Blood asymmetric dimethylarginine and nitrite/nitrate concentrations in short-stature children born small for gestational age with and without growth hormone therapy

Nagasaka, Hironori ; Morioka, Ichiro ; Takuwa, Mayuko ; Nakacho, Mariko ; Yoshida, Mayumi ; Ishida, Akihito ; Hirayama, Satoshi ; Miida,…

(Citation)
Journal of International Medical Research,46(2):761-772

(Issue Date)
2018-02

(Resource Type)
journal article

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/90004727

Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(2) 761–772

DOI: 10.1177/0300060517723183
journals.sagepub.com/home/imr
SAGE

Blood asymmetric dimethylarginine and nitrite/nitrate concentrations in short-stature children born small for gestational age with and without growth hormone therapy

Hironori Nagasaka[1,*], Ichiro Morioka[2,*], Mayuko Takuwa[1], Mariko Nakacho[1], Mayumi Yoshida[1], Akihito Ishida[3], Satoshi Hirayama[4], Takashi Miida[4], Hirokazu Tsukahara[5], Tohru Yorifuji[6] and Kazumoto Iijima[2]

Abstract

Objective: To investigate the basal amino acid metabolism and impact of growth hormone (GH) therapy in short-stature children born small for gestational age (short SGA children).

Methods: In this age-matched case-control study, the basal blood levels of amino acids, asymmetric dimethylarginine (ADMA), and nitrite/nitrate (NOx) were compared between 24 short SGA children and 25 age-matched normal children. Changes in these parameters were assessed for 12 months in 12 short SGA children initiating GH therapy (Group A) and 12 age-matched short SGA children without GH therapy (Group B).

Results: The arginine levels were significantly lower in the short SGA than in normal children. The ADMA levels were significantly higher and NOx levels were significantly lower in the short SGA than normal children. In Group A, the ADMA level was significantly lower and NOx level was

[1]Department of Pediatrics, Takarazuka City Hospital, Takarazuka, Japan
[2]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan
[3]Kobe Children's Primary Emergency Medical Center, Kobe, Japan
[4]Department of Clinical Laboratory Medicine, Juntendo University School of Medicine, Tokyo, Japan
[5]Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[6]Division of Pediatric Endocrinology and Metabolism, Children's Medical Center, Osaka City General Hospital, Osaka, Japan
[*]These authors contributed equally to this work.

Corresponding author:
Ichiro Morioka, Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 6500017, Japan.
Email: ichim@med.kobe-u.ac.jp

significantly higher at 6 months than at baseline. At 12 months, the ADMA level in Group A began to increase, but the NOx level remained the same. Group B showed no significant changes.
Conclusions: This study is the first to show that ADMA is promoted and nitric oxide is suppressed in short SGA children and that GH therapy affects the production of ADMA and nitric oxide.

Keywords
Asymmetric dimethylarginine, growth hormone treatment, nitrite/nitrate, short stature, small for gestational age, amino acids

Date received: 13 February 2017; accepted: 7 July 2017

Introduction

Growth hormone (GH) has been widely accepted as a treatment for primary and secondary GH deficiency.[1–4] In addition to increased height in childhood, GH therapy is associated with a reduced risk of cardiovascular events later in life.[1,4] GH therapy has also been administered to small-for-gestational age (SGA) children presenting with a short stature (short SGA children), and its impact on height catch-up has been demonstrated.[5–7]

Short SGA children are inclined to present with metabolic abnormalities predisposing to cardiovascular diseases such as hypercholesterolemia later in life as a consequence of relative GH deficiency.[5,6] However, limited evidence regarding other metabolic risk factors is available. To date, no details regarding the amino acid metabolism in short SGA children with and without GH therapy have been reported.

The production of nitric oxide (NO) and asymmetric dimethylarginine (ADMA) is strongly linked to amino acids such as arginine and citrulline (Figure 1). NO has many biological functions, including the maintenance of vascular tone, neurotransmitter function, and mediation of cellular defense.[8] ADMA competes with arginine for NO synthase and inhibits NO production; it also promotes superoxide production and enhances oxidative stress.[9] ADMA was recently employed as a surrogate risk marker for cardiovascular disease, renal disease, hypertension, diabetes mellitus, and other conditions in both adults and children.[10–12]

In the present study, we investigated the blood levels of amino acids, ADMA, and NOx[13,14] and the impact of GH therapy for 12 months in short SGA children.

Patients and Methods

Study design and patients

This age-matched case-control study was approved by the institutional review boards of Takarazuka City Hospital (reference number: 198), Osaka City General Hospital (reference number: 1611073), and Kobe University Graduate School of Medicine (reference number: 160089). Written informed consent was obtained from the parents of the children prior to study enrollment.

