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ARTICLE

Reversibility of pancreatic β-cells dysfunction after vitamin D and calcium supplementation: a pilot study in a population of obese and prepubescent North-African children

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ABSTRACT

The mechanisms of diabetogenesis in children remain largely obscure. This study aimed to determine the impact of vitamin D and calcium supplementation on pancreatic β-cells function in terms of insulin secretion and sensitivity. This was a quasi-experimental study involving 30 obese and prepubescent Tunisian children (57% boys). During three months, the children received calcium and vitamin D supplementation at therapeutic doses. An oral glucose tolerance test (OGTT) was performed at the beginning and at the end of the study. The following metabolic definitions were applied: **i)** hyperinsulinism: insulinemia sum > 300 μ UI/ml during OGTT, **ii)** insulin-resistance: homeostatic model assessment of insulin-resistance > 2, **iii)** normal glycaemic profile: normal plasma levels during OGTT without any spike, and **iv)** pancreatic β-cells dysfunction reversibility: disappearance of the aforementioned disorders. The means ± standard-deviation of age and body mass index were 10.87 ± 1.9 years, and 30.17 ± 4.99 kg/m^2, respectively. All children were at the stage of hyperinsulinism associated with insulin-resistance. These disturbances were noted even in children having a normal glycaemic profile at OGTT. After calcium and vitamin D supplementation, glycaemic profile as well as insulin-secretion improved significantly ($p < 0.0001$). Hyperinsulinism and insulin-resistance decreased significantly by 56.67% ($p < 0.0001$) and 70.00% ($p < 0.0001$), respectively. Complete reversibility of these two disorders was noted in 26.6% of children. To conclude, in obese and prepubescent children, vitamin D and calcium supplementation led to the reversibility of the pancreatic β-cells dysfunction.

1. Introduction

Prevalence of childhood obesity continues to increase throughout the world at an alarming rate [1]. Obesity childhood constitutes a real threat to health-promoting organizations [1]. Affecting one in three children in the USA [2], and costing 4.085 $ per child [3], childhood obesity is now considered one of the most serious and costly pandemics [2,3]. The main obesity-related diseases are type 2 diabetes-mellitus (T2DM) and endothelial dysfunction [4–6]. T2DM is an 'apocalyptic' pandemic, classified by the assembly of nations as 'an international public health challenge' [4]. About three new cases of T2DM are recorded every ten seconds and over a million new cases are recorded annually [7]. At this frantic pace, by 2040, more than one in ten adults will be affected [8]. Moreover, the scope with regard to the health and

economic impacts of T2DM complications is overwhelming, representing about 12% of the world's financial resources and more than 57% of health spending in North America [4]. It has long been regarded as the pathology of senescence; however, the epidemiological characteristics of T2DM are in full mutation since the disease is currently reported in much younger ages, affecting young adults, adolescents, and even children [4,7–9]. Yet, it is worth noting that data (*e.g.*; those linked to diagnosis and treatment) available so far on T2DM in children are incomplete [9,10]. Indeed, T2DM in children is a 'new' and 'rare' entity as it is largely underdiagnosed in favour of type 1 diabetes-mellitus [10]. In children, it is 'difficult' to make a clear distinction between the two types of diabetes-mellitus (*i.e.* type 1 diabetes-mellitus vs. T2DM) [10]. In fact, there is no clear consensus on

what defines T2DM in children/adolescents, since neither specific thresholds nor 'gold standards' has been developed [10]. In addition, the number of studies investigating T2DM diagnosis as well as its therapeutic side is limited [9,11]. The current conduct, consisting in extrapolating both the diagnostic and therapeutic schemas from adults to children [12], is a source of confusion. In fact, children/adolescents are not miniature adults and their disease phenotype is very distinct from that of adults [11]. Moreover, the inclusion of pubescent children in paediatric cohorts has long been viewed as a bias, which calls into question the published results [13].

As for T2DM treatment, results concerning the effect of T2DM drugs (*e.g.* metformin, rosiglitazone) on children's β-cells function restoration are divergent [11,12]. 'Possible reversibility' of the process of diabetogenesis is also suggested [14]. Nowadays, some new perspectives are advanced for T2DM treatment in adults [15]. For example, vitamin D, implicated in phosphocalcic homeostasis, has recently been introduced as a protective agent against the development of T2DM [16]. This finding is based on the presence of vitamin D receptors on pancreatic β-cells [16], and on its ability to modulate insulin-secretion and peripheral insulin sensitivity in vitro [16]. Moreover, it has been demonstrated that the addition of calcium (Ca) during vitamin D supplementation improves pancreatic β-cells function [17], and is therefore strongly recommended [18].

To the best of the authors' knowledge, no previous study has investigated the effects of vitamin D supplementation on pancreatic β-cells function in terms of insulin secretion and sensitivity in obese children. This pilot study was therefore conducted to assess the impacts of effective doses of vitamin D and Ca supplementation on pancreatic β-cells function in obese and prepubescent children. Our hypothesis was that combined vitamin D and Ca supplementation would induce 'reversibility' of the T2DM process.

2. Patients and methods

2.1. Study design

This was a quasi-experimental study performed from December 2017 to June 2019. It was a collaborative work between the laboratory of Physiology and Functional Explorations, and the Department of Paediatrics at Farhat HACHED Hospital, Sousse, Tunisia. This study was conducted in compliance with the world medical association Declaration of Helsinki. Approval for the study was obtained from Farhat HACHED Hospital Ethical Committee (approval number 2908FH/2017). Written consent was obtained from all the children's parents. Children also agreed verbally to participate in this study. Participation in

this study was free of pressure and free of charge. Parents were informed that they could withdraw their children from the study at any time. At the end of the protocol, all children received a copy of their biological examinations, and were referred to specialists to manage their obesity.

2.2. Sample size

The sample size was estimated according to the following formula [19]: $N = (Z_{\alpha/2})^2 \ s^2/d^2$, where '*s*' is the standard-deviation (SD), '*d*' is the accuracy of estimate of the main outcome (*e.g.*; homeostatic model assessment of insulin-resistance (HOMA-IR)), and '$Z_{\alpha/2}$' is the normal deviate for a two-tailed alternative hypothesis at a level of significance. Given the pioneering nature of this pilot study, '*s*' and '*d*' values were collected from a previous Turkish study, comparing some data (*e.g.*; insulin-resistance, fasting glucose, insulin-sensitivity indexes) determined by oral glucose tolerance testing (OGTT) in obese children [20]. The aforementioned study included 148 children (mean body mass index (BMI): $27.7 \pm 4.2 \ kg/m^2$), divided into two groups (93 non-insulin-resistant, and 55 insulin-resistant). In the insulin-resistant group, the mean ± SD of HOMA-IR was 4.9 ± 2.3 [20]. Injection of the above data in the formula, after fixing '$Z_{\alpha/2}$' to 2.58 (*i.e.*; error rate of 0.01%) gave a sample of 30 patients. Assumption of 70% for the non-inclusion criteria or absence during the second session gave a corrected total sample of 100 patients (100 = 30/ (1–0.70)).

2.3. Population

This study involved children attending primary schools in five geographically distinct areas in Sousse city (Tunisia). Selection of schools was made haphazardly. Recruitment of potential children was performed through regular medical school visits. Several meetings were held by two physicians (*MG* and *IK* in the authors' list) with children's parents to raise awareness about obesity complications. Once a final parents' agreement was obtained, a first morning appointment (*i.e.*; at 8 a.m.) was set at the Paediatrics Department to start the protocol. The children retained for this study were mainly selected based on the criterion of childhood obesity. The other two inclusion criteria were being prepubescent (stages I and II of Tanner classification) and free from chronic diseases. The presence of any acute or chronic disease, and medical intake were applied as non-inclusion criteria. The following exclusion criteria were applied: non-observance of the daily intake of vitamin D or Ca, absence during the second session, more than 10% variation in the initial weight during the three-month protocol period (in order to avoid bias in insulin-resistance variation [21,22]), and

change in ordinary life style, such as diet regimen or regular physical activity.

