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ORIGINAL ARTICLE

Age-related changes of muscle and plasma amino acids in healthy children

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Abstract The aim of the study was to explore if changes in muscle and plasma amino acid concentrations developed during growth and differed from levels seen in adults. The gradient and concentrations of free amino acids in muscle and plasma were investigated in relation to age in metabolic healthy children. Plasma and specimens from the abdominal muscle were obtained during elective surgery. The children were grouped into three groups (group 1: < 1 year, $n = 8$; group 2: 1–4 years, $n = 13$ and group 3: 5–15 years, $n = 15$). A reference group of healthy adults (21–38 years, $n = 22$) was included in their comparisons and reflected specific differences between children and adults. In muscle the concentrations of 8 out of 19 amino acids analysed increased with age, namely taurine, aspartate, threonine, alanine, valine, isoleucine, leucine, histidine, as well as the total sums of branched chain amino acids (BCAA), basic amino acids (BAA) and total sum of amino acids ($P < 0.05$). In plasma the concentrations of threonine, glutamine, valine, cysteine, methionine, leucine, lysine, tryptophane, arginine, BCAA, BAA and the essential amino acids correlated with age ($P < 0.05$). These results indicate that there is an age dependency of the amino acid pattern in skeletal muscle and plasma during growth.

Keywords Amino acids · Growth · Skeletal muscle · Plasma

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Introduction

Protein metabolism in adult and children has many similarities but also some major differences. The literature over free amino acid concentrations in skeletal muscle of children under the age of 15 years is sparse. Adult and children have different nutritional needs regarding amino acid intake in order to promote a normal protein homeostasis and in children growth (Imura and Okada 1998; Young and Borgonha 2000). In addition to the amino acids that are essential for adults, also histidine, arginine, cysteine, tyrosine and taurine are considered to be semi-essential amino acids in children for a normal development (Imura and Okada 1998). Changes of amino acid concentration are seen in different clinical and physiological conditions both in adults and in infants adapting to the amino acid metabolism to the metabolic and nutritional needs (Canepa et al. 2002; Gore and Jahoor 2000).

Children show a faster recovery and are more easily attaining equilibrium of nitrogen balance following a protein catabolic event such as trauma, operation and infection (Kien et al. 1978). The amino acid need for the infants has earlier been derived from levels of amino acids in plasma, which in some respects differ from the levels seen in adults (Brunton et al. 2000; Canepa et al. 2002). However, the

nutritional need of parenterally fed infants has been poorly defined leading to a risk of either inadequate or increased supply with potential adverse effects (Brunton et al. 2000). With a novel method, a minimally invasive amino acid oxidation model, the need and proportion of amino acids can be assessed in various conditions and during growth (Elango et al. 2008). This method has advantages in accuracy as compared to nitrogen balance studies also showing a higher estimate than earlier recommendations. Skeletal muscle is an important store for amino acids both regarding the bulk of muscle proteins and the large pool of free amino acid in the intracellular compartment. Skeletal muscle during growth is very sensitive to insulin and to the postprandial rise in amino acids (Davis and Fiorotto 2009). Changes in plasma amino acid pattern during growth have earlier been described, showing mainly lower values in younger children (Lepage et al. 1997), but no data exist regarding the change of muscle amino acid pattern in growing children. The aim of the study was to explore if amino acid concentrations in muscle and plasma changed during growth and differed compared to values obtained in an adult reference group. In the present study muscle and plasma amino acids were determined in muscle biopsies and plasma obtained in otherwise healthy children between the ages of 0–14 years undergoing elective surgery. The correlation between age and concentration was calculated. The values were compared between age groups and to a reference group of adult subjects.

