

## Micronutrients intake in patients with refractory epilepsy with ketogenic diet treatment

### Ingesta de micronutrientes en pacientes con epilepsia refractaria tratados con dieta cetogénica

Silvia Velandia<sup>a</sup>, Patricio Astudillo<sup>b</sup>, Keryma Acevedo<sup>c</sup>, Catalina Le Roy<sup>a,d</sup>

<sup>a</sup>Departamento de Gastroenterología y Nutrición Pediátrica, División de Pediatría, Facultad de Medicina, Pontificia Universidad Católica de Chile. Santiago, Chile.

<sup>b</sup>División de Pediatría, Facultad de Medicina, Pontificia Universidad Católica de Chile. Santiago, Chile.

<sup>c</sup>Sección de Neurología Pediátrica, División de Pediatría, Facultad de Medicina, Pontificia Universidad Católica de Chile. Santiago, Chile.

<sup>d</sup>Departamento de Pediatría y Cirugía Infantil, Campus Centro, Facultad de Medicina, Universidad de Chile. Santiago, Chile.

Received: September, 28, 2023; Approved: April 25, 2024

#### What do we know about the subject matter of this study?

The Ketogenic Diet (KD) is an effective therapy in the treatment of drug-resistant epilepsy (DRE) and some inborn errors of metabolism, but it is characterized by being restrictive in micronutrients. Its different modalities, depending on the proportion of macronutrients, can generate differences in micronutrient deficiencies.

#### What does this study contribute to what is already known?

Micronutrient sufficiency in different KD modalities is described in pediatric patients with DRE and GLUT1 deficiency syndrome. We found that the micronutrient intake is reduced for most of them, being the modified Atkins diet with special formula the one with the most adequate micronutrient intake. It is important to consider this information for the follow-up and planning of their supplementation.

#### Abstract

The Ketogenic Diet (KD) is a non-pharmacological strategy for drug-resistant epilepsy (DRE) and inborn errors of metabolism (Glut-1 deficiency) management. KD is characterized by being restrictive, affecting micronutrient intake. There are different modalities of KD in which food intake and nutritional deficiencies vary. **Objective:** To determine the micronutrient intake in different KD modalities. **Patients and Method:** Observational, cross-sectional study with patients diagnosed with DRE and Glut-1 deficiency. The dietary intake of 21 micronutrients was evaluated, and analyzed according to KD modality [Classic, Modified Atkins Diet (MAD)], use of special formula, and adequacy of recommended dietary intake (RDI) according to age and sex, defining < 75% as deficient. **Results:** 19 patients were evaluated, median age 62 months (IQR: 20.5-79), 12/19 (63.2%) male, 13/19 (68.4%) eutrophic, 5/19 (26.3%) gastrostomy users, 10 (52.6%) MAD modality, use of special

#### Keywords:

Epilepsy;  
Ketogenic Diet;  
Minerals;  
Supplementation;  
Vitamins

formula 7/19 (36.8%). Micronutrient deficiencies were found in 16/21 (76.2%) in the classic diet and 9/16 (42.9%) in the MAD. The intake of vitamin D, B2, B12, sodium, phosphorus, zinc, and selenium was significantly lower in the classic diet than in DMA, the median intake adequacy of vitamins A, C, D, E, and K was > 100%. The use of special formula manages to complete the requirements in MAD.

**Conclusions:** The micronutrient intake in the different KD modalities is low for most of them, being the MAD with formula the one that presented a more adequate micronutrient intake. These results should be considered in nutritional follow-up and supplementation planning.

## Introduction

The Ketogenic Diet (KD) is an effective non-pharmacological therapy in the treatment of Drug-Resistant Epilepsy (DRE) and in some inborn errors of metabolism (pyruvate dehydrogenase deficiency and GLUT1 deficiency syndrome)<sup>1-4</sup>. In the case of DRE, it has demonstrated a significant decrease in both focal and generalized seizure episodes, reduction of epileptic spasms, improvement in alertness, and positive change in cognitive, behavior, and functionality aspects<sup>5-8</sup>. Its anti-crisis mechanism of action is associated with the production of ketone bodies that stimulate the synthesis of  $\gamma$ -aminobutyric acid (GABA), reduce neuroinflammation, modulate the monoamine activity (serotonin, noradrenaline, dopamine, and adenosine), and promote the activity of  $K^+$  and  $Cl$  channels<sup>4,9,10</sup>.