2.4. Study protocol and examinations performed

The study included three sessions (Figure 1). The first session was performed at the beginning of the protocol. The second and the third sessions were conducted 45 and 90 days after receiving an effective dose of vitamin D and Ca supplementation, respectively. During the first and third sessions, the following tests were applied in a subsequent order: medical questionnaire, clinical examination including puberty stage evaluation and anthropometric measurements, fasting blood sample, and OGTT. At the end of the first session, all calculated doses of vitamin D and Ca supplementation were delivered to parents. The second session consisted in controlling Ca plasma level.

The medical questionnaire, habitually used in the Paediatrics Department, includes questions related to the family history of mellitus-diabetes and/or, arterial-hypertension, and/or obesity. The degree of heredity was defined as follows: the first degree for relatives includes parents, the second degree for grandparents, and the third degree for uncles and aunts [23]. The questionnaire also includes questions with regard to the following personal items: medical history (chronic medical diseases, obesity onset), practice of sport and its duration (hour/week), and use of sunscreens. Children were considered as non-active or active according to their sport activity, based on the response to the following question [24]: do you practice any sports activities outside of school?

Clinical examination consisted in measuring systolic and diastolic blood pressures (SBP, DBP, respectively) in a sitting position after 10 minutes of rest. The measurements were verified three times during the first and third sessions. A manual sphygmomanometer (Spengler Lian metal child pattern, Germany) with its adapted brassards were used. The puberty status was determined according to the Tanner classification, which includes five stages (I to V) [25].

Age was determined. Height and weight were measured, nearest to 0.1 cm and 0.1 kg, respectively, in underwear children without shoes using a mechanical column scale (Detecto 437 Eye Level
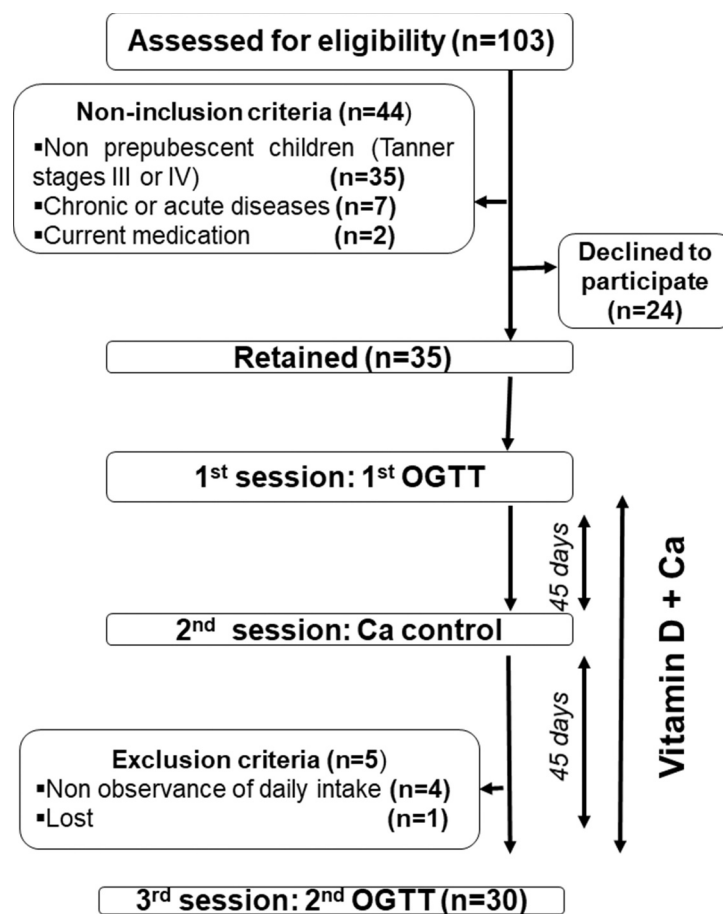

Figure 1. Study flow chart.

Ca: calcium. **OGTT**: oral glucose tolerance test.

Beam Physician scale 450 Ib x 4 oz, USA). Waist (m) and hip (m) circumferences were determined according to Lohman's anthropometric standardization reference manual [26]. The waist/hip circumferences ratio and the BMI (kg/m^2) were calculated [27]. Metabolic risk was evaluated according to the waist/hip ratio using an online calculator (URL: https://www.thecalculatorsite.com/health/whr-calculator.php, last visit 23 March 2022).

Before starting OGTT, an intravenous line was placed in the upper limb, and a fasting blood sample (after 10 to 12 hours of fasting) was taken and recorded as T_0 (T for time). The T_0 blood sample was divided into six tubes: a *fluoride tube* (for glycaemia), two *dry tubes* for lipid balance (total-cholesterol, high- and low- density lipoproteins cholesterol), triglycerides, and for hormonal assays (insulin, vitamin D), and two *lithium heparin tubes* with gel for C-reactive protein, phosphate, alkaline phosphatases, and without gel for Ca. An ethylene-diamine-tetra-acetic acid *tube* was reserved for glycated haemoglobin. T_0 blood biochemical data were performed using Cobas6000 analyser (Roche diagnostics GmbH, Germany) according to the appropriate method for each required type of dosage and without storage of the samples.

OGTT was performed in accordance with the recommendations specific to children [28]. Each child ingested, in less than five minutes, a solution of 1.75 grams of glucose per kg of weight, without exceeding 75 grams as the upper limit [28]. After ingestion of the glucose solution, four additional blood samples were taken at T_{30}, T_{60}, T_{90}, and T_{120} minutes in order to measure the biochemical (*i.e.*; glycaemia) and hormonal (*i.e.*; vitamin D and insulin) data. Samples for the hormonal assays were taken in a *dry tube*, rapidly centrifuged and frozen at −70°C, until the time of the assay. Insulinemia was assayed using chemiluminescent immunoassay technology via Liaison Diasorin assay kits whose detection threshold was estimated at 0.50 μUI/ml [29]. Determination of vitamin D follows the same principles as those described above for insulinemia. The detection threshold for this kit is estimated at 4 ng/ml [30].

In order to adhere to the general rule of increasing vitamin D doses in case of obesity, administered doses were multiplied by a factor of two in children with obesity classes I and II (800 IU/day), and by a factor of three in children with obesity class III (1200 IU/day) [31,32]. Vitamin D was administered on an empty stomach as daily drops to potentiate the action of insulin [33]. The drug used was Sterogyl 2000000 IU/ml/100 ml, drinkable solution, 20 ml vial. For each child, one tablet of elementary Ca [Albical® (dicalcium malate chelated), 200 mg tablet] was administered daily. Albical® is a generic drug marketed in Tunisia (https://covir-tn.com/categorie-produit/gamme-albion/ last visit: Mars 23, 2022). A control of the Ca plasma level at 45 days was performed during the second session.

2.5. Applied definitions

Obesity was defined as BMI ≥ 97th percentile according to the French reference curves, adapted with regard to the child's sex and age [34]. The following obesity classes were defined according to approaches taking into consideration age and sex [27,35]: I (BMI: 100–120% of the 95th percentile), II (BMI: 120–140% of the 95th percentile), III (BMI: 140–160% of the 95th percentile), and IV (BMI> 160th of the 95th percentile). In order to evaluate the effect of vitamin D and Ca supplementation on 'ΔVitamin D' blood concentration (*i.e.*; vitamin D blood concentration after minus before supplementation) based on the obesity status, two obesity groups were identified (group including classes 'I + II'; and group including classes 'III + IV'), and 'ΔBMI' (*i.e.*; BMI after minus before supplementation) was calculated.

Puberty stages I and II were characterized according to Tanner classification [25], which takes into account the description of pubic hair scale [stage I (no hair) and stage II (downy hair)], breast development scales for girls [stage I (no glandular breast tissue palpable) and stage II (breast bud palpable under the areola)], and external genitalia scale for boys [stage I (testicular volume < 4 ml or long axis < 2.5 cm), and stage II (4 ml-8 ml or 2.5 to 3.3 cm long)].

