Universitat de Girona

Glycemic control after vitamin D repletion in type 1 diabetes mellitus pediatric population

A clinical trial intervention

Author: Alfons Gasset Teixidor
Tutor: Dra. Pilar Ruiz- Cuevas García

11/11/2015

INDEX

1 ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my mentor Dra. Pilar Ruiz-Cuevas García for the continuous support on my Final Degree Project and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this protocol. I could not have imagined having a better advisor and mentor for my Final Degree Project.

This project consumed huge amount of work, research and dedication. Still, implementation would not have been possible if we did not have a support of many individuals. Therefore, I would like to extend our sincere gratitude to all of them.

- Dr. R. Cardona Hernández: pediatric endocrinologist in Sant Joan de Deu Hospital, Barcelona.
- Dra. G. Carreras Badosa: Institute of Biomedical Research of Girona - IDIBGI. Dr. Josep Trueta Hospital, Girona.
- Dra. MJ Ferri Iglesias: tutor at LLIR and coordinator of clinical sessions for physicians. Dr. Josep Trueta Hospital and University of Girona, Girona.
- Dr. A. López Bermejo: pediatric endocrinologist in Dr. Josep Trueta Hospital and University of Girona, Girona.
- Dra.M. Neras: chief of immunology and hormones department in Analysis Center Girona, Girona.
- Dr. R. Ramos: researcher in Translational Medicine Laboratory and Science of the Decision (Translab). University of Girona, Girona.
- Dr. D. Yeste: chief of Pediatric Endocrinology Unit at Vall d'Hebron Hospital, Barcelona.

2 ABREVIATIONS

1,25(OH)2 D	1,25 dihydroxivitamin D
25(OH) D	25-hydroxivitamin D
BMI	Body Mass Index
Ca	Calcium
CV	Cardiovascular
DM	Diabetes Mellitus
FPG	Fasting Plasma Glucose
HbA1c	Glycated hemoglobin
OGTT	Oral Glucose Tolerance Test
P	Phosphorus
PG	Plasma Glucose
PTH	Parathyroid Hormone
RCT	Randomized Controlled Trial
SD	Standard Deviation
SEEP	Sociedad Española de Endocrinología Pediátrica
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
UVB	ultraviolet B radiation
VDBP	Vitamin D Binding Protein
Vit D	Vitamin D

3 ABSTRACT

Background	It has been discovered that 3% of the human genome is regulated directly or indirectly by the vitamin D and that with adequate levels of vitamin D, if the established relations with cancer and others are correct, death rate would drop a 7%. Several studies show a high prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes, ranging between 43 and 91%. Currently, the screening and treatment of vitamin D deficiency is not considered standard of care for T1DM patients.
Justification	Vitamin D is required for and improves the production of insulin, and also improves insulin sensitivity. On this basis, vitamin D repletion should improve glycemic control in T1DM patients.
Purpose	The goal of the present work is to evaluate improvement in glycemic control after vitamin D repletion in T1DM pediatric population with vitamin D levels ranging below normality. Glycemic control will be evaluated on basis of glycated hemoglobin.
Design	This protocol is for a multicenter, prospective, randomized, double blind, placebo controlled 12 months clinical trial in pediatric patients with T1DM.
Participants	T1DM pediatric population with vitamin D levels ranging below sufficiency from Pediatric Endocrinology Unit in Hospital Josep Trueta, Hospital de Palamós, Hospital de Calella, Hospital de Figueres and Hospital de Blanes.
Method	158 T1DM patients with vitamin D levels below sufficiency (<30 µg/ml) will be randomized into treatment or placebo group (1:1) receiving 25.000 IU of cholecalciferol or placebo respectively. Measurements will be collected at month 0, 6 and 12, coinciding with T1DM follow up visit.
Key words	Type 1 Diabetes, vitamin D, deficiency, supplementation, repletion, trial.

4 INTRODUCTION

4.1 VITAMIN D

4.1.1 DEFINITION AND TYPES

Vitamin D or calciferol refers to a group of liposoluble substances, which actually consists of six compounds named from D2 to D7. Receives the name of "vitamin" because in case of lack of diet supplies or endogen cutaneous synthesis, can be cured through oral supplementation. But it is also considered a hormone, because its actions take place through a union to nuclear receptors inside a cell, while most vitamins act as cofactors in specific biochemical reactions.

The clinically relevant substances are vitamin D3 (cholecalciferol, "sun vitamin") and vitamin D2 (ergocalciferol, "diet vitamin") (1). Vitamin D3 or cholecalciferol is the main source of vitamin D in the nature and can be obtained endogenous through 7-dihydrocholesterol (a liver synthesized cholesterol derivate) deposited in the dermis or through diet. On the other hand vitamin D2 or ergocalciferol comes from plant's ergosterol and can only be obtained through diet (2,3).

4.1.2 SYNTHESIS AND CATABOLISM

Vitamin D requires 2 hydroxylation in order to become functional.

- First one, in the liver, turns vitamin D into 25-hydroxivitamin D or calcidiol (25(OH)D) which is the circulating form of the vitamin D and the one that should be taken into account to determine the levels of vitamin D.

- Second one, in the renal tubule, where it turns into 1,25-dihydroxyvitamin D or calcitriol, which is the active metabolite. This metabolite is ten times more powerful than 25(OH) D but the circulating concentration is 1000 times inferior.

1,25(OH)2 D levels are regulated by parathyroid hormone (PTH), which increases or decreases 1-α hydroxylase. Therefore, if PTH increases the action of 1-α hydroxylase, also does the synthesis of 1,25(OH)2 D, hence PTH is in charge of avoiding excessive production of 1,25(OH)2 D.

The half-life of the vitamin D varies depending on the metabolite. 25(OH) D usually lasts 15 days. On the other hand, 1,25(OH)2 D half-life is much shorter, a few hours. In non-deficient vitamin D states, 25(OH) D conversion is low, because most of the vitamin (50% approximately) is distributes in the muscular and fat compartment (3). Therefore, high doses of vitamin D are deposited in the fat tissue, which can last months. Due to this fact, 25(OH) D is the substance that should be measured in order to assess vitamin D levels (4).

Inactivation of vitamin D and its metabolites depends on a complex system of esterifying enzymes present in the microsomal liver cells. Some exogenous factors, such as anticonvulsants and barbiturates, can activate systems responsible for the degradation of vitamin D. For this reason, children receiving anticonvulsant or barbiturates treatment may end up suffering from a vitamin D deficiency. 1,25(OH)2 D would be the best biomarker of negative feedback, i.e., the higher the level of this metabolite, the more active is the enzyme (24-hydroxylase) to calcitroic acid.

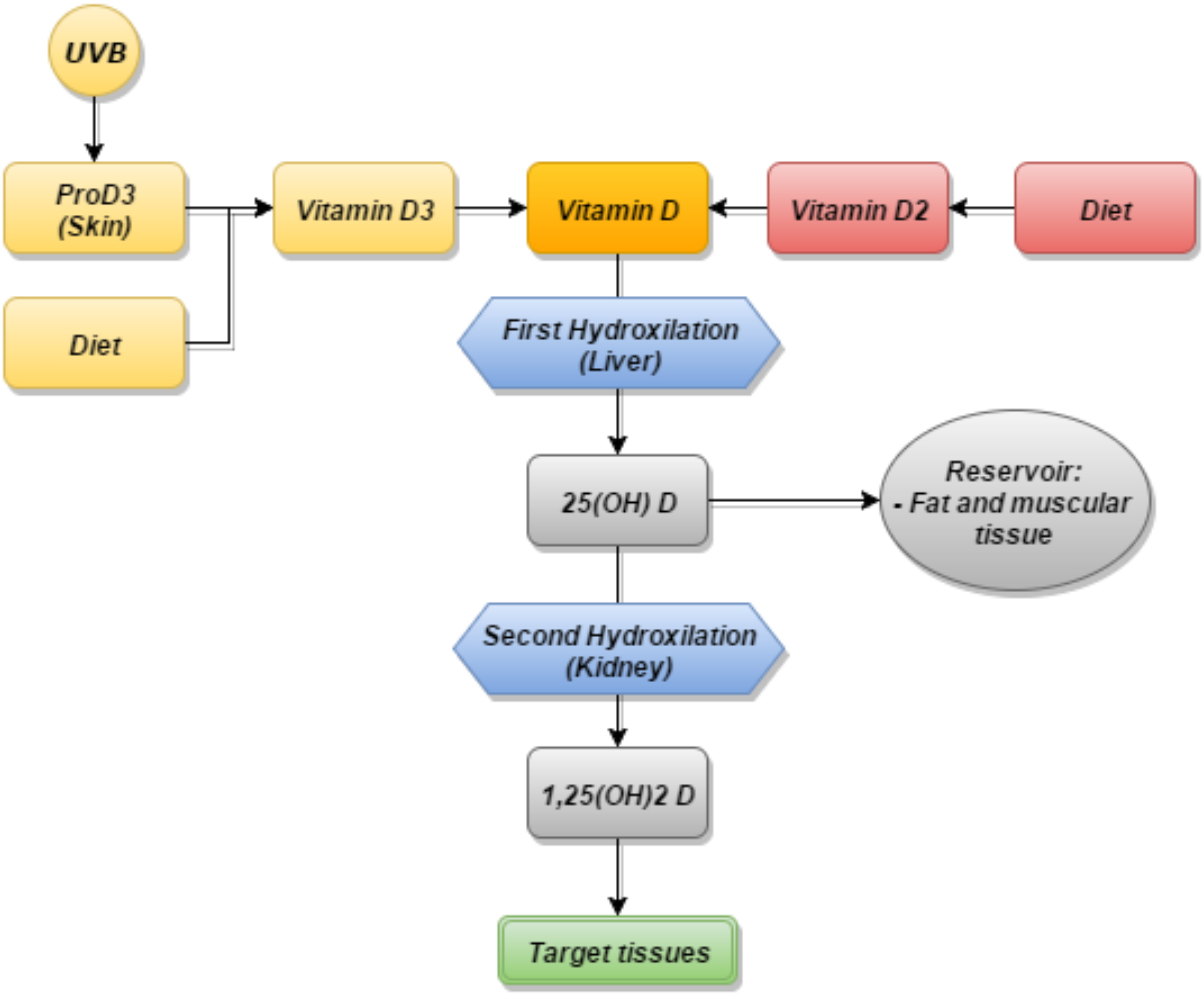


Figure 1. *Vitamin D metabolism*

4.1.3 MECHANISM OF ACTION

1,25(OH)2 D is characterized by acting as a steroid hormone because is secreted by an endocrine organ (kidney) and is transported to target tissues (intestine, bone, kidney, etc). There is unified with specific receptors, inducing protein synthesis and thereby, executing the physiological action of the hormone.

The vast majority of its effects are regulated through a nuclear transcription factor called vitamin D receptor (VDR). Discovered in 1969, VDR is present in over 30 tissues and organs and acts as a high affinity and specificity 1,25(OH)2 D receptor. 1,25(OH)2 D is associated with vitamin D binding protein (VDBP) in the blood, it

dissociates from its carrier in order to enter the cell. Thanks to its liposoluble nature, 1,25(OH)2 D crosses the cell membranes and the cytoplasm to reach the nucleus, where it binds to the VDR. As consequence, phosphorylation of calcitriol occurs, followed by the association of VDR-calcitriol complex to a protein, that acts as the retinoic acid X receptor (RXR) and, with the vitamin and the receptor, forms an heterodimer. The resulting set (VDR-calcitriol-RXR) unifies small DNA sequences known as elements of vitamin D response (VDREs), initiating multiple molecular interactions which finally modulate transcription of specific genes. It has been found that up to 200 genes in tissues throughout the body are regulated by 1,25(OH)2 D (5). Furthermore, the VDR has an affinity 1000 times greater with calcitriol than with calcidiol (3).

Table 1. *Tissues with VDR presence (3,4).*

Tissue distribution	
Adipose	Muscular, embrionary
Suprarenal	Muscle, smooth
Bone	Osteoblast
Bone marrow	Ovary
Brain	Pancreatic beta cell
Mammary gland	Parathyroid gland
Cancer cells	Parotid gland
Cartilage	Hypophysis
Colon	Placenta
Gland eggshell	Prostate
Epididymis	Retina
Pileus follicle	Skin
Intestine	Stomach
Kidney	Testicle
Liver (fetal)	Thymus
Lung	Thyroid gland
B & T lymphocytes	Uterus
Muscle, cardiac	Yolk sac (bird)

4.1.4 FUNCTION

Vitamin D2 and D3 have identical biological functions, because they are hydroxilated in the liver at the same time, becoming one substance, 25(OH) D. Nevertheless, studies suggest that vitamin D3 could be 2 or 3 times more powerful regarding the 25(OH) D levels maintenance. Moreover, 25(OH) D3 could bind to the transporting protein with more affinity than 25(OH) D2.

Traditionally, the function of vitamin D has been related to the regulation of the metabolism of calcium and phosphorus and maintaining a healthy bone mineralization. In the last years, other functions have been highlighted in several other physiological processes. Vitamin D receptors have been found all around in our organism and it is estimated that vitamin D directly regulates 200 genes and indirectly close to 2000. Furthermore, its precursor, 25(OH)D can be turned into calcitriol in other non-renal tissues, such as immune cells, bone, parathyroid gland and Langerhans islets in the pancreas, among others, performing specific paracrine and autocrine functions for those tissues. Therefore, vitamin D also modulates growing and cell differentiation in several tissues, endocrine activity of the pancreas and kidney and enhances innate immune system, among other functions (1).

4.1.5 SOURCES

80-90% of vitamin D is produced by the action of sun exposure, while through diet we receive only a 10-20% (1). Any solar cream with a SPF (Sun Protection Factor) of 15 or above absorbs 98% of UVB radiation, therefore, in the same amount, vitamin D production is reduced (1,3).

The capacity of the skin to produce vitamin D decreases as time goes by, because the concentration of 7-dehydrocholesterol in the skin decreases. By the time we become 70 years old, the capacity may be reduced to a 30% (1).

4.1.6 VITAMIN D BINDING PROTEIN

The main vitamin D carrier protein is VDBP, binding 85 to 90% of total circulating 25-OH D. The non-vitamin D-binding protein fraction (bioavailable 25(OH) D) consists primarily of albumin-bound 25(OH) D (10 to 15% of total 25(OH) D), with less than 1% of total 25-OH in the free form. Bioavailable 25(OH) D is defined as circulating 25(OH) D not bound to VDBP, which is analogous to the definition of bioavailable testosterone, of which formula was based vitamin D free equation (6). Clinical assays

measure the level of total 25(OH) D without distinguishing fractions bound to carrier proteins (see *Limitations* in *Table 6*).

When bound to VDBP, vitamin D may be unavailable to act on target cell and some actions of vitamin D would become inhibited. VDBP prolongs the half-life of 25(OH) D by serving as a reservoir and aiding in the reabsorption of filtered vitamin D through nephron in the kidney (6).

It was observed that black Americans consistently have lower levels of total 25-OH D than white Americans, and they are frequently given a diagnosis of vitamin D deficiency (6). However, when comparing PTH levels and bone densitometry, these parameters are found normal, or even better, in black Americans, which lead to the hypothesis that another factor was playing an important role in this scenario.

Common genetic polymorphisms in the VDBP gene produce variant proteins that differ in their affinity for vitamin D. The prevalence of these polymorphisms differs between racial groups. A study was conducted to determine whether VDBP genotypes and concentrations of circulating VDBP differ between black Americans and white Americans (6).

In the study, blacks were more likely than whites to have the T allele of the VDBP polymorphisms, which was associated with decreased levels of VDBP and total 25(OH) D; whereas whites were more likely to have the G allele at this locations, which relates to higher levels of VDBP. Moreover, allele A, which characterizes for with higher levels of VDBP both in black and whites, was less likely found in blacks than in whites, and in the last ones, allele A was related to decreased levels of total 25(OH) D.

In conclusion, black Americans may have lower levels of total 25(OH) D, but they also present lower levels of VDBP, resulting in levels of bioavailable 25(OH) D that are equivalent to those in whites.

Levels of total 25(OH) D are, in part, genetically determined. The effect of VDBP polymorphisms on total 25(OH) D concentrations appeared to be mediated by the concentration of VDBP; therefore it should be taken into account.

Nevertheless, levels of VDBP only partially explained racial differences in levels of total 25(OH) D; other factors including skin pigmentation and other polymorphisms, probably contribute to low levels of total 25(OH) D in blacks (6).

4.1.7 SUFICIENCY, INSUFICIENCY AND DEFICIENCY

The normal levels of vitamin D are defined from 30ng/ml (50nmol/l) to 100ng/ml, even though vitamin D intoxication is not usual. Under the lower limit we define:

Table 2. *Vitamin D levels correlation (1).*

SUFICIENCY	>30 µg/ml (>75 nmol/l)
INSUFICIENCY	20-30 µg/ml (50-75 nmol/l)
DEFICIENCY	<20 µg/ml (<50 nmol/l)

Thresholds for vitamin D sufficiency have been based on total 25-OH D levels at which calcium absorption declines or PTH levels increase (3,6).Nevertheless, experimental data are inconclusive; controversy surrounds the precise level of total 25-OH D at which these changes occur. Moreover, in 2010 the Institute of Medicine, Food and Nutrition board, redefined vitamin D insufficiency as 25(OH) D below 20 µg/ml (50 nmol/l) and below 16 µg/ml in infants (7). However, consensus hasn't been reached yet; therefore, previous vitamin D values will be applied in this protocol in order increase evidence whether definitions should be changed or not.

Vitamin D deficiency can be sub classified according to several biochemical analysis parameters into moderated or severe:

✓ *Moderate Vitamin D deficiency 10-20 ng/ml*

Ca	Normal	25-OH D	↓
P	↓	1,25 (OH)2 D	↑
AP	↑	PTH	↑

✓ *Severe Vitamin D deficiency (<10 ng/ml)*

Ca	↓	25-OH D	↓↓
P	↓↓	1,25 (OH)2 D	↓
AP	↑↑	PTH	↑↑

Figure 2. *A. Moderate Vitamin D deficiency (10-20 ng/ml). B. Severe Vitamin D deficiency (<10 ng/ml) (1).*

Only severe vitamin D deficiency (<10ng/ml) may lead to symptomatology, producing rickets, in children, and osteomalacia, in adults. Nevertheless, insufficient levels of vitamin D can be objectified, as discussed previously, in a lab analysis through the PTH determination, which rises to compensate the low levels of vitamin D. Therefore, even though insufficient levels of vitamin D may go by as asymptomatic through the bone/calcium function of the vitamin D due to the PTH compensation, the vitamin D levels are still low and that may have a negative effect in the immune function of vitamin D. However, as exposed previously, elevated levels of PTH are more common in white population than black, due to different levels of VDBP. Therefore, the relation between levels of PTH and total 25(OH) D may differ between blacks and whites (6).

How common is vitamin D deficiency in pediatrics?

Surprisingly, it has made resurgence in neonates and young children, in part because of the campaign to encourage all women to provide all of their infants' nutrition through breastfeeding. Because there is very little, if any, vitamin D in human milk, infants, especially infants of woman of color, are at high risk of developing vitamin D deficiency and rickets if they are not given vitamin D supplement (8,9).

It is important to recognize vitamin D deficiency not only because of the vital role that vitamin D plays in bone formation, but also its extra-skeletal effects. Vitamin D deficiency is being linked to broadening field of health problems including several types of cancer and autoimmune or metabolic diseases (6,10–12). When low vitamin D levels are present, the innate immune system dysfunctions, with defects in chemostaxis, phagocytosis and killing of bacteria and viruses, whereas the adaptive immune system seems hyperactive (12).

Without vitamin D, the small intestine absorbs no more than 10-15% of dietary calcium. In a person with vitamin D sufficiency, the small intestine absorbs, on average, 30% of dietary calcium; during growth, lactation and pregnancy, the efficiency increases to 80%. Due to this fact, vitamin D deficiency during bone development and growth causes the bone-deforming disease rickets, previously introduced (8).

Vitamin D deficiency often goes undiagnosed or, worse is misdiagnosed. There are 3 reasons for this. First, it is believed that both exposure to sunlight or dietary intake is adequate, therefore, that first world citizens are not at risk of vitamin D deficiency. Second, physicians who perform routine blood work-ups often obtain a blood calcium value. If they find it to be normal, they assume that the patient is vitamin D sufficient. Third, some physicians may erroneously order an analysis for the active form of vitamin D, 1,25(OH)2 D, to determine the vitamin D status of a patient. Unfortunately, 1,25 (OH)2 D not only is not a measure of vitamin D status, but its measurement also can mislead the physician into thinking that the patient is vitamin D sufficient. The reason for this is that, as a person becomes vitamin D deficient, there is an increase in the concentration of PTH, which increases the renal production of 1,25(OH)2 D, the circulating concentrations of which often become normal or even elevated (8).