It is characterized for being a restrictive diet with high lipid content, adequate or higher proportion of proteins, and low in carbohydrates. Its proportions vary according to the type of modality<sup>1</sup>. Among the varieties of classic KD are the 3:1 diet (recommended for younger infants because it allows a higher protein intake required for rapid growth at this stage) and the 4:1 diet (suggested for children between 2 and 4 years old)<sup>5,6</sup>. Other less restrictive modalities correspond to the medium-chain triglyceride (MCT) KD, Modified Atkins Diet (MAD) (70-75% fat), and low glycemic index KD (45-60% lipids)<sup>7,8</sup>, mainly used in schoolchildren and adolescents due to better adherence<sup>5,6</sup>.

The restrictive nature of KD compromises the recommended intake of all micronutrients, such as calcium, iron, zinc, selenium, magnesium, and B-complex vitamins, generating associated complications such as osteopenia, anemia, delayed growth, and psychomotor development, and in some cases, heart disease secondary to selenium deficiency<sup>7,11-13</sup>. Specialized clinical follow-up is key to planning permanent supplementation of potentially deficient micronutrients and/or the use of special fortified formulas<sup>1,2</sup>. Currently, commercial formulas of classic 4:1 and 3:1 fortified with vitamins, minerals, and electrolytes can be used to supplement KD, and in the case of patients under 6 months of age, or in children using a nasogastric tube or gastrostomy

tube (G-tube), or in special situations such as travel, it can be indicated as the sole source of nutrition since it covers the energy and macronutrient requirements, but the micronutrient intake must be calculated and pharmacologically supplemented, if necessary<sup>11</sup>. However, their high cost limits the accessibility to these products<sup>2,5</sup>.

Due to the therapeutic importance of KD and its high micronutrient restriction, the main objective of this research was to determine and compare the micronutrient and electrolyte intake of the different KD modalities in patients under follow-up by the KD team at a tertiary university healthcare center, and to relate it to the Dietary Reference Intakes (DRIs) established by the US National Academy of Sciences<sup>14</sup>.

## Patients and Method

Observational, descriptive, cross-sectional study.

### Patients

Data were obtained from the review of clinical histories of 25 patients who were under treatment with KD in the UC-Christus Healthcare Network from July 2015 to March 2020. Those who complied with regular check-ups (1, 3, 6, 9, and 12 months minimum) and who remained with the team (neurologist, dietitian, and pediatrician subspecialist in nutrition) were included. Patients who did not maintain nutritional indications, supplementation, or follow-up with the team were excluded.

### Anthropometric nutritional evaluation

Children under 2 years of age were weighed using an infant scale (precision: 100g), and their length was measured with an infant stadiometer. In patients over 2 years old, weight and height were measured in a standing position using a scale with an integrated stadiometer (SECA reference). For patients with reduced mobility, weight was measured using a digital chair scale (SECA), and length was determined by measuring the tibia length (estimated height = (tibia length x 3.26) + 30.8)<sup>15</sup>.

In children without special conditions, under 5 years of age, the WHO 2006 growth standards were used, and in patients older than 5 years of age, the WHO 2007 growth standards were used, according to the current Chilean ministerial norm<sup>16-18</sup>.

Children with Down syndrome were evaluated using the Zemel 2015 growth charts<sup>19</sup>, which classifies nutritional status by percentiles (p), considering underweight when the W/H parameter is below p10, normal between p10-90, and excess above p90. The H/A parameter is considered normal between p5-95 and under p5 as short stature.

The nutritional status of patients with cerebral palsy was determined using the 2011 Brooks curves designed for children aged 2-20 years, considering weight deficit when BMI  $\leq$  p10 and excess weight with BMI  $\geq$  p90; as for the H/A parameter, below the 5th percentile was defined as short stature, interpretation decided by the authors.

### Determination of dietary intake of micronutrients

The KD modality was decided mainly by age. Those younger than 2 years had classic 3:1 KD, patients between 2 and 4 years followed classic 4:1 KD, and those older than 5 years were with MAD.

The KD provided to each patient was designed by the pediatric dietitian, and individualized to meet the energy requirements based on characteristics such as age, sex, mobility, physical activity, and nutritional diagnosis. Each KD variety comprises menus for 7 days with alternatives of 4 to 5 handmade prepared meals or milk and/or special ketogenic formula. The diet was decided by agreement between the team and parents, according to some characteristics such as age, route of administration, and affordability. Parents were instructed to manage volumes and weights of food in milliliters and grams using a digital portable dietary scale (precision: 0.1 grams), syringes, and volume meters, respectively.

