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Amino Acid Plasma Concentrations and Urinary Excretion in Young Diabetics

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Additional information is available at the end of the chapter

Abstract

The aim of this study is to analyze amino acid plasma profile in a group of young diabetics and to evaluate its application as markers of metabolic control of the disease, as well as to analyze the urinary excretion of amino acids in these patients. A clinical assessment and metabolic study (amino acid serum concentrations and urinary excretion of amino acids) was accomplished in a group of 49 children diagnosed with diabetes, and a group of 48 healthy children (control group). The plasma levels of total amino acids as well as branchedchain, glucogenic and ketogenic amino acids were significantly higher ($p < 0.05$) in the diabetic group with respect to the control group. Total as well as branched-chain, glucogenic and ketogenic amino acids urinary levels were significantly lower ($p < 0.05$) in the diabetic group compared to the control group. The study of the amino acid plasma in the young diabetic reflect disturbances in protein/amino acid metabolism and, consequently, in metabolic control of the disease. The study of amino acid urinary excretion might have interest not only in the context of diabetic nephropathy, but also in the revealing of partial aspects of amino acid metabolism and, probably, in the metabolic control of the disease.

Keywords: amino acids, insulin-dependent diabetes mellitus, children, serum concentration, urinary excretion

1. Introduction

Most of the cells in our body are dependent on the anabolic effects of insulin, which enables the use and storage of different nutrients from the diet.

It has been experimentally proven that insulin deficiency involves a series of ultrastructural and/or functional changes at an intracellular level, within muscle as well as liver, which substantially inhibit protein synthesis and stimulate protein degradation. Therefore, being amino acidosis, the structural elements of proteins and its metabolism could be altered in diabetes mellitus [1, 2]. In fact, significant changes in amino acid plasma levels and urinary excretion have been described in diabetic ketoacidosis, as well as anomalies in postprandial plasma profile of such amino acids in diabetic patients, whose values will not even return to normal levels after intensive insulin therapy [3–6].

Diabetic nephropathy is one of the most frequent and severe late complications in infant-juvenile diabetes; its functional and structural pathology seems to be shaped from the early stages of the disease. Persistent microalbuminuria is a functional disruption that occurs in the emerging phases of diabetic nephropathy, whose early detection and monitoring is quite important due to its prognostic significance [7, 8].

An increased urinary excretion of low molecular weight proteins and lysosomal enzymes has been confirmed in diabetic patients in the absence of microalbuminuria, as a result of a disorder in renal tubular reabsorption; its significance in natural history of diabetic nephropathy would be interpreted as early markers of renal injury [9–11]. On the other hand, barely 2–3% of the total amount of amino acidosis filtered by the glomerulus is excreted in urine following a massive and active tubular reabsorption [12]. Hence, aminoaciduria in diabetic individuals might be conditioned by the degree of structural and/or functional integrity of the renal tubule.

The aim of this study is to analyze amino acid plasma profile in a group of young diabetic individuals and to evaluate its potential application as markers of metabolic control of the disease, as well as to analyze the urinary excretion of amino acids in the absence of microalbuminuria in these patients.

2. Material and methods

2.1. Participants

A clinical assessment and metabolic study was accomplished in 49 children diagnosed with insulin-dependent diabetes mellitus, aged 8.6–14.3 years, following conventional insulin therapy, and a group of 48 healthy children (control group) aged 7.4–14.8 years.

2.2. Clinical assessment

Information recorded from every patient/participant included age, weight and height, BMI, time and progress of the disease, and dosage of subcutaneous insulin.

Weight and height measurements were made in underwear while being barefoot. Weight was measured using the Año-Sayol scale (reading interval 0–120 kg and a precision of 100 g), and height was measured using the Holtain wall stadiometer (reading interval 60–210 cm, precision 0.1 cm). The Z-score values for the BMI were calculated using the epidemiologic data

contained within the program Aplicación Nutricional, from the Spanish Society of pediatric gastroenterology, hepatology, and nutrition (Sociedad Española de Gastroenterología, Hepatología y Nutrición Pediátrica, available at http://www.gastroinf.es/nutritional/). The graphics from Ferrández et al. (Centro Andrea Prader, Zaragoza) (2002) were used as reference charts.