The following definitions related to arterial-hypertension were applied [36]: *i)* Prehypertension: SBP or DBP ≥ 90th percentile but < 95th percentile; *ii)* Arterial-hypertension: SBP and/or DBP ≥ 95th percentile for sex, age, and height on three or more occasions. Arterial-hypertension was classified as stage I if SBP or DBP were between 95th-99th percentile plus 5 mmHg, and stage II if SBP or DBP were > 99th percentile plus 5 mmHg.

Hyperinsulinism was retained when a sum of insulinemia at T_0, T_{30}, T_{60}, T_{90}, and T_{120} ≥ 300 μUI/ml [37]. Peripheral insulin-resistance was retained when HOMA-IR > 2 [38]. As for T2DM diagnosis, it was retained in the presence of hyperglycaemia at T_{120} of OGTT (*i.e.*; ≥ 11.1 mmol/l) [39]. Impaired fasting glucose was defined when glycaemia at T_0 was between 5.6 and 6.9 mmol/l [39]. The normal values of the haematological and biochemical calculated data and the relative applied definitions are detailed in Box 1 [40–43].

Box 1. Biochemical data and applied definitions.

The following three definitions related to vitamin D status were retained [44]: *i)* Deficiency: vitamin D ≤ 20 ng/ml, *ii)* Insufficiency: vitamin D: 21–29 ng/ml, and *iii)* Sufficiency: vitamin D: 30–100 ng/ml.

The evaluation of insulin secretion and sensitivity after vitamin D and Ca supplementation was carried out by calculating various specific indexes as detailed in Box 2 [38,45–52]. The indexes evaluated insulin-sensitivity (*e.g.*; HOMA-IR, insulinogenic index (ISI)

Box 1 Biochemical data and applied definitions.

Data	Data (unit)	Sex	Age range	Normal values	Applied definitions	Ref
Liver data	ALP (UI/l)	B/G	6–10	135.4–537.2	High > 537.2	[40]
		G	11–13	50.2–414.8	High > 414.8	[40]
		B	11–13	92.0–549.3	High > 549.3	[40]
Lipid data	TC (mmol/l)	G	9.3–14.7	3.21–4.85	High > 4.85	[41]
		B	9.3–14.7	3.28–4.67	High > 4.67	[41]
	TG (mmol/l)	G	9.3–14.7	0.7 (0.53–0.92)	High ≥ 1.7	[41,42]
		B	9.3–14.7	0.58 (0.45–0.80)	High ≥ 1.7	[41,42]
	HDL-C (mmol/l)	G	9.3–14.7	1.28–1.90	Low < 1.03	[41,42]
		B	9.3–14.7	1.64–1.79	Low < 1.03	[41,42]
	LDL-C (mmol/l)	G	9.3–14.7	1.79–2.97	High > 2.97	[41,42]
		B	9.3–14.7	1.69–2.87	High > 2.87	[41,42]
Other data	Calcium (mmol/l)	B/G	6–10	2.3–2.6	High > 2.6	[40]
		B/G	11–13	2.2–2.6	High > 2.6	[40]
	Phosphate (mmol/l)	B/G	6–10	1.22–2.00	-	[40]
		G	11–13	0.97–1.86	High > 1.86	[40]
		B	11–13	1.21–1.96	High > 1.96	[40]
	HbA1c (%)	B/G	2–19	< 5.7	High ≥ 5.7	[43]
	CRP (mg/l)	B/G	6–10	4.76–11.81	BIS: > 12.0	[40]
		B/G	11–13	4.78–18.14	BIS: > 18.14	[40]

ALP: alkaline phosphatases. B: boys. BIS: biological inflammatory syndrome. CRP: c-reactive protein. G: girls. HbA1c: glycated haemoglobin A1c. HDL-C: high-density-lipoprotein-cholesterol. LDL-C: low-density-lipoprotein-cholesterol [=TC – (HDL-C) – (TG/5)]. TC: total-cholesterol. TG: triglycerides. Normal values were expressed as minimum-maximum, except for TG, which was expressed as mean (95% confidence interval).

Composite, ISI Stumvoll, ISI Stumvoll age, Matsuda, Belfiore, Quicki, Cederholm, Gutt) and secretion (*e.g.*; insulin/glycaemia ratio30 and 120, ISI30 and 120, corrected insulin response 30 and 120, area under curve insulin/glycaemia 30 and 120).

Box 2. Applied definitions of indexes of insulin-sensitivity (IS) and insulin-secretion.

2.6. Statistical analysis

Shapiro-Wilk normality test was used to evaluate quantitative data for the underlying assumptions of normality. Outcome data were determined to be distributed normally. Means ± SD (95% confidence interval) were therefore used as summary statistics. Categorical data were expressed as relative

Indexes	Definitions	Reflects the	Ref
IS indexes			
HOMA-IR	$GlyT_0 \times InsT_0$ (pmol/l)/135	Interaction between pancreatic β-cells and peripheral IS (essentially hepatic)	[38]
ISI composite	$10000/\sqrt{(GlyT_0 \times InsT_0 \times GlyT_{120} \times InsT_{120})}$	IS of the whole body	[45]
ISI Stumvoll	$0.156 - 0.0000459 \times InsT_{120} - 0.000321 \times InsT_0 - 0.00541 \times GlyT_{120}$	Whole body IS during the absorptive phase	[46]
ISI Stumvoll age	$0.222 - 0.00333 \times BMI - 0.0000779 \times InsT_{120} - 0.000422 \times Age$	Whole body IS during the absorptive phase taking into account the age and the BMI of each child	[46]
Matsuda	$10000/[\sqrt{(GlyT_0 \times InsT_0)} \times \sqrt{(mean\ Gly \times mean\ Ins)}]$	Whole body IS during the absorptive phase	[47]
Belfiore	$2/((((0.5 \times GlyT_0) + GlyT_{60} + (0.5 \times GlyT_{120}) \times (((0.5 \times InsT_0) + InsT_{60} + (0.5 \times InsT_{120}))/638)) + 1)$	Whole body IS during the absorptive phase	[48]
Quicki	$1/[Log(InsT_0) + Log(GlyT_0)]$	IS	[49]
Cederholm	$(75000 + (GlyT_0 - GlyT_{120}) \times 1.15 \times 180 \times 0.19 \times Weight/[120 \times log(mean\ Ins) \times mean\ Gly]$	IS to all target peripheral tissues (liver, muscle and adipose tissue)	[47]
Gutt	$75000 + (GlyT_0 - GlyT_{120}) \times 0.19 \times Weight/[120 \times log([InsT_0 + InsT_{120}]/2) \times [GlyT_0 + GlyT_{120}]/2]$	Whole body IS during the absorptive phase	[50]
Insulin-secretion indexes			
Ins/Gly ratio30	$InsT_{30}/GlyT_{30}$	Predictive value (the best one) of the first phase of insulin-secretion with a correlation still valid in case of obesity or over-added insulin-resistance	[51]
Ins/Gly ratio120	$InsT_{120}/GlyT_{120}$	Severity of peripheral insulin-resistance	[51]
ISI 30	$(InsT_{30} - InsT_0)/(GlyT_{30} - GlyT_0)$	Change of insulin in relation to blood glucose during the first 30 min following the administration of a glucose load	[51,52]
ISI 120	$(InsT_{120} - InsT_0)/(GlyT_{120} - GlyT_0)$	Change of insulin in relation to blood glucose after 120 min following the administration of a glucose load	[51]
CIR 30	$100 \times InsT_{30}/GlyT_{30} \times (GlyT_{30} - 3.89)$	β-cells secretory function at T_{30}	[51]
CIR 120	$100 \times InsT_{120}/GlyT_{120} \times (GlyT_{120} - 3.89)$	β-cells secretory function at T_{120}	[51]
AUC Ins/Gly 30	$(InsT_0 + InsT_{30})/(GlyT_0 + GlyT_{30})$	Total systemic exposure to glucose and corresponding insulin response between T_0 and T_{30}	[51]
AUC Ins/Gly 120	$[(0.5 \times InsT_0) + InsT_{60} + (0.5 \times InsT_{120})]/[(0.5 \times GlyT_0) + GlyT_{60} + (0.5 \times GlyT_{120})]$	Total systemic exposure to glucose and corresponding insulin response between T_0 and T_{120}	[51]