Twenty-four short SGA children aged 3 to 6 years who visited Takarazuka City Hospital and Osaka City General Hospital from 2010 to 2016 were enrolled. The birth weight and height standard deviation (SD) scores were determined using the standard curve according to gestational age for the Japanese population.[15]

At 3 to 6 years of age, the patients had visited hospitals in the vicinity of their homes because of parental concern

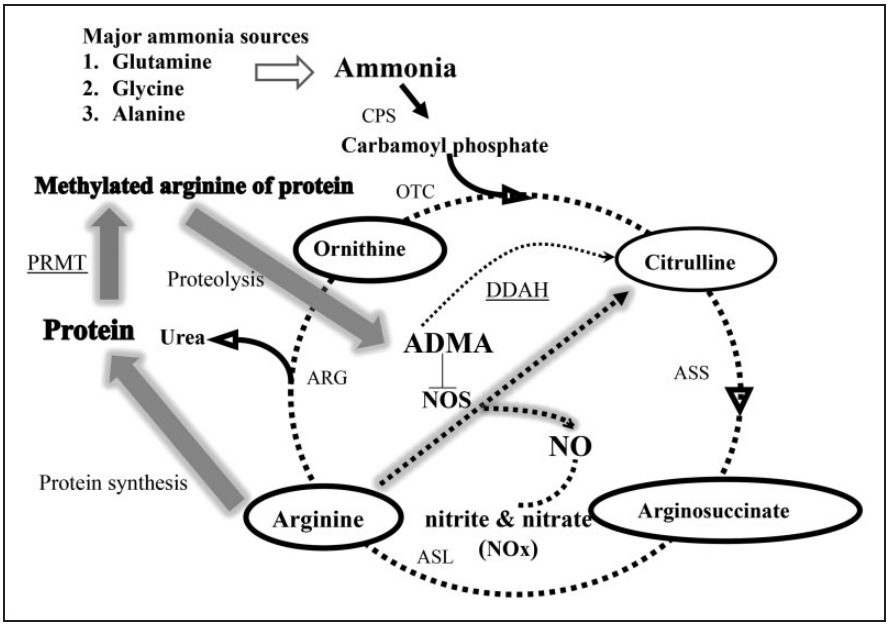

Figure 1. Coupling of urea cycle with nitric oxide cycle.

ADMA, asymmetric dimethylarginine; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS, carbamoyl phosphate synthetase; DDAH, dimethylarginine dimethylaminohydrolase; NO, nitric oxide; NOS, nitric oxide synthase; OTC, ornithine transcarbamylase; PRMT, protein arginine methyltransferase.

regarding the child's short stature. They were then referred to our hospital for thorough examinations. All patients showed normal peak plasma GH concentrations (>6 ng/mL on arginine, clonidine, and insulin tests). None of the enrolled patients had experienced serious episodes of conditions such as asphyxia, respiratory distress, or severe infection during the newborn or infancy period with the exception of two patients who had developed mild hypoglycemia that was resolved by intravenous glucose administration a few days after birth. The patients showed normal intellectual development with the exception of one patient who had been diagnosed with a mild learning disability.

After receiving a detailed explanation of GH therapy, the parents of 12 children requested to initiate GH therapy as soon as possible (Group A), and parents of the remaining 12 children requested to initiate GH therapy a few years later (Group B). Children in Group A received a subcutaneous injection of GH once daily at a dose of 0.23 to 0.25 mg/kg for the first 6 months and 0.34 to 0.36 mg/kg for the following 6 months. For both groups, the following data were monitored for 12 months: height, weight, and various biochemical parameters (amino acid profiles, the ADMA and NOx concentrations, and parameters linked to citrulline–arginine recycling, such as arginine) (Figure 1). Blood insulin-like growth factor-1 (IGF-1) was also monitored. Additionally, to evaluate the oxidative stress status that influences NO and ADMA production, the plasma concentrations of the antioxidants β-carotene and α-tocophenol were examined.[8,9]

A group of 25 age-matched healthy volunteer children (13 boys and 12 girls aged 3–6 years) was enrolled at the Kobe Children's Primary Emergency Medical Center to

provide comparison levels for all monitored parameters.

Determination of IGF-1 and antioxidant concentrations

The serum IGF-1 concentration was determined via an immunoradiometric assay using a somatomedin C-II kit (Mitsubishi Chemicals Co., Ltd., Tokyo, Japan). The IGF-1 SD scores were determined based on the age- and sex-specific standard values for the Japanese population.[16] The plasma β-carotene and α-tocophenol concentrations were measured using high-performance liquid chromatography.

Determination of serum ADMA, NOx, and amino acid concentrations

The serum ADMA concentration was determined using an enzyme-linked immunosorbent assay kit (DLD Diagnostika GmbH, Hamburg, Germany).[17,18] The serum concentration of NOx was assessed using the Griess method with an NOx colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA). The plasma concentrations of amino acids involved in citrulline–arginine recycling (Figure 1)[13,14] were determined using routine ion-exchange chromatography with an autoanalyzer (L822; Hitachi High-Technologies Corp., Tokyo, Japan).

