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Combination high-dose omega-3 fatty acids and high-dose cholecalciferol in new onset type 1 diabetes: a potential role in preservation of beta-cell mass

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Abstract. – Several studies have evaluated the role of inflammation in type 1 diabetes (T1D). The safety profile and anti-inflammatory properties of high dose omega-3 fatty acids combined with Vitamin D supplementation make this therapy a possible candidate for T1D intervention trials. Herein, we describe the case of a 14-year-old boy with new onset T1D treated with high dose Omega-3 and vitamin D_3. By 12 months, peak C-peptide increased to 0.55 nmol/L (1.66 ng/mL) corresponding to a 20% increment from baseline and AUC C-peptide was slightly higher compared to 9 months (0.33 vs. 0.30 nmol/L/min) although remaining slightly lower than baseline. Combination high-dose Omega-3 fatty acids and high-dose vitamin D_3 therapy was well tolerated and may have beneficial effects on beta-cell function. Randomized controlled trials could be of assistance to determine whether this therapy may result in the preservation of beta-cell function in patients with new onset T1D.

Key Words
Diabetes, Omega-3, Fatty acids, Inflammation, Vitamin D, Autoimmunity

Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by a progressive loss of pancreatic beta-cell mass ultimately leading to hyperglycemia and the need for exogenous insulin therapy[1]. Several clinical trials in patients with new onset T1D have tested a variety of immunomodulatory therapies aimed at preventing or delaying disease progression[2]. Although many interventions have not shown a clinical benefit, a few have shown promise[3-6] and several others are currently being tested[7]. Notably, the participation of children in prevention trials is typically limited by the toxicity profile of the therapeutic agents being studied. Thus, the identification of potentially effective "safe therapies" is of particular interest as it would allow for the broader inclusion of children in prevention trials.

Several studies have evaluated the role of inflammation in type 1 diabetes[8-10] and therapies aimed at blocking inflammatory cytokines have been tested in T1D intervention trials[11,12]. The anti-inflammatory properties of omega-3 long-chain polyunsaturated fatty acids (LCPUFA) [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] have been extensively reported in the literature[13-15]. Dietary supplementation with long chain omega-3 fatty acids has shown to suppress synthesis of interleukin-1β (IL-1 β) and tumor necrosis factor (TNF)[16] both of which have been implicated in beta-cell death[11,12]. Most importantly, the safety of omega-3 supplementation has been shown in children[17,18]. Also, the anti-inflammatory properties of vitamin D are well established. In particular, it has been shown that Vitamin D prevents both insulitis and type 1 diabetes mellitus in mouse models of T1D and retrospective studies have shown apparent beneficial effects of vitamin D supplementation in early life on successive lifetime risk of T1DM. Thus, the safety profile and anti-inflammatory properties of high

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dose omega-3 fatty acids combined with Vitamin D supplementation make this therapy a possible candidate for T1D intervention trials.

Herein, the case of a boy with new onset T1D who was started on high dose Omega-3 fatty acids and vitamin D_3 post-diagnosis is reported and data on glycemic control and measures of beta-cell function during the first 15 months post-diagnosis are provided.

Case description

A 14-year-old white boy, professional skier, with no past medical history presented with a few days of weakness, polydipsia, polyuria and weight loss of 3.0 kg. A urinalysis revealed 3+ ketones and glycosuria of 1,000 mg/dL. Blood tests were remarkable for glucose of 660 mg/dL and pH of 7.2. He was admitted to a local hospital with a diagnosis of diabetic ketoacidosis and treated with intravenous insulin as per hospital protocol. On admission, he weighed 50 kg and measured 5'7" (BMI 17.3). Additional testing showed a glycated hemoglobin (HbA1c) of 12.8% (116 mmol/mol) and C-peptide <0.1 nmol/L (<0.3 ng/mL). GAD65, IA-2, and islet cell antibodies (ICA) were positive. Following a 6 day hospitalization, weight increased to 52.6 kg and he was discharged on multiple daily insulin injections with Lantus® (glargine) at bedtime as basal insulin and Apidra® (glulisine) for mealtime coverage.

