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Favorable effects of omega-3 PUFAs on glucose control in an adolescent with type 1 diabetes using continuous glucose monitoring: a case report

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Abstract

Omega-3 polyunsaturated fatty acids (PUFAs) represent valuable dietary adjuncts for subjects with hypertriglyceridemia and at increased risk of cardiovascular disease, including subjects with type 1 diabetes (T1D). However, the exact role of omega-3 PUFAs in the regulation of glucose homeostasis and chronic complications in patients with diabetes remains uncertain. Herein, we report a 3-year follow-up of an athlete adolescent with T1D who started to use continuous glucose monitoring (CGM) two years after the diagnosis, and initiated omega-3 PUFA supplementation four years after the clinical onset of T1D. CGM metrics related to a 18-month period of omega-3 PUFA supplementation (50 mg/Kg/day of EPA and DHA) was compared to CGM metrics referring to a 18-month period prior to the nutrition intervention. Omega-3 PUFA supplementation resulted in a significant reduction of mean glucose and glucose variability, along with a significant increase in Time in range (TIR) 70-180 mg/dL. No significant changes were observed in daily insulin requirements between the two study periods. Future studies will be of assistance to better understand the exact impact of omega-3 PUFAs on the regulation of glucose homeostasis in patients with type 1 and its complications.

Abbreviations

ABG: Arterial blood gas analysis; AA: Arachidonic acid; AA/EPA ratio: Arachidonic acid/Eicosapentaenoic acid ratio; BE: Base excess; BMI: Body mass index; CGM: Continuous glucose monitoring; CV: Coefficient of variation; DKA: Diabetic ketoacidosis; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; HbA1c: glycated hemoglobin; HDL cholesterol: High Density Lipoprotein cholesterol; LDL cholesterol: Low Density Lipoprotein cholesterol; MDI: Multiple daily injections; Omega-3 PUFAs: Omega-3 polyunsaturated fatty acids; Omega-6 PUFAs: Omega-6 polyunsaturated fatty acids; SD: Standard deviation; SPMs: Specialized pro-resolving mediators; T1D: Type 1 diabetes; T2D: Type 2 diabetes; TAR: Time above range; TBR: Time below range; TIR: Time in range

Introduction

Type 1 diabetes (T1D) is an organ-specific autoimmune disease, which results from the immune-mediated destruction of insulin-producing pancre-

atic beta cells. Global prevalence and incidence of T1D are progressively rising, with the disease representing 5-10% of diabetes cases worldwide[1-4]. These epidemiological trends cannot be explained by genetic predisposition alone and may depend on parallel changes in environmental risk factors[5]. Although much progress has been made in T1D research during the last decade, a biological cure for this disease is still not available[6,7]. However, the past 5-10 years have witnessed tremendous advances in technology used for treatment of subjects with T1D[6], which paved the way for the advent of novel indicators of glucose control, namely: i) time in range (TIR), defined as the proportion of time that a person spends within a desired target glucose range (usually 70-180 mg/dL); ii) time below range (TBR), defined as the proportion of time spent below a given target glucose range (usually <70 mg/dL or <54 mg/dL); and iii) time above range (TAR), defined as the proportion of time spent above a given target glucose range (usually >180 mg/dL and >250 mg/dL)[8,9]. A tighter time in range 70-140 mg/dl (TIR 70-140 mg/dL) which is closer to normal glucose values, is considered as an additional CGM metric indicative of a near normal glucose control[8,9]. The primary goal for effective glucose control is to increase TIR and reduce TAR and TBR in order to minimize the number and frequency of hyperglycemic and hypoglycemic episodes, respectively[9]. For the majority of patients with T1D, a TIR 70-180 mg/dL >70%, a TBR <70 mg/dL <4%, a TBR <54 mg/dL <1%, a TAR >180 mg/dL <25%, and a TAR >250 mg/dL <5% have been established as the recommended targets[9]. However, for age <25 years, if the HbA1c goal is 7.5%, a less stringent TIR 70-180 mg/dl (approximately 60%) is recommended[9]. The International Consensus on TIR also recommends a CGM use of at least 14 days (the percentage of time CGM should be active during this period is at least 70%) and a coefficient of variation (CV) - an indicator of glycemic variability - of ≤36%[9]. Besides allowing patients to better tailor their insulin therapy and evaluate patterns, trends and percentages of time spent in or out of glucose targets, the use of continuous glucose monitoring (CGM) has been associated with improved glucose control and reduced time spent in hyperglycemia or hypoglycemia among children, adolescents and adults with T1D[10,11]. Importantly, the good correlation between glycated hemoglobin (HbA1c) and TIR may allow for the transition to TIR as the preferred indicator of the risk of diabetes complications[12], although further prospective studies are needed in this area. In addition, the use of CGM metrics as novel indicators of glucose control is more advantageous compared to HbA1c measurement, allowing for a better detection of glucose excursions and daily glucose patterns, which can help patients to make quick therapy decisions and lifestyle modifications. On the other hand, HbA1c has a number of limitations in clinical practice, including the lack of information about glucose excursions and the acute complications of hyperglycaemic and hypoglycaemic episodes[9]. HbA1c measurement can also be confounded by several diseases, such as anemia, iron deficiency, haemoglobinopathies and pregnancy[9,13].

Although insulin remains the mainstay of therapy for people with T1D, healthy dietary patterns play an important role in the management of glucose control and in the reduction of cardiovascular risk. Indeed, patients with T1D may more effectively achieve near-normal glucose levels and reduce the risk of micro- and macro-vascular complications by coordinating insulin therapy, diet, and physical activity[14,15]. In this context, omega-3 polyunsaturated fatty acids (PUFAs) represent valuable dietary adjuncts for subjects at risk of cardiovascular disease - including T1D patients[16]- in light of their well-established cardio-protective and triglyceride-lowering properties[17,18]. Indeed, omega-3 PUFAs can be safely administered in children with diabetes and hypertriglyceridemia[19,20]. Herein, we report the 36-month follow-up CGM data of an athlete adolescent with T1D who started to use CGM shortly after the onset of the disease, and initiated omega-3 PUFA supplementation approximately 4 years after the clinical onset of T1D. We compared data on CGM metrics (including those obtained during physical activity) and serum lipid profile between two 18 month-periods, before and after the initiation of omega-3 PUFA supplementation.

Case Report

The patient is a 16-year-old female with no family history of diabetes. At the age of 9 years and 4 months, she started to experience common symptoms of diabetic ketoacidosis (DKA), including polyuria, weight loss, drowsiness, confusion and vomiting, thus requiring hospitalization. Upon hospital admission, blood tests showed hyperglycemia (648 mg/dL) and arterial blood gas (ABG) analysis confirmed the presence of a severe DKA (pH 6.8,

PCO_2 13 mmHg, BE -3 mEq/L, K^+ 3.1 mEq/L, Na^+ 125 mEq/L). At admission, height was 126 cm and body weight was 18 Kg, which corresponded to the 10th and the 5th Italian percentiles during childhood, respectively. After a proper management and resolution of DKA, the patient started multiple daily injection (MDI) insulin therapy and was then discharged after seven days. The patient was diagnosed with T1D diagnosis, according to the *American Diabetes Association* criteria[21].

Blood tests also revealed fasting serum C-peptide levels of <0.1 ng/ml (<0.03 nmol/L), along with a HbA1c of 14.4% (134 mmol/mol). Autoantibodies against the transglutaminase 2 (TG2) were also detected, indicating the coexistence of celiac disease, ultimately confirmed by duodenal biopsy. Therefore, a gluten-free diet was prescribed shortly after the onset of diabetes, in combination with a Mediterranean dietary pattern, and according to the International Society for Pediatric and Adolescent Guidelines[22,23]. Thyroid autoantibody levels were found within the normal range, and laboratory findings did not reveal the presence of any other T1D-associated autoimmune disease. HLA genotyping revealed the presence of HLA alleles DQ2-DR3 (DQA1* 05:01, DQB1* 02:01, DRB1* 03).