21 micronutrients were chosen for analysis the following vitamins: A ( $\mu$ g), C (mg), D ( $\mu$ g), E ( $\mu$ g), K ( $\mu$ g), B1 (mg), B2 (mg), niacin (mg), B6 (mg), B12 ( $\mu$ g), folic acid ( $\mu$ g), pantothenic acid (mg); and minerals: sodium (mg), potassium (mg), calcium (mg), phosphate (mg), iron (mg), zinc (mg), selenium ( $\mu$ g), magnesium (mg), and copper ( $\mu$ g). The analysis of the micronutrient content of each KD was calculated using the food base of the food composition table of the Institute of Nutrition and Food Technology (INTA) of the Universidad de Chile and the US Department of Agriculture (USDA) food composition database<sup>21</sup>. Micronutrient contents that were not found in some of these databases were acquired from product labels, websites, or manufacturers.

The average daily intake (ADI) of each micronutrient was the weekly sum provided exclusively by the diets, without considering pharmacological supplementation, divided by seven, and compared to the recommended daily intake (RDI) according to age and sex<sup>14</sup>. The nutrient intake adequacy was classified as deficient when the percentage was  $< 75\%$  and excessive  $> 125\%$  of the RDI. The percentage of energy derived from the special formula in relation to the total calories was calculated for those children where it was possible to use it through the formula: calories from the special formula/total intake calories  $\times 100$ .

### Statistical analysis

For the description of numerical variables, the median and interquartile range (p25-p75) were used, and for the comparison between micronutrient intakes, the Mann-Whitney U test and Kruskal-Wallis test with Dunn's *post-hoc* test were applied. Categorical variables were analyzed using the two-tailed Fisher's exact test. The analyses were conducted using the SPSS software V25.0 (NY, USA). The association between energy and micronutrient intake according to RDI percentage with the different types of formulas was correlated by Spearman's correlation test with GraphPad software (Version 5.1). A significant p value  $< 0.05$  was considered.

This research was conducted under the Helsinki Declaration of Principles and was approved by the Research Ethics Committee of the School of Medicine of the Pontificia Universidad Católica de Chile (ID: 200830001).

## Results

### Demographic data

19 patients were evaluated, 12 (63.2%) were male with a male:female ratio of 1.7:1.9, 47.3% were younger than 36 months (table 1). 21% of the patients had Down syndrome (DS) and 42% cerebral palsy (CP) with severe motor compromise according to the Gross Motor Function Classification System (GMFCS). In relation to the diagnoses associated with KD indication, they were mainly encephalopathy of genetic cause (31.6%) and infantile epileptic spasms syndrome (26.3%). It should be noted that 2 patients in the group had GLUT1 deficiency syndrome. Regarding the nutritional status of the group, most patients were diagnosed as well-nourished (68.4%), 21% were undernutrition, and 10.5% had short stature. Additionally, 73.7% of the sample were feeding orally (table 1).

**Table 1. Demographic data of recruited patients (n = 19)**

| Demographic features                           | N (%)        |
|------------------------------------------------|--------------|
| Male sex, n(%)                                 | 12 (63.2)    |
| Age, months. Median (IQR)                      | 62 (20.5-79) |
| Diagnosis of Epilepsy, n(%)                    |              |
| Genetic epileptic encephalopathy               | 6 (31.55)    |
| Other drug-resistant epilepsies                | 6 (31.55)    |
| Infantile spasms epileptic syndrome            | 5 (26.3)     |
| Cortical dysplasia                             | 1 (5.3)      |
| Lennox-Gastaut syndrome                        | 1 (5.3)      |
| Nutritional Diagnosis, n(%)                    |              |
| Undernutrition                                 | 4 (21.1)     |
| Well-nourish                                   | 13 (68.4)    |
| Overweight                                     | 2 (10.5)     |
| Short Stature, n(%)                            | 2 (10.5)     |
| Type of ketogenic diet, n(%)                   |              |
| MAD                                            | 8 (42.1)     |
| Classic Ketogenic Diet + Special Formula       | 5 (26.3)     |
| Classic Ketogenic Diet without special formula | 4 (21.0)     |
| MAD + Special Formula                          | 2 (10.6)     |
| Feeding route, n(%)                            |              |
| Oral                                           | 14 (73.7)    |
| Gastrostomy tube                               | 5 (26.3)     |

IQR: Interquartile Range (p25-p75); DMA: Modified Atkins Diet.