Materials and methods

Metabolically healthy children ($n = 36$) aged 0–15 years undergoing elective surgery were studied. The characteristics of the patients are shown in Table 1. The patients were undergoing surgical procedures like hernia repair, orchidopexy, operation due to hydronephrosis and antireflux operation. All the children were well-nourished and within the normal range with regard to length and weight and considered otherwise healthy. Muscle biopsies were taken from the abdominal wall under general anaesthesia during surgery. The concentrations of the muscle amino acids were determined in the deproteinised tissue homogenates on an amino acid analyser. The children were grouped as follows: group 1 (0–1 year, $n = 8$), group 2 (1–4 years, $n = 13$), group 3 (5–15 years, $n = 15$). Since research in amino acid metabolism earlier has been done in adults changes during growth was compared to an adult reference group of healthy male volunteers, age between 21 and 38 years These had earlier been included in studies where muscle samples were obtained from the vastus lateralis muscle using the percutaneous needle biopsy technique ($n = 22$) (Hammarqvist et al. 1998, 2005).

Table 1 Characteristics of the children in the three study groups

Group	Age	Sex boy/girl	Length (cm)	Weight (kg)
1	<1	4/4	54.0 ± 13.2	4.4 ± 3.7
2	1–4	8/5	75.4 ± 5.2	10.3 ± 1.1
3	5–14	12/3	101.6 ± 7.5	17.6 ± 2.0

Values are mean ± SD

Plasma samples were drawn from the patients and volunteers except from the youngest group (0–1 year). Plasma samples were therefore obtained from only two children in the latter group, whereas it was possible to obtain plasma samples from the other groups. The possible risks and the study design were explained to the parents of the children and to the healthy volunteers putting emphasis on the fact that participation was (frivillig) before approval and inclusion in the study. The ethical committee of the University of Uppsala has prior approved the study.

Amino acid determination

Determinations of the concentrations of free amino acids in both plasma and muscle were performed by ion-exchange chromatography after deproteinisation. In brief, plasma was mixed with 2% sulphosalicylic acid (SSA) and the frozen muscle biopsy was homogenised in 4% SSA, containing nor-leucine as internal standard. The homogenate was centrifuged (3,000 × g for 15 min 4°C) and the supernatant was stored at −80°C. The free amino acids in the supernatant were determined using an automatic amino acid analyser (Alpha Plus, LKB, Bromma, Sweden) as described previously. The amino acids were separated on an Ultropae 8 Lithium ion exchange resin (Biochrom, Cambridge, UK) using lithium citrate buffers. The amino acids were detected and quantified by postcolumn derivatisation with *o*-phthaldialdehyde (OPA) and fluorescent detection. The concentrations of the amino acids in plasma and muscle were expressed as μmol/l and mmol/kg wet weight muscle, respectively.

Statistics

All values are given as mean ± SD except from the plasma values in group 0–1 year which was presented as range, due to only three samples being analysed. Analysis of variance was performed for each amino acid for all the groups regarding muscle amino acids, but only for group 1–4 years and 5–15 years regarding plasma amino acids and concentration gradient. Scheffe's test was used as a post hoc test. A P-value less than 0.05 was considered as statistically significant. The values of the reference group are presented as mean and 95% confidence interval for

observations. Calculations of correlation were performed using regression analysis (Snedecor and Cochran 1980).

Results

Muscle amino acids

In skeletal muscle higher concentrations were seen in group 5–15 years as compared with group 0–1 year for 6 out of 19 amino acids, namely taurine (105%), aspartate (210%), glutamate (178%,), alanine (121%), valine (67%) and histidine (101%; Table 2). Consequently statistical correlations were seen regarding the concentration of taurine ($R = 0.53$), aspartate ($R = 0.36$), threonine ($R = 0.44$), alanine ($R = 0.66$), valine ($R = 0.53$), isoleucine ($R = 0.39$), leucine ($R = 0.51$), histidine ($R = 0.69$), the total sum of branched chain amino acids (BCAA; $R = 0.52$), basic amino acids (BAA; $R = 0.36$) and total amino acids ($R = 0.42$; $P < 0.05$) and age. Carnosine, an amino acid not incorporated into protein, also increased during growth and correlated with age ($R = 0.76$). When comparing the amino acid concentrations of the reference group with the study groups the mean values of taurine, threonine, glutamate, glutamine, valine, isoleucine, leucine, lysine, histidine and the total amino acids were higher whereas the mean values of serine and cysteine, were lower as compared to the confidence interval of the reference group (Table 2). Figures are showing the correlation between the concentrations of muscle taurine ($R = 0.74$; Fig. 1), muscle glutamine ($R = 0.69$; Fig. 2), total muscle amino acids ($R = 0.55$; Fig. 3) and age.