After discharge, improved glucose control and regular psychophysical development were achieved (Figure 1). The patient initiated continuous glucose monitoring (GCM) in May 2015 through the use of the subcutaneous glucose sensor Enlite®, while remaining on MDI therapy. In June 2019, the sensor Enlite® was replaced with Dexcom G6®. The girl showed normal physical and mental growth, along with an excellent intellectual and academic performance. The menarche occurred at the age of 14. The patient was also regularly engaged (with

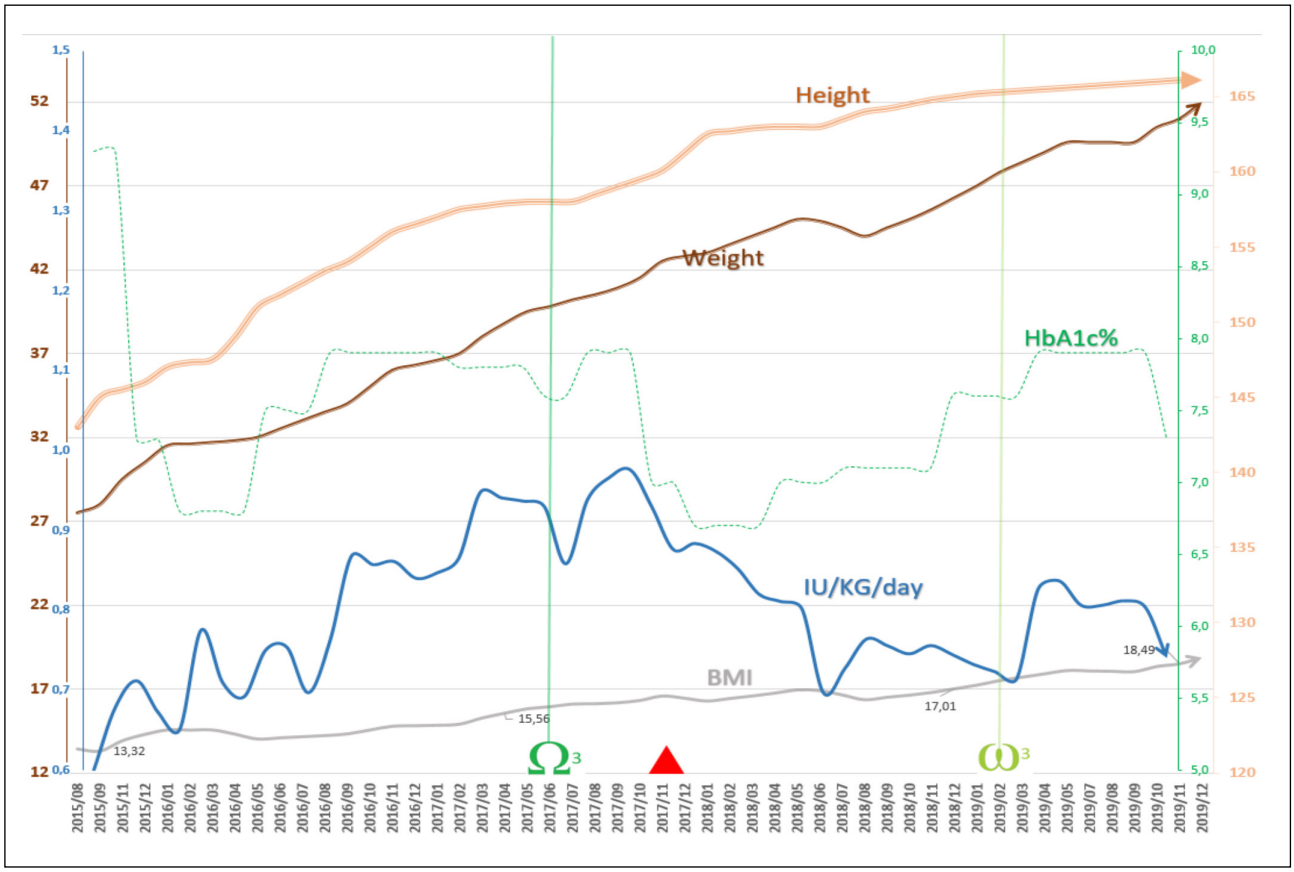

Figure 1. Anthropometric and metabolic parameters during the follow-up period. The blue line represents the daily insulin requirements (expressed as IU/Kg/day), which slightly decreased after the initiation of omega-3 PUFA supplementation. On the other hand, BMI (Kg/m^2; gray line), height (cm; orange line) and body weight (Kg; brown line) increased. The green line indicates the average HbA1c (expressed as percentage). The symbol Ω indicates the initiation of omega-3 PUFA supplementation, whereas the symbol ω indicates the intentional reduction of omega-3 PUFA daily dose. The red triangle represents the occurrence of menarche.

good performance) in physical activity, which consisted of athletic and gym activities for an average of 5 hours per week. Occasionally, physical activity consisted of high-intensity exercise, such as hiking and cycling (discussed later).

In 2015, the patient was also diagnosed with hypovitaminosis D (as evidenced by serum 25-hydroxyvitamin D levels of 28 ng/mL) and subsequently initiated vitamin D supplementation with vitamin D3 (cholecalciferol) at a dose of 1,000 IU/day. In June 2017, we also performed blood analysis to assess the balance between omega-3 PUFAs and omega-6 PUFAs, using AA/EPA ratio (arachidonic acid/eicosapentaenoic acid ratio, a surrogate marker of omega-6/omega-3 ratio), as previously described[24]. AA/EPA ratio values were 14.195, which are deemed to be outside the optimal range (1.5-3)[24,25]. Therefore, an omega-3 PUFA supplementation with ultra-refined fish oil (EnerZona RX Omega 3 Liquid®, containing EPA plus docosahexaenoic acid [DHA] in a ratio of 2:1) was started at a dose of 50 mg/Kg/day. In January 2018, AA/EPA ratio values decreased and reached the optimal range (2.27). Since February 2019, the omega-3 PUFA dose was intentionally reduced by the patient, and then, on January 2020 resumed in previous dosage. No side effects potentially related to omega-3 PUFA supplementation have been reported during the entire follow-up period. Coagulation testing during omega-3 PUFA supplementation showed normal values. Serum lipid profile (performed annually) showed normal values before and during the initial omega-3 PUFA supplementation, while triglycerides increased after the reduction of omega-3 PUFA administered dose (Table 1).

Figure 1 illustrates the anthropometric and metabolic parameters during the follow-up period, whereas Figure 2 illustrates average glucose data during the same period. After 24 months of omega-3 PUFA supplementation, serum levels of fasting C-peptide remained unchanged from baseline (0.13 ng/mL; 0.04 nmol/L).

Materials and Methods

We found 344,020 valid subcutaneous glucose data from CGM; each one was considered eligible up to a maximum of 5 minutes. Measurements shorter than 5 minutes were predominant (>98%).

The data covered an average of 86.1%±5.2 of the whole CGM use period. Missing data (13.9%) were due to fault detection in CGM sensor and time required for glucose sensor replacement. Since July 2015, CGM data have been continuously recorded.

Omega-3 PUFA supplementation was started in July 2017. Therefore, we compared the 18-month period before the initiation of omega-3 PUFA supplementation (from January 2016 to June 2017) and the 18-month period after the initiation of omega-3 PUFA supplementation (from July 2017 to January 2019). Glucose data were recorded by CGM through the use of subcutaneous glucose sensor Enlite® (July 2015 through June 2019) and Dexcom G6® (from July 2019 onwards)[26]. CGM data have been evaluated according to the recommen-

Table 1. Serum lipid profile during the entire follow-up.

Date	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol° (mg/dL)	Triglycerides (mg/dL)	AA/EPA ratio*
March 2015	164	64	91	46	na
February 2017	200	76	116	42	na
May 2017	216	69	na	na	14.19
August 2017	223	71	137	76	na
January 2018	218	75	133	49	2.27
May 2018	217	na	na	57	na
May 2019	225	58	128	212	na
January2020	206	74	122	48	3.40

Table legend: °LDL-cholesterol calculated with the Friedewald's formula: LDL-cholesterol (mg/dL) = total cholesterol (mg/dL) – [HDL-cholesterol (mg/dL) + triglycerides (mg/dL) / 5). Abbreviations: AA/EPA ratio, arachidonic acid/ eicosapentaenoic acid ratio; na, not available.

