Vitamin D trial to reduce cardiovascular disease risk markers in prepubertal and pubertal children with obesity

A Clinical Trial Intervention

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"La cultura es la libertad de los pueblos"

Miguel de Unamuno

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1. ABSTRACT

Background: Obesity and vitamin D deficiency in pediatric populations are two important and prevalent health problems. Obesity is a rising problem and according to literature vitamin D deficiency is more prevalent in obese population being vitamin D status an important point of investigation nowadays as regards its role in cardiovascular disease

Justification: Recent literature indicate an important role of vitamin D status in cardiovascular disease, in fact there are a large number of in vitro and animal studies which show an important relation between them; however experimental trials have failed to find causality. This may be explained by different dose regimes, treatment duration or inclusion criteria.

Objectives: Study the effect of vitamin D supplementation for one year on cardiovascular risk markers: Blood Pressure (BP), Carotid Intima media thickness (cIMT), Pulse wave velocity (PWV) and C-reactive protein (CPR) in pubertal obese children.

Participants: Subjects will be adolescents (10-15 years) with obesity (>95th centile), pubertal development (Tanner 2 or over) and Caucasian ethnicity located in the area of Gironès. 48 subjects will be selected (24 placebo group and 24 vitamin D group) according to inclusion criteria (Body mass index: +2.0 SD and +3.5 SD for age and gender). Vitamin D deficiency status: <12 ng/ml. Free vitamin D < 3.0 ng/ml. Several exclusion criteria are applied also to these subjects.

Main outcome measures: Primary outcomes**:** BP, cIMT, PWV and CPR. Secondary outcomes: glucose, fasting insulin, HOMA, lipid profile and HWM adiponectin.

Intervention and method: Intervention will be placebo or vitamin D, cholecalciferol (Deltius®) 25.000 IU / two week (equivalent to 1785 IU/day), with a 1:1 ratio and will be stratified by age, gender and BMI. Baseline measurements will include blood pressure, cIMT, PWV and CPR (additional parameters will also be studied). The intervention will last 12 months. The measures will be taken at the beginning, and at 6th and 12th months, being one year the estimated period to carry out this clinical trial.

Settings: The study will be performed in Hospital Dr. Josep Trueta (Idibgi).

Data analysis: Data analysis will be performed using SPSS version 12.0. Results for major variables will be performed using Mann-Whitney test (Continuous variable) and Fisher exact test (categorical variables). The analysis of response to treatment for endpoints variables (SBP, cIMT, PWV and CRP) will be performed by general lineal model (GLM) for repeated measures. A p value < 0.05 will be considered statistically significant.

Keyword: Vitamin D deficiency, cardiovascular risk marker, obesity, adolescent.

2. ABREVIATIONS

- **BMI**: Body mass index
- **HBP**: High blood pressure
- **VDR**: Vitamin D receptor
- **VDRE**: Vitamin D receptor elements
- **1,25(OH)D:** 1,25 Hidroxivitamin D
- **DBP**: Vitamin D binding protein
- **RxR**: Retinoid X receptor
- **PTH**: Parathyroid hormone
- **IU**: International units
- **CVD:** Cardiovascular disease
- **CKD**: Chronic kidney disease
- **RAAS**: Renin-Angiotensin-Aldosterone System.
- **cIMT**: Carotid intima media thickness
- **PWV**: Pulse wave velocity
- **CRP**: C-Reactive protein

3. INTRODUCTION

OBESITY-OVERWEIGHT

"Overweight and obesity are both labels for ranges of weight that are greater than what is generally considered healthy for a given height. The terms also identify ranges of weight that have been shown to increase the likelihood of certain diseases and other health problems"(1).

Obesity is commonly defined based on body mass index (BMI), which is calculated as *"weight (in kilograms) divided by the square of height (in meters)"*. In childhood, BMI changes with the age and gender so the measurement and diagnosis is different than adults(2).

Epidemiology

Childhood overweight and obesity is an important epidemic which affects the whole world. Being obese in children increases the likelihood of being an adult obese(3). More than 1.4 billion of adults are overweight (35% are overweight and 11% are obese). This results in at least 2.6 million people dying from overweight or obesity(4). This number is increasing each year particularly in metropolitan areas, being higher in developing countries where there is a controversial situation because of the coexistence of desnutrition and a high incidence of obesity in the population(4).

In Spain, two of ten adolescents are overweight and one of ten is obese (27.8% are overweight or obese in both sexes). If we compare Spain with the rest of European countries, Spain is ranked in the fourth position from the top in child obesity rates(5).

Diagnosis and risk factors

In childhood obesity is defined according to cutoff points of BMI:

- BMI ≥95th centile is considered obesity.
- BMI between the 85-94th centiles conform the term of overweight(2)

In adults BMI greater or equal to 25 is overweight and BMI greater or equal to 30 is obesity(4).

Imbalanced calorie intake and a sedentary lifestyle are the most important risk factors for obesity and overweight(4).However, there are other factors being the obesity a concept that

must be understood as the result of a mix of environmental, genetic and other factors (Illness or drugs)(6).

Health consequences and economic impact

Obesity and overweight increase all-cause mortality in white adults (7) in fact in the world there are more people dying from overweight than from underweight (4). Being obese in childhood is linked to a number of different diseases and consequences in adulthood (premature death, cardiovascular disease, diabetes, respiratory problems, vascular disease…)(4), although the normal presentation in childhood is asymptomatic, it may lead to different pathologies like high blood pressure, insulin resistance, breathing and musculo-skeletal problems or psychiatric disorders(2).

Estimated costs for obesity care are around 147 billion of Dollars in United States in 2008. This economic impact must be divided into direct and indirect costs (6).

Vitamin D and obesity

An important meta-analysis of cohort studies concluded that a high relation exits between obesity leading to vitamin D deficiency; however, the evidence was unclear regarding vitamin D deficiency causing obesity(8). The most accepted arguments supporting obesity leading to vitamin D deficiency are:

1. Adipose tissue traps vitamin D (fat soluble vitamin) so people with more adipose tissue are likely to have deficiency or insufficiency.
2. Psychological consequences of anxiety and mood which lead obese people not to leave from closed areas and not receive the umbral level to produce vitamin D from sunlight exposure(9,10)

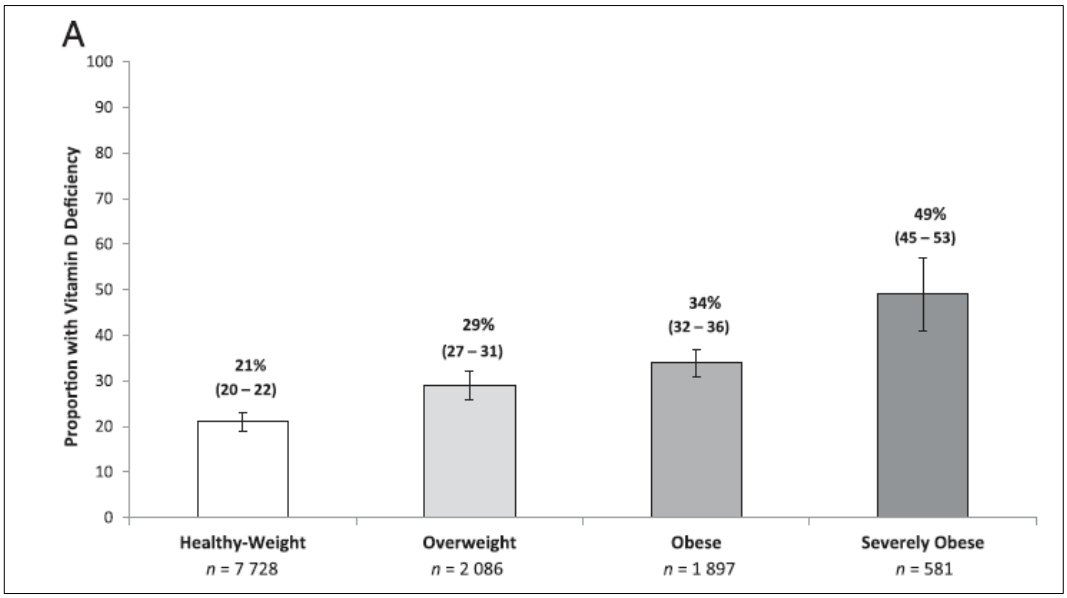


Figure 1: Vitamin D deficiency between US children according to BMI (23)

Literature compares the level of vitamin D and the BMI, age and season (11,12). However, it was not exactly the BMI which was inversely related with vitamin D status but the body fat percentage. The muscle mass was directly related with higher level of vitamin D(13).

VITAMIN D

History-Definition-Metabolism

McCullum and Davis in 1913 discovered the first vitamin (Vitamin D was the 4th vitamin discovered: D). Vitamin D is synthetized in the skin, it comes from cholesterol (steroid hormone) as a subtract (7-Dehidrocholesterol) to produce pre-vitamin D, which in turn isomerized to Vitamin D3. Vitamin D2 comes from the irradiation of Ergosterol. Vitamin D3 itself has no biological effects (there are patients with rickets but with normal level of vitamin D3), an activation is there for needed to the final active form: 1α,25-dihydroxyvitamin D3 thanks to 1α-hydroxylase located in the kidney. The level of 1,25-dihydroxivitamin D3 is the best biomarker of negative feedback, i.e., as the higher the level of this hormone, the more active the enzyme degrading it: 24-hidroxylase to calcitroic acid(14,15).

In 1969 the receptor of vitamin D (VDR) was discovered, which is located in over 30 tissue/organs and its expression is regulated by genomic and rapid response mechanisms (16). 1,25 (OH)D, which is associated with vitamin D binding protein (DBP) in serum, dissociates

from its carrier to enter the nucleus as a steroid hormone acting on the VDR. This binding triggers a group of biochemical reaction finally conforming a complex with the retinoid X receptor (RxR) – [complex VDR-RXR]that attaches to vitamin D response elements (VDRE) initiating the regulation of transcription on the cells where VDR are present thus making possible the function of vitamin D (17).

VITAMIN D METABOLISM

7 Dehydrocholesterol	Pro-vitamin D3	7DHC	Lipid in cell membranes
Cholecalciferol	Pre-vitamin D3	D3	Photosynthesized in skin or diet
Ergocalciferol	Pre-vitamin D2	D2	Diet, equivalent to vitamin D3 as precursor
Calcidiol	25-Hydroxyvitamin D	25(OH)D2-3	Best reflect vitamin D status
Calcitriol	1,25-Dihydrovitamin D	1,25 (OH)D2-3	Active form of vitamin D, tightly regulated
Calcitroic acid	1,24,25-Hydrovitamin D	1,24,25 (OH)D2-3	Catabolism metabolite

Functions related to calcium

The most known function of vitamin D is its action in the homeostasis of calcium serum level. Three are the mechanism to increase de calcium in serum:

1. Exogenous: Vit D induces synthesis of protein to absorb Ca^{2+} for active absorption in intestinal tube.
2. Endogenous: Activation of RANKL which induces osteclastogenesis and increase the number of osteoclast-producing bone resorption.
3. Endogenous: Distal renal tubule reabsorbs calcium.

In normal circumstances, the body prioritizes the exogenous Calcium. However if the Calcium level from the diet is minimum, the body activates all the internal biomechanisms to achieve a higher level(14).

Functions unrelated to calcium

VDR is not only found in enterocytes, bone or kidney. Significant expression of this receptor is also found in(14):

FUNCTIONS ⟶

1- Lymphocytes.
2- Colon cells
3- Pituitary gland
4- Bone marrow
5- Hair follicle
6- Pancreatic cells
7- Adipose tissue
8- Ovarian cells
9- Heart and vascular
10- Liver
11- Skeletal muscle

Vitamin D may also have an important function in multiple sclerosis, autoimmune diabetes, lupus, bowel disease, transplants rejection, allergy, asthma, infection, depression, psychiatric and pain(18).

Vitamin D status

The measurement of 25(OH)D level is commonly performed with radioimmunoassay, enzyme linked assays and liquid chromatography with mass spectrometry(19). The best assessment of vitamin D status is the 25-hidroxivitamin D. The next table shows the different status of vitamin D according to National Institute of Health in 2010 (20);

nmol/L	ng/ml	Health status	Vit D Status
<30	**<12**	Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults.	Deficiency
30-50	**12-20**	Generally considered inadequate for bone and overall health in healthy individuals.	Insufficiency
≥50	**20-50**	Generally considered adequate for bone and overall health in healthy individuals.	Adequate level
>125	**>50**	Emerging evidence links potential adverse effects to such high levels, particularly >150nmol/L (>60ng/mL)	Possibly harmful

**1 nmol/L = 0,4 ng/ml*

However these cut off points are already an important matter of debate nowadays. Adequate level is 30 ng/ml which is the level that suppresses parathyroid hormone (PTH) secretion.

There are many factor than influence 25(OH)D levels:

- Race
- Vitamin D intake
- Sun exposure
- Adiposity
- Age
- Physical activity(18)

These reasons lead to a situation where, as a result of the variability of 25(OH)D level, the variability of signs in population depending on the status and the lack of precision of assays create an important debate as to agree on which is the optimal status and the optimal diary intake(21).

Being outdoor and getting sun light are better predictors of 25(OH)D levels than dietary intake, in fact a 80% of total vitamin D in serum comes from the action of sunlight(9).

The most common presentation of vitamin D deficiency in children is rickets which is an alteration of the conformation of the structure bone. Osteomalacia is commonly associated with vitamin D deficiency in adults. However my study will not be focused on rickets and osteomalacia because it is not the aim of the trial(18,20).

Vitamin D in pediatrics

A recent study performed in 2014 in Spain (Barcelona) show that 58,3% of obese adolescent had vitamin D deficiency and 28,3% were insufficient (12). Although the most common presentation of vitamin deficiency in children is with no signs or symptoms, rickets being the most relevant and known clinical presentation of vitamin D deficiency in childhood (3-18 months).To prevent rickets and vitamin D deficiency it is recommended that children and adolescent get an intake of vitamin D of 400IU/day; this new recommendation however was changed in 2008 (replacing the 2004 recommendation of 200 IU/day)(20).