Gender, age, race and season of the year need to be taken into account due to variations in the reference levels:

Table 3. *Percentage of variability in 25(OH) D levels depending on (1):*

Gender	2%	Male/Female
Race	67%	White/Black
Age	33%	Young/Old
Season	8%	Winter/Summer

The supply of vitamin D is limited by the reduced exposure to solar UVB due to indoor living, clothing, and the use of sunscreen, as well as by the geographical and seasonal variations in UVB irradiance (12).

Obesity is often associated with vitamin D deficiency. It is now recognized that, whether vitamin D is ingested in the diet or obtained from exposure to sunlight, it is efficiently deposited in the large body fat stores and is not bioavailable (8,13).

4.2 DIABETES MELLITUS TYPE 1

4.2.1 DEFINITION AND CLASIFICATION

T1DM is a group of metabolic diseases characterized for hyperglycemia secondary to a partial or complete defect in insulin secretion, which produces also alteration of the lipid and protein metabolism leading to microvascular and macrovascular effect in different organs such as eyes, kidney, nerves, heart and vessels.

Previously known as insulin-dependent or juvenile diabetes mellitus in which the destruction of pancreatic beta cells lead to an absolute insulin deficiency, T1DM is now divided in T1DM A or autoimmune or T1DM B or idiopathic:

- T1DM A or autoimmune: autoimmune disease in which takes place a beta cell selective destruction mediated by activated T lymphocytes in HLA predisposed subjects. After a preclinical period of variable duration, in which the patient remains asymptomatic, when the insulin producer cell mass reaches a critical value the patient presents the classical symptomatology: polyuria, polydipsia, polyphagia, weight loss and progressive ketosis which can lead to ketoacidosis if treatment with exogenous insulin is not provided.
- T1DM B or idiopathic: contrary to T1DM A, T1DM B contains those patients with same or alike characteristics, but without any kind of autoimmune data or HLA predisposition. As it has been recently described, little is known about its etiology, evolution or prognosis.

4.2.2 EPIDEMIOLOGY

Incidence of T1DM in childhood is increasing at about 3% per year. T1DM is the most common metabolic disease in the young (12,14,15).

T1DM results from cellular-mediated autoimmune destruction of the beta cells of the pancreas. DM has been recognized as a main independent risk factor for cardiovascular diseases. Clinical studies indicate that most diabetic patients die due to cardiovascular

diseases, with atherosclerosis accounting for 8% to 10% of all diabetic deaths. DM is a complex, progressive disease, accompanied by multiple complications. Hyperglycemia has been accepted as being essential for the development of diabetic complications. The Diabetes Control and Complication Trial (DCCT) established that prolonged exposure to hyperglycemia is considered the primary factor associated with the development of diabetic microvascular and macrovascular complications in T1DM. The DCCT showed that improvement of glycemic control, as measured by reduction in glycosylated hemoglobin levels, significantly reduced the risk of development and/or progression of all diabetic complications and also reduced the mortality and morbidity due to cardiovascular diseases in T1DM patients (14,16).

Increased hypoglycemia is a well-known complication of improved glucose control, and an important goal of therapy is to minimize this risk. Therefore, hypoglycemic events should be registered and related, if there is the case, to insulin dosage requirements.

4.2.3 DIAGNOSIS

Table 4. *Criteria for the diagnosis of diabetes* (16)

HbA1c>6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

OR

FPG >126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*

OR

2-h PG >200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

OR

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose >200 mg/dL (11.1 mmol/L).

*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

4.2.4 GLYCEMIC CONTROL

The risk of arterial disease and microvascular complications in T1DM is related with an inadequate metabolic control sustained long terms. Glycated hemoglobin (HbA1c) has proved being a good measure regarding metabolic control, but, given a very strict control relates with an increased number of hypoglycemic events, it is important to identify the optimal value for this parameter which should guide the medical treatment for T1DM patients.

A randomized clinical essay published by *Diabetes Control and Complications Trial Research Group* and the follow up of that same cohort, which measured the apparition of microvascular complications, compared a group of 638 insulin-dependent patients receiving intensive therapy (7% HbA1c average level) vs. another group containing

638 diabetic patients receiving conventional therapy (8,8% HbA1c average level) (17). After a follow up of 6,5 years, it was observed that the group which followed an intensive therapy reduced the risk of retinopathy apparition a 75% and a 54% its progression, the risk of microalbuminuria decreased a 56%, and the risk of neuropathy apparition a 69% and its progression a 57%. However, 3 times more risk of hypoglycemia was observed, as well as a higher overweight rate, in the intensive therapy group than in the conventional therapy group.

In conclusion, intensive therapy with 7% HbA1c average levels reduces the risk of microvascular complications even though it increases the risk of hypoglycemia. HbA1c levels higher than 7% increase the risk of death due to cardiovascular event and all other causes.

Table 5. *Plasma Blood glucose and HbA1c goals for T1DM by age-group (16).*

Values by age (years)	Plasma blood glucose goal range (mg/dl)		HbA1c	Rationale
	Before meals	Bedtime/overnight		
Toddlers and preschoolers (0-6)	100-180	110-200	<8,5%	▪ Vulnerability to hypoglycemia ▪ Insulin sensitivity ▪ Unpredictability in dietary intake and physical activity ▪ A lower goal (<8,0%) is reasonable if can be achieved without excessive hypoglycemia
School age (6-12)	90-180	100-180	<8%	▪ Vulnerability of hypoglycemia ▪ A lower goal (<7,5%) is reasonable if can be achieved without excessive hypoglycemia
Adolescents and young adults (13-19)	90-130	90-150	<7,5%	▪ A lower goal (<7%) is reasonable if can be achieved without excessive hypoglycemia

Key concepts in setting glycemic goals:

- Goals should be individualized and lower goals may be reasonable based on benefit-risk assessment.
- Blood glucose goals should be modified in children with frequent hypoglycemia or hypoglycemia unawareness.
- Postprandial blood glucose values should be measured when there is a discrepancy between preprandial blood glucose values and HbA1c levels and to help assess glycaemia in those on basal-bolus regimens.

Clinical Practice Guideline of T1DM of the Sociedad Española de Endocrinología Pediátrica and *Diabetes Care* 2015 publication recommends informing the T1Dm patients and their families of the benefits of a long-term metabolic control with HbA1c levels lower than 7% (46 mmol/mol) without disabling hypoglycemic events. Therefore, the healthcare program should be designed to reach those targets. The objectives of the treatment must be individualized and negotiated with the patients, taking into account risks and benefits (14,16).

4.2.5 ROLE OF INSULIN RESISTANCE IN T1DM

Intensive diabetes management is associated with both improved insulin sensitivity and beta cell function (18,19). Investigators interested in the pathogenesis of T1DM have appropriately focused on the role of insulin secretion. However, the maintenance of normoglycemia is actually the result of the interplay of islet beta cell secretion and insulin sensitivity of the periphery and liver (with additional contributions from the effect of glucose to facilitate its own uptake).

Though insulin resistance is a prominent clinical feature in patients with T2DM, defects in insulin secretion must also be present in this population before glucose abnormalities ensue. In the face of a stable deficit in insulin secretion, therapies that reduce insulin resistance such as diet and exercise changes, and treatment with thiazolidinediones, result in improved glucose tolerance. However, in most patients with T2DM, progression of the beta cell defect eventually reduces de clinical benefit of this interventions and insulin therapy is required.

In contrast, defects in insulin secretion through both beta cell loss and dysfunction are the prominent feature of T1DM.However, the physiological relationship between secretion and resistance still holds and determinates the state of glucose tolerance. Thus, baboons with reduced beta cell mass induced by the beta cell toxin, streptozotin, have reduced insulin secretion but do not demonstrate markedly abnormal glucose tolerance until either additional loss of beta cells or induction of insulin resistance (20).

A study of 53 T1DM patients grouped at 2-10 years, 11-20 years, and >20 years from diagnosis demonstrated a significant reduction in insulin sensitivity as compared with control subjects and T1DM patients during the first year of disease. These subjects also had a decrease in fasting C-peptide, an increase in insulin dose, and an increase in HbA1c as compared with type 1 subjects during the first year of disease (21).

As insulin action is the net effect of circulating insulin and tissue sensitivity to insulin. Improvements in insulin sensitivity are unlikely to have significant clinical effect in subjects without residual beta cell function. However, early in the disease process, most

subjects maintain a limited capacity to secrete insulin and thus could theoretically benefit from treatments that increase insulin sensitivity. A small improvement in diabetes control and decrease in insulin requirement has been reported in two small studies using metformin in subjects with early T1DM, but also showed an increase of hypoglycemia. In addition, improvements in insulin resistance can be seen with changes in diet and exercise. Though most pronounced in T2DM in whom only a small change in weight and/or increase in exercise is beneficial, it is possible that these factors are important in some subjects with T1DM.

It has been observed an increased incidence of clinical T1DM during puberty and the anecdotal reports of a precedent illness, which could be attributed to the influence of relative insulin resistance during these time periods (19).

4.3 VITAMIN D AND DIABETES MELLITUS

Few literature can be found regarding the relation between vitamin D supplementation and improvement of glycemic control in T1DM, however that would not be the case between vitamin D repletion and T2DM (22–24). A systematic review of 8 observational cohort studies and 11 RCTs (25), concluded that an inverse association between the vitamin D status (25(OH) D or self-reported vitamin D intake) and the development of type 2 diabetes could be observed in the observational longitudinal studies. In RCTs, vitamin D supplementation did not show any beneficial effects on glycemic measures among persons with normal glucose tolerance, however there were beneficial effects among patients with glucose intolerance or insulin resistance at baseline.

As discussed previously, insulin resistance plays a larger role in the T1DM disease process than is commonly recognized. Subsets of people with mild manifestations of the T1DM could benefit from treatments aimed at improving the insulin-resistant state (2,18,19).

Several studies show a high prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes, ranging between 43 and 91% (2,10,26). In adults, low 25-OH D concentration is found to be associated with high risk of hyperglycemia, insulin resistance and T2DM (2,13). Vitamin D is required for and improves the production of insulin, and also improves insulin sensitivity (27). Insulin secretion is impaired when levels of vitamin D are low, but hypocalcemia that accompanies vitamin D deficiency also plays a role in this phenomenon (12,28).

In vitro, vitamin D acts as an immunosuppressive agent, reducing lymphocyte proliferation and cytokine production (9). On this basis, a 2015 clinical trial evaluated vitamin D repletion effect on T cell attack on beta pancreatic cell (29). Concluded that cholecalciferol supplementation improves suppressive capacity of T regulatory cells in T1DM patients, but not enough to stop beta cell downfall. Nevertheless, on the process, less insulin requirements were observed in vitamin D supplemented group. However,

the trial was designed for another purpose; therefore, conclusions should not be drawn but could be taken into account.

5 CURRENT STUDIES

Due to recent progress, vitamin D is a booming topic. When searching for "vitamin D" in http://www.ncbi.nlm.nih.gov/pubmed results in 65.374 items. Last year, another trial protocol was elaborated through University of Girona aiming to evaluate the relation between vitamin D and obesity in pediatric patients (30). Similarly, a 2014 high school research project objectified vitamin D insufficiency in 75% in 108 adolescents from Girona, which was the seed that originated this protocol (31).

Regarding current literature relating glycemic control in T1DM and vitamin D repletion, 19 studies were found through search in https://clinicaltrials.gov/ using as keywords: "vitamin D" AND "type 1 diabetes". Of those 19, only 3 met similar characteristics with the purpose this protocol proposes. None of them had results nor publications posted.

In the same way, a search was performed in https://www.clinicaltrialsregister.eu/ctr-search/search. 6 studies were found, none of which aimed to study the effect of vitamin D repletion in glycemic control in vitamin D insufficient T1DM patients.

Finally, same search was performed through http://www.ncbi.nlm.nih.gov/pubmed resulting in 426 studies. In order to pin down the search it was modified to: "vitamin D" AND "type 1 diabetes" AND "glycemic control", resulting into 17 studies, of which 4 met characteristics with this protocol. The studies found are summarized in the table 6 (2,10,32,33).

During the elaboration of this protocol, pediatric endocrinologists from Hospital Dr. Josep Trueta, Dra. Ruiz-Cuevas and Dr. López, attended the European Society of Pediatric Endocrinology (ESPE) in Barcelona. 3 study posters were presented, with similar features to this protocol. Even though, no publication of these studies has been yet released, information obtained from the posters has been included in the following table and added to Annex 5: Posters European Society of Pediatric Endocrinology (ESPE).

Table 6. Literature regarding the subject

TITLE – AUTHORS – YEAR (Bibliography)	*CHARACTERISTICS /*	*CONCLUSIONS*	*LIMITATIONS*
Glycemic changes after vitamin D supplementation in patients with type 1 diabetes mellitus and vitamin D deficiency - Aljabri K, Bokhari S, Khan M - 2010 (2)	Prospective, non-blinded, non-randomized trial involving 13-29 year old subjects, who were assigned to receive 4000 UI of cholecalciferol. N = 80	Inverse relationship between 25-OH vitD and HbA1c levels following 12 weeks of vitD therapy.	No control group, does not examine insulin dosage requirements, no observation between vitD levels and glycaemia, short duration (12 weeks), does not take into account nutritional factors or solar exposition and does not examine VDBP.
Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomized, placebo controlled trial. – von Hurst P, Stonehouse W, Coad J – 2010 (32)	Prospective, blinded, randomized, placebo controlled-trial involving 23-68 year old South Asian women who received 4000 UI/daily of cholecalciferol or placebo during 6 moths. N = 106	After 6 months of vitD therapy, significant improvements in insulin sensibility in comparison to placebo.	Does not examine VDBP, does not take into account nutritional factors or solar exposition.
Effects of vitamin D repletion on glycemic control and inflammatory cytokines in adolescents with type 1 diabetes. – Shih E, Mittelman S, Pitukcheewanont P, Azen C, Monzavi R – 2014 (10)	Prospective, non-blinded, randomized, crossover study involving adolescents T1DM for at least a year. Subjects received 20.000 UI/week cholecalciferol for 6 months, either immediately or after 6 months of observation. N = 25	63% subjects screened were vitamin D deficient. Treatment did not affect HbA1c, insulin dosage, CRP, IL-6, or TNFα.	Small sample, no control group, post-puberty: GH peak in adolescence promotes insulin resistance, only 6 months therapy, does not include nutritional factors or solar exposition, and does not examine VDBP.
Effect of Vitamin D3 Supplement in Glycemic Control of Pediatrics with Type 1 Diabetes Mellitus and Vitamin D Deficiency - Mohammadian S, Fatahi N, Zaeri N, Ali Vakili M – 2015 (31)	Prospective, non-blinded, non-randomized control trial involving 6 months old to 7 year old subjects, who were treated with 300.000 UI vitD if found vit D deficient or insufficient . N = 53	VitD supplement causes the improvement of HbA1c. Insulin requirement for each patient was not changed during study and pre and post vitamin D supplementation.	No control group, wide age range, does not take into account nutritional factors or solar exposition and does not examine VDBP.

TITLE – AUTHORS – YEAR (Bibliography)	*CHARACTERISTICS*	*CONCLUSIONS*	*LIMITATIONS*
Vitamin D status in Egyptian children with T1DM and the role of Vitamin D replacement in glycemic control – Hafez M, Hassan M, Musa N, Sharaf S, Abdel S – 2015 – Annex X: Posters European Society of Pediatric Endocrinology (ESPE) – No publication available	Prospective cohort study involving T1DM >1 year onset patients above 5 years old. Those with vit D deficiency were supplemented during 3 months. N = 50	Checking the serum 25(OH) D levels in children and adolescents with T1DM and providing replacement for children with low levels improved glycemic control at 3 and 6 months after therapy in those with low levels with no reduction in insulin requirements.	Compares results to supplemented sufficient patients, no observation between vitD levels and glycaemia, short duration (6 months), does not take into account nutritional factors or solar exposition, VDBP
Correlation of vitamin D levels with glycemic control, total daily insulin dose, BMI and ethnicity in pediatric patients with type 1 diabetes mellitus – Bianco M, Durazo R, Durazo-Arvizu R, Minutti C – 2015 – Annex 5: Posters European Society of Pediatric Endocrinology (ESPE) – No publication available	Cross-sectional study involving T1DM patients aged 3-20 years old in whom vitD levels, HbA1c and daily insuline requirement were measured. N = 162	Findings suggest that lower levels of vitD may contribute to the need for higher insulin doses, which may be related to insulin resistance and suboptimal glucose control in pediatric patients with T1DM.	Wide age range, no observation between vitD levels and glycaemia, does not take into account nutritional factors, solar exposition or VDBP.
Vitamin D deficiency in children with DM1A in northern Spain. – Diez I, Sarasua A, Lorente I. – 2015 – Annex X: Posters European Society of Pediatric Endocrinology (ESPE) – No publication available	Prospective open intervention, non-blinded, non-randomized involving T1DM patients with at least 12 months of evolution. Intervention consists on 3 months of outdoor activities and 3 months of 25.000 UI cholecalciferol supplementation (1 every 3 weeks). N = 57	Children with T1DM in Alava have an important deficiency of vitD. The outdoor activities in summer and the treatment with depot preparations is effective in correcting this deficit. Although no significant improvement in metabolic control has been observed.	No control group, does not examine insulin dosage requirements, no observation between vitD levels and glycaemia and does not take into account VDBP.

6 JUSTIFICATION

It has been discovered that 3% of the human genome is regulated directly or indirectly by the vitamin D and that with adequate levels of vitamin D, if the established relations with cancer and others are correct, death rate would drop a 7% (1). Moreover, it has been estimated that more than 30-50% of all children and adults are at risk of vitamin D deficiency (12,34). Literature suggests that T1DM patients may be even at a higher risk of vitamin D insufficient levels (23,26,35).

On this basis, 24 frozen serums from T1DM patients, ages ranging between 1 to 14 years old, were analyzed. The serums were obtained on the diabetic debut, before any medical intervention in the daily routine of the patients was applied. Informed consents were signed by each guardian of the patient and approved by the Ethic Committee of Hospital Dr. Josep Trueta, allowing the serums to be frozen and being used in any possible future investigation. The analysis results are printed in the following graphic:

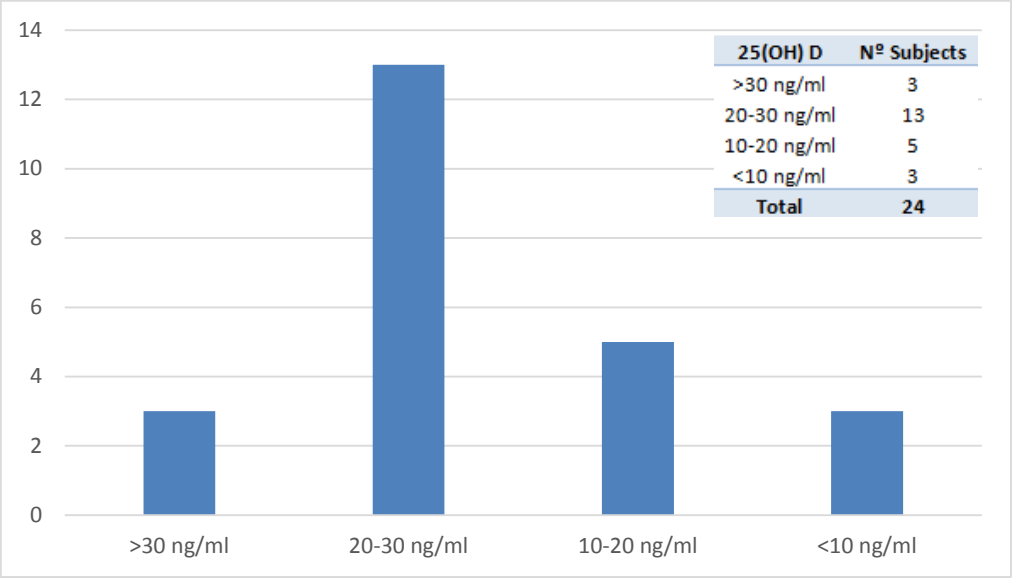


Figure 3. *25(OH) D levels in T1DM onset.*

Even though this data is not representative of T1DM population, because no sample was calculated in order to draw conclusions from the results, data reinforce the idea that vitamin D levels in T1DM pediatric population may be below normality.

