Amino Acids (2001) 21: 303–318

Amino
Acids

Conscientious metabolic monitoring on a patient with hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome undergoing anaesthesia

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Accepted July 1, 2000

Summary. Currently we know not more than 50 patients who show an interesting combination of increased plasma ornithine concentrations, postprandial hyperammonemia, and homocitrullinuria (HHH-syndrome). Since exact knowledge of this severe, although rare syndrome is important for any perioperative or intensive medical treatment concerning therapy and progression of the disease, we report a comprehensive study on a 32-year old woman with this rare multifaceted disorder who had to undergo general anaesthesia. For the first time amino acid status in plasma, urine, cerebrospinal fluid and especially polymorphonuclear leucocytes, which in the investigation showed to be valuable tool for evaluating amino acid metabolism in nucleated cells in HHH-syndrome, and further important pathophysiologic indicators of cellular and metabolic function have been conscientiously investigated and compared. The pathophysiological repercussions of our results as well as the recommendations for conscientious therapeutical management are discussed.

Keywords: Amino acids – HHH-syndrome – PMN – Liquor – Urine – Amino acids – Coagulation analyses

Introduction

Shih et al. (1969) have described for the first time a disease with a number of symptoms including periods of lethargy, vomiting, ataxia, choreoathetosis, delayed development, severe muscle spasticity and severe cerebral retardation. The analytical techniques developed using this "first" patient led to the discovery of an interesting combination of increased plasma ornithine

concentrations, postprandial hyperammonemia, and homocitrullinuria (HHH-syndrome; Shih et al., 1974). The paradoxical combination of hyperornithinemia and hyperammonemia has led to extensive studies on the biochemical mechanisms underlying this disorder (Oyanagi et al., 1983; Shih et al., 1982), but until now the pathophysiology of HHH-syndrome has not been fully understood. For example, the possibility that an enzymatic deficiency (i.e. urea cycle enzymes) causes this syndrome has been investigated, but no consistent or convincing defects have been demonstrated. Increased plasma ornithine concentrations differentiate the HHH-syndrome from other urea cycle disorders, and both hyperammonemia (especially postprandial) and homocitrullinuria distinguish this syndrome from gyrate atrophy in which major symptoms of progressive chorioretinal degeneration are expressed (Fukuda et al., 1983). The clinical symptoms are related to hyperammonemia and resemble those of urea cycle disorders. Although the symptoms generally appear in newborns and children, they may be delayed until late adulthood. Hyperammonemia results from the ingestion of high protein foods and may lead to vomiting, lethargy or even episodes of coma. After infancy, patients often opt for a low-protein diet spontaneously (milk etc. is avoided). Some patients survive to adulthood relatively free of symptoms but usually periods of vomiting, progressively deteriorating neurological symptoms (spastic paraparesis, choreoathetosis, ataxia), and delayed development in particular bring the patients to clinical attention (Nakajima et al., 1988). The mental state varies between barely subnormal to extreme retardation. We are currently aware of not more than 50 patients with this severe syndrome from a variety of ethnic backgrounds (Lemay et al., 1992).

To our knowledge, no comprehensive investigations have as yet been published, in which the influence of perioperative treatment on both amino acid metabolism as well as further extensive laboratory examinations has been investigated in patients with this severe clinical presentation. At the present state of knowledge in two patients only the influence of anaesthesia on patients with HHH-syndrome has been described, but any comprehensive monitoring of metabolic state and amino acid turnover has been failed (Michaelis et al., 1986; Noguchi et al., 1988). Moreover, till today, no investigations exist in which free intracellular amino acids turnover in polymorphonuclear leucocytes (PMN) and plasma, urine or cerebrospinal fluid amino acids have been conscientiously described and compared. Since exact knowledge of this severe, although rare syndrome is important for any perioperative anaesthesiological or intensive medical treatment concerning therapy and progression of the disease, we report on a 32-year old woman with this rare multifaceted disorder who had to undergo general anaesthesia in our hospital.

Materials and methods

Case report

Following an acute deterioration of neurological symptoms of unclear origin in a $32^{4}/_{12}$ years old woman (height: 160 cm, weight: 55 kg) suffering from HHH-syndrome, a

computer tomographic examination of the head and a spinal puncture requiring general anaesthesia, because of compromised cooperation in our patient, have been planned. The woman was first diagnosed with HHH-syndrome in 1982. She has two younger brothers of whom one also suffered from HHH-syndrome. The female patient developed normally after birth, having learned to walk after $1\frac{1}{2}$ years, and started to speak after 2 years. However, from the 5th to the 6th year a unfavourable development characterised by an increased dementia became apparent; although the patient had an IQ of 81 after 3 years, it was just 58 after 5 years. Gradually progressing disruptions in gait were also evident. From the 8th year the patient attended a special school for mentally handicapped individuals which she had to leave when she was 12 years because she was incapable of working with even the simplest of educational material any more. Until she was 20 years, the patient was still capable to eat and drink on her own, sitting on a stool, and standing up. Thereafter, however, the patient became both urinally and fecally incontinent. She could still walk on her own until she was 25 years. After this period she became more disabled, and for the last two years the patient has not been able to walk at all. The most recent physical examination revealed a reduced nutritional status, an increased lordosis of the lumbar spine, narrow, long fingers, and a peripheral circulatory disorder with acrocyanosis. The most recent neurological examination revealed a severe dementia as well as severe spasticity and paraparesis of the legs. Following a pain stimulus, the legs could only be withdrawn to a minimal extent. All ipsilateral reflexes increased (hyperreflexia), the reflex zones were clearly widened, and there were cloni on both feet. Babinski's phenomenon was positive on both sides. The patient has been kept on the following medication: citrulline 3×2 g/day, etilefrine 3×5 mg/day, baclofene 2×5 mg/day, and pipamperone 0–20–60 mg/day.