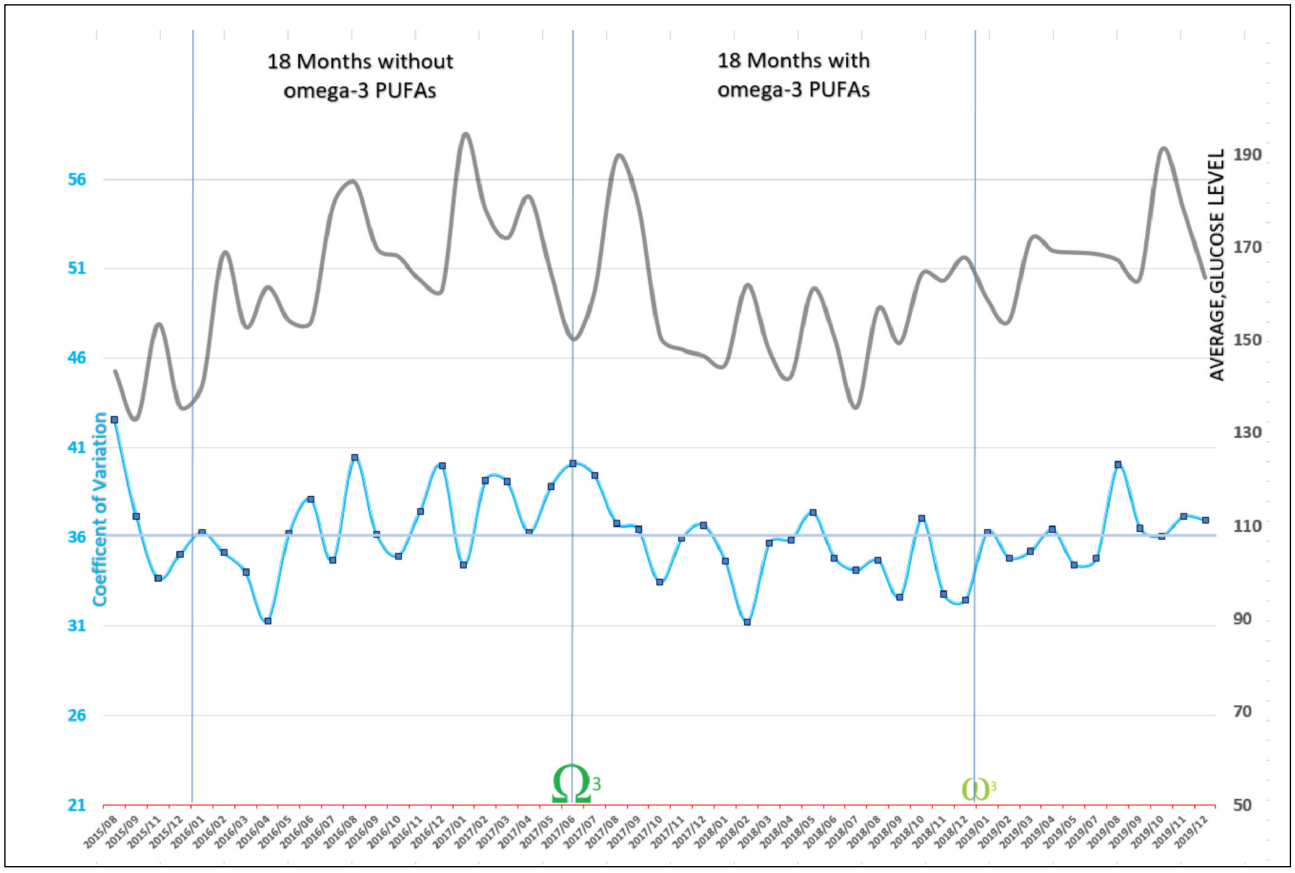

Figure 2. Average glucose levels and CV during the entire follow-up. The mean monthly glucose (mg/dL) level is represented by a gray line, whereas monthly coefficient of variation (CV) is represented by a blue line.

dations from the international consensus on time in range (TIR)[9]. We evaluated the following CGM metrics: i) standard deviation (SD), ii) coefficient of variation (CV, expressed as percentage), iii) mean glucose, iv) TIR 70-180 mg/dL [time spent within glucose range 70-180 mg/dL (3.9-10.0 mmol/L), expressed as percentage], v) TIR 70-140 mg/dL [time spent within the tighter glucose range 70-140 mg/dL (3.9-7.8 mmol/L), expressed as percentage], vi) TBR <70 mg/dL [time spent below glucose threshold of <70 mg/dL (<3.9 mmol/L), expressed as percentage], vii) TAR >180 mg/dL [time spent above glucose threshold of >180 mg/dL (>10.0 mmol/L), expressed as percentage], viii) TAR >250 mg/dL [time spent above glucose threshold of >250 mg/dL (>13.9 mmol/L), expressed as percentage]. Confirmatory blood glucose testing by glucometer (Contour® next USB) was performed upon detection of CGM glucose values of ≤70 mg/dL (≤3.9 mmol/L) (Table 2; Figures 2-3). Values of mean glucose, SD and CV were evaluated and compared before and after the initiation of omega-3 PUFA supplementation. CV was calculated according to the following formula: CV = SD/ mean glucose[27]. The amplitude and timing of glucose variability (as assessed by CV) was investigated through a Poincaré plot[27]. All mean hourly glucose values were depicted in a graph, on x axis n., and on y axis n. + 1. The difference (y – x) of the coordinates of each data point represents the glucose change between times ti and ti – 1, setting time to 1 hour[27]. The dispersion of data around the 45 ° axis indicates faster glucose fluctuations.

Whole blood fatty acid composition to determine AA/EPA ratio was measured as we previously described[24]. Anthropometric parameters (body weight, height), HbA1c (%), and daily insulin requirements (IU/Kg/day) were evaluated during the entire follow-up period. Data were elaborated trough Microsoft Visual Studio and graphics were derived from Microsoft Excel. Written informed consent was given by the patient and her parents for permission to publish this case report.

Table 2. Comparison of metabolic parameters and CGM metrics between the two study 18-month periods (before and after the initiation of omega-3 PUFA supplementation). Data are expressed as mean ± SD.

	Without omega-3 PUFAs	With omega-3 PUFAs	*p*-value
Height (cm)	153.7 ± 3.9	162. ± 2.2	<0.0001
Weight (Kg)	34 ± 2.9	43.5 ± 1.7	<0.0001
BMI (Kg/m^2)	14.7 ±0.57	16.5 ± 0.27	<0.0001
HbA1c%	7.55±0.43	7.14±0.40	0.05
Insulin requirements (IU/Kg/day)	0.81±0.09	0.83±0.84	0.63
Mean glucose	166.2±36.5	156.2±30.4	<0.0001
CV	36.8±2.5	35.1±2.07	0.07
TBR < 70 mg/dL	1.83±5.5	2.03±3.7	0.18
TIR 70-180 mg/dL	60.78±23.5	67.68±21.4	<0.0001
TIR 70-140 mg/dL	38.23 ± 22.5	42.44 ± 23.4	<0.01
TAR 180-250 mg/dL	26.52±16.7	23.75±18	<0.01
TAR >250 mg/dL	10.87±15.2	6.54±10.58	<0.0001