A *Diabetes Care* publication from 2015 exposed that there is no clear evidence of the benefit from vitamin or mineral supplementation in people with diabetes who do not have underlying deficiencies (16). Nevertheless, this conclusion does not contemplate whether T1DM patients with levels below sufficiency would benefit from vitamin D repletion.

Likewise, the publication reports a lack of evidence to support the routine use of micronutrients such as chromium, magnesium, and vitamin D to improve glycemic control in people with diabetes. However, this conclusion is drawn from 4 trials evaluating vitamin D supplementation in T2DM patients and another assessing the effect in diabetic subjects with normal serum 25(OH) D levels (36–40). Instead, this trial aims to evaluate the effect of vitamin D repletion in T1DM pediatric patients, given it has been observed a higher incidence of insufficient levels also in that population.

Currently, the screening and treatment of vitamin D deficiency is not considered standard of care for T1DM patients (14). When contacted, pediatric endocrinologists of Hospital Sant Joan de Déu and Hospital Vall d'Hebron, expressed interest in this protocol, and given the current evidence on the benefits of vitamin D, are considering include the determination of vitamin D in the diagnosis and monitoring protocol of children with DM1.

7 HYPOTHESIS

7.1 MAIN HYPOTHESIS

Vitamin D repletion in T1D pediatric patients with vitamin D deficiency improves glycemic control.

8 OBJECTIVES

8.1 MAIN OBJECTIVE

The goal of the present work is to evaluate glycemic control after vitamin D repletion in T1DM pediatric population with vitamin D levels ranging below normality. Glycemic control will be evaluated on basis of glycated hemoglobin.

8.2 SECONDARY OBJECTIVES

- To determine the effect of vitamin D repletion on insulin requirement of T1DM pediatric population.

- To evaluate levels of PTH after vitamin D repletion in T1DM pediatric population.

9 METHODOLOGY

9.1 STUDY DESIGN

This protocol is for a multicenter, prospective, randomized, double blind, placebo controlled 12 months clinical trial in pediatric patients with T1DM. The study will be conducted in Hospital Josep Trueta, Hospital de Palamós, Hospital de Calella, Hospital de Figueres and Hospital de Blanes, being the first one the center of reference. A principal investigator will be assigned in each of the involved centers, in order to form a Steering Trial Committee and enhance communication between the centers.

9.2 STUDY POPULATION

This is a multicentric study of pediatric T1DM subjects. The participating centers were consulted regarding the actual number of T1DM pediatric patients in order to evaluate the viability of this protocol:

- Dr. Josep Trueta Hospital: 202
- Hospital of Palamós: 26
- Hospital of Calella: 19
- Hospital of Figueres: 16
- Hospital of Blanes : 11

9.3 SAMPLE SELECTION

Table 7. *Inclusion & exclusion criteria.*

INCLUSION CRITERIA
✓ Vitamin D levels <30 µg/ml.
✓ Age ≥ 4 years old.
✓ Prepuberal Tanner <2.
✓ T1DM >12 months evolution.
✓ HbA1c 7-9%.
✓ Caucasian ethnicity.
EXCLUSION CRITERIA
× Currently receive vitamin D supplementation.
× Vitamin D severe deficiency defined as <10 µg/ml or Rickets
× Subjects with severe chronic disease.
× Other Disorders of calcium metabolism or hypercalcemia.
× Kidney dysfunction (serum creatinine >1,2 mg/dl).
× Hepatic dysfunction (AST and ALT >2,5 times the upper limit of normal.
× Known or suspected malabsorption (such as celiac disease).
× Subjects receiving chronic medical treatment or hyperglycemic drug (14,41):
o Sympatico mimetic hormones (Antihypertensive)
o Antiarrhythmic or antiepileptic
o Immunosuppressive or immunomodulative
o Antiviral
o Miscellany
× Subjects without signed informed consent.

9.3.1 INCLUSION CRITERIA JUSTIFICATION

- ***Vitamin D levels <30 µg/ml:***

As discussed earlier, even though the Institute of Medicine defined vitamin D insufficiency as <20 µg/ml, controversy still surrounds this topic. Therefore, in order to provide more evidence to the subject, previous values have been selected to define these criteria.

- ***Age ≥ 4 years old:***

Glycemic control targets differ depending on the age of the patient (see table 5), also there are factors, such as physical activity, which affect glycemic control and differ considerably between ages. Therefore, age will be taken into account during randomization in order to achieve balanced groups.

- ***Prepuberal Tanner <2*** (42)***:***

During adolescence, T1DM have showed a decrease of glycemic control due to hormonal changes to adulthood. For example, the secretion of growth hormone during adolescence relates to an increase of insulin resistance. In order to avoid heterogeneity produced by this hormonal instability it has been decided to exclude pubertal patients.

- ***T1DM >12 months evolution:***

10% to 30% of the functional beta-cell mass remains at the time of diagnosis (12). Therefore, during the initial period after diagnosis of T1DM, some insulin is still being produced, which lead to a decreased even no demand of exogenous insulin requirement with maintenance of relatively normal glucose levels. This period, usually peaking 3 months after diagnosis, is commonly known as “honeymoon remission phase”, which has been reported to occur in up to 50% of individuals within the first year after diagnosis (14,18,19). Whether is result from improved insulin secretion due to resolution of the acute inflammatory response or from improved insulin sensitivity is

still on debate. In order, to avoid confusing this situation with the effect of the vitamin D we will select research subjects who have passed this period.

- ***HbA1c 7-9% (53-75 nmol/mol):***

Only subjects within a HbA1c range (7-9%, 53-75nmol/mol) will be evaluated. Upper limit was selected in order to minimize effects of extremely poor control and avoid other variability produced by a heterogeneous sample. On the other hand, lower limit is the established by Clinical Practice Guidelines as target for a proper glycemic control, therefore once achieved little effect may be observed from vitamin D (14,16).

- ***Caucasian ethnicity:***

There is evidence that in Asian Indian there is increased 24-hydroxylase activity, which inactivates the hormone 1,25 (OH)2 D (43). Dr. Sánchez Muro, evaluated vitamin D levels in 310 children attending Primary HCS in Salt. Results show higher incidence of deficiency levels in immigrant population, fact the investigation relates more to cultural reasons that to nutritional ones (44). Furthermore, due to differences of VDBP levels, therefore total 25-OH D, between racial groups, it was decided to limit the study to the most prevalent one in order to avoid confusion produced by these factors (6).

9.3.2 WITHDRAWAL CRITERIA

Subjects may be withdrawn from the study for the following reasons:

- Patient is not willing to comply with the protocol.
- For medical reasons (adverse event) under investigator criteria.
- Participant retires consent to continue in the study.

Subjects withdrawn from the trial will not be replaced.

9.4 SAMPLE SIZE

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, **79** subjects are necessary in first group and **79** in the second to recognize as statistically significant a difference greater than or equal to 0.6 units. The common standard deviation is assumed to be 1.2 and the correlation coefficient between the initial and final measurement as 0.5. It has been anticipated a drop-out rate of 20%.
The source where the sample was calculated is:

http://www.imim.cat/ofertadeserveis/software-public/granmo/

A total of 158 patients should be recruited in order to perform this trial and Hospital Dr. Josep Trueta disposes of 202 patients, some of which may not be included inside the trial. Therefore, other centers were contacted, turning the trial into multicentric, in order to raise the patient selection pool to 274, value expected to fulfill the required sample size.

9.5 ENROLLMENT AND PATIENT SELECTION

Consecutive non-probabilistic sampling will be applied. Potential research subjects will be determined from the lists of T1DM pediatric patients in the hospitals participating in the study, based on inclusion criteria available in clinical history. An appointment will be scheduled, in which the investigator will inform the patient and guardians about the study and sign written informed consent (see Annex 4: Consents and Patient Informative Sheet).

Vitamin D status must be established before including participants in any group, in order to exclude vitamin D sufficient patients and exclude and treat those patients with severe deficiency. Therefore, 4 weeks before the start of the study, potential research subjects will meet the investigators for a complete history and physical examination and to have baseline laboratory measurements, including HbA1c, 25(OH) D, calcium, phosphorus, magnesium, parathyroid hormone and VDBP. Creatinine, AST and ALT will also be measured as they are exclusion criteria.

The estimated time of recruitment will be of 6 months. Due to variability vitamin D levels depending on the season, patients with levels close to insufficiency during spring-summer period may turn out insufficient during winter. Therefore, those patients with normal levels tested during spring-summer, will be retested during autumn-winter period. On the other hand, patients who test insufficient levels during spring-summer, will most probably test insufficient also during autumn-winter period, and hence will be directly included in the study if they meet the rest of inclusion criteria.

9.6 RANDOMIZATION

Eligible participants will be randomized 1:1 using block randomization into treated and placebo groups.

Because of the variability produced by age, sex and BMI in DM1 disease and vitamin D levels, to guarantee the randomization, the sample will be balanced by:

- Age (one group with ages between 4 and 8 years and second group with ages ≥ 9 years and Tanner <2).
- Sex (male and female).
- BMI (BMI-SDS over 2 SD will be balanced between treatment and placebo group).

9.7 VARIABLES AND INSTRUMENTATION

Table 8. *Variables and instrumentation*

	VARIABLE	TYPE	INSTRUMENTATION	UNITS
Independent	Vitamin D supplementation or placebo	DcQV	Deltius® Cholecalciferol 25000 IU/ 2,5 ml oral solution (Annex 3) or placebo	-
Dependent	HbA1c	CQV	Siemens DCA Vantage Analyzer	%
	Insulin dosage requirement	CQV	Total daily insulin dosage	Units/kg/day
	PTH	CQV	SEIMENSE chemiluminoscence	pg/ml
	BMI	CQV	Scale and stadiometer	SDS
Covariates	Geographical latitude	NQV	Cartographic map	-
	Sun exposure	NQV	Questionnaire (Annex 2)	Score
	Season	NQV	Follow up analysis date	-
	Age	CQV	Clinical exploration	-
	Sex	DcQV	Clinical exploration	-
	Weight	CQV	Calibrated scale (45)	Kg
	Height	CQV	Harpenden stadiometer (45)	Cm
	Waist circumference	CQV	Measuring tape	Cm
	Diet	NQV	Questionnaire (Annex 2)	Score
	Physical activity	NQV	Questionnaire (Annex 1)	Score
	Family history diabetes	NQV	Clinical medical history	-
	Laboratory	NQV	Lyason, Roche or DiaSorin (1)	-
	Vitamin D binding protein	CQV	Commercial enzyme linked immunosorvent assay (R&D Systems)	ng/mL
	Free vitamin D	CQV	Vitamin D equation (6)	%
	Hypoglycemic events (<72mg/dl)	DcQV	Accu-Check Aviva ® (46)	-
	Acute episode	DcQV	Clinical medical history	-
Safety	Urine calcium/creatinine ratio	CQV	Colorimetric method. Express plus analyzer (Chiron)	mg/mg

*DQV: Discrete quantitative variable. *CQV: Continuous quantitative variable. *NQV: nominal qualitative variable. *DcQV: Dichotomy qualitative variable

10 INTERVENTION

10.1 ORGANIZATION AND DATA COLLECTION

The intervention visits will take place during the routine follow-up visits for diabetic patients established every 6 months. By not adding new visits, the trial should not affect patients' routine; therefore, less drop outs are expected.

In order to optimize study adherence, guardians and subjects will receive text message or e-mail reminders on a weekly basis to take the vitamin D/placebo during the treatment period. Also, 1 week in advance, guardians and subjects in both groups will receive text message or e-mail reminders about their upcoming appointments. Treatment bottles will be tallied following completion of the treatment period, as an indicator of adherence.

At each visit, follow-up measurements below will be tallied:

Table 9. *Follow up measurements*

- HbA1c	- Insulin dosage requirement
- Hypoglycemic event	
- 25(OH) D	- PTH
- Sun exposure	- Season
- Diet	- Physical activity
- Weight	- Height
- Waist circumference	- BMI
- Calcium/creatinine ratio	

The follow-up measurements should not be realized when the patient presents acute symptomatology (such as any pulmonary infection, very common in pediatrics). Acute symptomatology increases the demand of insulin; therefore it needs to be taken into account when registering data.

As established by protocol, venous blood sampling will be drawn with the child in fasting state. All serum samples will be obtained between 8:00 a.m. and 10:00 a.m. in order to avoid variability produced by circadian hormones.

Measurements and questionnaires will be taken during follow up visit in all children and will be performed by the same observer who will be unaware of the subjects' clinical and laboratory vitamin D characteristics.

- Weight should be measured with the child wearing light clothing on a calibrated scale.

- Height should be measured with the child wearing no shoes on a Harpenden stadiometer.

- BMI will be calculated as weight divided by the square of height in meters. Age- and sex-adjusted z-score values for current weight, height, and body mass index [BMI] will be calculated using regional normative data (45).

- Waist circumference will be measured at the umbilical level with the child in the supine position with a measuring tape.

10.2 QUESTIONNAIRIES

Vitamin D questionnaire

Garabedian test (1999) provides information on the daily intake of vitamin D through a system score, by which a value of vitamin D is associated with each food according to the amount ingested. After the survey, all the points are added, obtaining a result expressed as IU of vitamin D per week. At this point, it is just required to pass that value to IU / day to obtain vitamin D obtained by nutrition daily. The drawback of the test is that Garabedian Scoring is based on the recommendations of the time (1999). However, recent recommendations have increased the intake of vitamin D in order to ensure benefits of vitamin D both bone and extra bone. For example, in the original test, an intake of vitamin D of 201 IU / day was considered a high amount, when nowadays would be considered low. In addition, some foods have changed the amount of vitamin D that provide, such as fortified milk, and for that reason, those values had to be modified. Current vitamin D content of the products was researched in order to establish new scores (7).

Garabedian test score:	**Modified Garabedian test score:**
Max. punctuation: 51,5 points.	Max. punctuation: 94,5 points.
1 point = 100 IU vitamin D/week.	1 point = 100 IU vitamin D/week.

Vitamin D intake recommendations in 1999 (44)**:**

- Low intake <5,6 (<80 IU/day)
- Medium intake 6,6-14 (80-200 IU/day)
- High intake >14 (>200 IU/day)

Current Vitamin D intake recommendations:

- Low intake <28 (<400 IU/day)
- Medium intake 28-42 (400-600 IU/day)
- High intake >43 (>600 IU/day)

Calcium questionnaire

Calcium assessment is included in this survey because it is one of the most important nutritional factors and is closely related with vitamin D. A calcium intake ensures proper bone remodeling and the absorption capacity depends not only on the amount of calcium offered, but the content of vitamin D in the body. Calcium intake is measured with the same scoring system that vitamin D, and therefore changes in the test are similar. The maximum score changes, and supplemented foods such as calcium fortified milk are added to the questionnaire. The results are obtained in milligrams of calcium per week, so must be converted to milligrams calcium/day in order to meet daily intake.

Garabedian test score:	**Modified Garabedian test score:**
Max. punctuation: 120 points.	Max. punctuation: 153 points.
1 point = 120 calcium mg/week.	1 point = 120 calcium mg/week.

Current recommendations for calcium intake for children between 9 and 18 of age have not changed so much as with vitamin D are considered a those adequate levels of around 75 points, which means an intake of 1300 mg / day.

Solar exposure questionnaire

This section discusses issues that assess time of sun exposure, season during which the individual is exposed to the sun, the time slot, location during exposure and finally, if the person wears sunscreen or not. For Garabedian, the maximum score is 9, and if the individual would be wearing a sunscreen with a protection factor (PF) greater than 15 in exposure, only 1 point of the total valuation would be deducted. Given that with a cream than 15 FP 94% of the sun's rays are not absorbed to vitamin D synthesis, the score is the total should be deducted (3). For this reason, the item regarding sunscreen was modified.

Modified Garabedian questionnaires can be found in Annex 2.

10.3 STUDY TREATMENT

10.3.1 INTERVENTION DRUG

Calcitriol or 1,25(OH)2 D, the active metabolite of vitamin D, would not be the best option as therapy drug. As commented before, 1,25(OH)2 D half-life is much shorter, therefore constant administration should be provided and levels tested. Also, as the active metabolite, ten times more powerful than 25(OH) D, could more easily lead to hypercalcemia.

On the other hand, 25(OH) D can deposit in fat and muscular compartments extending the time inside the organism from 15 days to months. Moreover, being an intermediate metabolite, and as the negative feedback of vitamin D metabolism is regulated by 1,25(OH)2 D, its accumulation will not develop a blockage of the calcitriol synthesis.

However, a 25(OH) D drug is not available, but substances prior to the hepatic hydroxylation that produces calcidiol are, such as, ergocalciferol (plant derived) and cholecalciferol (animal derived). Studies postulate that cholecalciferol may have higher affinity for VDBP, which translates into a higher potency than ergocalciferol (2,47,48). Moreover, Vitamin D2 has several unknown metabolites with unknown effects.

In conclusion, the selected experimental drug will be Deltius ®, produced by Italfarmaco S.A. (Madrid), consists in cholecalciferol 25.000 IU/ 2,5 ml oral solutions. Further information regarding Deltius® can be found in Annex 3: Deltius Public Datasheet.

10.3.2 VITAMIN D DOSAGE

In 2011, Food and Nutrition Board of the *Institute of Medicine* published a large actualized guide regarding vitamin D nutritional needs in the different stages of life (7):

Table 10. *Vitamin D intake recommendations*

Age group	EAR (IU/day)	RDA (IU/day)	Tolerable upper intake (IU/day)
0-6 months	400	400	1000
6-12 months	400	400	1500
1-3 years	400	600	2500
4-8 years	400	600	3000
9-18 years	400	600	4000

*EAR: Estimated Average Requirement *RDA: Recommended Dietary Allowances

The age of the subjects will range between 4 and 12, this last one depending on pubertal development state. Therefore, RDA should be 600 IU/day. However, in order to correct vitamin D levels faster and develop an easier routine for the subjects, the 25.000 IU of cholecalciferol will be given every month, which translates into 833,33 IU/day, value inside the safety range established by the *Institute of Medicine*.

Also, literature postulates that supplementing with ≤ 250 µg/week (≤ 10000 IU/week) can improve or maintain vitamin D status in healthy populations without the risk of hypercalciuria, but 24 h urinary Ca excretion should be evaluated in healthy persons receiving Vitamin D3 supplementation in weekly single doses of 1250 µg (50000 IU) (49). The study treatment (25000 IU/month or 833,33 IU/day) would be placed inside the range.

10.3.3 TREATMENT DURATION

In a 2015 clinical trial performed in the University of Graz (Austria), cholecalciferol supplementation was given to patients with new-onset T1DM during 12 months, in order to assess the suppressive capacity of regulatory T-cells (29). A cholecalciferol dose of 140 IU/kg/day were given the first 3 months, 70 IU/Kg/day afterwards, doses higher than those planned to give in this study. Results regarding the safety of 12 months cholecalciferol were: 50% of patients in the treatment group had a serum of 25(OH) D level above 150 nmol/L after 3 months, 64% after 6 months and 36% after 12

months. Nevertheless, calcium concentrations were similar in the treatment and placebo group during the whole study period and stayed within the normal range in all participants. In conclusion, it would be safe to assume that long term cholecalciferol supplementation would have little negative effects in study subjects.

In order to evaluate vitamin D3 repletion effect in glycemic control, a long period of sufficient vitamin D levels are required. Therefore, a 12 months supplementation was established. Moreover, difference in vitamin D levels between seasons has been observed (35,50), due to sun exposure differences. A 1 year duration may allow the gathering of enough data to compare between seasons, characteristic which some actual studies have not taken into account (2,10,33).

10.3.4 DRUG CHARACTERISTICS

CONTRAINDICATIONS

- Hypersensitivity to cholecalciferol or any of the excipients.
- Hypercalcemia or hypercalciuria.
- Hypervitaminosis D.
- Kidney stones (nephrolithiasis, nephrocalcinosis) in patients with chronic hypercalcemia.

INTERACTIONS

- **Antiepileptic drugs (phenytoin) or barbiturates**: hepatic enzyme-inducing drugs may reduce the effect of vitamin D3 through its metabolic inactivation.
- **Thiazide diuretics:** can reduce urinary calcium excretion, therefore monitoring of serum calcium concentrations is recommended.
- **Glucocorticoids:** can decrease the effect of vitamin D.
- **Medications containing digitalis or other cardiac glycosides:** administration of vitamin D may increase the risk of digitalis toxicity (arrhythmia). In these cases,

strict medical supervision is necessary as well as monitoring of serum calcium and, if necessary, electrocardiographic controls.

