Universitat de Girona
Facultat de Medicina

Supplements of calcium and vitamin D to improve bone mineral density of children with cerebral palsy

A quasi-experimental study

Final degree project

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1. ABSTRACT

Background	Osteoporosis is a bone disease characterized by low bone mineral density and abnormal bone architecture, that leads to bone fragility and increased risk of fractures, even spontaneously or with low-energy trauma. It affects children as well as adults, and it can be either a primary problem or secondary to various diseases (such as cerebral palsy), lifestyle issues and medications.
Justification	In this study we will focus on children with cerebral palsy that have a low bone mineral density, but do not meet osteoporosis criteria. Currently those children do not receive a treatment, and we think that they would beneficiate from supplements of calcium and vitamin D in order to prevent fractures, thus increasing their quality of life.
Main objective	The aim of this study is to evaluate the effect of supplements of calcium and vitamin D on densitometric results of children with cerebral palsy and low bone mineral density.
Design	It is designed as a longitudinal, prospective, analytic, quasi-experimental study
Participants	Children between the age of 4-14 that have cerebral palsy with low bone mineral density, objectified by a DXA and a blood test.
Variables	Calcium and Vitamin D, DXA scan results (Z-score)
Intervention and method	A densitometry and study of bone metabolism are performed. Then, supplements of calcium and vitamin D are given for one year, and after that the densitometry and the study of bone metabolism are repeated to compare the results. There will be a follow-up visit 6 months after the beginning of the treatment.
Settings	Hospital Universitari Dr. Josep Trueta de Girona
Key words	Pediatric osteoporosis, low bone mineral density, cerebral palsy, fractures in children, bone density, densitometry, DXA

2. ABBREVIATIONS

BMC = bone mineral content

BMD = bone mineral density

BMI = body mass index

BP = bisphosphonate

Ca = calcium

CEIC = “Comitè ètic d’investigació clínica”

cm = centimeters

CP = cerebral palsy

DXA = dual-energy x-ray absorptiometry (also called bone densitometry or bone density scanning)

Fe = iron

GMFCS = Gross Motor Function Classification System

HUJT = Hospital Universitari Dr. Josep Trueta

ID = identity document

ISCD = International Society for Clinical Densitometry

K = potassium

kg = kilograms

ml = milliliters

Mg = magnesium

ng = nanograms

OP = osteoporosis

P = phosphorus

iPTH = intact parathyroid hormone

SD = standard deviation

SPSS = Statistical Package for the Social Sciences

TBLH = Total body less head

VF = vertebral fractures

WHO = World Health Organization

yr = years

Zn = zinc

3. INTRODUCTION

3.1. OSTEOPOROSIS

Osteoporosis means “porous bone”. It is a metabolic bone disorder characterized by low bone mass and abnormal bone architecture, that leads to bone fragility and increased risk of fractures, even spontaneously or with low-energy trauma. (1)

3.1.1 Epidemiology

Osteoporosis is a major public health problem worldwide and its prevalence is increasing, affecting about 30% of women and 8% of men older than 50 in Europe. (2,3)
Although it is a well-established clinical problem for adults over 50 years of age, osteoporosis in children is rather new and increasingly recognized, with a lot of challenges. (2)

The prevalence or incidence of secondary osteoporosis in children is not known because the studies in children are only carried out in risk groups. However, it is known that childhood osteoporosis is an increasingly prevalent pathology due to the increased life expectancy of children with chronic pathologies and the use of medications that have a wide variety of direct and indirect effects on bone, such as glucocorticoids.(4,5)

Some researchers suggested that osteoporosis seen later in life may originate during childhood or adolescence years.(2) The skeletal growth is effectively completed with the closure of the epiphyses at the end of the adolescent growth period, and the peak bone mass achieved in the late teens will be an important determinant of future fracture risk and osteoporosis. If the hereditarily determined peak bone mass is not achieved during that time, the person can enter young adulthood with low bone density and have an

increased risk of fracture, especially when that person gets older and develops postmenopausal osteoporosis or involutional osteoporosis.(6)

As we can see from that, it is important to promote adequate bone formation in the early stages of life if we want the population to have a good quality of life in the long term. (4)

Figure 1: Causes of increase of secondary osteoporosis (7)

3.1.2. Pathogenesis and etiology

Osteoporosis (OP) can be classified as:

- **Primary OP**: caused by an underlying genetic disorder that cause bone fragility, such as osteogenesis imperfecta, hypophosphatasia and idiopathic juvenile osteoporosis. These patients typically have a family history of osteoporosis (8)
- **Secondary OP**: secondary to a chronic disease, medications, diet or lifestyle issues (9)

In this project we are going to focus on secondary osteoporosis. There is a wide variety of pathologies that can affect bone mass and lead to secondary osteoporosis (see Table 1).

Table 1. Causes of secondary osteoporosis. Adapted from (3)

Neuromuscular disorders	Cerebral palsy Duchenne muscular dystrophy Rett syndrome Myopathies Diseases resulting in long-term immobilization
Hematological diseases	Leukemias Hemophilia Thalassemia
Systemic autoimmune diseases	Juvenile systemic lupus erythematosus Juvenile dermatomyositis Systemic juvenile idiopathic arthritis Systemic sclerosis
Lung diseases	Cystic fibrosis
Gastrointestinal diseases	Celiac disease Inflammatory bowel disease Chronic liver disease Cow's milk protein allergy
Renal diseases	Nephrotic Syndrome Chronic renal failure
Psychiatric illnesses	Anorexia nervosa
Infectious diseases	HIV infection Immunodeficiencies
Endocrine diseases	Delayed puberty Hypogonadism Turner syndrome Klinefelter Syndrome Growth hormone deficiency Acromegaly Hyperthyroidism Diabetes Hyperprolactinemia Cushing syndrome Adrenal insufficiency Hyperparathyroidism Vitamin D metabolism disorders
Inborn errors of metabolism	Glycogen storage disease Galactosemia Gaucher disease
Skin conditions	Epidermolysis bullosa
Iatrogenesis	Systemic glucocorticoids Cyclosporine Methotrexate Heparin Anticonvulsants Radiation therapy

In children suffering from chronic diseases or receiving osteotoxic treatments for a prolonged period of time, there are usually several factors that increase bone resorption and decrease its formation, resulting in an increase in bone fragility.(3,10)

Two processes happen in the bone tissue of children: modeling, by which bone formation is not linked to resorption, and remodeling (common to children and adults) in which bone formation is linked to bone resorption. The balance between these processes is maintained through a series of intercellular signals.(4)

This balance can be altered by certain treatments (such as corticosteroids or antiepileptic drugs), chronic inflammation, immobility, malnutrition and low sun exposure, resulting in an increased resorption. Moreover, they decrease calcium absorption and the gain of muscle mass, both essential for bone development. (4)

3.1.3. Risk factors

There are numerous risk factors that can impact bone health in children and can lead to low bone mass density or osteoporosis. Some of these are modifiable, and others are not. (3) This classification is adapted from Galindo-Zavala et al. (3)

A. Modifiable

a. Nutritional
   i. Calcium intake
   ii. Phosphorus intake
   iii. Vitamin D
   iv. Caloric intake
   v. Protein intake
   vi. Others (vitamins K, Mg, K ...)

b. Lifestyle
   i. Solar exposure

ii. Physical exercise
iii. Tobacco
iv. Alcohol

B. Partly modifiable

a. High risk diseases
   i. (see Table 1)
b. Hormonal
   i. Treatment with glucocorticoids
   ii. Hyperparathyroidism
   iii. Hypogonadism

C. Non-modifiable

a. Genetics
b. Sex
c. Ethnicity

Calcium, phosphorus and vitamin D, among others, have a positive effect on bone health. Table 2 shows the daily nutrient requirements for a healthy child. (11)

Table 2. Daily calcium and vitamin D requirements according to age (for healthy children). Adapted from (4)

AGE	CALCIUM (mg)	Vitamin D (IU)
0-6 months	200	400
6-12 months	260	400
1-3 years	700	600
4-8 years	1000	600
9-18 years	1300	600

It is possible that children who suffer from chronic diseases (like cerebral palsy) or the ones who are taking medicines that alter intestinal absorption may need higher intakes of calcium and vitamin D. According to Galindo-

Zavala et al. (3), in those cases it is recommended to start with the recommended dose for healthy children (Table 2) and then modify it depending on the levels of intact paratohormone (iPTH), plasmatic 25(OH)D and calciuria.

