase [7–9], the peroxisomal β-oxidation of very long-chain fatty acid may be impaired in obese children.

5. Conclusion

Concentrations of C26:0 in whole blood in children had a close relationship with abdominal obesity, increased LDLC concentrations and high blood pressure, and then with the clustering of these cardiovascular risk factors. The elevation of C26:0 concentrations may be one of the metabolic features of cardiovascular risk factors in children.

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補足文章

【補足目的】

主論文「Concentrations of very long-chain fatty acid in whole blood are associated with cardiovascular risk factors in children」(Clinica Chemica Acta, 401巻, P141-143)を2009年3月に発表した後も，小児肥満とヘキサコサン酸(C26:0)との関連について追随する他の発表はない。本補足文章は成人も含めた同分野における研究状況について補足したうえで，主論文の現在の位置付けを明らかにすることを目的とした。

【ヘキサコサン酸研究の今】

PubMed検索[1]において，2009年3月以降「メタボリックシンドローム」を抄録文中に含む報告は50,623件であった。そのうち「心血管病リスク」も含む報告は11,450件，そのうち，「脂肪酸」も含む報告は650件，さらに，「極長鎖脂肪酸」を含む報告は54件，「C26:0」を含む報告はMiyazakiらの1件のみであった[2]。「小児」のC26:0については0件であった。

【メタボリックシンドロームとヘキサコサン酸の位置づけ】

2009年に本論文は初めて、小児においてC26:0が高値であることが心血管病リスクと相関することを証明し，かつ，C26:0の値と心血管病リスク因子の保

有数も正の相関関係にあることを明らかにした。

その後，2013年にMatsumoriらは成人おいて赤血球中の極長鎖脂肪酸がメタボリックシンドロームでは高値であり、心血管病リスク因子と関連することを発表した[3]。2014年にMiyazakiらはC26:0が高値であることは成人での冠動脈疾患の独立した危険因子であると報告した[2]。しかし、小児における心血管病リスクについての報告はなく、本論文報告が唯一かつ最新のエビデンスとなっている。

【小児肥満とヘキサコサン酸のこれから】

日本を含む先進国では，小児の肥満について注目が年々増している。2017年に日本肥満学会から小児肥満症診療ガイドライン2017が発表され，「肥満小児」と「肥満症」が定義された[4]。「肥満症」の新定義では2007年の小児期メタボリックシンドローム診断基準の項目である，腹囲，血圧，血清脂質，血糖に加え，内臓脂肪型肥満，早期動脈硬化，睡眠時無呼吸などの換気障害，非アルコール性肝障害，尿酸が健康障害として含まれている。C26:0は腹部肥満，高血圧，高コレステロール血症と相関していることから，最新の診断基準に照らしても，小児肥満症の発症に関与する1つの成因として高C26:0血症が関与すると考えられる。本論文の限界点として，小児のC26:0とトリグリセリド，

インスリン抵抗性に有意な相関は認めず，メタボリックシンドロームに認められる脂質異常症すべてとの相関は確認できていないことがある。小児は年齢による検査値の変化があるため，今後調査対象を幼児から学童まで広げることや，同一群での追跡調査が望まれる。また，本論文を含むこれまでの研究では，血中極長鎖脂肪酸心血管病リスクの関係が成人と小児において同等の意義を示すかは明らかにされていない。今後，追跡調査によって，小児の高C26:0血症が成人期に若年性の心血管病イベントを発症することに関与するのか，小児期の保健指導によって肥満が改善することでC26:0値も低下するのか，ひいては心血管病の発症率を低下することに寄与できるのか，を検証することで心血管病予防への貢献が望まれる。

【文献（補足文章内）】

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