Statistical analyses

Data distributions were assessed for normality using a one-sample Kolmogorov–Smirnov test. Data are presented as mean ± SD. Differences between the controls and patient groups with and without GH treatment were examined using an unpaired Student's two-tailed *t*-test or nonparametric Mann–Whitney U test. Pre- and post-treatment comparisons were performed using the Wilcoxon signed-rank test. Correlations between variables were estimated using Pearson's correlation tests. A p-value of <0.05 was considered statistically significant.

Results

Basal anthropometric and amino acid profiles in short SGA and normal children

The height and body weight SD scores of the short SGA children were significantly lower than those of the non-obese and non-short stature normal children ($p < 0.001$ vs. controls). The amino acid profile together with the total protein and albumin blood concentrations were not different between these two groups. However, the NOx and arginine concentrations were significantly lower ($p < 0.05$) and the ADMA levels were significantly higher ($p < 0.01$) in the short SGA children than in the normal children (Table 1). The NOx concentration exhibited a significantly positive correlation with the ADMA concentration in controls but a significantly negative correlation in the short SGA children (normal children, $R^2 = 0.76$, $p < 0.001$; short SGA children, $R^2 = 0.45$, $p < 0.01$) (Figure 2).

Changes in height and body weight SD scores

Consistent with SGA, the birth height and/or weight SD scores of the patients were less than −2 SD (Table 2).[5,6] The height and weight SD scores in infancy were determined based on the age- and sex-specific standard values for the Japanese population.[19] Consistent with the indications for GH therapy, the height SD score of the patients was less than −2.5 SD at the age of ≥3 years (Tables 1 and 2).[5,6] At 0 months, the height and body weight SD scores were not significantly different between Groups A and B. At 6 months, the height SD score in Group A had increased significantly from that at 0 months ($p < 0.05$ vs. basal SD score). At 12

Table 1. Backgrounds and amino acid profiles of short SGA children and age-matched controls.

	Short SGA children	Controls
Patients (male/female)	24 (13/11)	25 (13/12)
Age (years)	4.7 ± 1.2	5.0 ± 1.0
Height (cm)/SD score	93.2 ± 7.1***/ − 2.79 ± 0.18***	107.4 ± 8.2/0.15 ± 0.22
Weight (kg)/SD score	11.4 ± 1.5***/−2.46 ± 0.47***	17.7 ± 2.2/0.12 ± 0.33
Body mass index (kg/m^2)	14.9 ± 0.4	15.3 ± 0.5
Total protein (g/dL)	6.8 ± 0.2	6.9 ± 0.2
Albumin (g/dL)	3.7 ± 0.1	3.8 ± 0.1
NOx (μmol/L)	27.1 ± 7.1*	32.1 ± 10.2
ADMA (μmol/L)	0.712 ± 0.155**	0.622 ± 0.225
Arginine (μmol/L)	80.3 ± 11.6*	88.4 ± 12.4
Ornithine (μmol/L)	88.6 ± 10.3	90.8 ± 11.1
Citrulline (μmol/L)	25.2 ± 4.9	24.4 ± 4.8
Glutamine (μmol/L)	435 ± 85	399 ± 98
Glycine (μmol/L)	258 ± 39	240 ± 46
Alanine (μmol/L)	389 ± 57	413 ± 62
Lysine (μmol/L)	171 ± 39	183 ± 38

ADMA, asymmetric dimethylarginine; NOx, nitrite/nitrate as stable metabolites of nitric oxide; SD, standard deviation; SGA, small for gestational age.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with controls

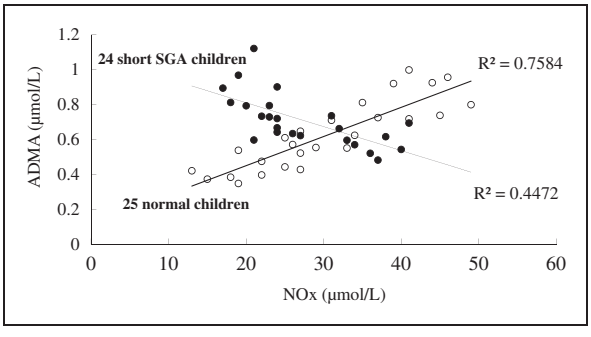

Figure 2. Correlation between NOx and ADMA levels in short SGA and normal children.
ADMA, asymmetric dimethylarginine; R^2, coefficient of determination; NOx, nitrite/nitrate; SGA, small for gestational age.

months, the height SD score had continued to increase in Group A ($p < 0.001$ vs. basal SD score), and the body weight SD score had also significantly increased from that at 0 months ($p < 0.05$ vs. basal SD score). In contrast, the height and weight SD scores in Group B did not significantly change during the 12-month study period (Table 2).

