Evaluation of bone health in children with intractable epilepsy treated with the ketogenic diet on a long-term basis

A combination of quantitative, retrospective longitudinal cohort and survey research at the Erasmus MC Sophia Children's Hospital

Bachelor thesis I.M. (Isabel) van Ruijven, January 2018

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Preface

In front of you lies my thesis 'Evaluation of bone health in children with intractable epilepsy treated with the ketogenic diet on a long-term basis' for the bachelor Nutrition and Dietetics at the Hague University of Applied Sciences, the Netherlands. I wrote this thesis commissioned by Elles van der Louw, paediatric dietitian and PhD student at the Erasmus MC Sophia Children's Hospital in Rotterdam, the Netherlands from August 2017 to January 2018. The results of this research were presented to the paediatric neurologists and dietitians of the Erasmus MC Sophia Children's Hospital.

This thesis is meant for all professionals that are allied to the ketogenic diet and everyone else with interest in bone health and the ketogenic diet. I hope that this research will contribute to the improvement of the ketogenic diet treatment and encourages to future research on this topic.

In the third year of my Bachelor, I did an abroad internship at the White and Yellow Cross Foundation in Saint Martin, where my interest for clinical dietetics originates. During my second internship, at the Erasmus MC, I got in contact with Elles van der Louw, who gave me the chance to write this thesis. I would like to thank her for providing me with feedback, inspiring and challenging me. Furthermore, I would like to thank Sanne Borckink, my supervising lecturer, for coaching me throughout the research process and providing me with feedback. This thesis could not have been written without their help.

I would like to thank all ketogenic diet professionals from SEIN Heemstede and Zwolle, Kempenhaeghe Heeze and Oosterhout, UMC Nijmegen Amalia Children's Hospital, UMC Groningen Beatrix Children's Hospital, UMC Utrecht Wilhelmina Children's Hospital and AMC Amsterdam for taking the time to complete the survey. Your experience has been very valuable to me.

Last but not least, a special thanks to my parents, family, friends and everyone that showed interest during my thesis and provided me with feedback.

This thesis has been a great challenge to me, but I have worked very hard the past weeks to make this a success. I am proud of the final product and hope you enjoy reading it!

Isabel van Ruijven,

The Hague, January 15th 2018

Samenvatting

Introductie: Refractaire epilepsie kan behandeld worden met het Ketogeen dieet (KD). Een van de bijwerkingen van het KD is slechte botgezondheid. Ervaring vanuit het Erasmus MC Sophia Kinderziekenhuis (EMCS) leert dat de status van de daadwerkelijke botdichtheid vaak onbekend is, wat het bepalen van de vitamine D en calcium suppletie-hoeveelheid voor de diëtist lastig maakt. Door de status van botgezondheid te onderzoeken kan vitamine D en calcium suppletie worden aangepast en kunnen er verbeteringen voor het behandelprotocol worden opgesteld. De hoofdvraag van dit onderzoek luidt: *'Wat is de status van botgezondheid in patiënten met refractaire epilepsie welke op lange termijn (>1jaar) worden behandeld met het Ketogeen dieet in de leeftijd 0 tot 18 jaar bij het Erasmus MC Sophia Kinderziekenhuis?'*

Methode: Deze scriptie bestaat uit kwantitatief retrospectief longitudinaal cohort en beschrijvend enquêteonderzoek. De onderzoekspopulatie werd samengesteld uit 136 patiënten in de leeftijd van 0 tot 18 jaar met refractaire epilepsie welke behandeld werden met het KD in het EMCS. Diverse patiëntkarakteristieken en variabelen werden verzameld waaronder de hoeveelheid calciumsuppletie. Voor zover beschikbaar werden botdichtheid scans en SD Z-scores van de gehele onderzoekspopulatie verzameld. Er werd gebruik gemaakt van diverse testen om te onderzoeken of de botdichtheid van de onderzoekspopulatie afweek van de referentiewaarde en of er sprake was van verlies van botdichtheid gedurende behandeling. Daarnaast werd er getest of er een correlatie was tussen patiëntkarakteristieken, variabelen en botdichtheid. Er werd een enquête gehouden onder KD-behandelaren. De enquête was gericht op het verkrijgen van informatie op het gebied van monitoring van botdichtheid en kijk op botgezondheid om zo een evaluatiekader voor het EMCS te creëren.

Resultaten: Botdichtheid was bekend in 39% van de onderzoekspopulatie *(N=18)* welke bestond uit 46 patiënten. De mediaan van botdichtheid was -2 (IQR 1,6) SD Z-score, wat gelijk is aan de referentiewaarde. De mediaan van calciumsuppletie was 36%/ADH (IQR 60). Er was geen significant verlies van botdichtheid gedurende behandeling met het KD, noch werd er een significante correlatie gevonden tussen patiëntkarakteristieken, variabelen en botdichtheid. Wel werd er een trend gevonden in calciumsuppletie (P0,075). Er werden 8 enquêtes ingevuld met een 100% respons. Immobiliteit, gebruik van anti-epileptica, getinte huid, beperkte blootstelling aan zonlicht en behandeling met het Modified Atkins KD werden gescoord als risicofactor voor een verlaagde botdichtheid. De mediaan van calciumsuppletie in andere instituten was 100%/ADH (IQR 94).

Conclusie en discussie: De status van botgezondheid kan worden beschouwd als 'net binnen de waarden'. Wel met een kritische noot, in verband met de grote spreiding (IQR 1,6) en kleine beschikbaarheid van botdichtheid metingen *(N=18)*. Er was geen significant verlies van botdichtheid gedurende behandeling, ondanks dat het onderzoek door Bergqvist *et al.* (2008) dit wel laat zien. Er werd geen significante correlatie gevonden tussen patiëntkarakteristieken, variabelen en botdichtheid, echter zijn er diverse studies die wel een significante correlatie aantonen. Andere instituten gaven tevens diverse risicofactoren voor een lage botdichtheid aan. Wel was er sprake van aanzienlijk lagere calciumsuppletie bij patiënten met een verlaagde botdichtheid (P0,075), tevens was calciumsuppletie aanzienlijk lager dan in andere instituten (36%/ADH versus 100%/ADH).

Abstract

Introduction: Intractable epilepsy can be treated with the Ketogenic diet (KD). One of the side-effects of the KD is poor bone health. Experience from the Erasmus MC Sophia Children's Hospital (EMCS) shows that the status of bone mineral density (BMD) is unknown in many cases. This makes it difficult for the dietitian to ascertain the required amount of vitamin D and calcium supplementation. By investigating the status of bone health, vitamin D and calcium supplementation can be adjusted and suggestions to improve the treatment protocol can be made. The main question of this research is: *'What is the status of bone health in patients with intractable epilepsy treated with the ketogenic diet on a long-term basis (>1 year) aged 0 to 18 years at the Erasmus MC Sophia Children's Hospital?'*

Method: This thesis exists of quantitative retrospective longitudinal cohort and descriptive survey research. The research population was derived from 136 patients aged 0 to 18 years with intractable epilepsy treated with the KD at the EMCS. Multiple patient characteristics and variables were collected among which the amount of calcium supplementation. BMD measurements and SD Z-scores were collected to the extent of availability. Multiple tests were used to research whether BMD of the research population deviated from the reference value and if there was loss of BMD during treatment. Furthermore, it was tested whether there was a significant correlation between patient characteristics, variables and BMD. A survey was held among KD-professionals. The survey focused on information regarding monitoring of BMD and their point of view on bone health to create an evaluation framework for the EMCS.

Results: BMD was known in 39% of the research population *(N=18)* which existed of 46 patients. The median BMD was -2 (IQR 1,6) SD Z-score, which is equal to the reference value. The median calcium supplementation was 36% of the Dutch RDA (ADH). There was no significant loss of BMD during treatment with the KD, neither was there a significant correlation between patient characteristics, variables and BMD. However, there was a trend found in calcium supplementation (P0,075). A total of 8 surveys were completed with a 100% response. Immobility, use of anti-epileptic drugs, darker skin, limited exposure to sunlight and treatment with the Modified Atkins KD were considered risk factors for decreased BMD. The median calcium supplementation in other institutes was 100%/ADH (IQR 94).

Conclusion and discussion: Status of bone health can be considered as 'just in range'. However, this deserves a critical note due to the wide spread (IQR 1,6) and little availability of BMD measurements *(N=18)*. There was no significant loss of BMD during treatment, although the study by Bergqvist *et al*. (2008) does show this. There was no significant correlation found between patient characteristics, variables and BMD, although there are multiple studies that show significant correlation. Other institutes also scored multiple risk factors for decreased BMD. Nevertheless, calcium supplementation was notably lower in patients with decreased BMD (P0,075). Furthermore, calcium supplementation was notably lower than in other institutes (36%/ADH versus 100%/ADH).

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List of abbreviations

25-OHD	25-Hydroxy vitamin D
1,25-OH2D	1,25-Dihydroxy vitamin D
ADH	Dutch recommended daily intake
AED	anti-epileptic drugs
BMD	bone mineral density
BMD-	group with BMD <-2 SD Z-score
BMD+	group with BMD >-2 SD Z-score
DEXA	dual energy x-ray absorptiometry
GLUT-1	glucose transporter-1 deficiency syndrome
IQR	inter quartile range
KD	ketogenic diet
LCT	long chain triglycerides
MCT	multi chain triglycerides
PDHC	pyruvate dehydrogenase complex
T0	at initiation of the KD
T1	after 1 month of treatment with the KD
T2	after 2 months of treatment with the KD
T3	after 3 months of treatment with the KD
T4	after 6 months of treatment with the KD
T5	after 1 year of treatment with the KD
T6	after 2 years of treatment with the KD
T7	after 3 years of treatment with the KD
T8	after 4 years of treatment with the KD
X-Hand	digital automated radiogrammatry of the hand

1. Introduction

Epilepsy affected around 180.000 people in the Netherlands in 2015(1). Every person can experience some form of epilepsy at any time in life, but groups at risk are children, the elderly and people with intellectual disabilities(2). Incidence of epilepsy is five times higher for people with intellectual disabilities, in comparison to people without(3-5). Half of this group arises from those with profound intellectual and multiple disabilities(6).