Blood pressure (BP) was measured in the right arm with the patient in the supine position using Visomat comfort 20/40 (Roche Diagnostics Inc.) digital blood pressure monitor, recording the lowest of three measurements.

2.3. Biochemical analysis

All participants (diabetic and control group) underwent blood testing after a 12-hour fast, in order to determine plasma glucose levels, glycosylated hemoglobin (Hb1Ac), creatinine and amino acid concentrations. In addition, a 24-hour urine sample was collected to determine albumin and amino acid concentrations and glomerular filtration rate (GFR).

The analyzed amino acids (in blood and urine) were the following: alanine (ALA), arginine (ARG), aspartic acid (ASP), cysteine (CYS), glutamine (GLN), glutamic acid (GLU), glycine (GLY), histidine (HIS), isolecucine (ILE), leucine (LEU), lisine (LYS), methionine (MET), phenylalanine (PHE), serine (SER), threonine (THR), tyrosine (TYR), valine (VAL), and taurine (TAU).

Measurements in plasma (glucose and creatinine) and urine (creatinine) were made using a Synchron CX5 (Beckman) analyzer. HbA1c was determined using Boehringer-Mannheim reagents.

The quantification of urinary albumin excretion (UAE) was made by nephelometry (Away Protein System-Beckman), and microalbuminuria was considered when values exceed 12 ug/min, being that a reason for exclusion. GFR was calculated using the endogenous creatinine clearance, and hyperfiltration was considered when values were over 145 ml/min/11.73 m^2.

The determination of urine and plasma amino acid concentrations was made by reversed-phase high pressure liquid chromatography (HPLC) with o-phthaldialdehyde precolumn derivatization.

2.4. Statistical analysis

Results are displayed as means (M) with corresponding standard deviations (SD). Statistical analysis (descriptive statistics, Student's T and Pearson's correlation) was done using the Statistical Packages for the Social Sciences version 20.0 (Chicago, IL, USA). Statistical significance was assumed when p value was lower than 0.05.

Parents and/or legal guardians were informed and provided verbal consent for the participation in this study in all cases. The study was approved by the Ethics Committee for Human Investigation at our institution (in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and later amendments).

3. Results

Table 1 shows the comparison of mean values for the clinical and biochemical characteristics (blood and urine) in the diabetic and control groups. Fasting glycaemia, Hb1Ac, and GFR were significantly higher ($p < 0.05$) within the diabetic group compared to the control group. There were not any significant differences in age, BMI Z-score, systolic and diastolic blood pressure, and urinary albumin excretion between both groups.

There was no correlation between glomerular filtration and Hb1Ac or urinary albumin excretion, not between blood pressure (systolic and diastolic blood pressure) and glomerular filtration or Hb1Ac. There was a positive correlation ($p < 0.05$) between diastolic blood pressure and the evolution of the disease (years) ($r = 0.515$).

Table 2 exposes and compares the mean values of amino acid plasma concentrations for the samples of the diabetic and control groups. Plasma concentrations of ARG, GLN, ILE, PHE, THR, TYR, VAL, and TAU were significantly higher ($p < 0.05$) within the diabetic group with respect to the control group.

Table 3 depicts and compares the mean values of plasma concentrations of different amino acids groups analyzed in the diabetic and control group. The plasma levels of total amino acids as well as branched-chain, glucogenic, and ketogenic amino acids were significantly higher ($p < 0.05$) in the diabetic group with respect to the control group.

There was no correlation between the single amino acid (or amino acid groups) plasma levels and the evolution of the disease (years) or Hb1Ac. There was a negative correlation ($p < 0.05$) among insulin dosage and amino acids THR ($r = -0.404$), MET ($r = -0.513$), PHE ($r = -0.456$), SER ($r = -0.442$), CYS ($r = -0.390$), GLY ($r = -0.451$), and TAU ($r = -0.479$), as well as a

Items	Diabetic group (n = 49)	Control group (n = 48)	p-Values
Age (years)	11.82 ± 1.78	12.05 ± 1.93	n.s.
BMI Z-score	0.05 ± 0.67	−0.01 ± 0.55	n.s.
Systolic BP	93.15 ± 8.85	89.0 ± 8.95	n.s.
Diastolic BP	55.0 ± 7.26	52.2 ± 8.36	n.s.
Evolution (years)	5.79 ± 2.67	—	—
Insulin (UI/kg/d)	0.82 ± 0.26	—	—
Glucose (mg(dl))	198.8 ± 55.5	89.57 ± 10.2	<0.01
Hb1Ac (%)	7.7 ± 1.68	4.56 ± 0.7	<0.05
GFR (ml/min/1.73 m^2)	135.65 ± 34.3	114.16 ± 9.13	<0.05
UAE (ug/min)	3.77 ± 1.8	3.42 ± 1.89	n.s.