Table 2 Skeletal muscle amino acids mmol/kg ww muscle

AA	Group 1 $n = 8$	Group 2 $n = 13$	Group 3 $n = 15$	Adult reference group ($n = 22$)	Correlation (R) with age within the study group (1–3)	Correlation (R) with age within all the groups (1–4)
Tau	2.70 ± 1.88	4.25 ± 2.06	5.55 ± 1.43 b	8.30 (7.48–09.17)*,⊕	0.53	0.74
Asp	0.46 ± 0.47	1.11 ± 0.90	1.43 ± 0.87 b	1.30 (1.09–1.51)	0.36	
Thr	0.26 ± 0.14	0.29 ± 0.10	0.37 ± 0.12	0.44 (0.41–0.48)*,⊕	0.44	0.53
Ser	0.66 ± 0.49	0.55 ± 0.19	0.56 ± 0.10	0.39 (0.35–0.43)*,⊕		0.39
Asn	0.30 ± 0.19	0.29 ± 0.13	0.31 ± 0.08	0.26 (0.23–0.30)		
Glu	0.85 ± 0.54	2.12 ± 1.36	2.37 ± 1.24 b	1.74 (1.51–1.98)*,⊕		
Gln	5.74 ± 4.12	6.28 ± 2.13	7.21 ± 1.67	11.21 (10.3–12.15)*,⊕		0.69
Gly	1.22 ± 0.72	0.78 ± 0.23 a	1.00 ± 0.23	0.93 (0.84–1.03)		
Ala	1.02 ± 0.66	1.52 ± 0.57	2.25 ± 0.75 b	1.92 (1.66–2.19)	0.66	
Val	0.12 ± 0.06	0.16 ± 0.02	0.20 ± 0.06 b	0.24 (0.21–0.26)*	0.53	0.59
Cys	0.19 ± 0.17	0.21 ± 0.15	0.11 ± 0.07	0.07 (0.04–0.10)*		0.44
Met	0.04 ± 0.01	0.03 ± 0.005	0.04 ± 0.01	0.04 (0.03–0.05)		
Ile	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.03	0.07 (0.06–0.08)	0.39	0.33
Leu	0.09 ± 0.05	0.10 ± 0.02	0.14 ± 0.06	0.15 (0.13–0.18)	0.51	0.40
Tyr	0.09 ± 0.05	0.06 ± 0.02	0.06 ± 0.02	0.05 (0.04–0.06)		0.32
Phe	0.08 ± 0.03	0.06 ± 0.02	0.07 ± 0.03	0.05 (0.04–0.07)		
Orn	0.39 ± 0.15	0.16 ± 0.06	0.16 ± 0.05	0.15 (0.12–0.18)		
Lys	0.29 ± 0.26	0.25 ± 0.12	0.31 ± 0.08	0.40 (0.32–0.49)*		0.33
His	0.15 ± 0.08	0.23 ± 0.07	0.30 ± 0.09 b	0.37 (0.33-0.40)*,⊕	0.69	0.65
Carn	0.87 ± 1.02	2.39 ± 1.28	4.25 ± 1.46 b, c	5.52 (4.62-6.41)*,⊕	0.76	0.77
Arg	0.26 ± 0.23	0.35 ± 0.22	0.42 ± 0.10	0.29 (0.24–0.35) ⊕		
BCAA	0.24 ± 0.23	0.35 ± 0.22	0.42 ± 0.10 c	0.46 (0.40–0.52)	0.52	0.34
BAA	0.70 ± 0.55	0.83 ± 0.39	1.04 ± 0.22	0.96 (0.81–1.10)	0.36	0.50
EAA	1.36 ± 0.80	1.53 ± 0.51	1.78 ± 0.38	1.16 (1.43–1.72)		
Tot AA	11.77 ± 7.08	14.43 ± 5.55	17.21 ± 3.54 b	19.84 (18.56–21.13)*,⊕	0.42	0.55