The word epilepsy is derived from the Greek *epilamambanein* which means to seize, attack(7). Epilepsy is a medical condition which occurs in the form of multiple seizures(8) that are caused by a sudden, temporary disturbance of the electrical stimulus transfer in the brain(9). This disturbance in the brain is usually caused by scar tissue, originated by an earlier condition like an infection, abscess, oxygen deficiency or surgery. Epilepsy can also be the result of a metabolic disease, genetic predisposition or abnormality in the blood vessels(10).

Treatment of epilepsy depends on factors like seizure types, age and gender. However, most treatments start by the use of medications called *anti-epileptic drugs* (AED). These AED can control seizures for 60% of people with epilepsy. If the epilepsy is resistant for three or more AED, we speak of intractable epilepsy. If AED are not sufficient, epilepsy surgery has to be taken into consideration. In case of non-operable intractable epilepsy, treatment with the *ketogenic diet* (KD) may be the solution(11-13).

The KD has come a long way. In the year 1911, two physicians found that starvation could be used as a treatment for epilepsy, resulting in less severe seizures(14). Years later, in 1921, Woodyatt found out that acetone and bèta-hydroxybutyric are present in both starvation and a diet high in fat and low in carbohydrate and protein(15). That same year, Dr. Wilder from the Mayo Clinic suggested that the metabolism of starvation could be obtained by putting the body in to ketosis, using a high in fat and low in carbohydrate and protein diet: the KD was used for the first time(16).

Since the KD is high in fat and low in carbohydrate and protein, the metabolic process changes to ketosis forcing the body to use ketone bodies as the predominant fuel source. The Cochrane systematic review of randomized controlled trials by Levy *et al.* (2012) and multiple other studies showed that this metabolic switch can completely eliminate seizures up to 55% and reduce seizures up to 85%(17-19). The exact mechanism of the KD is not clear. A wide range of suggestions to explain the efficacy have been described but there is no unanimous explanation(20). The KD is suited for both children and adults, although results of the diet are greater for children. This is expected to be due to their still developing brain(11).

There are different types of the KD. The most common diets are the *long chain triglycerides* (LCT) diet - which is considered to be the classic KD - and the *medium chain triglycerides* (MCT) diet. The difference between the LCT and MCT diet is the total fat rate. The classic KD originally exists of 90% energy derived from only LCT, 6% energy derived from protein and 4% energy derived from carbohydrate. MCT emulsions are absorbed more efficiently, leading to an increased ketogenic potential which means that there is less total fat needed for the MCT KD (60% MCT, 11% LCT, 10% protein and 19% carbohydrate)(18). In figure 1, the regular diet, classic KD and MCT KD are presented to give an impression of the big difference between the diets.

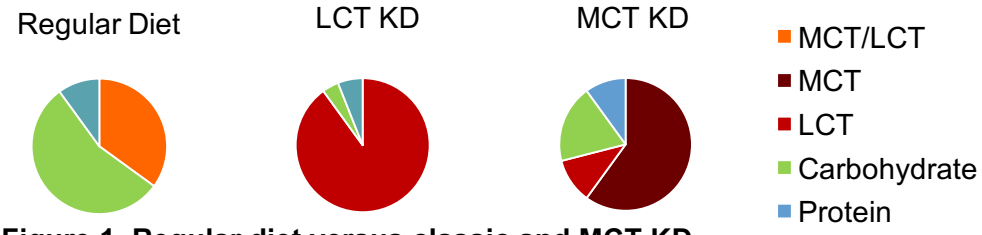

Figure 1. Regular diet versus classic and MCT KD

The diet is usually started in a specialized hospital under the guidance of a nurse, neurologist and a dietitian, who makes a diet plan that is adjusted to the patient's needs. It should be clear whether the diet is effective or not within 3 months. If the KD is found effective, treatment is often continued – if possible – up to two years. In case of epilepsy caused by a metabolic disorder (e.g. *glucose transporter 1 deficiency syndrome* (GLUT-1), *deficiency of pyruvate dehydrogenase complex* (PDHC)), the KD is the only therapy and can therefore be used on a much longer term than two years(18). The diet can even be effective up to six to twelve years(21).

The great benefit of the KD is the reduction of seizures. Unfortunately, the KD is associated with many side-effects as a result of the drastic changed energy metabolism(22). Side effects that were found to correlate with the KD are dehydration, hypoglycaemia(23-25), nephrolithiasis, hyperuricemia(26), hyperlipidaemia(27), weight loss, inadequate growth(28) and gastrointestinal complaints(29). Fractures are also a frequent occurring problem in children treated the KD on a long-term basis(21). Research has shown that 21% of children treated with the KD on a long-term basis develop fractures(30). Two studies showed that children treated with the KD had progressive loss of *bone mineral density* (BMD)(30,31), although poor bone health was already present at baseline. The same research showed that non-ambulatory children were most at risk for poor bone health(30).

Bone health is defined as ''a public health issue with an emphasis on prevention and early intervention to promote strong bones and prevent fractures and their consequences''(32). Determinants that affect bone health are calcium and vitamin D intake and uptake, AED, physical activity and body weight(32). Vitamin D and calcium are the building bricks of our bones. In case of the KD, the vitamin D and calcium metabolism is disturbed. Ketones in the blood lead to acidosis and since our kidneys always aim for homeostasis, they will try to alkalize the blood. Alkalization of the blood is done by withdrawing calcium from the bone, which might result in decreased BMD on the long term(30). As noted before, the incidence of epilepsy is significantly higher in those with profound intellectual and multiple disabilities(6), resulting in a high number of profound intellectual and multiple disabilities in patients treated with the KD. Unfortunately, incidence of decreased BMD is also significantly higher in people with profound intellectual and multiple disabilities(33). Ambulatory skills are often limited in this group(34), which has adverse effects on BMD(32), making them even more at risk.

AED are often used besides the KD. The review of randomized controlled trials by Verrotti *et al.* (2010) showed that some AED have adverse effects on bone density. AED that have adverse effects on bone density are benzodiazepine, carbamazepine, phenytoin, phenobarbital, valproic acid, gabapentin and oxcarbazepine(35). The use of AED, combined with ketosis and profound intellectual and multiple disabilities make this group even more at risk for decreased BMD. Nevertheless, treating BMD during ketosis might be challenging since lowering ketosis might also result in decreased effectiveness of the KD. It is therefore recommended to supplement calcium and vitamin D to individual needs and have periodic *dual energy x-ray absorptiometry* (DEXA) or *digital automated radiogrammatry of the hand* (Hand-X) screening done to monitor bone health(18).

The Erasmus MC Sophia Children's Hospital is a center of expertise concerning treatment with the KD in both children and adults. Experience from practice in the Erasmus MC Sophia Children's Hospital points out that the status of bone health is unknown in many cases, due to missing data. This makes it difficult for the dietitian to ascertain the required amount of vitamin D and calcium supplementation, whilst these vitamins and minerals play an important role in bone metabolism. The above findings raise interest to evaluate the actual status of bone health in children with intractable epilepsy treated with the KD on a long-term basis, since they are at high risk for poor bone health. By investigating the status of bone health in this complex patient group and the extent to which monitoring is done (looking at improvement), vitamin D and calcium supplementation can be adjusted. Thereby, suggestions to improve the international treatment protocol for children treated the ketogenic diet can be made and – hopefully – fractures can be prevented.

1.1. Research questions

The main question of this research is: *'What is the status of bone health in patients with intractable epilepsy treated with the ketogenic diet on a long-term basis (>1 year) aged 0 to 18 years at the Erasmus MC Sophia Children's Hospital?'*

Sub questions that helped get the answer to the main question are:

I. *What is the status of bone mineral density in the research population?*
II. *What is the course of bone mineral density during treatment with the ketogenic diet?*
III. *What is the daily supplementation of vitamin D and calcium in the research population?*
IV. *What are vitamin D and calcium serum levels in the research population?*
V. *Is the level of ketosis a determinant for bone mineral density?*
VI. *What are the characteristics of patients with decreased bone mineral density?*
VII. *How well is monitoring done in comparison to other institutes?*

Reader's guide

Sub questions I to VI were investigated in a quantitative cohort research. Sub question VII was investigated in a quantitative survey research. Distinction between these research will be made in the following chapters.

2. Methods for cohort research

Data for the cohort research was collected from September to November 2017 at the Erasmus MC Sophia Children's Hospital. An anonymized data set is available on request, with permission of Elles van der Louw, but may not leave the Erasmus MC Sophia Children's Hospital. Full output of all performed tests is available on request.

To gain more insight in the treatment and thereby the variables that need to be collected, the following paragraph describes the monitoring of bone health affecting determinants – discussed in the introduction – according to the protocol and experience from practice at the Erasmus MC Sophia Children's Hospital.

2.1. Treatment according to protocol(12)

Screening on BMD should be done at baseline in case of risk factors (profound intellectual and multiple disabilities, immobility and some AED), once every 2 years and more often in case of risk factors or spontaneous fractures. Methods to be used are DEXA or Hand-X. Decreased BMD is defined as >-2 *standard deviation* (SD) Z-score. Treatment in case of decreased BMD is noted as analysis of vitamins and minerals in the diet and supplementation in case of deficiencies, advice on physical activity (if possible) and sunlight exposure. At the Erasmus MC Sophia Children's Hospital, BMD measurements were done cross-sectional in some children treated with the KD next to the protocol around January 2016, which could have affected the results.