Changes in ADMA, NOx, and amino acid concentrations

At 0 months, the ADMA concentrations in Groups A and B were not significantly different from each other, but they were significantly higher than those in the control group ($p < 0.001$). In Group A, the ADMA

Table 2. Height and body weight at birth and during the study period in short SGA children with/without GH.

	Group A (with GH)	Group B (without GH)
Patients (male/female)	12 (7/5)	12 (6/6)
Age at entry (years)	4.6 ± 1.1	4.7 ± 1.2
Gestational age (weeks)	38.9 ± 1.6	38.6 ± 1.9
Height (cm): SD score		
At birth	44.3 ± 1.9: −2.09 ± 0.32	43.9 ± 2.1: −2.14 ± 0.29
At entry	93.5 ± 7.0: −2.82 ± 0.24	92.9 ± 6.5: −2.76 ± 0.16
At 6 months	98.1 ± 7.1: −2.48 ± 0.26*	95.0 ± 6.9: −2.87 ± 0.18
At 12 months	102.6 ± 7.2: −2.07 ± 0.32**	98.3 ± 6.4: −2.92 ± 0.26
Weight (kg): SD score		
At birth	2.26 ± 0.35: −2.24 ± 0.19	2.31 ± 0.32: −2.20 ± 0.26
At entry	11.2 ± 1.7: −2.46 ± 0.48	11.6 ± 1.8: −2.35 ± 0.45
At 6 months	12.0 ± 1.8: −2.36 ± 0.49	12.1 ± 1.9: −2.32 ± 0.48
At 12 months	12.0 ± 1.8: −1.93 ± 0.57*	12.9 ± 2.1: −2.20 ± 0.44

In Group A, the dosage of GH was 0.23 to 0.25 mg/kg/week for the first 6 months and 0.34 to 0.36 mg/kg/week for the next 6 months.
*$p < 0.05$, **$p < 0.001$ vs. basal (entry) levels. GH, growth hormone; SD, standard deviation; SGA, small for gestational age.

concentration at 6 months was significantly lower than that at 0 months ($p < 0.01$) and comparable with that in the control group. At 12 months, the ADMA concentration was higher than that at 6 months but significantly higher than that at 0 months ($p < 0.05$). In contrast, Group B exhibited no significant changes in the ADMA concentration during the 12-month study period (Table 3).

The basal NOx concentrations in Groups A and B were comparable with each other and significantly lower than those in the control group ($p < 0.01$). Thereafter, in Group A, the NOx concentration significantly increased compared with basal levels ($p < 0.01$), with the NOx concentrations at 6 and 12 months comparable with those in the control group. In Group B, the NOx concentration remained at the basal level throughout the study period (Table 3).

The basal arginine concentrations in Groups A and B were significantly lower than those in the control group ($p < 0.05$). After the initiation of GH therapy, the arginine level significantly increased in Group A ($p < 0.05$ at 6 months and $p < 0.01$ at 12 months), demonstrating a concentration that was almost comparable with that in the control group. In contrast, the arginine concentration remained unchanged in Group B (Table 3). Among the many remaining amino acids examined, only the glutamine concentration in Group A was significantly lower at 6 and 12 months compared with basal levels ($p < 0.05$) (Table 3).

Changes in IGF-1 and antioxidant concentrations

The basal IGF-1 concentrations in Groups A and B were not significantly different and were within the normal range (105 vs. 120 ng/mL) (Table 4). The SD score of the basal IGF-1 concentration in Groups A and B was less than −0.5. In Group A, the IGF-1 concentration significantly increased to more than the 0.5 SD score (154 ng/mL, $p < 0.001$) at 6 months and to more than the 1.0 SD score (190 ng/mL, $p < 0.001$) at 12 months. However, no dramatic increases were seen in Group B (120 ng/mL at

Table 3. Changes in NOx, ADMA, and amino acid concentrations during the 12-month study period.

	Group A (n = 12)			Group B (n = 12)			
Sampling time	0 mos	6 mos	12 mos	0 mos	6 mos	12 M	Controls (N = 25)
NOx (μmol/L)	26.8 ± 6.6††	31.4 ± 7.6**	32.5 ± 6.6**	27.8 ± 8.1††	26.5 ± 6.0	25.2 ± 6.7	31.6 ± 10.2
ADMA (μmol/L)	0.707 ± 0.156†††	0.605 ± 0.153**	0.640 ± 0.140*	0.716 ± 0.146†††	0.716 ± 0.178	0.679 ± 0.176	0.622 ± 0.225
Arginine (μmol/L)	78.9 ± 14.5†	89.8 ± 18.2*	93.4 ± 15.5**	81.8 ± 11.9†	78.9 ± 13.4†	80.0.0 ± 15.6†	88.4 ± 12.4
Ornithine (μmol/L)	87.3 ± 12.9	89.4 ± 14.5	94.3 ± 15.6	89.8 ± 9.3	91.0 ± 13.0	87.0 ± 9.8	90.8 ± 11.1
Citrulline (μmol/L)	24.3 ± 5.4	25.3 ± 4.4	27.0 ± 4.8	26.0 ± 6.6	23.5 ± 5.3	23.9 ± 5.0	24.4 ± 4.8
Glutamine (μmol/L)	459 ± 80	375 ± 77*	362 ± 76*	492 ± 93	459 ± 54	443 ± 84	399 ± 69

ADMA, asymmetric dimethylarginine; NOx, nitrite/nitrate as stable metabolites of nitric oxide
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. basal (0-mo) levels
†$p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. control levels

Table 4. Changes in IGF-1 and antioxidant concentrations during the 12-month study period.