Vitamin D participates in the absorption calcium. This is the classification of serum levels of **25(OH)D**: (3)

- >20 ng/mL → normal
- 10-20 ng/mL → insufficient
- <10 ng/mL → deficient

Daily exposure to sunlight as well as a healthy diet with food enriched with vitamin D (like fruits and vegetables) is necessary in order to have adequate levels of vitamin D and other essentials nutrients (such as proteins, phosphate, K, Mg, Fe, Zn, and vitamin A, C and K) that will help to maintain healthy bones. Moreover, extreme thinness and adiposity, are associated with lower BMD and increased fracture risk.(3)

On the other hand, exercise is considered the best method to maximize peak bone mass during childhood, lowering the risk of fractures. Exercise also helps to reduce the risk of falling, and that would also mean less fractures.(12) The benefits to the bone obtained from physical activity during adolescence persist into adulthood(12) The best exercises to increase the BMD are the ones with high impact and low frequency, like jumping, running or resistance training. (7)

Furthermore, the optimal control of the primary disease is the most effective way to prevent and treat secondary osteoporosis.

3.1.4. Clinical features

A common presentation of childhood osteoporosis is recurrent fractures, particularly secondary to low-energy trauma. According to their location, these fractures are classified as (2,4):

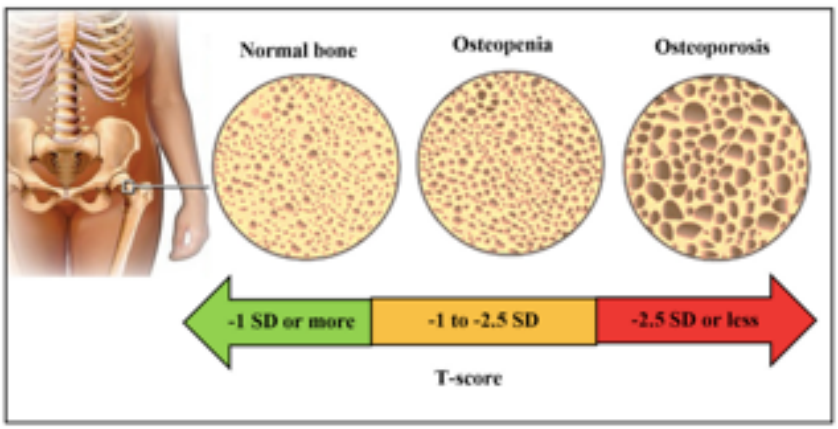
- **Vertebral compression fractures**, which can be asymptomatic and unnoticed or symptomatic with back pain and potential spinal deformity. When vertebral fractures are asymptomatic they may only be identified when a spinal X-ray is performed in a child who is being investigated for a low bone density or any other reason. (2,4)
- **Non-vertebral fractures**, which usually affect long bones and present with pain, deformity and functional impotence.(4)

We have to keep in mind that symptomatic osteoporosis may be the first manifestation of an underlying chronic disease (8)

3.1.5. Diagnosis

The most commonly used method for evaluating bone mineral density (BMD) and bone mineral content (BMC) is the dual-energy X-ray absorptiometry (DXA). The diagnostic of osteoporosis in **adults** is made by comparing the areal BMD of a specific person to the mean areal BMD of healthy young people of their same sex, expressed as standard deviations (SDs). This is called **T-score**. A T-score below -2.5 SD is indicative of osteoporosis and a T-score of -1.0 to -2.5 SD indicates osteopenia. (13)

Figure 2. Comparative view of normal bone, osteopenia, and osteoporosis (14)



Nonetheless, the diagnostic guidelines used in adults cannot be directly extrapolated to children because their BMD is affected by growth and hormonal development, so the diagnostic is more challenging.
The areal BMD and BMC results in children have to be compared to a reference population (with the same age, gender and ethnicity), expressed as standard deviations. This is called **Z-score**. As opposed to adults, you cannot make a diagnosis with this value. (13)

The Official Pediatric Positions of the ISCD published in 2013, and revised and updated in 2019 by the International Society for Clinical Densitometry, consider two possible conditions to make the **diagnosis of childhood osteoporosis**(15):

- Presence of one or more vertebral compression fractures (VF) in absence of local disease or high-energy trauma
- Z-score of bone mineral density (BMD) ≤ -2 and a history of a clinically significant fracture, which is 1 or more of the following:
    - two or more long bone fractures occurring by age 10 yr
    - three or more long bone fractures at any age up to 19 yr

Imaging tests

Although the diagnosis of childhood osteoporosis is based on the presence of fragility fractures, a **dual-energy x-ray absorptiometry** (DXA) is recommended to make a comprehensive skeletal health assessment. (15)

DXA is the method of choice to determinate the quality of the bone, thanks to its availability, reproducibility, speed and low exposure to ionizing radiation (0.001 mSv, which is equivalent to 2-3 hours of solar exposure). (4)

According to the 2019 ISCD Official Position, Lumbar spine (L1-L4) and total body less head (TBLH) are the preferred skeletal locations to perform DXA as they are the most accurate and reproducible areas in children. It may be difficult in some cases to perform a DXA scan in these locations, due to mechanical positioning issues, especially in cerebral palsy patients. In these

cases, we can use proximal femur, lateral distal femur or distal third of the radius. (15)

In short children with a size below the percentile 3 or the ones with growth delay, lumbar spine and TBLH Z-score results should be adjusted using the height Z-score. (15) The ISCD guidelines also say that bone fragility can be present even with a Z-score > -2 (15).

They also add that DXA should not be performed if the child cannot stay in an appropriate and safe position. (15)

Apart from the DXA, there are other techniques that we could use to evaluate the quality of the bone: a peripheral quantitative computed tomography and an ultrasound. Nonetheless, they are not frequently used because there are not enough on children to recommend their use on a regular basis. (3)

Laboratory tests

These are the recommended parameters to analyze when you have to evaluate a child with suspected or established secondary osteoporosis (4):

- Blood count
- Blood chemistry: calcium, ionized calcium, phosphorus, magnesium, total proteins, creatinine, urea, glucose, 25-hydroxyvitamin D_3, iPTH, TSH, free T4
- Urine screening: Ca/Creatinine (sample from a single urination, preferably first one in the morning)
- Bone turnover makers: Total alkaline phosphatase

In case we have a clinical suspicion, we can also analyze other parameters(3):

- Anti-transglutaminase IgA antibodies

- Homocysteine
- Cortisol
- Prolactin
- FSH, LH, testosterone
- Immunoglobulins
- Genetic studies (genes related to osteogenesis imperfecta and disorders characterized by bone fragility)

3.1.6. Follow-up

In children who are suffering from a chronic disease or receiving treatments that are osteotoxic for a prolonged period of time, there is an increase in bone resorption and a decrease in bone formation that results in increased bone fragility. For this reason, in those children with risk factors it is important to assess bone health during follow-up and adopt adequate preventive measures. In those cases, a laboratory test to analyze the bone mineral metabolism should be done periodically. (3)

Every case has to be individualized according their chronic disease, their diet, presence of fractures, lifestyle, etc. (4)

In cases when a follow-up DXA scan is indicated, the minimum interval between scans should be 12 months.(3) With this follow-up we will see the trajectory of the BMD in each patient, and this will inform about the situation of the patient.(4)

3.1.7. Treatment

The approach of secondary osteoporosis has to be multidisciplinary.