One week after discharge, mean daily capillary blood glucose was 109±36 mg/dL (n=8). Nine days post-diagnosis, after obtaining informed consent from parents and assent from patient, he was started on ultra-refined omega-3 EPA/DHEA concentrate (EnerZona® Omega 3 Rx; EPA 400 mg + DHA 200 mg per 1 g capsule) using an EPA dose of 800 mg (2 capsules) given 3 times daily for a total daily dose (TDD) of EPA 2,400 mg (6 capsules) aimed to achieve a target AA/EPA ratio between 1.0-2.0. The AA/EPA ratio was derived from the fatty acid profile obtained from a finger stick drop of blood which was analyzed by gas chromatography using dried blood spot testing.

In addition, he was noted to be vitamin D deficient (vitamin D 25-OH was 16 ng/mL) and was started on cholecalciferol 25,000 units PO weekly 9 days post-diagnosis.

To assess the impact of high dose omega-3 therapy intervention on beta-cell function, 2 hr mixed meal tolerance tests (MMTT) were performed at 2 (baseline), 4, 6, 9 and 12 months post-diagnosis (most recent follow-up). Meritene®Drink, NestlèHealthScience, was used at a dose of 6 ml/kg (maximum 360 mL). Rapid acting insulin was held on the morning of the test and the reported mean fasting glucose for samples obtained prior to each MMTT was 112±3 mg/dL. Fasting, peak, and area under the curve (AUC) C-peptide were analyzed and rate of decline assessed by evaluating the slope of the regression line of each variable using linear regression (GraphPad Prism version 6, GraphPad Software Inc., San Diego, CA, USA).

By one month post-diagnosis HbA1c had improved to 7.6% (60 mmol/mol), insulin total daily dose (TDD) was 11 units (0.2 units/kg/day), and fasting C-peptide was 0.26 nmol/L (0.78 ng/mL). At 2 months, fasting C-peptide was 0.20 nmol/L (0.6 ng/mL), peak C-peptide 0.46 nmol/L (1.4 ng/mL) and AUC C-peptide 0.39 nmol/L/min. By 3 months post diagnosis, HbA1c had decreased to 6.0% (42 mmol/mol). A modest decline in C-peptide was observed up to 6 months followed by an improvement at 9 months with values for fasting, peak, and AUC increasing by 63.6%, 9.6%, and 15.4%, respectively (Table I).

By 12 months, peak C-peptide increased to 0.55 nmol/L (1.66 ng/mL) corresponding to a 20% increment from baseline and AUC C-peptide was slightly higher compared to 9 months

Table I. C-peptide variables derived from serial 2 hr MMTT.

Months post diagnosis	C-peptide variables from 2 hr MMTT		
	Fasting (nmol/L)*	90 min (nmol/L)*	AUC (nmol/L/min)*
2	0.20	0.43	0.39
4	0.14	0.34	0.30
6	0.11	0.30	0.26
9	0.18	0.31	0.30
12	0.05	0.55	0.33

*Divide units in nmol/L by 0.333 to convert to ng/mL.

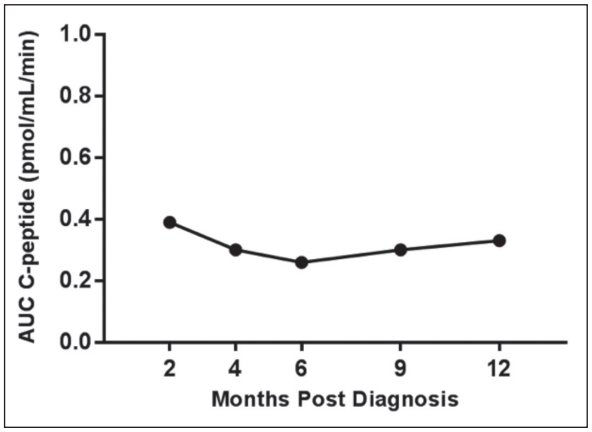

Figure 1. Decline in C-peptide area under the curve for the first 12 months following diagnosis of type 1 diabetes.

