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Urinary Excretion of Amino Acids in Children with Type 1 Diabetes

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Abstract

***Objective:** The aim of this study is to analyze the urinary excretion of amino acids in a group of children with type 1 diabetes without microalbuminuria.*

***Methods:** A clinical assessment and metabolic study (HB1AC, GFR, urinary albumin excretion and urinary excretion of amino acids) was accomplished in a group of 49 children diagnosed with type 1 diabetes, aged 8.6 to 14.3 years, and a group of 48 healthy children (control group), aged 7.4 to 14.8 years.*

***Results:** Branched-chain (54.5±26.5 vs. 101.1±36.7 µmol/m^2), glucogenic (252.5±178.7 vs. 943.7±370.5 µmol/m^2) and ketogenic (236.4±121.1 vs. 530.6±215.7 µmol/m^2) 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 (0.34±0.09 vs. 0.50±0.07) 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 (0.33±0.16 vs. 0.28±0.12) between both groups. There was no correlation between the levels of each amino acid (or amino acids group) in urine and the time of evolution, Hb1Ac, urinary albumin excretion, GFR and blood pressure.*

***Conclusions:** 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. Children. Type 1 diabetes. Urinary excretion.*

Introduction

Diabetic nephropathy in 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 [1,2].

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 [2-5]. 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 [6]. Hence, aminoaciduria in diabetic individuals might be conditioned by the degree of structural and/or functional integrity of the renal tubule. It should be emphasized that we have barely found studies available about urinary excretion of amino acids in young diabetics.

The aim of this study is to analyze the urinary excretion of amino acids in a group of children diagnosed with type 1 diabetes in the absence of microalbuminuria.

Material and Methods

Participants

A clinical assessment and metabolic study was accomplished in 49 children diagnosed with type 1 diabetes, aged 8.6 to 14.3 years, under treatment with three or four administrations (injections) per day consisting of a mixture of human long-acting insulin (insulin glargine) and rapid-acting insulin (insulin lispro or insulin aspart). At the authors´ institution, meal planning in diabetic patients is based on a combination of carbohydrate counting and the traditional exchange system. A group of 48 healthy children (control group) aged 7.4 to 14.8 years was recruited. They came from external consultations of the different paediatric subspecialities and no pathologies were previously detected

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 to 120 kg and a precision of 100 g), and height was measured using the Holtain wall stadiometer (reading interval 60 to 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.

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) and creatinine. 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 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 amino acid concentrations was made by reversed-phase high pressure liquid chromatography (HPLC) with o-phthaldialdehyde precolumn derivatization.

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 of Navarra Hospital Complex (in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and later amendments).

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.

Table1. *Clinical and biochemical characteristics of the diabetic and control groups (M ± SD)*

Items	Diabetic group (n=49)	Control group (n=48)	p-values
Age (years)	11.7±1.7	12.1±1.9	N.S.
BMI Z-score	0.05±0.67	-0.01±0.55	N.S.
Systolic BP (mmHg)	93.1±8.8	89.0±8.9	N.S.
Diastolic BP (mmHg)	55.0±7.2	52.2±8.3	N.S.
Evolution (years)	3.7±2.6	---	---
Insulin (UI/kg/d)	0.79±0.26	---	---
Glucose (mg/dl)	198.8±55.5	89.5±10.2	<0.01
Hb1Ac (%)	7.0±1.6	4.1±0.9	<0.05
GFR (ml/min/1.73m2)	135.6±34.3	114.1±9.1	<0.05
UAE (ug/min)	3.7±1.8	3.4±1.8	N.S.

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

Fifteen patients of diabetic group patients (30.6%) presented glomerular hyperfiltration, and the evolution of the disease in these patients were significantly lower (p <0.05) than in the rest of the diabetic group (2.6±0.6 vs. 1.9±0.4 years).

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

Table2. *Urinary levels of amino acids (μmol/m^2) in the diabetic and control groups (M±SD)*

Amino acids	Diabetic group (n=49)	Control group (n=48)	p-values
ALA	53.9±36.6	118.1±45.2	<0.001
ARG	2.4±1.9	4.5±2.9	<0.05
ASP	4.9±3.2	8.8±4.2	N.S.
CYS	19.2±11.0	61.9±29.1	<0.05
GLN	7.8±4.5	95.0±32.1	<0.001
GLU	16.6±9.6	35.8±15.7	<0.05
GLY	23.0±15.0	192.4±121.5	<0.001
HIS	74.1±48.5	233.9±89.3	<0.01
ILE	18.9±10.3	29.8±18.4	N.S.
LEU	12.4±8.1	22.1±9.1	<0.05
LYS	223.6±150.7	525.3±196.3	<0.05
MET	19.1±8.0	86.0±56.0	<0.05
PHE	40.6±22.5	51.6±13.7	N.S.
SER	10.6±7.6	25.4±13.6	<0.01
THR	14.4±10.9	63.2±19.3	<0.05
TYR	26.6±16.2	79.1±21.1	<0.001
VAL	25.6±11.9	45.1±13.6	<0.05
TAU	115.1±56.7	172.3±107.1	<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 3 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.

Table3. *Urinary level of amino acid groups (µmol/m²) in the diabetic and control groups (M±SD)*

Amino acid groups	Diabetic group (n=49)	Control group (n=48)	p-values
Total	754.9±427.1	1868.4±662.3	<0.05
Branched-chain	54.5±26.5	101.1±36.7	<0.05
Glucogenic	252.5±178.7	943.7±370.5	<0.05
Ketogenic	236.4±121.1	530.6±215.7	<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.

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).

Discussion

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 [7, 8], 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 [9]. 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 [5, 6]. 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 [10]. Hence, any tubular malfunction might condition significant qualitative and/or quantitative aminoaciduria and, therefore, it could have a potential clinical application in 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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