All the preventive measures mentioned above should be used, such as physical activity, balanced diet, sun exposure, etc. When these measures are not enough there are two possible treatments:

- **Ca and vitamin D supplementation**: they do not seem to have a clinically significant effect on BMD in studies that had been done in healthy children, but some studies propose that they could work in patients with chronic pathologies that have a low BMD or OP. It has not been studied what dose children with chronic diseases need, but it is expected to be higher than the dose for healthy children, since these children may have disorders that modify calcium metabolism or interfere with intestinal absorption. It is advised to start with the dose that corresponds to their age (Table 2), and then adapt it depending on the results of the laboratory test (serum 25(OH) D, iPTH and calciuria). No side effects of this treatment have been reported.(3,16)

- **Bisphosphonates (BPs):** they inhibit bone resorption and increase BMD in areas of high remodeling rates. Currently, BPs are only prescribed when there is a diagnosis of osteoporosis (so when a fracture has already occurred) and the aim is to prevent new fractures. An informed consent is needed for this treatment because BPs are used **off label** in childhood osteoporosis.(4,7) They have some side effects that have to be taken into account (mainly gastrointestinal and ocular)(16).

  There are two options of treatment: intravenous (IV) and oral. Intravenous are usually preferred for childhood OP, and oral BPs are only used for patients with mild forms of OP, without vertebral fracture, during the maintenance phase or when it is contraindicated to use the IV administration. The duration of the treatment has not been clearly defined. There is a study that propose discontinuing or progressively decreasing BPs dosing in patients who have not had any fractures during the last year and have a Z-score > -2 (3).

Table 3: doses and dosing intervals for the most commonly used BPs in pediatrics. Adapted from (3)

Drug	Administration	Dose
Pamidronate (2nd generation)	Intravenous (dilute in 100-250 ml physiological saline solution, in 3–4 hours)	**< 1 year**: 0. 5 mg/kg every 2 months **1–2 years:** 0. 25-0. 5 mg/kg/day 3 days every 3 months **2–3 years:** 0.375–0.75 mg/kg/day 3 days every 3 months **> 3 years:** 0. 5–1 mg/kg/day 3 days every 4 months **Maximum dose**: 60 mg/dose and 11. 5 mg/kg/year
Neridronate (3rd generation)	Intravenous (dilute in 200–250 ml physiological saline solution, in 3 hours)	1–2 mg/kg/day every 3–4 months
Zolendronate (3rd generation)	Intravenous (dilute in 50 ml physiological saline solution, in 30-45 min)	0.0125–0.05 mg/kg every 6–12 months **Maximum dose**: 4 mg
Alendronate (2nd generation)	Oral	1–2 mg/kg/week **< 40 kg:** 5 mg/day or 35 mg/week **> 40 kg**: 10 mg/day or 70 mg/week **Maximum dose:** 70 mg/week
Risendronate (3rd generation)	Oral	**< 40 kg**: 15 mg/week **> 40 kg**: 30 mg/week **Maximum dose:** 30 mg/week

3.2. CEREBRAL PALSY AND LOW BONE MINERAL DENSITY

Cerebral palsy (CP) is a non-progressive permanent encephalopathy that affects the ability to control the muscles. Therefore, it can be difficult to move and maintain balance and posture. (2) CP is caused by abnormal brain development or damage to the developing brain.(17)

Children with moderate and severe cerebral palsy and low BMD seem to present an increased risk of fracture even with low-energy trauma, affecting their quality of life(18) Low BMD can also predispose the children to fractures later in life.(19)

Epidemiology

CP is the most common motor disability in childhood.(17) The average incidence rate of CP is 2.7 per 1000 live births.This number is increasing because there is a higher survival rate of babies with low birth weight and also an improved neonatal care, which leads to less children dying but more children with cerebral palsy and other pathologies. (20)

Children with CP have an incidence of fractures between 5 to 30% (21)

Causes of bone fragility in patients with CP

There are several situations than can cause bone fragility in children with CP, such as reduced mobility, vitamin D deficiency, nutritional disorders anticonvulsants and a poor diet. The most important one is the **reduced mobility**, which leads to a low BMD and abnormal bone architecture. When that happens, even low-energy trauma can cause fractures, and their bones can break during transfers, when holding something that is heavy or even with muscle contractures associated with a convulsion. (2)

Clinical features

People with CP have problems with movement and posture, and they can also have intellectual disability, spine problems (such as scoliosis), joint problems (such as contractures), seizures and vision, hearing, or speech problems. (17)

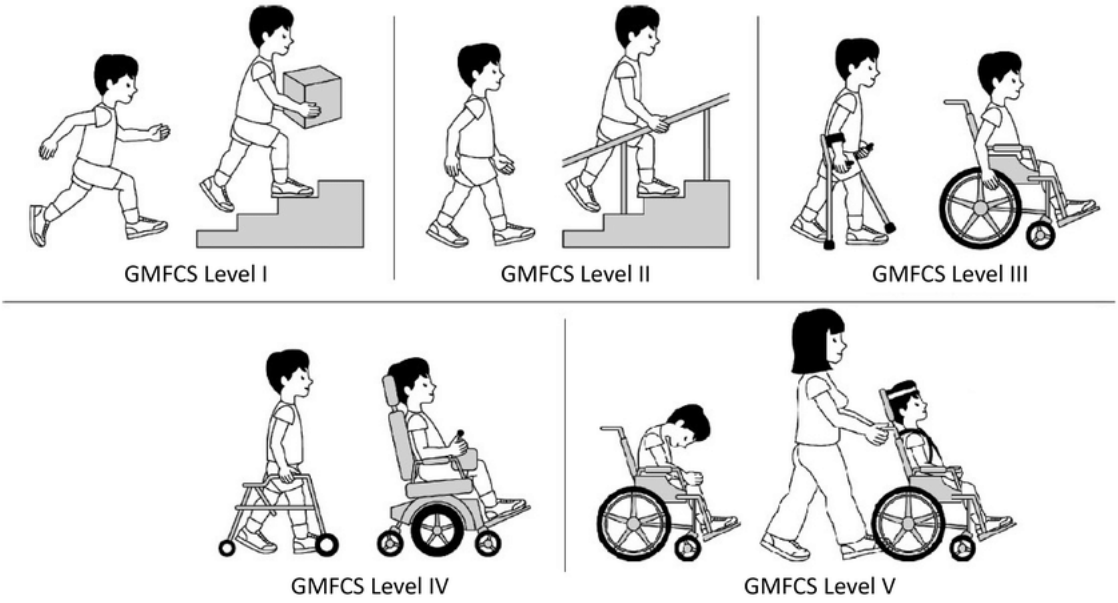
Classification

In order to classify the ambulation/functional status of children with cerebral palsy, we can use the Gross Motor Functional Classification System (GMFCS),which is a 5 level scale (22) :

- **Level 1**: they can walk indoors and outdoors, climb stairs without railing, run and jump, but their coordination, balance and speed are limited.