Values are given as mean (SD. Values in the reference group are shown as means and 95% confidence interval for the observations

Online alphabets denote statistical significant difference (<0.05); (a) between group 1 and 2, (b) between group 1 and 3, (c) between group 2 and 3

* The mean values in the groups 1–3 are outside the 95% confidence interval of the reference group

⊕ Significant difference between group 3 and the reference group

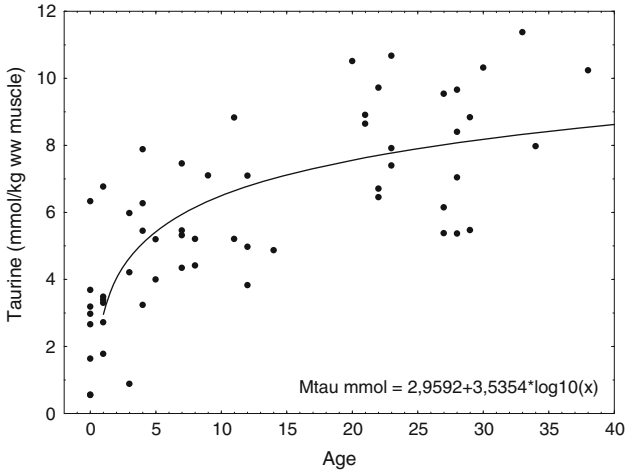

Fig. 1 Correlation ($R = 0.74$) between *age* and the muscle *taurine* concentration, taking the study group and the reference group into consideration

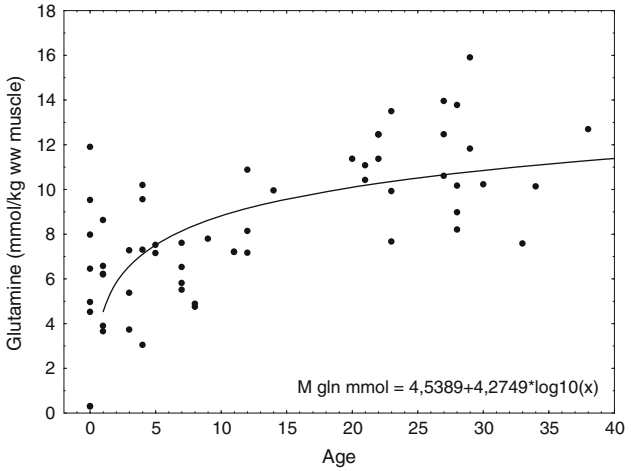

Fig. 2 Correlation ($R = 0.69$) between *age* and the muscle *glutamine* concentration, taking the study group and the reference group into consideration

Plasma amino acids

Due to a low number of observations in group 1 ($n = 3$), these were not included in the comparisons between the groups. In plasma, higher concentrations of threonine (46%), asparagine (26%), glutamine (20%), valine (31%), cysteine (26%), leucine (45%), lysine (35%), tryptophane (43%), BCAA (39%), BAA (23%), essential amino acids (EAA; 31%) and total sum of amino acids (27%) were seen in group 5–15 years compared to group 1–4 years ($P < 0.05$; Table 3). Correlations with increasing age were seen regarding threonine ($R = 0.53$), valine ($R = 0.41$), cysteine ($R = 0.66$), methionine ($R = 0.54$), leucine ($R = 0.49$), ornithine ($R = 0.52$), lysine ($R = 0.65$), tryptophane ($R = 0.67$), arginine ($R = 0.43$), BCAA ($R = 0.45$), BAA ($R = 0.57$) and the EAA ($R = 0.62$) correlated with age