AUC: area under curve. BMI: body mass index. CIR: corrected insulin response. Gly: glycaemia. HOMA-IR: homeostatic model assessment of insulin-resistance. Ins: insulinemia. ISI: insulinogenic index. mean Gly: mean glycaemia at all times of oral glucose tolerance test (OGTT). mean Ins: mean insulin levels at all times of OGTT. T_x: corresponding time (min) at OGTT.

frequency. Wilcoxon test and two-sided Chi2 tests were utilised to compare quantitative and categorical data, respectively (*e.g.*; session 1 vs. session 3). Student T test was utilised to compare the 'ΔVitamin D' blood concentration values between the obesity two groups (*i.e.*; 'I + II' vs. 'III + IV'). The correlation-coefficient between 'ΔVitamin D' and 'ΔBMI' was determined. Analyses were carried out using the Statistica software (Statistica Kernel version 6; StatSoft, Paris, France). Significance level was set at 0.05.

3. Results

At the beginning of the protocol, a list of 103 potentially eligible children was established. After application of the non-inclusion and exclusion criteria, 35 children were retained. At the end of the protocol, data of five children were excluded from the analysis. Therefore, 30 children (14 males) were involved in the study (Figure 1).

Table 1 illustrates the children's characteristics. The main results were: *i)* 17%, 87%, and 50% of the children were free from family antecedent of diabetes-mellitus, arterial-hypertension, and obesity, respectively; *ii)* 33.3% of the children had obesity class III or IV; and *iii)* 40% of the children had a high metabolic risk.

Table 2 describes the effects of vitamin D and Ca supplementation on biochemical data. High- and low- density lipoproteins cholesterol values decreased significantly between sessions 1 and 3. However, no change was observed for the biochemical profile.

Table 3 illustrates the effects of vitamin D and Ca supplementation on anthropometric and blood pressure data. Weight and hip circumference values significantly increased between sessions 1 and 3, by 1.20 kg and 1.25 cm, respectively. No change was observed for the blood pressure data or profile.

Table 4 describes the effects of vitamin D and Ca supplementation on OGTT data, and vitamin D blood concentration and status. The main results were: *i)* Only glycaemia at T_{30}, T_{60}, and T_{120} decreased between sessions 1 and 3, by 0.88, 1.18, and 1.05 mmol/l, respectively; *ii)* All insulinemia values decreased between sessions 1 and 3; *iii)* The percentages of children having hyperinsulinism and insulin-resistance decreased significantly between sessions 1 and 3 (100 vs. 51.8%; and 96.6 vs. 65.5%, respectively); *iv)* Vitamin D blood concentration increased by 6.52 ng/ml between sessions 1 and 3; and *v)* The percentages of children with vitamin D deficiency or sufficiency changed significantly between sessions 1 and 3 (80.0 vs. 44.8%, 0.0 vs. 13.8%, respectively).

Table 1. Characteristics of the obese and prepubescent children (n = 30).

Data	Unit	
Anthropometric data		
Sex	(male)	14 (46.66)
Age	(year)	10.76 ± 1.85 (10.07 to 11.45)
Height	(m)	1.51 ± 0.12 (1.46 to 1.55)
Weight	(kg)	70.95 ± 19.78 (63.56 to 78.33)
Body mass index	(kg/m²)	30.65 ± 5.21 (28.70 to 32.59)
Waist circumference	(cm)	97.20 ± 12.70 (92.46 to 101.94)
Hip circumference	(cm)	103.83 ± 11.27 (99.63 to 108.04)
Ratio waist/hip	(absolute value)	0.94 ± 0.06 (0.92 to 0.96)
Familial antecedents of		
Diabetes-mellitus	No Heredity	5 (16.7)
	Heredity degree 1	8 (26.7)
	Heredity degree 2	12 (40.0)
	Heredity degree 3	5 (16.7)
Arterial-hypertension	No Heredity	26 (86.7)
	Heredity degree 1	1 (3.3)
	Heredity degree 2	2 (6.6)
	Heredity degree 3	1 (3.3)
Obesity	No Heredity	15 (50.0)
	Heredity degree 1	7 (23.3)
	Heredity degree 2	4 (13.3)
	Heredity degree 3	4 (13.3)
Obesity data		
Obesity classes	(I)	8 (26.7)
	(II)	12 (40.0)
	(III)	7 (23.3)
	(IV)	3 (10.0)
Obesity groups	(classes 'I + II')	20 (66.7)
	(classes 'III + IV')	10 (33.3)
Obesity onset	(year)	4.85 ± 3.49 (3.50 to 6.20)
Other data		
Puberty stage	(I)	13 (43.3)
	(II)	17 (56.7)
Sport practice	(non-active)	30 (100.0)
	(volume(hour/week))	1.03 ± 0.80 (0.64 to 1.08)
Use of sunscreen	(yes)	30 (100.0)
Metabolic risk	(low)	10 (33.3)
	(moderate)	8 (26.7)
	(high)	12 (40.0)

Categorical and quantitative data were expressed as number (%) and mean ± standard-deviation (95% confidence interval), respectively.
Note: Obesity onset data were lacking in 2 children.

Figure 2 illustrates the effect of vitamin D and Ca supplementation on 'ΔVitamin D' blood concentration for obesity groups 'I + II' (n = 20) and 'III + IV' (n = 10). There was no statistical significant difference between the two groups' 'ΔVitamin D' blood concentrations (mean ± SD: 5.26 ± 6.17 vs. 8.91 ± 7.90 ng/ml, respectively for classes 'I + II' and 'III + IV'). There was no significant correlation between 'ΔVitamin D' and 'ΔBMI' (correlation-coefficient = −0.13; p = 0.4986).

Figure 3 illustrates the percentages of children having hyperglycaemia at different times of OGTT during the two sessions. Significant lower percentages of children with hyperglycaemia were noted at T_{30} and T_{60}.

After vitamin D and Ca supplementation, all indexes of insulin-sensitivity and secretion significantly improved (Table 5).

Table 2. Effects of vitamin D and Ca supplementation on biochemical data of obese and prepubescent children (n = 30) .

Data Numerical data	Unit	Session 1	Session 3	Δ Session	p-value
ALP	(UI/l)	198.40 ± 55.11(177.82 to 218.98)	192.52 ± 57.16(170.77 to 214.26)	−3.59 ± 32.88(−16.1 to 8.92)	0.8198
TC	(mmol/l)	4.19 ± 1.41 (3.66 to 4.71)	4.01 ± 1.17 (3.58 to 4.45)	−0.18 ± 0.61 (−0.4 to 0.05)	0.1528
TG	(mmol/l)	1.13 ± 0.53 (0.94 to 1.33)	1.22 ± 0.53 (1.02 to 1.41)	0.08 ± 0.52 (−0.1 to 0.27)	0.2410
HDL-C	(mmol/l)	1.13 ± 0.23 (1.04 to 1.22)	1.05 ± 0.21 (0.96 to 1.14)	−0.09 ± 0.16 (−0.2 to −0.03)	0.0072*
LDL-C	(mmol/l)	2.58 ± 1.26 (2.11 to 3.06)	2.29 ± 1.09 (1.85 to 2.73)	−0.20 ± 0.45 (−0.4 to −0.01)	0.0348*
Ca	(mmol/l)	2.38 ± 0.10 (2.35 to 2.42)	2.37 ± 0.10 (2.33 to 2.41)	−0.02 ± 0.10 (−0.1 to 0.02)	0.7456
PO4	(mmol/l)	1.54 ± 0.21 (1.46 to 1.62)	1.53 ± 0.18 (1.46 to 1.60)	−0.01 ± 0.2 (−0.1 to 0.06)	0.6474
HbA1c	(%)	5.34 ± 0.27 (5.24 to 5.44)	5.27 ± 0.29 (5.16 to 5.38)	−0.06 ± 0.23 (−0.1 to 0.02)	0.1230
CRP	(mg/l)	4.10 ± 0.80 (3.80 to 4.40)	6.07 ± 4.23 (3.63 to 8.52)	0.29 ± 3.97 (−2.0 to 2.58)	0.7007
Biochemical profile					
High ALP		0 (0.0)	0 (0.0)	-	-
High TC		5 (16.7)	5 (16.7)	-	-
High TG		3 (10.0)	5 (16.7)	-	0.7926
Low HDL-C		12 (41.4)	15 (57.7)	-	0.2273
High LDL-C		6 (20.7)	3 (11.5)	-	0.7328
High Ca		0 (0.0)	0 (0.0)	-	-
High PO4		0 (0.0)	0 (0.0)	-	-
High HbA1c		2 (6.7)	3 (10.0)	-	0.8978
High CRP		1 (3.3)	1 (7.1)	-	0.8459

