Vitamin D and fatty acids Ω3: role in the "honeymoon" of the Type 1 Diabetes of the child

The weight of diabetes on the child, on the family and in society

Type 1 diabetes (T1D) is an autoimmune disease in which there is a progressive reduction of insulin-secreting β-cells that involves the need to undertake replacement therapy with exogenous insulin.

The therapy consists of the administration of insulin in multiple administrations per day by pen or continuous infusion under the pump and requires frequent monitoring of capillary blood glucose or continuous glycemic monitoring with medical devices. T1D is burdened by important long-term complications: neurological, ocular, renal microangiopathic, which make diabetes the leading cause of chronic renal failure and dialysis, and acquired blindness. In addition, the pathology determines diabetic macroangiopathy, which leads to an increased risk of heart attack, macrovascular damage especially in the lower limbs with consequent diabetic foot and atherosclerotic complications of specific cardiac, cerebral and genital districts. These micro and macro-angiopathic complications, together with the continuous increase in the incidence and prevalence of diabetic pathology, cause a huge social and individual cost that currently represents a significant resource commitment, and will become an unsustainable health care expenditure in the years to come.

Data from the Pediatric Regional Register (0-15 years), reported in Literature, provide a precise assessment of the incidence of T1D in the Piedmont Region: from these data we can see an increase of 3.3% annually, with a consequent increase in direct costs of diabetes (in Italy for the NHS in 2012 direct costs over € 9 billion, 57% for hospital access, 30% for drugs and devices, 13% for outpatient care) and indirect and immaterial, which are even higher, even if hardly quantifiable. There was also a greater increase in incidence in pre-school and immigrant children, especially in relation to the dark color of the skin that limits the vitamin D biosynthesis.

The previous consideration, together with the prevalence of widespread vitamin D deficiency usually detected at the onset of T1D, led to a causal relationship between vitamin D deficiency and the genesis of diabetes. The essential role of this vitamin consists in the immunoregulatory activity on T-lymphocytes. Today, vitamin D supplementation in every child with diabetes, with 1000 IU / day (corresponding to the current indications of the Endocrine Society, IOS and SIPPS), and the monitoring of the actual serum concentration [as 25 (OH) D3] in the range 30-50 ng / ml, is a therapeutic act that is consolidating in the therapy of T1D.

In addition to a genetic condition of polygenic risk, which finds the greatest risk related to specific predisposing HLA alleles, there are no known causes of this autoimmune pathology, ie an event, presumably environmental, conditions the increased incidence of the disease especially in fast-moving countries socio-economic evolution. By logical deduction, genetic predisposition is stable, and the increase in incidence of T1D must be related to environmental factors (pollutants, infections, especially viral, or food changes), since the increase in T1D is observed in countries of all over the world, and in particular in developing countries. There are many possible environmental causes of the increase of T1D.

A vitamin D and omega 3 (Ω-3) deficiency status is reported in the general population, including the pediatric population. The lack of Ω-3 in our power supply could represent an environmental cause or an

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amplifying factor a trigger event. In any case, the ratio between Ω-6 and Ω-3 has shifted from 1: 1 a few decades ago, to a current 10: 1 or 25: 1. In particular, a case-control study of Stene et all. in Norway (2000) showed that the intake of cod liver oil by the mother during pregnancy or during the first year of life was associated with a reduced risk of developing T1D. Stene concluded that vitamin D and long fatty acids omega-3 (Ω-3), eicosapentenoic acid (EPA) and docosahexaenoic acid (DHA) have a protective effect against the development of T1D. In a further study, Stene and Joner (2003) found that dietary supplementation of cod liver oil during the first year of life was significantly associated with a lower risk of developing T1D during childhood (OR 0.7, $p < 0.001$). This is consistent with a protective role played by vitamin D and Ω-3 during pregnancy and early childhood.