The oral version of the KD lacks vitamin D and calcium due to product restriction, although ketogenic specific dietary foods like formula and tube-feeding (e.g. KetoCal) contains added vitamins and minerals. At the Erasmus MC Sophia Children's Hospital, supplementation of calcium and vitamin D is given in the form of Fruitivits – a low-in-carbohydrate vitamin, mineral and trace elements supplement –, Davitamon totaal, since this is also a low-in-carbohydrate supplement, and Davitamon oil-based vitamin D drops.

Supplementation of vitamin D and calcium is adjusted after reviewing the diet. The required amount of vitamin D and calcium is described as 'sufficient', but no amount is given according to protocol. Neither is there information available on the amount of supplementation for those with profound intellectual and multiple disabilities.

No information on monitoring of vitamin D (*25-Hydroxy vitamin D* (25-OHD) *and 1,25-Dihydroxy vitamin D* (1,25-OH2D)) serum levels is described. Calcium serum levels need to be reviewed at baseline, after 3 months, once or twice a year and more often if indicated.

Ketosis is measured in the blood. During the fine-tuning period, ketosis should be measured 3 times a week and more often if indicated. When the ketosis is stabilized, it should be measured once a month and more often if indicated. The normal range for ketosis (during treatment with KD) in the blood is 2,5-6,5 mmol/L.

At the Erasmus MC Sophia Children's Hospital, regular check-ups take place *at initiation of treatment with the KD* (T0), *after 1 month* (T1), *after 2 months* (T2), *after 3 months* (T3), *after 6 months* (T4), *after 1 year* (T5), *after 2 years* (T6), *after 3 years* (T7), *after 4 years* (T8) of treatment with the KD – and so on.

2.2. Research population

The research population was derived from a group of 136 patients with intractable epilepsy in the range 0 to 18 years treated with the ketogenic diet at the Erasmus MC Sophia Children's Hospital. Patients who started the diet before 2008 were excluded, due to lack of information in the patient file in the absence of a structured KD protocol. Furthermore, patients were excluded if there was no information available regarding BMD, calcium serum levels/intake or vitamin D serum levels/intake. This was due to too little available information.

2.3. Data collection

The following patient characteristics were collected: gender, date of birth, ethnicity (on account of vitamin D supplementation), mobility scored by using the Gross Motor Function Classification Scale (36), level of mental retardation scored by DSM IV (37), underlying cause of the epilepsy, presence of an epilepsy syndrome and presence of glucose transporter-1 deficiency syndrome or Rett(-like) syndrome (since they are already at risk for decreased BMD (38)). The above characteristics were collected to give an impression of the research population. It was also registered when a patient was deceased, since this can clarify any missing data.

The start and stop date of the KD was registered to evaluate the total time of treatment with the KD. In case of ongoing treatment, the total time of treatment until December 1st 2017 was registered.

The total number of AED used at initiation of the KD was gathered to give insight in the severity of the epilepsy. The use of BMD decreasing AED (benzodiazepine, carbamazepine, phenytoin, phenobarbital, valproic acid, gabapentin and oxcarbazepine) prior to or during treatment with the KD was also gathered.

Growth during treatment with the KD was monitored by gathering the height for age SD Z-score and weight for height SD Z-score. Growth was monitored at T0, T3, T4, T5, T6, T7 and T8. All growth data was scored by the use of growth curves made available by the World Health Organization and TNO in 2010(39).

It was not achievable to collect the exact intake of vitamin D and calcium because the nutritional composition of ketogenic specific dietary foods like formula and tube-feeding have changed throughout the years. Therefore, computations with the current nutritional values would not be reliable. Supplementary intake of vitamin D and calcium of T0, T3, T4, T5, T6, T7 and T8 was registered down in % of the *Dutch recommended daily intake* (ADH)(40),(41).

The ADH was used since there are no specific recommendations given in the protocol. The ADH of vitamin D for all healthy Dutch children in the age 0-3 is 10 micrograms. From the age 4-49 the recommended daily intake is 10 micrograms for everyone with a darker skin or those that are not often exposed to the sun (40), which is often the case in this population. The recommended daily intake of calcium for healthy Dutch children is presented in table 1.

Table 1. Recommended daily intake of calcium for healthy Dutch children(41)

	Age	Calcium (mg)
	1 - 3 years	500
	4 - 8 years	700
Female	9 - 18 years	1100
Male	9 - 18 years	1200

To complete the overview of total intake of vitamins and minerals the way of feeding was registered and divided in ‘orally’, ‘partially tube/bottle’ and ‘fully tube/bottle’.

Serum levels of 25-OHD (ng/mL) and 1,25-OH2D (pg/mL) were gathered to the extent of availability, including the total time on diet in months at the moment of measurement. The 25-OHD reflects the body’s vitamin D stores and 1,25-OH2D is the most powerful vitamin D metabolite, stimulating calcium absorption in the intestine(42). The 25-OHD serum levels are found to be in range between 20-100 ng/mL and 1,25-OH2D serum levels between 24-86 pg/mL(43). Serum levels were scored as ‘too low’, ‘in range’, or ‘too high’.

Serum levels of calcium (mmol/L) were gathered to the extent of availability, including the date of measurement and the total time on diet in months at the moment of measurement with a maximum of 3 measurements. Calcium serum levels are found to be in range between 2,1-2,6 mmol/L(43). Serum levels were scored as ‘too low’, ‘in range’, or ‘too high’.

To investigate the influence of ketosis on BMD, ketone levels in the blood were gathered in exact numbers at T1, T2, T3, T4, T5, T6, T7 and T8.

BMD measurements were gathered to the extent of availability. The date of the measurement and the total time on diet in months at the moment of measurement was gathered so that the course of BMD during treatment could be evaluated. The way of measurement was registered, divided in DEXA-scans and Hand-X. BMD was scored by exact SD Z-scores using boneXpert software.

Data was collected from the electronic patient record file (HiX) and registered down in a case record form (appendix I) and IBM SPSS 20. Missing data was registered down as 999. If the diet ended before the moment of measuring, this was registered as 998. If a patient passed away before the moment of measuring, this was registered as 997. Missing data in growth data due to the wrong age/measurement ratio was registered as 996. Missing data in BMD measurements due to a, for the boneXpert software, non-readable scan was registered as 995. A summary of all collected data and their variables is presented in appendix II.

2.4. Data processing

Data was analysed by the use of IBM SPSS 20. Graphs and tables were made by the use of Microsoft Word. The distribution of all used data was tested by the use of the Shapiro-Wilk test, which is best suitable for a research population under 50 patients(44). Skewness and kurtosis below 1,96 in the Shapiro-Wilk test was considered as a normal distribution(44). In case of nominal or ratio-level variables with no normal distribution, medians *and inter quartile range* (IQR) were used(44). All descriptive statistics of characteristics and variables of the total cohort were first presented in a table.

Specific variables are presented mainly descriptive Analysis with the One Sample Wilcoxon Signed Rank Test was used to test if there was a significant difference between the BMD SD Z-score of the research population and the reference value. The One-Sample Wilcoxon Signed Rank Test was also used to investigate whether there was a significant difference between patients with a <-2 BMD SD Z-score, >-2 BMD SD Z-score and the reference value.

The course of BMD was showed by using a line graph. Analysis with the Two Sample Wilcoxon Signed Rank Test was used to test if there was a significant difference between the 1st, 2nd, 3rd, 4th and 5th BMD measurement.

Since ketogenic specific dietary foods like formula and tube-feeding contain added vitamins and minerals, the correlation between the way of feeding (oral, partially tube/bottle, fully tube/bottle), 50%/ADH/100%/ADH of vitamin D and 50%/ADH/100%/ADH of calcium supplementation was tested with the Chi Square Test. This test was done to check for adequate supplementation between the different ways of feeding. Since there is no specific recommendation for this specific group it was evaluated whether 50% or 100%/ADH was supplemented. Significant results were presented in a table.

The cohort was then divided in a subgroups BMD <-2 SD (BMD-) and BMD >-2 SD (BMD+). Variables were reviewed per group and presented in a table. Significant differences between both groups and variables on ratio level were tested with the non-parametric Mann-Whitney Test in case of no normal distribution. Calcium supplementation of each group was then presented by using a boxplot. Analysis with the Chi Square Test was used to test differences between both groups and variables on nominal and ordinal level.

A p-value <0,05 was considered as ‘significant’. The Fisher’s Exact Test was used in case of analysis with the Chi Square test in a group <5 patients. It is important to notice that the gathered data, especially in different T’s, may be derived from different patients. Due to missing data, patients ‘enter’ and ‘leave’ the research population in some longitudinal collected data. This was registered in the results as ‘*missing*’.

2.5. Quality aspects

This research has been designed in close collaboration with the commissioning company, Elles van der Louw, to ensure the usability for practice. Thereby, there has never been research done on BMD in children treated the KD in the Netherlands, which makes a first evaluation very valuable.

Validity of this research was increased by peer-examination done by a co-author. Measurements of BMD were scored by the use of boneXpert software, which is validated for research(45).

The co-author also randomly verified data after collection of 10 patients was done and after 40 patients to increase the reliability. Thereby, all SPSS tests were performed twice to ensure the right outcomes.

Data was collected at the Erasmus MC Sophia Children's Hospital and saved on the secured V-disk of the dietetic department. Data analysis was done at multiple locations by using a anonymized data set. All filled out case record forms were and will be kept in a closed closet at the dietetic department office location Sophia. A declaration of no objection was issued by the Erasmus MC Medical Examination Board.

Reader's guide

Results of the cohort research will be presented in chapter 4.

3. Methods for survey

A survey was held to map monitoring of bone health in other institutes, gain understanding in their point of view on bone health and to create an evaluation framework for the results of this research. The survey was conducted from October to December 2017. An anonymized document of all completed surveys is available on request with the permission of Elles van der Louw, but may not leave the Erasmus MC Sophia Children's Hospital.