The investigation was approved by the local ethics committee of the Justus-Liebig-University, Giessen. One day prior to anaesthesia (08:00 a.m., $\mathbf{T_0}$), immediately before anaesthesia (08:05 a.m., $\mathbf{T_1}$), 35 min after the beginning of anaesthesia ($\mathbf{T_2}$), 1 hour after completion of anaesthesia ($\mathbf{T_3}$) on the next morning (08:00 a.m., $\mathbf{T_4}$), and two days after general anaesthesia (08:00 a.m., $\mathbf{T_5}$) blood (PMN, plasma, serum) and urine were withdrawn for amino acid analysis and further comprehensive laboratory screening. Cerebrospinal fluid (CSF) was withdrawn on only one occasion during general anaesthesia ($\mathbf{T_2}$).

General anaesthesia

Our patient had received no premedication. Anaesthesia was started with 5 mg·kg^{-1} body weight thiopentone, 25 μg·kg^{-1} body weight vecuronium bromide and 1 mg·kg^{-1} body weight suc-cinylcholine bromide. Anaesthesia was then maintained using sufentanil (Σ = 25 μg) and isoflurane (up to 1 MAC = minimum alveolar concentration isoflurane; $\leq$0.5 vol% end-tidal) in $N_2O:O_2 = 2:1$. End-tidal concentrations of isoflurane have been monitored continuously using a multigas (Sirecust®, Siemens, München) monitor. Muscle relaxation was discontinued. The ventilator was adjusted to maintain normocapnia with the help of continuous recordings of end-expiratory (end-tidal) CO_2-concentrations. Volume substitution and nutrition during periods of fasting was accomplished by adding cristalloid solutions containing no lactate (2 ml/kg body weight/h; Ringer's solution®, Pharmacia, Erlangen) supplemented with glucose ($1{,}14 \cdot 10^3$ calories/kg body weight/h; B. Braun, Melsungen). Peripheral oxygen saturation, non-invasive blood pressure, heart rate and rectal temperature were registered on a continuous basis. Anaesthesia lasted approximately 70 min. A high value was set on a calorically adequate diet with strict protein restriction ($<$1,2 g protein/kg body weight/day) during the postoperative course. We supplemented the low protein diet with increased citrulline doses (5×2 g) in order to lower blood ammonia levels perioperative.

Highly selective separation of polymorphonuclear leucocytes (PMN) from whole blood

Precise details of our PMN-separation technique have been described previously (see Mühling et al., 1999). 10 ml of whole blood (lithium-heparinate plastic tubes; central venous catheter) were withdrawn. Before further processing, all fractions were immediately cooled in an ice water bath at 4°C and 100 μg/ml phenyl-methyl-sulfonyl-fluoride, 10 μg/ml leupeptin, 10 μg/ml pepstatin, as well as 10 μg/ml antipain (all acquired from Sigma, U.S.A.) have been added to each plastic heparin tube; these additions served to inhibit proteases.

Orotic acid

Orotic acid in urine was determined as described by Hommes et al. (1986) and Rogers et al. (1968).

Amino acid analysis in plasma, urine and cerebrospinal fluid (CSF)

Plasma: 4 ml blood was withdrawn (Lithium-heparinate plastic tubes; central venous catheter) and centrifuged (4°C) for 10 minutes. 200 μl samples of the supernatants were then stored immediately (−80°C). *Urine* and *CSF*: 2 ml of urine (bladder catheter) and 1 ml CSF were withdrawn and 200 μl fractions were stored immediately (−80°C), too. After withdrawal and preparation, all froozen plasma, urine and CSF samples were lyophilized (freeze drying at −80°C) in the same manner as PMN samples.

Chromatographic amino acid analysis

Amino acids in plasma, PMN, CSF and urine were quantified using previously described high-performance liquid chromatography (HPLC) methods which fulfill the strict criteria required for ultra-sensitive, precisely validated and comprehensive amino acid analysis – especially concerning measurement of free intracellular amino acids in PMN – during continuous surveillance of severe diseases and organ dysfunctions in particular. The coefficients of variations for both the method reproducibilities as well as the retention times were as described elsewhere (Mühling et al., 1999).

Further extensive laboratory investigations

All further extensive laboratory determinations were carried out in the Department of Clinical Chemistry and Pathobiochemistry as well as in our Department of Anaesthesiology and Intensive Care Medicine (Justus-Liebig-University, Giessen) following known and described procedures.