GFR: glomerular filtration rate, UAE: urinary albumin excretion.

Table 1. Clinical and biochemical characteristics of the diabetic and control groups (M ± SD).

Amino acids	Diabetic group (n = 49)	Control group (n = 48)	p-Values
ALA	144.83 ± 36.32	134.84 ± 36.67	n.s.
ARG	49.01 ± 16.78	22.62 ± 6.94	<0.01
ASP	0.33 ± 0.95	1.34 ± 2.18	n.s.
CYS	34.77 ± 11.61	31.56 ± 10.90	n.s.
GLN	243.23 ± 59.42	187.84 ± 56.83	<0.01
GLU	18.28 ± 9.69	20.70 ± 10.22	n.s.
GLY	46.53 ± 22.3	35.34 ± 10.23	n.s.
HIS	133.62 ± 43.59	147.09 ± 53.89	n.s.
ILE	90.83 ± 19.37	66.54 ± 15.27	<0.001
LEU	74.74 ± 17.37	68.65 ± 14.65	n.s.
LYS	60.70 ± 27.32	57.15 ± 28.61	n.s.
MET	31.57 ± 10.68	29.18 ± 13.05	n.s.
PHE	81.84 ± 19.54	65.64 ± 16.45	<0.01
SER	53.93 ± 26.03	66.04 ± 22.04	n.s.
THR	73.26 ± 27.90	57.90 ± 18.07	<0.05
TYR	60.25 ± 27.18	38.25 ± 12.47	<0.05
VAL	190.46 ± 48.01	148.91 ± 35.31	<0.01
TAU	99.69 ± 36.82	75.66 ± 37.01	<0.05

ALA: alanine, ARG: arginine, ASP: aspartic acid, CYS: cysteine, GLN: glutamine, GLU: glutamic acid, GLY: glycine, HIS: histidine, ILE: isolecucine, LEU: leucine, LYS: lisine, MET: methionine, PHE: phenylalanine, SER serine, THR: threonine, TYR tyrosine, VAL: valine, TAU: taurine.

Table 2. Plasma concentrations of amino acids (nmol/ml) in the diabetic and control groups (M ± SD).

positive correlation (p < 0.05) among glycaemia and amino acids VAL (r = 0.545) and LEU (r = 0.648).

Table 4 displays and compares the mean values of urinary concentrations of different amino acids quantified in the diabetic and control group. The urinary level of amino acids, except for ASP, ILE, and PHE, was significantly lower (p < 0.05) in the diabetic group with respect to the control group.

Table 5 outlines and compares the mean values of urinary levels of the different amino acids groups in the diabetic and control group. Total as well as branched-chain, glucogenic and ketogenic amino acid urinary levels were significantly lower (p < 0.05) in the diabetic group compared to the control group. The mean values of the glucogenic/total amino acid ratio were significantly lower (p < 0.05) in the diabetic group with respect to the control group. There were no significant differences in the ketogenic/total amino acid ratio between both groups.

Amino acid groups	Diabetic group (n = 49)	Control group (n = 48)	p-Values
Total	1383.70 ± 353.67	1198.46 ± 261.16	<0.05
Branched-chain	347.65 ± 58.76	285.20 ± 45.20	<0.01
Glucogenic	1252.74 ± 236.82	1053.69 ± 211.19	<0.001
Ketogenic	441.62 ± 57.09	354.13 ± 53.45	<0.05

Table 3. Plasma concentrations of amino acids (nmol/ml) in the diabetic and control groups (M ± SD).