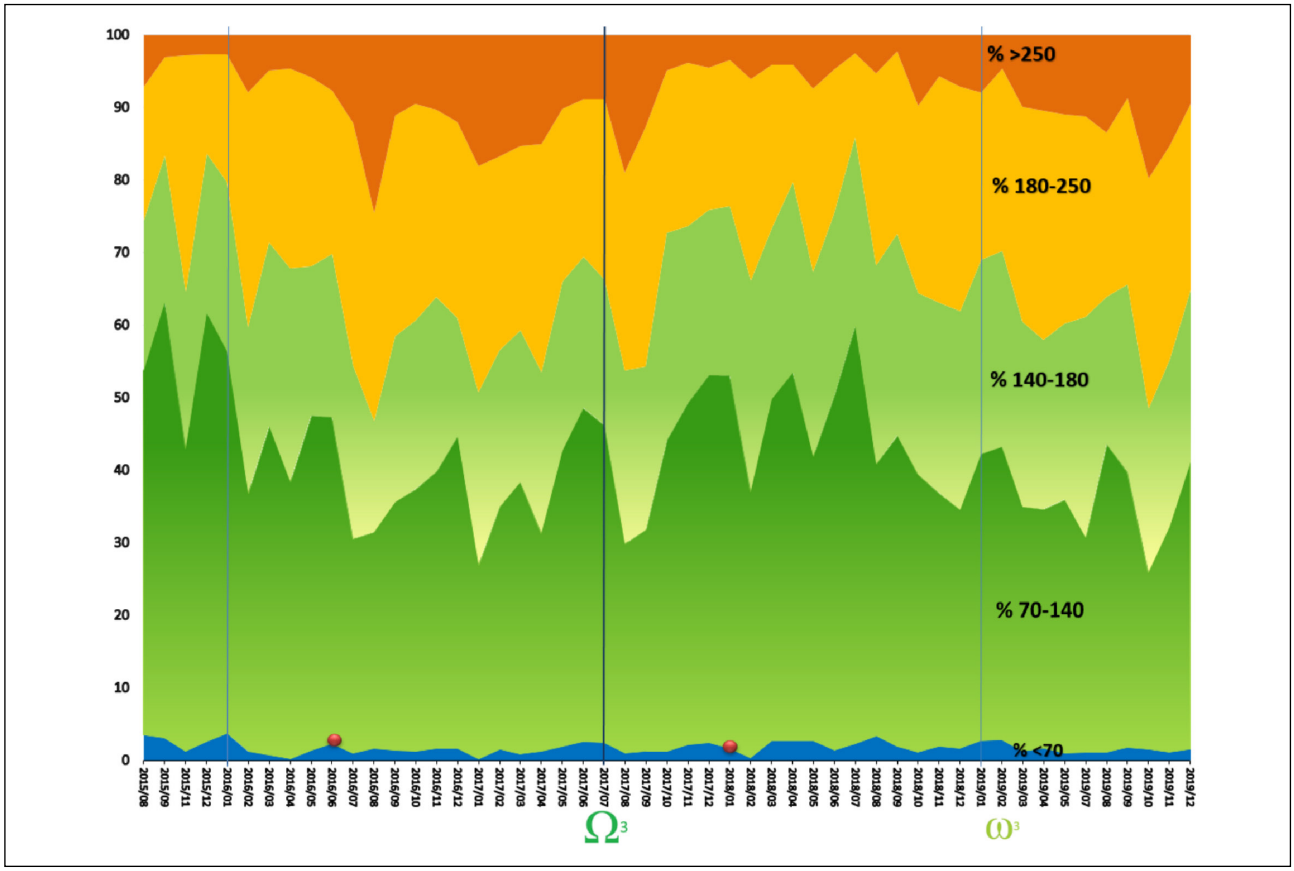

Figure 3. CGM metrics during the entire follow-up period. Different colored areas represent: Time below range (TBR) <70 mg/dL, Time in range (TIR) 70-140 mg/dL, and TIR 140-180 mg/dL, Time above range (TAR) >180 mg/dL, and TAR ≥250 mg/dL. Glucose levels ≤54 mg/dL were confirmed through blood glucose testing by glucometer (represented by the red circles). Vertical lines separate the two 18-month study periods. The start of omega-3 PUFA supplementation is indicated by the symbol Ω, while the symbol ω represents the time point of the intentional reduction of omega-3 PUFA daily dose.

Statistical analysis

Data were expressed as mean ± SD, or as percentages. The assessment of variability in metabolic parameters and CGM metrics before and after the initiation of omega-3 PUFA supplementation was performed through repeated measures ANOVA. Statistical significance was expressed as a *p*-value <0.05. All statistical analyses were performed using SPSS for Windows, version 22.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA).

Results

As we previously mentioned, we compared two different, consecutive18-month periods, namely: i) the 18-month period before the initiation of omega-3 PUFA supplementation (from January 2016 to June 2017), and ii) the 18-month period after the initiation of omega-3 PUFA supplementation (from July 2017 to January 2019). The patient showed a significant increase in body weight (34±2.9 *vs.* 43.5±1.7 Kg, $p<0.0001$), height (153.7±3.9 *vs.* 162.1±2.2 cm, $p<0.0001$) and BMI (14.7±0.57 *vs.* 16.5±0.27 Kg/m^2, $p<0.0001$), with a parallel reduction in HbA1c (7.5±0.4% *vs.* 7.14±0.4%, $p=0.05$) and no significant changes in daily insulin requirements (expressed as IU/Kg/day) between the two overall periods (0.81±0.09 *vs.* 0.83±0.84, $p=0.632$) (Figure 1). It is also worth noting that the entire 3-year follow-up period included the pubertal development.

After the initiation of omega-3 PUFA supplementation, CGM metrics showed a significant reduction in mean glucose (166.2±36.5 *vs.* 156.2±30.4 mg/dL, $p<0.0001$) and monthly CV (36.8±2.5 *vs.* 35.1±2.07, $p<0.07$) (Table 2, Figure 2), along with an increase in TIR 70-180 mg/dL (60.78±23.5 *vs.* 67.68±21.4, $p<0.0001$) and a decrease in TAR 180-250 mg/dL (26.52±16.7 *vs.* 23.75±18, $p<0.01$) and TAR >250 mg/dL (10.87±15.2 *vs.* 6.54±10.58, $p<0.0001$), without significant changes in TBR ≤70 mg/dL (1.83±5.5 *vs.* 2.03±3.7, $p<0.183$). Only two events of very low glucose levels (<54 mg/dL) were detected (one during the first 18-month period and one during the second 18-month period). Moreover, the tighter TIR (70-140 mg/dL) significantly increased during the second 18-month period compared to the previous 18-month period (38.23 ± 22.5 *vs.* 42.44 ± 23.4, $p<0.01$) (Table 2, Figures 3-4).

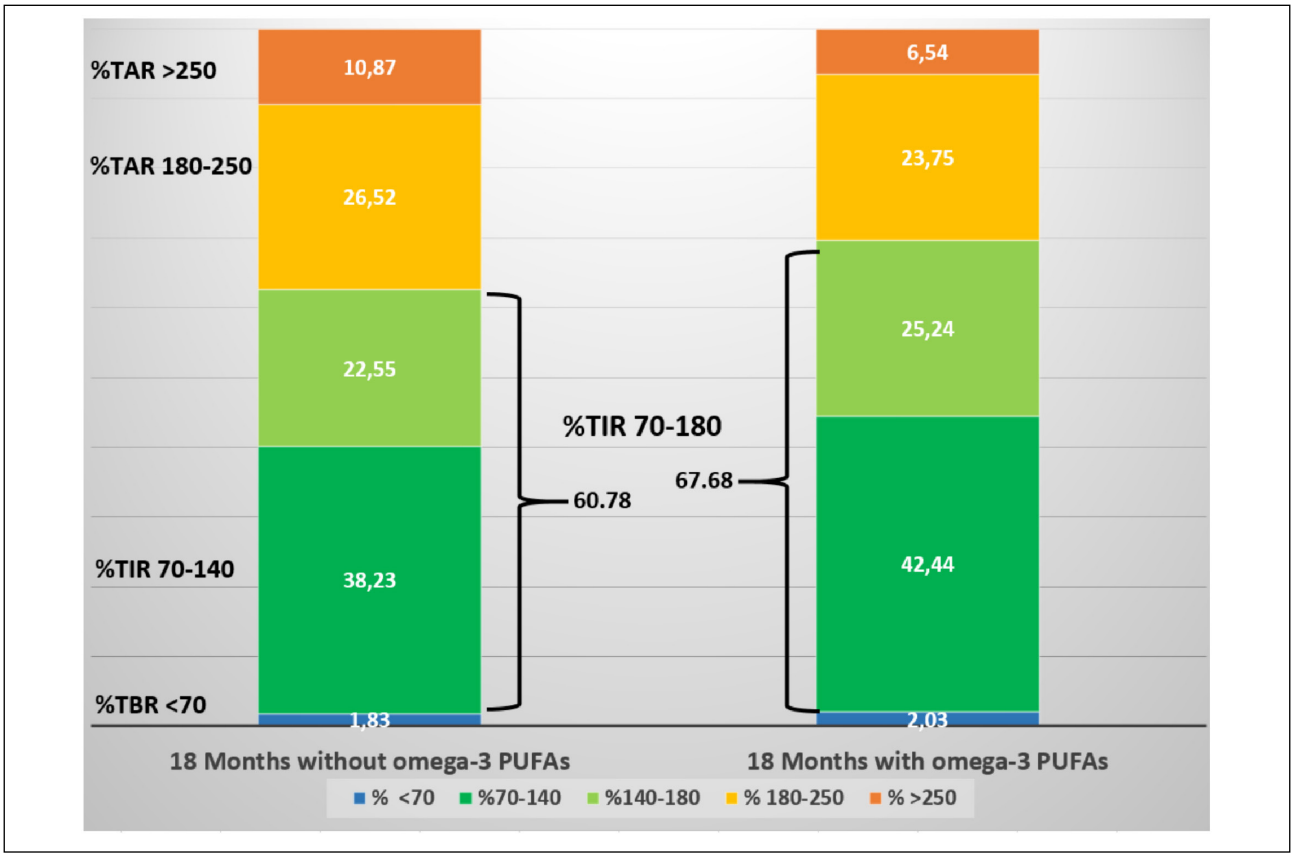

Figure 4. Schematic diagram of CGM metrics before and after the initiation of omega-3 PUFA supplementation. The stacked bars represent the proportion of time (expressed as percentage) spent within specific target glucose range.