Results

Amino acid evaluations

Regarding free amino acid profiles in PMN (Table 1) HHH-syndrome caused increased levels of ornithine and citrulline while decreased concentrations of histidine, methionine, alanine and phenylalanine, compared to healthy volunteers (see Mühling et al., 1999 for physiological ranges), have been

Table 1. Free amino acids, protein, NH_3 concentrations and GOT (glutamate-oxalacetate-transaminase), GPT (glutamate-pyruvate-transaminase), γ-GT (gamma-glutamyl-transferase) and LDH (lactate-dehydrogenase) activities in PMN-cells found in our patient perioperatively. Time points (T) are explained in text

PMN-Probes [pMol/PMN-cell]						
Amino acids	T_0	T_1	T_2	T_3	T_4	T_5
aspartate	1.22	1.29	1.33	1.25	1.21	1.28
glutamate	4.23	4.13	4.21	3.98	4.14	4.26
asparagine	0.51	0.48	0.46	0.53	0.45	0.49
serine	1.53	1.45	1.61	1.47	1.58	1.46
glutamine	3.53	4.09	3.94	3.89	3.63	3.88
histidine	0.15	0.16	0.14	0.14	0.15	0.15
glycine	1.82	1.91	1.86	1.99	1.87	1.97
threonine	0.79	0.76	0.75	0.71	0.81	0.73
citrulline	0.31	0.24	0.27	0.26	0.26	0.30
arginine	0.28	0.25	0.22	0.23	0.26	0.27
taurine	37.9	36.3	38.5	35.4	35.8	37.2
alanine	0.99	1.10	0.92	1.02	1.03	1.09
tyrosine	0.48	0.40	0.45	0.42	0.41	0.46
α-aminobutyrate	0.22	0.21	0.22	0.23	0.22	0.19
tryptophane	0.11	0.11	0.12	0.12	0.11	0.13
methionine	0.10	0.10	0.11	0.10	0.12	0.12
valine	0.38	0.34	0.30	0.36	0.35	0.38
phenylalanine	0.29	0.31	0.34	0.33	0.29	0.34
isoleucine	0.30	0.34	0.31	0.33	0.38	0.36
leucine	0.40	0.44	0.46	0.41	0.45	0.44
ornithine	1.61	1.47	1.54	1.52	1.59	1.57
lysine	0.52	0.51	0.55	0.55	0.57	0.65
homocitrulline	0.09	0.08	0.09	0.09	0.08	0.08
protein [pg/PMN-cell]	38	41	38	39	43	43
NH_3 [fMol/PMN-cell]	0.85	0.78	0.69	0.72	0.79	0.80
GOT [nU/PMN-cell]	44.1	45.3	43.8	44.6	45.7	45.8
GPT [nU/PMN-cell]	84.7	86.2	85.8	85.7	84.2	84.5
γ-GT [nU/PMN-cell]	0.25	0.24	0.24	0.24	0.25	0.26
LDH [nU/PMN-cell]	12.9	12.3	12.8	13.4	13.3	13.1

observed in our patient. Interestingly homocitrulline – which is not detectable in healthy volunteers, has been found to a small extent in PMN, too. PMN-NH_3 concentrations as well as GOT, GPT, γ-GT and LDH activities showed no data beyond physiological ranges (Table 1). Furthermore, HHH-syndrome caused a typical plasma amino acid pattern in our patient (Table 2). We observed increased levels of ornithine and asparagine while profiles of aspartate, glutamate, taurine, tryptophane and lysine were decreased. Homocitrulline was found to a small extent in plasma, too. NH_3, protein, albumine, and glucose levels in plasma have also been quantified (Table 2). Concerning these parameters, slightly elevated NH_3, as well as decreased albumine concentrations have been evaluated. In urine (Table 3) HHH-syndrome decreased concentrations of isoleucine and leucine. Moreover, in

Table 2. Free amino acids as well as NH_3, protein, albumine, glucose, creatinine, urea, and uric acid concentrations in plasma found in our patient perioperatively. Time points (T) are explained in text