	Group A (n = 12)			Group B (n = 12)			
Sampling time	0 mos	6 mos	12 mos	0 mos	6 mos	12 M	Controls (N = 25)
IGF-1 (ng/mL)	105 ± 24	154 ± 25**	190 ± 32** $^{\#}$	120 ± 29	121 ± 24	129 ± 22	148 ± 35
α-tocophenol (mg/dL)	0.67 ± 0.17	0.79 ± 0.15*	0.77 ± 0.14*	0.63 ± 0.15	0.66 ± 0.16	0.68 ± 0.18	0.81 ± 0.18
β-carotene (mg/dL)	33.3 ± 12.5	40.4 ± 12.7*	38.9 ± 11.1*	31.9 ± 14.4	31.7 ± 12.3	33.5 ± 14.1	42.7 ± 13.3

IGF-1, insulin-like growth factor-1
*$p < 0.05$, **$p < 0.001$ vs. basal (0-mo) levels; $^{\#}p < 0.01$ vs. 6-mo levels

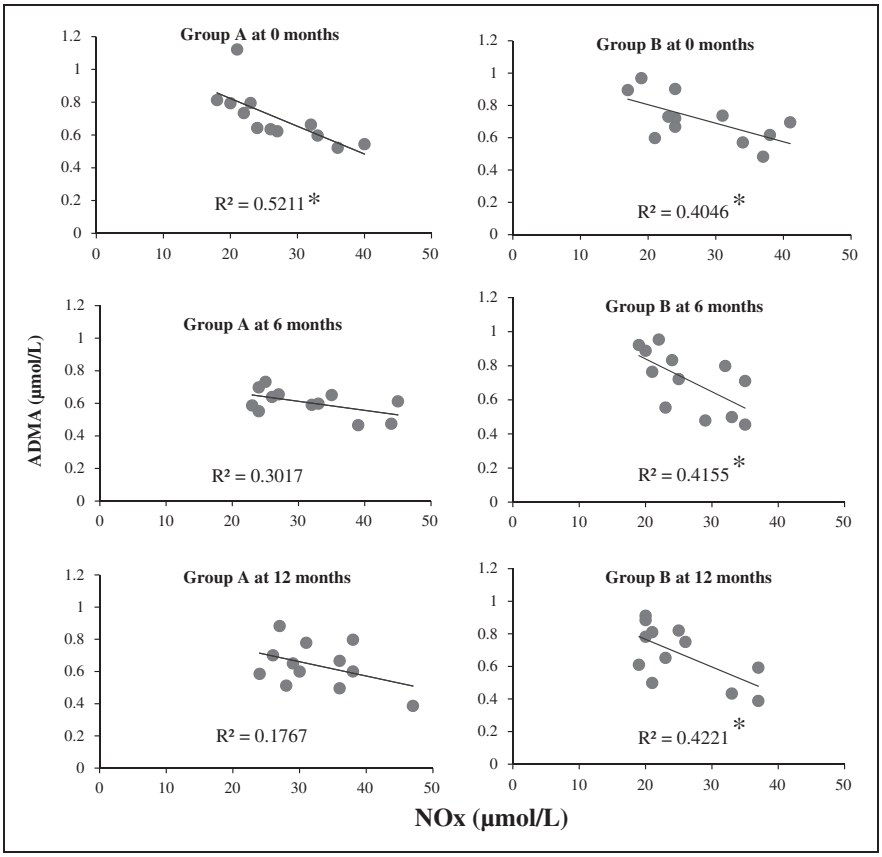

Figure 3. Scatter graphs displaying NOx against ADMA for short SGA children with/without GH therapy. ADMA, asymmetric dimethylarginine; R^2, coefficient of determination; GH, growth hormone; NOx, nitrite/nitrate; SGA, small for gestational age. *$p < 0.05$ (Pearson's correlation test)

baseline, 121 ng/mL at 6 months, and 129 ng/mL at 12 months). The basal β-carotene and α-tocophenol concentrations in the short SGA children were lower than those in the age-matched controls. These levels were significantly higher at 6 and 12 months compared with the basal levels in Group A but not in Group B (Table 4).