### Comparison between types of ketogenic diets

The most used KD modality was MAD without a special formula (42.1%) and the use of classic diets predominated in those younger than 36 months ( $p = 0.001$ ). 55.6% of the patients with classic KD received a special formula compared to the group receiving MAD where it was only 20%. There were no differences in relation to energy intake according to the special formula ( $p = 0.5$ ) (table 2).

When analyzing the intake of micronutrients and electrolytes, the classic KD had a lower intake of vitamin D, B2, B12, sodium, phosphate, zinc, and selenium compared to the MAD, however, the vitamin D intake in the classic KD and MAD was adequate with 130% and 283%, respectively (table 2). It is important to highlight that in the classic KD, 76.2% of micronutrients were deficient compared to the MAD, where 42.9% of its micronutrients were deficient ( $p = 0.002$ ). It was also shown that the MAD had 38.1% of its micronutrients with adequate intake, while in the classic KD, only 9.5% of its micronutrients were adequate ( $p = 0.003$ ) (table 2).

Table 3 shows the adequacy of RDI according to the KD modality and the use of a special formula. When comparing the 5 modalities, significant differences were found for vitamins A, C, D, K, B1, B6, pantothenic acid, and selenium. In the Classic 3:1 KD without the use of special formula, there was a deficient supply of all micronutrients, except for vitamins E and K. In the case of the Classic KD with the use of a special formula, the intake adequacy percentage was deficient for B1, B2, niacin, B12, folic acid, pantothenic acid, sodium, potassium, calcium, phosphate, and iron (table 3).

The MAD without formula showed deficient adequacy for B1, niacin, folic acid, pantothenic acid, calcium, zinc, magnesium, sodium, and potassium. In contrast, the MAD with a special formula showed optimal adequacy for all micronutrients and minerals except potassium (46.3%) (table 3). When evaluating the intake of micronutrients according to age, it was observed that pediatric patients younger than 36 months had higher intakes of vitamin C and K compared to patients older than 36 months (figure 1). The MAD without formula had a higher intake of vitamin D and selenium compared to the Classic KD without formula (Figure 2a and 2b), but when comparing the Classic KD with the MAD, both with special formula, there were significant differences in the intake of most of the micronutrients (Figure 2c and 2d).

Finally, a correlation was observed between the daily energy intake in each type of KD with the micronutrient intake according to RDI, showing a moderate to strong negative correlation ( $r > -0.5$ ) with vitamins B1, B2, niacin, B6, folic acid, pantothenic acid, and of the minerals only magnesium and copper. In the classical diets, a positive correlation was obtained with vitamin E and a negative one with vitamin K and folic acid (Supplementary table 1, available in the online version).

### Discussion

The KD as a restrictive and unbalanced dietary therapy involves micronutrient deficits and there is still no evidence available to estimate and compare the net nutritional intake (without supplementation) of vitamins and minerals of the different KD modalities<sup>5,8,12,13</sup>. The KD modality was performed considering age-specific recommendations, mainly using the classic 3:1 KD in infants, and 4:1 in preschool children (2-5 years), while the MAD was used for those older than 4 years and adolescents<sup>4,7</sup>. Family circumstances, cognitive compromise, autonomy, use of G-tube, and type of epilepsy were considered<sup>5,7,22-24</sup>.

**Table 2. Adequacy of recommended dietary micronutrient intake according to type of ketogenic diet: classic and modified Atkins diet**