Plasma probes [μMol/l]						
Amino acids	$\mathbf{T_0}$	$\mathbf{T_1}$	$\mathbf{T_2}$	$\mathbf{T_3}$	$\mathbf{T_4}$	$\mathbf{T_5}$
aspartate	1.55	1.63	1.13	1.66	1.59	1.58
glutamate	18.3	19.8	17.1	17.2	19.2	18.9
asparagine	38.6	39.3	39.5	35.2	34.9	36.8
serine	129.8	134.2	138.7	127.2	134.8	136.7
glutamine	654.7	596.9	629.5	592.4	628.8	639.4
histidine	62.4	65.2	69.3	70.5	67.6	72.3
glycine	298.2	309.7	308.2	288.2	299.8	301.6
threonine	79.3	83.8	87.9	78.5	73.6	78.2
citrulline	27.8	22.9	23.1	22.3	30.1	29.6
arginine	80.4	86.3	88.4	78.7	82.4	89.4
carnosine	11.9	12.9	11.7	12.6	12.8	11.1
taurine	36.7	39.4	30.8	33.8	34.6	38.9
alanine	285.8	271.2	252.7	238.2	278.7	281.4
tyrosine	29.5	30.3	30.9	28.4	26.5	32.8
α-aminobutyrate	10.8	11.7	11.8	10.9	10.5	10.9
tryptophane	31.4	35.2	27.2	28.5	29.9	32.3
methionine	20.6	20.1	19.8	18.4	19.7	21.6
valine	112.8	118.5	120.6	113.8	106.1	115.3
phenylalanine	48.8	43.6	43.2	40.7	41.6	49.3
isoleucine	39.9	43.6	43.5	40.8	39.7	42.6
leucine	72.8	69.5	70.9	66.4	70.1	72.3
ornithine	735.4	700.3	693.2	656.1	712.1	740.3
lysine	34.2	38.4	38.7	34.7	36.2	41.5
homocitrulline	6.89	6.73	6.86	6.78	6.69	6.81
NH_3 [μMol/l]	42	34	35	36	38	40
protein [g/l]	60	59	58	63	61	66
albumine [g/l]	33	32	31	34	34	37
glucose [mg/dl]	88	74	72	76	85	89
creatinine [mg/dl]	0.8	0.7	0.7	0.8	0.7	0.7
urea [mg/dl]	18	17	19	20	19	20
uric acid [mg/dl]	4.8	4.5	4.6	4.6	4.7	4.7

our patient elevated urinary concentrations of serine, glutamine, glycine, citrulline, alanine, α-aminobutyrate and lysine excretion have been found. Moreover, HHH-syndrome highly increased urinary ornithine and homocitrulline levels in our patient, too. Protein, albumine, glucose, creatinine, creatine, urea, uric acid and orotic acid levels in urine are given in Table 3, too. As seen in our patient high orotic aciduria and subnormal creatine excretion have also been connected with HHH-syndrome. The free amino acid concentrations in CSF, withdrawn once during general anaesthesia ($\mathbf{T_2}$), are given in Table 4; the calculated plasma-CSF-ratios, urine-CSF-ratios, urine-plasma-ratios (at $\mathbf{T_2}$) as well as NH_3, protein, albumine, and glucose levels are given additionally (Table 4). In CSF especially elevated ornithine and NH_3 as well as homocitrulline concentrations have been found.

Table 3. Free amino acids as well as orotic acid, creatinine, urea, urea-N, uric acid, protein, albumine, glucose concentrations and creatinine-clearance in urine found in our patient perioperatively. Time points (T) are explained in text

Urine probes [μMol/l]						
Amino acids	T_0	T_1	T_2	T_3	T_4	T_5
aspartate	4.67	4.72	4.66	4.02	4.34	4.58
glutamate	39.4	38.5	36.2	33.5	35.7	39.9
asparagine	101.8	108.5	102.9	98.7	106.7	109.4
serine	668.4	686.3	646.1	627.8	663.9	654.2
glutamine	1489.5	1320.2	1460.4	1497.2	1399.8	1523.1
histidine	604.7	570.9	589.2	534.3	562.1	595.6
glycine	2797.1	2546.8	2146.2	2209.8	2662.8	2732.9
threonine	285.4	264.6	238.6	221.7	231.7	278.9
citrulline	63.7	49.5	55.6	58.9	59.2	64.7
arginine	177.3	167.0	136.4	151.9	179.3	174.8
carnosine	368.5	321.3	340.2	305.5	337.7	347.3
taurine	56.8	50.5	47.8	45.7	46.8	59.2
alanine	678.2	618.7	616.8	629.7	688.9	694.5
tyrosine	95.6	83.3	94.3	101.9	99.8	86.3
α-aminobutyrate	562.8	525.3	510.1	502.9	546.7	556.2
tryptophane	149.7	133.7	106.7	110.1	126.7	156.7
methionine	288.6	226.9	246.1	235.9	249.8	265.8
valine	36.7	35.7	31.6	30.1	32.7	34.2
phenylalanine	58.3	51.7	63.8	57.4	59.9	58.3
isoleucine	26.7	22.9	22.7	19.9	23.1	25.4
leucine	29.6	27.1	28.0	27.3	26.9	28.4
ornithine	1565.4	1435.1	1414.6	1396.7	1559.3	1599.7
lysine	272.3	259.8	229.2	268.1	265.1	269.7
homocitrulline	903.1	899.9	863.4	837.8	910.6	919.2
orotic acid [μMol/l]	802.9	751.7	789.7	773.2	699.9	708.6
creatinine [mg/dl]	48	50	42	40	44	47
creatine [mg/dl]	7.1	6.9	5.9	5.5	6.3	6.4
urea [g/l]	15	12	11	13	13	14
uric acid [g/l]	0.36	0.41	0.38	0.30	0.29	0.31
protein [mg/l]	68	69	68	62	64	63
albumine [mg/l]	14	14	15	12	15	16
glucose [mg/dl]	14	11	10	11	14	15
creatinine-clearance: 98 [ml·min^{-1}/1.73 m^2] (T_1 to T_4)						

Laboratory and coagulation evaluations

Further conscientious laboratory investigations are given in Table 5; a comprehensive perioperative coagulogram is given in Table 6. Except for elevated GPT-levels in plasma neither laboratory evaluations nor coagulation analyses obtained from our patient showed data beyond physiological ranges.