- **Ion exchange resins or laxatives:** gastrointestinal absorption of Vitamin D can be reduced by simultaneous treatment.
- **Some cytotoxic agents such as actinomycin and imidazole antifungals:** interfere with vitamin D activity of inhibiting the conversion of 25(OH) D to 1,25(OH)2 D by renal 25(OH) D hydroxylase enzyme.

ADVERSE EFFECTS

Frequencies of adverse reactions are defined as:

Uncommon (> 1 / 1,000 to <1/100):

- Metabolism and nutrition disorders: hypercalcemia and hypercalciuria

Rare (> 1 / 10,000 to <1 / 1,000):

- Disorders of the skin and subcutaneous: itching, rash and urticaria.

It is important to report suspected adverse drug reactions after approval. This allows continuous monitoring of the benefit / risk of the drug. If suspected new adverse reactions are detected, they will be reported through the Spanish Pharmacovigilance System for Medicinal Products for Human Use:

www.notificaRAM.es

OVERDOSE

Deltius treatment should be stopped when the serum calcium exceeds 10.6mg / dl (2.65 mmol / l) or calciuria exceeds 300mg / 24 hours in adults or 46 mg / kg / day in children.

Overdose is manifested by hypercalcemia and hypercalciuria, whose symptoms include:

- Nausea
- Vomiting
- Thirst
- Constipation
- Polyuria
- Polydipsia
- Dehydration

Chronic overdoses can lead to vascular and organ calcification as a result of hypercalcemia.

TREATMENT IN CASE OF OVERDOSE

Discontinue Deltius® and initiate rehydration.

Further information can be found in Deltius® Public Datasheet (Annex 3: Deltius Public Datasheet) or via web (51).

10.4 TREATMENT MONITORING

Vitamin D overdose can be monitored through three different methods: 24 hour calcium in urine, urine calcium/creatinine ratio or serum calcium. In 24h urine calcium, urine sample must be collected along the day several times, meanwhile, more practically, urine calcium/creatinine ratio and serum calcium requires to collect a single sample once. However, serum calcium metabolism interferes in the masking of the trial, because treated patients would increase their calcium levels due to increase of absorption. In conclusion, the best method to monitor vitamin D overdose would be through urine calcium/creatinine ratio, which will be measured at 0, 6 and 12 months. Urine will be collected from the second urination of the morning the day of the visit.

Normal limit of calcium/creatinine ratio is:
- Children ≥ 4 years: < 0,20 mg/mg (52).

If the value of this coefficient is higher, they will be diagnosed as having hypercalciuria.

The treatment will be discontinued in the case of any of the adverse effects previously described and/or ratio calcium/creatinine >0,20 mg/mg.

10.5 MASKING

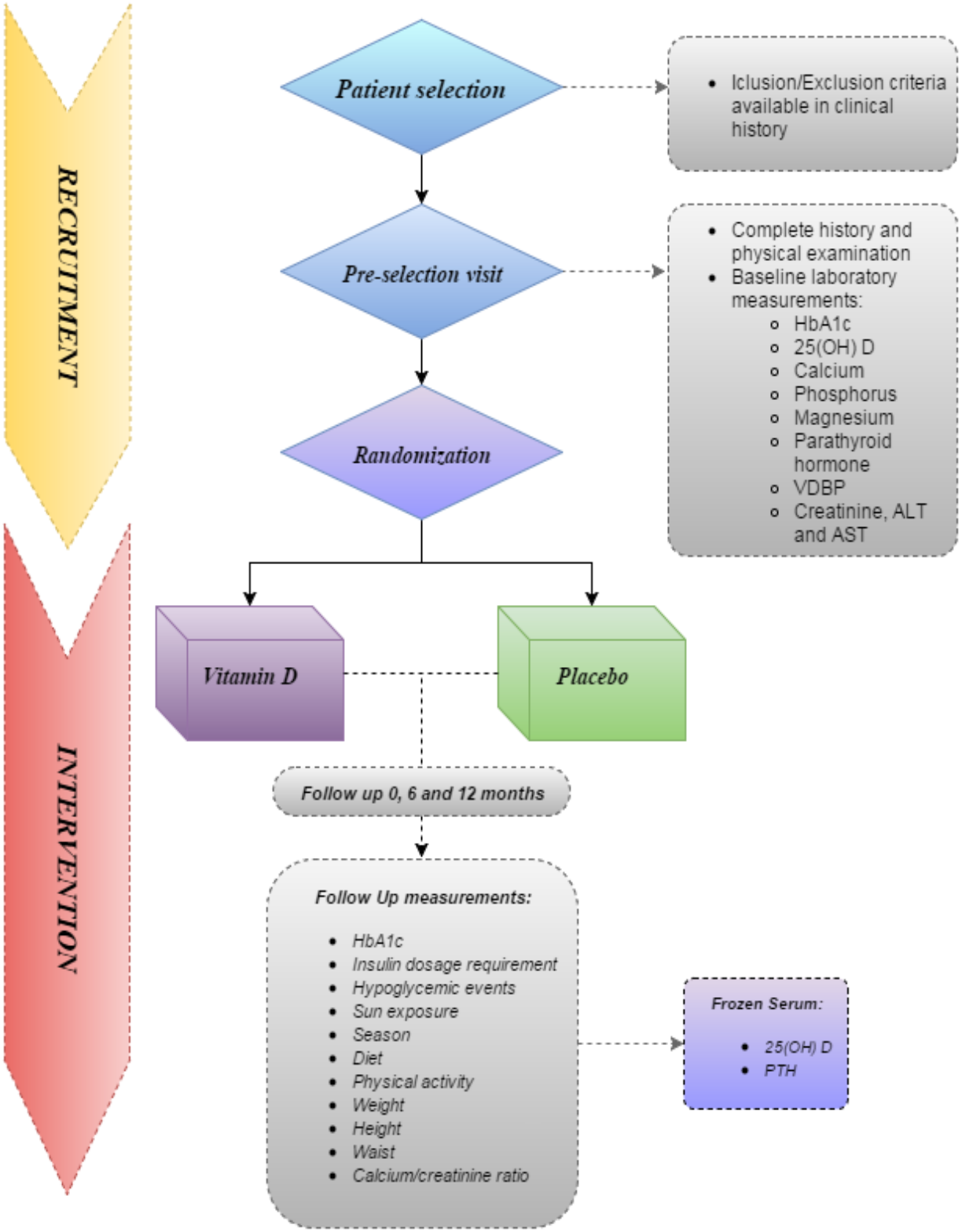
10.5.1 PATIENT MASKING: PLACEBO

Control group will receive placebo. Same price and excipients formula than Deltius® will shape placebo, but without the active ingredient. Communications have been initiated with Italfarmaco S.A. in order to synthesize this placebo.

10.5.2 INVESTIGATOR MASKING: FROZEN SERUM

If, in follow up measurements, 25(OH) D levels and PTH were established, it would become clear which subjects were receiving treatment and which were not. Therefore, serum samples will be frozen, in order to be analyzed at the end of the study in Hospital Josep Trueta, to where will be sent through message service. This way, several advantages are gained. The most clear one being blinding the investigators, but also, analyzing serum in badges and in one single laboratory will reduce variability produced by different lab techniques.

11 FLOW CHART



12 STATISTICAL ANALYSIS

UNIVARIANT DESCRIPTIVE ANALYSIS

Results for quantitative variables assuming a normal distribution will be summarized as mean ± standard deviation (SD), and those for variables without a normal distribution will be expressed as median and interquartile range (IQR). Non-parametric variables will be mathematically transformed to improve symmetry. Frequencies (n) and percentages for each category will be used to describe categorical variables.

BIVARIANT ANALYSIS

Results for major variables at baseline will be compared using Mann-Whitney test (for continuous unpaired variables) and Fisher exact test (for categorical unpaired variables) in order to ensure that comparable treatment groups were produced through the randomization process.

MULTIVARIANT ANALYSIS

General linear model (GLM) will be applied for repeated measures in order to perform the analysis of response to treatment for endpoint variables (HbA1c, Insuline requirement, PTH and BMI) between independent treatment and control groups (main objective of the proposal). The interaction term among the intervention variable and the endpoint variables of the study will be applied in order to highlight differences in 1-year changes across intervention groups. Models will be adjusted for potential confounders (see “covariates” in ***Table 8***). “Intention-to-treat” (ITT), “as treated” and “per protocol” analyses will be applied, in the case of:

- Protocol violations.
- Losses to follow up.
- Withdrawals from the study.
- Non-compliance.
- Refusal of the allocated treatment.

- Other deviations from established protocol.

Imputation of missing values for endpoints variables will be performed using the latest observed values for each variable and subject.

A p value <0.05 will be considered statistically significant.

13 ETHICAL CONSIDERATIONS

The trial will be sent the local independent ethics committee of Hospital Josep Trueta (Girona), in order to be reviewed and approved, before any further procedure.

As actual recommendations postulate, the trial will be registered with an International Standard Randomized Controlled Trial Number (http://www.controlled-trials.com) and will be submitted to ClinicalTrials.gov (http://clinicaltrials.gov.com).

The trial will be conducted in accordance with the four principles for medical research involving human subjects defined by the Declaration of Helsinki.

Before any trial-related activities, all patients and guardians, as all will be under 18 years of age, will be provided with all needed information. Written informed consent will be provided their guardians. However we will obtain informed assent from all children, in order to enroll their participation in the study.

The information of clinical history, names and surnames, will be confidential, guaranteeing the anonymity of the patients involved in the study according to “Ley Orgánica 15/1999, 13 de Diciembre, Protección de Datos de Carácter Personal.

The protocol must be approved by the European Medical Agency (EMA) and Asociación Española de Medicamentos y Productos Sanitarios (AEMPS) before final approval of the clinical trial.

14 LIMITATIONS

Several limitations should be acknowledged:

- Regarding the patient selection, a consecutive non-probabilistic method is going to be applied. Instead of waiting for the follow-up visit to introduce the study to the patients, an appointment will be scheduled. It is expected to reach all patients. Therefore, a non-response rate should be taken into account.

- Regarding inclusion criteria:
    - Honeymoon phase: Even though this period can sometimes last 2 years, we plan to avoid heterogeneity produced by this event through the next inclusion criteria (HbA1c 7-9%), therefore those who are already in "honeymoon" phase (characterized by low or no insulin requirement with correct metabolic control) will not affect the data.
    - Establishing >7% HbA1c as a criteria, limits the applicability of the results of the study in those below the limit. But literature suggests that they may benefit of other aspects of the study, such as a lower insulin dosage requirement.

- Regarding sun exposure, the most precise method to measure would be to ask the patients to wear a meter, not only impractical for the patients but also expensive. Therefore, it was decided to apply a questionnaire, even though it may be subject to survey bias, it would be enough to assess this variable.

- Regarding vitamin D variability, polymorphism of VDBP is not taken into account. Nevertheless, direct measures of VDBP will be performed, which can be related to the different polymorphisms.

- Differences between measurement techniques could cause lack of comparability between the different centers participating in the study. Therefore, as discussed in blinding paragraph, frozen serum analyses will be performed only in Hospital

Josep Trueta. Moreover, variability between glucose testers will be controlled providing the same tester for all subjects.

- Regarding questionnaires, last validated questionnaire to assess vitamin D intake, calcium intake and solar exposure is Garabedian Test (1999). However, this test, as discussed in questionnaires section, is outdated and has been modified in order to adapt current data. Validation of the questionnaires should be sought if possible before application to the trial.

15 WORK PLAN

Personnel:

- Investigators: as exposed previously, a principal investigator will be assigned in each of the participating centers: Hospital Universitari de Girona Josep Trueta, Hospital de Palamós, Hospital de Figueres and Hospital de Blanes. They will form the Trial Steering Committee (TSC). TSC is responsible for trial overall supervision. Research associates (RA) will be selected in each center.
- Collaborators: statistician (ST), pharmacist (PH), laboratory (LA), nursery staff (NU) and data manager (DA).

The trial has been designed in 5 phases:

PHASE 1 - Coordination phase. Development of theoretical framework (6 months): TSC and RA center will be involved in this phase.

- PROTOCOL ELABORATION (Accomplished): objectives will be established in order to answer a formulated hypothesis. A comprehensive literature search will be conducted, followed by a detailed definition of study variables and study design. Theoretical methodology of data collection will be elaborated.

- ORGANIZATIONAL MEETING will be held in order to schedule a study chronogram and establish a timeline. Data collection circuits and communication systems between the involved centers will be set up.

- PILOT TEST will take place in order to evaluate the protocol, to identify problems and to apply corrections.

- AUTHORIZATIONS: administrative and ethical authorization will be asked before any further activity.

PHASE 2 - Field research (18 months): All the study staff from each center will be involved.

- RECRUITMENT PERIOD (6 months): sample selection will be made applying inclusion and exclusion criteria. A member in charge of patient flow analysis evaluates the recruitment time in order to prove that the rate of inclusion of patients is being fulfilled. Informative documents regarding the study must be exposed to subjects and informed consent signed before any further activity in the trial.

- GROUP ASSIGNMENT: randomization procedure, masking method and allocation type will be performed.

- INTERVENTION (12 months): study variables will be recorded in a common database between the involved centers. In each follow up visit, serum samples of the patients will be frozen in order to evaluate and enter in database dependent variables at the end of the trial.

PHASE 3 – Database processing and statistical analysis (4 months): TSC and RA from each center along with ST and DA will be involved.

- DATABASE PROCESSING (2 months): data results will be evaluated looking for possible errors in the data collection circuits.
- STATISTICAL ANALYSIS (2 months): appropriate statistical test will be applied for all collected data. Descriptive and bivariate analysis will be performed first, followed by the multivariate analysis.

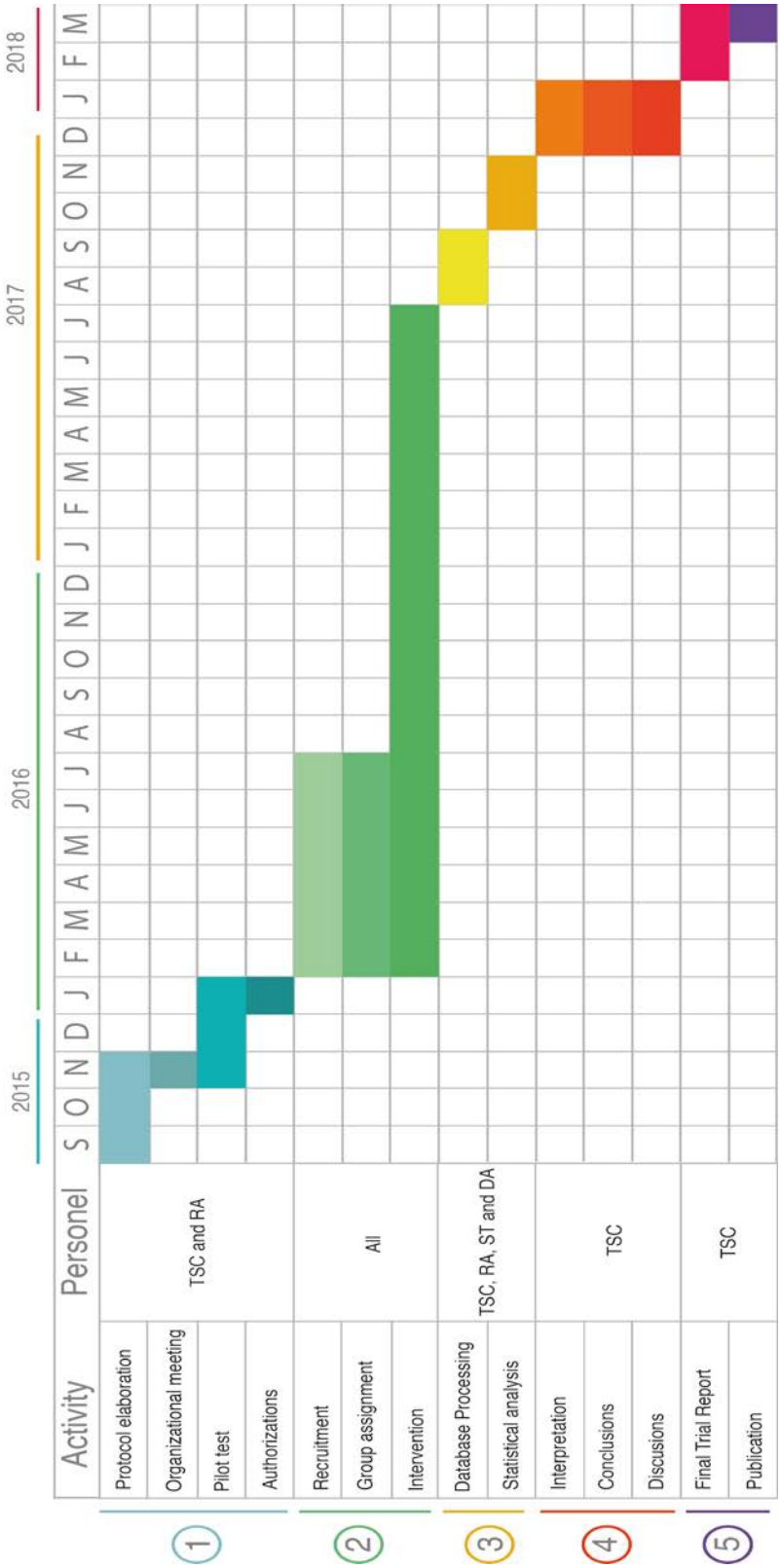
PHASE 4 - Analysis of the results (2 Months): TSC will be involved.

The outcomes of the trial will be interpreted and conclusions will be drawn and discussed.

PHASE 5 - Finalization and results publication (3 Months): TSC will be involved.

A final clinical trial resolution report will be elaborated. Whether results support or not initial hypothesis will not affect publication of the results. Dissemination strategy consists on an open access online publication and study results presentation in Sociedad Española de Endocrinología Pediatrica Conference in 2018.

16 TIMELINE


Activity
Personel
2015
2016
2017
2018
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
Protocol elaboration
Organizational meeting
Pilot test
Authorizations
TSC and RA
Recruitment
Group assignment
Intervention
All
Database Processing
Statistical analysis
TSC, RA, ST and DA
Interpretation
Conclusions
Discusions
TSC
Final Trial Report
Publication
TSC
1
2
3
4
5

17 BUDGET

	QUANTITY	COST	TOTAL
1. Staff costs			
- Statistical consulting	100 h	35 €/h	3.500 €
- Data manager	80 h	20 €/h	1.600 €
- Meetings organization	28 h	50 €/h	4.200 €
SUBTOTAL			9.300€
2. Implementation costs			
Inventory material costs:			
- Accu-Check Aviva ®	158	39,95 €	6.312,1 €
Services and disposable items costs:			
- Laboratory parameters:			
o Calcium (Urine)	474	4.44 €/u	2104,56 €
o Creatinine (Urine)	474	1,274 €/u	603,88 €
o Phosphorus (Serum)	474	3.181 €/u	1.507,79 €
o PTH (Serum)	474	10,795 €/u	5.116,83 €
o Total 25(OH) D (Serum)	474	20,948 €/u	9.929,35 €
o Vitamin D-binding protein (Serum)	158	9,075 €/u	1.433,85 €
Liability insurance	1	7.000 €	7.000 €
Drug purchase (Cholecalciferol and placebo pills)	474 packs	3,90 €/pack	1848,6 €
Administrative permits (AGEMED)	-	3.948,1 €	3.948,1 €
MRW messenger to send serum samples to Hospital Dr. Josep Trueta (Girona)	-	-	350,0 €
SUBTOTAL			40.155,06 €
3. Dissemination of the results			
Congress of the Spanish Society of Pediatric Endocrinology 2016:			
- Inscription	2 pers.	500 €/pers.	1.000 €
- Travel expenses	2 pers.	100 €/pers.	200 €
- Accommodation expenses	2 pers.	200 €/pers.	400 €
Publication fees			
- Translation services	50 h	30 €/h	1.500 €
- Open access	-	1500 €	1.500 €

Software and bibliography	-	1.000 €	1.000 €
SUBTOTAL			5.800 €
4. Subcontracting of professional services			
- Contracting of nursing services			1.720 €
SUBTOTAL			1.720 €
5. Indirect and added costs*			11.051,01 €
TOTAL COSTS			**66.306,07 €**

*IDIBGI research center receives a 20% of the total expenses, in order to cover the account management services at the research institute and for the use of research offices, examination rooms and laboratory.

BUDGET SPECIFIC ASPECTS

- **T1DM management costs:**

T1DM management is included in Spanish Social Healthcare System, therefore no costs will result from related consumables.