| Variable                                                  | Classic Diet<br>(n = 9) | MAD<br>(n = 10)  | p-Value |
|-----------------------------------------------------------|-------------------------|------------------|---------|
| Male sex, n(%)                                            | 5 (55.6)                | 4 (40)           | 0.6     |
| Age, months. Median (IQR)                                 | 17 (11-24)              | 79 (58-142)      | 0.001*  |
| Use of special formula, n(%)                              | 5 (55.6)                | 2 (20)           | 0.17    |
| Energy contribution from special formula, %, median (IQR) | 50 (28.9-50.8)          | 44.1 (39.5-44.8) | 0.5     |
| Micronutrient, % RDI. Median, IQR                         |                         |                  |         |
| Vitamin A                                                 | 100 (40-139)            | 108.8 (84-203)   | 0.4     |
| Vitamin C                                                 | 100 (40-139)            | 115.4 (55-239)   | 1.0     |
| Vitamin D                                                 | 130 (67-162)            | 283 (171-356)    | 0.003*  |
| Vitamin E                                                 | 225 (183-532)           | 345 (265-552)    | 0.32    |
| Vitamin K                                                 | 389 (206-1791)          | 279 (196-445)    | 0.36    |
| Vitamin B1                                                | 48 (22-83)              | 45.7 (38.3-81)   | 0.66    |
| Vitamin B2                                                | 63 (27-89)              | 105 (71.4-131)   | 0.04*   |
| Niacin                                                    | 60 (18-80)              | 71.5 (51-109)    | 0.24    |
| Vitamin B6                                                | 69 (40-93)              | 93 (65-143)      | 0.11    |
| Vitamin B12                                               | 66 (42-83)              | 78.6 (55-105)    | 0.03*   |
| Folic acid                                                | 53 (31-85)              | 67.8 (46.6-97)   | 0.16    |
| Pantothenic acid                                          | 37 (20-77)              | 60.1 (30-82)     | 0.11    |
| Sodium                                                    | 25 (16-31)              | 31.8 (28-44.9)   | 0.04*   |
| Potassium                                                 | 20 (17-44)              | 31.7 (28-45)     | 0.09    |
| Calcium                                                   | 49 (32-53)              | 58.5 (26-109)    | 0.5     |
| Phosphorus                                                | 58.5 (47-64)            | 102 (73-121)     | 0.01*   |
| Iron                                                      | 45 (13-67)              | 57.6 (50-91.5)   | 0.13    |
| Zinc                                                      | 63 (31-95)              | 97 (77.4-143)    | 0.04*   |
| Selenium                                                  | 62 (43-113)             | 130(113-218)     | 0.04*   |
| Magnesium                                                 | 57 (29-91)              | 68 (45-147)      | 0.6     |
| Copper                                                    | 70 (52-81)              | 82 (53-144)      | 0.28    |
| Number of micronutrients, n (%)                           |                         |                  |         |
| Deficient (< 75% RDI)                                     | 16 (76.2)               | 9 (42.9)         | 0.002*  |
| Adequate (75-125% RDI)                                    | 2 (9.5)                 | 8 (38.1)         | 0.003*  |
| Excess (> 125%)                                           | 3 (14.3)                | 4 (19)           | 0.5     |
| Feeding route, n(%)                                       |                         |                  |         |
| Oral                                                      | 5 (55.6)                | 9 (90)           | 0.14    |
| Gastrostomy tube                                          | 4 (44.4)                | 1 (10)           |         |

\*Significant,  $p < 0.05$ , Mann-Whitney U test. IQR: Interquartile Range (p25-75); MAD: Modified Atkins Diet.

However, no protocol determines the number of daily meals or percentage distribution of these in each type of KD. In the elaboration of the KD, a special formula for KD was included in some patients who could afford it, administered orally or via G-tube as a complement.

Due to the strong restriction of carbohydrates in

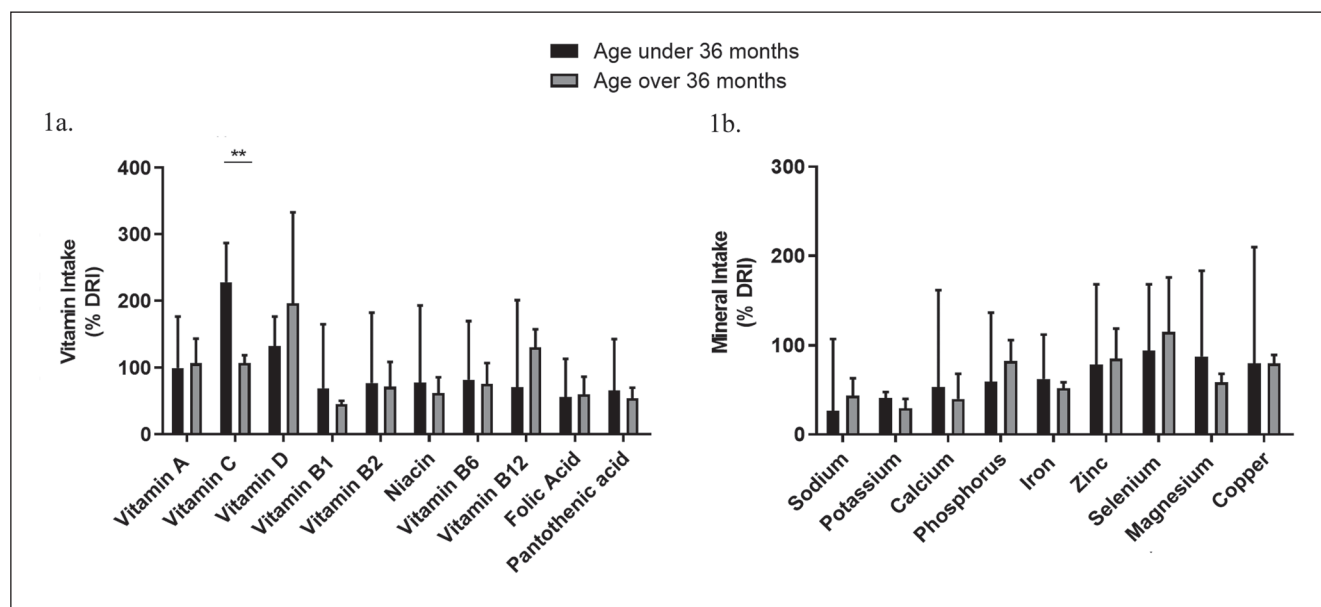
the classic KD, the intake of fruits and whole grains is almost non-existent. When analyzing the micronutrient intake of the different KD administered to our patients, the classic 4:1 and 3:1 KD without ketogenic formula, presented very low adequacy in almost all vitamins except for vitamins E and K which showed very high intake values.