Correlations between NOx and ADMA concentrations after initiation of GH therapy

At 0 months, the NOx concentration demonstrated a significant negative correlation with the ADMA concentration in Groups A and B ($p < 0.05$) (Figure 3). Group B continued to exhibit this significant negative correlation, but no significant correlations were found at 6 and 12 months in Group A.

Discussion

GH has been widely administered to patients with GH deficiency, particularly to young children with idiopathic GH insufficiency.[1–4] For the last 10 years, GH has also been increasingly administered to short SGA children, although the doses are

pharmacological and much higher than those used for treatment of physiological GH deficiency.[5,6] This is consistent with the higher IGF-1 concentrations in short SGA children undergoing treatment with GH as shown in Table 4. The strong effect of GH in increasing height has been shown in many patients. In addition, the metabolic effects of GH on lipids and carbohydrates have been previously reported.[1,4–6] Specifically, GH lowers the low-density lipoprotein cholesterol concentration, and a higher dose of GH decreases insulin sensitivity, predisposing patients to diabetes mellitus.[1,4–6] Nevertheless, the impact of GH on amino acid metabolism has been scarcely examined.[20–25] Furthermore, few data exist on amino acid metabolism in short SGA children.

The results of the present study also suggest that basal ADMA production is promoted in short SGA children and that in contrast to ADMA, NO production is suppressed. Almost all cells and tissues produce ADMA as a product of protein degradation. ADMA is formed from arginine residues in proteins and competes with arginine for each of the three isoforms of NO synthase (NOS) (Figure 1).[9] ADMA is mainly metabolized into citrulline and dimethylamine by the liver, kidney, and endothelium via dimethylarginine dimethylaminohydrolase.[9,26,27] In the present study, the serum NOx concentration showed a significant negative correlation with the serum ADMA concentration in short SGA children without GH treatment in contrast to normal children, who exhibited a significant positive correlation. Accordingly, increased ADMA production is likely to suppress NO production in short SGA children. The mechanistic explanation for the increase in ADMA remains unknown. Oxidative stress stimulates ADMA production by suppressing dimethylarginine dimethylaminohydrolase activity and promoting protein arginine methyltransferase (Figure 1).[28–30] The oxidative stress status in short SGA children was not fully evaluated in the present study. However, the blood concentration of the antioxidants β-carotene and α-tocophenol were decreased in the short SGA children and restored by GH treatment (Table 4). These results suggest that enhanced oxidative stress contributes to suppression of NO production in short SGA children.

The influence of GH on the NOS-ADMA pathway has been rarely studied. Only a few reports have described ADMA and NO production during GH treatment.[20–25] According to these earlier reports,[20–23] GH is thought to exhibit generally favorable effects on the NOS-ADMA pathway in GH deficiency, possibly leading to reduced risk of cardiovascular events. Most reports have described decreased blood ADMA concentrations in GH-deficient adults and increased basal ADMA concentrations in prepubertal to pubertal children undergoing GH therapy.[20–24] Önder et al.[25] reported decreased ADMA in prepubertal to pubertal GH-deficient children, whose basal ADMA concentrations were comparable with those of the age-matched controls, by GH therapy. The effect of GH on ADMA production may differ according to age. The results of the present study suggest that suppressed NO production in short SGA children is, at least in part, restored by GH therapy because the serum NOx concentration (reflecting NO production) was increased at dosages of 0.23 to 0.25 and 0.34 to 0.36 mg/kg/week. In contrast, the serum ADMA concentration decreased under the former dosage but increased under the latter dosage. In general, our results are largely consistent with those described in earlier reports.[20–24]

The results of the present study also showed that the concentrations of the amino acids involved in the urea cycle and citrulline–arginine recycling changed with GH treatment.[13,14] Specifically, the basal serum arginine concentration in the short

SGA children decreased and was restored after the initiation of GH treatment; the arginine-to-ornithine ratio significantly increased by GH therapy, and in contrast to arginine, increased levels of glutamine (a major source of ammonia) decreased with GH therapy. Based on these data, we speculate that urea cycle function is promoted by GH treatment to some degree, resulting in increased arginine production (Figure 1). Considering that arginine is a substrate for NO, the increase of arginine may contribute to the increased NO production, at least in part.

IGF-1 increases NOS activity by interacting with a tyrosine kinase membrane receptor linked to insulin receptor substrate.[31,32] This receptor complex activates phosphatidylinositol 3-kinase, which activates the serine/threonine kinase Akt signaling pathway, promoting NOS activity and NO production.[29,30] From this context, we infer that the considerably increased IGF-1 concentration by GH is attributable to the restoration of NO production.

Short SGA children exhibit a decrease in muscle volume and an increase in visceral fat mass volume.[33–35] The accumulation of visceral fat is sometimes considerable in affected patients.[33,34] GH treatment has been shown to substantially increase muscle mass and decrease adipose tissue.[1,2,4] Furthermore, a recent study demonstrated that GH therapy also reduces the liver fat content in adults with GH deficiency.[36] Such changes in body composition by GH therapy may also contribute to the favorable changes in the NOS-ADMA pathway as well as endothelial and liver function, including urea cycle function.