Obese children are suggested to have vitamin D deficiency, in fact there is a higher level of fractures, Blount disease and slipped capital femoral epiphysis among obese adolescent. In

addition, cardiovascular diseases risk factors, insulin resistance and abnormal lipid profile are also associated with vitamin D deficiency and are more common among obese children than in control subjects(12,22).

Vitamin D insufficiency has been related to elevated inflammatory markers. C-reactive protein is a predictor of higher inflammation which increases the risk of CVD, obesity and metabolic syndrome(23).

Prevention

1. Exposure to sunlight. (Principal source of vitamin D).
2. Fortification of food with vitamin D (Meat or fish are natural source): Milk and others manufactured products can be fortified.
3. Supplements as cholecalciferol in oral solutions.

All these prevention measures must be considered depending on the age, skin color and season (24,25).

Monitoring

It is important to measure calcium, phosphorus and alkaline phosphatase (ALP) levels 1 month after initiating the therapy. After 3 months, is important to obtain calcium, phosphorus, magnesium, ALP, 25(OH)D and PTH levels. To our study, it is relevant to determine de calcium/creatine ratio which is an easy, cheap and comfortable way to monitorize the therapy(24).

VITAMIN D BINDING PROTEIN

Vitamin D binding protein (DBP) is originally known as a group specific component (Gc-globulin). DBP gene is a member of a family gene that includes albumin, α-fetoprotein and others [DBP-ALB-AFP telomere]. It is synthetized in the liver and it contains two binding areas and its function is to regulate the bioavailability of the 25(OH)D, acting as a transporter from the liver to the kidney for the synthesis of the active metabolite.

There are various known genetic variants as a result of two common polymorphisms. The functions of this protein are currently being studied and more studies are needed, however up to day there are observations which provide enough information to confer DBP an attractive clinical role as a relevant biomarker of vitamin D status. The relation and the impact of serum DBP and 25(OH)D concentration have been demonstrated, the levels of vitamin D being different depending on the polymorphism of the DBP: Gc1F and Gc1S [D432E] did not differ in vitamin levels; however Gc2 [T436K]showed lower levels of DBP and 25(OH)D.

As previously commented, vitamin D may have an important role in inflammation and immunity. DBP by its own may have a direct function in the inflammatory cascade (acting on the C5a complement factor) and on the innate immune regulation (Actin)(26).

DBP binds 85-90% of total 25(OH)D, the remainder (10-15%) being found as albumin-bound vitamin; less than 1% is presented as the free form. According to the literature, black people have vitamin D deficiency in comparison with white people, however their probability of bone fracture is lower and their bone mineralization is not deteriorated; Vitamin D binding protein may provide the clue. Black people have low levels of vitamin D. However their quantity of vitamin D binding protein is also lower than in white people. As a result, their free vitamin D level is similar. This affirmation was proved when black people were shown to have more prevalence of the T Allele of the DBP polymorphism, which is related to a lower level of DBP and total 25(OH)D than white people who usually have the G allele, characterized to have more DBP and more total 25(OH)D This confirms than the genetics and the polymorphism of

DBP may have a very important consequence on 25(OH)D status, black people being diagnosed as having vitamin D deficiency and receiving supplements which are not needed because their bioavailable 25(OH)D is adequate. For this reason, not only total vitamin D should be measured, DBP is also necessary to correctly determine the vitamin D status. Direct detection of bioavailable 25(OH)D is also possible(27).

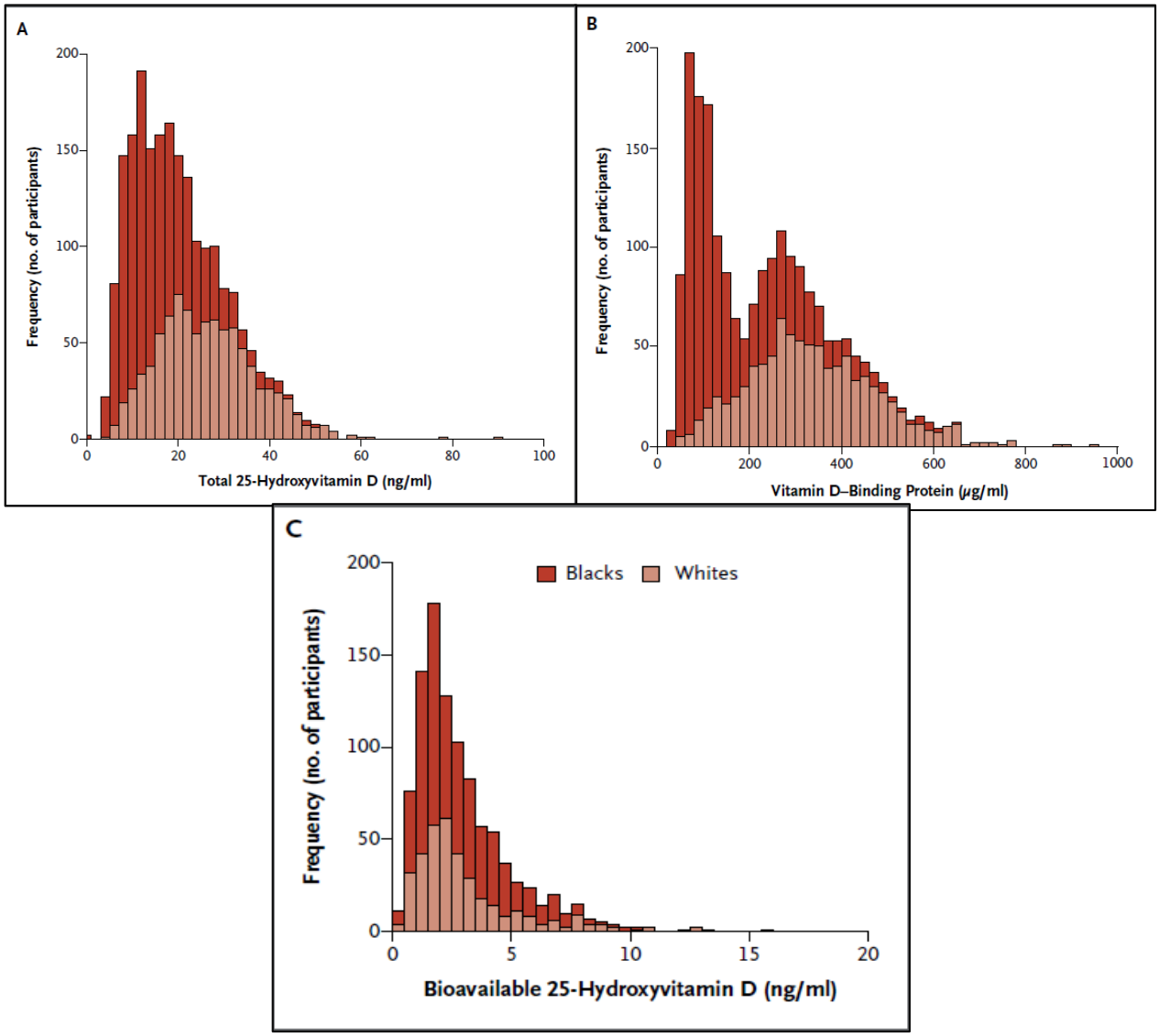


Figure A: Total 25(OH)D. Figure B: Total DBP. Figure C: Bioavailable 25(OH)D. Comparation between black and white people (27)

VITAMIN D AND CARDIOVASCULAR RISK

A large number of studies have investigated the relationship between Vitamin D and cardiovascular disease. VDR and 1-α-hidroxylase are found in cardiovascular tissues(28). In vitro and animal studies have shown an important relation between vitamin D and vascular function, however the experimental trials are failing to find causality(9,29). Vitamin D deficiency is implicated as an independent risk factor with an increasing mortality in cardiovascular and oncology disease(30,31).

We can divide the effect of vitamin D on cardiovascular tissue in the next areas: Inflammation and immunity, endothelial, heart and RAAS.

Vitamin D and inflammation

The relation between vitamin D and inflammation has been largely studied in chronic kidney disease (CKD). This has been proven in childhood population where there is less comorbidity. In these patients it was observed that mortality was mainly due to cardiovascular disease instead of renal outcomes. Patients with CKD suffer from vitamin D deficiency even in early stages (kidney accomplishes an important role in vitamin D metabolism). The explanation of why CKD patients suffer from CDV disease is related with atherosclerosis phenomenon and its relation with systemic disease. To study the relation between vitamin D and inflammation, non-invasive methods such as pulse wave velocity or brachial arterial distensibility have been used.

According to pathophysiological mechanisms, there are potential ones which can explain the link between Vitamin D and Inflammation;

1. The leukocyte telomere length is reduced in CKD patients. This element has an important role in oxidative stress and inflammation accelerating age process.
2. Vitamin D suppresses the activation of TNF-α converting enzyme (TACE) which has an important function increasing renal fibrosis and activating the Renin-Angiotensin-Aldosterone system (RAAS).

3. Vitamin D deficiency is related to increased levels of proteinuria and albuminuria which are factors linked to all-cause mortality and cardiovascular mortality(16,32).

Endothelial function and microvascular activity assessed as brachial-artery flow mediated dilatation and reactive hyperemia index, respectively, were independently correlated with 25(OH)D level(16).

Vitamin D and vascular: endothelial cells

VDR is expressed also in endothelial cells. Endothelial dysfunction is a hallmark on the physiology of arterial vasculature and it is an important target for prevention and early recognition of subclinical cardiovascular disease(16,17). Vitamin D inhibits cytokine mediated endothelial cell activation as well as TNF-αthat, as previously discussed, has an important role in atherosclerosis(33). Therefore, among all the functions of vitamin D, we herein highlight the reduction of the synthesis of adhesion of molecules in endothelial cell and a presumable important action on atherosclerosis(16,17).

Vitamin D and Heart: Cardiomyocytes

1,25(OH)D2 is related to the cell cycle of cardiomyocytes. The deficiency of vitamin D is associated with increased ventricular mass, increased store of fibrotic matrix, ventricular dilation, electromyocardial interference, higher systolic pressure and serum creatine phosphokinase. All these effects are reverted with vitamin D analogues(16).

Vitamin D and hypertension and RAAS axis

It is the medical condition most commonly related to vitamin D deficiency and its metabolism. Vitamin D has a direct connection with the RAAS axis regarding the activation of RAAS and its relation with its action on CYP27B1, which may have an important role in the paracrine regulation of 1,25(OH)D(34). RAAS activation followed by Angiotensin II synthesis causes hypertension and both processes are predictors of CDV risk. The administration of cholecalciferol downregulates the RAAS(16).

When 25-OH-D level is low, there are alterations in arterial wave reflection and systemic stiffening; these pathological measures were corrected after correcting their 25-OH-D levels(16,34).

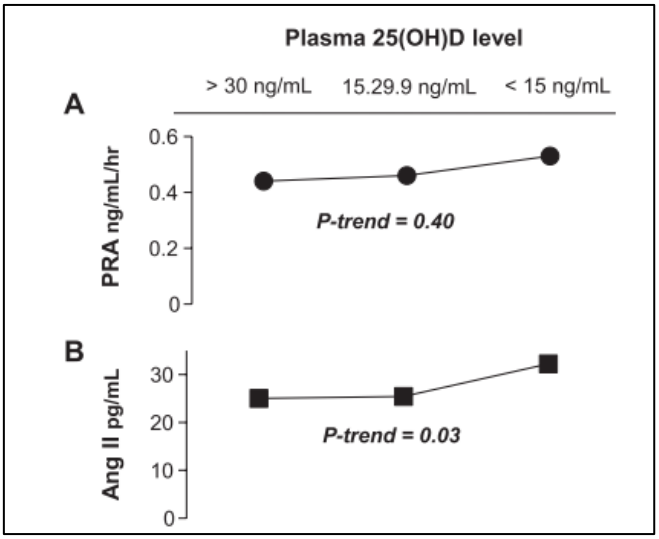


Figure 5: Relation between plasma renine activity (PRA) and Angiontensine II (Ang II) to level of vitamin D(34).

CARDIOVASCULAR TEST PROCEDURES

The American Heart Association (AHA) guidelines (2013) stablishes different methods to assess asymptomatic adults in clinical practice(35)

Serum markers	Cardiac and vascular test
Lipoprotein and apolipoprotein	Resting electrocardiogram
Natriuretic peptides	Transthoracic echocardiography
C Reactive protein	Carotid intima-media thickness
Hemoglobin A1C	Brachial flow mediated dilation
Lipoprotein associated phospholipase A2	Specific measures of arterial stiffness
	Ankle-Brachial index
	Exercise electrocardiography
	Stress echocardiography
	Myocardial perfusion imaging

Blood pressure

Lurbe et al. determined that the younger the hypertension is detected and ambulatory monitored, the better the prevention programs to solve future cardiovascular events and end-organ damages and its consequences on morbidities and mortality(36).

Structural and functional measures

As shown above, there are several tests to control cardiovascular status in clinical practice. However, according to *Shroff et al*. a wide range of methods are available to determine CVD damage in adults. Nevertheless, an adaptation and standardization followed by validation are required to use them in pediatric studies. cIMT (a structural parameter) and PWV (a functional parameter) are the most widely used tests, and therefore these results must be taken with caution (37).

Dalla Pozza et al and *Barsalou et al* determine cIMT, PWV and Flow Mediated dilatation (a more invasive method) as good indicators of subclinical cardiovascular damage in adolescents and pediatric population, paying special attention to the practical guideline of the European Pediatric Cardiology Society in order to standardize the results (38–40).