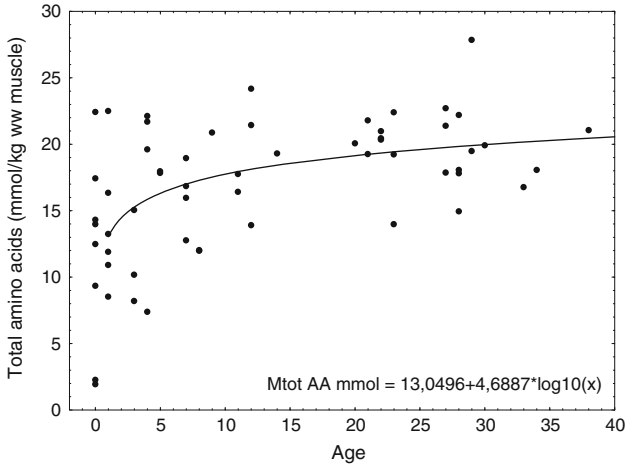

Fig. 3 Correlation ($R = 0.55$) between *age* and the total muscle *amino acid* concentration, taking the study group and the reference group into consideration

($P < 0.05$). When comparing the amino acid concentrations of the reference group with the study group higher levels were found regarding threonine, asparagine, glutamine, alanine, valine, methionine, isoleucine, leucine, lysine, tryptophane, arginine, BCAA, BAA, EAA and the total sum of amino acids. Lower levels were found regarding taurine, aspartate and glutamate.

Gradients

Due to a low number of observations in group 1 ($n = 3$), these were not included in the comparisons between the groups. The gradients between the concentrations in muscle and plasma for the individual amino acids did not change between the different age groups for all amino acids except cysteine and ornithine, which showed a lower gradient in group 5–15 years (Table 4).

When taking both the study group and the reference group into account decreases of the gradients were seen regarding serine, asparagine, alanine, valine, cysteine, tyrosine, phenylalanine, ornithine, lysine, arginine, the BCAA, BAA and the essential amino acids, while increases were seen regarding taurine, aspartate, glutamate, glutamine and histidine during growth.

Discussion

In this study the concentrations of muscle and plasma amino acids pattern changed in relation to age among children. The most pronounced difference was between the infants (< 1 year) and the older children (5–15 years) with the young children (1–5 years) in between. The general pattern was an increase in concentrations in both plasma

Table 3 Plasma amino acids µmol/l

AA	Group 1 $n = 3$	Group 2 $n = 13$	Group 3 $n = 15$	Adult reference group ($n = 22$)	Correlation (R) with age within the study group (1–3)	Correlation (R) with age within all the groups (1–4)
Tau	53–183	74 ± 37	66 ± 23	49 (43–55)*		
Asp	6–13	6 ± 6	5 ± 2	3 (3–4)*,⊕		0.41
Thre	84–94	67 ± 26	98 ± 22 c	133 (112–153)*,⊕	0.53	0.50
Ser	101–176	96 ± 15	114 ± 26	108 (95–121)		
Asn	48–68	38 ± 10	48 ± 7 c	74 (66–79)*,⊕		0.66
Glu	50–95	56 ± 17	63 ± 16	25 (20–30)*,⊕		0.68
Gln	484–589	422 ± 60	508 ± 65 c	581 (528–634)*,⊕		0.47
Gly	166–315	178 ± 47	205 ± 34	224 (202–247)		
Ala	248–268	197 ± 69	248 ± 88	356 (313–399)*,⊕		0.55
Val	85–185	149 ± 31	195 ± 42 c	223 (202–244)*	0.41	0.50
Cys	60–62	68 ± 10	84 ± 15 c	92 (76–107)	0.66	0.35
Met	2–13	12 ± 3	16 ± 4	26 (23–28)*,⊕	0.54	0.70
Ile	18–46	39 ± 7	51 ± 16	64 (57–71)*,⊕		0.51
Leu	56–83	74 ± 13	107 ± 29 c	128 (115–140)*,⊕	0.49	0.56
Tyr	58–62	36 ± 7	46 ± 7	58 (40–64)⊕		0.51
Phe	37–88	39 ± 5	43 ± 9	54 (49–59)⊕		0.35
Orn	54–59	30 ± 6	48 ± 14	58 (51–66)	0.52	0.45
Lys	44–63	40 ± 7	54 ± 8 c	103 (87–119)*,⊕	0.65	0.68
His	76–79	66 ± 15	75 ± 10	72 (63–82)		
Try	13–23	21 ± 4	30 ± 4 c	44 (40–49)*,⊕	0.67	0.70
Arg	40–47	47 ± 11	59 ± 11	90 (80–100)*,⊕	0.43	0.64
BCAA	76–130	114 ± 20	159 ± 45 c	415 (276–454)*,⊕	0.45	0.84
BAA	169–182	153 ± 25	188 ± 20 c	275 (247–302)*,⊕	0.57	0.66
EAA	559–670	571 ± 90	746 ± 100 c	865 (794–936)*,⊕	0.62	0.57
Tot AA	1,876–2,177	1,650 ± 250	2,041 ± 194 c	2,502 (2,300–2,705)*,⊕		0.62