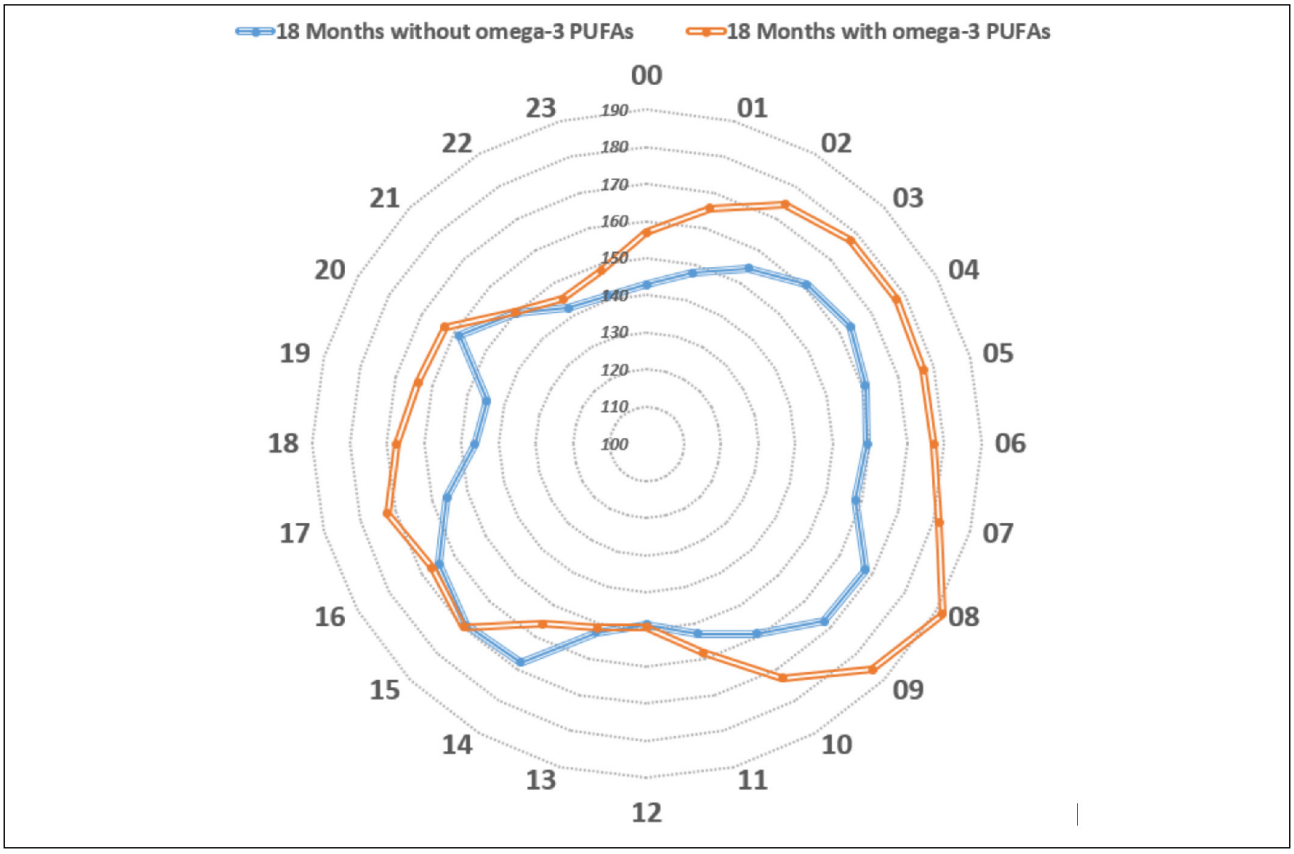

Figure 5. Distribution of the mean glucose values over 24 hours. The orange line represents mean glucose values during the 18 month-period prior to the initiation of omega-3 PUFA supplementation, whereas the blue line represents mean glucose values during the 18 month-period following the initiation of omega-3 PUFA supplementation. The blue line encircles a smaller area compared to the orange line. From midnight to midday constant lower mean glucose values were observed upon omega-3 PUFA supplementation, while glucose values appear to be similar between the two study periods from midday onwards at mealtime (lunch, afternoon snack, and dinner).

Interestingly, we also found a non-random circadian rhythm of CGM glucose data, with a pronounced reduction in glucose levels from midnight to midday during the 18 months of omega-3 PUFA supplementation compared to previous 18-month period without supplementation (Figure 5). Similarly, the distribution of TIR over 24 hours increases in the range 70-180 mg/dL from midnight to noon, rather than at meals, in the period with omega-3 PUFA supplementation (Figure 6).

A further decrease of mean glucose and SD during physical activity was also observed after omega-3 PUFA supplementation (see the section "*Physical activity and Sport*").

CV was assessed by Poincaré plot. After embedding mean hourly glucose values in graph A (10,900 dots related to the 18 months before the initiation of omega-3 PUFA supplementation), and as many values after intervention in graph B, two comparable areas were depicted around a central 45° axis. The dots were slightly more concentrated in graph B than graph A (Figure 7).

The overall representation of glucose values over time and along the day showed several glucose peaks before omega-3 PUFA supplementation, and less frequent and less pronounced glucose peaks after the initiation of omega-3 PUFA supplementation (Figure 8).

With regard to the serum lipid profile, a reduction in HDL-cholesterol and a parallel increase in triglycerides were observed after the intentional reduction of omega-3 PUFA dose (Table 1).

Discussion

Several remarks arise from this case report. After omega-3 PUFA supplementation**,** we observed: i) a decrease of mean glucose (assessed by CGM) and HbA1c , ii) a reduction of glucose variability (ex-

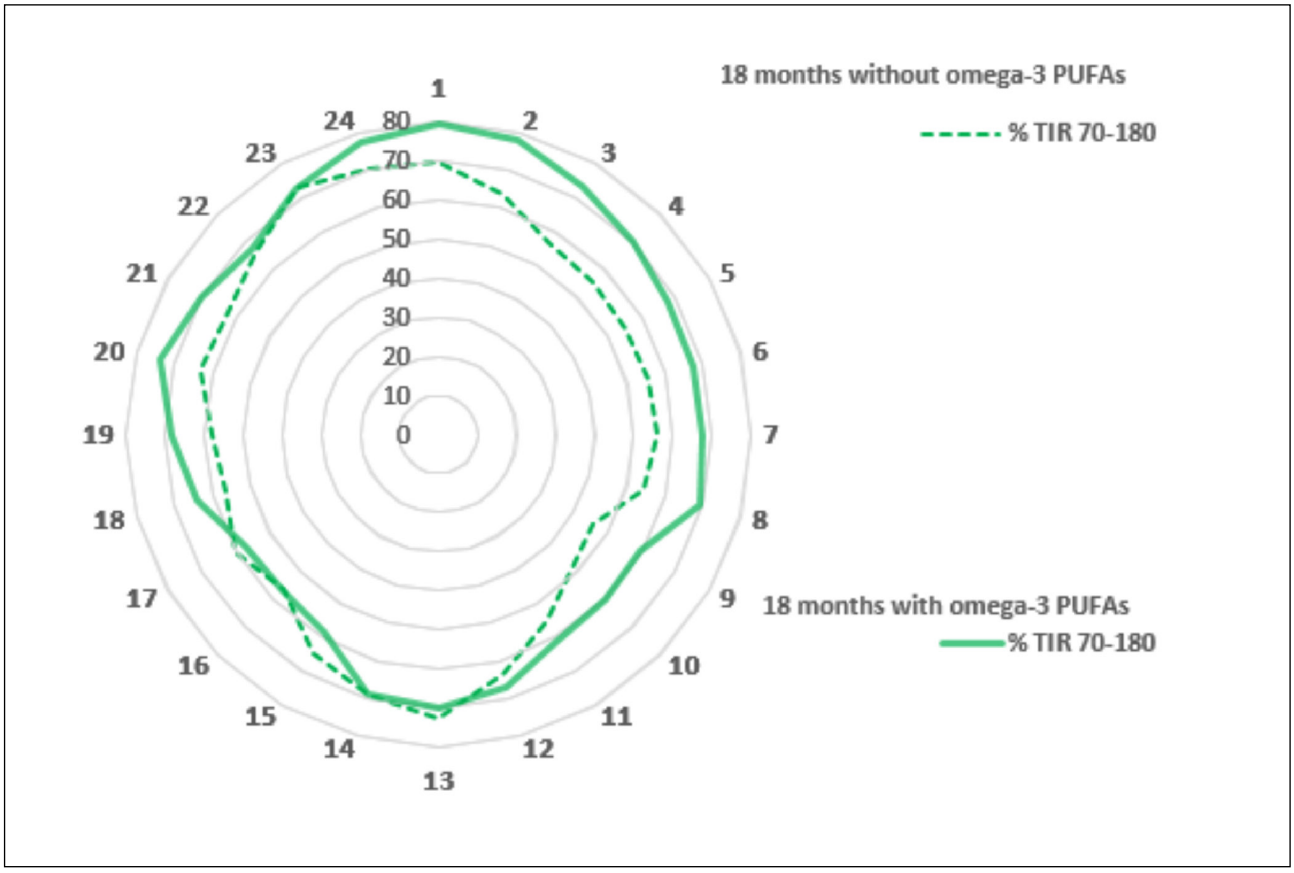

Figure 6. Distribution of TIR (70-180 mg/dL) over 24 hours. The graph shows the mean 24-hour glucose values within the TIR range (70-180 mg/dL) during the two study periods, without (dotted green line) and with (solid green line) omega-3 PUFA supplementation.