Table 4. Free amino acids, NH_3, protein, albumine and glucose concentrations in CSF from our patient withdrawn during general anaesthesia ($\mathbf{T_2}$). Calculated ratios (at $\mathbf{T_2}$): P/L-Ratio (= plasma-liquor-ratios), U/L-Ratio (= urine-liquor-ratios) and U/P-Ratio = (urine-plasma ratios) are given additionally

Liquor-probe and ratios				
Amino acid	μMol/l	P/L-Ratio	U/L-Ratio	U/P-Ratio
aspartate	0.10	10.8	44.6	4.1
glutamate	2.83	6.0	12.8	2.1
α-aminoadipinate	0.12	6.9	213.5	31.0
asparagine	4.26	9.3	25.4	2.7
serine	18.8	7.4	36.5	4.9
glutamine	127.5	4.9	10.4	2.1
histidine	13.4	5.2	42.6	8.2
glycine	5.27	58.5	483.1	8.3
threonine	13.4	6.6	19.8	3.0
citrulline	11.8	2.1	4.6	2.2
arginine	24.9	3.5	6.7	1.9
carnosine	1.43	8.2	224.7	27.4
taurine	3.28	9.4	15.4	1.6
alanine	16.7	15.1	436.9	2.4
γ-aminobutyrate	0.08	–	–	–
tyrosine	3.47	8.9	23.9	2.7
α-aminobutyrate	0.98	11.9	535.9	44.7
tryptophane	0.97	27.9	137.3	4.9
methionine	2.20	9.1	102.9	11.4
valine	5.59	21.6	6.37	0.3
phenylalanine	5.37	8.1	9.5	1.2
isoleucine	2.14	20.4	10.7	0.5
leucine	3.34	21.3	8.1	0.4
ornithine	89.4	7.8	16.1	2.1
lysine	2.98	12.8	86.9	6.8
homocitrulline	2.40	2.9	131.1	374.3
NH_3 [μMol/l]	41	0.85	–	–
protein [mg/l]	278	208	0.28	1.3×10^{-3}
albumine [mg/l]	179	173	0.08	4.8×10^{-4}
glucose [mg/dl]	61	1.18	0.16	0.14

Anaesthesiological treatment

Perioperative treatment – with respect to our comprehensive laboratory (Table 5), coagulation (Table 6) and amino acid evaluations (Tables 1 to 4) –

GOT Glutamate-oxalacetate-transaminase; *GPT* glutamate-pyruvate-transaminase; *GLDH* glutamate-dehydrogenase; *γ-GT* gamma-glutamyl-transferase; *APH* alkaline phosphatase; *CHE* cholinesterase; *LAP* leucine-aminopeptidase; *LDH* lactate-dehydrogenase; *HBDH* α-hydroxy-butyratdehydrogenase; *CK* creatin kinasis; *TSH* thyroid stimulating hormone; *TBG* thyroxine binding globulin.

Table 5. Laboratory evaluations made routinely one day prior to anaesthesia ($\mathbf{L_1}$) as well as one day ($\mathbf{L_2}$) and two days ($\mathbf{L_3}$) after completion of anaesthesia

Laboratory – evaluations				
Parameters	$\mathbf{L_1}$	$\mathbf{L_2}$	$\mathbf{L_3}$	
hemoglobin	11.8	10.9	11.1	(g/l)
hematocrit	0.35	32	36	(l/l)
erythrocytes	4.7	4.5	4.8	(tera/l)
leucocytes	5.0	4.6	4.0	(giga/l)
thrombocytes	115	105	122	(giga/l)
neutrophils	1.9	2.0	1.8	(giga/l)
lymphocytes	2.3	2.4	2.3	(giga/l)
eosinophils	0.05	0.05	0.05	(giga/l)
basophils	0.01	0.01	0.01	(giga/l)
monocytes	0.40	0.39	0.40	(giga/l)
sodiu	144	141	143	(mMol/l)
potassium	4.4	3.9	4.4	(mMol/l)
calcium	2.2	2.2	2.1	(mMol/l)
phosphate	1.3	1.2	1.2	(mMol/l)
chloride	117	112	112	(mMol/l)
magnesium	0.95	0.91	0.93	(mMol/l)
C-reactive protein	<4.0	<4.0	<4.0	(mg/l)
GOT	16	18	12	(U/l)
GPT	25	22	20	(U/l)
GLDH	4.2	5.2	4.8	(U/l)
γ-GT	10	10	11	(U/l)
APH	105	108	105	(U/l)
CHE	4991	5221	5456	(U/l)
LAP	29	26	27	(U/l)
LDH	195	205	202	(U/l)
HBDH	96	92	102	(U/l)
bilirubine	0.6	0.6	0.7	(mg/dl)
CK	57	60	68	(U/l)
amylasis	55	52	62	(U/l)
lipasis	156	148	150	(U/l)
T_3	2.0	1.9	2.1	(ng/ml)
T_4	12.1	12.0	11.8	(μg/ml)
free T_4	1.4	1.6	1.5	(ng/dl)
free T_3	4.8	5.3	5.1	(pg/ml)
TSH	0.81	0.87	0.9	(mU/l)
TBG	23	21	22	(μg/ml)
pre-albumine	34	31	32	(mg/dl)
protein-electrophoresis:				
albumine	61		58	(%)
α_1-globulin	4		3	(%)
α_2-globulin	9		10	(%)
β-globulin	13		12	(%)
γ-globulin	13		17	(%)
cholesterin	133	116	122	(mg/dl)
triglycerides	119	97	134	(mg/dl)
HDL-cholesterin	22	16	11	(mg/dl)
LDL-cholesterin	127	123	137	(mg/dl)
lipoprotein – a	12	11	13	(mg/dl)
lipoprotein Apo-A	142	125	132	(mg/dl)
lipoprotein Apo-B	57	49	52	(mg/dl)
lipoprotein Apo-E	9.3	8.5	8.9	(mg/dl)