ADMA not only inhibits NOx activity but may also enhance oxidative stress, predisposing to insulin resistance and cardiovascular disease.[9,37] In contrast, NO exerts multifarious biological functions such as vasodilation, anti-inflammation, apoptosis, and regulation of neurological systems.[8] In this context, more attention should be paid to ADMA and NO production before and after GH therapy.

The main limitations of this study are that the number of enrolled short SGA children was relatively small, the production of intracellular ADMA and NO was not examined, and we were unable to determine the daily intake of arginine, glutamine, and other amino acids that might have influenced the results. Throughout this study, however, we evaluated the patients' appetite and dietary pattern by interviewing their mothers, and these parameters seemed almost unchanged. Despite these limitations, the present study provides evidence that GH treatment influences ADMA and NO production as well as relevant amino acid concentrations in short SGA children. Recent meta-analyses have revealed that the onset of cardiovascular disease and stroke are associated with high ADMA concentrations, suggesting that ADMA is an independent risk factor for such diseases.[38,39] More extensive and long-standing studies are required to gain better knowledge regarding the metabolic effects and resultant clinical benefits of GH treatment in short SGA children.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

Funding

This work was supported by Grants-in-Aid for Young Scientists and Scientific Research of JSPS KAKENHI in Japan (Nos. 23791224, 2011-2013; Nos. 26461644, 2014-2016; Nos. 15K08624, 2015–2017).

References

1. Muller N and Jorgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocr Rev* 2009; 30: 152–157.

2. Kuromaru R, Kohno H, Ueyama N, et al. Long-term prospective study of body composition and lipid profiles during and after growth hormone treatment in GH deficiency: gender-specific metabolic effects. *J Clin Endocrinol Metab* 1998; 83: 3890–3896.
3. Ciresi A, Amato MC, Criscimanna A, et al. Metabolic parameters and adipokine profile during GH replacement therapy in children with GH deficiency. *Eur J Endocrinol* 2007; 156: 353–360.
4. Sas T, Mulder P and Hokken-Koelega A. Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone treatment in children with short stature born for gestational age either with or without GH deficiency. *J Clin Endocrinol Metab* 2000; 85: 3786–3792.
5. Lebl J, Lebenthal Y, Kolouskova S, et al. Metabolic impact of growth hormone treatment in short children born small for gestational age. *Horm Res Paediatr* 2011; 76: 254–261.
6. Tanaka T, Yokoya S, Fujieda K, et al. Efficacy and safety of up to 8 years of long-term growth hormone treatment in short children born small for gestational age in Japan: Analysis of the subpopulation according to the Japanese guideline. *Clin Pediatr Endocrinol* 2012; 21: 57–68.
7. Itabashi K, Mishina J, Tada H, et al. Longitudinal follow-up of height up to five years of age in infants born preterm small for gestational age; comparison to full-term small for gestational age infants. *Early Hum Dev* 2007; 83: 327–333.
8. Moncada S, Palmer RMJ and Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; 43: 109–142.
9. Cook JP. ADMA: its role in vascular disease. *Vasc Med* 2005; 10: S11–S17.
10. Bouras G, Deftereos S, Tousoulis D, et al. Asymmetric Dimethylarginine (ADMA): a promising biomarker for cardiovascular disease? *Curr Top Med Chem* 2013; 13: 180–200.
11. Anderssohn M, Schwedhelm E, Lüneburg N, et al. Asymmetric dimethylarginine as a mediator of vascular dysfunction and a marker of cardiovascular disease and mortality: an intriguing interaction with diabetes mellitus. *Diab Vasc Dis Res* 2010; 7: 105–118.
12. Tain YL and Huang LT. Asymmetric dimethylarginine: clinical applications in pediatric medicine. *J Formos Med Assoc* 2011; 110: 70–77.
13. Nagasaka H, Komatsu H, Ohura T, et al. Nitric oxide synthesis in ornithine transcarbamylase deficiency: possible involvement of low NO synthesis in clinical manifestation of urea cycle defect. *J Pediatr* 2004; 145: 259–262.
14. Pearson DL, Dawling S, Walsh WF, et al. Neonatal pulmonary hypertension urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. *N Engl J Med* 2001; 344: 1832–1838.
15. Itabashi K, Miura F, Uehara R, et al. New Japanese neonatal anthropometric charts for gestational age at birth. *Pediatr Int* 2014; 56: 702–708.
16. Isojima T, Shimatsu A, Yokoya S, et al. Standardized centile curves and reference intervals of serum insulin-like growth factor-I (IGF-I) levels in a normal Japanese population using the LMS method. *Endocr J* 2012; 59: 771–780.
17. Tsukahara H, Ohta N, Tokuriki S, et al. Determination of asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor, in umbilical blood. *Metabolism* 2008; 57: 215–220.
18. Schulze F, Wesemann R, Schwedhelm E, et al. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. *Clin Chem Lab Med* 2004; 42: 1377–1383.
19. Ministry of Health, Labour and Welfare in Japan. Charts of standard growth in Japanese infants in 2000 (In Japanese). http://www.mhlw.go.jp/houdou/0110/h1024-4.html (2002, Accessed 18 June 2017).
20. Böger RH, Skamira C, Bode-Böger SM, et al. Nitric oxide may mediate the hemodynamic effects of recombinant growth hormone in patients with acquired growth hormone deficiency. A double-blind, placebo-controlled study. *J Clin Invest* 1996; 98: 2706–2713.