- **Level 2**: they can walk indoors and outdoors, but not long distances. They may need some type of assistance to walk long distances. They can climb stairs holding to a railing.
- **Level 3**: they can walk using a hand-held mobility device indoors, but mainly use a wheelchair when traveling long distances.
- **Level 4**: They may walk short distances with physical assistance, but they mainly use a manual or a powered wheelchair.
- **Level 5:** they do not have independent motor function even for basic antigravity postural control. They are transported in a manual wheelchair.

Figure 3: Gross Motor Functional Classification System (22)



Prevention of OP

In order to prevent osteoporosis in children with CP it is recommended to maintain ambulation and weight bearing (being this one the most important one), eat a variate healthy diet with an adequate intake of calcium and vitamin D, minimize iatrogenic causes of loss of bone mass and ensure well timed pubertal development. (2)(20)

Treatment

When they have established osteoporosis (so they already have fractures), they will be treated with bisphosphonates.(2)

4. JUSTIFICATION

Osteoporosis is a bone disease characterized by a decrease in its density, resulting in the appearance of fractures (1). The past few years, patients with chronic diseases have increased their life expectancy; thus, increasing osteoporosis.

In general, osteoporosis in adults is well studied as it is a very common disease, especially in women over 50 years. But in the case of children, there is very little research done, and although there is currently an increased interest in this area of study, there is still a lot of research to do. On many occasions, we end up extrapolating what is known from adults to children, even though it has not been studied on pediatric patients.

Currently, most of the studies about bone mineral density done in children are focused on a subtype of the population with a specific pathology, and it is understandable, as it is known that the risk of osteoporosis is highly increased in children who suffer from a chronic disease or undergoing certain treatments (5). One of the most common pathologies associated with osteoporosis in children and that has been given considerable importance in recent studies is cerebral palsy. (20)

There are several studies that claim that children with cerebral palsy (especially the severe cases) have a lower bone mineral density and a higher risk of fracture, even with low-energy trauma, with the consequences that this implies in their quality of life (20,23). One factor involved in the reduction of the BMD is the reduced mobility of these children, among others. (24)

According to a study published in the *Translational Pediatrics* journal, 2-3 out of every 1000 live births have cerebral palsy, and this number is increasing due to the increase of children born underweight that are able to survive, among other factors (23), so it is a relatively frequent pathology.

Currently, patients with cerebral palsy who have osteoporosis criteria (low bone mineral density (≤-2DS) in DXA and have a fracture (vertebral and/or long bone)) are treated with bisphosphonates, but it has been postulated that early treatment should be done to avoid the onset of fractures. Moreover, it should also be considered that nowadays only patients with criteria for deficiency in blood tests receive treatment with Vit D, leaving without treatment those who have insufficient values(25). All that added to the fact that this group of patients tend to have a lower bone mineral density(26), we think that it would be very interesting to study the degree of bone mineralization of this patient. We would do this study starting with a bone densitometry (lumbar and total body less head) and a blood and urine test to analyse parameters of bone metabolism. If low bone mineral density is present, we would prescribe early treatment with Vit D and calcium supplements and then repeat the DXA and blood and urine test after one year of treatment to evaluate whether this intervention can improve the degree of bone mineralization in our patients. Patients with osteoporosis criteria would be excluded from the study, as treatment with bisphosphonates should be initiated in those patients.

5. HYPOTHESES

5.2. MAIN HYPOTHESIS

Supplements of calcium and vitamin D improve densitometric results of children with cerebral palsy and low bone mineral density.

5.3. SECONDARY HYPOTHESES

Children taking anticonvulsants have lower BMD compared to those who do not take anticonvulsants.

Children taking anticonvulsants have a higher improvement of the densitometric results after taking supplements of calcium and vitamin D compared to the ones that do not take anticonvulsants.

Children with higher levels of GMFCS (levels IV-V) have a higher improvement of the densitometric results after taking supplements of calcium and vitamin D compared to children with lower levels (levels I-III).

6. OBJECTIVES

6.1. MAIN OBJECTIVE

The aim of this study is to evaluate the effect of supplements of calcium and vitamin D on densitometric results of children with cerebral palsy and low bone mineral density.

6.2. SECONDARY OBJECTIVES

To analyze if children that take anticonvulsants have lower BMD compared to those who do not take anticonvulsants.

To evaluate the effect of supplements of calcium and vitamin D on densitometric results of children that are taking anticonvulsants compared to the ones that do not take anticonvulsants.

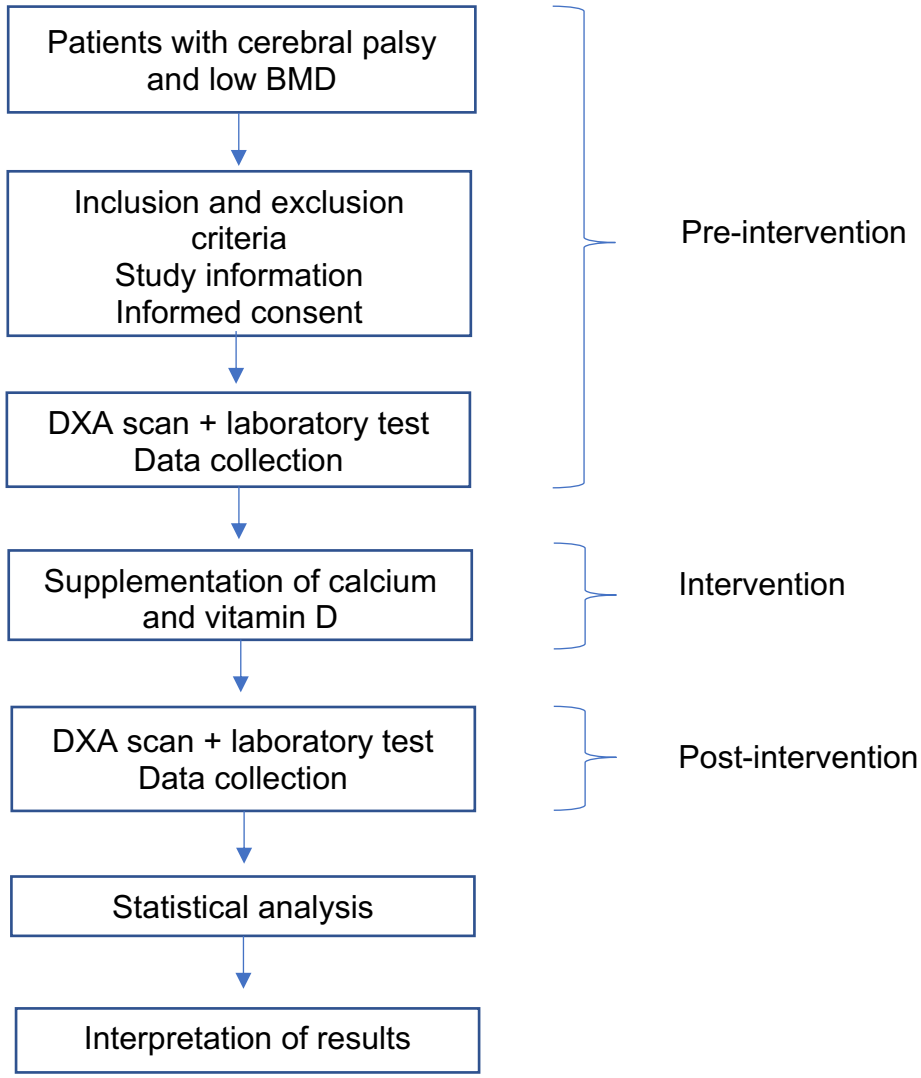
To evaluate the effect of supplements of calcium and vitamin D on densitometric results of children with higher levels of GMFCS (levels IV-V) compared to children with lower levels (levels I-III).