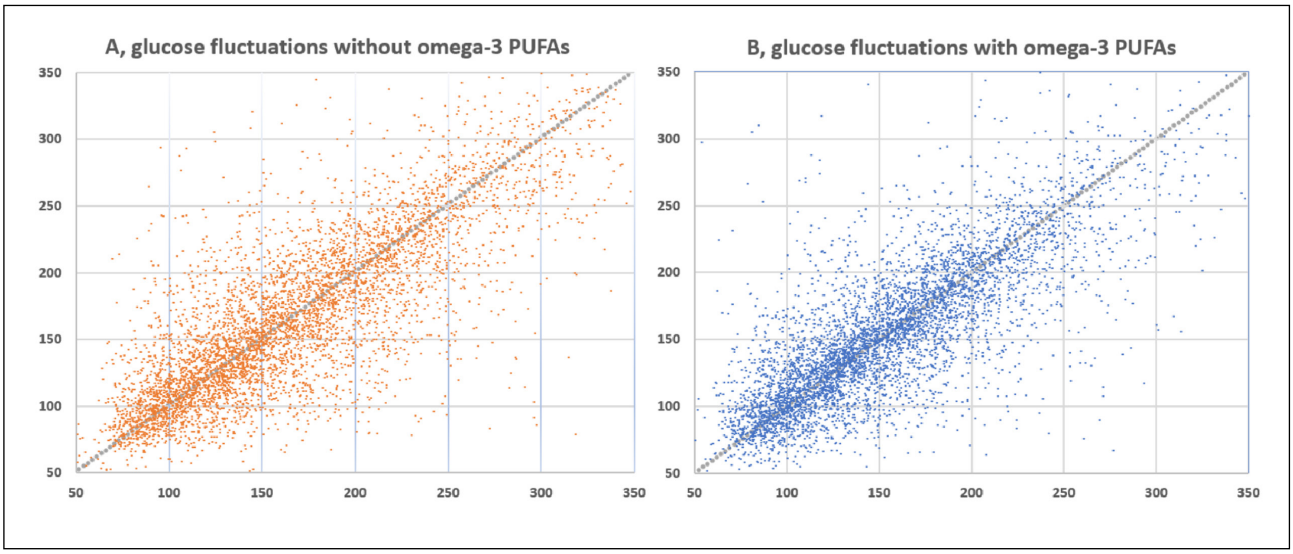

Figure 7. Glucose fluctuations (Poincaré plot). Graph A shows the mean hourly glucose values (10,900 points) related to the 18 month-period before the initiation of omega-3 PUFA supplementation; graph B shows as many values as graph A, which are related to the 18 month-period after the initiation of omega-3 PUFA supplementation. As a result, two comparable areas depicted around the central 45° axis indicate the hourly fluctuations of glucose. The data were slightly more thickened in graph B than graph A, making the oblique axis less perceptible.

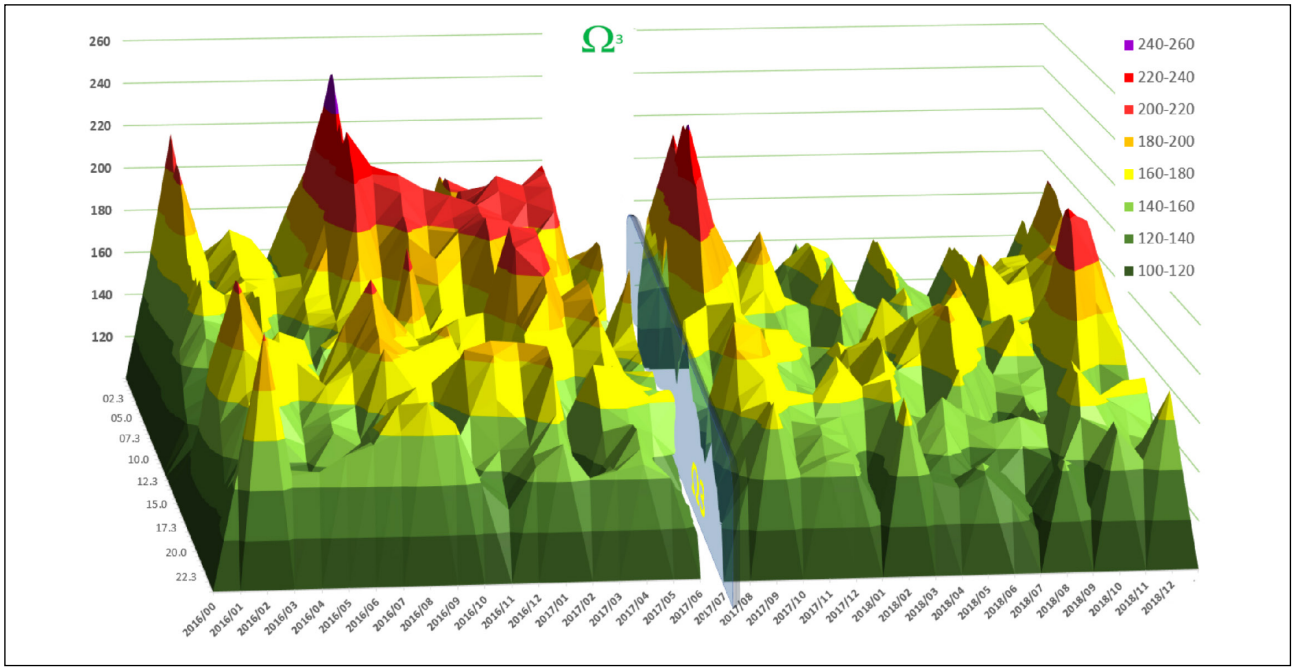

Figure 8. Three-Dimensional (3D) model of CGM values before and after initiation of omega-3 PUFA supplementation. Glucose values are plotted on the vertical axis, while time is plotted on the horizontal axis. The depth represents daily glucose values distributed over 24 hours. The vertical glass-like bar in the middle indicates the time when omega-3 PUFA supplementation was initiated.

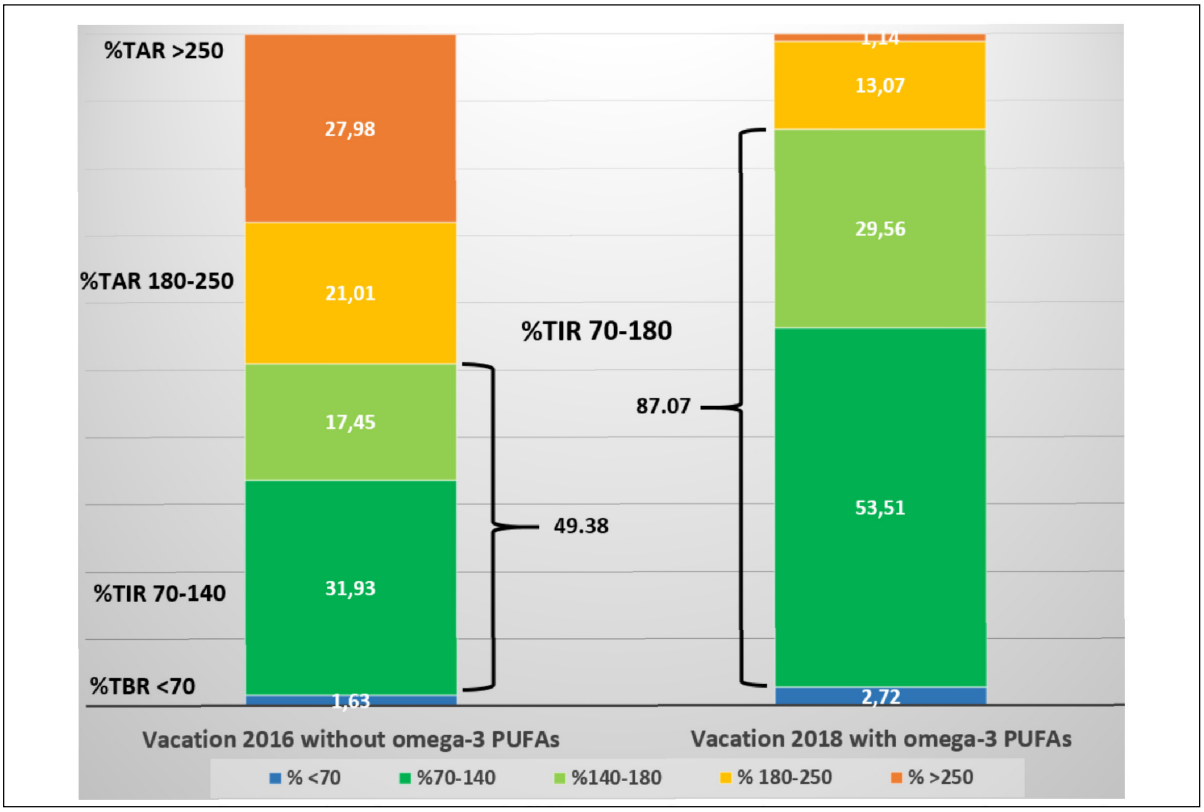

Figure 9. Schematic diagram of CGM metrics during two weeks of mountain vacation before and after the initiation of omega-3 PUFA supplementation, respectively. The stacked bars represent the proportion of time (expressed as percentage) spent within specific target glucose range, respectively before and after the initiation of omega-3 PUFA supplementation.