**Table 3. Adequacy of recommended dietary micronutrient intake according to type of ketogenic diet: classic and modified Atkins diet, with and without use of special formula**

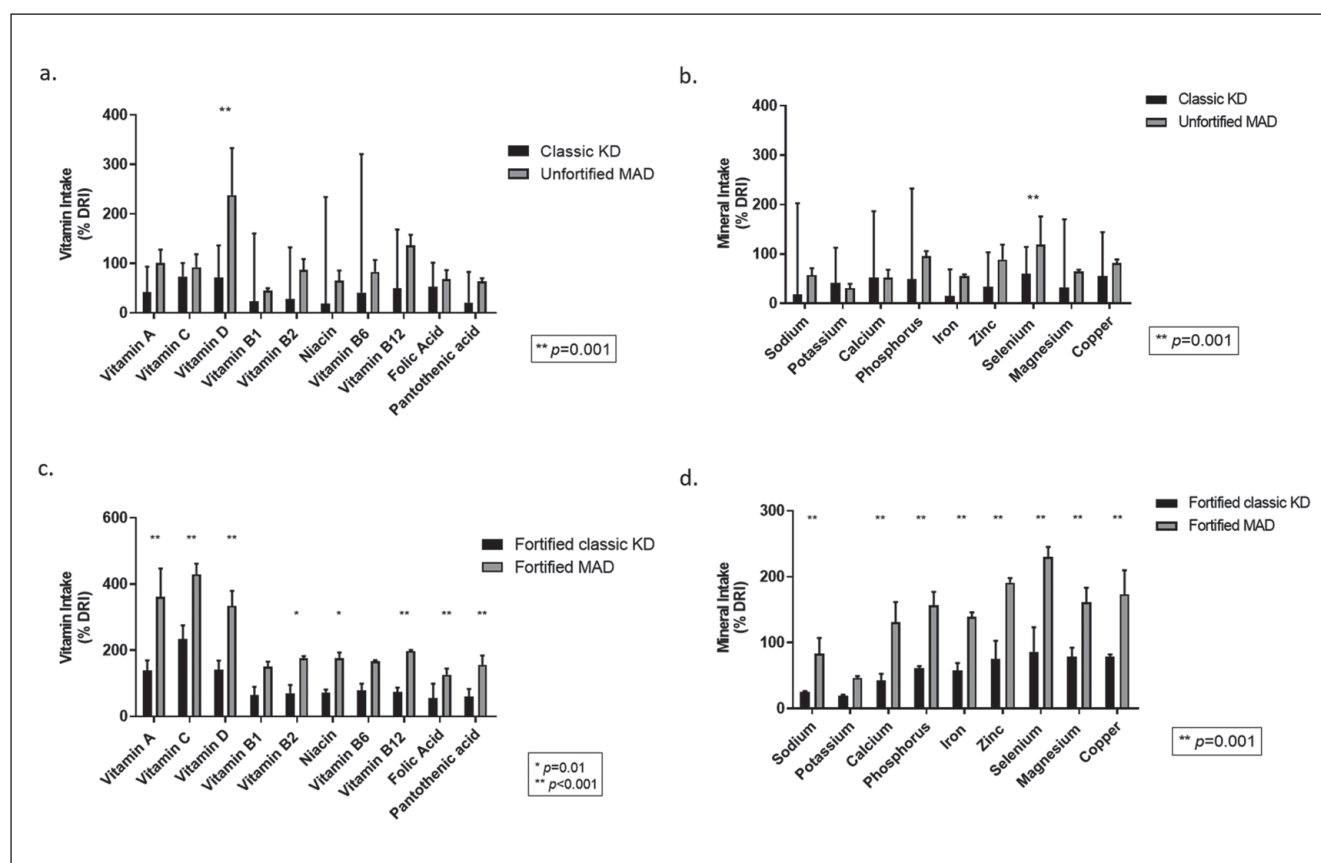
| Micronutriente <sup>a</sup> | Classic 4:1<br>(n = 2) | Classic 3:1<br>(n = 3) | Classic+Formula<br>(n = 4) | MAD<br>(n = 8)      | MAD+Formula<br>(n = 2) | p-Value <sup>b</sup> |
|-----------------------------|------------------------|------------------------|----------------------------|---------------------|------------------------|----------------------|
| Vitamin A                   | 66 (44.4-55.2)         | 41.7 (39.5-59.7)       | 138.7 (122.1-155)          | 101.3 (84.7-111.5)  | 360.8 (317.5-404)      | 0.04*                |
| Vitamin C                   | 95.8 (84.8-112.2)      | 45.3 (42.3-64.6)       | 234 (196.1-251.6)          | 92.5 (54.7-116.9)   | 429.7 (413-445.7)      | 0.03*                |
| Vitamin D                   | 51.1 (28.2-39.7)       | 71.1 (67-123.8)        | 141 (131.9-155.9)          | 238.3 (170.8-304)   | 334.2 (311.4-357)      | 0.04*                |
| Vitamin E                   | 740 (736.9-746.5)      | 224.7 (204.6-278.1)    | 196.3 (178.9-226)          | 295 (265-366.8)     | 457 (329.7-389.6)      | 0.08                 |
| Vitamin K                   | 111.8 (97.3-1054.6)    | 2280 (1791-2525.7)     | 347.6 (297-409.9)          | 238.7 (196.9-428.5) | 149.7 (141.8-157)      | 0.04*                |
| Vitamin B1                  | 22.1 (19.8-20.9)       | 23.3 (21.9 -158.7)     | 64.4 (56.6-76.1)           | 45.2 (38.3-45.7)    | 149.7 (141.9-146)      | 0.04*                |
| Vitamin B2                  | 45.2 (33.3-39.3)       | 28.6 (26.8-112.3)      | 70.2 (61.1-83.1)           | 87.1 (71.4-103.9)   | 175.2 (171.5-173)      | 0.14                 |
| Niacin                      | 16.9 (16.6-17.3)       | 22.9 (20.5-234)        | 72.7 (65.6-78.8)           | 65.3 (50.9-79.3)    | 175.9 (167-171.6)      | 0.1                  |
| Vitamin B6                  | 39.4 (31.7-35.5)       | 41.1 (39.9-314.6)      | 77.8 (70.1-78.9)           | 83.3 (65.4-90.1)    | 166.9 (165-166)        | 0.03*                |