ALP: alkaline phosphatases. **Ca**: calcium. **CRP**: c-reactive protein. **HbA1c**: glycated haemoglobin A1c. **HDL-C**: high-density-lipoprotein-cholesterol. **LDL-C**: low-density-lipoprotein-cholesterol. **PO4**: phosphate. **Session 1**: before vitamin D and Ca supplementation. **Session 3**: after vitamin D and Ca supplementation. **TC**: total-cholesterol. **TG**: triglycerides. **Δ session** = session 3 value minus session 1 value. Categorical and quantitative data were expressed as number (%) and mean ± standard deviation (95% confidence interval), respectively.
*p-value < 0.05 (2-tailed Wilcoxon test or 2-sided Chi2 test): session 3 vs. session 1.
Notes:
Lacking data during **session** 1: 1 LDL-C, 1 HDL-C.
Lacking data during **session** 3: 16 CRP; 4 LDL-C, 4 HDL-C; 1 ALP.

Table 3. Effects of vitamin D and Ca supplementation on anthropometric data, blood pressure data and profile of obese and prepubescent children (n = 30).

Data Anthropometric data	Unit	Session 1	Session 3	Δ Session	p-value
Weight	(kg)	70.95 ± 19.78(63.56 to 78.33)	72.14 ± 19.65(64.80 to 79.48)	1.20 ± 2.05(0.43 to 1.96)	0.0067*
Body mass index	(kg/m^2)	30.65 ± 5.21(28.70 to 32.59)	30.40 ± 5.38(28.39 to 32.41)	−0.25 ± 1.21(−0.70 to 0.20)	0.2452
Waist circumference	(cm)	97.20 ± 12.70(92.46 to 101.94)	98.55 ± 13.04(93.68 to 103.42)	1.35 ± 7.87(−1.59 to 4.29)	0.9840
Hip circumference	(cm)	103.83 ± 11.27(99.63 to 108.04)	105.08 ± 11.20(100.90 to 109.26)	1.25 ± 2.74(0.23 to 2.27)	0.0232*
Ratio waist/hip	(absolute value)	0.94 ± 0.06(0.92 to 0.96)	0.94 ± 0.09(0.91 to 0.97)	−0.00 ± 0.09(−0.03 to 0.03)	0.1072
Obesity classes	(I)	8 (26.7)	11 (36.7)	-	0.6459
	(II)	12 (40.0)	9 (30.0)	-	0.6360
	(III)	7 (23.3)	7 (23.3)	-	-
	(IV)	3 (10.0)	3 (10.0)	-	-
Obesity groups	(classes 'I + II')	20 (66.7)	20 (66.7)	-	-
	(classes 'III + IV')	10 (33.3)	10 (33.3)	-	-
Metabolic risk	(low)	10 (33.3)	11 (36.7)	-	0.8705
	(moderate)	8 (26.7)	5 (16.7)	-	0.6761
	(high)	12 (40.0)	14 (46.6)	-	0.7351
Blood pressure data and profile					
Systolic blood pressure	(mmHg)	110.16 ± 8.85(7.05 to 11.90)	109.86 ± 8.04(6.40 to 10.81)	−0.30 ± 7.09(5.64 to 9.53)	0.9840
Diastolic blood pressure	(mmHg)	72.60 ± 7.31(5.82 to 9.83)	72.50 ± 6.40(5.09 to 8.60)	−0.10 ± 6.07(4.83 to 8.16)	0.8807
Pre arterial-hypertension		7 (23.3)	7 (23.3)	-	-
Arterial-hypertension	(I)	0 (0.0)	1 (3.3)	-	0.3135
stage	(II)	2 (6.7)	0 (0.0)	-	0.1493

Ca: calcium. Δ session: session 3 value minus session 1 value. Session 1: before vitamin D and Ca supplementation. Session 3: after vitamin D and Ca supplementation. Categorical and quantitative data were expressed as number (%) and mean ± standard deviation (95% confidence interval), respectively. *p-value < 0.05 (2-tailed Wilcoxon test or 2-sided Chi2 test): session 3 vs. session 1.

4. Discussion

The two main findings of this pilot study evaluating the impacts of effective doses of vitamin D and Ca supplementation on the function of pancreatic β-cells in North-African obese and prepubescent children were the early onset of T2DM process, and its possible 'reversibility'.

4.1. Effects of vitamin D and Ca supplementation on anthropometric, blood pressure, and biochemical data

In the absence of data specific to paediatric age, those of adults indicate that vitamin D supplementation has no significant short-term impact on both anthropometric parameters [53] and blood pressure profile

Table 4. Effects of vitamin D and Ca supplementation on OGTT data and vitamin D status of obese and prepubescent children (n = 30).

	Time/Status/Unit	Session 1	Session 3	Δ Session	p-value
Glycaemic data					
Glycaemia (mmol/l)	T_0	4.86 ± 0.42 (4.70 to 5.02)	4.90 ± 0.63 (4.66 to 5.13)	0.03 ± 0.50 (0.40 to 0.68)	0.5026
	T_{30}	8.12 ± 1.91 (7.40 to 8.83)	7.24 ± 1.45 (6.70 to 7.78)	−0.88 ± 1.63 (1.30 to 2.19)	0.0128*
	T_{60}	8.24 ± 1.58 (7.65 to 8.83)	7.06 ± 1.28 (6.58 to 7.54)	−1.18 ± 1.18 (0.94 to 1.59)	0.0001*
	T_{90}	6.53 ± 1.64 (5.91 to 7.14)	6.09 ± 1.11 (5.68 to 6.51)	−0.43 ± 1.43 (1.14 to 1.93)	0.1535
	T_{120}	6.78 ± 1.40 (6.25 to 7.30)	5.72 ± 0.78 (5.43 to 6.01)	−1.05 ± 1.20 (0.95 to 1.61)	0.0001*
Insulin data					
Insulinemia (UI/l)	T_0	24.88 ± 14.45 (19.48 to 30.27)	15.56 ± 13.03 (10.60 to 20.51)	−9.43 ± 14.14 (11.22 to 19.12)	0.0010*
	T_{30}	223.03 ± 109.78 (181.27 to 264.79)	128.56 ± 109.19 (87.02 to 170.09)	−94.79 ± 82.91 (65.55 to 112.86)	0.0001*
	T_{60}	229.41 ± 112.39 (186.66 to 272.16)	115.32 ± 91.50 (79.84 to 150.80)	−112.75 ± 80.69(63.80 to 109.83)	0.0001*
	T_{90}	182.11 ± 112.84 (139.19 to 225.03)	83.51 ± 51.49 (63.54 to 103.47)	−99.86 ± 82.88 (65.52 to 112.81)	0.0001*
	T_{120}	138.45 ± 107.09 (98.46 to 178.44)	57.22 ± 47.09 (38.96 to 75.48)	−88.06 ± 91.02 (71.96 to 123.89)	0.0001*
Glycaemic abnormalities, hyperinsulinism and insulin-resistance					
IFG	Yes	2 (6.6)	1 (3.3)	-	0.5538
T2DM	Yes	2 (6.6)	0 (0.0)	-	0.1505
Hyperinsulinism	Yes	29 (100.0)	14 (51.8)	-	0.0001*
Insulin-resistance	Yes	29 (96.6)	19 (65.5)	-	0.005*
Vitamin D blood concentration and status					
Blood concentration	ng/ml	15.88 ± 5.59 (13.79 to 17.97)	22.51 ± 8.86 (19.14 to 25.88)	6.52 ± 6.90 (3.89 to 9.15)	0.0002*
Deficiency	Yes	24 (80.0)	13 (44.8)	-	0.0052*
Insufficiency	Yes	6 (20.0)	12 (41.4)	-	0.0743
Sufficiency*	Yes	0 (0.0)	4 (13.8)	-	0.0351*