Circulating biomarkers of CVD disease

Apolipoproteins (VLDL, LDL, IDL or HDL) and triglycerides would define the lipid profile of a given patient (35). C-Reactive protein is also recommended, its role in chronic inflammation and atherosclerosis has been widely studied(41). Furthermore, its relation with overweight and obesity has been clearly determined(35,42,43). Adiponectin also has been related with mortality and cardiovascular disease(44,45).The insulin resistance status (insulin and Hemoglobin A1C) are additional parameters in the study of future cardiovascular events (35).

A number of procedures are accepted in children(46). All of them are non-invasive procedures. This is important because our sample selection is composed of pediatric patients and aggressive procedures can be contraproductive. These techniques enable us to know the arterial status of pubertal obese children with vitamin D deficiency and their longitudinal study over one year will allow us to know how vitamin D treatment can influence these markers.

LATEST OUTCOMES

To a large extent, literature is focused on vitamin D and their secondary biological actions, apart from muscular-skeletal effects. To sum up, the next reviews show how vitamin D has been proven to have an important relation with cardiovascular risk parameters in *in vitro* and in animal studies (9,10,16,29). Large cohorts studies show controversial results, nevertheless a role for vitamin D in CVD may be plausible on the basis of results from important and relevant studies, such as NHANES (47), The Framingham offspring study (48) (which have shown an important inverse association 25(OH)D > 20 ng/dl with CV outcomes) or a meta-analysis performed by *Kunutsor et al* (49)showing a reduction of 30% of hypertension in people with correct status of vitamin D (n= 283.537 participants). In addition, vitamin D deficiency has related with increase of all causes of mortality(30). However the administration at the same time of vitamin D and Calcium supplementation have increase mortality. (50)

The largest randomized trial of vitamin D therapy was carried out by The Women's Health Initiative which population was 36.282 postmenopausal women and 7 years of follow up showing no differences between treatment and placebo group. In addition, most prospective and interventional trials have failed to find causality between them. Three meta-analyses were performed recently; *Pittas et al* showed a review where ten clinical trials failed to prove the effectiveness of vitamin D supplementation in promoting blood pressure decrease(51). Other authors like *Witham et* al and *Wu et al* confirmed what *Pittas et al* published(29). For the reasons stated above, we should always keep in mind the possibility of bias that may complicate the interpretation of the results of these studies:

- Cardiovascular markers were not the main objective in these studies(29).
- Doses of vitamin D administration maybe are too low to be able to elicit cardiovascular effects. Indeed, an important study is being carried out named VitalE (it is still enrolling patients) which tries to show the relation between the administration between vitamin

D (2000 IU/Day) and omega-3 acid with CVD. During 5 years, 20.000 US people are being treaten with vitamin D and cardiovascular markers are one of the endpoints of the study(52).

- Most of the interventional trials were performed in a short period of time, this is an important bias because as I explained in the introduction, the level of 25(OH)D is modified depending on the season, ethnicity, age, physical activity, comorbidities, etc. Most of these studies were limited to a period shorter than a year.
- Vitamin D binding protein has been recently identified as an important factor of vitamin D status. The most recent literature highlights newer studies considering this parameter (and possibly also bioavailable vitamin D) which is commonly erroneously considered as the total vitamin D. Also the study depending on the polymorphism of the protein is required(26,27).
- Methodological problems with the standardization of the techniques used to assess cardiovascular risk parameters may complicate the studies in pediatric populations. Data interpretation may be also a bias, considering the different rules used to interpret the results of the different studies (38).

4. JUSTIFICATION

Obesity is an important and crescent problem nowadays. According to the literature, obesity is related to vitamin D deficiency due to diverse mechanisms and, in addition, vitamin D deficiency is related with cardiovascular disease. This is why vitamin D status and its role as a predictor of cardiovascular disease are gaining an important relevance. So, it is important to find alternative strategies to weight loss programs (they are failing) in order to prevent future cardiovascular events. Furthermore, the use of antihypertensive or lipid-lowering drugs in young ages may have a relevant economical consequence and a wide range of side effects in comparison with early administration of vitamin D supplements.

Results from *in vitro* studies and animal studies show an important and a relevant role of vitamin D status in cardiovascular disease; in addition, VDR activation may have a beneficial effect on the cardiovascular system.

Furthermore, according to observational studies, vitamin D may have an important role in the cardiovascular disease. However, in the interventional studies, results do not support the causality. This paradox may be due to the diversity of low doses of vitamin D administration, diet, inclusion/exclusion criteria, short term duration, Vitamin D binding protein not taken into account and differences in VDR activity or genotypes among interventional trials.

The innovation of this study is to perform a clinical trial being the free vitamin D (not bind to vitamin D protein) the status in obese children. We will consider vitamin D deficiency status defined as: total vitamin D < 12 ng/mL and DBP < 3 mg/dL. Up to now, the level of vitamin D used to perform trials was total vitamin D; however newer studies show that vitamin D binding protein may have an important role in vitamin D status in patients with vitamin D deficiency. The duration, doses and age of participant enrolled in the study also fulfill an innovation in comparison with randomized clinical trial completed until nowadays.

Higher doses are recommend in new trials to be able to elicit cardiovascular effects (52), long term duration to avoid changes in different seasons as previously described and the need to search for possible new effects of vitamin D, as recommended by *Zittermann et al* (53). In addition the absence of clinical trials in pediatric populations searching causality between cardiovascular and vitamin D as a main objective may fill an empty gap in the literature.

5. HYPHOTESIS

Main hypothesis

The administration of Vitamin D reduces cardiovascular risk markers in pubertal obese children.

Secondary hypothesis

The administration of Vitamin D improves metabolic markers in pubertal obese children.

6. OBJECTIVES

Main objective

Study the effect of vitamin D supplementation for two years on cardiovascular risk markers in pubertal obese children with vitamin D deficiency.

- Blood pressure
- cIMT (Carotid intima media thickness)
- Pulse wave velocity
- C reactive protein

Secondary objectives

Study the effect of vitamin D supplementation for two years on metabolic parameters:

- BMI
- Waist
- Serum lipids
- Fasting Insulin
- HOMA
- HMW-adiponectin

7. METHODOLOGY

7.1. STUDY DESIGN

This study is a protocol of a phase II/III Clinical Trial which tries to prove the importance role of vitamin D in cardiovascular risk markers. Two groups of patients will be treated (one with vitamin D and other one with placebo) and will be studied during one year at three time points (0, 6 and 12 months). The final purpose is to assess if vitamin D administration improves cardiovascular and metabolic risk markers in obese pubertal children.

The center which will carry out the next clinical trial;

Hospital General Josep Trueta of Girona.

- Principal investigator: St Carlos Jiménez Padilla and Dr. Abel López Bermejo
  - Servicio de Endocrinología Pediátrica (Pediatric Endocrinology)
- Hospital Dr. Josep Trueta
  - Avda. Francia s/n
  - 17007 Girona
  - Tel.: 972 940200

STUDY

Study type	Interventional: Clinical Trial.
Health Care Centre	Single center
Allocation	Randomized
Control type	Placebo
Intervention model	Parallel assignment
Masking	Double blind (Subject, investigator)
Primary purpose	Prevention

7.2. POPULATION

Girona has a total population of 749.191 persons(54), the area of Gironès has a total of 97.292(55). Our population study are children between 10-14 years old from the Gironès' area which comprises 4.967 subjects(56).

In addition we are going to study obese children; According to *"Encuesta Nacional de Salud 2011 (ENS)"* 27.8% of children are overweight or obese (57);

$$4.967 * \frac{27.8}{100} = 1.380{,}87\ children.$$

A recent study performed in 2014 in Spain (Barcelona) show that 58,3% of obese adolescent had vitamin D deficiency

$$1.381 * \frac{58{,}3}{100} = 805\ children$$

$$\mathbf{805\ obese\ and\ vitamin\ D\ deficient\ children\ aged\ 10-15\ years.}$$

POPULATION

Girona province	749.191 people
Gironès area	97.292 people
Children 10-15 years	4.967 children
Children 10-15 years with obesity or overweight	1381 children
Children 10-15 years with obesity or overweight and with Vitamin D deficiency	**805 children**

7.3. PATIENT SELECTION AND STUDY VISITS

The study population will comprise 48 Caucasian obese pubertal children with vitamin D deficiency who will be recruited from the primary health care centers of Girona (region in northern Spain).

All children attending well-child checkup visits at the primary care clinics in this region will be informed about the study by their primary care pediatricians **(Annex 6)**. All of the children will be apparently healthy, other than being obese.

To guarantee the randomization, the sample will be balanced by: Age (one group with ages between 10 and 12.5 years and second group with ages between 12.5 and 15 years), gender (male and female) and BMI (one group with BMI-SDS between +2 SD and +2.75 SD and a second group with BMI between+2.75 SD and 3.5 SD) **(BMI Tables: Annex 1)**.

Patient selection

Performed from a census of possible candidates. In Girona, 805 children are possible candidates based on age, obesity and vitamin D deficiency. However, only 48 patients will be included in our study, of these 48 patients; 24 will be girls and 24 will be boys, in additions these two groups will be further stratified according to age and BMI categories as indicated above.

First visit (Pre-selection visit)

One month after the first contact, patients and their families will be informed (informed consent) **(Annex 6)** regarding the participation in the clinical trial. After giving consent (signing the informed consent), an appointment will be scheduled for the next step. They will be asked about the features of the most recent clinical history. **(Questionnaires: Annexes 2-3)**. These questionnaires must be approved by CEIC of HJT.

Second visit (Selection visit)

Fifteen days before the selection visit, anthropometry parameters will be measured and laboratory tests will be obtained. At this selection visit, all results will be revised and the

patients will be assessed in order to see if they meet the inclusion and exclusion criteria to participate in the trial. Additional tests may be taken if is necessary.

An identification code will be assigned to all patients included in the study which will remains constant during all the process and will be the identification key in the *"CRD – Cuaderno de recogida de datos-"*. **(Annex 4)**

As mentioned above, all patients included in the study will receive treatments in a random way balanced by age, gender and BMI. These data will be completely confidential and they only can be known by the pharmaceutic agent (Masking: double bind – subject and investigator-). This information can only be revealed in an emergency situation.

Patients included in the study will be scheduled for subsequent visits at 6 months and 12 months for follow-up measurements and investigations as described below:

Parameter	Basal	6 m	12 m
Weight, height and BMI	x	x	x
Blood pressure	x	x	x
cIMT	x	x	x
Pulse wave velocity	x	x	x
C Reactive protein	x	x	x
Glucose	x	x	x
Fasting insulin	x	x	x
Lipid profile	x	x	x
HOMA	x	x	x
HMW-Adiponectin	x	x	x
Diet/Ph act questionnaires	x	x	x

After the end of our trial, patients included in the study will be scheduled for an end-of-trial appointment at 3 months of the end of trial to assess for possible side effects of the treatment and inform about them if it is required to the pertinent authorities.

7.4. SAMPLE SELECTION

SAMPLE SELECTION	
INCLUSION CRITERIA	**EXCLUSION CRITERIA**
- BMI: Between +2.0 SD and +3.5 SD for age and gender. - Vitamin D deficiency defined as < 12 ng/ml - Free vitamin D < 3.0 ng/ml - Pubertal development (10-15 years old) – Tanner 2 or over - Caucasian ethnicity	<u>CURRENTLY RECEIVE;</u> - Vitamin D supplementation - Concomitant drugs <u>MEDICAL HISTORY;</u> - Early puberty or delayed puberty - 25-OH vitamin D level l > 50 ng/ml - Morbid obesity (BMI ≥ 3.5 SD) - Any endocrine disease other than obesity like thyroid disease or growth hormone deficiency. - Genetic syndrome associated with obesity - Arterial hypertension - Dyslipidemia - Type 1 or 2 diabetes mellitus - Glucose intolerance - Other metabolic disease (Galactosemia) - Liver or kidney disease (Calcium nephrolithiasis) - Rickets

INCLUSION CRITERIA

BMI: Between +2.0 SD and +3.5 SD for age and gender

Obesity has been related with vitamin D deficiency (22). In our study, selected subjects will be between +2.0 SD and +3.5 SD for BMI, compared with the rest of population which is the diagnosis of non-morbid pediatric obesity (58,59).

Vitamin D deficiency defined as < 12 ng/ml

According to the Medicine Health Institute(20) < 12 ng/mL defines vitamin D deficiency and it is related with all-cause mortality, being CDV disease the most prevalence disease when vitamin D level is <17.8 ng/mL (47).

Free vitamin D < 3.0 ng/ml

Free vitamin D will be calculated with a complex formula as described(60). Measuring free vitamin D directly is a very expensive method and not available in our research center, thus free vitamin D will be calculated considering the values of total vitamin D and vitamin D binding protein. According to new studies and emergent role of vitamin D binding protein, the level of normal status of free vitamin D is 3 ng/ml; therefore our cut-off point for subject selection in our study will be those patients with level of free vitamin D level < 3 ng/ml (who will also have vitamin D level < 12 ng/mL (true vitamin D deficiency) (26,27).

Pubertal development (10-15 years old) - Tanner 2 or over:

The age has an important role in vitamin D metabolism and the management of vitamin D deficiency is different according to the age of the patient in pediatric population(24). Our population will be composed by pubertal children (adolescents) with a tanner 2 stage or over in their sexual development screening.

Caucasian ethnicity

Depending on the ethnicity, the metabolism of vitamin D and its diverse features change (DBP polymorphism, receptors, genotypes, etc.)(27); that is why only Caucasian ethnicity will be included trying to avoid a heterogeneous group and limiting confounding variables.

7.5. SAMPLE SIZE

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, **24.0** subjects are needed in the first group and **24.0** in the second to detect as statistically significant a difference greater than or equal to 5.73 units (1 SD). The common standard deviation is assumed to be 5.73 (61) and the correlation coefficient between the initial and final measurement is assumed to be 0.4. A drop-out rate of 20% has been anticipated.