(0.33 *vs.* 0.30 nmol/L/min) although remaining slightly lower than baseline. Fasting glucose and C-peptide were lower at one year compared to baseline (78 mg/dL *vs.* 119 mg/dL and 0.05 nmol/L [0.16 ng/mL] *vs.* 0.20 nmol/L [0.6 ng/mL], respectively). At 15 months, HbA1c was 6.7% (50 mmol/mol), BW and height increased to 66 kg and 5'9.3" (BMI 21.3), and insulin TDD was 19 units (0.29 units/kg/day).

At 12 weeks post-diagnosis the AA/EPA ratio was 2.3 on 3,600 mg EPA TDD. At 4 months, a ratio of 1.75 was achieved remaining between 1.7-1.9 during the first year on 4,800 mg EPA TDD (12 capsules) and increasing to 2.86 by 15 months.

By 3 months, 25OH vitamin D levels were up to 41.9 ng/mL and remained within normal limits at 12 months (32.2 ng/mL) and 15 months (34.2 ng/mL).

The rate of decline over 12 months for fasting C-peptide was -0.0102 ± 0.0062 (95% CI -0.0302 ± 0.0097), for peak C-peptide 0.0078 ± 0.0134 (95% CI -0.0347 to 0.0504), and for AUC C-peptide -0.0036 ± 0.0067 nmol/L/month (95% CI -0.0249 to 0.0177) (Figure 1).

Discussion and literature review

The patient reported herein demonstrated a slow rate of decline in C-peptide during the first year with a higher stimulated C-peptide by 12 months compared to baseline. In addition, the C-peptide rate of decline is slower than observed in placebo groups from intervention studies[19]. However, the reported decline in stimulated C-peptide during the first year following diagnosis of T1D is highly variable with some investigators having reported reductions of up to 58% whereas others showing no change or even an increase compared to baseline[20]. Nonetheless, the degree of stability in beta-cell function demonstrated by our patient and the improvement in stimulated C-peptide are encouraging.

Derivatives from omega-3 LCPUFA have been associated with marked anti-inflammatory effects whereas arachidonic acid (AA), an omega-6 LCPUFA, is the precursor of eicosanoids associated with pro-inflammatory properties[15,21]. Thus, a high AA/EPA ratio would suggest that the balance is shifted towards a pro-inflammatory state. Treatment with omega-3 fatty acids aimed at lowering the AA/EPA ratio may then prove to be beneficial particularly in diseases associated with a high inflammatory sate.

The bioactive mediators resolvins and protectins which are derived from omega-3 fatty acids have been postulated as the molecular basis for the anti-inflammatory properties of omega-3 fatty acids[22]. These mediators act by several mechanisms including reduction of neutrophil infiltration, attenuation of TNF-stimulated NF-κB activation, inhibition of IL-1β production, reduction in TNF and interferon-γ secretion, inhibition of T-cell migration and regulation of T-cell apoptosis.

Clinical studies have shown that high dose administration of omega-3 LCPUFA may result in clinical benefits in cardiovascular disease and depression[23,24]. The JELIS study demonstrated that long-term intake of high dose EPA led to a reduction in major coronary events in Japanese hypercholesterolemic patients treated with statins[24]. The AA/EPA ratio was 1.6 at baseline and decreased to 0.8 at 5 years.

Bellenger et al reported that transgenic fat-1 mice, a model of endogenous omega-3 LCPUFA synthesis, were protected against streptozotocin-induced diabetes[25]. This protection was associated with a reduction in pro-inflammatory cytokines with a concomitant increase in anti-inflammatory cytokines.