7. MATERIAL AND METHODS

7.1. STUDY DESIGN

This study is designed as a longitudinal, prospective, analytic, quasi-experimental study. There will be a before-and-after evaluation of the intervention.

***Figure 4**. Diagram representing the study*



7.2. STUDY SETTING

This study will be conducted in the pediatric neurology outpatient clinic of the *Hospital Universitari Dr. Josep Trueta de Girona*, which is the most important hospital of the demarcation and area of reference. The Pediatric Service of this hospital is the reference center for all urgent and highly complex pediatric pathology in the Health Region of Girona, with a population of about 800,000 inhabitants, more than 140,000 of those in pediatric age (0 to 14 years).

7.3. STUDY POPULATION

The study population will be composed of children with a **cerebral palsy**, that fulfil de following requirements:

7.3.1. Inclusion criteria

- Cerebral palsy
- Age ≥4 and ≤14 years old
- At least one (lumbar spine, proximal femur or TBLH) BMD Z-score ≤ -2.0 SD
- Parents/tutor agree to participate and sign the informed consent

7.3.2. Exclusion criteria

- History of vertebral fractures, low-energy fractures or 2 or more fractures of long bones if younger than 10yr, or 3 or more fractures if older than 10yr
- Serum 25(OH)D levels < 10 ng/mL
- Children diagnosed with other bone metabolic diseases, such as rickets, celiac disease, etc.
- Children with alterations of the phospho-calcium metabolism from other causes
- Parents/tutor do not agree to participate and/or do not sign the informed consent

7.3.3. Withdrawal criteria

- If any of these happen: vertebral fracture, low-energy fracture or 2 or more fractures of long bones if younger than 10yr, or 3 or more fractures if older than 10yr
- Poor compliance of the treatment or follow-up

7.4. SAMPLE

7.4.1. Sample selection

A non-probabilistic consecutive method of recruitment will be used in this study. All patients with cerebral palsy who come to the pediatric neurology outpatient consultation at the HUJT that meet the inclusion criteria and none of the exclusion criteria will be asked to participate. They will receive the study information document (Annex 1), as well as the informed consent (Annex 2). The physician will explain what the study consists of and all the process that will take place if they participate. At any moment, the parents and the children themselves will be able to ask any question that they may have. If they agree to participate, a parent will sign the informed consent. The physician has to make sure that they understand that it is voluntary and anonymous, and that they can withdraw their informed consent and leave the study at any time.

7.4.2. Sample size

In order to calculate the sample size, we need to know which is the mean of the Z-score of these patients. The results of a study carried out in Spain in 2020 that studied the bone health of pediatric patients with cerebral palsy concluded that the mean bone mineral density Z-score was -2.1 (95% CI -2.5, -1.7). (18)

It has been anticipated a drop-out rate of 20% because of the possibility of data collection sheet misplacing, incomplete collection of the information, withdrawal of consent, no follow-up…

With that information the sample size has been calculated.

In a two-sided test, accepting an alpha risk of 5%, a statistical power of 80% and a minimum difference to detect equal to 0.5 standard deviations, the sample size would be 99 children. If, in addition, we assume drop-outs of 20%, the sample size would be **118 children**.

The calculations were made with the software of Prof. Dr. Marc Saez, from the package 'pwr' of the free statistical software environment R (version 4.0.3).

7.4.3. Time of recruitment

The pediatric neurology outpatient clinic of HUJT attends a little more than a hundred patients with cerebral palsy per year. Therefore, it is estimated that between one and one and a half years will be necessary to reach the number of patients needed for this study. The recruitment will stop when the number of patients recruited reach the needed number of patients (118).

7.5. VARIABLES

7.5.1. Independent variable

The treatment, consisting of supplements of calcium + Vitamin D. It is a dichotomous qualitative variable, being "no" before the intervention and "yes" after the intervention.

7.5.2. Dependent variable

The BMD **Z-score** results of the DXA scan. This is a continuous quantitative variable. The data will we obtained through the data collection sheets.

7.5.3. Covariates

The following covariates will be taken into account, as they may act as confounding factors:

- **Age:** measured as a discrete quantitative variable, expressed in years (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). It will be collected from the ID card of the patient or another official documentation.
- **Gender:** is a nominal qualitative variable, expressed as male, female or unknown. It will be collected from the ID card of the patient or another official documentation.
- **Gross Motor Function Classification System (GMFCS):** is an ordinal qualitative variable, expressed as levels (level I, level II, level III, level IV, level V). The level will be determined by the physician with ana anamnesis and physical exploration
- **Height:** it is a continuous quantitative variable, expressed in cm. If the child is able to stand, it will be measured with a height board. If the child is unable to stand, it will be measured with the length board (lying down).
- **Weight:** it is a continuous quantitative variable, expressed in kg. It will be measured with a medical scale. If the child is unable to stand, measure the weight of the parent and the child together, and then measure the weight of the parent and subtract it from the weight of the two together:

  Child weight = (Parent + child weight) – parent weight
- **Anticonvulsants:** measured as a Dichotomous qualitative variable, expressed as yes /no (and add the name).
- **Serum 25(OH)D levels**: measured as a nominal qualitative variable.

  Expressed as:

  -Insufficiency: if serum 25(OH)D <20 ng/ml

  -Sufficiency: if serum 25(OH)D > 20 ng/ml

Table 4. Variables of the study

Variables/ covariables	Type of data	Categories or values	Measure Instrument
Calcium + Vit D	Dichotomous qualitative	Yes/No	-
BMD Z-score	Continuous quantitative	Standard deviation	DXA scan
Age	Discrete quantitative	Years (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14)	Patient's ID card or another valid document
Gender	Nominal qualitative	Male/female/unknown	Patient's ID card or another valid document
GMFCS	Ordinal qualitative	Level I, level II, level III, level IV, level V	Anamnesis and physical exploration
Height	Continuous quantitative	cm	Height board or length board
Weight	Continuous quantitative	kg	Scale
Anticonvulsant	Dichotomous qualitative	Yes/No	Anamnesis
Serum 25(OH)D levels	Nominal qualitative	Stratified as: • Insufficiency • Sufficiency	Blood test

This data will be collected by physicians in data collection sheets.

7.6. METHODS OF MEASUREMENT

DXA

For this study, we will need DXA measurements of lumbar spine (L1-L4), proximal femur and total body less head (TBLH) before and after the intervention, so before and after giving the supplements of calcium and vitamin D. Patients with an initial DXA with a Z-score ≤-2 SD result (in the lumbar spine and / or TBLH and / or femur) will be included in the study.

The aim of this study is to see if the Z-score improves with the treatment with calcium and vitamin D. We will compare the results of both DXAs, assuming a significant improvement when there is an increase greater than 0.5 SD.
In addition, the corresponding DXA reports already contain the percentage of objectifiable change between the previous test and the current one for each location. The trajectory followed by the patient will also be taken into account (if it has remained the same, it has improved and / or worsened).