| Vitamin B12                 | 29.9 (27.1-28.5)       | 64.3 (53-101.5)        | 73 (70-78.9)               | 136.7 (124.3-154)   | 197.2 (195.3-196)      | 0.09                 |
| Folic acid                  | 27.1 (26-26.5)         | 53 (52.9-101.5)        | 55.3 (48.9-70.7)           | 68.2 (54.7-77.3)    | 126.1 (117-139.8)      | 0.07                 |
| Pantothenic acid            | 18.6 (17.4-17.9)       | 22.2 (21-82.8)         | 60.1 (50-71.6)             | 63.8 (46.6-66.7)    | 156.1 (142-149.1)      | 0.008*               |
| Sodium                      | 26.4 (22.4-24.44)      | 13 (11.7-192)          | 25.7 (23.6-26.4)           | 57.3 (30.5-66.12)   | 83 (70.9-76.9)         | 0.29                 |
| Potassium                   | 13.4 (12.1-12.7)       | 47.5 (44.2-112.8)      | 19.4 (18.8-20)             | 30.8 (28.2-34.7)    | 46.3 (44.9-48.3)       | 0.06                 |
| Calcium                     | 27.5 (25.2-31.0)       | 53 (52.3-186.6)        | 42.3 (35.2-50.2)           | 51.8 (26.2-62.3)    | 131.2 (116-123.6)      | 0.13                 |
| Phosphorus                  | 50.6 (46.6-48.63)      | 48.6 (47.4-227.3)      | 61.6 (57.9-63.9)           | 95.4 (73.1-104.6)   | 156.7 (146.6-172)      | 0.07                 |
| Iron (mg)                   | 20 (17.4-18.7)         | 10.6 (10.3-61.3)       | 57.3 (50.3-64.5)           | 95.4 (73-104.6)     | 138.8 (135.1-137)      | 0.08                 |
| Zinc (mg)                   | 33.8 (31.3-32.6)       | 33.3 (31.5-100.7)      | 75.2 (69.5-86.2)           | 55.5 (49.6-58.3)    | 191.2 (183.7-189)      | 0.07                 |
| Selenium (ug)               | 59.9 (59.7-60.1)       | 26.6 (25.9-97.3)       | 86.3 (74.2-103.9)          | 119.1 (113.2-126.4) | 229.9 (222-226)        | 0.04*                |
| Magnesium (mg)              | 30.4 (33.3-36.9)       | 31.9 (29.7-165.7)      | 79.1 (67.1-89.3)           | 64.1 (45.8-67.9)    | 161.3 (150-155.8)      | 0.14                 |
| Copper (mg)                 | 40.6 (25.3-27.8)       | 57.1 (53.6-144.1)      | 78.9 (76.1-80.6)           | 81.4 (84.6-111.1)   | 173.5 (155.2-164)      | 0.19                 |