3.1. Research population

The research population was derived from professionals (dietitians, nurses and neurologists) active in treatment with the KD in all 9 KD-offering hospitals and expertise centres in the Netherlands, which are:

- Erasmus MC Sophia Children's Hospital
- UMC Groningen Beatrix Children's Hospital
- UMC Nijmegen Amalia Children's Hospital
- UMC Utrecht Wilhelmina Children's Hospital
- AMC Amsterdam
- Stichting Epilepsie Instellingen Nederland (SEIN) Zwolle
- Stichting Epilepsie Instellingen Nederland (SEIN) Heemstede
- Academisch Centrum voor Epileptologie Kempenhaeghe Maarheeze
- Academisch Centrum voor Epileptologie Kempenhaeghe Oosterhout

Participants were recruited by e-mail.

3.2. Data collection

The questions were based on the topics presented in table 2. The complete survey, , can be found in appendix III with open multiple choice questions formulated in Dutch language. The survey was spread out by the use of www.enquetesmaken.nl and e-mail.

Table 2. Topics questioned in survey

First- and last name, Profession and institution
Total number of patients treated with the ketogenic diet
Patients who are most at risk to their opinion/experience
Preventive vitamin and/or mineral intake
Measurement of BMD (how, when, practicability)
Cut-off value for decreased BMD
Additional research and treatment in case of decreased BMD

3.3. Data processing

An overview of all given answers was created in Microsoft Excel. Graphs and tables were then created with Microsoft Word. A bar chart was created with the characteristics of high-at-risk patients, vitamin D and calcium supplementation and the BMD SD Z-score that was defined as 'decreased'. Moments of BMD measurements and treatment in case of decreased BMD were presented in a table.

3.4. Quality aspects

By obtaining information from other institutes, the recommendations in this thesis were thereby placed in perspective which increased the external validity and usability. All participants independently completed the survey which prevents influencing of answers and improved the validity of this research.

Reader's guide

Results of the survey will be presented in chapter 5.

4. Results for cohort research

A total of 46 patients complied with the in- and exclusion criteria. The inclusion process is presented in figure 2.

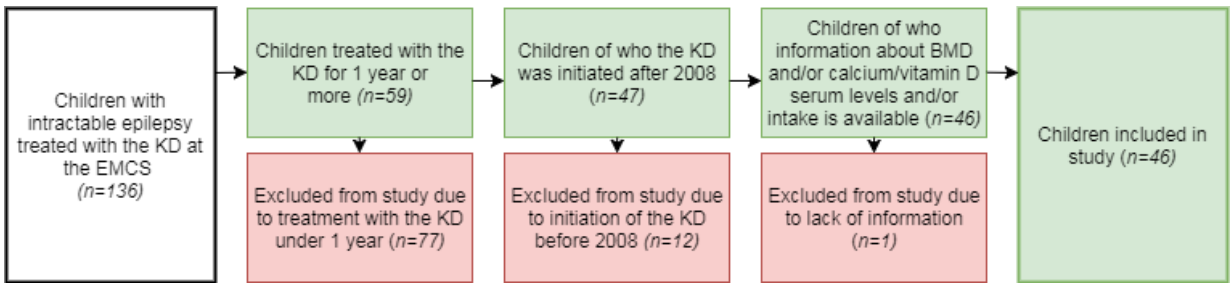

Figure 2. In- and exclusion flowchart

The research population existed of 39% female and 61% male patients. Median age at initiation of the KD was 4 (IQR 6). All patient characteristics and variables for the total cohort that were tested are presented in table 3. The main results will be described in the following text.

Table 3. Characteristics and variables of the total cohort

N		46
Female/male	%	39/61
Age at initiation of KD	Median (IQR)	4 (6)
Ethnicity	Afro-American /other (%)	2/98
Level of mental retardation	Prevalence	
None		2
Low ability (IQ 70-90)		6
Mild mental retardation (IQ 50-70)		6
Moderate mental retardation (IQ 35-50)		5
Severe and profound mental retardation (IQ<35)		27
Gross Motor Function Classification Scale	Prevalence	
None		3
I. Limitation of gross motor function only		13
II. Walking without tools, more difficult outdoors		3
III. Walking with tools		4
IV. Self-propelling with tools		6
V. Not self-propelling		17
Height for age SD Z-score	Median (IQR)	-0,8 (2,1)
Height for weight SD Z-score	Median (IQR)	-0,1 (2,3)
Etiology of disorder	Prevalence	
Genetic		5
Structural/metabolic		15
Unknown cause		24
Electronical syndromes	Prevalence	
Non-syndromic		29
Lennox-Gastaut		2
Dravet		1
West		9
ESES/CSWS		1
Otahara		1
Doose		2
Myoclonic epilepsy in infancy		1
GLUT-1	No/yes	44/2
Rett(-like) syndrome	No/yes	44/2
Number of AED at initiation of KD	Median (IQR)	3 (1)

Use of BMD decreasing AED during KD or in history	No/yes (%/cohort)	31/15 (32,6%)
Benzodiazepine		31/15 (32,6%)
Carbamazepine		40/6 (13%)
Phenytoin		34/12 (26,1%)
Phenobarbital		6/40 (87%)
Valproic acid		100/0 (0%)
Gabapentin		38/8 (17,4%)
Oxcarbazepine		
Number of BMD decreasing AED at initiation of KD	Median (IQR)	2 (2)
Time on KD in months	Median (IQR)	27 (46)
Way of feeding	Prevalence	
Oral		21
Partially tube/bottle		8
Fully tube/bottle		17
BMD SD Z-score *(N=18, 28 missing)*	Median (IQR)	-2 (1,6)
Vitamin D supplementation (%/ADH) *(N=44, 2 missing)*	Median (IQR)	45,7 (37)
Calcium supplementation (%/ADH *(N=45, 1 missing)*	Median (IQR)	36 (60)
25-OHD serum levels *(N=12, 34 missing)*	Prevalence	
Too low *(<20 ng/mL)*		7
In range *(20-100 ng/mL)*		5
Too high *(>100 ng/mL)*		0
1,25-OH2D serum levels *(N=8, 38 missing)*	Prevalence	
Too low *(<24 pg/mL)*		1
In range *(24-86 pg/mL)*		4
Too high *(>86 pg/mL)*		3
Calcium serum levels *(N=44, 2 missing)*	Prevalence	
Too low *(<2,1 mmol/L)*		0
In range *(2,1-2,6 mmol/L)*		44
Too high *(>2,6 mmol/L)*		0
Ketosis serum levels (mmol/L) *(N=46)*	Median (IQR)	3,8 (1,7)

BMD status

BMD was measured in 48% *(N=22, 24 missing)* of the research population. Four of these measurements were non-readable for the BMD-measuring software 'BoneXpert' due to a deviating position of the hand, resulting in a known BMD in 39% *(N=18, 28 missing)* of the research population. DEXA-scan was performed in 2% of the measurements, Hand-X in 98% of the measurements.

Median BMD SD Z-score is -2 (IQR 1,6). The One-Sample Wilcoxon Signed Rank Test showed no significant difference with the reference value (P0,916). BMD below -2 SD Z-score was found in 5 patients. A BMD above -2 SD Z-score was found in 13 patients. Analysis with the One-Sample Wilcoxon Signed Rank Test showed a significant difference between the group with BMD SD Z-score below -2 (P0,042) as well as the group with BMD SD Z-score above -2 (P0,012) and the reference value.

BMD course

Multiple BMD measurements were done in 13 patients. Two patients had non-readable scans, resulting in known BMD in 11 patients. Decreasing BMD during the course of treatment with the KD was seen in 5 patients. Increasing BMD during the course of treatment with the KD was seen in 6 patients. The course of BMD during treatment per patient is presented in figure 3. A table with exact numbers is presented in appendix IV.

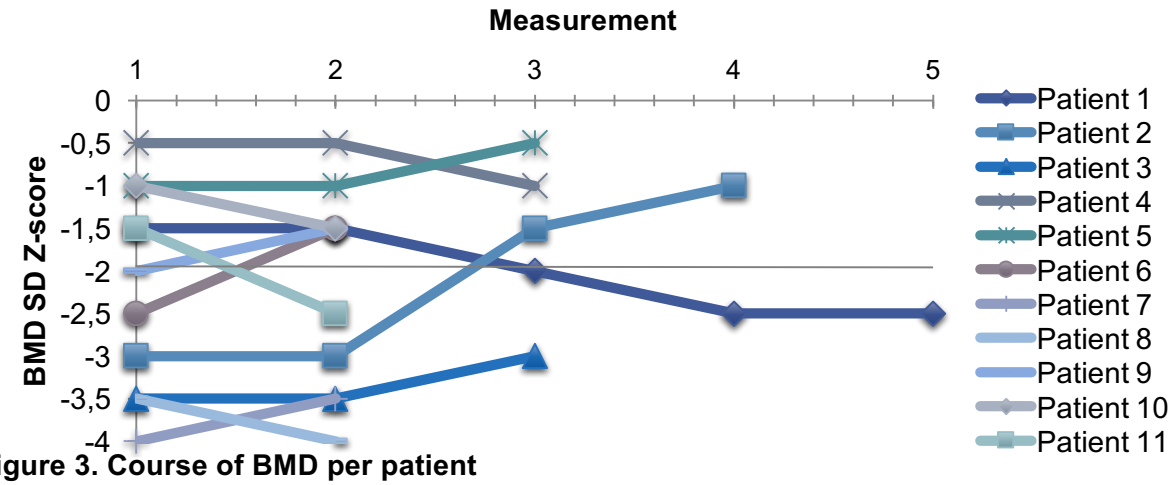

Figure 3. Course of BMD per patient

The Two-Related Samples Wilcoxon Signed Rank Test showed no significant difference between the 1st and 2nd (P1,000), the 1st and 3rd (P0,705) or the 1st and 4th (P0,655) available measurements.