The Diabetes Autoimmunity Study in the Young (DAISY) conducted in children at high risk for T1D showed that dietary intake of omega-3 fatty acids was associated with a reduced risk of islet autoimmunity but not with conversion to T1D[18,26]. However, the study was not designed to adjust dietary intake of omega-3 fatty acids to target specific AA/EPA ratios and it is unclear whether reaching specific ratios may translate into improved clinical outcomes. A pilot study in established T1D (>4 years duration) evaluating the impact of omega-3

fatty acids on glycemic control failed to show an improvement after 4 weeks of therapy[27]. This may suggest that anti-inflammatory therapies may need to be introduced early in the course of the disease in order to have an impact on prevention of beta-cell loss. In addition, considering that T1D is a chronic autoimmune and inflammatory disease, anti-inflammatory strategies may need to be administered over a prolonged period of time in order to observe a clinical benefit.

The AA/EPA ratio observed at study entry in Japanese participants from the JELIS study was 1.6. This low ratio is not unexpected as in Japan the average fish consumption is one serving of 85 g (3 oz; 900 mg EPA and DHA) per day and 90% of individuals eat fish at least once a week[28]. Considering that high dietary intake can result in AA/EPA ratios below 2.0 as observed in this Japanese population, we chose to aim for AA/EPA ratio between 1.0 and 2.0. However, this is an arbitrary range as specific AA/EPA ratios associated with improved clinical outcomes have not been documented in the literature.

Vitamin D has been linked to several immunomodulatory effects. Studies[29,30] in new onset type 1 diabetes have shown that treatment with cholecalciferol (vitamin D_3) results in both an increase in the percentage and the suppressive capacity of regulatory T-cells. A recent study[31] in adults with vitamin D deficiency showed that daily treatment with 4,000 IU of vitamin D_3 significantly reduced CD4+ T-cell activation compared to treatment with 400 units of vitamin D_3. However, the role of vitamin D in the preservation of beta-cell function in new onset T1D is controversial. Two studies showed no significant effect of therapy with calcitriol ($1,25(OH)_2D_3$) whereas one study using 2,000 IU of cholecalciferol daily showed a higher stimulated C-peptide at 18 months compared to placebo[30].

We hypothesize that combination therapy with high-dose omega 3 LCPUFA, aimed at maintaining narrow AA/EPA ratios, and high-dose cholecalciferol at T1D onset may have resulted in a significant decrease in the pancreatic beta-cell inflammatory response leading to preservation of beta-cell mass and inhibition of adaptive and cell-mediated immune system responses. Our patient was treated with high-dose omega-3 LCPUFA and achieved the target AA/EPA ratio by 4 months which was maintained throughout and his vitamin D deficiency was promptly corrected and he was maintained on high dose vitamin D_3 supplementation. Most importantly, the therapy was well tolerated without adverse events. Cytokine profiles were not monitored in this subject, but it could be useful to assess biochemical changes in inflammatory markers in follow-up studies.

Although we cannot establish a cause-effect association, the results observed in this subject are encouraging showing preservation of stimulated C-peptide at one year. Longer follow-up and randomized controlled trials with additional mechanistic studies, including assessment of cytokine profiles and other inflammatory markers, may be of assistance to further determine the effect of the proposed combination for the preservation of beta-cell mass and function in new onset T1D.

Conclusions

Use of combination high-dose Omega-3 fatty acids and high-dose vitamin D_3 therapy was well tolerated and may have beneficial effects on beta-cell function. Randomized controlled trials are required to determine whether this therapy may result in the preservation of beta-cell function in patients with new onset T1D and thus suggest that anti-inflammatory strategies may be of assistance in intervention trials using combination therapies to reverse autoimmunity.

Conflict of Interests

Camillo Ricordi is a member of the Scientific Advisory Board of Zone Labs.

The other Authors declare that they have no conflict of interests.

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