Our experience in this regard was initially focused on the case of an 8-year-old boy with T1D who, after the classic onset of the disease, has progressively reduced his insulin needs. To date, at 24 months from the onset, persists a state of excellent metabolic compensation with a minimum and single administration of insulin a day equal to 3 U. To this child, after the diagnosis, once achieved a good metabolic compensation, were administered Ω-3 and vitamin D. This result is considered to be different from the usual course of the disease that entails, a frequent (but not constant) reduction of the insulin requirement (so-called "honeymoon period") to less than 0.5 IU / Kg / day, then followed, invariably by a progressive increase in insulin necessary for the metabolic compensation. The "honeymoon" phenomenon underlies a resumption of the secretive function of the pancreatic tissue residual to the destruction of β-cells mediated by cells infiltrating the pancreas *insulae*, and by a series of tissue cytokines.

Some children with T1DM in the wake of the published anecdotal case, have already spontaneously begun to supplement with omega 3.

At the onset of T1D about 10-30% of β-cellular tissue is still active; the usual increase in insulin needs following onset, corresponds to the further depletion of this residual quota, due to the progression of the inflammatory autoimmune process (insulitis), potentially counterbalanced by administration of immunosuppressive drugs. However, immunosuppressive drugs can not be used in the pediatric age, due to possible side effects, and even in young adults, the research trials have so far not yielded significant results. There are no side effects in the controlled administration of vitamin D, nor in food supplements such as Ω-3 fatty acids.

Usually the honeymoon phenomenon is exhausted within a year, even if a residual amount of secretion persists at least up to two years from the onset. The endogenous insulin secretion is quantifiable as C-peptide, which represents the conjugation peptide between chain A and B of pro-insulin, which after free cleavage in the portal circle molecules of C-peptide and insulin in molar ratio 1: 2. Unlike insulin that is rapidly degraded in the liver and tissues, the C-peptide passes the liver and is filtered by the unchanged renal glomeruli in the urine. C-peptide is not present in exogenous insulin of pharmacological origin. The endogenous insulin secretion is quantifiable as C-peptide. A persistence of C-peptide (also dosages 0.2 ng / ml) reduces the risk of complications, improves the metabolic compensation and reduces the number of hypoglycemic episodes, so at least one of its maintenance would be an important objective to preserve.

The study is preliminary and aimed at identifying whether a therapy with Ω-3 could be of support in T1D especially in the first years of illness, when a persistence of a residual β-cell mass is presumable and it is possible to act better on the pro-inflammatory component (insulitis).

Purpose of the study

The aim of the study is to evaluate whether a controlled administration of vitamin D and Ω-3, in the early years of the onset of T1D, can preserve the residual β-cell mass, evaluable with a reduced insulin need for a long time, with a persistent level of C-peptide, a lower glycemic variability compared to children who do not carry out this supplementation.

Secondary assessment is to perform a baseline assessment of the AA / EPA ratio in children with type 1 diabetes.

Subjects and Methods

The subjects will be recruited at the Diabetology Clinic of the Pediatric Clinic of the AOU "Maggiore della Carità" of Novara.

In particular, two groups of subjects will be included, subject to the signing of the informed consent:

1°,2°,3° the groups, with T1D onset in 2014, 2015, 2016, retrospectively were evaluated as historical controls before 2017.

4 group°: all children with T1D with disease onset in the years 2017, will receive omega 3 supplement (EPA + DHA 60 mg / kg / of), and will be compared from a metabolic point of view (in particular for the needs of insulin and glycated hemoglobin) according to the scheme outlined with the pre-supplementation data, deduced from the medical records retrospectively.

1°,2°,3° groups: alter 2017 were allowed to enter in the study and were prospectively evaluated

supplementation omega-3

↓

Group 1	Group 2	Group 3	Group 4 new onset	
	vs Group 1	vs Group 2	vs Group 3	
		vs Group 1	vs Group 2	

			vs Group 1	
2014	2015	2016	2017	2018

The analysis will allow to quantify the effects of omega-3 at different distance from the onset in terms of metabolic advantage (insulin units / Kg / die, glycated hemoglobin, etc.) and to evaluate if and how much supplementation after the onset may give metabolic advantages compared to other patients not yet supplemented.