Ca: calcium. IFG: impaired fasting glucose. OGTT: oral glucose tolerance test. T2DM: type 2 diabetes-mellitus. T_x: corresponding time (min) at OGTT. Δ session: session 3 value minus session 1 value. Session 1: before vitamin D and Ca supplementation. Session 3: after vitamin D and Ca supplementation.

Categorical and quantitative data were expressed as number (%) and mean ± standard-deviation (95% confidence interval), respectively.
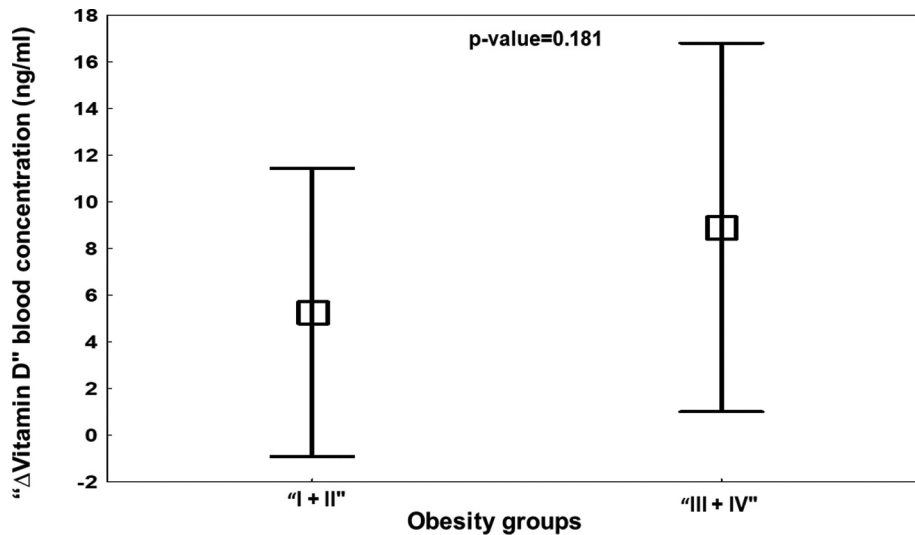
*p-value < 0.05 (2-tailed Wilcoxon test or 2-sided Chi2 test): session 3 vs. session 1

Notes:

Lacking data during session 1: 1 T_{30}, 1 T_{60}, 1 T_{90}, 2 hyperinsulinism.

Lacking data during session 3: 1 T_0, 1 T_{30}, 1 T_{60}, 1 T_{90}, 1 T_{120}, 3 hyperinsulinism, 1 insulin-resistance, 1 Vitamin D

Figure 2. Effect of vitamin D and calcium supplementation on 'ΔVitamin D' blood concentration in obese and prepubescent children according to obesity groups: 'I + II' (n = 20) vs. 'III + IV' (n = 10).

'ΔVitamin D' blood concentration = vitamin D blood concentration after minus before vitamin D and calcium supplementation.Data were mean (:) and standard deviation ().p-value: Student T test (group 'I + II' vs. group 'III + IV').
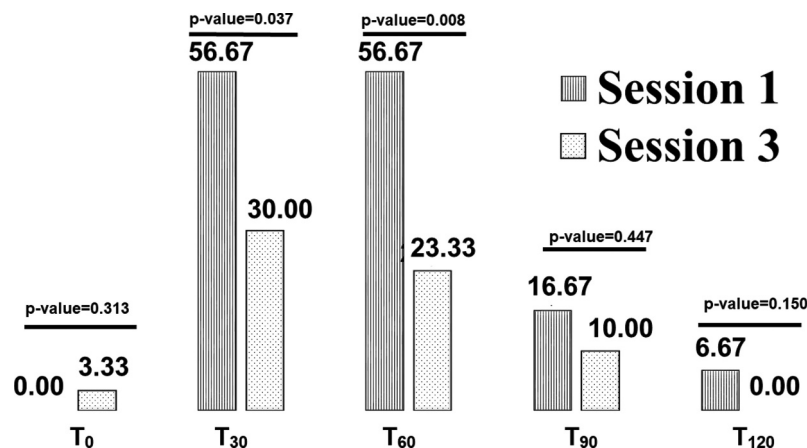
Figure 3. Percentages of obese and prepubescent children (n = 30) having hyperglycaemia at different times (T_x) of OGTT before (session 1) and after (session 3) vitamin D and calcium supplementation.

OGTT: oral glucose tolerance test. **T_x**: corresponding time (min) at OGTTData were percentages. *p-value < 0.05 (Chi2 test): session 1 vs. session 2.

[53,54]. However, this supplementation could explain the improvement in the lipid profile via insulin action [55], even in the short term.

4.2. Effects of vitamin D and Ca supplementation on OGTT data, hyperglycaemia at different times of OGTT, and indexes of insulin-sensitivity and insulin-secretion

The natural evolution to T2DM requires the concomitant presence of two major defects. They are insulin-secretion dysfunction, and the defect of its action [14,56,57]. Even in the presence of 'severe' peripheral insulin-resistance, the functional integrity of β-cells is perfectly capable to counteract the defect in the insulin action [57,58]. Disturbance in insulin-secretion and insulin-resistance are considered as independent factors, but with an 'additive' effect [56,59]. These phenomena appear at an early stage. Indeed, they were found, in this study, even in children having a normal glycaemic profile at OGTT. According to the literature, glucose-intolerant patients are more obese and more insulin-resistant than their 'normal-tolerant' counterparts [56]. Obesity is significantly reported in patients 'progressing' to more serious stages of diabetes. These patients are called 'The progressors' [56]. In obese children, decline in pancreatic β-cells function is an established fact despite its varied clinical manifestation [57]. It is recommended to search for this decline, even when the glycaemic profile is considered 'normal' at OGTT [57]. Abnormalities in the first phase of insulin-secretion remain its most explicit evidence [57]. The decline in the first phase of insulin-secretion is a premonitory step in the establishment of glucose intolerance according to the World Health Organization [47].

Table 5. Effect of vitamin D and Ca supplementation on insulin-sensitivity and insulin-secretion indexes on obese and prepubescent children (n = 30).