Laboratory test

In order to study the bone mineral metabolism, we will analyse:

- Blood count
- Blood chemistry: calcium, ionized calcium, phosphorus, magnesium, total proteins, creatinine, urea, glucose, 25-(OH)D, iPTH, TSH, free T4.
- Urine screening: Ca/Creatinine (sample from a single urination, preferably first one in the morning)
- Bone turnover makers: Total alkaline phosphatase

7.7. STUDY INTERVENTIONS

The study intervention will consist of giving supplements of calcium and vitamin D for one year to patients with low mineral density, to study what happens to their BMD. There will be a DXA scan before starting the treatment and another one after finishing it (so one year after the first one), and 3 laboratory tests (one before starting the supplementation, one after six months, and the last one after finishing it). Those tests are described in the previous section: *7.6. Methods of measurement.*

The recommended dose of vitamin D for children over one year of age is 600 IU. The recommended dose of calcium for children 4 to 8 years is 1000 mg and for children 9 to 18 years is 1300 mg (see Table 2). In the case of children with cerebral palsy who may possibly have swallowing problems, we should provide medication that is easy for them to swallow, such as oral solutions or sachets. We did not find any medication available that had 1300 mg of calcium in this format. For this reason, we decided to use 1000 mg of calcium for all of them. We have to keep in mind that most of these patients will weigh less than they should for their age. Therefore, in case of children between 4 and 8 years old who weigh less than the 3rd percentile by age, we will look at which age group their weight correspond to the 50th percentile. If it was the case of being in the lower group (1-3 years) we would lower the dose to 700 mg of calcium.
We are using the recommended dose for healthy children because there are no studies that specify what dose is needed for children with cerebral palsy.

The medicines that we recommend (and the ones that we used to calculate the budget) are the following ones:

- Calcium: CAOSINA SUSPENSION (60 sachets)
- Vitamin D: VITAMIN D3 KERN PHARMA 2.000 UI/ml ORAL SOLUTION (10 ml bottle with dropper)

We recommend them because they are easy to swallow, cheaper than the others, and they have the correct dose. Nonetheless, other medicines with the same dose can be used.

7.8. DATA COLLECTION

Information of the patients will be collected during approximately 30 months using data collection sheets (Annex 3). There will be a first period of data collection before the intervention, and then another period after the intervention.

When a potential patient is found, the neuropediatrician will inform the patient and the parents/tutor about the study and they will have to sign the informed consent if they accept.

After that, the physician will order a DXA scan and a blood test. When this is done, the neurologic pediatrician will look at the results, and 2 situations can happen:

- The children can't participate in the study because he/she is not meeting all the inclusion and none of the exclusion criteria. Then the neuropediatrician will treat the child as she would normally do.
- The children can participate because he/she is meeting all the inclusion and none of the exclusion criteria. Then the neuropediatrician will send the child to the pediatric rheumatologist and she will explain the treatment and fill the data collection sheets.

Data collection sheets will be used to register:

- Identification number to ensure confidentiality
- Date of birth (this way there will not be a misunderstanding of the age of the patient at every moment)
- Gender
- Height
- Weight
- Pathological antecedents
- Anticonvulsant: name and dose
- Other medications
- Fractures
- GMFCS level

- BMD Z-score of lumbar spine, TBLH and proximal femur
- Laboratory test results:
    - Blood count
    - Blood chemistry: calcium, ionized calcium, phosphorus, magnesium, total proteins, creatinine, urea, glucose, 25(OH)D, iPTH, TSH, free T4
    - Urine screening: Ca/Creatinine (sample from a single urination, preferably first one in the morning)
    - Bone turnover makers: Total alkaline phosphatase

There will be a follow-up visit 6 months after the beginning of the treatment. A urine and blood test will be repeated. In this visit the physician will ask if any fractures happened since the beginning of the treatment. If the answer is affirmative and the child now has osteoporosis criteria (described in *3.1.5.Diagnosis*), the child will leave the study and start the corresponding treatment (bisphosphonates).

After one year of the first visit, another DXA and blood and urine test will be needed, and after that the patients will have another visit with the rheumatology pediatrician, and this one will fill another data collection sheet. The document will be the same as the first time, and the physician will fill all the information with what the child and parents say and with the results of the DXA and blood test.

Data collection sheets (Annex 3) will be in Catalan because all the physicians participating in the study understand Catalan.

8. STATISTICAL ANALYSIS

The statistician will carry out the statistical analysis using the Statistical Package for the Social Sciences (SPSS).
Results will be considered statistically significant if p-value ≤ 0.05.

8.1. DESCRIPTIVE ANALYSIS

A descriptive analysis of the variables will be performed as follows:
The quantitative variables will be expressed as a mean and the standard deviation if they have a normal distribution or as a median and quartiles if they do not have a normal distribution. Quantitative variables are BMD Z-score, age, height and weight.

The qualitative variables will be expressed as a proportion (percentage) and a confidence interval of 95%. Qualitative variables are calcium + vitamin D supplements, gender, GMFCS, anticonvulsant and initial serum 25(OH)D levels.

8.2. BIVARIATE ANALYSIS

The bivariate analysis will be used to compare the covariates with the Z-score.
The chi-square test will be used to co-relate qualitative variables.
The t-Test will be used to compare quantitative variables if there is normal distribution, and the Mann-Whitney U test if there is no normal distribution.

8.3. MULTIVARIATE ANALYSIS

The analysis of the BMD Z-score of the patients before and after the intervention (supplements of calcium and vitamin D) will be performed using a multiple lineal regression.
The analysis will be adjusted for all the covariates.

9. ETHICAL CONSIDERATIONS

This study will follow the ethical principles for medical research established by the *World Medical Association* in the *Declaration of Helsinki for Ethical Principles for Medical Research Involving Human Subjects* (last revision in the 64th General Assembly, Fortaleza, Brazil, in October 2013).

The present project will be submitted to the *Clinical Research Ethical Committee* (CEIC, "Comitè Ètic d'Investigació Clínica") of HUJT. Recommendations given by the CEIC will be considered before the study begins.

This study will respect the *Spanish Legislative Royal Decree 1/2015, del 24 de Julio 1090/2015*, which regulates the use of medications and sanitary products.

According to *Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales*, all patients personal and clinical information collected in this study will be anonymous, and the database will use a randomized code instead of the names of patients to guarantee the confidentiality of the patients' information. All the data collected will only be used for research purposes and it will be treated in a homogeneous and no-discriminative way, preserving the ethical principle of justice. This study will also preserve the principles of beneficence and non-maleficence.

A physician will inform the patients and parents/legal tutor, and he/she will give them a document with all the information regarding the study (Annex 1). The physician has to make sure that they understand that it is voluntary to participate in this study, and that they are also allowed to access, modify or delate all the data collected at any given moment. They can also withdraw the consent and leave the study whenever they want. After asking all their questions, understanding it all, and only if they want to, the parents/legal tutor will sign the informed consent (Annex 2). Both of these documents will be available in Catalan and Spanish.

Investigators of this study declare that there are no conflicts of interest.

10. LIMITATIONS

There are several potential limitations that should be considered. As this is a quasi-experimental study, there is no randomization to pick the sample. The sample selection method is consecutive (non-probabilistic), so there can be a bias of selection.

It is likely that patients with a higher level of GMFCS (Gross Motor Functional Classification System), so the ones with a more severe cerebral palsy, have lower levels of bone mineral density. That means that if there are a lot of children in the sample that are in the same level of GMFCS, but not as many children in other levels, the results could be biased.