21. Setola E, Monti LD, Lanzi R, et al. Effects of growth hormone treatment on arginine to asymmetric dimethylarginine ratio and endothelial function in patients with growth hormone deficiency. *Metabolism* 2008; 57: 1685–1690.
22. Thum T, Fleissner F, Klink I, et al. Growth hormone treatment improves markers of systemic nitric oxide bioability via insulin-like growth factor-1. *J Clin Endocrinol Metab* 2007; 92: 4172–4179.
23. Langen J, Kayacelebi AA, Beckmann B, et al. Homoarginine (hArg) and asymmetric dimethylarginine (ADMA) in short stature children without and with growth hormone deficiency: hArg and ADMA are involved differently in growth in the childhood. *Amino Acids* 2015; 47: 1875–1883.
24. De Marco S, Marcovecchio ML, Caniglia D, et al. Circulating asymmetric dimethylarginine and lipid profile in pre-pubertal children with growth hormone deficiency: effect of 12-month growth hormone replacement therapy. *Growth Horm IGF Res* 2014; 24: 216–220.
25. Önder A, Aycan Z, Koca C, et al. Evaluation of asymmetric dimethylarginine (ADMA) levels in children with growth hormone deficiency. *J Clin Res Pediatr Endocrinol* 2014; 6: 22–27.
26. Nijveldt RJ, Teerlink T, Siroen MP, et al. The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). *Clin Nutr* 2003; 22: 17–22.
27. Siroen MP, van der Sijp JR, Teerlink T, et al. The human liver clears both asymmetric and symmetric dimethylarginine. *Hepatology* 2005; 41: 559–565.
28. Sydow K and Munzel T. ADMA and oxidative stress. *Atheroscler Suppl* 2003; 4: 41–51.
29. Jia SJ, Jiang DJ, Hu CP, et al. Lysophosphatidylcholine-induced elevation of asymmetric dimethylarginine level by the NADPH oxidase pathway in endothelial cells. *Vasc Pharmacol* 2006; 44: 143–148.
30. Forbes SP, Druhan LJ, Guzman JE, et al. Mechanism of 4-HNE mediated inhibition of hDDAH-1: Implication in no regulation. *Biochemistry* 2008; 47: 1819–1826.
31. Schini-Kerth VB. Dual effects of insulin-like growth factor-1 on the constitutive and inducible nitric oxide (NO) synthase–dependent formation of NO in vascular cells. *J Endocrinol Invest* 1999; 22: 82–88.
32. Withers DJ, Burks DJ, Towery HH, et al. IRS-2 coordinates IGF-1 receptor–mediated β-cell development and peripheral insulin signalling. *Nat Genet* 1999; 23: 32–40.
33. Biosca M, Rodríguez G, Ventura P, et al. Central adiposity in children born small and large for gestational age. *Nutr Hosp* 2011; 26: 971–976.
34. Labayen I, Moreno LA, Ruiz JR, et al. Small birth weight and later body composition and fat distribution in adolescents: the Avena study. *Obesity (Silver Spring)* 2008; 16: 1680–1686.
35. Cho WK and Suh BK. Catch-up growth and catch-up fat in children born small for gestational age. *Korean J Pediatr* 2016; 59: 1–7.
36. Matsumoto R, Fukuoka H, Iguchi G, et al. Long-term effects of growth hormone replacement therapy on liver function in adult patients with growth hormone deficiency. *Growth Horm IGF Res* 2014; 24: 174–179.
37. Brinkmann SJ, de Boer MC, Buijs N, et al. Asymmetric dimethylarginine and critical illness. *Curr Opin Clin Nutr Metab Care* 2014; 17: 90–97.
38. Willeit P, Freitag DF, Laukkanen JA, et al. Asymmetric dimethylarginine and cardiovascular risk: Systematic review and meta-analysis of 22 prospective studies. *J Am Heart Assoc* 2015; 4: e001833.
39. Meinitzer A, Seelhorst U, Wellnitz B, et al. Asymmetric dimethylarginine independently predicts total and cardiovascular mortality in individuals with angiographic coronary artery disease (the Ludwigshafen Risk and Cardiovascular Health Study). *Clin Chem* 2007; 53: 273–283.