The source where sample size is calculated is;

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

7.6. VARIABLES

VARIABLES		TYPE	INSTRUMENTATION	Units
Independent	Vitamin D Colecalciferol- Deltius 25.000 UI/2,5 ml oral solution or placebo.	DcQV		UI/mL
Dependent	Blood pressure	CQV	Manual/Electronic sphygmomanometer- (Dinamap Pro 100, GE Healthcare, Chalfont St Giles, United Kingdom).	mmHg
	criteri(Intima media thickness)	CQV	High-resolution ultrasonography (MyLab25; Esaote, Firenze, Italy).	mm
	Pulse wave velocity (PWV)	CQV	The SphygmoCor system (AtCor Medical, Australia)	m/seg
	C Protein reactive	CQV	Ultrasensitive latex immunoassay(CRP Vario; Sentinel Diagnostics, Abbott Diagnostics Europe, Milan, Italy	mg/L
Covariables	Age	CQV	Clinical exploration	-
	Gender	DcQV	Clinical exploration	-
	Weight	CQV	Calibrated scale (SECA, Hamburg, Germany)	Kg
	Height	CQV	Harpendenstadiometer (Holtain Ltd, Crymych, UK)	cm
	Waist	CQV	Measuring tape	cm
	BMI	CQV	Scale and stadiometer	SD
	Glucose	CQV	Hexokinase method(AEROSET c8000)	mg/dL
	Fasting insulin	CQV	Insulin-automated assay IMMULITE 2000 System (Siemens, Healthcare Diagnostics, Deerfield, IL, USA)	mIU/l
	HOMA	CQV	Homeostasis model assessment for insulin resistance.	mg/dL
	Triglycerides and HDL-cholesterol	CQV	Cobas Integra 711 model (Roche Diagnostics, Indianapolis, IN, USA)	mg/dL,
	HMW-adiponectin	CQV	Radioimmunoassay (RIA) (LincoResearch, Inc., St. Charles, Missouri, EE. UU.)	mg/L
	Free/unbound 1, 25 hidroxyvitamin D	CQV	Formula using total vitamin D and binding protein	ng/mL
	Vitamin D– binding protein	CQV	Commercial enzyme-linked immunosorbent assay (R&D Systems)	ng/mL
	Diet	NQV	Nutrition questionnaires	Score
	Physical activity	NQV	Physical activities questionnaires	Score
	Family history	NQV	Clinical medical history	-
Safety	Calcium/creatinine ratio	CQV	Colorimetric method. Express Plus Analyzer (Chiron)	-

*__DQV__: Discrete quantitative variable. *__CQV__: Continuous quantitative variable. *__NQV__: Nominal qualitative variable. __*DcQV__: Dichotomy qualitative variable

8. INTERVENTION

8.1. VITAMIN D DOSIS

Calcitriol is not preferred for the therapy. It is expensive, it has got a short life, doses are not very efficacy and can induce side effects as hypercalcemia. Among other drug presentations such as ergocalciferol (plant derived) or cholecalciferol (animal derived), studies have shown than cholecalciferol may be at least three times more potent than ergocalciferol (these differences are related to the affinity binding DBP) that is why cholecalciferol therapy is preferred (24,25).

The experimental drug is Deltius® - Cholecalciferol 25.000 IU/ 2.5 ml oral solutions - (Italfarmaco S.A., Madrid). Doses in our trial will be 25.000 IU/ two weeks during one year of duration. All public data is contained in datasheet of Deltius®**(Datasheet-Annex 4)**.

8.2. PLACEBO

Placebo will be received in the control group. Italfarmaco S.A. will prepare this placebo which contain the same excipients formula and price than Deltius® but not the active ingredient.

8.3. JUSTIFICATION OF DOSIS AND TREATMENT DURATION

According to the *Institute of Medicine of the National Academies* (20) the estimated average requirement (EAR), recommend dietary allowance (RDA) and upper tolerable intake (UL) of vitamin D in people between 10-15 years are:

- EAR: 400 IU/day
- RDA: 600 IU/day
- UL: 4.000 IU.

Deltius® contains 25.000 IU and we will be given as an oral vial every two weeks.

$$\frac{25.000\ IU}{14\ DAYS} = 1.785,7\ \text{IU/DAY}$$

Thus, according to tolerable upper intake level which is 4.000 IU/day, the selected dose of 1.785,7 IU/day is in a safety range.

25.000 IU/ two week

In one study, the administration of 3332 IU/day of cholecalciferol in patients with vitamin D deficiency (<12ng/ml) resulted in a statistically significant reduction in total cholesterol and LDL (cardiovascular markers)(62). The most important current study which is already enrolling patient is VITALE trial which recommends 2000 IU/day to achieve additional effects on the cardiovascular system(63). Other authors like *Dong et al*. and *Harris et al*. showed that the administration of 2.000 IU/ day improves endothelial function(64,65). In addition, there are no differences in 25(OH)D status improvement between the daily administration of 2000 IU as compared to bolus administration of 10.000-50.000 IU/week (66). For this reason and for the easier management of treatment and to facility the compliance, 25.000 IU/ two week will be the dose chosen to carry out this clinical trial.

1 year

In a study, vitamin D supplements were administered during one year (12 months). The supplementation had no results reducing weight in an adverse way, however it had beneficial effects on cardiovascular markers in overweight subjects (62). The justification of the treatment duration will be one year, a shorter time may be insufficient to observe changes in cardiovascular markers and treat during more than 12 months may be over treat in obese children and may expose them to unnecessary side effects. In addition one year duration may provide us with enough results to compare between different seasons, which may help eliminate an important bias in most studies which have only presented data of short-term duration, given that 25(OH)D levels differ depending on the season and sunlight exposure.

8.5. RANDOMIZATION

Randomization of treatments (vitamin D or placebo) will be performed according to a randomization list generated by nQuery Advisor 7.0 (Statistical Solutions Ltd., Cork, Ireland), in which patients remain into 8 blocks of 6 subjects balanced for age, gender, BMI and treatment. This will ensure the comparability of treatment groups by the above mentioned baseline characteristics (Age, gender and BMI). By stratifying by age, we also expect the treatment groups to be comparable in terms of pubertal development.

The pharmacist will be responsible for randomly dispensing the intervention according to the abovementioned randomization list and he/she will also be responsible for masking the intervention. It will not be possible for either the patient or the researchers to know at any time during the trial the interventional drug assigned to any of the patients. It may only be disclosed in case of emergency provided that this is necessary for the patient's treatment.

PATIENT	AGE	GENDER	BMI	TREATMENT
10001	≥10 and<12.5 years	Male	≥2 SDS and<2.75 SDS	?
10002	≥10 and<12.5 years	Male	≥2 SDS and<2.75 SDS	?
10003	≥10 and<12.5 years	Male	≥2 SDS and<2.75 SDS	?
10004	≥10 and<12.5 years	Male	≥2 SDS and<2.75 SDS	?
10005	≥10 and<12.5 years	Male	≥2 SDS and<2.75 SDS	?
10006	≥10 and<12.5 years	Male	≥2 SDS and<2.75 SDS	?
20001	≥10 and<12.5 years	Male	≥2.75 SDS and <3.5 SDS	?
20002	≥10 and<12.5 years	Male	≥2.75 SDS and <3.5 SDS	?
20003	≥10 and<12.5 years	Male	≥2.75 SDS and <3.5 SDS	?
20004	≥10 and<12.5 years	Male	≥2.75 SDS and <3.5 SDS	?
20005	≥10 and<12.5 years	Male	≥2.75 SDS and <3.5 SDS	?
20006	≥10 and<12.5 years	Male	≥2.75 SDS and <3.5 SDS	?
30001	≥10 and<12.5 years	Female	≥2 SDS and<2.75 SDS	?
30002	≥10 and<12.5 years	Female	≥2 SDS and<2.75 SDS	?
30003	≥10 and<12.5 years	Female	≥2 SDS and<2.75 SDS	?
30004	≥10 and<12.5 years	Female	≥2 SDS and<2.75 SDS	?
30005	≥10 and<12.5 years	Female	≥2 SDS and<2.75 SDS	?
30006	≥10 and<12.5 years	Female	≥2 SDS and<2.75 SDS	?
40001	≥10 and<12.5 years	Female	≥2.75 SDS and <3.5 SDS	?
40002	≥10 and<12.5 years	Female	≥2.75 SDS and <3.5 SDS	?
40003	≥10 and<12.5 years	Female	≥2.75 SDS and <3.5 SDS	?
40004	≥10 and<12.5 years	Female	≥2.75 SDS and <3.5 SDS	?
40005	≥10 and<12.5 years	Female	≥2.75 SDS and <3.5 SDS	?
40006	≥10 and<12.5 years	Female	≥2.75 SDS and <3.5 SDS	?
50001	≥12.5 and<15 years	Male	≥2 SDS and<2.75 SDS	?
50002	≥12.5 and<15 years	Male	≥2 SDS and<2.75 SDS	?
50003	≥12.5 and<15 years	Male	≥2 SDS and<2.75 SDS	?
50004	≥12.5 and<15 years	Male	≥2 SDS and<2.75 SDS	?
50005	≥12.5 and<15 years	Male	≥2 SDS and<2.75 SDS	?
50006	≥12.5 and<15 years	Male	≥2 SDS and<2.75 SDS	?
60001	≥12.5 and<15 years	Male	≥2.75 SDS and <3.5 SDS	?
60002	≥12.5 and<15 years	Male	≥2.75 SDS and <3.5 SDS	?
60003	≥12.5 and<15 years	Male	≥2.75 SDS and <3.5 SDS	?
60004	≥12.5 and<15 years	Male	≥2.75 SDS and <3.5 SDS	?
60005	≥12.5 and<15 years	Male	≥2.75 SDS and <3.5 SDS	?
60006	≥12.5 and<15 years	Male	≥2.75 SDS and <3.5 SDS	?
70001	≥12.5 and<15 years	Female	≥2 SDS and<2.75 SDS	?
70002	≥12.5 and<15 years	Female	≥2 SDS and<2.75 SDS	?
70003	≥12.5 and<15 years	Female	≥2 SDS and<2.75 SDS	?
70004	≥12.5 and<15 years	Female	≥2 SDS and<2.75 SDS	?
70005	≥12.5 and<15 years	Female	≥2 SDS and<2.75 SDS	?
70006	≥12.5 and<15 years	Female	≥2 SDS and<2.75 SDS	?
80001	≥12.5 and<15 years	Female	≥2.75 SDS and <3.5 SDS	?
80002	≥12.5 and<15 years	Female	≥2.75 SDS and <3.5 SDS	?
80003	≥12.5 and<15 years	Female	≥2.75 SDS and <3.5 SDS	?
80004	≥12.5 and<15 years	Female	≥2.75 SDS and <3.5 SDS	?
80005	≥12.5 and<15 years	Female	≥2.75 SDS and <3.5 SDS	?
80006	≥12.5 and<15 years	Female	≥2.75 SDS and <3.5 SDS	?

8.6. SIDE EFFECTS

- Side effect: It is any non-desirable experience which happens in a subject in a clinical trial, whether it is considered or not to be related with the drugs under investigation.
- Severe side effect: It is a potentially deathly side effect or that which can cause severe clinical symptoms. It can induce permanent incapacity, hospitalization or aggravate these complications.
- Unexpected side effect: It is an effect not described in the bibliography or in the datasheet of the drug under investigation.

The administration of Deltius® *(Vitamin D - Cholecalciferol- Deltius 25.000 UI/2,5 ml oral solution)* to pregnant or lactating women is not deleterious to the fetus or infant; however it is recommend to be used in low doses.

Side effects are well described in the data sheet of Deltius®;

- Infrequent: Hypercalcemia and hypercalciuria.
- Very infrequent: Rash, urticarial effects and pruritus.

Drugs that can interact with Deltius® are:

- Antiepileptic (Phenytoin)
- Barbiturates
- Diuretics (thiazides)
- Glucocorticoids
- Digoxin
- Colestiramin, colestipol, orlistat and paraffin oil.
- Actinomicyn or antifungals (imidazoles).

For further information, see datasheet (Annex) or via web(67).

Monitoring the drug

For this study, it is relevant to determine the calcium/creatinine ratio, which is an easy, cheap and non-invasive way to monitorize the therapy and control for possible side effects, and therefore, it will be measured at 0, 6, 12 and15 months as screening for possible side effects.

Upper level of calciuria in children > 2 years (and adults) is > 4mg/kg/day. This value can be calculated collecting urine during 24 hours or, more practically, calculating calcium/creatinine ratio in isolated urine samples (our election will be the second option).

Normal limit of calcium/creatinine ratio is:

- Children > 2 years: < 0.21 mg/mg. If the value of this coefficient is higher, they will be diagnosed as having hypercalciuria.

Urine will be collected from the second urination of the morning the day of the visit.

The treatment with cholecalciferol will be discontinued if there exist one of the following clinical presentations:

- Dysuria, polaquiuria, urine incontinence, hematuria, recurrent abdominal pain.
- Recurrent urine tract infection.
- Dark urine
- Any bone metabolic disease
- Lithiasis (it is the most common clinical manifestation of hypercalciuria in childhood)
- Poor growth
- Calcium/creatinine > 0.21 mg/mg
- Side effects described in the Deltius® datasheet.