Values are given as mean (SD (range for group 1). Values in the reference group are shown as means and 95% confidence interval for observations

Online alphabet denotes statistical significant difference between group 2 and 3 (< 0.05)

* The mean values in the groups 2 and 3 are outside the 95% confidence interval of the reference group

⊕ Significant difference between group 3 and the reference group. The values of group 1 ($n = 3$) was not included in the comparison between the groups but were included in the correlation analysis

and muscle tissue during growth, leaving the gradient unaltered. Moreover, in comparison to adults, there were also clear differences in the amino acid pattern of the older children (5–15 years).

Muscle tissue was obtained through surgical biopsies in the children and percutaneous biopsies in the reference group. Skeletal muscle is an important organ system both from a functional point of view and since it comprises a large percentage of the body mass, also constituting an important store for substrates. Muscle amino acid metabolism both in health and during illness has extensively been studied in adults (Bergström et al. 1974; Roth et al. 1982; Stehle et al. 1989). In adults characteristic changes in the muscle amino acid pattern has been described during catabolic conditions such as a decrease in muscle glutamine and increased concentrations of the branched chained and aromatic amino acids (Vinnars et al. 1975).

The total sum of free amino acids in skeletal muscle increased in relation to age in children, and was still higher in the reference group of adult subjects and exhibited correlation to age. In plasma this pattern was not obvious for the total amino acid concentrations, although all the three age groups of children were lower as compared to the adults. Muscle taurine and carnosine, amino acid compounds that are end products, show the most marked increase. On the other hand, plasma taurine was lower in the reference group as compared to the combined child group.

Among non-essential amino acids amino acids with a high muscle–plasma gradient muscle aspartate increased