Table 6. Comprehensive coagulation analysis one day prior to anaesthesia ($\mathbf{L_1}$) as well as one day ($\mathbf{L_2}$) and two days ($\mathbf{L_3}$) after completion of anaesthesia

Coagulation – Analysis				
Parameters	$\mathbf{L_1}$	$\mathbf{L_2}$	$\mathbf{L_3}$	
INR	0.9	0.9	1.0	–
Quick	110	106	108	(%)
PTT	24	22	26	(s)
TT	18	17	20	(s)
reptilasis-time	18	18	19	(s)
fibronectin	290	311	306	(mg/l)
fibrinogen	328	319	338	(mg/dl)
Factor II	102	106	101	(%)
Factor V	81	86	78	(%)
Factor VII	79	85	82	(%)
Factor VIII	116	111	121	(%)
Factor VIII: C	124	131	119	(%)
Factor IX	72	85	82	(%)
Factor X	98	102	96	(%)
Factor XI	104	101	106	(%)
Factor XII	107	111	107	(%)
Factor XIII	99	98	104	(%)
PKK	89	86	88	(%)
HMWK	78	81	79	(%)
RCF	83	79	81	(%)
AT III	82	93	87	(%)
protein C	111	108	107	(%)
protein S	99	92	95	(%)
plasminogen	103	107	105	(%)
α_2-antiplasmin	94	100	99	(%)
α_2-makroglobulin	96	98	90	(%)
C_1-esterasis-inhibitor	103	108	106	(%)
α_1-proteinasis-inhibitor	107	105	109	(%)
D-Dimer	112	123	118	(ng/ml)
β-Thromboglobulin	38	32	41	(ng/ml)
platelet – factor 4	4.9	5.1	5.0	(ng/ml)

INR International normalized ratio; *Quick* thromboplastin time; *PTT* partial throboplastin time, *TT* thrombin time; *PKK* pre-kallikrein; *HMWK* high-molecular-weight-kininogen; *AT III* anti-thrombin III; *F VIII: Ag* factor VIII associated protein; *RCF* ristocetin cofactor.

was well tolerated by the patient, too; no immediate or delayed adverse metabolical abnormalities – compared with preoperative status – have been observed during the entire investigation. Moreover, anaesthesia was well tolerated by our patient and no adverse events have been observed during and after the entire course. Heart rate, blood pressure, end-tidal pCO_2, arterial oxygen saturation, and rectal temperature all remained constant during the whole general anaesthesia. Careful post-operative physical as well as neurological examinations revealed no further aggravation of the disease.