If any new side effects are described, we will be contact; *https://www.notificaram.es*

9. DATA COLLECTION

The following describe the medical procedures for this study:

1. Clinical examination and venous blood sampling will be performed in the morning, with the child in the fasting state.
2. Blood pressure will be measured before venous blood sampling with the child supine on the right arm after a 10-minute rest, using an electronic sphygmomanometer (Dinamap Pro 100, GE Healthcare, Chalfont St Giles, United Kingdom) with a cuff of appropriate size for the child's arm circumference.
3. cIMT will be measured by high-resolution ultrasonography (MyLab25; Esaote, Firenze, Italy). For cIMT, diastolic images will be obtained using a linear 12-MHz transducer on the right side at the level of the distal common carotid artery, 1 cm away from its bifurcation. Averages of 5 cIMT measurements on the far wall of the artery will be used in the study. The intra-subject coefficient of variation (CV) for ultrasound measurements is <6%.
4. PWV: the PWVcf (carotid-femoral) will be measured by SphygmoCor. SphygmoCor PWVcf path length will be measured from the suprasternal notch to the femoral pulse at the point of deadening. Measurements will be rejected if the SD of the mean transit time exceed 6% (automatically flagged by SphygmoCor). Measurements will be done in triplicate whenever possible. All measurements will be made by one experienced trained observer.(68)
5. Levels of total 25-hydroxyvitamin D (D2 and D3) Levels of vitamin D– binding protein will be measured by means of a commercial enzyme-linked immunosorbent assay (R&D Systems) that uses two monoclonal antibodies in a sandwich format (interassay coefficient of variation, 7.2%).
6. Serum levels of hsCRP will be measured using an ultrasensitive latex immunoassay (CRP Vario; Sentinel Diagnostics, Abbott Diagnostics Europe, Milan, Italy). The lower limit of detection is 0.2 mg/L, and the intra-assay and interassay CVs were both <3%.

All measurements will be taken during a separate visit in all children and will be performed by the same observer who will be unaware of the subjects' clinical and laboratory characteristics. All serum samples will be obtained between 8:00 a.m. and 9:00 am under fasting conditions.

1. Weight will be measured with the child wearing light clothing on a calibrated scale, and height will be measured with a Harpenden stadiometer. BMI will be calculated as weight divided by the square of height in meters.
2. Age- and sex-adjusted z-score values for current weight, height, and body mass index [BMI] will be calculated using regional normative data.
3. Waist circumference will be measured at the umbilical level with the child in the supine position with a measuring tape.
4. Serum glucose will be analyzed by the hexokinase method (AEROSET c8000). Insulin will be measured by immunochemiluminiscence (IMMULITE 2000; Diagnostic Products, Los Angeles, California). The lower limit of detection is 0.4 mIU/L, and the intra-assay and interassay CVs are both <10%.
5. Fasting insulin sensitivity will be estimated from fasting insulin and glucose levels using the homeostasis model assessment for insulin resistance (HOMA-IR): (fasting insulin, mU/L) = (fasting glucose, mg/dL)/405.
7. HMW-adiponectin will be measured using a Radioimmunoassay (RIA) (Linco Research, Inc., St. Charles, Missouri, EE. UU.), samples will be diluted 500 times before the analysis. The sensibility of the method is 2ng/ml. Intraassay and interassay variation coefficients are below 5%.
6. Lipid profile: High-density lipoprotein (HDL)-cholesterol and total serum triglyceride level will be quantified by Cobas Integra 711 model (Roche Diagnostics, Indianapolis, IN, USA).

10. STATISTICAL ANALYSIS

Univariant

All statistical analyses will be performed using SPSS version 12.0 (SPSS Inc, Chicago, Illinois). Results for variables with a normal distribution will be expressed as mean and SD, and those for variables without a normal distribution will be expressed as median and 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

Comparisons of results for major variables at baseline will be performed using Mann-Whitney test (for continuous unpaired variables) and Fisher exact test (for categorical unpaired variables) to ensure that the randomization process yields comparable treatment groups.

Multivariant

The analysis of response to treatment for endpoint variables (SBP, cIMT, PWC and CRP) between independent treatment and control groups (main objective of the proposal) will be performed by general linear model (GLM) for repeated measures. Differences in 1-year changes across intervention groups will be tested by the interaction term among the intervention variable and the endpoint variables of the study. Models will be adjusted for potential confounders (age, gender, BMI, waist, fat mass, puberty, physical activity, nutrition score, serum lipids, HMW-adiponectin, and family history for CVD). An intention-to-treat analysis and a per-protocol analysis will both be performed. Imputation of missing values for endpoints variables will be performed using the latest observed values for each variable and subject.

Similarly, a GLM for repeated measures will be used to test whether vitamin D improves metabolic markers in obese pubertal children, computing each of the metabolic markers as the dependent variable, and adjusting for similar confounding variables. A p value <0.05 will be considered statistically significant.

11. ETHICAL CONSIDERATIONS

Ethical procedures are required in our study. This protocol will follow;

1. The four principles for medical research involving human subjects stablished by *Declaration of Helsinki* **(Annex 7)**.
2. It will be submitted to the *"Comité de Ética de investigación clínica (CEIC) del Hospital Universitario Dr Josep Trueta*" following the guidelines subscribed;
   a. *"Art 18 of Real Decreto 223/2004, de 6 de Febrero por el que se regulan los ensayos clínicos con medicamentos"*.
   b. "*Ley 14/2007, de 3 de Julio, de investigación biomédica"*.
   c. All patient data will be stored and studied under the fulfillment of the *"Ley Orgánica 15/1999 of 13th of December* whose goal is to guarantee the confidentiality and fundamental rights of physical people involved in the study.
3. All patients will be provided all needed information documents, the investigator will ascertain that all information is understood by the patient before signing an informed consent **(Annex 6)** approved by *Ministerio de Sanidad, Servicios Sociales e Igualdad*. Informed consent must be signed by the patient or by the child's legal tutor.
4. Our study will include pediatric subjects, for this reason all procedures must follow the ethical and legal instruction of Pediatric investigation plan included in "*Regulation No 1901/2006 and 1902/2006 of the European parliament and of the council of 12 Dec 2006 on medicinal products for pediatric use and amending Regulation"*.
5. Before approval our clinical trial, two organisms must approve our study: *European Medicaments Agency (EMA)* and *Asociación Española de Medicamentos y Productos Sanitarios (AEMPS)*.

12. LIMITATIONS

1. Dynamic measures of vascular function, such as flow mediated dilatation and glyceryl trinitrate mediated dilatation of the brachial artery will not be performed in our study because they are more invasive techniques and may cause harm to children.
2. We do not take into account the single nucleotide polymorphism of VDR. We acknowledge that this would be of additional interest for our study but the number of subjects needed to take into account this additional variable would be much greater.
3. We do not take into account the polymorphism of DBP. However, we will have direct measures of DBP instead of estimates of the effect on polymorphism on protein concentration.
4. Seasonality is an important variable to take into account. The enrolment of patients depending on the season can modify their vitamin D status. However, our study will have a short recruitment time (less than 3 months) and seasonality will not be major confounding factors in our subjects.
5. Differences between vitamin D diagnostic assays may cause lack of comparability or the results of our study with those from other studies.

13. WORK PLAN

Activity nº	Description	Months involved	Professionals

PHASE 0- PROTOCOL PLAN (ACCOMPLISHED)

- Activity 1- Hypothesis and objectives approach. (M1). CJP-ALB
- Activity 2- Bibliography research, professionals involved and methodology planning (Identify study design and variables.) (M1-3). CJP-ALB
- Activity 3- Pilot test and evaluation. (M4-5). CJP-ALB
- Activity 4- Problems identified and changes in the proposal. (M5-6). CJP-ALB
- Activity 5- Definitive protocol and CEIC/AEMPS delivery. (M6-7). CJP-ALB

PHASE 1- DATA COLLECTION

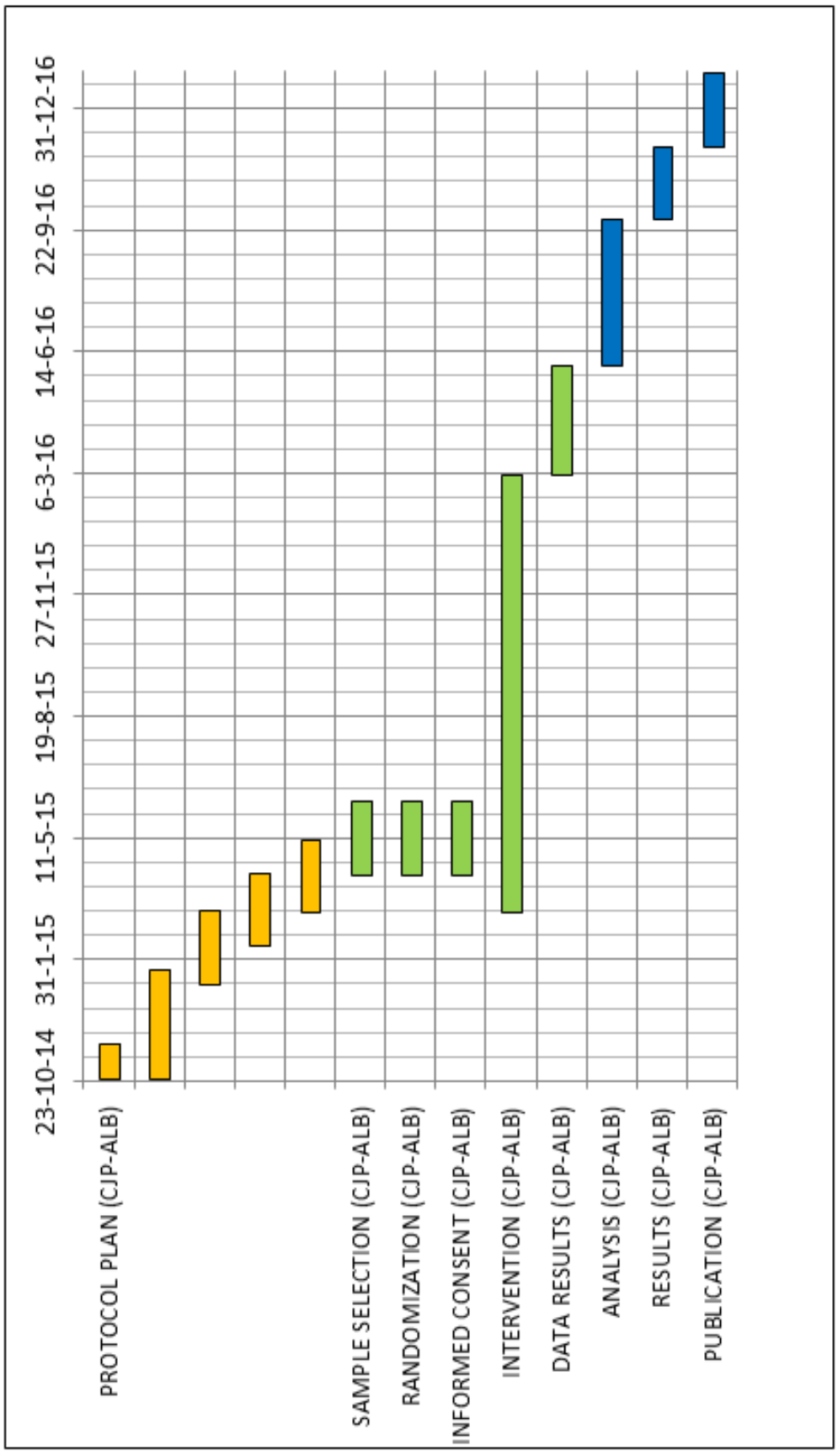
- Activity 6- Population identification and sample selection (M7-8). CJP-ALB.
- Activity 7- Control sample selection, randomization, masking method and allocation type. (M7-8). CJP-ALB.
- Activity 8- Information documents, informed consent signature. (M7-8). CJP-ALB.
- Activity 9- Inclusion, data collection, data base collection and statistical analysis. (M6-18). CJP-ALB.
- Activity 10- Data results and correction of possible errors. (M18-20). CJP-ALB.

PHASE 2- OUT COMES ANALYSIS AND CONCLUSION

- Activity 11- Statistical analysis. (M18-21). CJP-ALB.
- Activity 12- Results discussion and conclusion. (M21-23). CJP-ALB.
- Activity 13- Final clinical trial resolution, data diffusion and publication in a journal. (M23-26). CJP-ALB.

CJP: Carlos Jiménez Padilla

ALB: Abel López Bermejo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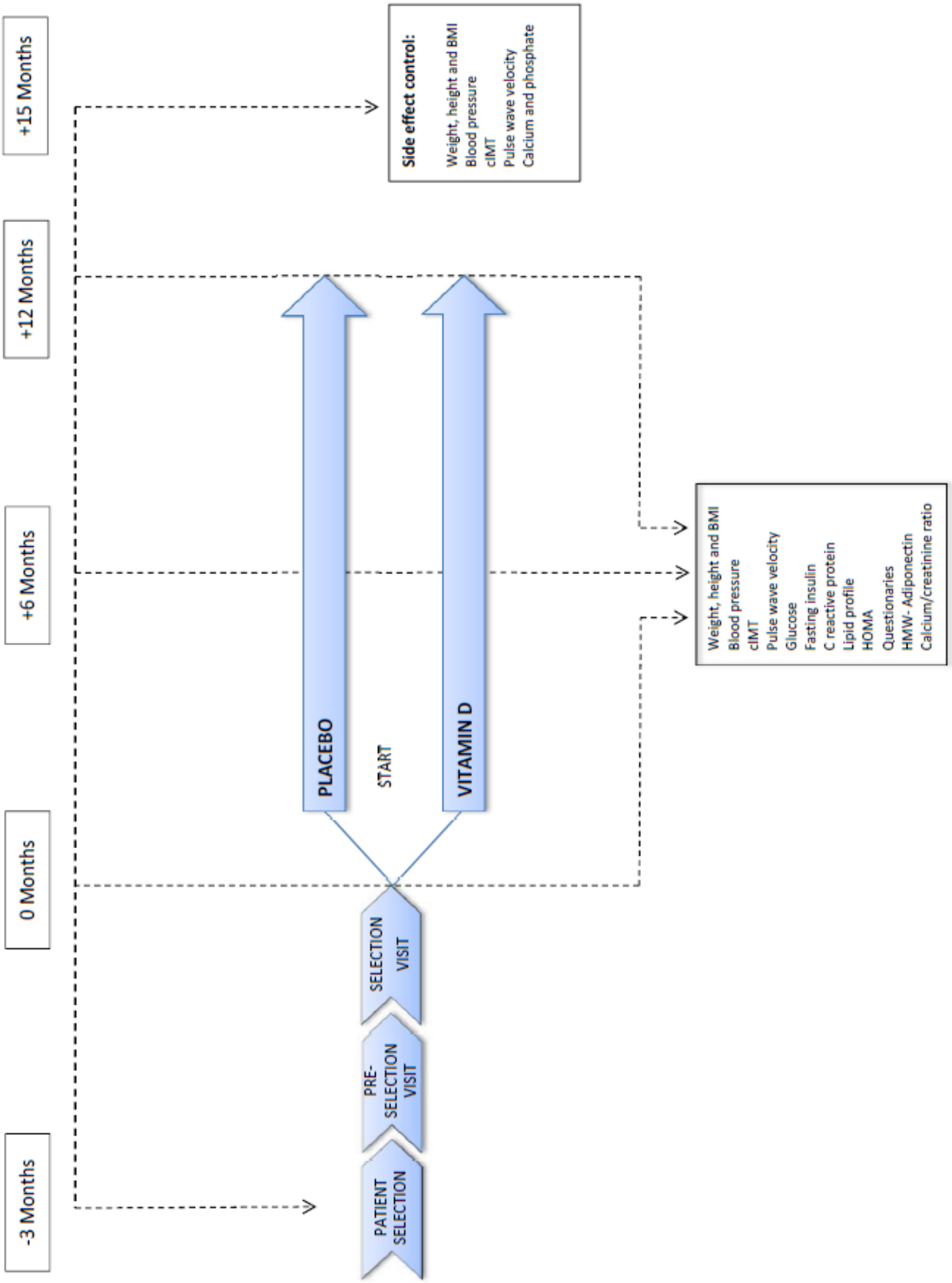
31-1-15
11-5-15
19-8-15
27-11-15
6-3-16
14-6-16
22-9-16
31-12-16
PROTOCOL PLAN (CJP-ALB)
SAMPLE SELECTION (CJP-ALB)
RANDOMIZATION (CJP-ALB)
INFORMED CONSENT (CJP-ALB)
INTERVENTION (CJP-ALB)
DATA RESULTS (CJP-ALB)
ANALYSIS (CJP-ALB)
RESULTS (CJP-ALB)
PUBLICATION (CJP-ALB)