- **Accu-check ® Aviva:**

This glucometer allows the user to download the data into a database directly, facilitating further data management. Moreover, as explained in Limitations section, by using the same glucometer with all patients, machine variability in glucose measurements is avoided.

- **Laboratory parameters quantity:**

Samples of the 158 patients will be performed drawn at 0, 6 and 12 month (158 patients * 3 measurements = 474). Therefore, 474 samples will need to be analyzed.

- **Vitamin D Binding Protein measurement:**

VDBP only needs to be measured once (Quantity = 158). Measurement can only be done in a research laboratory, such as the disposing one in Hospital Dr. Josep Trueta. Determinations of VDBP are performed in badges of 80 samples and cost 726 €, IVA included (726 € / 80 samples = 9,075 €/sample).

- **Drug and placebo quantity:**

Deltius® pack has a cost of 3,90 €, which includes 4 vials for administration. Placebo is believed to have the same cost and presentation. Patients will be instructed to take one vial per month; therefore a pack will be needed every 4 months. Taking into account the intervention phase will take 12 months and 158 patients will be receiving the drug or placebo, 474 packs will be needed.

- **Frozen serums costs:**

Frozen serums cost is included in the indirect costs, which covers the expenses of IDIBGI research facilities utilization.

18 HEALTH IMPACT OF THE PROJECT

As discussed previously, poor glycemic control is considered to be the main factor to develop complications in T1DM patients. These complications have been related to death in T1DM patients and account for the bulk of economic overload that causes T1DM in the healthcare system (14). A Vitamin D level below sufficiency is a common situation in T1DM, which could be corrected easily. If vitamin D repletion would translate into improvement in glycemic control, it is assumed that microvascular and macrovascular complications should decrease, resulting, both short and long term, into a decrease in mortality and an improvement in quality of life.

Moreover, given the new extra-bone functions discovered in vitamin D more advantages could be gained from normal levels of vitamin D. Vitamin D deficiency has been related to the treatment and pathogenesis and/or progression of several disorders including cancer, hypertension, multiple sclerosis, rheumatoid arthritis, osteoporosis or muscle weakness (53).

The introduction of vitamin D measurement into T1DM patients follow up control would result in advantages regarding T1DM disease control, but also regarding other aspects: hypertension, CV diseases, etc. Moreover, it would also translate in a decrease of resources spent in order to treat this chronic disease.

19 VITAMIN D POTENTIAL TARGETS

Relation between vitamin D and T1DM has been discussed since the 90s, even stablished. In animal models of T1DM (in particular the NOD mouse), vitamin D deficiency in early life leads to an increase in risk for development type 1 diabetic disease in later life. When these NOD mice received 1,25(OH)D3 throughout their life, their risk of developing T1DM was reduced by 80%. In humans, epidemiology also points to this correlation (12).

On this basis, Elina Hyppönen promoted a large scale (n=10.821) birth-cohort study in Finland, which proved that vitamin D supplementation during first year reduced risk of developing T1DM by about 80% compared with those receiving less (9). Nowadays, newborns receive vitamin D supplementation during their first year of life. However, vitamin D is not taken into account further on.

During the literature check performed for this protocol, some of the results aimed to assess the effect of vitamin D supplementation on autoimmune pancreatic cell destruction during T1DM onset. But given the high prevalence that seems to be affecting the whole population, it may be interesting to study the effect of vitamin D repletion in reducing incidence of T1DM, by expanding vitamin D supplementation to all pediatric ages.

20 BIBLIOGRAPHY

1. Busturia Jimeno MÁ. Vitamina D: visión desde el laboratorio. Rev Esp Endocrinol Pediatr. 2012;3(Suppl):39–45.

2. Aljabri KS, Bokhari SA, Khan MJ. Glycemic changes after vitamin D supplementation in patients with type 1 diabetes mellitus and vitamin D deficiency. Ann Saudi Med [Internet]. 2010 [cited 2015 Sep 8];30(6).

Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2994161/

3. Yeste D, Carrascosa A. Vitamina D. Fisiología e implicaciones clínicas [Internet]. Barcelona: Hospital Universitari Vall d'Hebrón; 2012 [cited 2015 Jul 1].

Available from:

http://aula-endocrino-pediatrica.com/descargas/vitamina_D.pdf

4. Norman AW. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr [Internet]. 2008 Aug 1 [cited 2015 Aug 1];88(2):491S – 499S.

Available from:

http://ajcn.nutrition.org/content/88/2/491

5. Infante Pina D, Yeste Fernandez D. Health and vitamin D: an incomplete puzzle. An Pediatr (Barc) [Internet]. 2012 [cited 2015 Jul 12];77(1):1–4.

Available from:

http://www.analesdepediatria.org/es/salud-vitamina-d-un-

puzle/articulo/S169540331200166X/

6. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D–Binding Protein and Vitamin D Status of Black Americans and White Americans. N Engl J Med [Internet]. 2013 [cited 2015 Oct 3];369(21):1991–2000.

Available from:

http://www.nejm.org/doi/full/10.1056/NEJMoa1306357

7. Ross C, Taylor CL, Yaktine AL, Del Valle H. Dietary Reference Intakes for Calcium and Vitamin D - Institute of Medicine Food and Nutrition Board [Internet]. Washington: National Academy Press; 2011 [cited 2015 Oct 6].

Available from:

http://www.nap.edu/catalog/13050/dietary-reference-intakes-for-calcium-and-vitamin-d

8. Holick MF. Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr [Internet]. 2004 [cited 2015 Sep 20];79(3):362–71.

Available from:

http://ajcn.nutrition.org/content/79/3/362.long

9. Hyppönen E, Läärä E, Reunanen A, Järvelin M-R, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet [Internet]. 2001 Nov [cited 2015 Sep 6];358(9292):1500–3.

Available from:

http://www.sciencedirect.com/science/article/pii/S0140673601065801

10. Shih EM, Mittelman S, Pitukcheewanont P, Azen CG, Monzavi R. Effects of vitamin D repletion on glycemic control and inflammatory cytokines in adolescents with type 1 diabetes. Pediatr Diabetes [Internet]. 2014 Dec 18 [cited 2015 Sep 16];

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/25524404

11. Takiishi T, Van Belle T, Gysemans C, Mathieu C. Effects of vitamin D on antigen-specific and non-antigen-specific immune modulation: Relevance for type 1 diabetes. Pediatr Diabetes [Internet]. 2013 [cited 2015 Sep 8];14(2):81–9.

Available from:

http://onlinelibrary.wiley.com/doi/10.1111/j.1399-5448.2012.00923.x/epdf

12. Mathieu C. Vitamin D as a Modulator of the immune system: the case of type 1 diabetes. Rev Esp Endocrinol Pediatr. 2012;3(Suppl):47–8.

13. Arslanian S. Vitamin D, adiposity, insulin sensivity and beta cell function in children. Rev Esp Endocrinol Pediatr. 2012;3(Suppl):49–50.

14. Gaztambide Sáenz S. Guía de Práctica Clínica sobre Diabetes Mellitus Tipo 1 [Internet]. Plan de Calidad para el Sistema Nacional de Salud del Ministerio de Sanidad y política Social. Vitoria-Gasteiz; 2012 [cited 2015 Sep 11].

Available from:

http://www.guiasalud.es/GPC/GPC_429_Diabetes_2_Osteba_compl.pdf

15. Barrio R, García B, Gómez AL, González I, Hermoso F, López MJ, et al. Lo que debes saber sobre la diabetes en la edad pediátrica

[Internet]. 3rd ed. Madrid: Sociedad Española de Endocrinología Pediátrica (SEEP); 2008 [cited 2015 Oct 4]. 108 p.

Available from:

http://www.seep.es/privado/gdiabetes/libro_diabetes_infantil.pdf

16. Standards of Medical Care in Diabetes - 2015. Diabetes Care [Internet]. 2015 [cited 2015 Oct 17];38(Suppl 1):S1–2.

Available from:

http://care.diabetesjournals.org/cgi/doi/10.2337/dc15-S001

17. Delahanty L, Simkins SW, Camelon K. Expanded role of the dietitian in the Diabetes Control and Complications Trial: implications for clinical practice. The DCCT Research Group. J Am Diet Assoc [Internet]. 1993 Jul [cited 2015 Nov 8];93(7):758–64, 767.

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/8320401

18. Greenbaum CJ. Insulin resistance in type 1 diabetes. Diabetes Metab Res Rev [Internet]. 2002 [cited 2015 Oct 23];18(3):192–200.

Available from:

http://onlinelibrary.wiley.com/doi/10.1002/dmrr.291/pdf

19. Xu P, Cuthbertson D, Greenbaum C, Palmer JP, Krischer JP. Role of insulin resistance in predicting progression to type 1 diabetes. Diabetes Care [Internet]. 2007 [cited 2015 Oct 23];30(9):2314–20.

Available from:

http://care.diabetesjournals.org/content/30/9/2314.full.pdf+html

20. McCulloch DK, Kahn SE, Schwartz MW, Koerker DJ, Palmer JP. Effect of nicotinic acid-induced insulin resistance on pancreatic B cell function in normal and streptozocin-treated baboons. J Clin Invest [Internet]. 1991 [cited 2015 Oct 22];87(4):1395–401.

Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC295182

21. Yki-Jarvinen H, Koivisto V. Natural Course of Insulin Resistance in Type I Diabetes. N Engl J Med [Internet]. 1976 [cited 2015 Oct 23];

Available from:

http://www.nejm.org/doi/full/10.1056/NEJM198607243150404

22. Huang Y, Li X, Wang M, Ning H, A L, Li Y, et al. Lipoprotein lipase links vitamin D, insulin resistance, and type 2 diabetes: a cross-sectional epidemiological study. Cardiovasc Diabetol [Internet]. 2013 [cited 2015 Sep 25];12:17.

Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3570389

23. Janner M, Ballinari P, Mullis PE, Flück CE. High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. Swiss Med Wkly [Internet]. 2010 [cited 2015 Sep 16];140(oct):34.

Available from:

http://www.smw.ch/content/smw-2010-13091/

24. Brekke HK, Ludvigsson J. Vitamin D supplementation and

diabetes-related autoimmunity in the ABIS study. Pediatr Diabetes [Internet]. 2007 [cited 2015 Oct 27];8(1):11–4. Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1399-5448.2006.00223.x/pdf

25. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. Eur J Clin Nutr [Internet]. 2011 [cited 2015 Sep 11];65(9):1005–15.

Available from:

http://www.nature.com/ejcn/journal/v65/n9/full/ejcn2011118a.html

26. Svoren BM, Volkening LK, Wood JR, Laffel LMB. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J Pediatr [Internet]. 2009 Jan [cited 2015 Oct 6];154(1):132–4.

Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2635941

27. Schwalfenberg G. Vitamin D and diabetes: improvement of glycemic control with vitamin D3 repletion. Can Fam Physician [Internet]. 2008 Jun 1 [cited 2015 Sep 16];54(6):864–6.

Available from:

http://www.cfp.ca/content/54/6/864.full

28. Al-Sofiani ME, Jammah A, Racz M, Khawaja RA, Hasanato R, El-Fawal HAN, et al. Effect of Vitamin D Supplementation on Glucose Control and Inflammatory Response in Type II Diabetes: A Double Blind, Randomized Clinical Trial. Int J Endocrinol Metab [Internet]. 2015 Jan 1 [cited 2015 Sep 16];13(1):e22604.

Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4338666

29. Treiber G, Prietl B, Fröhlich-Reiterer E, Lechner E, Ribitsch A, Fritsch M, et al. Cholecalciferol supplementation improves suppressive capacity of regulatory T-cells in young patients with new-onset type 1 diabetes mellitus - a randomized clinical trial. Clin Immunol [Internet]. 2015 Aug 12 [cited 2015 Aug 20];

Available from:

http://www.sciencedirect.com/science/article/pii/S152166161530019X

30. Jiménez Padilla C. Vitamin D trial to reduce cardiovascular disease risk markers in prepubertal and pubertal children with obesity [Internet]. Girona: Facultat de Medicina; 2015 [cited 2015 Sep 15].

Available from:

http://dugi-doc.udg.edu//handle/10256/10823

31. Escuder V. La epidemia desconocida [trabajo de investigación]. Girona: CCE Montessori-Palau; 2014.

32. Mohammadian S, Fatahi N, Zaeri H, Vakili MA. Effect of vitamin d3 supplement in glycemic control of pediatrics with type 1 diabetes mellitus and vitamin d deficiency. J Clin Diagn Res [Internet]. 2015 Mar [cited 2015 Sep 15];9(3):SC05–7.

Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4413124/

33. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial. Br J Nutr [Internet]. 2010

Feb [cited 2015 Oct 10];103(04):549.

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/19781131

34. Greer RM, Portelli SL, Hung BS-M, Cleghorn GJ, McMahon SK, Batch J a, et al. Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes [Internet]. 2013 [cited 2015 Sep 17];14(1):31–41.

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/22913562

35. Chakhtoura M, Azar ST. The role of vitamin D deficiency in the incidence, progression, and complications of type 1 diabetes mellitus. Int J Endocrinol [Internet]. 2013 [cited 2015 Oct 9];2013.

Available from:

http://www.hindawi.com/journals/ije/2013/148673/

36. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. Eur J Nutr [Internet]. 2009 Sep [cited 2015 Nov 4];48(6):349–54.

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/19370371

37. Patel P, Poretsky L, Liao E. Lack of effect of subtherapeutic vitamin D treatment on glycemic and lipid parameters in Type 2 diabetes: A pilot prospective randomized trial. J Diabetes [Internet]. 2010 [cited 2015 Sep 27];2(1):36–40.

Available from:

http://friedmanfellows.com/assets/pdfs/elibrary/VitDdeficiency.DrLiao.pdf

38. Parekh D, Sarathi V, Shivane VK, Bandgar TR, Menon PS, Shah NS. Pilot study to evaluate the effect of short-term improvement in vitamin D status on glucose tolerance in patients with type 2 diabetes mellitus. Endocr Pract [Internet]. 2010 Jan 29 [cited 2015 Nov 4];16(4):600–8.

Available from:

http://journals.aace.com/doi/10.4158/EP09300.OR?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed&

39. Soric MM, Renner ET, Smith SR. Effect of daily vitamin D supplementation on HbA1c in patients with uncontrolled type 2 diabetes mellitus: a pilot study. J Diabetes [Internet]. 2012 Mar [cited 2015 Sep 24];4(1):104–5.

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/22018074

40. Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, Kalayi A, et al. Daily consumption of vitamin D- or vitamin D + calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. Am J Clin Nutr [Internet]. 2011 Apr 1 [cited 2015 Nov 4];93(4):764–71.

Available from:

http://ajcn.nutrition.org/content/93/4/764.full

41. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and

classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med [Internet]. 1998 [cited 2015 Sep 14];15(7):539–53.

Available from:

http://apps.who.int/iris/bitstream/10665/66040/1/WHO_NCD_NCS_99.2.pdf

42. Herramientas endocrinología - Asociación Española de Pediatría [Internet]. Madrid; 2015 [cited 2015 Oct 10].

Available from:

http://continuum.aeped.es/toolareas/inicio2/0/11#.Vj-pbrcvfcs

43. Awumey E, Mitra D, Hollis B, Kumar R, Bell N. Vitamin D metabolism is altered in Asian Indians in the southern United States: a clinical research center study. J Clin Endocrinol Metab [Internet]. 1998 [cited 2015 Sep 6];

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/9435436

44. Sánchez Muro J. Estudio Nutricional Comparativo entre una población infantil caucásica y una inmigrante [thesis]. Universidad Autónoma de Barcelona; 2013.

45. Estudio Español de Crecimiento - Gráficos Talla, Peso y BMI [Internet]. Barcelona: Asociación Española de Pediatría; 2010 [cited 2015 Sep 9].

Available from:

www.aeped.es/noticias/estudios-espanoles-crecimiento-2010

46. El sistema Accu-Chek ® Aviva. [cited 2015 Oct 26];

Available from:

https://www.accu-chek.es/documents/Manual_Accu-Chek_Aviva.pdf

47. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics [Internet]. 2008 [cited 2015 Oct 12];122(2):398–417.

Available from:

http://pediatrics.aappublications.org/content/122/2/398.full-text.pdf

48. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics [Internet]. 2008 [cited 2015 Sep 26];122(5):1142–52.

Available from:

http://pediatrics.aappublications.org/content/122/5/1142.full-text.pdf

49. Zwart SR, Parsons H, Kimlin M, Innis SM, Locke JP, Smith SM. A 250 µg/week dose of vitamin D was as effective as a 50 µg/d dose in healthy adults, but a regimen of four weekly followed by monthly doses of 1250 µg raised the risk of hypercalciuria. Br J Nutr [Internet]. 2013 Nov 28 [cited 2015 Sep 15];110(10):1866–72.

Available from:

http://www.ncbi.nlm.nih.gov/pubmed/23595003

50. Jacobsen R, Abrahamsen B, Bauerek M, Holst C, Jensen CB, Knop J, et al. The influence of early exposure to vitamin D for development of diseases later in life. BMC Public Health [Internet]. 2013 [cited 2015 Sep 29];13(1):515.

Available from:

http://www.scopus.com/inward/record.url?eid=2-s2.0-84878260560&partnerID=tZOtx3y1

51. Deltius 25.000 UI Cholecalciferol - Italfarmaco S.A. [Internet]. Ministerio de Sanidad; p. 1–14.

Available from:

http://www.italfarmaco.es/documents/8447302/17311473/FT+Ministerio+Deltius+Rev01(08.2013)-+25.000UI+Viales.pdf/0df57eb1-762f-4a6b-977b-c45f59a29fc4

52. Rodr F, Huertes B. Evaluación básica de la función renal en pediatría [Internet]. Barcelona; 2014 [cited 2015 Nov 2].

Available from:

https://www.aeped.es/sites/default/files/documentos/02_evaluacion_basica_fr.pdf

53. Zhang R, Naughton D. Vitamin D in health and disease: Current perspectives. Nutr J [Internet]. 2010 [cited 2015 Sep 11];9(1):65.

Available from:

http://www.nutritionj.com/content/9/1/65/abstract\nhttp://www.nutritionj.com/content/pdf/1475-2891-9-65.pdf

54. Hábitos en actividad física de la población escolar Cuestionario para los niños/as. [Internet]. Madrid: Ministerio de Educación y Ciencia; 2007 [cited 2015 Nov 4].

Available from:

http://www.perseo.aesan.msssi.gob.es/docs/docs/cuestionarios/cuestionario_1_exploracion_alumnado.pdf

21 ANNEX

- **Annex 1:** Physical Activity Questionnaire (54).
- **Annex 2:** Modified Garabedian Test (44).
- **Annex 3:** Deltius ® Public Datasheet (51).
- **Annex 4:** Consents and Patient Informative Sheet.
- **Annex 5:** Posters European Society of Pediatric Endocrinology (ESPE).

ANNEX 1: Physical Activity Questionnaire

Algunas preguntas sobre la actividad física y el deporte[1]

La actividad física es cualquier actividad que aumenta tu ritmo cardiaco y te hace perder el aliento en parte. Se puede practicar como deporte, en actividades del colegio, jugando con amigos o cuando vas andando al colegio.

Algunos ejemplos de actividad física son correr, andar rápido, patinar, andar en bici, bailar, monopatín, nadar, fútbol, baloncesto o hacer surf.

E1 ¿Cómo vienes al colegio habitualmente?

- ☐ Andando
- ☐ En bici
- ☐ En transporte público
- ☐ En coche
- ☐ En autobús escolar
- ☐ No se

E2 ¿Participas en actividades de deporte organizado, en el colegio o fuera del colegio (fútbol, baloncesto, aeróbic...)?

- ☐ Sí
- ☐ No

E3 En caso afirmativo, ¿Qué actividad practicas?

_____

_____

E4 ¿Qué sueles hacer durante los recreos del colegio la mayor parte de los días? (puedes señalar más de una respuesta)

- ☐ Estoy sentado/a (hablando, leyendo, internet, gameboy, muñecas...)
- ☐ Juego en los columpios
- ☐ Juego al balón, correr, cuerda, a la goma, patines...
- ☐ Otros (especificar):
- ☐ No se

E5 Habitualmente, <u>FUERA DE LAS HORAS DE CLASE</u>: ¿Cuántas horas <u>a la semana</u> practicas algún deporte o haces ejercicio jugando hasta sudar o cansarte?

- ☐ Ninguna
- ☐ Alrededor de 1 hora a la semana
- ☐ Alrededor de 2 horas a la semana
- ☐ Alrededor de 3 horas a la semana
- ☐ Alrededor de 4 horas a la semana
- ☐ 5 horas a la semana o más

E6 ¿Cuántas horas <u>al día</u> sueles ver la televisión y vídeos?