Discussion

Metabolical considerations concerning HHH-syndrome

As seen in our patient, the free amino acids in plasma, urine CSF in HHH-syndrome often show a typical pattern. Especially with regard to free intracellular amino acids in PMN-cells there are no further data available. The ornithine plasma, and CSF-levels were clearly above normal levels during the entire period of investigation and especially PMN ornithine levels were also high. Plasma ornithine concentrations published in the literature (Bender et al., 1985) range between 200 and 1020 μmol/l for an unlimited diet, and as seen in most cases urinary excretion of free ornithine in our patient was also markedly increased. Until now, the exact pathophysiologal sequence of HHH-syndrome has not been fully understood, an autosomal recessive inheritance has been suggested, but our findings seem to corroborate the earlier investigations of Fell et al. (1974) who considered that the primary defect is related to ornithine transport across the inner mitochondral memebrane into the mitochondria. According to this, ornithine accumulates in the cytoplasm, and intramito-chondrial ornithine is reduced so that ureagenesis is impaired and orotic aciduria occurs (Inoue et al., 1988). Therapeutical promises support these hypothesis: increasing the cytosolic urea cycle intermediates might drive transmitochondral ornithine, arginine or citrulline transport and improve urea cycle function. Indeed, Fell et al. (1974) clearly proved that ornithine treatment increased and significantly reduced plasma ammonium levels. However, in some patients, ornithine or arginine supplementation have had little or even deleterious effects and today consensus has been reached that only if decreased transport of ornithine is the primary defeciency the patients may benefit from supplementation. Similary, dietary citrulline supplementation in our patient clearly increased PMN citrulline concentrations (without affecting ammonium concentrations). Interstingly, plasma citrulline levels remained normal which in our opinion mainly was the result of an increased urinary citrulline excretion. In close relation to former findings, urinary homocitrulline excretion in our patient has shown to be highly elevated, too (Nakajima et al., 1988). Moreover, we found homocitrulline in low concentrations in plasma, CSF and, to a small extent, in PMN, too. Especially with regard to PMN homocitrulline profiles, there are no studies available which described intracellular findings of these "branch-line" amino acid in leucocytes. The origin of homocitrulline is uncertain but our hypothesis for the pathophysiology of HHH-syndrome predicts that lysine uptake into mitochondria might be undisturbed and that the elevated lysine : ornithine-ratio in the mitochondrium leads to conversion of lysine into homocitrulline catalyzed by transcarbamylase. Indeed, Carter et al. (1984) which studied transcarbamylation of lysine in digitonin-treated rat liver mitochondria suggested that two separate carbamylases exist, one for ornithine and another for lysine. However, till today important questions concerning homocitrulline metabolism are unanswered and especially regulation of homocitrulline excretion in urine remains unclear. Interestingly, in one HHH-syndrome patient a poor correlation between homocitrulline excretion and lysine ingestion has

been found because dietary lysine supplementation was followed by increased homocitrulline excretion and concerning these findings it has been suggested that lysine might have be therapeutical value in HHH-syndrome (Hommes et al., 1983). However, elevated homocitrullinuria was simultaneously accompanied by increasing plasma ammonium levels and in the author's opinion these findings illustrate that exact knowledge of this severe, although rare syndrome is important for any perioperative anaesthesiological or intensive medical treatment. Additionally, although various investigators suggest that renal tubular amino acid reabsorbation in HHH-syndrome seems to be normal and is not affected by citrulline or ornithine loads, we observed increased excretion in further amino acids (i.e. serine, glutamine, glycine, citrulline, alanine, α-aminobutyrate and lysine) leaving several important questions about pathophysiology of urinary amino acid excretion in these patients. For example, especially regarding subnormal excretion of creatine which was also connected with high glycinuria in our patient, we suggest that accumulated ornithine inhibits glycine transaminidase (the first enzyme in the creatine biosynthetic pathway). In accordance with former findings in several patients orotic acid concentration in urine was increased although plasma ammonium levels stayed within normal range. The authors believe that orotic aciduria in HHH-syndrome reflects carbamylphosphate accumulation and we suggest that intramitochondral carbamyl phosphate might be underused. Our findings imply that orotic aciduria may be a better indicator of the impairment of ureagenesis – compared to plasma ammonium levels – in patients with HHH-syndrome. Threonine and valine concentrations in plasma and PMN, as valuable indicators of adequate protein supply, as well as plasmatic and cellular protein levels (although albumine levels in plasma were slightly decreased) were considered to be adequate and demonstrated no perioperative changes. Interstingly PMN histidine profiles, which was fomerly selected to predict intracellular amino acid synthesis in adults, and PMN methionine levels, which are also known to be involved in the initiation states of protein synthesis, were markedly decreased (Metcoff, 1986). Regarding these findings we suggest that especially intracellular histidine and methionine would be better indicators of inadequate protein metabolism in HHH-syndrome. Levels of ammonium in plasma, CSF and PMN following fasting as well as during the postoperative course were within physiological ranges even though mean values often increased in HHH-patients. Protein restriction diet in the perioperative period connected with calorically adequate glucose supplementation prevented postprandial hyperammonemia, although concentrations of plasma, CSF and PMN ornithine did not decrease. Morevover, as mentioned above our patient derived benefit from dietary citrulline supplementation and indeed various investigators described that ammonia accumulation can be reduced by increasing urea cycle intermediates. Interestingly, following supplementation with 5×2 g citrulline/day, the plasma citrulline level remained normal in our patient which in our opinion mainly was due to citrullinuria.

The arginine concentrations in plasma, CSF, PMN and urine were normal (as has been in most other patients investigated until now). This consistency with earlier studies was also apparent for plasma and CSF lysine levels, of

which the former was below normal (as it was in most of the earlier HHH patients investigated; Botschner et al., 1989). Interestingly, despite of increased urinary lysine excretion and decreased plasma lysine profiles, PMN lysine levels were normal during the entire period. Further interesting findings demonstrated that alanine levels in plasma were elevated while PMN alanine profiles were decreased. This in our opinion may be due to following: PMN and plasmatic alanine provide substrates for plasmatic accumulation of waste nitrogen. Additionally, regarding glutamine metabolism Shigeto et al. (1992) have been suggested that elevation of glutamine and glutamate in CSF may cause dysfunctions of the neuronal system, especially degeneration of spinal anterior horn cells, but in our investigation imbalances of these amino acids neither in CSF or plasma nor in PMN have been demonstrated in our patient.