Amino acids	Diabetic group (n = 49)	Control group (n = 48)	p-Values
ALA	53.97 ± 36.68	118.07 ± 45.24	<0.001
ARG	2.45 ± 1.91	4.59 ± 2.97	<0.05
ASP	4.98 ± 3.23	8.84 ± 4.22	n.s.
CYS	19.25 ± 11.07	61.91 ± 29.17	<0.05
GLN	7.84 ± 4.52	95.02 ± 32.18	<0.001
GLU	16.62 ± 9.61	35.83 ± 15.77	<0.05
GLY	23.00 ± 15.05	192.41 ± 121.59	<0.001
HIS	74.12 ± 48.50	233.95 ± 89.36	<0.01
ILE	18.98 ± 10.31	29.83 ± 18.42	n.s.
LEU	12.46 ± 8.17	22.16 ± 9.13	<0.05
LYS	223.65 ± 150.75	525.32 ± 196.31	<0.05
MET	19.11 ± 8.01	86.04 ± 56.06	<0.05
PHE	40.64 ± 22.58	51.67 ± 13.71	n.s.
SER	10.64 ± 7.64	25.40 ± 13.65	<0.01
THR	14.47 ± 10.96	63.29 ± 19.36	<0.05
TYR	26.65 ± 16.28	79.08 ± 21.15	<0.001
VAL	25.61 ± 11.94	45.11 ± 13.67	<0.05
TAU	115.17 ± 56.70	172.31 ± 107.12	<0.01

ALA: alanine, ARG: arginine, ASP: aspartic acid, CYS: cysteine, GLN: glutamine, GLU: glutamic acid, GLY: glycine, HIS: histidine, ILE: isolecucine, LEU: leucine, LYS: lisine, MET: methionine, PHE: phenylalanine, SER serine, THR: threonine, TYR tyrosine, VAL: valine, TAU: taurine.

Table 4. Urinary levels of amino acids ($\mu mol/m^2$) in the diabetic and control groups (M ± SD).

Amino acid groups	Diabetic group (n = 49)	Control group (n = 48)	p-Values
Total	754.94 ± 427.16	1868.42 ± 662.36	<0.05
Branched-chain	54.58 ± 26.51	101.13 ± 36.76	<0.05
Glucogenic	252.51 ± 178.75	943.79 ± 370.50	<0.05
Ketogenic	236.40 ± 121.16	530.69 ± 215.78	<0.05
Ratio G/T	0.34 ± 0.09	0.50 ± 0.07	<0.05
Ratio K/T	0.33 ± 0.16	0.28 ± 0.12	n.s.

G/T: glucogenic/total, K/T: ketogenic/total.

Table 5. Urinary level of amino acid groups in the diabetic and control groups (M ± SD).

There was no correlation between the levels of each particular amino acid and/or group of amino acids in urine and the time of evolution, Hb1Ac, urinary albumin excretion, GFR, and blood pressure (systolic and diastolic blood pressure).

4. Discussion

4.1. Plasma concentrations

In diabetes mellitus, the deficiency in insulin, and in large part, the effects of the counter regulatory hormones would stimulate the synthesis of glucose—other than the glycogenolysis

pathway—through the glyconeogenesis [1, 2, 13]. This might explain the differences found in the amino acid plasma levels within the diabetic and control group that, in general, would indicate that there is an increase in the bioavailability of glycogenic substrates in diabetic patients, even in basal conditions.

Insulin leads to a decrease in amino acid plasma levels through the stimulus of protein synthesis and the inhibition of proteolysis [2, 14]; this would largely explain the negative correlation found between insulin dosage and plasma level of the different amino acids. Therefore, the significantly high plasma concentrations of the different amino acids–particularly glucogenic amino acids–in the diabetic group with respect to the control group (probably as a consequence of the insulinopenia that characterizes diabetes), could be useful as plasma markers of a deficient metabolic control.

An increase in postprandial branched-chain amino acid (valine, leucine, and isoleucine) plasma concentrations has been described in diabetic patients, in relation to the metabolic control of the disease [4, 15]. This is probably due to a deficient peripheral metabolism of these amino acids (they undergo basically muscle metabolism). Since it has not been possible to prove an increase in the release of branched-chain amino acids from muscle and/or liver in diabetic patients during fasting [1, 2], and being conscious that biological effects of insulin are deficient in diabetes, it can be assumed that the increased branched-chain amino acid plasma levels in the diabetic group in comparison to the control group would be due to a low stimulation (by insulin) in amino acid transportation inside the cell. Even though no correlation has been found between branched-chain amino acids and metabolic control or time of evolution of diabetes, a positive correlation has been detected between valine and leucine and fasting glycaemia, a fact that would support the hypothesis of a more intense relationship among basal plasma concentration of these amino acids and single determination glycaemia rather than with mediumterm metabolic control.