Table 4 Gradients between amino acids in muscle and plasma

AA	Group 1 $n = 3$	Group 2 $n = 13$	Group 3 $n = 15$	Adult reference group ($n = 22$)	Correlation (R) with age within the study group (1–3)	Correlation (R) with age within all the groups (1–4)
Tau	16.1–60.2	69.3 ± 44.1	92.0 ± 31.6	202.5 (161.9–243.0)*,⊕		0.78
Asp	23.1–198.2	234.6 ± 248.3	332.8 ± 213.3	396.2 (346.9–445.6)*		0.38
Thr	4.3–5.0	4.6 ± 2.0	3.9 ± 0.9	3.6 (3.1–4.1)		
Ser	3.7–13.9	5.5 ± 1.9	5.1 ± 1.6	3.8 (3.4–4.2)*,⊕		0.43
Asn	6.0–6.6	7.7 ± 3.2	6.7 ± 1.4	3.7 (3.2–4.1)*,⊕		0.62
Glu	11.0–14.4	37.1 ± 22.8	42.1 ± 25.3	89.5 (65.6–113.4)*,⊕		0.51
Gln	10.3–16.2	14.7 ± 5.5	14.2 ± 3.7	20.1 (17.4–22.8)*,⊕		0.48
Gly	4.2–6.0	4.5 ± 1.8	5.0 ± 1.0	4.5 (3.7–5.3)		
Ala	6.0–8.0	8.9 ± 4.6	9.6 ± 2.4	5.8 (4.8–6.7)*,⊕		0.39
Val	1.3–1.4	1.1 ± 0.3	1.1 ± 0.3	1.1 (1.0–1.2)		0.50
Cys	4.6–4.9	3.0 ± 2.3	1.4 ± 0.8	0.9 (0.4–1.3)*	0.46	0.35
Met	3.5–30.0	2.6 ± 1.1	2.9 ± 2.5	1.7 (1.3–2.0)⊕		0.70
Ile	2.2–2.4	1.4 ± 0.5	1.3 ± 0.5	1.1 (0.9–1.3)		
Leu	1.8–2.5	1.4 ± 0.5	1.3 ± 0.5	1.2 (1.0–1.4)		
Tyr	1.5–3.6	1.6 ± 0.7	1.4 ± 0.5	0.9 (0.8–1.1)*,⊕		0.53
Phe	1.1–3.1	1.4 ± 0.3	1.5 ± 0.4	1.0 (0.8–1.1)*,⊕		0.51
Orn	5.7–8.2	5.0 ± 2.0	3.5 ± 1.3	2.7 (2.2–3.3)*	0.42	0.48
Lys	6.0–11.6	6.7 ± 3.4	5.9 ± 1.3	4.0 (3.3–4.7)*,⊕		0.48
His	2.8–3.7	3.8 ± 1.7	4.1 ± 1.0	5.5 (4.6–6.4)*,⊕		0.48
Arg	6.1–14.3	8.2 ± 4.9	7.4 ± 2.2	3.4 (2.6–4.1)*,⊕		0.58
BCAA	3.4–4.2	2.9 ± 0.8	2.6 ± 0.9	1.1 (1.0–1.3)*,⊕		0.74
BAA	4.6–8.8	5.8 ± 3.2	5.6 ± 1.2	3.6 (3.0–4.2)*,⊕		0.45
EAA	3.2–3.9	2.8 ± 1.3	2.4 ± 0.6	1.9 (1.7–2.0)*,⊕		0.47
Tot AA	7.4–8.0	8.7 ± 3.7	8.5 ± 1.8	8.2 (7.2–9.3)		

Values are given as means (SD (range for group 1). Values in the reference group are shown as means and 95% confidence interval for observations

* The mean values in the groups 2 and 3 are outside the 95% confidence interval of the reference group

⊕ Significant difference between group 3 and the reference group

while plasma values were lower in the reference group. Muscle glutamate did not change, although the reference group showed higher values in muscle and lower in plasma. Muscle glutamine was higher in the reference group while it was unchanged in plasma. Both muscle and plasma alanine increased during growth.

In general the essential amino acids did not show marked changes during growth. Skeletal muscle threonine, valine, isoleucine, leucine and histidine increased. When comparing the reference group with the study group increased concentrations were seen regarding threonine, valine, lysine and histidine, while serine and cysteine levels were lower. In plasma, threonine, valine, cysteine, methionine, leucine, tryptophane, ornithine and lysine arginine increased during growth. Increased concentrations were seen in the reference group regarding plasma threonine, asparagine, valine, methionine, isoleucine, leucine, lysine, tryptophane, arginine, while taurine, aspartate and glutamate levels were lower.

Changes of amino acids in human muscle have not earlier been reported during growth although data regarding plasma amino acids are available (Lepage et al. 1997). Data from children regarding muscle amino acid pattern during health and disease is also sparse. Some information exists regarding muscle and plasma amino acid pattern in healthy children compared to those found after kidney transplantation (Canepa et al. 2002; Perfumo et al. 1994). The levels of the individual amino acids in muscle and plasma in healthy children of age between 3 and 16 years in the cited studies were comparable to those found in the present study among the children in groups 2 and 3.