Therapeutical considerations concerning HHH-syndrome

There were no changes in the metabolic state due to the therapeutical treatment. Since both a protein loading and an interference with liver function could potentially have disadvantageous effects on the metabolic function in HHH-syndrome, attention has to be paid towards therapeutical management. It is important to avoid potentially fatal hyperammonemia. Moreover an excessive fasting period in patients with HHH-syndrome ($>$10h) has to be avoided during the preoperative preparation phase, since hypercatabolism due to fasting may lead to an endogenous protein overloading of the patient. In this connection, especially anaesthetics in isotonic oil-water emulsions (i.e. propotol) cannot be recommended because galenic preparations of this anaesthetic contain 10% soya bean oil, 1.2% ovian phosphatides, and 2.25% glycerol. This is important because lipids are not transported in a free form in human blood, but in the form of special lipoproteins (apolipoproteins, apoproteins). The synthesis of lipoproteins occurs in the mucosal cells of the intestinal epithelium and it is dependent on the lipid uptake in the liver. An excessive intake of lipids might therefore result in an increase in endogenous protein metabolism and thereby an increase in NH_3 levels. Moreover, especially regarding invasive surgery as long as it is ethically feasible, transfusions (i.e. blood and plasma) should be avoided and be substituted by cristalloid solutions: this prevents a protein overloading, too. Even plasma substitutes (dextrans, hydroxyethylstarch, or gelatin solutions) should only be infused after careful consideration, since patients suffering from HHH-syndrome could have associated with a deficiency in blood coagulation factors VII and X, as has been shown in four patients from three different ethnic backgrounds (Smith et al., 1992; Dionisi et al., 1987). As seen in our patient, a careful perioperative coagulation analysis could be of help in evaluating potential risks of intraoperative bleeding and may point to possible impairments in liver function. Anaesthetics and volume load should be optimised by avoiding hypotension with the danger of a reduced liver perfusion. Although many studies failed to show a significant difference in postoperative/

post-anaesthesiological changes in liver function dependent on the chosen anaesthetic procedure, we preferred to use an opiate/isoflurane anaesthesia (Leaman et al., 1978). Isoflurane seems to exert a positive influence on liver perfusion (Gelman et al., 1984). Another advantage of isoflurane is the lower metabolic rate compared with other anaesthetics (Gelman et al., 1987). N_2O which has been used on our patient is having only minor effects on resistance vessels of the area liver perfusion and it is metabolised only to a very small extent, too (Cooperman et al., 1968). Moreover, in our opinion, local anaesthetic procedures could not be considered because of 1) decreases in blood pressure as well as reduction in liver perfusion (due to the lack of autoregulation in the sphlanchnic area), and 2) compromised cooperation in these patients. Additionally, any anaesthetic procedure in patients presenting with HHH-syndrome should be selected with respect to pareses, neurological spasticity and should prevent any further neurological damage.

When substitution with protein and amino acid-containing infusions is absolutely necessary (i.e. for enteral nutrition), measurements of ammonia levels, although orotic aciduria may be a better indicator of the impairmant of ureagenesis, as well as plasma amino acids are important during the perioperative course. Protein restriction to less than 1.2 g–1.5 g/kg/day prevents hyperammonemia and may result in decreased concentrations of plasma ornithine. Furthermore, if decreased intramitochondral transport is the primary defect patients may benefit from ornithine supplementation, if decreased transport of ornithine into the mitochondria is the primary abnormality in this syndrome (Gordon et al., 1987). Apart from oral ornithine supplementation (0.5–1 mmol/kg/day, i.e. 66–132 mg/kg/day), oral dosing of arginine (6–7 g/day) or citrulline (3–10 g/day) are also effective and present further possibilities to prevent ammonia intoxication due to their degradation by the urea cycle, and in this way increase their activity (Dionisi et al., 1987).

In conclusion: **1)** for the first time it is reported on the amino acid status in plasma, urine, cerebrospinal fluid and especially free intracellular amino acids in single polymorphonuclear leucocytes as well as on further extensive laboratory and coagulation analyses, as additional indicators of cellular and metabolic function, of a single patient with HHH-syndrome who had to undergo general anaesthesia. **2)** As seen in our patient, free amino acid profiles in PMN, plasma, urine and CSF show a typical pattern and especially regarding free intracellular amino acids in PMN, the authors suggest that PMN, are important valuable tool for evaluating amino acid metabolism in nucleated cells as well as monitoring of therpeutical managemant in patients with these rare multifaceted disorder. **3)** Concerning our findings, the authors corroborate earlier investigations which described that the primary defect is related to ornithine transport into the mitochondria. **4)** Moreover, increased PMN ornithine profiles and intracellular findings of homocitrulline in PMN connected with hyperonithinemia and homocitrullinuria more precisely differentiate the HHH-syndrome from other urea cycle disorders (i.e. gyrate atrophy). **5)** Further important findings imply that orotic aciduria may be a better indicator of the impairmant of ureagenesis, compared to plasma ammonium levels, in patients with HHH-syndrome. **6)** Additionally, as seen in our patient,

glutamine as well as glutamate are not responsible for dysfunctions of the neuronal system.

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Received September 9, 1999