14. CURRENT STUDIES

TITLE	CL. TRIAL Nº	DATE/LOCALIZATION	STUDY DESIGN	DURATION	AGES	BRIEF DESCRIPTION
Effect of Vitamin D Supplementation on Endothelial Function in Obese Adolescents.	NCT01746264	May 2014. United States (Mayo Clinic)	Interventional, single group assignment, open label and prevention.	13 months	12-18	In this study, they administrated 100.000 IU/ month for 3 month. Their outcomes are related to flow mediated dilatation.
Serum 25-hydroxyvitamin D, Vascular Functioning, and Insulin Sensitivity in Adolescent girls	NCT01041365	January 2014. United States (University of Alabama at Birmingham)	Observational study (cohort study), cross sectional	4 years	14-18	The main outcome is the insulin sensibility being flow mediated dilatation and arterial stiffness secondary outcome measures.
Are Bone, Nutritional and Cardiovascular Status of French Children and Adolescents, Correlated With Their Vitamin D Status?	NCT01832623	December 2014. France (Hospices Civils de Lyon)	Interventional, single group assignment, open label trial.	3 months	10-17	The main outcome is study the bone however in the secondary outcomes (cardiovascular parameters) are included and they have measured: BP, CIMT, Extra-media thickness, lipid profile, endothelial function (iontophoresis of acetilcoline), inflammatory status with CPR
Effect of Vitamin D Supplementation on Inflammation and Cardiometabolic Risk Factors in Obese Adolescents.	NCT01217840	August 2012. United States (Stanford University)	Interventional, randomized, parallel assignment, double blind, treatment.	12 weeks	11-17	Their main outcomes are study the cytokines and secondary lipid profile, glucose metabolism and blood pressure (not CIMT, PWV and C reactive protein).
Influence of Vitamin D on Vascular Function in Adolescents and Young Adults With Type 1 Diabetes.	NCT01103817	January 2011. Canada (Health Canada).	Interventional, non-randomized, efficacy study, parallel assignment, open label, treatment.	10 months	12-18	Vascular status will be measured in this study with peripheral arterial tonometry each three months in diabetic population
The Effects of Vitamin D Supplementation on Glycemic Control and Proinflammatory Markers Involved in Microvascular Complications in Adolescents With Type 1 Diabetes	NCT01697228	June 2014. United States (Children's Hospital Los Angeles)	Interventional, randomized, crossover assignment, open label , prevention	1 year and 8 months.	13-21	The main outcome is to measure HbA1c and secondary outcomes is to know pro-inflammatory markers (CRP, IL-6, TNF-α) in diabetic population.
Use of Vitamin D to Improve Glucose Metabolism and Reduce Inflammation	NCT00994396	April 2013. United States (University of Missouri-Columbia)	Interventional, randomized, efficacy study, parallel assignment, double blind , treatment	3 years and 6 months	9-19	The main outcomes are to study vitamin D status, inflammatory markers (IL-6, TNF-α, CPR), Hb1Ac, glucose and insulin in obese adolescent.

15. FLOW CHART



16. BUDGET

STUDY BUDGET	COST (euros)
1. Staff costs	
2. Implementation costs	
Inventory material costs - The SphygmoCor system (AtCor Medical, Australia)	 22.500
Consumables: - Laboratory parameters (in 144 samples): - Glucose x144 - Fasting insulin x144 - HOMA x144 - Lipid profile (TAG and HDL) x144 - HMW-adiponectin x144 - Total 25 hidroxyvitamin D x144 - Vitamin D– binding protein x144 - C Protein reactive x144	 182,88 1.005,12 - 921,6 4.000 3.016,8 4.000 948,96
- Liability Insurance	6.000
- Drug purchase (Cholecalciferol and placebo pills)	6.739,2
- Administrative permits (AGEMED)	3.948,1
- Publication fees	2.500
- Software and bibliography	1.000
SUBTOTAL	**56.762,63**
3. Travel	
- Congress of the Spanish Society of Pediatric Endocrinology 2015	1.500
- Congress of the European Society for Paediatric Endocrinology 2015	1.500
SUBTOTAL	**3.000€**
4. Subcontracting of professional services	
- Contracting of nursing services	1.720
- Contracting of echography services (20 € x 144 studies)	2.880
- Subcontracting (Contract Research Organization)	4.800
SUBTOTAL	**9.400€**
5. Direct additional general costs	**-**
6. Indirect and added costs	**14.501,65**
TOTAL COSTS	**83.664,28€**

Staff cost

No staff cost is necessary to carry out this clinical trial.

Implementation cost:

1. The SphygmoCor system (AtCor Medical, Australia): It is a required device to measure different variables such as pulse wave velocity. In Idibgi research center there is no units of this system. The SphygmoCor device and software have a total cost of 22.500€.
2. Serum parameter levels: In this study we will perform a total of 144 measures of each parameter. We will assess them at 0, 6, 12 months in a total of 48 subjects (48 patients * 3 measures = 144). These serum parameters are: PCR, TAG, HDL cholesterol, glucose, fasting insulin, total 25 hidroxivitamin D.
3. Vitamin D binding protein and HWL adiponectin are special markers which have an increased cost because of special features of their measurement.
4. Drug purchase: Deltius ® has a purchase cost of 3.90€ and placebo will be considered as having the same price. Each Deltius ® pack includes 4 administration units. Patients will be administered with one unit each two week so, the utilization of a unit of Deltius® pack will be two/month. Taking into account that the study will last one year (12 months) and there will be 48 patients receiving the treatment or placebo, the total cost of drug purchase will be: 6.739,2.
5. Administrative AEGMED
6. Publication fees, software and bibliography are considered as general expenses.

Travels

Two travels are included in the budget: *Congress of the Spanish Society of Pediatric Endocrinology 2015* and *Congress of the European Society for Paediatric Endocrinology 2015.*

Professional services

Professional services from a nurse, an ultrasonography expert and a Contract Research Organization are needed to be hired for the study.

Direct additional general costs

No additional costs are requested for the study

Indirect and added costs

Idibgi research center receives a 20% of the total expenses. Indirect costs are requested to cover the account management services at the research institute and for the use of research offices, examination rooms and laboratory.

17. MEANS AVAILABLE TO DEVELOP THE STUDY

Hospital of Girona Dr. Josep Trueta: The Department of Pediatrics of the Hospital of Girona Dr. Josep Trueta with several clinical offices will be the physical framework for inclusion and monitoring of patients. The center has the equipment necessary to perform the investigations herein proposed.

The center has specific research laboratories and research support units, and the corresponding Institutional Review Board (Committee of Ethics in Clinical Research). The researchers are supported by previous experience in conducting similar studies and clinical trials in pediatric patients.

18. PROJECT IMPACT ON THE NATIONAL HEALTH SERVICE

Obesity is related to all-cause mortality and morbidity in our health system. Prevent its health consequences might reduce a significant number of deaths, complications and may result in an important reduction of health costs to the authorities.

Vitamin D deficiency is a common problem which can be easily solved. Investing time and efforts to avoid this pathological status can reduce future cardiovascular events. Indeed, the screening of vitamin D deficiency in pediatric populations could solve future cardiovascular diseases, such as hypertension, dyslipemia, myocardial ischemia, among others, which compose the most relevant health issues to solve in adults in developed countries.

Vitamin D administration to obese adolescents may be a good prevention program to avoid having to treat them with antihypertensive, statins, diuretics, beta blockers or other drugs when these adolescents grow up and health consequences develop. The side effects of cholecalciferol are fewer than those of the drugs previously discussed.

We also believe that free Vitamin D (25-OH) should be taken into account as a more accurate parameter, compared to total vitamin D, when assessing vitamin D status in patients. Further studies are nevertheless needed to support this statement.

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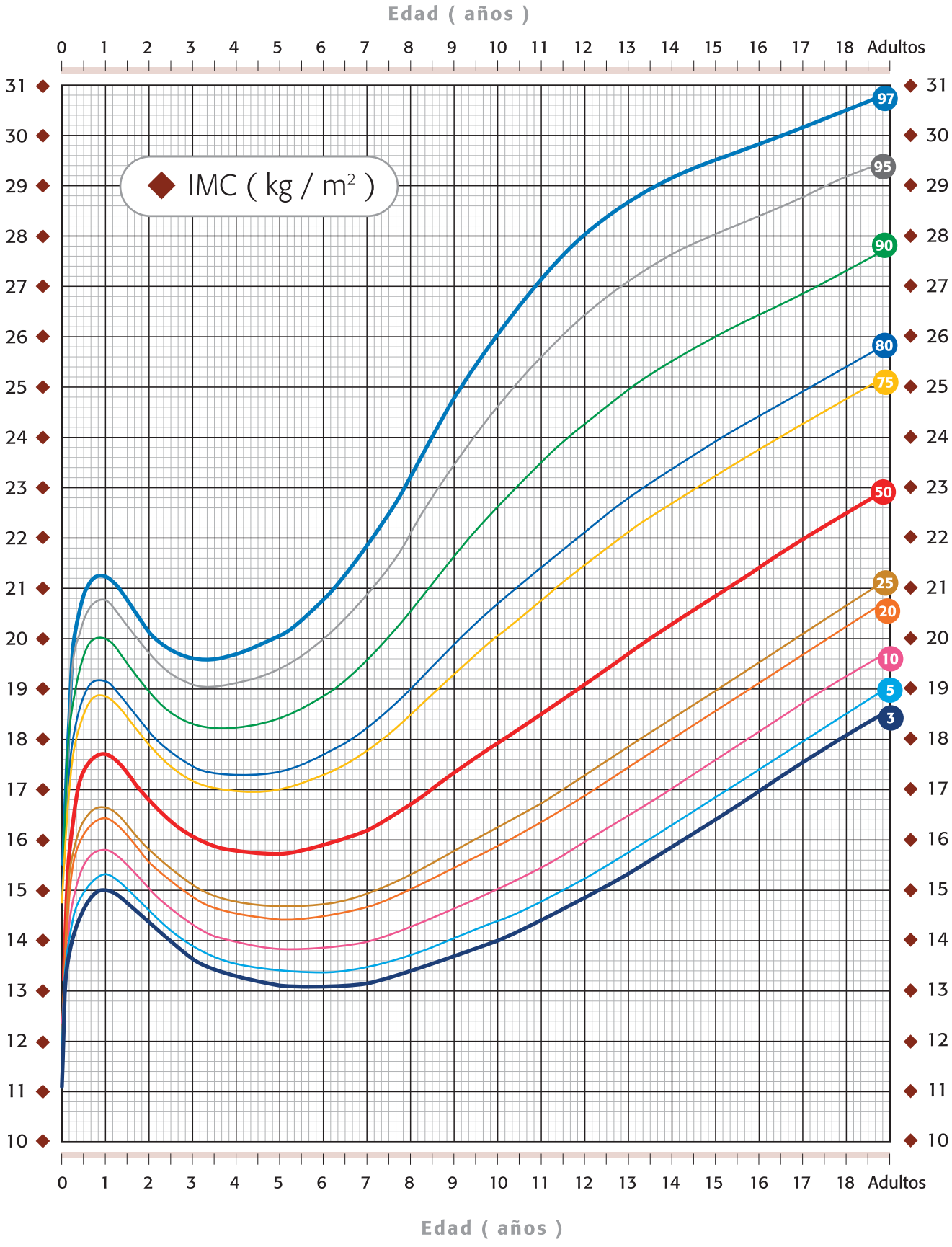
ANNEXES