The skeletal muscle of the newborn amounts to 25% of the body weight, while it represents a larger proportion in adults, 30–40% (Dickerson and Widdowson 1960; White et al. 1991). This corresponds to a water-free protein proportion of 12.5% in the full term baby compared to 18% in the adult, an increase that mainly is explained by the increase in skeletal muscle mass (Friis-Hansen 1971).

Also the composition of muscle fibres changes with an increased proportion of glycolytic Type 2b fibres during growth (Kriketos et al. 1997). Important differences in the regulation of muscle protein synthesis has been described during growth (Davis and Fiorotto 2009).

During early growth the proportion of water decreases in parallel to an increase in protein content. This process slows down 6 months after birth whereafter the proportion of water and protein is comparable to the muscle composition seen in adults (Dickerson and Widdowson 1960). The extracellular volume decreases from 44% in the newborn to 26% at 1 year of age, and then remains almost constant (Friis-Hansen 1971). In older individuals an increased proportion of extracellular water is seen in skeletal muscle biopsies that parallels increased contents of sodium and chloride (Möller et al. 1979). The intracellular volume on the other hand increases from 33% at birth to around 37% at 4 months of age, whereafter it increases slightly during adolescence followed by a decrease at older ages (Friis-Hansen 1971). In the present study total muscle water and the distribution of extra- and intracellular water was not measured. Since the water content and water distribution is similar already at young age compared to adults (Friis-Hansen 1971) this could not be a main explanation for the alterations seen during growth regarding the muscle amino acids, in particular when changes were not uniform, some increasing, while other decreased and the remaining did not change.

Changes in muscle and plasma amino acid concentrations during growth may be explained by changes in protein synthesis, in tissue composition as well as to metabolic alterations. The amino acid concentration in the intracellular space is the net-effect of the combination of protein synthesis, breakdown, deamination, amination and transmembrane transportation (Fürst 1983). In the growing individual substrate availability, the influences of growth factors and the activity of amino acid transportation are of importance. The differences in amino acid and protein metabolism between children and adults following trauma is reflected by unchanged body protein flux, protein synthesis, amino acid oxidation and protein degradation, measured with isotope technique, in children (Powis et al. 1998) as compared to adults (Waterlow et al. 1977).

A few studies have focused on amino acid metabolism during catabolic events in children.

In critically ill neonates undergoing operation for necrotising enterocolitis increased levels of BCAA, phenylalanine and histidine were shown, the extent of which correlated with the severity of the disease which in turn mirrored the degree of liver dysfunction and deranged amino acid metabolism in the tissue (Kamata et al. 1995). In uremic children lower levels of plasma and muscle BCAA has been shown compared to healthy individual reflecting a condition of malnutrition (Canepa et al. 2002). Glutamine levels decrease in severe catabolic conditions, such as burn injuries, in children, that is explained by a decreased peripheral glutamine production (Gore and Jahoor 2000). In the present study muscle concentrations of glutamine were lower in the children compared to the reference group of older individuals.

Glutamine is considered to be "conditionally essential" in very-low birth weight infants and in septic neonates (Pierro 2002). Glutamine has an important role for the immune system, for the gut mucosa, and has a specific role in catabolic conditions to promote the function of immune system and the gastrointestinal tract (Neu et al. 2002). These properties make it an important compound during severe catabolic conditions with potential beneficial effects during sepsis in neonates (Eaton 2003). The risk of sepsis is reduced in low weight neonates when enteral glutamine is administered (Neu et al. 1997). This was accompanied by an earlier tolerance to enteral feeding in low weight neonates. On the other hand parenteral glutamine has in a multicenter study not shown to reduce the risk of necrotising enterocolitis in neonates (Pointdexter et al. 2004).

The present study shows that muscle and plasma amino acids change during growth. This may be explained by a combination of increase in protein stores, an increased uptake of amino acids for protein synthesis, maturation of amino acid transport system, at least in low age differences in tissue hydration state. In particular concentrations of amino acids that either are end products, such as taurine and carnosine, or synthesised in skeletal muscle, such as glutamine, show a correlation with increasing age. It is probably important to take age dependent differences into account when nutritional therapies are designed in growing individuals.

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