Even though amino acids are appropriate substrates for hepatic and/or renal synthesis of glucose (gluconeogenesis), glutamine and especially alanine are the most important glucogenic amino acids in quantitative terms [16]. However, while glutamine plasma levels in basal conditions were significantly higher in the diabetic group in comparison to control group, alanine plasma levels did not differ in those groups.

During fasting, alanine release does not exclusively correspond to a mechanism of proteolysis and posits muscle synthesis of new molecules of alanine from the glucose captured by the muscle or glucose alanine cycle [2, 16]. Nevertheless, since glucose uptake by the muscle is lowered due to insulinopenia in the diabetic patient, the conversion of glucose into alanine would be decreased, and consequently, this would explain why alanine plasma levels in the diabetic group do not differ from the control group.

Amino acid metabolism in insulin-dependent diabetes mellitus appears to be intrinsically disrupted, since insulin deficiency and to a great extent, the effects of the counter regulatory hormones imply an increase in hepatic gluconeogenesis and muscle proteolysis, as well as a deficient peripheral use and/or disturbance in hepatic amino acid metabolism; this would result in a plasma profile characterized by an increase of total amino acids, at the expense

mainly of branched-chain and glucogenic amino acids. In this way, the study of the amino acid plasma profile in diabetic patients would be worthwhile, since it would reflect disturbances in protein and/or amino acid metabolism and consequently, in metabolic control of the disease.

4.2. Urinary excretion

Diabetic nephropathy is preceded by a window period, which might show different renal functional and/or structural disturbances, even in the early stages of the disease [7, 8]. In fact, the results obtained, in line with other researchers [17, 18], reveal significantly higher glomerular filtration in the diabetic group in comparison to the control group, and especially in those patients with a shorter period of disease and regardless of metabolic control of the disease. In addition, even when the whole diabetic group had normal blood pressure measurements, the existing correlation between diastolic blood pressure and the time of evolution of the disease suggests a situation of window period in diabetic nephropathy in this group of young diabetics, and highlights the importance of periodic blood pressure measurements in diabetics from the early stages of the disease. This allows for the beginning of a dietary and/or medical treatment earlier than was recommended until now [19]. However, it can be concluded that the structural integrity of the glomerulus in these diabetic patients would be relatively well preserved, since the urinary excretion of albumin was similar in both groups.

On another note, several researchers have noted a higher beta 2 microglobulin and lysosomal enzyme urinary excretion in diabetic patients in the absence of microalbuminuria, as a sign of functional disorder in the proximal tubule with no glomerular lesion, from the early stages of the disease [10, 11]. In this context, the study of amino acid urinary excretion in the diabetic could be of great interest, since different mechanisms of specific tubular reabsorption for different amino acids have been described on an experimental basis [20]. Hence, any tubular malfunction might condition significant qualitative and/or quantitative aminoaciduria, and therefore, it could have a potential clinical application in the early detection of tubular lesion and/or silent diabetic nephropathy.

All the same, and according to the results obtained, urinary excretion of each single amino acid (except for isoleucine, aspartic acid, and taurine), as well as each amino acid groups analyzed were significantly lower in the diabetic group with respect to the control group. This may seem paradoxical; however, the difference observed in the relation glucogenic and total amino acid (G/T ratio) between both groups reveals that the lower amino acid urinary excretion in the diabetic would greatly be at the expense of glucogenic amino acids, probably because the glomerular filtration is also lower, as a consequence of a greater organic use of these amino acids in the endogenous synthesis of glucose. No correlation has been found between aminoaciduria and time of evolution, glomerular filtration, blood pressure, and metabolic control.

In sum, the study of amino acid urinary excretion in the young diabetic might have interest not only in the context of diabetic nephropathy, but also in the revealing of partial aspects of amino acid metabolism, and probably, in the metabolic control of the disease.

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