ANNEX 1

Estudio Transversal Español de Crecimiento 2010

VARONES

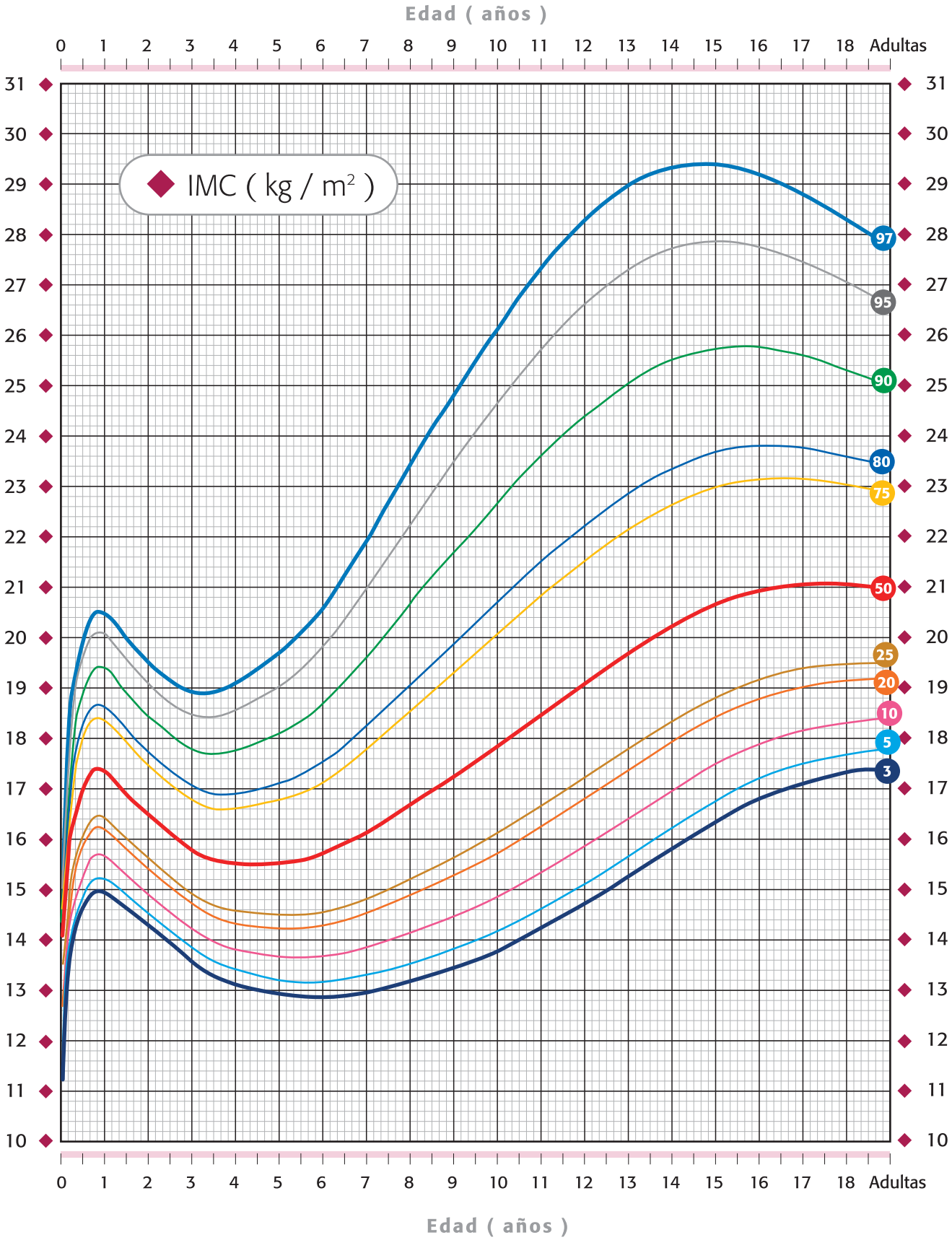


Carrascosa A., Fernández JM., Fernández C., Ferrández A., López D., López-Siguero JP., Sánchez E., †Sobradillo B., Yeste D. y Grupo Colaborador Español

An Pediatr (Barc) 2008;68:552-69. An Pediatr (Barc) 2010; en prensa.

Estudio Transversal Español de Crecimiento 2010

MUJERES



Carrascosa A., Fernández JM., Fernández C., Ferrández A., López D., López-Siguero JP., Sánchez E., †Sobradillo B., Yeste D. y Grupo Colaborador Español

An Pediatr (Barc) 2008;68:552-69. An Pediatr (Barc) 2010; en prensa.

ANNEX 2

Código: | | | | |

UNIVERSIDAD MIGUEL HERNANDEZ

Dpto. Salud Pública

Epidemiología

CUESTIONARIO DE FRECUENCIA ALIMENTARIA

A PARTIR DE AHORA COMIENZA EL CUESTIONARIO

En primer lugar se le pregunta con qué frecuencia ha consumido los alimentos **en los últimos 12 meses**. Por favor, marque su respuesta para todos los alimentos incluidos, incluso si no los come (marcando entonces la opción, "**nunca o menos de 1 vez al mes**). Siga las instrucciones, y trate de responder lo mejor posible con la ayuda de los ejemplos, teniendo en cuenta el consumo del alimento aislado, así como el añadido a otros platos.

Por ejemplo:

Huevos: considere los consumidos solos (ej. frito o cocido) y los de otros platos (ej. tortilla, revueltos)
Pollo: considere el que come en plato único y el que come en platos mixtos como la paella, guisos, etc.
La paella se considera dentro del consumo de **arroz**.
Aceite: tenga en cuenta el que añade en la mesa a ensaladas, al pan y a otros platos como verduras y huevos fritos.

Cuando un alimento se consume solo en temporada, como algunas **frutas** o **helados**, deberá indicar el número de meses que lo consume en el recuadro sombreado, además de su frecuencia de consumo. Si el tipo, cantidad o tamaño indicado para un alimento no coincide con el que toma habitualmente, trate de adaptar su respuesta al máximo, ampliando o disminuyendo la frecuencia de consumo o seleccionando los alimentos apropiados como en los ejemplos (ver ejemplo para leche semidesnatada). Si tiene dudas, pida la colaboración de un familiar o amigo, o llame a los teléfonos indicados arriba.

Por último, señale con un aspa (☒) las respuestas. Si se equivoca en la respuesta, táchela completamente (■) ó bórrela, y marque de nuevo la opción correcta (☐, ☒)

LEER LAS INSTRUCCIONES APARECIDAS EN EL CUESTIONARIO !!

Para cada alimento, consignar cuantas veces como media ha tomado la cantidad que se indica durante el año pasado. Tenga en cuenta las veces que lo toma solo y las que lo añade a otros alimentos o platos (Ej.: La leche del café, huevos en las tortillas, etc.)

I. LACTEOS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
1. Leche entera (1 vaso o taza, 200 cc)									
2. Leche descremada (1 vaso, 200cc)									
3. Leche condensada (1 cucharada)									
4. Yogurt (Uno, 125 gramos)									
5. Requesón, cuajada, queso blanco o fresco (100g)									
6. Queso cremoso o en porciones (Una porción)									
7. Queso curado o semicurado: Manchego (1 trozo, 50 g)									
8. Natillas, flan, puding (uno)									
9. Helados (1 cucurucho, vasito o bola)									
II. HUEVOS, CARNES, PESCADOS	**Nunca ó <1 mes**	**1-3 por mes**	**1 por sem**	**2-4 por sem**	**5-6 por sem**	**1 por día**	**2-3 por día**	**4-5 por día**	**6+ por día**
10. Huevos de gallina (uno)									
11. Pollo con piel (1 plato o pieza)									
12. Pollo sin piel (1 plato o pieza)									
13. Carne de ternera, cerdo, cordero como plato principal (1 plato o pieza)									
14. Carne de caza: conejo, codorniz, pato (1 plato)									
15. Hígado de ternera, cerdo o pollo (1 plato)									
16. Vísceras: callos, sesos, mollejas (1 ración, 100 g)									
17. Embutidos: jamón, salchichón, salami, mortadela (1 ración, 50g)									
18. Salchichas y similares (una mediana)									
19. Patés, foie-gras (media ración, 50 g)									
20. Hamburguesa (una, 100 g)									
21. Tocino, bacon, panceta (2 lonchas, 50 g)									
22. Pescado frito variado (un plato o ración)									
23. Pescado hervido o plancha: merluza, lenguado, sardinas, atún. (1 ración)									
24. Pescados en salazón: bacalao, anchoas (media ración, 50 g)									
25. Pescados en conservas: atún, sardinas, arenques (1 lata)									
26. Almejas, mejillones, ostras (1 ración, 100 g)									
27. Calamares, pulpo (1 ración, 100 g)									
28. Marisco: gambas, langosta y similares (1 ración, 100 g)									

(Si no se especifica, los platos para carnes y pescado son de tamaño mediano)

III. VERDURAS Y LEGUMBRES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
29. Espinacas cocinadas (1 plato)									
30. Col, coliflor, brocoles cocinadas (1 plato)									
31. Lechuga, endivias, escarola (1 plato)									
32. Tomates (uno mediano)									

Para alimentos que se consumen por temporadas, calcular el consumo medio para todo el año. Por ejemplo, si un alimento como la sandía se come 4 veces a la semana durante todo el verano (3 meses), entonces el consumo medio al año se marcaría en "1 vez por semana".

III. VERDURAS Y LEGUMBRES (Continuación)	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
33. Cebolla (una mediana)									
34. Zanahoria, calabaza (una o plato pequeño)									
35. Judías verdes cocinadas (1 plato)									
36. Berenjenas, calabacines, pepinos (uno)									
37. Pimientos (uno)									
38. Espárragos (una ración o plato)									
39. Champiñones, setas (1 plato)									
40. Legumbres cocinadas: lentejas, garbanzos, judías pintas o blancas (1 plato mediano)									
41. Guisantes cocinados (1 plato)									
IV. FRUTAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
42. Naranjas, pomelo, mandarinas (Una)									
43. Zumo de naranja natural (un vaso pequeño, 125 cc)									
44. Plátano (uno)									
45. Manzana, pera (una mediana)									
46. Fresas (1 plato o taza de postre)									
47. Cerezas (1 plato o taza de postre)									
48. Melocotón, albaricoques (uno mediano)									
49. Higos frescos (uno)									
50. Sandía, melón (1 tajada o cala, mediana)									
51. Uvas (un racimo mediano o plato de postre)									
52. Aceitunas (tapa o plato pequeño, aprox. 15 unidades pequeñas)									
53. Frutas en almíbar: melocotón, peras, piña (2 mitades o rodajas)									
54. Frutos secos: piñones, almendras, cacahuetes, avellanas (1 plato o bolsita pequeña)									
V. PAN, CEREALES Y SIMILARES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
55. Pan blanco (Una pieza pequeña o 3 rodajas de molde, 60 g)									
56. Pan integral (Pieza pequeña o 3 rodajas de molde)									
57. Picos, roscos y similares (una unidad, 3,5 g)									
58. Patatas fritas (1 ración, 100 g)									
59. Patatas cocidas, asadas (1 patata mediana)									
60. Bolsa de patatas fritas (1 bolsa pequeña, 25-30 g)									
61. Arroz cocinado (1 plato mediano)									
62. Pastas: espagueti, macarrones y similares (1 plato)									
VI. ACEITES Y GRASAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
63. Aceite de oliva (1 cucharada)									
64. Otros aceites vegetales: girasol, maíz, soja (1 cucharada)									
65. Margarina añadida al pan o la comida (1 cucharada o untada)									
66. Mantequilla añadida al pan o la comida (1 cucharada o untada)									
67. Manteca (de cerdo) añadida al pan o la comida (1 cucharada o untada)									

Para cada alimento, marcar la casilla apropiada para su consumo medio durante el año pasado. Por ejemplo si toma 1 cucharada de mermelada cada dos días, entonces debe marcar la casilla "2-4 veces por semana"

VII. DULCES Y PASTELESRES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
68. Galletas tipo María (1 galleta)									
69. Galletas con chocolate (1 galleta doble)									
70. Croissant, donuts (uno)									
71. Magdalena, bizcocho (uno)									
72. Pasteles, tarta (unidad o trozo mediano)									
73 Churros (masa frita), 1 ración									
74. Chocolate, bombones (una barrita o dos bombones, 30 g)									
75. Chocolate en polvo y similares (1 cucharada)									
VIII. BEBIDAS	**Nunca ó <1 mes**	**1-3 por mes**	**1 por sem**	**2-4 por sem**	**5-6 por sem**	**1 por día**	**2-3 por día**	**4-5 por día**	**6+ por día**
76. Vino blanco, tinto o rosado (1 vaso, 125 cc)									
77. Cerveza (una caña o botellín 1/5, 200 cc)									
78. Brandy, ginebra, ron, wiskey, vodka, aguardientes 40º (1 copa, 50 cc)									
79. Refrescos con gas: cola, naranja, limón (ej. cocacola, fanta, etc) (Uno, 250 cc)									
80. Zumo de frutas envasado (1 lata pequeña o vaso, 200 cc)									
81. Café (1 taza)									
82. Café descafeinado (1 taza)									
83. Té (1 taza)									
IX. PRECOCINADOS, PREELABORADOS Y MISCELANEAS	**Nunca ó <1 mes**	**1-3 por mes**	**1 por sem**	**2-4 por sem**	**5-6 por sem**	**1 por día**	**2-3 por día**	**4-5 por día**	**6+ por día**
84. Croquetas (una)									
85. Palitos o delicias de pescado fritos (una unidad)									
86. Sopas y cremas de sobre (1 plato)									
87. Mayonesa (1 cucharada)									
88. Salsa de tomate (media taza)									
89. Picantes: tabasco, pimienta, guindilla (1/2 cucharadita)									
90. Sal (1 pizca o pellizco con dos dedos)									
91. Ajo (1 diente)									
92. Mermeladas, miel (1 cucharada)									
93. Azúcar (ej. en el café, postres, etc.) (1 cucharadita)									

1. ¿Qué hace Vd. con la grasa visible cuando come carne? 1. La quito toda 2. Quito la mayoría 3. Quito un poco 4. No quito nada

2. ¿Cada cuanto tiempo come comidas fritas, fuera o dentro de casa? 1. A diario 2. 4-6 veces/semana 3. 1-3 veces/semana 4. Menos de 1 vez/semana

3. ¿Qué clase de grasa o aceite usa para:

	Manteca/Mantequilla	Margarina	Aceite oliva	Otros ac. vegetales
ALIÑAR	____	_____	_____	_____
COCINAR/FREIR	____	_____	_____	_____

4. ¿Toma Vd. algún producto de vitaminas? 1. Sí 2. No Si es sí ¿Cual? _____

5. ¿Hace algún tipo de dieta? 1. Sí 2. No Si es sí ¿Cual? _____

6. ¿Ha cambiado su dieta durante el año pasado? 1. Sí 2. No

7. ¿Cuánto pesa usted? (descalzo y desnudo o a lo sumo con ropa ligera) _____ Kgs.

