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## Research Article

# Amino Acid Plasma Profile in Children with Type 1 Diabetes

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### ABSTRACT

**Background:** Insulin deficiency inhibits protein synthesis and stimulates protein degradation, and therefore amino acid metabolism could be altered in diabetes mellitus.

**Objective:** To analyze amino acid plasma profile in a group of children with type 1 diabetes, and to evaluate its potential application as a marker of metabolic control for the disease.

**Methods:** A clinical assessment and metabolic study (amino acid plasma concentrations) was performed 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:** 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. Likewise, plasma concentrations of branched-chain ( $347.65 \pm 58.76$  vs.  $285.20 \pm 45.20$  nmol/ml), glucogenic ( $1252.74 \pm 236.82$  vs.  $1053.69 \pm 211.19$  nmol/ml) and ketogenic amino acids ( $441.62 \pm 57.09$  vs.  $354.13 \pm 53.45$  nmol/ml) 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 HbA1c levels.

**Conclusion:** The study of changes in amino acid plasma profile in the young diabetic, probably as a consequence of insulinopenia, could have interest as a marker of metabolic control for the disease.

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## 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 ultra-structural 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 acids the structural elements of proteins, their 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]. It should be

emphasized that we have barely found studies available on amino acid plasma profile in young diabetics. The aim of this study is to analyze amino acid plasma profile in a group of children diagnosed with type 1 diabetes, and to evaluate its potential application as markers of metabolic control for the disease.

## Subjects and Methods

### I Subjects

A clinical assessment and metabolic study were accomplished in 49 children diagnosed with type 1 diabetes mellitus, 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)

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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 subspecialties and no pathologies were previously detected.

## II 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 [7].

## III 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 amino acid plasma concentrations. 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), isoleucine (ILE), leucine (LEU), lysine (LYS), methionine (MET), phenylalanine (PHE), serine (SER), threonine (THR), tyrosine (TYR), valine (VAL) and taurine (TAU). Measurements of glycemia were made using a Synchron CX5 (Beckman) analyzer. HbA1c was determined using Boehringer-Mannheim reagents. The determination of amino acid plasma concentrations was made by Reversed-phase high pressure liquid chromatography (HPLC) with o-phthalaldehyde precolumn derivatization.

## IV 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.

## Results

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

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 1:** 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)	11.82±1.78	12.05±1.93	n.s.
BMI Z-score	0.05±0.67	-0.01±0.55	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

**Table 2:** 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	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: isoleucine, LEU: leucine, LYS: lysine, MET: methionine, PHE: phenylalanine, SER: serine, THR: threonine, TYR: tyrosine, VAL: valine, TAU: taurine.

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

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 depicts and compares the mean values of plasma concentrations of the different amino acids' groups analysed 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 acids (or amino acid groups) plasma concentrations 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 positive correlation ( $p < 0.05$ ) among glycaemia and amino acids VAL ( $r = 0.545$ ) and LEU ( $r = 0.648$ )

## Discussion

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 gluconeogenesis [1, 2, 8]. 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 glucogenic substrates in diabetic patients, even in basal conditions. Insulin induces a decrease in amino acid plasma levels through the stimulus of protein synthesis and the inhibition of proteolysis; this would largely explain the negative correlation found between insulin dosage and plasma level of the different amino acids [2, 9]. A significantly high plasma concentration of the different amino acids –particularly glucogenic amino acids- in the diabetic group with respect to the control group- has been detected. It is probably a consequence of the insulinopenia or deficient metabolic control, as it seems that happened in these patients (HbA1c:  $7.7 \pm 1.68\%$ ), and it could be useful as 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, 10]. 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 and being conscious that biological effects of insulin are deficient in diabetes, it can be assumed that the increased branched-chain amino acid serum 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 [1, 2]. Even though no correlation has been found between branched-chain amino acid plasma concentrations and metabolic control or time of evolution of diabetes, a positive correlation has been detected between valine and leucine and fasting glycaemia. This fact would support the hypothesis of a more intense relationship among basal plasma concentrations of these amino acids and single determination glycaemia rather than with medium-term metabolic control [6].

Even though amino acids are appropriate substrates for hepatic and/or renal synthesis of glucose (gluconeogenesis), glutamine, and especially alanine is the most important glucogenic amino acids in quantitative terms [11]. 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, 11]. Nevertheless, since glucose uptake by the muscle is lowered due to insulinopenia or deficient metabolic control 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 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, it would be interesting to perform screening of amino acid plasma profile in each child with newly diagnosed type 1 diabetes. Therefore, the study of the amino acid plasma profile in diabetic patients might be of interest since if we consider that these disturbances in proteins and/or amino acid metabolism were secondary to the insulinopenia that characterizes these patients, in a sense it would be reflecting the degree of metabolic control of the disease.

## Statement of Ethics

Parents and/or legal guardians were informed and provided written 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).

## Conflicts of Interest

None.

## Funding

None.

## Author Contributions

TDT and FGV participated in study design and data analysis and wrote the first draft of the manuscript. PMG, MMC, LAM and MUM participated in data collection and analysis. All authors participated in manuscript preparation and approved its final version.

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