Indices	Session 1	Session 3	Δ Session	p-value
Insulin-sensitivity indexes				
HOMA-IR	6.21 ± 3.58 (2.85 to 4.81)	3.87 ± 3.18 (2.53 to 4.31)	−2.35 ± 3.64 (2.89 to 4.92)	0.0016*
ISI Composite	47.90 ± 35.52 (28.29 to 47.75)	113.59 ± 81.30 (64.28 to 110.67)	68.05 ± 74.34 (58.77 to 101.18)	0.0001*
ISI Stumvoll	0.10 ± 0.01 (0.01 to 0.02)	0.11 ± 0.01 (0.01 to 0.01)	0.01 ± 0.01 (0.01 to 0.01)	0.0001*
ISI Stumvoll age	0.10 ± 0.02 (0.01 to 0.02)	0.11 ± 0.02 (0.01 to 0.02)	0.01 ± 0.01 (0.01 to 0.01)	0.0006*
Matsuda	36.25 ± 20.08 (15.93 to 27.15)	84.40 ± 59.56 (47.09 to 81.08)	47.96 ± 53.73 (42.48 to 73.14)	0.0001*
Belfiore	0.30 ± 0.15 (0.12 to 0.20)	0.70 ± 0.29 (0.23 to 0.40)	0.39 ± 0.22 (0.18 to 0.31)	0.0001*
Quicki	0.22 ± 0.03 (0.02 to 0.04)	0.27 ± 0.08 (0.06 to 0.11)	0.04 ± 0.07 (0.06 to 0.10)	0.0009*
Cederholm	17.63 ± 4.45 (3.53 to 6.03)	24.21 ± 6.58 (5.20 to 8.95)	16.90 ± 11.24 (8.89 to 15.31)	0.0001*
Gutt	27.35 ± 7.38 (5.88 to 9.93)	37.12 ± 9.34 (7.38 to 12.71)	10.28 ± 7.83 (6.19 to 10.66)	0.0001*
Insulin-secretion indexes				
Ins/Gly ratio 30	27.43 ± 12.26 (9.73 to 16.58)	17.01 ± 11.19 (8.88 to 15.14)	−10.27 ± 10.72 (8.48 to 14.60)	0.0001*
Ins /Gly ratio 120	19.31 ± 11.67 (9.29 to 15.68)	9.47 ± 6.68 (5.28 to 9.10)	−10.72 ± 9.49 (7.50 to 12.92)	0.0001*
ISI 30	115.05 ± 274.36 (217.73 to 371.06)	1.72 ± 204.67 (162.42 to 276.80)	−115.03 ± 337.67 (266.97 to 459.62)	0.0012*
ISI 120	68.18 ± 76.95 (61.28 to 103.44)	32.85 ± 72.66 (57.44 to 98.90)	−38.13 ± 65.94 (52.13 to 89.76)	0.0083*
CIR 30	11,632.98 ± 7329.30(5816.38 to 9912.52)	6236.71 ± 6954.97(5519.32 to 9406.27)	−5483.21 ± 5436.83(4298.46 to 7400.27)	0.0001*
CIR 120	6333.84 ± 6783.91(5402.76 to 9119.72)	2036.82 ± 2271.61(1795.98 to 3091.98)	−4635.72 ± 6009.94(4751.58 to 8180.35)	0.0001*
AUC Ins/Gly 30	18.97 ± 8.28 (6.57 to 11.20)	11.52 ± 7.53 (5.97 to 10.18)	−7.42 ± 6.75 (5.34 to 9.19)	0.0001*
AUC Ins/Gly 120	21.68 ± 9.27 (7.36 to 12.54)	11.77 ± 6.79 (5.37 to 9.25)	−9.89 ± 6.53 (5.16 to 8.89)	0.0001*

Ca: calcium. Δ session: session 3 value minus session 1 value. Session 1: before vitamin D and Ca supplementation. Session 3: after vitamin D and Ca supplementation. For the remaining abbreviations, see Box 2.
*p-value < 0.05 (2-tailed Wilcoxon test or 2-sided Chi2 test): session 3 vs. session 1.

Indeed, 50% of children with a glycaemic peak at T_{30} of OGTT are considered at 'high risk' for progression to T2DM [47]. This phenomenon is physiologically explained by the lack of insulin action during the first phase of secretion, inducing excessive reactive secretion during the second phase that could continue beyond 120 min [47]. Moreover, alteration in the first phase of secretion (T_{30}) is significantly correlated with the imbalances observed at T_{120} of OGTT (reflection of late secretion) and with the progression to diabetes [59]. Excess fat is statistically associated with T2DM in children, as the disease prevalence increases from 10.4% in overweight young children to 79.4% in obese ones [57]. Obesity induces peripheral insulin-resistance. The latter secondarily increases the basal insulin circulating levels with concomitant alteration of pancreatic β-cells function [13]. Once established, insulin-resistance is the most important factor in the onset of diabetes within 10 to 20 years [57,60] and in the onset of degenerative complications around the age of 40 years [60]. In children, the severity of hyperinsulinism is more correlated with peripheral insulin-resistance than with BMI [57]. In addition, there is no association between the stage of obesity and fasting glycaemia [57], since the increase in the latter occurs only in late stages [57]. Furthermore, it is worth noting that BMI does not provide specific indications on the distribution of the fatty mass [57] or on the histological nature of the fatty deposits (hypertrophic or hyperplastic character). The impact of puberty on insulin-sensitivity is widely documented [13]. Pubertal development is physiologically associated with an estimated 25–30% decrease in peripheral insulin-sensitivity [13]. This consequently induces compensatory hyperinsulinism [13]. This decrease in insulin-sensitivity is observed in all children, both slim and obese ones [13]. However, its impact on pancreatic β-cells function is potentiated by the concomitant presence of obesity [60]. Obesity duration is considered as a proven risk factor for T2DM regardless of BMI severity [52,60]. Although the physio-pathological mechanisms of diabetogenesis follow the same pattern in adults and children, the speed of the process is far from being the same [8]. In fact, paediatric age is characterized by a much faster or even an accelerated progression and a greater severity compared to adulthood [8,60]. Decline in pancreatic β-cells function is the main anomaly observed in obese children [57,61]. This decline is very rapid, with a possible loss of pancreatic β-cells function reaching 20–35% annually [8]. Cases of conversion from 'normal glycaemia' to glucose intolerance or even diabetes have been reported over a period ranging from 12 to 21 months [60,61]. The same figures were reported by Weiss et al. [62], where in a population of 117 obese children, one-third progressed from glucose intolerance to diabetes within 24 months. The decline speed is also related to the severity of the associated insulin-resistance [8]. In this regard, it should be noted that we only have part of the truth concerning the mechanisms explaining the decline of pancreatic β-cells function in children with T2DM [57]. This 'decline' cannot be measured objectively, and it is therefore reflected indirectly via the indexes of insulin-secretion and insulin-sensitivity. In practice, the decrease in the potency of insulin-secretion or insulin-sensitivity may be the result of either a malfunction affecting the conserved pancreatic β-cells, or a drastic decrease (greater than 80% [57]) of pancreatic-cell mass [57]. In fact, the very few data available from post-mortem autopsy studies in adults show that T2DM is accompanied by a variable loss of pancreatic-cell mass, ranging from 0 to 65% [57]. The little attention paid so far to this 'anatomical detail' can be secondary to the fact that loss of pancreatic-cell mass, up to 50%, does not necessarily have a clinical manifestation and can therefore remain silent [57]. The primary focus of the therapeutic component of T2DM is to preserve pancreatic β-cells function and to 'slow' its decline [11]. The main objective of the medication is to potentiate insulin-sensitivity and reduce 'insulin-demand', thus providing pancreatic β-cells with some 'rest' [11,58]. Concerning children, studies carried out on this age group are scarce [11]. Moreover, the disease phenotype appears to be very distinct from that in adults. Therefore, what is applicable to adults cannot be generalized to children [11]. The association between vitamin D deficiency and the risk of T2DM is well established [63,64]. Similarly, the severity of diabetes is closely associated with the severity of hypovitaminosis D [64], which is much more marked in diabetic patients in case they are affected by obesity [64]. This is largely explained by the physical effect of vitamin D blood dilution and its sequestration in the fatty deposits [64].It should be noted that although the Mediterranean region is characterized by an almost permanent sunny climate, hypovitaminosis D is a common biological disorder in the population [65]. This is largely explained by the avoidance behaviour [66]. Despite its beneficial character, which is 'experimentally proven' in vitro and sometimes in vivo [67], there is so far no consensus on the benefits of vitamin D supplementation for diabetes-mellitus [67]. The multiple suggestions regarding the protective role of vitamin D are still considered insufficient and the results are inconclusive [63]. This controversy is partly due to several biases affecting the experimental approaches, such as the non-standardization of the administered doses (sub-optimal doses), the short duration of supplementation, the low enrolment in most studies, the heterogeneity of the selected cohorts, and the existence of associated comorbidities [67]. Moreover, these contradictions confirm the