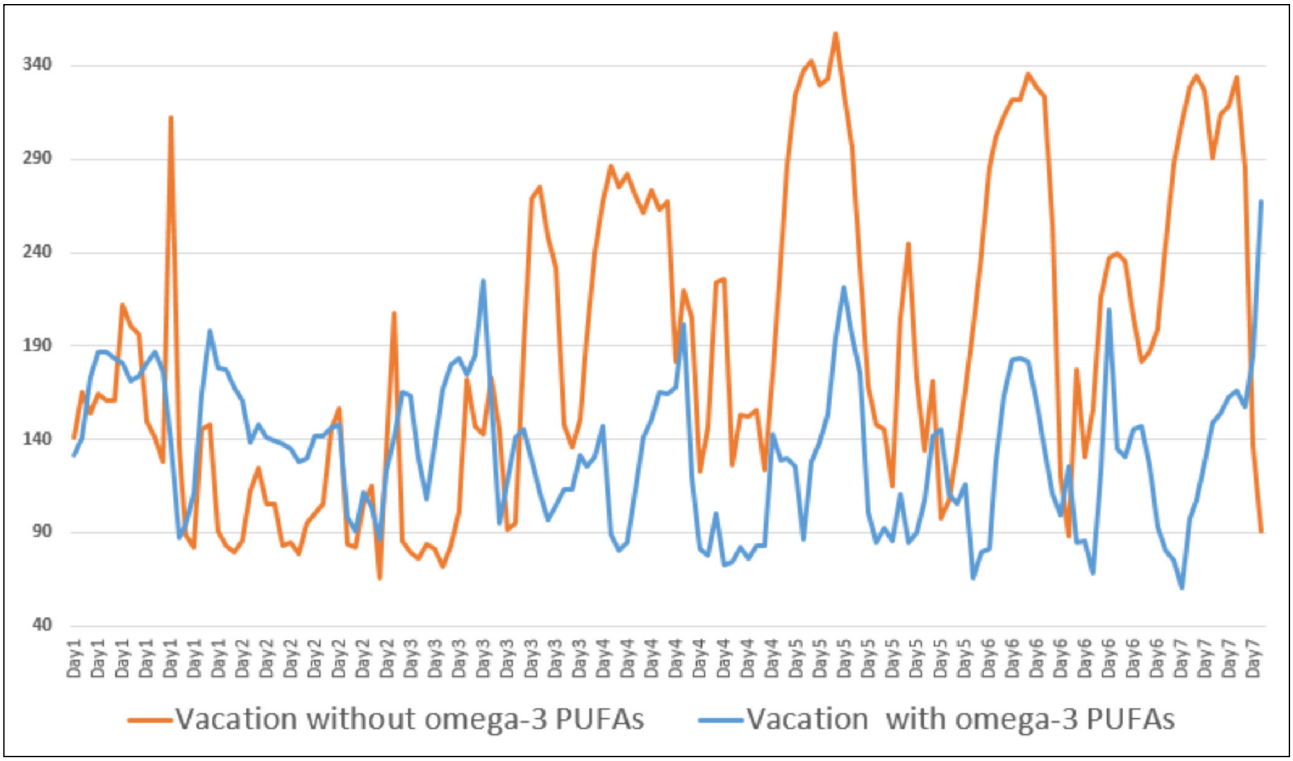

Figure 10. Comparison of average glucose levels referring to 2 weeks of mountain vacation with a daily physical activity consisting of walking, climbing and cycling. August 2016 (without omega-3 PUFAs) and August 2018 (with omega-3 PUFAs).

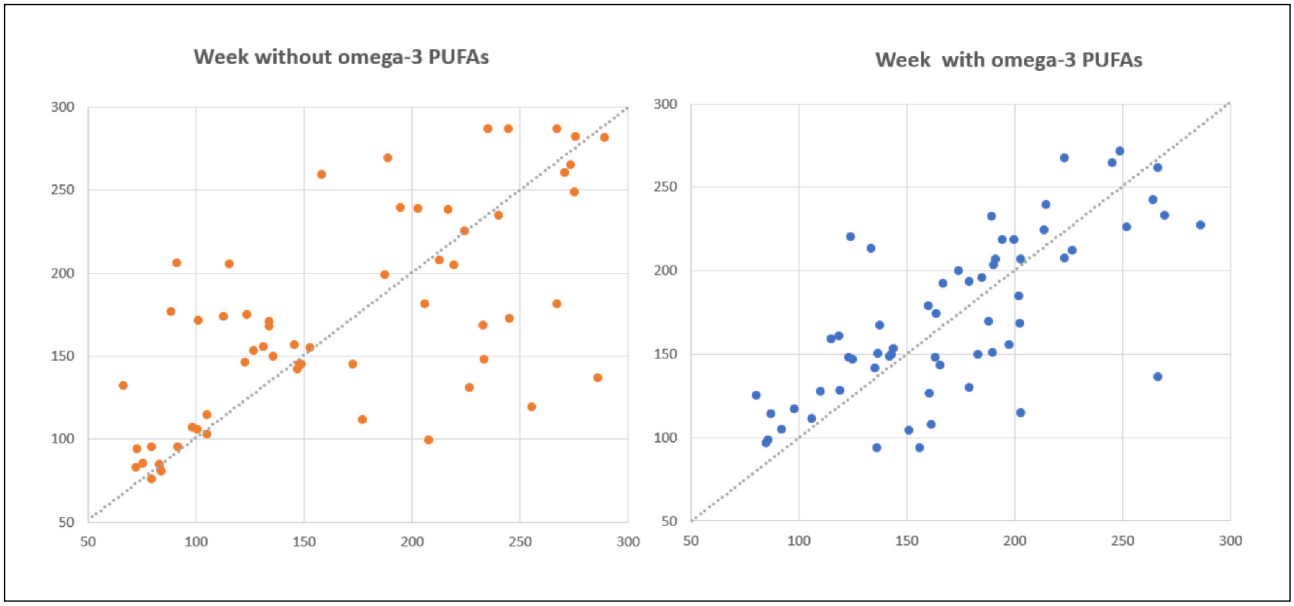

Figure 11. Poincaré plot showing CGM data related to the 2-week vacation periods, before and after the initiation of omega-3 PUFA supplementation. Graph A shows hourly mean glucose values referring to the week without omega-3 PUFA supplementation (142 dots), whereas graph B shows hourly mean glucose values referring to the week during omega-3 PUFA supplementation (122 dots). Dots appear more concentrated in graph B than graph A, indicating a reduction in glucose fluctuations.

Table 3. Comparison of metabolic parameters and CGM metrics between the two study 18-month periods (before and after the initiation of omega-3 PUFA supplementation, respectively). Data are expressed as mean ± SD.

	Mean glucose (mg/dL)	CV (%)	TBR <70 mg/dL (%)	TIR 70-140 mg/dL(%)	TIR 70-180 mg/dL (%)	TAR 180-250 mg/dL (%)	TAR >250 mg/dL (%)
Cycling							
Without omega-3 PUFAs	131.58±29.3	22	0.00	66.67±40.5	91.43±22.0	8.57±22	0.00
With omega-3 PUFAs	104.83±24.3	23	7.50±16.9	85.83±24.9	92.5±16.9	0.00	0.00
p-value	0.005	–	–	0.05	0.05	–	–
Hiking							
Without omega-3 PUFAs	167.35±50.1	30	0	40.63±45.1	59.38±49.9	34.78±42.6	5.84±10.9
With omega-3 PUFAs	120.51±20	16.6	0	71.88±35.9	100±0.0	0	0.00
p-value	<0.0001	-	-	0.05	0.05	-	-
Weeks of mountain vacation							
Without omega-3 PUFAs	190.35±81.1	42.59	1.63±7.2	31.93±38.8	49.38±45.1	21.01±32.8	27.98±42.4
With omega-3 PUFAs	134.35±41.1	30.56	2.72±12.3	53.51±43.1	83.07±32.1	13.07±29.5	1.14±10.2
p-value	<0.0001	-	0.341	<0.0001	=0.002	<0.05	<0.0001

pressed as SD and CV), iii) an increase in TIR, and iv) a reduction of TAR, accompanied by a non-significant increase in TBR <70 mg/dl. Only a severe hypoglycaemic episode (≤54 mg/dl) occurred. Moreover, we observed a significant reduction in mean daily glucose during fasting hours and physical activity, without significant changes in daily insulin requirements (expressed as IU/Kg/day). Fasting serum C-peptide levels did not change 12 months after the initiation of omega-3 PUFA supplementation, whereas stimulated C-peptide was not evaluated.