To the T0, T3, T6 and T12 will be detected anthropometric parameters (weight, height, BMI, Tanner Stages, PAOS / PAOD), insulin therapy in progress (IU / Kg / die) with MDI (multiple daily injection) or CSII (continuous subcutaneus infusion) and metabolic parameters (C-peptide, coagulation, lipid profile, basal blood sugar, glycated hemoglobin, vitamin D). The C-peptide in particular allows us to evaluate the residual cell function, which provides a "direct" measurement of endogenous insulin secretion. The fasting C-peptide / glycemia ratio will also be evaluated, as an estimate of residual insulin secretion. Glycated hemoglobin is an expression of blood sugar in the last three months. A value of less than 7.5% is a sign of good compensation.

In addition, the glucometer data will be downloaded at each visit to evaluate the glycemic variability parameters (mean glycemia, DS). A DS of less than 50% of the mean blood glucose represents an index of stability. The use by all patients of the same meter, delivered at the time of inclusion in the study, will eliminate the bias given by the diversity of the medical device. Indication will be given to perform pre-meal glycemic controls three times a day, and occasionally after 2 hours from the meal, in order to calculate the glycemic variability.

An initial dosage of the blood levels of AA, EPA and DHA (and relative ratio) will be carried out by capillary sampling and application of a few drops of blood on absorbent paper for all subjects. Fatty acids are measurable in whole blood, with a lipidogram, in which each component of FFA is selectively differentiated, and then quantified the individual fractions. In particular we will evaluate the ratio between 20: 4 Ω-6 arachidonic acid (AA), precursor of pro-inflammatory cytokines, and EPA, precursor of anti-inflammatory cytokines; this ratio represents a balance between the effector pre-metabolites of inflammation, which determine the apoptotic degeneration of β-cells, and the anti-inflammatory ones, which preserve a cellular share with an insulin secretive function. This value AA / EPA <3 is proposed in Literature for adults or patients with other diseases, but has also been used for children.

The measurement will be carried out free of charge at the Department of Pharmacological and Biomolecular Sciences (DiSFEB), Faculty of Pharmaceutical Sciences, University of Milan.

An assessment of the dietary intake of EPA and DHA fatty acids will be carried out for all the subjects included in the study through an interview with the dietician and the compilation of a frequency food questionnaire. The collection of the food questionnaire will take place at the time of enrollment (T0) and 12 months after supplementation with omega 3. All healthy control subjects will be submitted to the food questionnaire. Nutritional indications will be given to all families in order to optimize an optimal dietary introduction of fatty acids as indicated by the LARNs.

Supplementation

The intervention under study is only the administration of omega-3, to all patients with onset in recent years (2014-2018) with a contribution of 60 mg / kg / day of the sum of EPA and DHA (corresponding respectively to acid eicosapentenoico and docaesoenoico). The AA / EPA ratio will be monitored (arachidonic acid, Ω-6 / eicosapentenoic acid Ω-3), to reach the optimal range <3, as indicated in the literature.

The chosen preparation will be omega-3, of extraction from fish, with a high concentration and purified to exclude mercury and lead, commercially available in stores and supermarkets. EnerZona. Equipe Enervit SrL will provide free Ener Zone, in capsules, from EPA 400 mg + DHA 200 mg, or capsules from EPA 200 mg + DHA 100 mg, or liquid solution, for subjects unable to swallow capsules or intolerant gluten capsules themselves; this will allow us to provide precise delivery and the same preparation to each patient.

All of our patients are supplemented with vitamin D. As vitamin D also has an immunomodulatory role it will be monitored in the study, and being frequently low at onset will be given at 1000 IU / day, as indicated in prophylaxis for children and adolescent; its level [as 25 (OH) D] will be controlled to reach the optimal values (established by Endocrine and other scientific societies) of 40-50 ng / ml, and possibly increased or reduced.

Inclusion criteria:

All children with autoimmune T1D, debuted in the years selected at the Pediatric Clinic of Novara. Some children with T1D in the wake of the published anecdotal case, have already spontaneously begun to supplement with omega 3, and will be excluded from the study. We have primarily considered our series of children with T1D onset according to the ADA, ISPAD and SIEDP criteria of the years 2015, 2016 and 2017, since insulin secretive activity persists for at least two years from the onset.

Exclusion criteria:

pathologies such as:

• coagulation defects

• renal diseases (nephrotic syndrome, calculosis, renal cysts, etc.)