Way of feeding

The cohort was then divided in 3 subgroups, based on the way of feeding (oral *(N=21)*, partially tube/bottle *(N=8)* and fully tube/bottle *(N=17)*). The Chi Square Test showed a significant correlation between the way of feeding and 50%/ADH calcium supplementation at T4, T6, T7 and T8, which is presented in table 4.

Table 4. P-Values of the Chi Square Test for way of feeding and 50%/ADH calcium supplementation

T0	0,164
T3	0,061
T4	0,046
T5	0,065
T6	0,003
T7	0,045
T8	0,045

Correlation between way of feeding and 100%/ADH calcium supplementation, 50%/ADH and 100%/ADH vitamin D supplementation was tested as well but showed no significant results. Results are presented in appendix V.

BMD- and BMD+ group

The cohort was then divided in 2 subgroups, based on BMD SD Z-score. BMD- group *(N=5)* has a BMD SD Z-score below -2. BMD+ group *(N=13)* has a BMD SD Z-score above -2. All characteristics and variables per group are presented in table 5.

Table 5. Characteristics, variables and P-values for Mann-Whitney and Chi Square test of BMD- and BMD+ group

		BMD-group	BMD+ group	P-value
N		5	13	
BMD SD Z-score	Median (IQR)	-3,3 (1)	-1,8 (0,8)	
Female/male	%	40/60	69/31	0,272[2]
Age at initiation of the KD	Median (IQR)	2 (5)	5 (8)	0,208[1]
Ethnicity	Afro-American /other (%)	0/100	0/100	1,000[2]
Level of mental retardation	Prevalence			0,575[2]
None		0	0	
Low ability (IQ 70-90)		0	2	
Mild mental retardation (IQ 50-70)		0	2	
Moderate mental retardation (IQ 35-50)		1	2	
Severe and profound mental retardation (IQ<35)		4	7	
Gross Motor Function Classification Scale	Prevalence			0,207[2]
None		0	0	
I. Limitation of gross motor function only		0	5	
II. Walking without tools		1	0	
III. Walking with tools		0	1	
IV. Self-propelling with tools		2	2	
V. Not self-propelling		2	5	
Height for age SD Z-score	Median (IQR)	-0,4 (2,7)	-1,1 (2,1)	0,387[1]
Weight for height SD Z-score	Median (IQR)	-0,5 (1,6)	-0,1 (2,1)	0,208[1]
Etiology of disorder	Prevalence			0,758[2]
Genetic		0	1	
Structural/metabolic		2	6	
Unknown cause		3	6	
Electronical syndromes	Prevalence			0,107[2]
Non-syndromic		2	9	
Lennox-Gastaut		0	2	
Dravet		0	0	
West		3	1	
ESES/CSWS		0	1	
Otahara		0	0	
Doose		0	0	

Myoclonic epilepsy in infancy		0	0	
GLUT-1	No/yes	5/0	12/1	0,722[2]
Rett(-like) syndrome	No/yes	5/0	13/0	1,000[2]
Number of AED at initiation of KD	Median (IQR)	3 (1)	2 (1)	0,443[1]
Use of BMD decreasing AED during KD or in history	No/yes (%/group)			
Benzodiazepine		3/2 (40%)	10/3 (30%)	0,433[2]
Carbamazepine		5/0 (0%)	8/5 (38%)	0,150[2]
Phenytoin		5/0 (0%)	13/0 (0%)	1,000[2]
Phenobarbital		3/2 (40%)	11/2 (15%)	0,299[2]
Valproic acid		0/5 (100%)	2/11 (85%)	0,510[2]
Gabapentin		5/0 (0%)	13/0 (0%)	1,000[2]
Oxcarbazepine		4/1 (20%)	12/1 (8%)	0,490[2]
Number of BMD decreasing AED at initiation of KD	Median (IQR)	2 (1)	1 (2)	0,336[1]
Time on KD in months	Median (IQR)	63 (36)	57 (48)	1,000[1]
Way of feeding	Prevalence			0,121[2]
Oral		1	8	
Partially tube/bottle		1	0	
Fully tube/bottle		3	5	
Vitamin D supplementation (%/ADH)	Median (IQR)	43 (34)	50 (44)	0,624[1]
Calcium supplementation (%/ADH)	Median (IQR)	10 (35)	44 (60)	0,075[1]
25-OHD serum levels	Prevalence			0,500[2]
Too low *(<20 ng/mL)*		1	3	
In range *(20-100 ng/mL)*		2	2	
Too high *(>100 ng/mL)*		0	0	
1,25-OH2D serum levels	Prevalence			0,167[2]
Too low *(<24 pg/mL)*		0	0	
In range *(24-86 pg/mL)*		2	0	
Too high *(>86 pg/mL)*		0	2	
Calcium serum levels				1,000[2]
Too low *(<2,1 mmol/L)*		0	0	
In range *(2,1-2,6 mmol/L)*		4	13	
Too high *(>2,6 mmol/L)*		0	0	
Ketosis serum levels (mmol/L)	Median (IQR)	4,1 (1,2)	3,7 (1,3)	0,387[1]

[1] *P-value obtained by using the Mann-Whitney test*
[2] *P-value obtained by using the Chi Square test*

Analysis with the Chi Square test showed no significant difference in variables on nominal or ordinal level between the BMD- and BMD+ group, which is presented in table 5.

Analysis with the Mann-Whitney U test showed no significant difference in variables on ratio level between the BMD- and BMD+ group, which is presented in table 5. A trend (P0,075) was seen on calcium supplementation (%/ADH), which is also presented in figure 3.

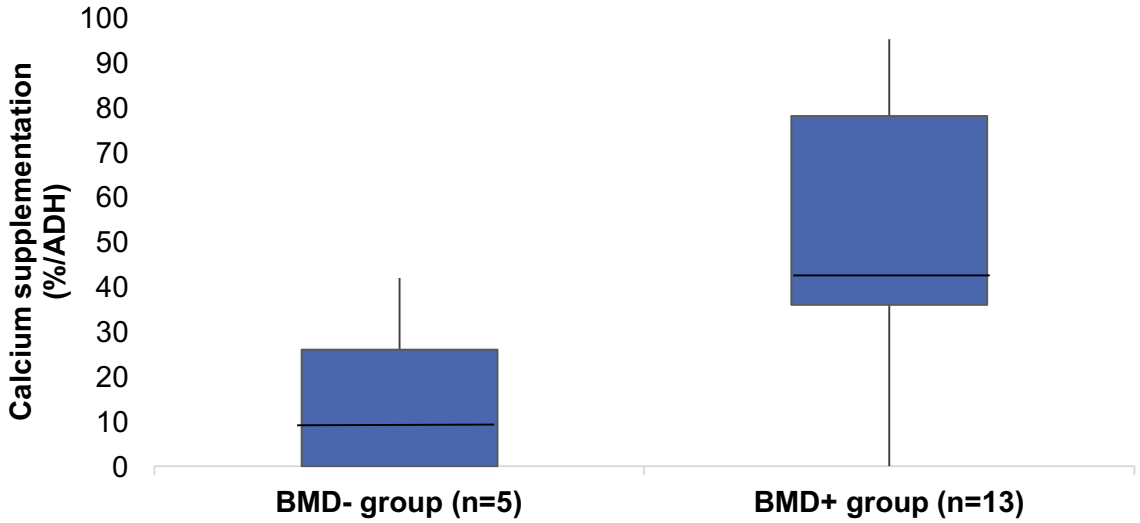

Figure 3. Calcium supplementation in %/ADH of BMD- and BMD+ group

5. Results for survey

Two institutes completed the survey together, resulting in a total of 8 completed surveys with a 100% response rate. The surveys were completed by (combined) 9 dietitians, 2 nursing specialists and 2 paediatric neurologists. The median number of children treated with the KD at all institutes until now was 60 (IQR 165).

Participants were asked to name characteristics of patients that were, in their opinion, high at risk for decreased BMD. One participant stated that patients treated with the Modified Atkins KD were considered 'high at risk', because they are often older, therefore make their own food-related choices and regret taking multivitamins, which contain calcium. All answers are presented in figure 4.

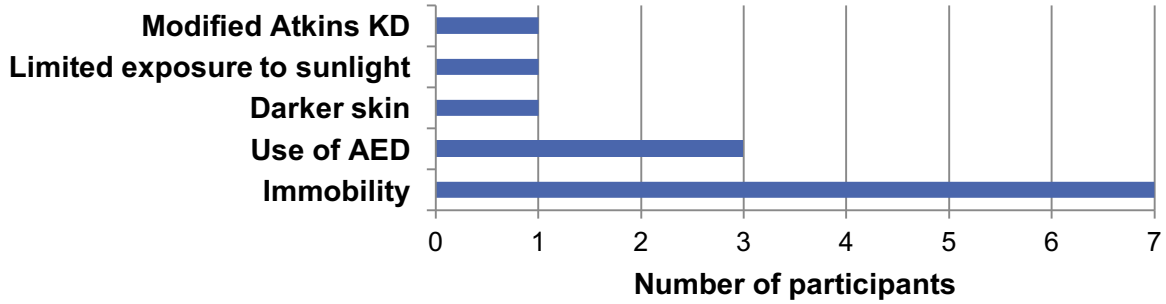

Figure 4. Characteristics of patients that were found high at risk for decreased BMD

'Decreased BMD is not very common within 'walking' children but it is very common for those that are wheelchair dependent.'

- **Quote Participant 8**

Participants were asked whether high-at-risk patients received a preventive modified vitamin- and mineral intake. The median vitamin D supplementation is 113%/ADH (IQR 50). The median calcium supplementation is 100%/ADH (IQR 94). One participant did not answer the question, resulting in 7 useful answers. One participant stated that supplementation was based on the status of vitamin D and calcium serum levels and another participant stated that it is risky to supplement calcium due to high acidity in the blood resulting in kidney stones. Figure 5 shows an overview of all given answers.