With regard to serum lipid profile, the patient showed a remarkable rise in triglyceride levels following the intentional reduction of omega-3 PUFA dose. Thereafter, triglycerides returned to normal levels after resumption of the initially prescribed omega-3 PUFA dose, as a likely consequence of triglyceride-lowering properties of omega-3 PUFAs when administered at proper doses[24,25,28]. In this regard, assessment of AA/EPA ratio could represent a valuable tool aimed to properly tailor omega-3 PUFA dose in clinical settings[24].

The aforementioned improvements in glucose control and CGM metrics may be explained, at least in part, by different mechanisms of actions exerted by omega-3 PUFAs beyond their well-known lipid-lowering properties. Of note, omega-3 PUFAs are the precursors of a group of lipid mediators known as "specialized pro-resolving mediators" (SPMs), which are involved in the resolution of inflammation[25,29]. Interestingly, emerging evidence from mice and clinical studies has shed light on the potential beneficial effects of omega-3 PUFAs and SPMs in the prevention and treatment of various autoimmune diseases (including T1D) in light of their anti-inflammatory and immunomodulatory properties[29,30]. In this regard, we previously showed a potential role of omega-3 PUFAs and vitamin D co-supplementation in preserving residual endogenous insulin secretion in subjects with newly diagnosed T1D[19,31-37]. The ongoing POSEIDON study (ClinicalTrials.gov Identifier: NCT03406897) will address whether this therapeutic approach has an impact on beta-cell function and/or glucose homeostasis in patients with newly diagnosed and established T1D[38,39]. Interestingly, it has been recently suggested that lower systemic or tissue levels of omega-3 PUFAs and omega-3/omega-6 ratio may be associated with the micro- and macrovascular complications of diabetes[40,41]. In addition, a study conducted in mice by Wang *et al*. showed that omega-3 PUFAs led to a rapid improvement of blood glucose levels and suppression of postprandial glycaemic fluctuations through inhibition of amino acid gluconeogenesis[42].

Various studies have focused on the potential influence of omega-3 PUFAs on insulin sensitivity in animal models fed with high-fat diets[43-44] or in humans with overweight and/or metabolic syndrome, which typically exhibit insulin resistance as well as a higher risk of developing type 2 diabetes (T2D)[45,46]. These studies reported an increase in insulin sensitivity and an improvement in lipid profile as the main outcomes of omega-3 PUFA supplementation[47]. The beneficial effects of

omega-3 PUFAs on peripheral insulin sensitivity may be mediated by the anti-inflammatory properties exerted by their derivative compounds SPMs, which potentially account for the reduction of adipose tissue inflammation and insulin resistance mediated by chronic low-grade inflammation[46]. In addition, pre-clinical studies suggest that omega-3 PUFAs can increase adiponectin concentration[43] and reduce mitochondrial dysfunction and endoplasmic reticulum stress[44] either directly or by microRNA[43]. These findings suggest further putative mechanisms underlying the beneficial role of omega-3 PUFAs in the prevention or reduction of insulin resistance.

Our case report suggests potential favourable effects of omega-3 PUFAs on glucose homeostasis, consisting of a significant reduction in mean glucose levels and glucose variability (determined by SD and CV). These effects were clearly appreciable during fasting and physical activity.[47]

In our case, omega-3 PUFA supplementation resulted in remarkable improvement in glucose control and serum lipid profile, regardless of daily insulin requirements. No side effects related to the use of omega-3 PUFAs were observed. Importantly, the CGM use allowed for a more reliable evaluation of glucose control compared to HbA1c, providing a better assessment of the patterns of glucose regulation and glucose variability. The reduction in glucose variability after the initiation of omega-3 PUFA supplementation was also observed during physical activity. Additionally, the reduction in mean daily glucose levels occurred particularly during the fasting hours even during sport activities (see Section "*Physical activity and Sport*").

An evocative image was represented in Figure 6 by a three-dimensional model of CGM values showing several glucose peaks before omega-3 PUFA supplementation, and less frequent and less pronounced glucose peaks after the initiation of omega-3 PUFA supplementation.

These findings suggest that omega-3 PUFAs could act: i) by reducing gluconeogenesis and hepatic glucose production, and/or ii) by reducing peripheral insulin resistance by virtue of their anti-inflammatory and lipid-lowering properties. With regard to the latter mechanism, omega-3 PUFA supplementation may have contributed to counteract, at least partly, the reduction in peripheral insulin sensitivity, which physiologically occurs during puberty [48].

Conclusion

Omega-3 PUFA supplementation in a T1D adolescent using CGM led to a reduction in mean glucose levels and glucose variability, as well as to an increase in TIR. The current availability of advanced CGM devices will certainly provide more insights into the role of different nutrition approaches - including omega-3 PUFA supplementation - in the management of type 1 diabetes[49]. However, mechanistic studies are warranted to better understand the role of omega-3 PUFAs in the regulation of glucose homeostasis in patients with type 1 diabetes.

SECTION – PHYSICAL ACTIVITY AND SPORT

Effects of omega-3 PUFA supplementation on glucose levels during long-term physical activity

Among the data recorded during the entire 3-year follow-up period, daily and weekly CGM data were collected during prolonged sport and physical activities performed with similar high-intensity of effort and duration. Pairwise comparisons of selected GCM data were performed between the two 18-month study periods, prior and after the initiation of omega-3 PUFA supplementation. Specifically we considered two different types of sport (cycling, hiking) and two weeks of mountain vacation. Every single event of cycling and hiking activity, both with and without omega-3 PUFA supplementation, was performed under the administration of a single reduced insulin bolus dose by halving the usual insulin to carbohydrate ratio at breakfast time.

Materials

Cycling: A 14-hour, 140-km-long bicycle tour (on September 2016, without omega-3 PUFAs) was compared with a 8-hour, 80-km long bicycle tour (on May 2018, with omega-3 PUFAs).

Hiking: On April 2017, a 6-hour and 30-min-hiking activity was performed at 300 meters above sea level, when patient was not on omega-3 PUFA supplementation. This activity was compared with a 6-hour and 30-min-hiking activity performed at 355 meters above sea level on August 2017, when the patient was on omega-3 PUFA supplementation.

Weeks of vacation: Two 7-day periods of mountain vacation with a daily physical activity

consisting of walking, climbing, and cycling were compared: August 2016(without omega-3 PUFAs) vs. August 2018 (with omega-3 PUFAs).

Results

CGM data related to physical activities performed during the two study periods (before and after the initiation of omega-3 PUFA supplementation) were compared. A lower mean glucose level and a reduced SD were found during sport and physical activities upon omega-3 PUFA supplementation. Accordingly, significant improvements in TIR 70-180 mg/dL and TIR 70-140 mg/dL were observed during omega-3 PUFA supplementation. Significant reductions in TAR 180-250 mg/dL and TAR >250 mg/dL were also reported, in presence of non-significant increases in TBR. Hypoglycemic events ≤54 mg/dL were not reported during exercise, both with and without omega-3 PUFA supplementation. See Tables 3, Figure 7, 8.

Moreover the CGM data related to the 2-week vacation periods, before and after the initiation of omega-3 PUFA supplementation, were analyzed using a Poincaré plot as previously described. Graph A shows hourly mean glucose values referring to the week without omega-3 PUFA supplementation (142 dots), whereas graph B shows hourly mean glucose values referring to the week during omega-3 PUFA supplementation (122 dots). Dots were slightly more concentrated in graph B than graph A (see Figure 9).

Discussion

The lack of significant changes in daily insulin requirements between the two periods (before and after the initiation of omega-3 PUFA supplementation) suggests that the improvements in glycemic control and reduction in glucose variability during exercise may be related, at least partly, to the omega-3 PUFA supplementation.

Author Contributions:

Conceptualization, F.C., R.I., C.R.; Data recovery, E.P., R.I.; Formal analysis, S.S., R.I.; Funding acquisition, F.C., A.M.R., G.B.; Investigation, F.C., A.M.R., S.S., G.M., E.P.; Methodology, F.C., R.I., C.R., A.M.R.; Project administration, F.C., C.R., R.I..; Supervision, S.S., C.R., G.B.; Writing—original draft preparation, F.C.; Writing—review and editing, S.S., C.R., A.M.R., M.S.

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Informed Consent:

Written informed consent was given by the patient and her parents for permission to publish this case report.

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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