8. ¿Cuánto mide usted? (descalzo) _____ cm.

9. ¿Ha cambiado su peso en el último año? 1. Igual 2. Aumentado 3. Disminuido

ANNEX 3

Cuestionario Mundial sobre Actividad Física
(GPAQ)

Departamento de Enfermedades crónicas y Promoción de la Salud
Vigilancia y Prevención basada en la población
Organización Mundial de la Salud
20 Avenue Appia, 1211 Ginebra 27, Suiza
Para más información: www.who.int/chp/steps

Organización Mundial de la Salud

Actividad física

A continuación voy a preguntarle por el tiempo que pasa realizando diferentes tipos de actividad física. Le ruego que intente contestar a las preguntas aunque no se considere una persona activa.

Piense primero en el tiempo que pasa en el trabajo, que se trate de un empleo remunerado o no, de estudiar, de mantener su casa, de cosechar, de pescar, de cazar o de buscar trabajo *[inserte otros ejemplos si es necesario]*. En estas preguntas, las "actividades físicas intensas" se refieren a aquéllas que implican un esfuerzo físico importante y que causan una gran aceleración de la respiración o del ritmo cardíaco. Por otra parte, las "actividades físicas de intensidad moderada" son aquéllas que implican un esfuerzo físico moderado y causan una ligera aceleración de la respiración o del ritmo cardíaco.

	Pregunta	Respuesta	Código
En el trabajo			
49	¿Exige su trabajo una actividad física intensa que implica una aceleración importante de la respiración o del ritmo cardíaco, como [*levantar pesos, cavar o trabajos de construcción*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P 4*	P1
50	En una semana típica, ¿cuántos días realiza usted actividades físicas intensas en su trabajo?	Número de días ⌊__⌋	P2
51	En uno de esos días en los que realiza actividades físicas intensas, ¿cuánto tiempo suele dedicar a esas actividades?	Horas : minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P3 (a-b)
52	¿Exige su trabajo una actividad de intensidad moderada que implica una ligera aceleración de la respiración o del ritmo cardíaco, como caminar deprisa *[o transportar pesos ligeros*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P7*	P4
53	En una semana típica, ¿cuántos días realiza usted actividades de intensidad moderada en su trabajo?	Número de días ⌊__⌋	P5
54	En uno de esos días en los que realiza actividades físicas de intensidad moderada, ¿cuánto tiempo suele dedicar a esas actividades?	Horas : minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P6 (a-b)
Para desplazarse			
En las siguientes preguntas, dejaremos de lado las actividades físicas en el trabajo, de las que ya hemos tratado. Ahora me gustaría saber cómo se desplaza de un sitio a otro. Por ejemplo, cómo va al trabajo, de compras, al mercado, al lugar de culto [*insertar otros ejemplos si es necesario*]			
55	¿Camina usted o usa usted una bicicleta al menos 10 minutos consecutivos en sus desplazamientos?	Sí 1 No 2 *Si No, Saltar a P 10*	P7
56	En una semana típica, ¿cuántos días camina o va en bicicleta al menos 10 minutos consecutivos en sus desplazamientos?	Número de días ⌊__⌋	P8
57	En un día típico, ¿cuánto tiempo pasa caminando o yendo en bicicleta para desplazarse?	Horas : minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P9 (a-b)
En el tiempo libre			
Las preguntas que van a continuación excluyen la actividad física en el trabajo y para desplazarse, que ya hemos mencionado. Ahora me gustaría tratar de deportes, fitness u otras actividades físicas que practica en su tiempo libre [inserte otros ejemplos si llega el caso].			
58	¿En su tiempo libre, practica usted deportes/fitness intensos que implican una aceleración importante de la respiración o del ritmo cardíaco como [*correr, jugar al fútbol*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P 13*	P10
59	En una semana típica, ¿cuántos días practica usted deportes/fitness intensos en su tiempo libre?	Número de días ⌊__⌋	P11
60	En uno de esos días en los que practica deportes/fitness intensos, ¿cuánto tiempo suele dedicar a esas actividades?	Horas : minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P12 (a-b)

SECCIÓN PRINCIPAL: Actividad física (en el tiempo libre) sigue.			
Pregunta		**Respuesta**	**Código**
61	¿En su tiempo libre practica usted alguna actividad de intensidad moderada que implica una ligera aceleración de la respiración o del ritmo cardíaco, como caminar deprisa, [*ir en bicicleta, nadar, jugar al volleyball*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P16*	P13
62	En una semana típica, ¿cuántos días practica usted actividades físicas de intensidad moderada en su tiempo libre?	Número de días ⌊__⌋	P14
63	En uno de esos días en los que practica actividades físicas de intensidad moderada, ¿cuánto tiempo suele dedicar a esas actividades?	Horas : minutos ⌊__⌋ : ⌊__⌋ hrs mins	P15 (a-b)
Comportamiento sedentario			
La siguiente pregunta se refiere al tiempo que suele pasar sentado o recostado en el trabajo, en casa, en los desplazamientos o con sus amigos. Se incluye el tiempo pasado [ante una mesa de trabajo, sentado con los amigos, viajando en autobús o en tren, jugando a las cartas o viendo la televisión], pero no se incluye el tiempo pasado durmiendo. *[INSERTAR EJEMPLOS] (UTILIZAR LAS CARTILLAS DE IMÁGENES)*			
64	¿Cuándo tiempo suele pasar sentado o recostado en un día típico?	Horas : minutos ⌊__⌋ : ⌊__⌋ hrs mins	P16 (a-b)

Organización Mundial de la Salud

ANNEX 4

Hospital Dr. Josep Trueta, Av. França s/n 17007 Girona	CUADERNO DE RECOGIDA DE DATOS	Ensayo clínico con vitamina D para medir los marcadores de riesgo cardiovascular en niños de edad puberal que presentan obesidad.

Código Paciente	Número de censo	Fecha
/___/___/___/	/___/___/___/___/	/___/___/ /___/___/ /___/___/___/___/ día mes año

CUADERNO DE RECOGIDA DE DATOS

Protocolo de Ensayo Clínico

Título: ENSAYO CLÍNICO COM VITAMINA D PARA MEDIR LOS MARCADORES DE RIESGO CARDIOVASCULAR EN NIÑOS ENTRE 10-15 AÑOS QUE PRESENTAN OBESIDAD.

Código del Protocolo: HJT- CDV-VIT-D Versión 3 final de fecha 15-01-15

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Responsable de la Monitorización: St. Carlos Jiménez Padilla.
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Hospital Dr. Josep Trueta, Av. França s/n 17007 Girona	CUADERNO DE RECOGIDA DE DATOS	Ensayo clínico con vitamina D para medir los marcadores de riesgo cardiovascular en niños de edad puberal que presentan obesidad.

Código Paciente	Número de censo	Fecha
/___/___/___/	/___/___/___/___/	/___/___/ /___/___/ /___/___/___/___/ día mes año

Periodo Basal

Sexo: Varón ☐ Mujer ☐

Raza: Caucásico ☐ Otras ☐

Peso actual: ___,___ Kg

Talla actual: ___ cm

IMC: ___,___ Kg/m^2

Cintura: ___,___ cm

Fat mass: ___,___ kg

Fat mass: ___,___ %

Edad: Años ___

Fecha Nacim. Día ___ Mes ___ Año ___

Fecha Diagn. Día ___ Mes ___ Año ___

Tensión arterial: Primera mesura

Sistólica ___ mm Hg

Diastólica ___ mm Hg

cIMT	Derecha				mm
	Izquierda				mm

PWV			

Laboratorio	Valores actuales	Unidades
C protein reactive		mg/L
Fasting insulin		mIU/L
HOMA		mg/dL
HMW-Adiponectin		mg/L
Lipid profile		mg/L

Al nacimiento Peso: ___,___ Kg Longitud: ___ cm Edad gestacional ___ Semanas

Historia familiar:

- Genetic syndrome associated with obesity, cardiovascular familiar disease, endocronological familiar disease (thyroid, dyslipemia, DM, metabolic disease), liver, kidney disease.

C o n f i d e n c i a l

Hospital Dr. Josep Trueta, Av. França s/n 17007 Girona	CUADERNO DE RECOGIDA DE DATOS	Ensayo clínico con vitamina D para medir los marcadores de riesgo cardiovascular en niños de edad puberal que presentan obesidad.

Código Paciente	Número de censo	Fecha
/___/___/___/	/___/___/___/___/	/___/___/ /___/___/ /___/___/___/___/ día mes año

Visita 6 meses

Sexo: Varón ☐ Mujer ☐

Raza: Caucásico ☐ Otras ☐

Peso actual: ___,___ Kg

Talla actual: ___ cm

IMC: ___,___ Kg/m^2

Cintura: ___,___ cm

Fat mass: ___,___ kg

Fat mass: ___,___ %

Edad: Años ___

Fecha Nacim. Día ___ Mes ___ Año ___

Fecha Diagn. Día ___ Mes ___ Año ___

Tensión arterial: Primera mesura

Sistólica	___ mm Hg
Diastólica	___ mm Hg

cIMT	Derecha				mm
	Izquierda				mm

PWV			

Laboratorio	Valores actuales	Unidades
C protein reactive		mg/L
Fasting insulin		mIU/L
HOMA		
HMW-Adiponectin		mg/L
Lipid profile		mg/L

Observaciones:

_____

_____

_____

_____

C o n f i d e n c i a l

Hospital Dr. Josep Trueta, Av. França s/n 17007 Girona	CUADERNO DE RECOGIDA DE DATOS	Ensayo clínico con vitamina D para medir los marcadores de riesgo cardiovascular en niños de edad puberal que presentan obesidad.

Código Paciente	Número de censo	Fecha
/___/___/___/	/___/___/___/___/	/___/___/ /___/___/ /___/___/___/___/ día mes año

Visita 12 meses

Sexo: Varón ☐ Mujer ☐

Raza: Caucásico ☐ Otras ☐

Peso actual: ___ , ___ Kg

Talla actual: ___ ___ ___ cm

IMC: ___ , ___ Kg/m^2

Cintura: ___ , ___ cm

Fat mass: ___ , ___ kg

Fat mass: ___ , ___ %

Edad: Años ___ ___

Fecha Nacim. Día ___ ___ Mes ___ ___ Año ___ ___

Fecha Diagn. Día ___ ___ Mes ___ ___ Año ___ ___

Tensión arterial:

	Primera mesura	
Sistólica	___ ___ ___	mm Hg
Diastólica	___ ___ ___	mm Hg

cIMT	Derecha				mm
	Izquierda				mm

PWV			

Laboratorio	Valores actuales	Unidades
C protein reactive		mg/L
Fasting insulin		mIU/L
HOMA		
HMW-Adiponectin		mg/L
Lipid profile		mg/L

Observaciones:

_____

_____

_____

_____

C o n f i d e n c i a l

ANNEX 5

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 POR ESCRITO DEL NIÑO (MAYOR DE 12 AÑOS)

TITULO DEL ESTUDIO: Efectos endocrino-metabólicos, sobre la adiposidad visceral y parámetros de riesgo cardiovascular de la administración de vitamina D en niños con obesidad.

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 que puedo retirarme del estudio cuando quiera, sin que esto repercuta en los cuidados médicos y sin tener que dar explicaciones.

....

Firma del participante Fecha

....

Firma del médico que dio la información Fecha

CONSENTIMIENTO POR ESCRITO DE LOS PADRES/TUTORES

TITULO DEL ESTUDIO: Efectos endocrino-metabólicos, sobre la adiposidad visceral y parámetros de riesgo cardiovascular de la administración de vitamina D en niños con obesidad.

Yo.....

(nombre y apellidos)

En calidad de .....

(relación con el participante)

de,.....

(nombre del participante)

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 que puedo retirarme del estudio cuando quiera, sin que esto repercuta en los cuidados médicos y sin tener que dar explicaciones.

....

Firma del niño (mayor de 12 años) Fecha

....

Firma del representante Fecha

....

Firma del médico que dio la información Fecha

ANNEX 6

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

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- 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.

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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.

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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,

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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

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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 30º 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.

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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

Enero 2014

10. FECHA DE LA REVISIÓN DEL TEXTO

Agosto 2013

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ANNEX 7

Declaración de Helsinki de la AMM – Principios éticos para las investigaciones médicas en seres humanos

Adoptada por la
18ª Asamblea Médica Mundial, Helsinki, Finlandia, junio 1964
y enmendada por la
29ª Asamblea Médica Mundial, Tokio, Japón, octubre 1975
35ª Asamblea Médica Mundial, Venecia, Italia, octubre 1983
41ª Asamblea Médica Mundial, Hong Kong, septiembre 1989
48ª Asamblea General Somerset West, Sudáfrica, octubre 1996
52ª Asamblea General, Edimburgo, Escocia, octubre 2000
Nota de Clarificación, agregada por la Asamblea General de la AMM, Washington 2002
Nota de Clarificación, agregada por la Asamblea General de la AMM, Tokio 2004
59ª Asamblea General, Seúl, Corea, octubre 2008
64ª Asamblea General, Fortaleza, Brasil, octubre 2013