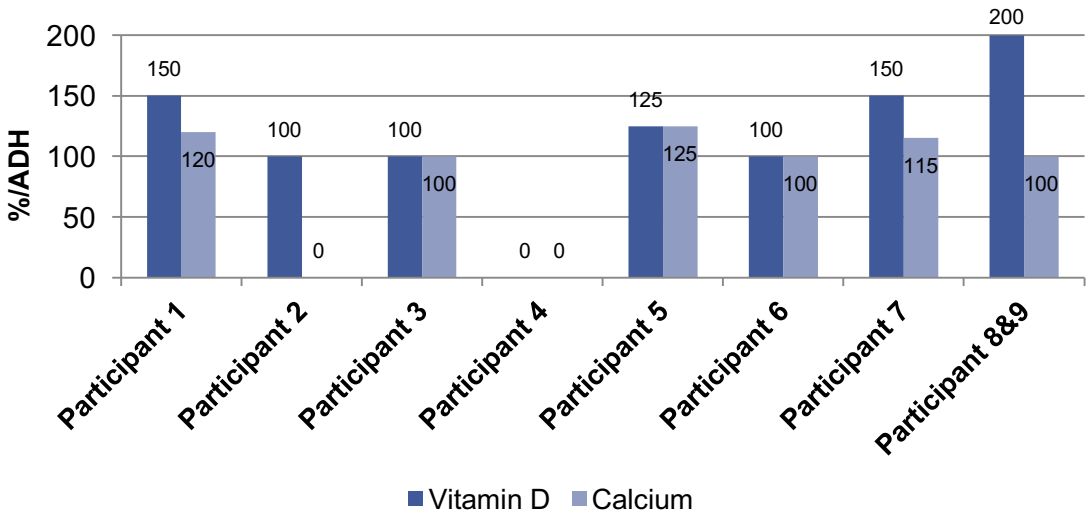

Figure 5. Given vitamin- and mineral intake

One institution did not measure BMD at all, but no reason was given. Two-yearly BMD measurements were done in 5 of 8 institutes. All moments of BMD measurements per institute are presented in table 6. DEXA was used in 6 institutes, Hand-X was used in 1 institute.

Table 6. Moments of BMD measurements

Participant	1	2	3	4	5	6	7	8&9
Unknown						X		
At baseline							X	
Every year								
Every two year		X	X		X		X	X
When indicated		X		X	X			

'According to protocol, BMD measurements should be done at initiation of the KD, but this does not apply in practice. First measurement is done when children are 2 years treated with the KD.'

- **Quote Participant 4**

Participants were asked at which SD Z-score they state that there is actual decrease in BMD. Four participants could not answer the question because they were not familiar with SD Z-scores for BMD. These are scored as 'unknown'. All answers are presented in figure 6.

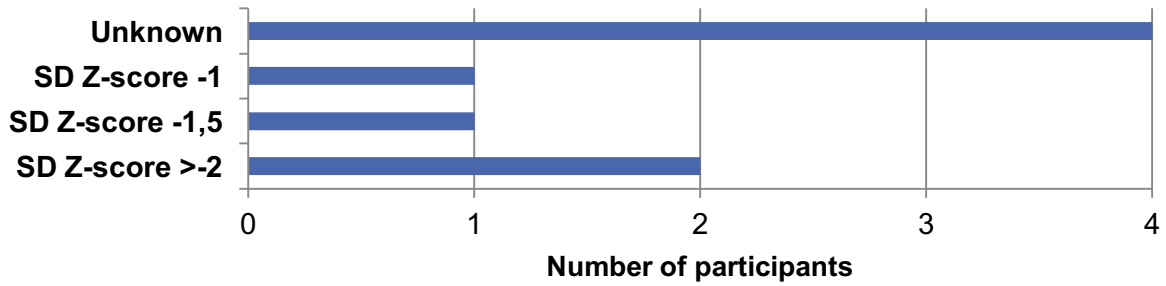

Figure 6. Rating of decreased BMD by SD Z-score

Participants were asked what treatment they applied in case a patient has decreased BMD. One participant did not change the treatment in response to decreased BMD. All applied treatments are presented in table 7.

Table 7. Treatment in case of decreased BMD

Participant	1	2	3	4	5	6	7	8&9
None	X							
Depending on the cause		X						
Evaluation of vitamin D and calcium			X	X	X	X	X	X
Pamidronate					X		X	

6. Conclusion, discussion and recommendations for future research

The status of bone health in patients with intractable epilepsy treated with the KD on a long-term basis (>1 year) aged 0 to 18 years at the Erasmus MC Sophia Children's Hospital can be considered as 'just in range' with a BMD measured at a median -2 SD Z-score (IQR 1,6), which is equal to the reference value. However, this deserves a critical note due to both the wide spread and little availability of BMD measurements *(N=18)*. Decreased BMD (>-2) was found in 28% of patients.

This research started as a retrospective longitudinal cohort research. Upfront, it was expected that there would be missing data regarding the retrospective character of the research. Indeed, this turned out to be the case, which unfortunately lowered the usability of this research. Although there is missing data, the Erasmus MC Sophia Children's Hospital has one of the largest population of children treated with the KD in the Netherlands. Therefore, this might be, with the currently available information, a suitable population to evaluate the bone health of children treated with the KD in the Netherlands. By repeating this research in a prospective way with pro-active collection of data, the liability will be increased.

There were no significant differences in consecutive BMD measurements which states that there is no loss of BMD during treatment with the KD, although the study by Bergqvist *et al*. (2008) showed a progressive loss of BMD during the KD in a population of 25 patients. The course of BMD in this research was known in only 11 patients and there was no measurement done at baseline, in opposition to the study by Bergqvist *et al*. (2008), which may explain the difference in outcomes(30). Furthermore, DEXA scans were used in the study by Bergqvist *et al*. (2008)(30). This is in contrast with the vast majority of Hand-X measurements in this research, which might have affected outcomes, although Hand-X is found to be a more precise technique(46), especially in children with profound intellectual and multiple disabilities(47).

The median vitamin D supplementation of the cohort was 45,7%/ADH (IQR 37). Vitamin D supplementation in BMD- group was 42,5%/ADH (IQR 34) and 50%/ADH (IQR 44) in BMD+ group. There was no significant difference between the vitamin D and calcium supplementation of both groups (P0,624). either a significant correlation between the way of feeding and vitamin D supplementation. Median vitamin D supplementation in other institutes is 113%/ADH (IQR 50). This is remarkably higher than vitamin D supplementation in the research population.

The median calcium supplementation of the cohort is 36%/ADH (IQR 60). Calcium supplementation in BMD- group was 10%/ADH (IQR 35) and 44%/ADH (IQR 60) in BMD+ group. Although no significant difference (P0,075) was found between the calcium supplementation of BMD- and BMD+ group, calcium was notable lower in the BMD- group. The wide range indicates calcium supplementation might be inadequate. However, tube feeding and formula contain added vitamins and minerals which were not included into the calculation. Therefore, the correlation between the way of feeding and calcium supplementation was tested. The Chi Square Test showed a significant correlation in 4 of 8 T's (P0,46, P0,003, P0,045 and P,045) and a trend in 2 of 8 T's (P0,061 and P0,065) at 50%/ADH. This suggests that calcium supplementation is not inadequate but adjusted for the way of feeding and therefore widely spread. As previously indicated, the consistency and thereby added vitamins and minerals of tube feeding and formula has been changed throughout the years. By performing a prospective research in the future, the exact intake of vitamin D and calcium can be evaluated. The median calcium supplementation in other institutes is 100%/ADH (IQR 94). This is remarkably higher than calcium supplementation in the research population.

Vitamin D and calcium supplementation were compared to the guidelines for healthy Dutch children since concrete recommendations for this specific group is missing. The Zorgpad Ketogeen Dieet (2015) advises to supplement 'sufficient' minerals and vitamins but no amount is given(12). The werkboek 'Zorg voor kinderen met een ernstige meervoudige beperking' (2016) advises to supplement 10-20 mcg of vitamin D in case of decreased BMD, and 'sufficient' calcium, but again, no amount was given(48). Future research should focus on evaluation of the effect of different levels of supplementation to make it possible for dietitians to tailor supplementation of calcium and vitamin D in this group.

Calcium serum levels were in range in all, to the extent of availability *(N=44)* patients. Serum levels of 25-OHD serum levels were known in 12 patients of who 7 had too low and 5 had in range levels. However, there was no difference (P0,500) between BMD- and BMD+ group. Tests showed no correlation between too low 25-OHD levels and low vitamin D supplementation. It will be of great value to further research the underlying cause of too low 25-OHD serum levels. The review of randomized controlled trials by Christakos *et al*. (2011) shows that vitamin D increases intestinal calcium absorption(49). By attaining a healthy 25-OHD serum level, calcium might be absorbed more efficiently leading to improved BMD. Serum levels of 1,25-OH2D were known in 8 patients of who 1 had too low, 4 had in range and 3 had too high levels.

Tests showed no correlation between hyper ketosis and BMD (P0,387).

This study showed no characteristics with a significant correlation to decreased BMD. This is remarkable, since the study by Babayigit *et al*. (2006), case-controlled study by Stephen *et al*. (1999), meta-analysis by Zhang *et al*. (2015) and multiple other studies confirm the correlation between particular AED and decreased BMD(50-52). This was also confirmed by 3 of 8 institutes. Likewise, both the study by Finbråten *et al*. (2015) and Shin *et al*. (2017) confirm the correlation between a lower ambulatory status and decreased BMD(53,54). Furthermore, 7 of 8 institutes stated that immobility is a characteristic of those with increased risk for decreased BMD. Darker skin, limited exposure to sunlight and the Modified Atkins KD were scored as characteristics of those with increased risk for decreased BMD by only 1 institute. The correlation of darker skin, limited exposure to sunlight and bone mineral density is confirmed by the study by Wacker and Holick (2013) and multiple other studies(55).