complex nature of the biological interactivities in vivo. The exact mechanisms by which vitamin D deficiency influences blood glucose balance are still unclear. However, three main mechanisms are suggested. First, vitamin D acts by improving insulin-secretion through a direct effect via its receptor, which is expressed on the surface of pancreatic β-cells, or indirectly by modulating the intra-cytoplasmic calcium flow in pancreatic β-cells [63]. The second mechanism consists in reducing peripheral insulin-resistance by promoting insulin receptor expression in the target tissues [63]. Thirdly, vitamin D complements the insulin action by stimulating the intracellular transport of glucose through a more important externalization of insulin-dependent glucose transporters [63]. Finally, vitamin D has an inflammation modulating action (inhibition of certain cytokines), and an anti-apoptotic effect on pancreatic β-cells [63]. Consequently, lack of vitamin D results in insulin secretory dysfunction associated with faster pancreatic β-cells degeneration and peripheral insulin-resistance [68]. Based on the current state of knowledge and according to 'the standard of care for diabetes', established by the American diabetes association in 2017, vitamin D supplementation alone cannot currently be considered as a 'medicine' for diabetes-mellitus [63]. It is rather a therapeutic 'option' that can help improve the glycaemic balance of patients with diabetes-mellitus [63]. However, the most recent publications show that these concepts are undergoing radical change [17,67]. Although the overall message conveyed is encouraging towards the therapeutic potential of vitamin D [69], results of the few studies conducted on children are inconclusive. To the best of the authors' knowledge, this is the first study, proposing vitamin D supplementation at effective therapeutic doses adapted to BMI, which showed a clear improvement in both biological and hormonal parameters, with reestablishment of insulin-secretion in pancreatic β-cells and optimization of peripheral insulin-sensitivity. These results could be partly explained by the homogeneity of our cohort involving only children in the prepubescent phase. It could also be explained by the potentiating effect of concomitant vitamin D and Ca supplementation. The results of this work also revealed the potentially 'reversible' character of the process of diabetogenesis. This is a very recent concept whose physio-pathological bases have reversed the acquired knowledge and paved the way for encouraging prospects with regard to future management of this disease [14]. According to this theory, 'remission' of T2DM is possible because pancreatic β-cells are characterized by a 'plasticity' allowing them to modulate their activity according to the severity of the aggression they undergo [58]. As a result, pancreatic β-cells mass is in reality preserved but it is temporarily non-functional in diabetes-mellitus. The great challenge consists in discovering the mechanisms allowing the 'awakening' of pancreatic β-cells [14,58].

4.3. Effects of vitamin D and Ca supplementation on vitamin D blood concentration and status

In this study, after vitamin D and Ca supplementation, the vitamin D status was improved, and its blood concentration increased by 6.52 ng/ml (Table 4). However, the 'ΔVitamin D' blood concentration was not influenced by the obesity group (Figure 2), and no significant correlation exists between 'ΔVitamin D' and 'ΔBMI'. To the best of the authors' knowledge, the effects of vitamin D and Ca supplementation on vitamin D status and blood concentration taking into consideration the obesity status was not detailed in literature, and children' data are scarce. The vitamin D deficiency is obesogenic since its lowest concentration is recorded in most severe obesity classes [70]. In adults, it appears that after vitamin D supplementation, the vitamin D response profile in terms of blood concentration remains unclear [70]. After vitamin D supplementation, it is expected that 'ΔVitamin D' will be inversely correlated with 'ΔBMI'. This was not the case in the present study. Our results are in line with these of Stein et al. [70] who reported that severe obesity classes do not affect the rise of vitamin D blood concentrations. As previously advanced by some authors [70], the unequal distribution of obese in study groups (*e.g.*; 20 classes 'I + II' vs. 10 classes 'III + IV') could explain the 'apparent' contradiction and the lack of correlation.

4.4. Study strong points

The strongest point of this study is the homogeneity of the selected cohort, which is seldom reported in the simulated studies. Moreover, it was a therapeutic adaptation of vitamin D and Ca supplementation based on the children obesity status. In addition, we have been careful to avoid variations in children's BMI during the protocol in order to rule out confounding factors such as the action of adipokines [21,22].

4.5. Study limitations

This study presents two limitations. The first is related to the lack of a control-group. This work could have been more methodologically perfectible if a control-group receiving no supplementation was involved. This was not, however, possible due essentially to logistic and ethical reasons. The second limitation concerns the lack of consensual thresholds of normality for children. This issue has arisen for most

of the definitions adopted in this study. First, it was not easy to decide which obesity criteria to adopt [71]. Second, OGTT data were interpreted based on the response criteria observed in adults [72]. Third, the biological parameters, such as HOMA-IR are very disparate (*e.g.*; with regard to early detection, the threshold initially established by Matthews et al. [38] for children was set at 2, but currently, the values vary from 1.65 to 3.82 in girls and from 1.95 to 5.22 in boys [13,38,73]). Fourth, insulin-resistance is a condition that depends as much on the pubertal stage [73] as on sex and ethnicity [13]. Fifth, there are two American 'guidelines' to define the status of deficiency and insufficiency in vitamin D [74]. Sixth, there is no clear consensus concerning vitamin D supplementation modalities [31], and the recommended intake is variable [31,32]. On the one hand, the 'desirable' daily intake for adults and children (without distinction) having a deficiency in vitamin D and insufficient sun exposure, should not exceed 2000 IU/day [32]. On the other hand, according to the latest recommendations for vitamin D supplementation in obese adults, doses of 'relevant dietary allowance' should be multiplied by a factor of two or three taking into account both the age and the stage of obesity [31]. Finally, there are no clear guidelines for the vitamin D maximum doses to be given to obese children. In this context, caution was taken in this study despite the fact that the risk of vitamin D intoxication is very low or non-existent [31]. For the above reasons, we chose the options that seemed to be best suited to the physiognomy of our Mediterranean population, while respecting as much as possible the environmental particularities of our region [75].

4.6. Clinical practice

Taking into account our findings, it is interesting to depict the insulin-resistance by OGTT in early ages, and it 'seems' beneficial to supplement obese children with sufficient vitamin D doses according to their BMI. However, the importance of the direct interaction of insulin with vascular endothelial allowing the glucose uptake should not be overlooked [76]. This central role of endothelial integrity is often underestimated while it is recognized that microvascular lesions are a precursor event and constant in different categories of risk for T2DM such as insulin-resistance states [76]. Obesity is associated with insulin-resistance and endothelial dysfunction via fat-derived metabolic products, hormones, and cytokines [5], as well as genetic factors [6]. At the same time, vitamin D deficiency is related to endothelium dysfunction [77]. This suggests that complex, multi-directional, and dynamic mechanisms closely link obesity, insulin-resistance, and vitamin D.

To conclude, this quasi-experimental pilot study is the first to report, in a group of obese and prepubescent North-African children aged 10 to 12 years, the early onset of T2DM process, and its possible 'reversibility' after three months of effective vitamin D and Ca supplementation.

Abbreviations list

BMI: body mass index
Ca: calcium
DBP: diastolic blood pressure
HOMA-IR: homeostatic model assessment of insulin resistance
ISI: insulinogenic index
OGTT: oral glucose tolerance test
SBP: systolic blood pressure
T2DM: type 2 diabetes-mellitus

Establishments where the work was performed

Laboratory of Physiology and Functional Explorations and the Department of Paediatrics at Farhat HACHED Hospital, Sousse, Tunisia.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Authors' contributions

MG and RK conceptualized and designed the study.
MG, HBS, and *IL* drafted and revised the initial manuscript.
MG, IL, NJ and, MS designed the data collection instruments, collected data, and carried out the initial analyses.
IL and BL coordinated and supervised data collection.
HBS and *MG* critically reviewed the manuscript for important intellectual content.
HBS and JM supervised statistical analyses.
All authors approved the final manuscript as submitted. They agree to be accountable for all the aspects of the work.

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