- ☐ Ninguna
- ☐ Menos de 1 hora al día
- ☐ Alrededor de 1 hora al día
- ☐ Alrededor de 2 horas al día
- ☐ Alrededor de 3 horas al día
- ☐ Alrededor de 4 horas al día
- ☐ Alrededor de 5 horas o más al día

E7 ¿Cuántas horas <u>al día</u> sueles utilizar el ordenador (para jugar, mandar correo electrónico, chatear o navegar en Internet) en tu tiempo libre?

- ☐ Ninguna
- ☐ Menos de 1 hora al día
- ☐ Alrededor de 1 hora al día
- ☐ Alrededor de 2 horas al día
- ☐ Alrededor de 3 horas al día
- ☐ Alrededor de 4 horas al día
- ☐ Alrededor de 5 horas o más al día

E8 ¿A qué hora te sueles ir a dormir entre semana?

_____

E9 ¿A qué hora te sueles levantar entre semana?

_____

1 Adaptado del test de evaluación rápida Kreceplus (estudio enKid)

Algunas preguntas sobre la actividad física y el deporte (amplio)

Para las dos siguientes preguntas, suma todo el tiempo que pasas haciendo actividad física cada día.

AF1.- Durante la semana pasada, en cuántos días hiciste actividad física en total durante al menos 60 minutos (1 hora) al día?

- ☐ Ninguno (cero días)
- ☐ 1 día
- ☐ 2 a 3 días
- ☐ 4 días o mas

AF2.- En una semana típica normal, cuántos días a la semana haces actividad física en total durante 60 minutos al día?

- ☐ Ninguno (cero días)
- ☐ 1 día
- ☐ 2 a 3 días
- ☐ 4 días o mas

AF3.- Habitualmente, alrededor de cuántas horas a la semana participas en actividades deportivas o haces actividad física (que te hace perder el aliento o sudar más de lo habitual):

... en el colegio, en tu tiempo libre (por ejemplo, después de comer, en el recreo)?

- ☐ Nunca
- ☐ Menos de 1 hora a la semana
- ☐ 2 a 3 horas a la semana
- ☐ 4 a 6 horas a la semana
- ☐ 7 o mas horas a la semana

AF4.- Habitualmente, alrededor de cuántas horas a la semana participas en actividades deportivas o haces actividad física (que te hace perder el aliento o sudar más de lo habitual):

… en el colegio, en las horas de clase?

- ☐ Nunca
- ☐ Menos de 1 hora a la semana
- ☐ 2 a 3 horas a la semana
- ☐ 4 a 6 horas a la semana
- ☐ 7 o mas horas a la semana

AF5.- Habitualmente, alrededor de cuántas horas a la semana participas en actividades deportivas o haces actividad física (que te hace perder el aliento o sudar más de lo habitual):

… fuera del colegio, en deporte extraescolar u otro tipo de clases o actividades deportivas?

- ☐ Nunca
- ☐ Menos de 1 hora a la semana
- ☐ 2 a 3 horas a la semana
- ☐ 4 a 6 horas a la semana
- ☐ 7 o mas horas a la semana

AF6.- Habitualmente, alrededor de cuántas horas a la semana participas en actividades deportivas o haces actividad física (que te hace perder el aliento o sudar más de lo habitual):
… fuera del colegio, cuando juegas solo o con tus amigos en actividades no organizadas?

- ☐ Nunca
- ☐ Menos de 1 hora a la semana
- ☐ 2 a 3 horas a la semana
- ☐ 4 a 6 horas a la semana
- ☐ 7 o mas horas a la semana

Algunas preguntas sobre tu actividad física AYER

AF7 Ayer, ¿fue un día de colegio?

☐ Si ☐ No

Si la respuesta es **NO**, pasa a la pregunta **AF12**

AF8 ***Si fue un día de colegio*****, ¿cómo viniste al colegio?**

☐ Andando
☐ En bici
☐ En transporte público
☐ En coche
☐ En autobús escolar
☐ No se

AF9 ¿A qué jugaste o qué hiciste en el recreo...,?

	por la mañana	**después de comer**	**por la tarde**
Estuve sentado/a (hablando, leyendo, internet, gameboy, muñecas...)			
Jugué en los columpios			
Jugué al balón, correr, cuerda, a la goma, patines...			
Otros (especificar):			
No se			

AF10 ¿Tuviste clase de educación física o deporte en el colegio ayer?

☐ Si ☐ No

AF11 Si tuviste clase de educación física o deporte:

1 ¿cuánto tiempo duró?
2 ¿Qué hiciste en clase de educación física o deporte?

AF12 Ayer, <u>en tu tiempo libre</u>, alrededor de cuánto tiempo participaste en actividades deportivas o hiciste actividad física (que te hizo perder el aliento o sudar más de lo habitual):

1 ...jugando solo o con amigos

☐ Nada
☐ Menos de 30 minutos
☐ Entre 30 minutos y menos de 1 hora
☐ Entre 1 hora y 1 hora y media
☐ Entre 1 hora y media y 2 horas
☐ 2 horas o más

2 ...jugando, haciendo ejercicio o deporte con tu familia

☐ Nada
☐ Menos de 30 minutos
☐ Entre 30 minutos y menos de 1 hora
☐ Entre 1 hora y 1 hora y media
☐ Entre 1 hora y media y 2 horas
☐ 2 horas o más

3 ...en deporte extraescolar

☐ Nada
☐ Menos de 30 minutos
☐ Entre 30 minutos y menos de 1 hora
☐ Entre 1 hora y 1 hora y media
☐ Entre 1 hora y media y 2 horas
☐ 2 horas o más

Algunas preguntas sobre tu actividad física AYER (sigue)

AF13 Ayer ¿cuántas horas viste la televisión, vídeos o videojuegos?

- ☐ No vi TV o videos ni jugué con videojuegos
- ☐ Menos de 1 hora
- ☐ Entre 1 y 2 horas
- ☐ Entre 3 y 4 horas
- ☐ Entre 5 y 6 horas
- ☐ 7 o mas horas

AF14 Ayer, ¿cuántas horas pasaste con el ordenador, jugando a videojuegos, con el correo electrónico, chateando, navegando en internet, etc.?

- ☐ No utilicé el ordenador
- ☐ Menos de 1 hora
- ☐ Entre 1 y 2 horas
- ☐ Entre 3 y 4 horas
- ☐ Entre 5 y 6 horas
- ☐ 7 o mas horas

AF15 ¿A qué hora te levantaste ayer por la mañana?

_____

AF16 ¿A qué hora te fuiste a dormir ayer?

_____

¡Muchas gracias por tu colaboración!

Observaciones (Personal de Salud)

ANNEX 2: MODIFIED GARABEDIAN TEST (44)

EVALUACIÓN DEL APORTE DE VITAMINA D

		RESULTADOS
1	**LECHE Y DERIVADOS** Leche no suplementada (1 vaso→ 250 cc) No ☐ (0 puntos) 1 -2 vasos/día ☐ (1 punto) 3-4 vasos/día ☐ (2 puntos) >4 vasos/día ☐ (3 puntos) Leche suplementada con Vit D (1 vaso→250 cc) No ☐ (0 puntos) 1 vaso/día ☐ (6 puntos) 2 vasos/día ☐ (12 puntos) 3 vasos/día ☐ (18 puntos) ≥4 vasos/día ☐ (24 puntos) Queso manchego (30 gramos) No ☐ (0 puntos) 1 /día ☐ (2 puntos) 2 /día ☐ (4 puntos) 3 /día ☐ (6 puntos) ≥4 /día ☐ (8 puntos)	☐ ☐ ☐
2	**PESCADOS FRESCOS O CONGELADOS, MARISCO** Sardina, salmón, arenque, trucha No ☐ (0 puntos) 1-2/mes ☐ (2 puntos) 1/semana ☐ (6 puntos) ≥ 2/semana ☐ (12 puntos) Angula, ostras (docena), bacalao, atún No ☐ (0 puntos) 1-2/mes ☐ (1 punto) 1/semana ☐ (2 puntos) ≥ 2/semana ☐ (4 puntos)	☐ ☐
3	**PESCADOS AHUMADOS O MARINADOS** Salmón No ☐ (0 puntos) 1-2/mes ☐ (2 puntos) 1/semana ☐ (4 puntos) ≥ 2/semana ☐ (8 puntos) Arenque, bacalao, trucha No ☐ (0 puntos) 1-2/mes ☐ (1 punto) 1/semana ☐ (2 puntos) ≥ 2/semana ☐ (4 puntos)	☐ ☐
4	**PESCADOS EN CONSERVA** Sardina, arenque No ☐ (0 puntos) 1/semana ☐ (2 puntos) 2/semana ☐ (4 puntos) ≥ 3/semana ☐ (8 puntos) Atún, bacalao, anchoa No ☐ (0 puntos) 1/semana ☐ (2 puntos) 2/semana ☐ (4 puntos) ≥ 3/semana ☐ (8 puntos)	☐ ☐
5	**HUEVOS** (nº consumido) <2/semana ☐ (0 puntos) 2-5 semana ☐ (2 puntos) 6-10/semana ☐ (5 puntos) >10/semana ☐ (7 puntos)	☐
6	**ALIMENTOS QUE CONTIENEN HUEVO** Sándwiches, pastelería, bollería.. <2/semana ☐ (0 puntos) 2-5/semana ☐ (2 puntos) 6-10/semana ☐ (5 puntos) >10/semana ☐ (7 puntos)	☐
7	**CHARCUTERIA, DESPOJOS-VISCERAS** Hígado, patés, jamón ahumado, tocino, salchichas, carne de cerdo No ☐ (0 puntos) 1-3/semana ☐ (0,5 puntos) 4-6/semana ☐ (1 punto) >6/semana ☐ (1,5 puntos)	☐
8	**CHAMPIÑONES** No ☐ (0 puntos) 1/mes ☐ (1 punto) 1/semana ☐ (3 puntos)	☐
	1 punto→ 100 UI vitamina D/semana. Bajo <28 puntos (<400UI/día), Medio: 28-42 (400-600 UI/día); Alto > 43 (>600UI/día)	TOTAL ☐

EVALUACIÓN DEL APORTE DE CALCIO

		RESULTADOS
1	**LECHE** Leche no suplementada No ☐ (0 puntos) 1 vaso/día ☐ (17 puntos) 2 vasos/día ☐ (34 puntos) 3 vasos/día ☐ (51 puntos) ≥4 vasos/día ☐ (68 puntos) Leche suplementada con calcio No ☐ (0 puntos) 1 vaso/día ☐ (23 puntos) 2 vasos/día ☐ (46 puntos) 3 vasos/día ☐ (69 puntos) ≥4 vasos/día ☐ (92 puntos)	☐ ☐
2	**YOGUR, HELADOS DE CREMA (2 bolas), Flan (125cc)** No ☐ (0 puntos) 1/semana ☐ (1 punto) 2-5 /semana ☐ (5 puntos) ≥ 6/semana ☐ (9 puntos) **PETIT SUISSES** No ☐ (0 puntos) 1/semana ☐ (0,5 punto) 2-5 /semana ☐ (2 puntos) ≥ 6/semana ☐ (4 puntos)	☐ ☐
3	**QUESOS** Queso tierno (ejemplo: Burgos) No ☐ (0 puntos) 1/semana ☐ (1 punto) 2-5 /semana ☐ (3 puntos) ≥ 6/semana ☐ (6 puntos) Queso tipo Camembert o crema (ejemplo: Philadelphia)(1 porción→30g) No ☐ (0 puntos) 1/semana ☐ (1 punto) 2-5 /semana ☐ (4 puntos) ≥ 6/semana ☐ (7 puntos) Queso seco tipo manchego o gruyère (1 porción→30 gramos) No ☐ (0 puntos) 1/semana ☐ (2 puntos) 2-5 /semana ☐ (8 puntos) ≥ 6/semana ☐ (15 puntos)	☐ ☐ ☐
4	**HUEVOS, CARNES, PESCADOS (1 porción→100g)** < 3 /semana ☐ (0 puntos) 3-7 /semana ☐ (0,5 puntos) ≥ 8/semana ☐ (1 punto)	☐
5	**CHOCOLATE CON LECHE O BLANCO (1 tableta→100g)** No ☐ (0 puntos) 1-2 tabletas /semana ☐ (3 puntos) 2-4/semana ☐ (6 puntos) >4/semana ☐ (9 puntos)	☐
6	**PAN (1 porción→100 gramos); FRUTAS (150g)** < 3 semana ☐ (0 puntos) 3-7/semana ☐ (1 punto) ≥8/semana ☐ (2 puntos)	☐
7	**LEGUMBRES (1 porción→200g)** No ☐ (0 puntos) 1-3 /semana ☐ (2 puntos) 3-7/semana ☐ (4 puntos) ≥ 8/semana ☐ (8 puntos)	☐
	Balance aporte de calcio: 1 punto→120 mg calcio/semana Recomendaciones diarias de calcio entre los 9 y los 18 años de edad: **75 puntos: 1300mg/día**	TOTAL ☐

EVALUACIÓN DE LA EXPOSICIÓN SOLAR

1	**Te has expuesto al sol durante LA PRIMAVERA Y EL OTOÑO entre las 10:00 y las 15:00 horas, durante al menos 15 minutos, teniendo el rostro y parte de los brazos al descubierto ?** No ☐ 1-2 días/semana ☐ 3-4 días/semana ☐ 5-7 días/semana ☐ (0 puntos) (2 punto) (4 puntos) (6 puntos)	**RESULTADOS** ☐
2	**Te has expuesto al sol durante EL VERANO (no en primavera ni en otoño) entre las 10:00 y las 15:00 horas, durante al menos 15 minutos, teniendo el rostro y parte de los brazos al descubierto?** No ☐ 1-2 días/semana ☐ 3-4 días/semana ☐ 5-7 días/semana ☐ (0 puntos) (1 punto) (2 puntos) (3 puntos)	☐
3	**LUGAR DE EXPOSICIÓN** Ciudad ☐ Campo ☐ Montaña-Mar ☐ (1 puntos) (2 puntos) (3 puntos)	☐
4	**¿ SE HA UTILIZADO UNA CREMA SOLAR CON IP>15?** Si ☐ No ☐ A veces ☐ (Se resta el total) (0 puntos) (Se resta la mitad)	☐
	PUNTUACIÓN MÁXIMA= 12 PUNTOS	**TOTAL** ☐

COMENTARIOS:

ANNEX 3: DELTIUS TECHNICAL DATA SHEET

FICHA TECNICA

1. NOMBRE DEL MEDICAMENTO

DELTIUS 25.000 UI/2,5ml solución oral.

2. COMPOSICIÓN CUALITATIVA Y CUANTITATIVA

1 frasco unidosis de 2,5ml de solución oral contiene 25.000 UI de colecalciferol (vitamina D), equivalentes a 0,625mg.
1 ml de solución oral contiene 10.000 UI de colecalciferol (vitamina D), equivalentes a 0,25mg.
Para consultar la lista completa de excipientes, ver sección 6.1.

3. FORMA FARMACÉUTICA

Solución oral
Solución oleosa transparente, de incolora a amarillo-verdosa, sin partículas sólidas visibles y/o precipitados.

4. DATOS CLÍNICOS

4.1. Indicaciones terapéuticas

Prevención y tratamiento de la deficiencia de vitamina D.

Como adyuvante en el tratamiento específico de la osteoporosis en pacientes con deficiencia de vitamina D o en riesgo de insuficiencia de vitamina D.

4.2. Posología y forma de administración

4.2.1. Posología

Población pediátrica

- Prevención de la deficiencia entre 0 y 1 años: 25000 UI (1 frasco) cada 8 semanas.

- Prevención de la deficiencia entre 1 y 18 años: 25000 UI (1 frasco) cada 6 semanas.

- Tratamiento de la deficiencia entre 0 y 18 años: 25000 UI (1 frasco) cada 2 semanas durante 6 semanas, (seguido de un tratamiento de mantenimiento de 400-1000 UI/día).

Pacientes embarazadas o en período de lactancia:

- Esta formulación de alta dosis no está recomendada

Adultos

- Prevención de la deficiencia de vitamina D: 25000 UI/mes(1 frasco). Podrían ser necesarias dosis mayores en determinadas situaciones, ver más adelante.

- Como adyuvante en el tratamiento específico de la osteoporosis: 25000 UI/mes(1 frasco).

- Tratamiento de la deficiencia de vitamina D (< 25ng/ml) 50000 UI/semana (2 frascos) durante 6 a 8 semanas, seguido de un tratamiento de mantenimiento (1400-2000 UI/día según requerimientos); Con objeto de comprobar que se ha alcanzado el nivel deseado, a los 3-4 meses del inicio del tratamiento de mantenimiento debería realizarse un control de seguimiento de la 25(OH)D.

Poblaciones de alto riesgo de deficiencia en vitamina D, estas poblaciones podrían requerir dosis mayores y monitorización de la 25(OH)D sérica:

- Pacientes institucionalizados u hospitalizados

- Personas de piel oscura

- Personas con exposición limitada al sol debido al uso de prendas protectoras o al uso continuado de cremas de protección solar.

- Personas obesas

- Pacientes en evaluación por sospecha de osteoporosis

- Pacientes en tratamiento concomitante con algunos medicamentos (por ejemplo; antiepilépticos o glucocorticoides)

- Pacientes con síndromes de malabsorción, incluyendo enfermedad inflamatoria intestinal o enfermedad celiaca.

- Pacientes tratados recientemente por deficiencia de vitamina D que requieran tratamiento de mantenimiento.

4.2.2. Forma de administración

Se debería advertir a los pacientes para que tomen Deltius 25.000 UI/2,5 ml solución oral preferiblemente con las comidas (ver sección "5.2 Propiedades farmacocinéticas, Absorción").

El producto debe agitarse antes de usar.

Deltius 25.000 UI/2,5ml solución oral tiene sabor a aceite de oliva. Deltius puede tomarse directamente del frasco o mezclado con una pequeña cantidad de comida fría o templada inmediatamente antes de su ingesta. El paciente debe asegurarse de tomar la dosis completa.

En niños, Deltius 25.000 UI/2,5ml solución oral puede mezclarse con una pequeña cantidad de alimento para niños, yogur, leche, queso u otros productos lácteos. Los padres deberían ser advertidos para que no incorporen Deltius a biberones de leche u otros recipientes con alimentos que el niño no vaya a ingerir por completo, a fin de evitar que el niño no tome la dosis completa. Los padres deberían asegurarse de que el niño tome la dosis completa. En caso de niños que hayan superado la etapa de lactancia, la dosis prescrita debe ser administrada junto con alguna comida principal.

Para consultar las instrucciones de uso del medicamento antes de la administración, ver sección 6.6.

4.3. Contraindicaciones

- Hipersensibilidad al colecalciferol o a alguno de los excipientes incluidos en la sección 6.1.
- Hipercalcemia o hipercalciuria.
- Hipervitaminosis D.
- Cálculos renales (nefrolitiasis, nefrocalcinosis) en pacientes con hipercalcemia crónica

4.4. Advertencias y precauciones especiales de empleo

Deterioro de la función renal:
La vitamina D debería ser utilizada con precaución en pacientes con deterioro de la función renal y se debería monitorizar su efecto sobre los niveles de calcio y fosfato. Se debería tener en cuenta el riesgo de calcificación de los tejidos blandos.

Enfermedades cardiovasculares:
Es necesario tener precaución con los pacientes en tratamiento por enfermedades cardiovasculares (ver sección "4.5. Interacción con otros medicamentos y otras formas de interacción", en especial la información sobre glicósidos cardiacos que contengan digitalina).

Sarcoidosis:
Deltius debería prescribirse con precaución en pacientes con sarcoidosis, debido a un posible aumento del metabolismo de la forma activa de la vitamina D. En estos pacientes deberían monitorizarse los niveles de calcio en suero y orina.

En caso de tratamiento con otros productos que contengan vitamina D o ingesta de alimentos enriquecidos con vitamina D (incluida leche enriquecida) o dependiendo del grado de exposición solar, se permite un margen de tolerancia en la dosis total de vitamina D. Adicionalmente, se debería tener en cuenta el nivel de exposición solar antes de establecer la posología.

No hay evidencia clara sobre la relación entre suplementación con vitamina D y aparición de cálculos renales, aunque dicha relación es plausible, especialmente en caso de que la suplementación sea simultánea. La necesidad de suplementación adicional con calcio debería ser considerada de forma individual en cada paciente. La suplementación con calcio debería efectuarse bajo estrecha supervisión médica.