7. Recommendations for the work field

The advice is to better adhere to the consensus protocol regarding monitoring of BMD. Missing BMD measurements mean blanc information on bone health, which makes prevention, treatment and supplementary modifying unattainable. With the information we have now, X-Hand seems to be the most feasible BMD measurement technique for this specific patient group(47).

To the dietitians at the Erasmus MC Sophia Children's Hospital it is advised to evaluate the vitamin D and calcium supplementation of all patients treated with the KD. Despite missing recommendations for this complex patient group, supplementation 100%/ADH in case of patients fed orally, 50%/ADH in case of patients fed partially by tube/bottle and 0% in case of fully tube/bottle of both vitamin D and calcium should be considered as legitimate.

Considering the fact that the majority of the research population has too low 25-OHD status and inadequate 25-OHD limits calcium absorption, it is advised to add this test to the baseline and two-yearly check-up. In this way, preventive modifications in the diet can be made. Special attention on vitamin D and calcium supplementation should be given at initiation of the KD to patients with risk factors; limited mobility, use of AED during treatment with the KD or in history (benzodiazepine, carbamazepine, phenytoin, phenobarbital, valproic acid, gabapentin, oxcarbazepine), darker skin, limited exposure to sunlight and limited intake of vitamin D and calcium. These recommendations are brought together in table 8.

Table 8. Risk profile for poor bone health

Considerations	Determination
- Limited mobility - Use of AED during treatment with the KD or in history; benzodiazepine, carbamazepine, phenytoin, phenobarbital, valproic acid, gabapentin, oxcarbazepine - Darker skin - Limited exposure to sunlight - Limited intake of vitamin D and calcium	- Measurement of BMD with Hand-X at baseline and periodically after 2 years of treatment with the KD - Lab; 25-OHD and 1,25-OH2D In case of decreased BMD (>-2 SD Z-score); evaluation of vitamin and mineral intake, repeat BMD measurement

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Appendix

I. Case record form

T0 = at start of the KD
T1 = after 1 month treated with the KD
T2 = after 2 months treated with the KD
T3 = after 3 months treated with the KD
T4 = after 6 months treated with the KD
T5 = after 1 year treated with the KD
T6 = after 2 years treated with the KD
T7 = after 3 years treated with the KD
T8 = after 4 years treated with the KD
missing data will be registered as 999

PID no.		# in study		
Name				
Gender		0 = male	1 = female	
Date of birth				
Ethnicity		0 = Afro-American	1 = other	9 = unknown
Gross Motor Function Classification Scale		0 = none 1 = limitation of rough motor skills 2 = walking without tools, more difficult outdoors 3 = walking with tools 4 = self-propelled with tools 5 = not self-propelled		
Mental retardation		0 = none 1 = low ability (IQ 70-90) 2 = mild mental retardation (IQ 70-90) 3 = moderate mental retardation (IQ 35-50) 4 = severe and profound mental retardation (IQ <35)		
Intake	0 = oral	1 = (partially) tube/PEG	9 = unknown	

Start of KD		
Stop KD/last follow-up		
Age at the start of KD in months		
Time on diet in months		
Deceased	0 = no	1 = yes

Underlying type of cause	0 = genetic 1 = structural/metabolic 2 = unknown cause
Electronical syndromes	0 = non syndromic 1 = Lennox-Gastaut 2 = Dravet 3 = West 4 = ESES/CWSWS 5 = Otahara 7 = Doose 8 = MEI 9 = FS+
GLUT-1 deficiency	0 = no 1 = yes

Growth	T0	T3	T4	T5	T6	T7	T8	N/A
Weight for age (<1 year) SD								
Height for age (>1 year) SD								
Weight for height (>1 year) SD								

Diet type	T1	T2	T3	T4	T5	T6	T7	T8
-1 = deceased								
0 = classic								
1 = MCT								
2 = combi								
3 = atkins								
7 = diet ended before t								
8 = t is in future								
9 = unknown								

Ketosis	T1	T2	T3	T4	T5	T6	T7	T8
-1 = deceased								
0 = <2,5 mmol/l								
1 = 2,5-4 mmol/l								
2 = 4,1-6,5 mmol/l								
3 = >6,5 mmol/l								
7 = diet ended before t								
8 = t is in future								
9 = unkown								

AED	T0	T1	T2	T3	T4	T5	T6	T7	T8
# AED									
Phenytoin									
Phenobarbital									
Valproic acid									
Oxacarbazepin									
Gabapentin									

BMD measurement	Date	Date	Date
Time on diet in months			
Way of measurement 0 = DEXA 1 = X-Bone 2 = boneXpert 9 = unknown			
SD -2 = not usable			
Treatment			

BMD measurement	Date	Date	Date
Time on diet in months			
Way of measurement 0 = DEXA 1 = X-Bone 2 = boneXpert 9 = unknown			
SD -2 = not usable			
Treatment			

Reasons for missing data of BMD	

Vitamin D	T0	T3	T4	T5	T6	T7	T8
Total intake (mcg)							
Supplementation (mcg)							
In diet (mcg)							
RDA							
%RDA							

Calcium	T0	T3	T4	T5	T6	T7	T8
Total intake (g)							
Supplementation (g)							
In diet (g)							
RDA							
%RDA							

Serum level 25-OHD	Date	Date	Date
Time on diet in months			
0 = too low 1 = in range 2 = too high 9 = unknown			

Serum level 1,25-OH2D	Date	Date	Date
Time on diet in months			
0 = too low 1 = in range 2 = too high 9 = unknown			

Serum level calcium	Date	Date	Date
Time on diet in months			
0 = too low 1 = in range 2 = too high 9 = unknown			

II. Collected data and their variables

Variable		Measurement level	
Name		Scale	-
Date of birth		Scale	Date
Ethnicity		Nominal	0 = Afro-american 1 = other
GMFCS		Ordinal	0 = none 1 = limitation of rough motor skills 2 = walking without tools, more difficult outdoors 3 = walking with tools 4 = self-propelled with tools 5 = not-self propelled
Level of mental retardation		Ordinal	0 = none 1 = low ability (IQ 70-90) 2 = mild mental retardation (IQ 50-70) 3 = moderate mental retardation (IQ 35-50) 4 = severe and profound mental retardation (IQ <35)
Etiology cause		Nominal	0 = genetic 1 = metabolic/structural 2 = unknown cause
Electroclinical syndromes		Nominal	0 = non-syndromic 1 = Lennox-Gastaut 2 = Dravet 3 = West 4 = ESES/CSWS 5 = Otahara 6 = Doose 7 = MEI 8 = FS+
GLUT-1 deficiency syndrome		Nominal	0 = no 1 = yes
Rett(-like) syndrome		Nominal	0 = no 1 = yes
Start KD		Scale	Date
Stop KD		Scale	Date
Total time on diet in months		Scale	-
Age in years at initiation of the KD		Scale	-
Way of feeding	T0	Ordinal	0 = oral 1 = partially tube/bottle 2 = fully tube/bottle
Way of feeding	T3	Ordinal	0 = oral 1 = partially tube/bottle 2 = fully tube/bottle
Way of feeding	T4	Ordinal	0 = oral 1 = partially tube/bottle 2 = fully tube/bottle
Way of feeding	T5	Ordinal	0 = oral 1 = partially tube/bottle

			2 = fully tube/bottle
Way of feeding	T6	Ordinal	0 = oral 1 = partially tube/bottle 2 = fully tube/bottle
Way of feeding	T7	Ordinal	0 = oral 1 = partially tube/bottle 2 = fully tube/bottle
Way of feeding	T8	Ordinal	0 = oral 1 = partially tube/bottle 2 = fully tube/bottle
Supplementation of vitamin D (mcg)	T0	Scale	-
Supplementation vitamin D %/ADH	T0	Scale	%
Supplementation of vitamin D (mcg)	T3	Scale	-
Supplementation vitamin D %/ADH	T3	Scale	%
Supplementation of vitamin D (mcg)	T4	Scale	-
Supplementation vitamin D %/ADH	T4	Scale	%
Supplementation of vitamin D (mcg)	T5	Scale	-
Supplementation vitamin D %/ADH	T5	Scale	%
Supplementation of vitamin D (mcg)	T6	Scale	-
Supplementation vitamin D %/ADH	T6	Scale	%
Supplementation of vitamin D (mcg)	T7	Scale	-
Supplementation vitamin D %/ADH	T7	Scale	%
Supplementation of vitamin D (mcg)	T8	Scale	-
Supplementation vitamin D %/ADH	T8	Scale	%
Mean supplementation vitamin D %/ADH of T0, T3, T4, T5, T6, T7 and T8		Scale	%
Supplementation of calcium (mg)	T0	Scale	-
Supplementation calcium %/ADH	T0	Scale	%
Supplementation of calcium (mg)	T3	Scale	-
Supplementation calcium %/ADH	T3	Scale	%
Supplementation of calcium (mg)	T4	Scale	-
Supplementation of calcium %/ADH	T4	Scale	%
Supplementation of calcium (mg)	T5	Scale	-
Supplementation of calcium %/ADH	T5	Scale	%
Supplementation of calcium (mg)	T6	Scale	-
Supplementation of calcium %/ADH	T6	Scale	%
Supplementation of calcium (mg)	T7	Scale	-
Supplementation of calcium %/ADH	T7	Scale	%
Supplementation of calcium (mg)	T8	Scale	-
Supplementation of calcium %/ADH	T8	Scale	%
Mean supplementation calcium %/ADH of T0, T3, T4, T5, T6, T7, T8		Scale	%
Weight for age SD Z-score (<1 year)	T0	Scale	-
Weight for age SD Z-score (<1 year)	T3	Scale	-
Weight for age SD Z-score (<1 year)	T4	Scale	-
Weight for age SD Z-score (<1 year)	T5	Scale	-
Weight for age SD Z-score (<1 year)	T6	Scale	-
Weight for age SD Z-score (<1 year)	T7	Scale	-
Weight for age SD Z-score (<1 year)	T8	Scale	-
Height for age SD Z-score (>1 year)	T0	Scale	-
Height for age SD Z-score (>1 year)	T3	Scale	-
Height for age SD Z-score (>1 year)	T4	Scale	-
Height for age SD Z-score (>1 year)	T5	Scale	-
Height for age SD Z-score (>1 year)	T6	Scale	-