The data collection sheet can have several limitations, such as being incorrectly filled, incomplete or even misplaced, therefore leading to an information bias. On the other hand, there will be only 2 neuropediatricians and one rheumatologic pediatrician filling the data collection sheets, so that means that the interobserver variability will be low.

It has already been assumed a drop-out rate of 20% when calculating the size of the sample because it is expected that we will lose part of the sample.

There are several confounding factors that may influence the association between the supplementation and an improve of the mineral bone density. Therefore, a multivariate analysis will be used to analyze all the variables.

11. WORK PLAN

The research team will be composed by the general coordinator of the study (Dra. Montse Gispert-Saüch), who is a pediatric rheumatologist, a rheumatologist, two neuropediatricians, the statistician, and a data manager.

The study will be performed during 4 stages, and it will last about 45 months, which is the same as 3 years and 9 months.

STAGE 0: STUDY DESIGN AND PREPARATION (4 months)

1. **First meeting (Nov 2020)**
   This meeting was made in order to think about this project and what gaps of information there were in this area of study.
2. **Study protocol development (Nov 2020- Jan 2021)**
   A lot of bibliographic research has been done, as well as the redaction of the protocol.
3. **Ethical evaluation (Feb 2021)**
   Presentation and evaluation of the protocol by the CEIC of HUJT.
4. **Determination and meeting of the professionals collaborating in the study (Feb 2021)**
   The general coordinator will contact the neuropediatricians, the rheumatologist, the statistician and the data manager, and will explain all they need to know about the study and how to fill the data collection sheets.

STAGE 1: SAMPLE COLLECTION, INTERVENTION AND DATA COLLECTION (31 months)

5. **Patient recruitment (Mar 2021 – Aug 2022)**
   118 children with CP and BMD Z-score ≤ 2 recruited by a consecutive sampling are needed for this study. They will have to meet all the inclusion and none of the exclusion criteria, and the parents/tutor have to sign the informed consent. The recruitment will last until we have

118 children that meet the criteria. Those children will be sent to the pediatric rheumatologist.

6. **Pre-intervention data collection (Mar 2021 – Aug 2022)**

   The neuropediatrician will record all the information collected in the first visit using the data collection sheet (Annex 3).

7. **Intervention (Mar 2021 – Aug 2023)**

   Vitamin D and calcium supplements will be given to all the children in the sample for one year (every child will start the treatment after they are included in the study, so not all of them will be taking the supplements at the same time)

8. **Generation of the database (Sep 2022)**

   This will be made by the data manager, introducing all the information of the data collection sheets into the database.

9. **Follow-up visit (Sep 2021 – Feb 2023)**

   6 months after the beginning of the treatment. There will be another blood and urine test, but not DXA. The pediatric rheumatologist will fill another data collection sheet (Annex 3) with the information of this visit.

10. **Tests II (Mar 2021- Aug 2023)**

    After the year of treatment, there will be another DXA and laboratory test.

11. **Post-intervention data collection (Sep 2022 -Aug 2023)**

    The pediatric rheumatologist will record all the information collected in the visit after the tests using the data collection sheet (Annex 3).

12. **Data introduction in the database (Sep 2023)**

    This will be made by the data manager, using all the information in the data collection sheets of the follow-up visit and last visit.

STAGE 2: DATA ANALYSISI AND INTERPRETATION (2 months)

13. **Statistical analysis (Sep 2023)**

    Performed by a statistician, who will analyze all the data collected according to the variables of the study and obtain the results.

14. **Interpretation of results (Oct 2023)**

    There will be a meeting with the whole team to evaluate the results.

STAGE 3: PUBLICATION OF RESULTS (8 months)

15. **Study writing and revision (Nov - Dec 2023)**

    The main researchers will make the redaction of the study with all the results and conclusions.

16. **Publication and dissemination (Jan – Jun 2024)**

    The written study will be published as a journal article and the results will be disseminated at a national congress.

Table 5: chronogram of the study

		2020	2021													2022												2023											2024					
		N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
STAGE 0	First meeting																																												
	Study protocol development																																											
	Ethical evaluation																																											
	Determination of professionals																																											
STAGE 1	Patient recruitment																																											
	Pre-intervention data collection																																											
	Intervention																																											
	Post-intervention data collection																																											
	Data introduction in the database																																											
STAGE2	Statistical analysis																																											
	Interpretation of results																																											
STAGE3	Paper elaboration and revision																																											
	Publication and dissemination																																											

12. BUDGET

The estimated budget for this study is detailed in the following table.

Table 6: Budget of the study.

Type of cost	Unit cost	Hours or units	Total
Staff/personnel			
Pediatricians, nurses, pharmacists, radiologists… [1]	0 €/h		0 €
Data manager: -Creation of the database -Data introduction	40 €/h	40 h	1,600 €
Statistician	50 €/h	48 h	2,400 €
Subtotal			**4,000 €**
Supplements			
Calcium: CAOSINA SUSPENSION (60 sachets)	5.5 € / unit	1 box lasts almost 2 months. Thus, 7 boxes are needed for a year of treatment 7x 118 children = 826 units	4,543 €
Vitamin D: VITAMIN D3 KERN PHARMA 2.000 UI/ml ORAL SOLUTION (10 ml bottle with dropper)	3.12 € / unit	1 bottle lasts aprox. 33 days. Thus, 12 bottles are needed for a year of treatment 12 x 118 children = 1416 units	4,417.92 €
Subtotal			**8,960.92 €**
Printings			
Study information document	0.03 €	120 (x 3 pages)	10.8 €
Informed consent	0.03 €	120	3.6 €

Data collection sheets	0.03 €	120	3.6 €
Subtotal			**18 €**
Publication and diffusion costs			
Linguistic correction			150 €
Article publication			1,500 €
National congress -registration -travel -accommodation -diet	 500 € 150 € 100 € 50 €	 2 people 2 people 2 people, 2 nights 2 people, 3 days	 1,000 € 300 € 400 € 300 €
Subtotal			**3,650 €**
TOTAL COST			**16,628.92 €**

The budget does not include the cost of the physicians and other staff that will visit and attend the participants of the study because they are already part of the National Health System.

DXA scans and laboratory tests are not included in the budget because the physicians would do those procedures anyway, so we are not spending extra money on those tests for this study.

The cost of the supplements is stablished by the Ministerio de Sanidad of the Spanish government.

13. IMPACT ON THE NATIONAL HEALTH SYSTEM

This study could have a big impact on the National Health System. On one hand, if we could increase the BMD of children with cerebral palsy, that would result in less fractures, resulting in a big increase of their quality of life. We have to keep in mind that these children may have difficulties to move, and it would be even more difficult to move if they have a fracture. Also, in the most severe cases (so the ones who have less mobility), it would be really difficult for the people taking care of them to move them if they have a broken bone.

It is also important to remember that these children have to go to the hospital very frequently because they are usually controlled by different specialists, so it would be better if we can prevent fractures from happening, so they don't have to go to the hospital also for this reason. Furthermore, bisphosphonates can cause side effects, so it would be better if they don't need to take BPs.

On the other hand, we could also reduce costs because less fractures would mean less visits to the hospital and less treatments, as well as not having to take bisphosphonates, which are more expensive than supplements of calcium and vitamin D.

In conclusion, supplements of calcium and vitamin D would have a good impact on the life of children with cerebral palsy and low BMD and on the National Health System as well.

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15. ANNEXES

Annex 1. Information document of the study.

FULL D'INFORMACIÓ SOBRE L'ESTUDI

Benvolgut / da Sr / a.