Se ha comunicado un aumento del riesgo de fracturas en personas de edad avanzada asociado a la administración oral de dosis ultra-altas de vitamina D (500.000 U.I. en una toma única anual), siendo dicho riesgo mayor durante los 3 primeros meses posteriores a la toma única.

4.5. Interacción con otros medicamentos y otras formas de interacción

El uso concomitante de antiepilépticos (como fenitoína) o de barbitúricos o, posiblemente, de otros medicamentos inductores de enzimas hepáticos, puede reducir el efecto de la vitamina D_3 mediante su inactivación metabólica.

Se recomienda la monitorización de la concentración de calcio sérico en caso de de tratamiento con diuréticos tiazídicos, ya que éstos pueden reducir la eliminación de calcio en orina.

El uso concomitante de glucocorticoides puede disminuir el efecto de la vitamina D.

La administración de vitamina D puede aumentar el riesgo de toxicidad por digitalina (arritmia), en caso de tratamiento con medicamentos que contengan digitalina u otros glicósidos cardiacos. En estos casos es necesaria una supervisión médica estricta, así como la monitorización de las concentraciones de calcio sérico y, si fuera necesario, controles electrocardiográficos.

La absorción gastrointestinal de vitamina D puede verse reducida por el tratamiento simultáneo con resinas intercambiadoras de iones, tales como colestiramina, hidrocloruro de colestipol, orlistat o algunos laxantes como el aceite de parafina.

Algunos agentes citotóxicos como la actinomicina y los antifúngicos imidazólicos interfieren con la actividad de la vitamina D inhibiendo la conversión de 25-hidroxivitamina D a 1,25-dihidroxivitamina D mediante el enzima renal 25-hidroxivitamina D-1-hidroxilasa.

4.6. Fertilidad, embarazo y lactancia

Durante el embarazo y la lactancia, no se recomiendan las dosis altas de vitamina D y deberían utilizarse, por el contrario, formulaciones con dosis bajas.

4.6.1. Embarazo

Hay muy pocos datos sobre los efectos del colecalciferol en mujeres embarazadas. Se ha demostrado toxicidad reproductiva en estudios con animales (ver sección "5.3. Datos preclínicos sobre seguridad"). La ingesta diaria recomendada en mujeres embarazadas es de 400 UI, sin embargo, en caso de mujeres con deficiencia de vitamina D, se puede requerir una dosis mayor (hasta 2000 UI/día – 10 gotas de la presentación de gotas en solución oral). Las mujeres embarazadas deberían seguir el consejo de su médico, ya que los requerimientos pueden variar en función de la gravedad de su enfermedad y de su respuesta al tratamiento con vitamina D y sus respectivos metabolitos que se eliminan por leche materna.

4.6.2. Lactancia

Si es necesario, se puede prescribir vitamina D en mujeres en período de lactancia. Esta suplementación no sustituye a la administración de vitamina D en el neonato.
No se han observado sobredosis inducidas por madres suplementadas con vitamina D durante el período de lactancia en bebés; sin embargo, cuando se prescriba vitamina D a un lactante, el médico debe tener en cuenta la dosis adicional de vitamina D que está tomando la madre.

4.7. Efectos sobre la capacidad para conducir y utilizar máquinas

No hay datos sobre los efectos de Deltius sobre la capacidad para conducir y utilizar máquinas. No obstante, un efecto en este sentido parece improbable.

4.8. Reacciones adversas

Las frecuencias de las reacciones adversas se definen como: poco frecuentes (>1/1.000 a <1/100) ó raras (>1/10.000 a <1/1.000).

Trastornos del metabolismo y la nutrición:
Poco frecuentes : hipercalcemia e hipercalciuria

Trastornos de la piel y subcutáneos:
Raros : prurito, rash y urticaria.

Notificación de sospechas de reaciones adversas
Es importante notificar sospechas de reacciones adversas al medicamento tras su autorización. Ello permite una supervisión continuada de la relación beneficio/riesgo del medicamento. Se invita a los profesionales sanitarios a notificar las sospechas de reacciones adversas a través del Sistema Español de Farmacovigilancia de Medicamentos de Uso Humano: www.notificaRAM.es

4.9. Sobredosis

El tratamiento con Deltius debería interrumpirse cuando la calcemia supere 10.6mg/dl (2.65 mmol/l) o si la calciuria supera los 300mg/24 horas en adultos o los 4-6 mg/kg/día en niños.

La sobredosis se manifiesta mediante hipercalcemia e hipercalciuria, cuyos síntomas son: náuseas, vómitos, sed, estreñimiento,

poliuria, polidipsia y deshidratación.
La sobredosis crónica puede dar lugar a calcificación vascular y orgánica como consecuencia de la hipercalcemia.

Tratamiento en el caso de sobredosis:
Interrumpir el tratamiento con Deltius e iniciar la rehidratación.

5. PROPIEDADES FARMACOLÓGICAS

5.1. Propiedades farmacodinámicas

Grupo farmacoterapéutico: vitamina D y análogos, colecalciferol
código ATC: A11CC05.

5.1.1. Mecanismo de acción

La vitamina D, en su forma biológicamente activa, estimula la absorción intestinal de calcio, la incorporación de calcio en el osteoide y la liberación de calcio del tejido óseo. En el intestino delgado, promueve la captación de calcio, tanto rápida como diferida.
Además, estimula el transporte activo y pasivo de fosfato. A nivel renal, inhibe la excreción de calcio y fosfato al favorecer la reabsorción tubular. La forma biológicamente activa de la vitamina D_3 inhibe directamente la producción de hormona paratiroidea (PTH) en la glándulas paratiroides. La secreción de PTH es inhibida, además, debido al aumento en la absorción de calcio que la forma biológicamente activa de la vitamina D provoca en el intestino delgado.

5.2. Propiedades farmacocinéticas

La farmacocinética de la vitamina D es bien conocida.

Absorción:

La vitamina D se absorbe fácilmente en el tracto gastro-intestinal en la presencia de sales biliares, por lo que su administración con las principales comidas puede facilitar su absorción.

Distribución y biotransformación:

Inicialmente, la vitamina D se hidroxilada en el hígado dando lugar a 25-hidroxi-colecalciferol. Posteriormente es hidroxilada de nuevo en los riñones dando lugar al metabolito activo, 1,25-dihidroxi-colecalciferol (calcitriol).

Eliminación:

Los metabolitos de la vitamina D circulan en el torrente sanguíneo unidos a una globulina plasmática específica, α – globina .
La Vitamina D y sus metabolitos se excretan principalmente en la bilis y en las heces.

Poblaciones especiales:

Pacientes con Alteraciones de la Función Renal :

Se ha comunicado una disminución de un 57% en la tasa de aclaramiento metabólico en pacientes con deterioro de la función renal, en comparación con voluntarios sanos.

Pacientes con síndrome de malabsorción

Puede producirse una reducción de la absorción y un aumento de la eliminación de la vitamina D.

Personas obesas

Para las personas obesas es más difícil poder mantener los niveles de vitamina D con la exposición solar y, en consecuencia, pueden necesitar mayores dosis orales de vitamina D para compensar el déficit.

5.3. Datos preclínicos sobre seguridad

Los estudios pre-clínicos llevados a cabo en varias especies animales revelaron que los efectos tóxicos en animales tienen lugar a dosis mucho más altas que las requeridas para uso terapéutico en humanos.

En los estudios de toxicidad a dosis repetidas, los acontecimientos adversos más frecuentes fueron aumento de la calciuria y disminución de la fosfaturia y de la proteinuria.

Se ha observado hipercalcemia a dosis altas. En estados de hipercalcemia prolongada, se han dado casos de alteraciones histológicas (calcificación); principalmente en riñones, corazón, aorta, testículos, timo y mucosa intestinal.

Se ha demostrado el efecto teratogénico del colecalciferol a dosis altas en animales. Sin embargo, no se observó ningún efecto teratogénico cuando se les administraron dosis dentro del rango terapéutico humano.

El colecalciferol no ha demostrado potencial mutagénico ni carcinogenético.

6 . DATOS FARMACÉUTICOS

6.1. Lista de excipientes

Aceite de oliva refinado

6.2. Incompatibilidades

En ausencia de estudios de compatibilidad, este medicamento no debe mezclarse con otros.

6.3. Periodo de validez

4 años.

6.4. Precauciones especiales de conservación

No conservar a temperatura superior a 30° C.
No refrigerar o congelar.
Conservar en el envase original para protegerlo de la luz.

6.5. Naturaleza y contenido del envase

Frascos de vidrio tipo III color topacio de 5ml de capacidad, conteniendo 2,5ml de solución oral y sellados con un tapón de polipropileno y polietileno.

Envases unidosis de 1 y 4 frascos.

Puede que solamente estén comercializados algunos tamaños de envases.

6.6. Precauciones especiales de eliminación y otras manipulaciones

Se debería administrar Deltius preferiblemente durante una de las comidas principales (ver sección "5.2, propiedades farmacocinéticas, Absorción").

No almacenar ningún producto, ni alimento que contenga Deltius para ser utilizado posteriormente o en la comida siguiente (ver sección "4.2. Posología y forma de administración").

La eliminación del medicamento no utilizado y de todos los materiales que hayan estado en contacto con él se realizará de acuerdo con la normativa local.

7. TITULAR DE LA AUTORIZACIÓN DE LA COMERCIALIZACIÓN

ITALFARMACO, S.A.
San Rafael, 3 – 28108 Alcobendas (Madrid). España
Tel.: 916572323

8. NÚMERO(S) DE AUTORIZACIÓN DE LA COMERCIALIZACIÓN

9. FECHA DE LA PRIMERA AUTORIZACIÓN/ RENOVACIÓN DE LA AUTORIZACIÓN

10. FECHA DE LA REVISIÓN DEL TEXTO

Agosto 2013

ANNEX 4: Consents and Trial Informative Sheet

FULL D'INFORMACIÓ PER AL PACIENT

UTILITZACIÓ DE MOSTRES BIOLÒGIQUES ROMANENTS D' UN PROJECTE D'INVESTIGACIÓ I CONSERVACIÓ FINAL AL BIOBANC- IDIBGI

Al Hospital Josep Trueta (HJT), l'Institut d'Investigació Biomèdica de Girona (Idibgi) i/o altres centres hospitalaris adscrits es realitza investigació biomèdica, a més de l'assistència als pacients. La finalitat d'aquesta investigació és progressar en el coneixement de les malalties, la seva prevenció, diagnòstic, pronòstic i tractament. Aquesta investigació biomèdica requereix recollir dades clíniques i mostres biològiques dels pacients o donants per analitzar-les amb l'objectiu de conèixer millor i avançar en el diagnòstic i/o tractament de les malalties.

En aquest sentit, les mostres obtingudes per al diagnòstic o control de les malalties, un cop utilitzades amb aquesta finalitat, resulten també molt útils i necessàries per a la investigació. De fet, molts dels avenços científics obtinguts en els darrers anys en medicina són fruit d'aquest tipus de mostres. Si no fossin cedides per a investigació, aquestes mostres biològiques sobrants o excedents del procés assistencial serien destruïdes.

D'acord amb les normes de bioètica i la legislació vigent, sol·licitem la vostra autorització per a la cessió de les mostres biològiques i la informació clínica associada per prosseguir amb la investigació biomèdica, una vegada hagi finalitzat el projecte d'investigació següent:

..... *(indiqueu la ref. del projecte).*

Seguint el que estableix la Llei 14/2007 de Recerca Biomèdica, la Llei Orgànica 15/1999 de Protecció de Dades Personals i les seves normes de desenvolupament (RD 1716/2011 i RD 1720/2007, respectivament), us demanem que llegiu detingudament aquest document d'informació i el consentiment informat que s'adjunta al final perquè el pugueu signar, si s'escau.

FINALITAT DE LA INVESTIGACIÓ: progressar en el coneixement de les malalties

La finalitat de la investigació és millorar el nostre coneixement de les malalties. Les mostres, les dades clíniques i analítiques i les proves d'imatge s'utilitzaran per a la recerca biomèdica.

Tot això permetrà progressar en el coneixement de la prevenció, diagnòstic, pronòstic i/o tractament de les malalties.

MOSTRES BIOLÒGIQUES I DADES CLÍNIQUES: una vegada finalitzat el projecte d'investigació es custodien i conserven al Biobanc fins a la seva extinció

És a les vostres mans decidir si una vegada finalitzat el projecte d'investigació abans descrit, les dades clíniques recollides i les mostres biològiques sobrants d'aquest projecte passen a ser custodiades i conservades al biobanc (banc de mostres biològiques), fins a la seva extinció.

Aquest biobanc és un establiment legalment autoritzat, sense ànim de lucre, acull col·leccions organitzades de mostres biològiques i informació associada a les condicions i garanties de qualitat i seguretat que exigeix la legislació ja referida i els codis de conducta aprovats pels comitès d'ètica. Aquestes mostres i la seva informació associada queden disponibles per a aquells centres o institucions de recerca nacionals o internacionals que ho sol·licitin oficialment al biobanc.

Qualsevol estudi d'investigació per al qual se sol·liciti la utilització d'aquestes dades o mostres ha de disposar sempre de l'aprovació del Comitè d'Ètica de la Investigació (CEI) competent, que vetlla perquè els investigadors desenvolupin els seus estudis seguint sempre les més estrictes normes ètiques i legals, i perquè l'aprovi un comitè científic que en garanteixi la utilitat científica.

A partir de les mostres donades, en els casos en què la investigació ho requereixi, es realitzaran estudis genètics, i a partir d'aquests es pot obtenir informació sobre la vostra salut i la dels vostres familiars. Sempre s'actuarà vetllant per la protecció d'aquesta informació (vegeu l'apartat de protecció de dades i confidencialitat).

En cas de ser necessària alguna mostra addicional, la institució sanitària es podria posar en contacte amb vosaltres per sol·licitar novament la vostra col·laboració.

PROTECCIÓ DE DADES I CONFIDENCIALITAT: les mostres es conserven codificades

Les dades personals que es recullin seran obtingudes, tractades i emmagatzemades complint en tot moment el deure de confidencialitat, d'acord amb la legislació vigent en matèria de protecció de dades de caràcter personal.

La identificació de les mostres biològiques del biobanc és sotmesa a un procés de codificació. A cada mostra se li assigna un codi d'identificació, que és el que utilitzen els investigadors. Únicament el personal autoritzat pel biobanc pot relacionar la vostra identitat amb els esmentats codis. Mitjançant aquest procés, els investigadors que sol·licitin mostres al biobanc no podran conèixer cap dada que reveli la vostra identitat. Així mateix, encara que els resultats obtinguts de la investigació realitzada amb les vostres mostres es publiquin en revistes científiques, la vostra identitat no és facilitarà.

Les dades clíniques i la informació de les mostres biològiques dels donants passen a formar part del fitxer del biobanc, inscrit en l'agència de protecció de dades sota la responsabilitat de Idibgi.

Aquestes dades són tractades i cedides amb l'única i exclusiva finalitat de dur a terme recerca biomèdica. Les dades de les mostres, sense dades personals, podran ser compartides en el si de xarxes cooperatives de biobancs i grups cooperatius de recerca.

Podreu exercir els vostres drets d'accés, rectificació, cancel·lació i oposició (ARCO) de les vostres dades dirigint-vos a la Direcció del Biobanc Idibgi per correu electrònic (biobanc@idibgi.cat) o via postal a l'adreça següent:

DIRECTOR DEL BIOBANC IDIBGI Hospital Josep Trueta Planta -9	Av. França s/n 17007 Girona Tel. 972 94 02 82 biobanc@idibgi.cat

En cas de dubte o impossibilitat per dur a terme el procediment, podeu contactar a través del telèfon d'atenció indicat.

CARÀCTER ALTRUISTA DE LA DONACIÓ: la cessió de mostres biològiques que realitzeu al Biobanc Idibgi és gratuïta

No obtindreu cap benefici econòmic directe per la cessió de la mostra i dades associades ni per participar en els estudis d'investigació. Tampoc tindreu drets sobre possibles beneficis comercials dels descobriments que es puguin aconseguir com a resultat de la investigació biomèdica.

PARTICIPACIÓ VOLUNTÀRIA: la vostra negativa no afectarà la vostra assistència mèdica, present o futura

La vostra participació és totalment voluntària. Podeu negar-vos a participar o retirar el vostre consentiment en qualsevol moment posterior a la signatura sense haver d'explicar els motius. Això no repercutirà negativament en la vostra assistència mèdica, present o futura.

REVOCACIÓ DEL CONSENTIMIENT: si decidiu firmar aquest consentiment, també podreu cancel·lar-lo lliurement. Això comportarà la destrucció de les vostres mostres

Si en un futur volguéssiu anul·lar o cancel·lar el vostre consentiment, les mostres biològiques serien destruïdes i les dades associades a aquestes serien retirades del biobanc. També podríeu sol·licitar que les mostres siguin anònimes, la qual cosa significa que s'eliminaria la relació entre les vostres dades personals (que revelen la vostra identitat) i les mostres biològiques i dades clíniques associades. Els efectes d'aquesta cancel·lació o anonimat no es podrien estendre a la investigació que ja s'hagués dut a terme. Si desitgeu cancel·lar el consentiment, hauríeu de sol·licitar-ho per escrit a la direcció del Biobanc Idibgi, a l'adreça indicada anteriorment.

INFORMACIÓ SOBRE ELS RESULTATS DE LA INVESTIGACIÓ: se us proporcionarà informació si la desitgeu rebre

En cas que ho demaneu expressament, el biobanc us pot proporcionar informació sobre quines són les investigacions en què s'han utilitzat les vostres mostres i dels resultats globals d'aquestes investigacions, excepte en cas de cancel·lació o anonimat.

Els mètodes utilitzats en investigació biomèdica solen ser diferents dels aprovats per a la pràctica clínica, per la qual cosa no els heu de considerar amb valor clínic. Però, en cas que aquestes investigacions proporcionin dades que poguessin ser tant clínicament com genèticament rellevants per a la vostra salut o la de la vostra família, se us comunicarien si així ho creieu oportú. Així mateix, podríeu obtenir informació rellevant per a la vostra família. Us correspon a vosaltres decidir si voleu o no que us les comuniquem. Si voleu que sigui així, ho heu de consignar a la casella que apareix al final d'aquest full.

Si no desitgeu rebre aquesta informació, tingueu en compte que la llei estableix que quan la informació obtinguda sigui necessària per evitar un greu perjudici per a la salut dels vostres familiars biològics, un comitè d'experts estudiarà el cas i haurà de decidir si és convenient informar els afectats o els seus representants legals.

Si teniu qualsevol dubte, ara o en el futur, en relació amb aquest consentiment, no dubteu a preguntar el que calgui al personal sanitari que us ha donat aquesta informació. També podeu comentar els dubtes amb el vostre metge, que us posarà en contacte amb el personal sanitari autoritzat.

Moltes gràcies per la vostra col·laboració.

Biobanc Idibgi

Format de full d'informació del pacient adaptat de Institut d'Investigació Biomèdica de Bellvitge (HUB -ICO -IDIBELL)

CONSENTIMIENTO INFORMADO

(PADRES/TUTORES)

Versión 1 fecha 11 de noviembre de 2015

TITULO DEL ESTUDIO: Glycemic control after vitamin D repletion in T1DM pediatric population.

Yo,.....

(Nombre y apellidos)

Como tutor legal de,.....

(Nombre y apellidos)

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido respuestas satisfactorias a mis preguntas.
He recibido suficiente información sobre el estudio.

He hablado con,.....

(Nombre del investigador)

Acepto voluntariamente que mi hijo/hija participe en el ensayo clínico y autorizo el uso de la información relacionada con el ensayo.
Comprendo que puedo retirarlo/la del estudio cuando quiera, sin que ello repercuta en los cuidados médicos y sin tener que dar explicaciones.

....

Firma del participante **Fecha**

....

Firma del médico que dio la información **Fecha**

CONSENTIMIENTO INFORMADO

(MAYOR DE 12 AÑOS)

Versión 1 fecha 11 de noviembre de 2015

TITULO DEL ESTUDIO: Glycemic control after vitamin D repletion in T1DM pediatric population.

Yo,.....

(Nombre y apellidos)

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido respuestas satisfactorias a mis preguntas.
He recibido suficiente información sobre el estudio.

He hablado con,.....

(Nombre del investigador)

Comprendo que la participación es voluntaria y autorizo el uso de la información relacionada con el ensayo.
Comprendo que puedo retirarme del estudio cuando quiera, sin que ello repercuta en los cuidados médicos y sin tener que dar explicaciones.