Height for age SD Z-score (>1 year)	T7	Scale	-
Height for age SD Z-score (>1 year)	T8	Scale	-
Mean height for age SD Z-score (> 1 year) of T0, T3, T4, T5, T6, T7 and T8		Scale	-
Height for weight SD Z-score (>1 year)	T0	Scale	-
Height for weight SD Z-score (>1 year)	T3	Scale	-
Height for weight SD Z-score (>1 year)	T4	Scale	-
Height for weight SD Z-score (>1 year)	T5	Scale	-
Height for weight SD Z-score (>1 year)	T6	Scale	-
Height for weight SD Z-score (>1 year)	T7	Scale	-
Height for weight SD Z-score (>1 year)	T8	Scale	-
Mean height for weight SD Z-score (>1 year) of T0, T3, T4, T5, T6, T7 and T8		Scale	-
Number of AED at initiation of KD	T0	Scale	-
Use of benzodiazepine (during KD or in history)?		Nominal	0 = no 1 = yes
Use of carbamazepine (during KD or in history)?		Nominal	0 = no 1 = yes
Use of phenytoin (during KD or in history)?		Nominal	0 = no 1 = yes
Use of phenobarbital (during KD or in history)?		Nominal	0 = no 1 = yes
Use of valproic acid (during KD or in history)?		Nominal	0 = no 1 = yes
Use of gabapentin (during KD or in history)?		Nominal	0 = no 1 = yes
Use of oxcarbazepine (during KD or in history)?		Nominal	0 = no 1 = yes
Number of BMD decreasing AED (during KD or in history)?		Scale	-
Ketosis serum level (mmol/L)	T3	Scale	-
Ketosis serum level (mmol/L)	T4	Scale	-
Ketosis serum level (mmol/L)	T5	Scale	-
Ketosis serum level (mmol/L)	T6	Scale	-
Ketosis serum level (mmol/L)	T7	Scale	-
Ketosis serum level (mmol/L)	T8	Scale	-
Mean ketosis serum level (mmol/L) of T3, T4, T5, T7, T7 and T8		Scale	-
Date of 1st 25-OHD measurement		Scale	-
Time on diet in months at 1st 25-OHD measurement		Scale	-
Serum at 1st 25-OHD measurement		Ordinal	0 = too low 1 = in range 2 = too high
Date of 2nd 25-OHD measurement		Scale	-
Time on diet in months at 2nd 25-OHD measurement		Scale	-
Serum at 2nd 25-OHD measurement		Ordinal	0 = too low 1 = in range 2 = too high
Date of 3th 25-OHD measurement		Scale	-
Time on diet in months at 3th 25-OHD measurement		Scale	-
Serum at 2nd 25-OHD measurement		Ordinal	0 = too low

		1 = in range
		2 = too high
Mean 25-OHD serum of 1st, 2nd and 3th measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Date of 1st 1,25-OH2D measurement	Scale	-
Time on diet in months at 1st 1,25-OH2D measurement	Scale	-
Serum at 1st 1,25-OH2D measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Date of 2nd 1,25-OH2D measurement	Scale	-
Time on diet in months at 2nd 1,25-OH2D measurement	Scale	-
Serum at 2nd 1,25-OH2D measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Date of 3th 1,25-OH2D measurement	Scale	-
Time on diet in months at 2nd 1,25-OH2D measurement	Scale	-
Serum at 3th 1,25-OH2D measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Mean 1,25-OH2D of 1st, 2nd and 3th measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Date of 1st calcium measurement	Scale	-
Time on diet in months at 1st calcium measurement	Scale	-
Serum at 1st calcium measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Date of 2nd calcium measurement	Scale	-
Time on diet in months at 2nd calcium measurement	Scale	-
Serum at 2nd calcium measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Date of 3th calcium measurement	Scale	-
Time on diet in months at 3th calcium measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Serum at 3th calcium measurement	Ordinal	0 = too low
		1 = in range
		2 = too high
Mean calcium serum of 1st, 2nd and 3th measurement	Scale	-
Date of 1st BMD measurement	Scale	-
Time on diet in months at 1st BMD measurement	Scale	-
Way of measurement at 1st BMD measurement	Nominal	0 = DEXA
		1 = X-Hand
BMD SD Z-score at 1st BMD measurement	Scale	-

Treatment after 1st BMD measurement	Nominal	0 = none 1 = evaluation of calcium and vitamin D intake
Date of 2nd BMD measurement	Scale	-
Time on diet in months at 2nd BMD measurement	Scale	-
Way of measurement at 2nd BMD measurement	Nominal	0 = DEXA 1 = X-Hand
BMD SD Z-score at 2nd BMD measurement	Scale	-
Treatment after 2nd BMD measurement	Nominal	0 = none 1 = evaluation of calcium and vitamin D intake
Date of 3th BMD measurement	Scale	-
Time on diet in months at 3th BMD measurement	Scale	-
Way of measurement at 3th BMD measurement	Nominal	0 = DEXA 1 = X-Hand
BMD SD Z-score at 3th BMD measurement	Scale	-
Treatment after 3th BMD measurement	Nominal	0 = none 1 = evaluation of calcium and vitamin D intake
Date of 4th BMD measurement	Scale	-
Time on diet in months at 4th BMD measurement	Scale	-
Way of measurement at 4th BMD measurement	Nominal	0 = DEXA 1 = X-Hand
BMD SD Z-score at 4th BMD measurement	Scale	-
Treatment after 4th BMD measurement	Nominal	0 = none 1 = evaluation of calcium and vitamin D intake
Mean BMD SD Z-score of 1st, 2nd, 3th and 4th measurement	Scale	-
BMD Group	Ordinal	0 = group with BMD SD Z-score below -2 1 = group with BMD SD Z-score above -2

III. Survey

Naam	
Functie	
Instelling	

1. **Hoeveel kinderen zijn er in totaal met het Ketogeen dieet behandeld in uw instelling?**
2. **Hoeveel Kinderen zijn er het afgelopen jaar gestart met het Ketogeen dieet in uw instelling?**
3. **Welke kinderen in uw populatie hebben volgens u het grootste risico op een verlaagde botdichtheid en waarom?**
4. **Krijgen de patiënten met verhoogd risico ter preventie aangepaste vitamine- en/of mineralen intake?** ***Meerdere antwoorden mogelijk, kruis aan wat van toepassing is***
   □ Vitamine D: ……%/ADH
   □ Calcium: …….%/ADH
   □ Anders, namelijk:……
5. **Hoe vaak wordt botdichtheid gecontroleerd tijdens de Ketogeen dieet behandeling in uw instelling?** ***Meerdere antwoorden mogelijk, kruis aan wat van toepassing is***
   □ start Ketogeen dieet
   □ Jaarlijks
   □ Tweejaarlijks
   □ Op indicatie
   □ Anders, namelijk:……
6. **Welke meetmethode past u toe?** ***Meerdere antwoorden mogelijk, kruis aan wat van toepassing is***
   □ DEXA-scan
   □ X-Hand
   □ Anders, namelijk:……
7. **Is het mogelijk om bij elke patiënt de botdichtheid daadwerkelijk te meten?** ***Wilt u uw antwoord toelichten?***
8. **Wanneer vindt u dat er daadwerkelijk sprake is van verlaagde botdichtheid?**
   □ -1 SD Z-score
   □ -1,5 SD Z-score
   □ >-2 SD Z-score
   □ Anders, namelijk:……
9. **Hoe vaak komt naar uw verwachting verlaagde botdichtheid voor in uw populatie?**
   □ 0-25%
   □ 26-50%
   □ 51-75%
   □ 76-95%
   □ 96-100%
   □ Anders, namelijk:……
10. **Welke aanvullende onderzoeken verricht u wanneer er sprake is van verlaagde botdichtheid?**
11. **Welke behandeling past u toe bij verlaagde botdichtheid?**

IV. Course of BMD

Table 2. Multiple BMD measurements of patients

#	Measurement 0-2 years	Measurement 2-4 years	Measurement 4-6 years	Measurement 6-8 years	Measurement 8-10 years
1	N/A	-1,5 / -1,5	-2	N/A	-2,5 / -2,5
2	N/A	-3	non-readable / -1,5	N/A	-1
3	non-readable	-3,5	-3	N/A	N/A
4	-0,5	non-readable	-1	N/A	N/A
5	non-readable / -1	-0,5	N/A	N/A	N/A
6	non-readable / non-readable	non-readable	N/A	N/A	N/A
7	N/A	-2,5	-1,5	N/A	N/A
8	N/A	non-readable / non-readable	N/A	N/A	N/A
9	N/A	-4 / -3,5	N/A	N/A	N/A
10	-3,5	-4	N/A	N/A	N/A
11	N/A	-2 / -1,5	N/A	N/A	N/A
12	N/A	-1	-1,5	N/A	N/A
13	N/A	-1,5	N/A	N/A	-2,5

V. Chi Square test for way of feeding versus vitamin D/calcium supplementation

P-Values of the Chi Square Test for way of feeding versus vitamin D supplementation

	P-value way of feeding x 100%/ADH	P-value way of feeding x 50%/ADH
T0	0,490	0,062
T3	0,032	0,496
T4	0,193	0,126
T5	0,553	0,572
T6	0,009	0,311
T7	0,363	0,305
T8	0,682	0,652

P-Values of the Chi Square Test for way of feeding versus calcium supplementation

	P-value way of feeding x 100%/ADH
T0	0,460
T3	0,201
T4	0,775
T5	0,690
T6	0,036
T7	0,318
T8	1,000