• sarcoidosis, histoplasmosis

• endocrinological diseases (hyperparathyroidism)

• tumoral pathologies or tuberculosis

Gluten intolerance (CD) or thyroiditis in T1D are not exclusion criteria.

Chronology of investigations and time of execution of the study

T.0 Signature of informed consent. Baseline auxological and metabolic evaluation (IU / Kg / day exogenous insulin) by means of examination, assessment of dietary intake through food questionnaire and interviews with dietician, blood tests (vitamin D, AA / EPA, C-peptide, basal glycemia, glycated hemoglobin, lipid profile, coagulation). Discharge of glucometer data for glycemic variability. Start supplementation with vitamin D, in case of deficiency, if subject not already supplemented. Begin supplementation with EPA + DHA, in case of deficiency, if willingness to recruit.

T.3 at 3 months from the beginning of the supplementation, only for the subjects supplemented with omega 3. Auxological and metabolic evaluation (IU / Kg / day exogenous insulin) by examination, blood tests (AA / EPA, C-peptide, basal glycemia, glycated hemoglobin, lipid profile, coagulation). Discharge of glucometer data for glycemic variability.

T.6 to 6 months from start of supplementation, only for subjects supplemented with omega 3. Auxologic and metabolic evaluation (IU / kg / exogenous insulin) by examination, blood tests (AA / EPA, C-peptide, basal blood sugar, glycated hemoglobin, lipid profile, coagulation). Discharge of glucometer data for glycemic variability.

T.12 At 12 months from the beginning of the study, will be re-evaluated for all subjects, all the previous auxological, metabolic and biochemical parameters and a new evaluation of the dietetic dike of fatty acids will be carried out.

Study security and caution of the subject included

The safety of the therapy with supplements is reflected in the extensive experience of using cod liver oil which in the past has been a routine supplement for children for several years.

High doses of Ω-3 over 5 g / day could result in a longer bleeding time, which is why coagulation monitoring is required, even if supplementation is required at doses of less than 5 g / day. With the use of Ω-3 a reduction of triglyceride levels is observed, which could represent a clinical advantage for the subject and a precise therapeutic objective often sought by the practitioners. A triglyceride evaluation will be performed pre-supplementation and at 12 months from the beginning of the same.

Financial resources and expected costs

Enervit supplies Ener Zone Omega 3 RX in capsules containing each EPA 400 mg + DHA 200 mg and in liquid formulation for younger children and / or with concomitant celiac disease. Ascensia Diabetes Care supplies Conturnext USB and Conturnext strips, to allow frequent blood glucose measurements, with accuracy and precision.

The blood chemistry tests are part of the normal T1D treatment routine. The dosage of the different metabolic and hormonal parameters at the time T1 will be borne by the Pediatric Clinic. The AA / EPA report will be determined by the Department of Pharmacological and Biomolecular Sciences DiSFEB, Faculty of Pharmaceutical Sciences of the University of Milan free of charge. Other possible findings as coagulation will be calculated as expenditure "study use" of the Pediatric Clinic.

DATA PROTECTION

to. Blood samples collected on Guthrie cardboard sent to Milan will be collected with the informed consent of the parents.

b. For the protection of the subject and clinical data, withdrawals will be identified with a code.

c. The clinical data will be managed by clinical referents inside Pediatrics (Dr. F. Cadario, Dr. S. Savastio) and the name of the subject will be identified by initial name and surname

d. The withdrawals will be stored in the Laboratory of Milan Pharmacy University of the Maggiore Hospital under the exclusive control of the laboratory referents (Dr. Ssa Rizzo A.M.)

Statistic analysis

The data will be expressed as a mean ± SD. For continuous variables, the variations between the groups are compared by means of the averages calculated with the non parametric Wilcoxon and Mann-Whitney U tests. The variability of the biochemical parameters over time will be evaluated by means of tests for repeated measurements. A correlation analysis will be performed with the Pearson correlation test, after adequate logarithmic transformation of the parameters when necessary. Multiple regression analysis will be used to evaluate associations between variables. Statistical significance will be assumed for values of $p < 0.05$. All statistical analyzes will be performed with the SPSS program for Windows version 22.0 (SPSS INC; Chicago, IL, USA).