....

Firma del participante **Fecha**

....

Firma del médico que dio la información **Fecha**

HOJA DE INFORMACIÓN PARA EL PACIENTE

Nombre del estudio:

EFECTO DE LA NORMALIZACIÓN DE LOS NIVELES DE VITAMINA D EN EL CONTROL GLICEMICO DE PACIENTES PEDIÁTRICOS DIABÉTICOS TIPO 1.

Agradecemos su interés respecto a su colaboración en el estudio realizado por las unidades de endocrinología pediátrica de los hospitales Dr. Josep Trueta, de Palamós, de Calella, de Figueres y de Blanes. A continuación, le explicamos en que consiste el estudio. Además, puede dirigirse al equipo de investigación para cualquier duda o cuestión que le pueda surgir.

¿Cuál es el objetivo del estudio?

Este estudio tiene como principal objetivo evaluar si la normalización de los niveles de vitamina D se traduce en un mejor control glicémico para el paciente con diabetes mellitus tipo 1 con niveles por debajo de la normalidad. Actualmente, la determinación de vitamina D no se encuentra incluida en el protocolo de control de los pacientes diabéticos. Estudios recientes son sugestivos de que niveles por debajo de la normalidad se relacionan con un aumento de la resistencia insulínica, dificultando el control glicémico.

¿Qué pasará si participo?

En primer lugar, 4 semanas antes de iniciar la suplementación con vitamina D se les citará para realizar una analítica y establecer los niveles de vitamina D. De estar por debajo de la normalidad, no asociarse comorbilidades o tratamientos farmacológicos crónicos y cumplir criterios de inclusión, se les ofrecerá participar en el estudio. De aceptar, su hijo será asignado de forma aleatoria en un grupo para recibir suplementación con vitamina D o placebo.

Su hijo/a seguirá con el control habitual de su diabetes mellitus. La suplementación se realizará cada mes de forma autónoma mediante unas ampollas que podrán recoger en la farmacia del hospital. En las dos próximas visitas de seguimiento, establecidas cada 6 meses, se les pedirá que retornen dichas ampollas para valorar la adherencia al tratamiento. Se les realizará una valoración de calcio y creatinina para asegurar que el tratamiento no excede los niveles de toxicidad de vitamina D. Se añadirán los parámetros relacionados con la vitamina D (calcidiol total, hormona paratiroidea) en la analítica de control, que incluye la hemoglobina glicosilada para valorar el control. Además, se les realizarán unos cuestionarios para valorar la actividad física, la ingesta de vitamina D y calcio y la exposición solar.

¿Y si a mi hijo/a le toca el grupo placebo?

Niveles de vitamina D por debajo de la normalidad pero por encima de deficiencia severa no han mostrado afectación clínica a corto plazo. Por esta razón, de estar los niveles de vitamina D de su hijo por debajo del rango de deficiencia severa, éste será tratado directamente sin incluirse en el estudio. Le recordamos la importancia de cumplir el placebo a lo largo del estudio, que permite objetivar el beneficio que supone la suplementación de vitamina D para los pacientes diabéticos.

¿La participación es obligatoria?

La participación en el estudio es totalmente voluntaria. Si decide participar, se le pedirá que firme el consentimiento informado según el cual usted conoce y entiende todo lo que implica la participación en este estudio. Contrariamente, si decide no participar, ello no afectará ni modificará el plan asistencial establecido para su hijo.

¿Qué debo realizar para participar?

Para llevar a cabo este estudio y acorde a las disposiciones legales vigentes, le solicitamos que rellene el consentimiento informado. Recuerde que, antes y después de firmar el documento, puede usted preguntar todo lo que le parezca conveniente al personal sanitario responsable del estudio.

¿Se manejarán los datos de mi hijo/a de forma confidencial?

Sí. La información recogida para este estudio será tratada y regulada en concordancia a la Ley Orgánica de Protección de Datos de Carácter Personal (15/1999) según la cual, sus datos serán manejados de forma confidencial y solamente serán utilizados con finalidad de investigación. La identificación personal de su hijo estará codificada a través de una serie numérica aleatorizada.

¿Qué pasará si cambio de opinión durante el estudio?

Si renuncia a seguir participando en el estudio una vez éste iniciado, no supondrá ningún castigo ni penalización ni para usted ni para su hijo/a. Se le pedirá que continúe con los controles y seguimientos habituales.

¿Qué se realizará con la información obtenida en el estudio?

Los resultados serán publicados en revistas de interés científico relacionadas con el área de conocimiento correspondiente a la patología endocrinológica pediátrica, con el fin de que otros centros y pacientes puedan beneficiarse de los resultados de este estudio. Recuerde que todos los datos de su hijo/a de carácter personal serán anonimizadas mediante una serie numérica aleatorizada.

Muchas gracias por su atención,

Comité Directivo del Ensayo Clínico

ANNEX 5: Posters European Society of Pediatric Endocrinology (ESPE)

Vitamin D status in Egyptian children with T1D and the role of Vitamin D replacement in glycemic control.

Mona Hafez[1], Mona Hassan[1], Noha Musa[1], Sahar Sharaf[2], Sally Abdel Azim[1]

[1] Diabetes, Endocrine and Metabolism Pediatric Unit, Cairo University

[2]Department of Chemical Pathology, Cairo University

Disclosure: Authors have nothing to disclose

BACKGROUND

Many epidemiological studies have found high prevalence of vitamin D deficiency in children with T1D **[1,2]**. 1,25 $(OH)_2$ D is a potent immune-modulator that also enhances the production and secretion of several hormones, including insulin **[3]**. The association of low serum 1, 25$(OH)_2$ D levels with high glucose level and diminished insulin sensitivity suggests that vitamin D may modulate insulin metabolism **[4]**.

OBJECTIVES

To screen for vitamin D deficiency in pediatric patients with T1D and to study the effect of vitamin D supplementation on glycemic control and insulin requirements in those patients.

METHODS

This study was a prospective cohort study that included 50 patients with T1D above 5 years of age with onset of T1D > 1 year, with no hepatic or renal problems or any drug therapy that may affect vitamin D metabolism. 25-hydroxyvitamin D (25(OH)D) level was assessed initially and after 3 months of vitamin D3 supplementation (in those with vitamin D deficiency) in a dose of 4000 IU/d. Glycemic control (HbA1c) and insulin requirements were studied at 0, 3 and 6 months of vitamin D therapy. The study protocol was approved by the Research Ethics Committee of Cairo University and patients were included after obtaining informed consents from their legal guardians.

RESULTS

- This study was a prospective cohort study, where 50 patients (23 females and 27 males) with T1D were recruited from Diabetes, Endocrinology and Metabolism Paediatric Unit (DEMPU), Cairo University. Mean age of included patients was (10.24 ± 3.46) years with a range of 5.2 - 16.9 years. Their age at onset of diabetes ranged from 1 to 14 years with mean of (6.16 ± 3.4), whereas their diabetes duration range was 1.3 to 11.5 years of mean (4.11± 2.34). Vitamin D levels in the study group measured in the form of 25(OH)D ranged from 0.2 to 33 (ng/ml) with a mean of (11.246 ± 5.716).
- When we correlated the basal vitamin D status of the 50 patients with other parameters (before vitamin D supplementation; there was a significant correlation with insulin dose, magnesium levels ($p<0.05$) and HbA1c % levels ($p=0.00$). However, there was no correlation between Vitamin D and calcium, phosphorus or alkaline phosphatase levels ($p> 0.05$).
- The thirty five (n=35) vitamin D-deficient patients were allocated to vitamin D supplementation for 3 months. Among the 33 patients allocated to vitamin D supplementation (2 patients were excluded due non-compliance), 12 (36.4%) were males while 21 (63.6%) were females. Their age ranged from 5.2 to 16.9 yrs with a mean of (10.389 ± 3.53 yrs). The supplemented group had significant improvement in their vitamin D levels after 3 mo of therapy with a mean of (31.44 ± 11.57 ng/ml) ($p=0.000$). Also, patients with low calcium and phosphorus levels showed normalisation of these levels after 3 mo of Vitamin D supplementation [table 2]. As for insulin requirements, no significant difference was noted at 0, 3 and 6 mo of Vitamin D supplementation ($p= 0.354$). However, there was significant improvement in HbA1c in the supplemented group ($p=0.000$) [table 3].

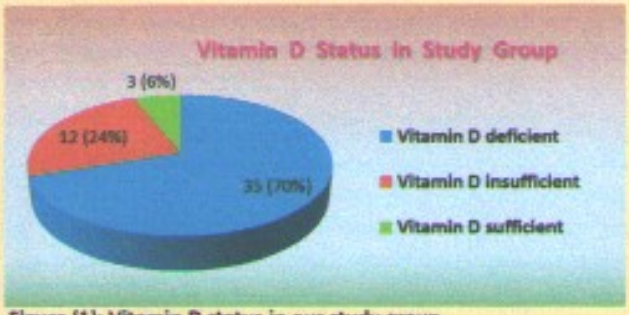


Figure (1): Vitamin D status in our study group.

Table (1): Calcium homeostasis, insulin requirements and HbA1c in relation to vitamin D status within the study group.

		Vitamin D deficient (n=35)	Vitamin D insufficient (n=12)	Vitamin D sufficient (n=3)
Calcium (mg/dl)	Low	6	0	1
	Normal	29	12	2
Phosphorus (mg/dl)	Low	1	0	0
	Normal	34	12	3
ALP (U/L)	High	0	0	0
	Normal	35	12	3
PTH (pg/ml)	High	1	0	0
	Normal	34	12	3
HbA1c (%)	< 7 (good)	3	3	0
	7-9 (fair)	13	5	3
	>9 (poor)	19	4	0
Insulin requirements (IU/kg/d)	< 0.5	1	1	0
	0.5 - 1	13	7	1
	>1	21	4	2

Table (2): The biochemical features of vitamin D deficient patients before and after vitamin D supplementation.

		Pre ttt		Post ttt (3 mo)		Pvalue
		mean	range	mean	range	
Calcium	(mg/dl)	9.13±0.77	7.8-10.4	9.27±0.47	8.2-10.5	0.400
Phosphorus	(mg/dl)	4.47±0.645	2.4-6.1	4.83±0.745	3-6.2	0.047
ALP	(U/L)	196.39±78.5	5-405	190.45±59.7	54-354	0.626
25(OH)D	(ng/ml)	8.71±3.17	0.2-33	31.44±11.57	13-58	0.000

Table (3): HbA1c and insulin requirements in vitamin D deficient patients before and after vitamin D supplementation.

	Before ttt		After 3 mo of ttt		After 6 mo of ttt		P value
	mean	range	mean	range	mean	range	
Insulin (U/Kg/d)	1.218±0.38	0.3-2	1.18±0.41	0.37-2.16	1.22±0.42	0.33-2.2	0.354
HbA1c %	9.413±1.97	5.7-14	8.78±1.58	6.2-13.4	9.53±1.7	6.9-13.0	0.000

CONCLUSION

Checking the serum 25(OH) D levels in children and adolescents with T1D and providing replacement for children with low levels improved glycemic control at 3 and 6 months after therapy in those with low levels with no reduction in insulin requirements.

REFERENCES

1- Janner M et al (2010) High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. Swiss Med Wkly Sep 3;140:w13091

2- Greer RM et al (2013) Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes Feb;14(1):31-41

3- Bock G et al (2011) The effect of vitamin D supplementation on peripheral regulatory T cells and β cell function in healthy humans: a randomized controlled trial.Diabetes Metab Res Rev. Nov;27(8):942-5

4- Giulietti A et al (2004) Vitamin D deficiency in early life accelerates T1D in non-obese diabetic mice. Diabetologia 47, 451–462

DOI: 10.1530/endoabs.39.P745

Correlation of vitamin D levels with glycemic control, total daily insulin dose, BMI and ethnicity in pediatric patients with type 1 diabetes mellitus.

Bianco, M[1], Durazo, R, Durazo-Arvizu, R[1], & Minutti, C[2]
[1]Department of Pediatrics, Loyola University Medical Center, Ronald McDonald Children's Hospital, Maywood, IL
[2]Department of Pediatrics, Division of Pediatric Endocrinology, Rush University Medical Center, Chicago, IL

LOYOLA HEALTH SCIENCES DIVISION

Ronald McDonald Children's Hospital LOYOLA UNIVERSITY MEDICAL CENTER

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune and inflammatory process. Vitamin D (VD) is thought to reduce inflammation and prevent autoimmune destruction. Research has shown that the VD receptor is present in osteoblasts, small intestine/colon, T and B lymphocytes, β-islet cells and most organs in the body. The presence of VD receptors on β-islet cells has prompted studies on the effect of VD levels on insulin sensitivity and requirements. Studies have shown that VD has an effect on insulin secretion and sensitivity in rats[1]. It has also been shown that adult type 2 diabetics with normal VD levels have decreased insulin requirements[2]. Another study showed that 1 year-old VD deficient children have been reported to be at a fourfold higher risk of developing type 1 diabetes than VD sufficient children[3].

Objectives

In this study, we aimed to determine if there is a significant correlation between VD levels and HbA1c, daily insulin requirement, BMI, and ethnicity in pediatric T1DM. Our hypothesis was that patients with low VD levels will have increased daily insulin requirement. Two prior studies in Turkey looked at the relationship between VD levels and daily insulin requirement in T1DM pediatric patients with contradictory results[4-5].

Methods

One hundred sixty two T1DM pediatric patients ages 3-20 years old were included in this study. Age, gender, ethnicity, BMI, HgA1c, VD level (1,25(OH)D), and total daily insulin requirement in units/kg/day were obtained through a retrospective chart review. VD levels were divided into 3 groups based on the Academy of Pediatrics recommendations on cut-off levels for states of VD[6]: <20 ng/ml was considered deficient/insufficient, 20-29.9 ng/ml low sufficient, and >30 ng/ml was high sufficient. Multivariate linear regression analysis (using STATA13.1) was used to assess the association between insulin requirement and VD levels adjusting for the following confounders: age, gender, ethnicity, BMI, and HA1c.

Demographics

Gender	Number in Study
Female	84
Male	78

AGE	Years Old
Minimum	3
Maximum	20
Mean	13

Ethnicity	Number in Study
Caucasian	99
Hispanic	27
African-American	30
Not specified	6

Results

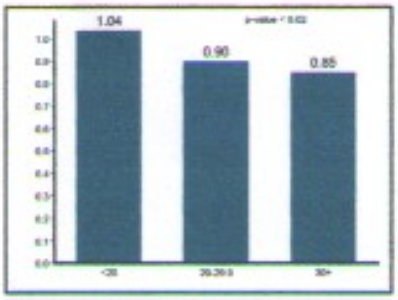


Figure 1:
Correlation between daily insulin requirements and VD levels. VD levels were divided into 3 groups: 1)<20ng/mL (deficient/insufficient), 2) 20-29.9 ng/mL (low sufficient), and 3) >30 ng/mL (high sufficient). There is a significant relation between daily insulin requirement and VD level. Daily insulin requirement was higher in those with lower vitamin D levels with a p-value of <0.02.

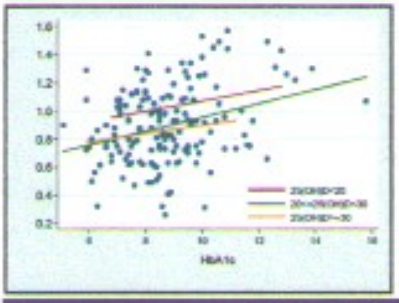


Figure 2:
Multivariate linear regression analysis showed an association between insulin requirement and VD levels adjusting for the following confounders: age, gender, ethnicity, BMI, and HA1c. Significant increase in insulin requirement was only seen in the deficient/insufficient group. Low sufficient and high sufficient did not show a significant difference in insulin requirement.

Discussion

The study included 84 girls (52%), 78 boys (48%), 30 African Americans (19%), 27 Hispanics (17%), and 99 Caucasians (61%). Mean age was 13. Analysis of the data showed that patients with deficient/insufficient VD levels had a statistically significant (P=0.02) increase in insulin requirement. Using multivariate linear regression analysis we were able to look at the relationship between daily insulin requirement and VD levels independent of HA1c levels, age, gender, ethnicity, and BMI.

Conclusion

These findings suggest that lower levels of VD may contribute to the need for higher insulin doses, which may be related to insulin resistance and suboptimal glucose control in pediatric patients with T1DM. These results indicate that VD is related to T1DM control. We suggest checking VD levels and replacing VD in patients that are deficient/insufficient to improve glucose control and decrease insulin requirements.

References

1.Bourlon PM., Billaudel B., Faure-Dusser A. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on dencovo insulin biosynthesis in the islets of the rat endocine pancreas. J Endocrinol. 1999;160:87-95.

2.Pittas, A., Lau, J., Hu, F., and Dawson-Hughes, B. The role of vitamin D and calcium in type 2 diabetes. A systemic review and meta-analysis. J Clin Endocrinol Metab. 2007;92:2017-2029.

3.Hypponen, E., Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001; 358(9292):1500-1503.

4.Mutlu, A., Mutlu, GY., Ozsu, E., Cizmecioglu, FM., Hatun, S. Vitamin D Deficiency in Children and Adolescents with Tryge 1 Diabetes. J Clin Res Pediatr Endocrinol, 2011;3(4): 179-183.

5.Tunc, O., Cetinkaya, S., Kiziltgun, M., and Aycan, Z. Vitamin D status and isulin requirements in children and adolescents with type 1 diabetes. J Pediatr Endocr Met. 2011, 24(11-12):1037-1041.

6.Misra, M., Pacaud, D., Petryk, A., Collett-Solberg, PF., and Kappy, M. Vitamin D Deficiency in Children and Its Management: Review of Current Knowledge and Recommendations. Pediatrics. 2008; 122(2):398-417.

54th Annual Meeting 1-3 October
ESPE 2015 BARCELONA
European Society for Paediatric Endocrinology

Vitamin D deficiency in children with DM1A in northern Spain

I.Diez López[1], A. Sarasua Miranda[1], I.Lorente Blázquez[1]
Sección Endocrinología Infantil, Servicio de Pediatría. Hospital Universitario Araba. Sede Txagorritxu.Vitoria. Vitoria/Álava

INTRODUCTION:

- 1,000 million people worldwide present vitamin D deficiency. In children, the prevalence of vitamin D deficiency is referred up to 80% in certain countries, especially at high latitudes (above37).
- Vitamin D role in the inmune system and in DM1 clinical variability has been described.

OBJECTIVE:

- To study deficit of VITD in children with DM 1 living in Alava (Location: 42º 51'north latitude 2°41'west longitude) and check its influence in the metabolic control.

MATERIAL & METHODS:

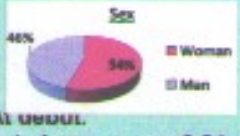
- Prospective open intervention.
- **Inclusion**: Patients with DM type 1a with at least 12 m. of evolution
  - First step of study (Abril-May 2014): **intervention** 6 months (sun exposure 3 m + treatment 3 m):
    - Intervention 3 m at summer: activities "outdoors"
    - Then treatment 3 months with 25.000 UI of colecalciferol (vitamin D), equivalent to 0,625mg (DELTIUS®)/1 every 3 weeks.

Revaluation:
November-December2014.

- **25-OH-Vitamin D levels** : insufficiency <30ng/ml.
- Statistic test study for parity (n<30) (Student t), with a confidence interval of 95%. SPSS 19.0

RESULTS:

- **57 cases**:



- **At debut:**
  - Average age: 8.24 years (DS 4.27 [0.3-15])
  - HbA1c debut 11.11% (DS 2.37[8-15.5])
- During this study:
  - Average age: 11.5 años (DS 3.67 [2-17])
  - HbA1c media 7.95%SDS1.16 [5.8-9.6]
  - No differences between sex
  - 93% with bolus-basal (4/57 ISCI).
- **VITAMIN D:**
  - **98% had deficit of vitamin D.**
  - 25-OH vitamin D levels: media 18 ngr/ml (DS[10-28]).
  - Normal: 1 case ; 12 years old European female (37 ngr/ml).

Treatment in 56 cases

- **After treatment with VitD:**
  - HbA1c: 7.68% (SDS1.18 [5.6-9.2] p:0.12)
  - 25OHD media 33 ngr/ml (DS[26-52] p:0.01)
  - Improve: 52 cases (93%)

CONCLUSIONS:

- **Children with DM1 in our region have an important deficiency of VitD.**
- The outdoor activities in summer and the treatment with depot preparations is effective in correcting this deficit. Although no significant improve in metabolic control has been observed.