Introducció

Ens dirigim a vostè per informar-li sobre un estudi que s'està duent a terme, en el qual invitem al seu fill/a a participar. Aquest estudi ha estat aprovat pel comitè d'ètica de l'Hospital Universitari Dr. Josep Trueta.

Volem que rebi tota la informació necessària per què pugui avaluar si desitja o no que el seu fill/a hi participi. Abans de confirmar la participació, es prega que llegeixi aquest full informatiu detingudament i feu totes les preguntes que li puguin sorgir.

Descripció de l'estudi

Els nens amb paràlisi cerebral tenen una menor densitat mineral òssia (els ossos són més febles) i, per tant, tenen més risc de patir osteoporosi i fractures degut a aquesta menor densitat òssia.

Creiem que si a nens amb baixa densitat mineral òssia se'ls donés suplements de calci i vitamina D, podríem millorar-los-hi la densitat mineral òssia, i s'intentaria evitar en certa mesura que arribessin a desenvolupar una osteoporosi i patir fractures. Per tal d'estudiar si això és cert, volem dur a terme el següent estudi:

"Supplements of calcium and vitamin D to improve bone mineral density of children with cerebral palsy"

Aquest estudi el durà a terme l'investigador / a: Carla Ragués López i Dra. Montserrat Gispert-Saüch.

Objectiu de l'estudi

El principal objectiu és avaluar l'efecte dels suplements de calci i de vitamina D en els resultats densitomètrics de nens amb paràlisi cerebral i baixa densitat mineral òssia

Participació voluntària

És important que entengui que la participació és completament voluntària i la seva decisió no influirà en la seva atenció mèdica. A més, pot decidir retirar el consentiment i sortir de l'estudi en qualsevol moment.

Nombre de pacients i durada estimada de la participació dels pacients

En aquest estudi es preveu la participació d'un total de 118 nens atesos a consultes externes de l'HUJT. La participació serà d'uns 13 mesos aproximadament.

Procediments de l'estudi

Es compararan els resultats de la densitometria i els anàlisis clínics en un any de diferència. Durant aquest any el seu fill s'haurà de prendre un tractament diari amb suplements de calci i vitamina D. Hi haurà una visita de seguiment entremig, on també es farà l'anàlisi de sang i orina, però no una densitometria.

Beneficis i riscos associats a la participació

El benefici serà que es possible que la densitat mineral òssia del seu fill augmenti degut a la suplementació que donarem. Aquest augment disminuirà el risc de fractures del seu fill, la qual cosa tindrà un bon impacte en la seva qualitat de vida. És important tenir en compte que com millor sigui la densitat mineral òssia (DMO) en la joventut, millor serà en l'edat adulta, ja que el pic màxim de DMO s'assoleix després de l'adolescència. No hi ha cap risc esperat derivat de la participació en l'estudi més enllà de que el seu fill no toleri el suplement.

Compensació econòmica

Els investigadors no obtindran cap tipus de benefici econòmic procedent d'aquest estudi.

De la mateixa manera, ni vostè ni el seu fill/a rebran remuneració pel fet de

participar-hi, però sí que es beneficiaran d'uns suplements sense d'haver-se de fer càrrec del cost (serà gratuït).

Confidencialitat

D'acord amb la Llei Orgànica 15/1999, de 13 de desembre, de protecció de dades de caràcter personal (LOPD) i Reial Decret 1720/2007, les dades personals i de salut que es recopilin per aquest estudi només seran utilitzades amb finalitats d'investigació. Aquestes dades seran identificades mitjançant un codi amb una sèrie numèrica aleatoritzada per garantir la confidencialitat de la seva identitat, impedint que pugui ser reconeguda.

Les dades que es recullin amb motiu d'aquest estudi, entre els quals es trobaran dades personals i de salut seran processades i analitzades amb la finalitat d'avaluar-les científicament. Si vostè decideix participar en aquest estudi estarà consentint expressament el tractament de les seves dades personals i de salut pel promotor.

Vostè podrà exercitar en qualsevol moment els seus drets d'accés, rectificació, cancel·lació i oposició dirigint-se al metge que l'atén en aquest estudi el qual ho ha de posar en coneixement del promotor.

Així mateix, els resultats de l'estudi poden ser comunicades a les autoritats sanitàries i eventualment a la comunitat científica a través de congressos i publicacions sense que la seva identitat sigui revelada en cap moment.

Preguntes / Informació

Per fer alguna pregunta o aclarir algun tema relacionat amb l'estudi, o si necessita ajuda per qualsevol problema de salut relacionat amb aquest estudi, si us plau, no dubti en posar-se en contacte amb:

Dr/a.: _____

Telèfon: _____

L'investigador li agraeix la seva inestimable col·laboració.

Annex 2: Informed consent document

CONSENTIMENT INFORMAT PER PARTICIPAR A L'ESTUDI:

"Supplements of calcium and vitamin D to improve bone mineral density of children with cerebral palsy"

Jo, _____ , amb DNI _____,
com a mare, pare o tutor legal de _____ declaro que:

□ He Llegit el full informatiu sobre l'estudi que se m'ha entregat.
□ He pogut preguntar tots els dubtes relacionats amb l'estudi.
□ He estat informat/da de les implicacions i objectius de l'estudi.
□ Entenc que es respectarà la confidencialitat de les dades del meu fill/a.
□ Entenc que la participació del meu fill/a a l'estudi es voluntària.
□ Entenc que puc retirar el consentiment quan vulgui, sense necessitat de justificació i sense que això repercuteixi en la meva assistència mèdica

Conforme l'establert a *L.O. 3/2018, de 5 de desembre, de Protección de Datos Personales y garantía de los derechos digitales,* declaro haver estat informat:

- De que existeix un fitxer o tractament de dades de caràcter personal, de per què es recullen aquestes dades, i dels destinataris de la informació.
- De que pot accedir, rectificar, oposar-se i cancel·lar-la dirigint-se per escrit al titular del fitxer de les dades.

Vull rebre informació via telefònica o per correu electrònic dels resultats
Contacte:
Número de telèfon _____
Direcció de correu electrònic _____

Per tot això, **OTORGO EL MEU CONSENTIMENT** per participar a aquest estudi i estic d'acord en que la informació obtinguda pugui ser utilitzada en investigacions futures.

Signatura del pare/ mare/ tutor Signatura de l'investigador

Girona, ____ de _____ de 20____

Annex 3: Data collection sheet

FITXA DE RECOLLIDA DE DADES

Dades del pacient

Número aleatoritzat d'identificació: _____

Data de naixement: _____ Edat: _____

Telèfon pare/mare/tutor: _____

Sexe: □Masculí □Femení □No especificat

Pes: _____

Alçada: _____

Nivell GMFCS: □N. I □N. II □N. III □N. IV □N. V

Tractament anticonvulsiu: □Sí □No

Nom i dosi: _____

Antecedents patològics d'interès: _____

Altres tractaments i dosi: _____

Fractures: _____

Anàlisis sang:

Hemograma: _____ Creatinina: _____

25(OH)D: _____ Urea: _____

Calci: _____ Glucosa: _____

Calci ionitzat: _____ PTHi: _____

Fòsfor: _____ TSH: _____

Magnesi: _____ T4 lliure: _____

Proteïnes totals: _____ Fosfatasa alcalina total: _____

Anàlisi d'orina (única mostra):

Ca/Creatinina: _____

Densitometria (si és primera i última visita)

BMD Z-score TBLH: _____

BMD Z-score columna L1-L4: _____

BMD Z-score fèmur proximal: _____

Data: _____ Metge responsable: _____

Centre: _____ Signatura metge responsable: