CHILDREN WHO DEVELOP TYPE 1 DIABETES EARLY IN LIFE HAVE LOW LEVELS OF CARNITINES AND AMINO ACIDS AT BIRTH: DOES THIS FINDING SHED LIGHT ON THE ETIOPATHOGENESIS OF THE DISEASE?

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Introduction

At the time of diagnosis of Type 1 Diabetes (T1D), patients showed reduced circulating levels of free and total carnitine, evidence that has also been confirmed in long standing insulin-treated patients. No investigations have analysed circulating carnitine in individuals before the onset of this disease. We have thus hypothesized that carnitine impairment might be evident before the clinical appearance of T1D, possibly from birth.

To clarify this point, we have carried out a retrospective case-control study, aimed at investigating the metabolic profiles of carnitine and amino acids in the first days of life, and their relationship with the future onset of T1D.

Materials and Methods

Diabetic patients were identified within the Unit of Diabetes at the Bambino Gesù Paediatric Hospital in Rome, Italy. Controls were selected from 1,650 children, who were HLA typed at birth for genetic susceptibility to develop T1D.

As criteria of inclusion, children had be born in Lazio between January 2000 and December 2002 and T1D had to be developed by the age of 4 years. These criteria were adopted in order to: a) have the possibility to retrieve dry spot samples; b) reduce the time elapsing from birth and assays; and c) identify controls matched for age, sex and genetic HLA risk categories (high, moderate and low), defined as described in Table I.

Overall, 11 diabetic children fulfilled the criteria and 44 matched controls were identified. Informed consent was obtained from parents of all children. Information regarding birth weight, gestational age at birth and feeding in the first week of life was also recorded.

Blood dry spots were then retrieved from the 2 centers in charge for neonatal screenings in the same region. All spots were collected within the first days after birth and stored at room temperature up to the analysis. Once identified, samples were coded and no information regarding T1D status was available up to results were obtained.

In each blood spot, concentrations of total (TC), free (FC) and acylcarnitines (AC) and of 13 amino acids (listed in Table II) were determined with a tandem mass spectrometer (Sciex API 365, PE Sciex Instrument, Concord, ON, Canada), as routinely made for neonatal mass screening.

TC, FC, AC and AC/FC ratio were analyzed. Amino acids were also studied as the algebraic sum of essential, non essential and total amino acid concentrations.

Table I: Genetic screening of individuals at risk of developing T1D (HLA DRB1, DQB1)

High risk (N=4 T1D patients, N=16 controls)	Low risk (N=1 T1D patients, N=4 controls)
DRB1*03 / *04 (not 0403), DQB1 0302	All others
Moderate risk (N=6 T1D patients, N=24 controls)	
DRB1*04 (not 0403) / *04 (not 0403), DQB1 0302	
DRB1*04 (not 0403) / X, DQB1 0302 / not 0602-3	
DRB1*03 / *03	
DRB1*03 / X, DQB1 not 0602-3, not 0301, not 0503	**X ≠ *03, *04, 0403**

Table II. Carnitine and amino acid concentrations (μmol/L) in blood dry spots

	Diabetic patients (N=11)	Control children (N=44)	Significance P
Total carnitine (TC)	29.9 (14.8-38.9)	39.5 (19.7-96.1)	0.004
Free carnitine(FC)	19.0 (6.8-25.6)	24.7(11.7-68.9)	0.009
Acylcarnitine(AC)	10.7 (7.8-16.3)	16.5 (8.0-27.2)	0.009
AC/FC ratio	0.7 (0.4-1.2)	0.6 (0.3-1.2)	0.556
Alanine (Ala)	82.5 (50.7-174.4)	140.8 (50.1-526.7)	0.037
Arginine (Arg)	15.1 (10-34.6)	17.4 (7.3-49.0)	0.599
Aspartate (Asp)	35.9 (15.8-70.4)	42.9 (18.0-152.9)	0.461
Citrulline (Cit)	4.6 (2.9-13.3)	6.8 (2.4-22.1)	0.064
Glycine (Gly)	80.9 (49.8-131.2)	122.1 (66.0-354-7)	0.002
Glutamate/Glutamine (Glu/Gln)	223.8 (114.4-332.1)	314.0 (211.2-578.1)	0.002
***Leucine/Isoleucine (Leu/Ile)**	60.9 (42.2-84.6)	86.9 (51.5-191.2)	< 0.001
***Methionine (Met)**	3.6 (1.9-8.2)	4.4 (1.3-10.9)	0.326
Ornitine (Orn)	6.7 (4.0-16.8)	11.0 (3.4-34.8)	0.001
***Phenylalanine (Phe)**	15.2 (3.9-29.8)	24.9 (12.4-54.8)	0.001
Proline (Pro)	45.3 (24.4-73.7)	73.9 (34.2-128.5)	0.002
Thyrosine (Thy)	26.4 (14.1-65.2)	32.3 (13.7-80.3)	0.323
***Valine (Val)**	59.8 (29.8-150)	67.8 (10-250)	0.192
Essential amino acids	134 (91-242)	177 (120-426)	0.003
Non essential amino acids	549 (424-852)	781 (465-1805)	0.003
Total amino acids	677 (515-1016)	954 (618-2230)	0.003

Values are expressed as median (range) * Essential amino acids

Figure 1. Total carnitine (μmol/L)

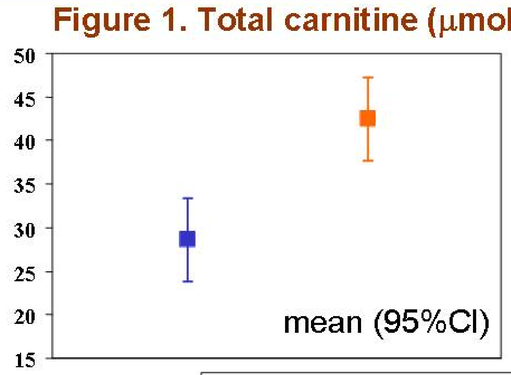
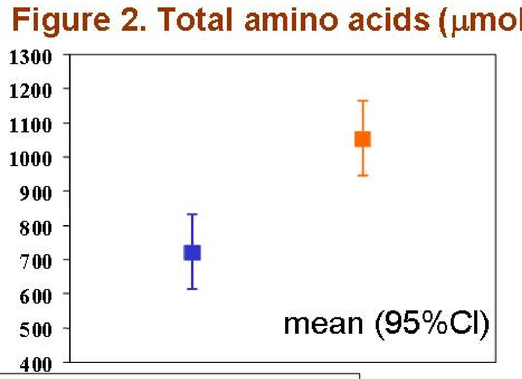


Figure 2. Total amino acids (μmol/L)



Diabetic patients (N=11) Control children (N=44)

Results

Among the 11 diabetic children, 4 were at high HLA genetic risk of developing T1D, 6 were at moderate risk and 1 was at low risk (Table I). The median age at the onset of T1D was 2.7 years, ranging from 1.1 to 3.8 years. No differences were seen in reference to gestational age at birth, birth weight and type of feeding in the first week of life (P=0.438, P=0.408 and P=0.522, respectively). The time elapsing from the collection of blood spots to the assays was comparable in the two groups (P=0.191).

Circulating TC, FC and AC concentrations were significantly lower in diabetic patients compared to controls, but no differences were detected in the AC/FC ratio (Table II, Figure 1). Diabetic patients showed significant lower levels of Ala, Gly, Glu/Gln, Leu/Xle, Orn, Phe and Pro (Table II). Overall, total aminoacid concentrations were significantly lower in diabetic patients than in controls (Table II, Figure 2), even when clustered in essential and non essential subgroups.

Conclusion

We conclude that children who later developed T1D early in life have reduced levels of circulating carnitine and total amino acids soon after birth.

How low carnitine fractions and low amino acid levels relate altogether? And, how these two patterns are linked with T1D? The data available so far do not allow us to definitively answer to these questions. The fact that these alterations are evident in the first days of life, when thymic selection of autoreactive and regulatory T cells is fully active, let us to speculate that mechanisms determining central tolerance are the main candidate for future investigations. However, given the broad influences that both carnitine and amino acids exert directly and indirectly on cellular metabolisms, including pancreatic beta cells, we can not rule out that other mechanisms, including peripheral tolerance, might be involved.

The determination of these compounds is easy to do and is routinely applied in population screenings. The evaluation of carnitine fractions and amino acids in blood dry spots might thus represent an additional tool for prospectively predicting T1D and theoretically open new perspectives for its prevention from birth.