<sup>a</sup>Values reported as median (Interquartile Range: p25-p75). <sup>b</sup>p-value according to Kruskal-Wallis test





**Figure 1.** Comparison of micronutrient intake between patients aged over or under 36 months undergoing KD treatment, categorized by vitamins (1a) and minerals (1b). Values presented as median and interquartile range. Statistical analysis was performed using the Mann-Whitney U test. A  $p < 0.05$  is considered significant.



**Figure 2.** Comparison of micronutrient intake between classic KD and MAD without the use of special formula according to vitamins (2a) and minerals (2b), and between classic KD and MAD with the use of special formula according to vitamins (2c) and minerals (2d). Values presented as median and interquartile range. Statistical analysis was performed using the Mann-Whitney U test. A  $p < 0.05$  was considered significant.

In the case of vitamin K, it can be attributed to the frequent intake of vegetables such as chard and spinach, the main dietary sources of vitamin K, and frequently used in the preparation of meals in children aged under 2 years<sup>21</sup>. However, it is not found in older children, where the consumption of this food group is below WHO recommendations despite the availability, variety, and accessibility of fruits and vegetables in most regions of the country<sup>25</sup>. Regarding vitamin E, this could be explained by the widespread use of canola and sunflower oils to meet the high lipid percentage in classic KD 3:1 and 4:1 (87% and 90%, respectively), and MAD (75%). These oils are rich in long-chain polyunsaturated fatty acids (100 ml of canola oil and sunflower oil provide 17µg and 41µg of vitamin E, respectively)<sup>21</sup>. Previously, Christodoulides et al. reported elevated plasma levels of vitamin E in the classical KD group compared with another KD modality, attributed because of the high intake of long-chain fatty acids<sup>22</sup>.

Classical KD without formula was deficient in almost all micronutrients, mainly in vitamin D, B12, sodium, phosphate, zinc, and selenium compared with MAD, coinciding with the above-mentioned inadequate intakes of vitamins and minerals such as phosphate, calcium, magnesium, and folic acid in classical KD<sup>11,26,27</sup>. Other studies have described phosphate and folic acid intakes below the RDI in KD without supplementation and even with pharmacological supplementation<sup>26,28</sup>. Likewise, calcium, vitamin D, and phosphate, important in the process of bone mineralization, are poorly provided by classical KD<sup>11,24,26,27</sup>.

This research describes that the level of calcium adequacy is 27.5%, 53% in classic KD without formula 4:1 and 3:1, 42.3% in classic KD with formula, and 51.8% in MAD without formula, while in MAD with formula, the adequacy was 131.2%. This result reflects the calcium intake mainly from the special formula, added to the calcium content in MAD foods such as milk, yogurt, cheese, and eggs (especially yolk)<sup>21</sup>.

In relation to selenium, classic KD with formula had an adequacy of 86.3%, compared to classic 3:1 and 4:1 KD without formula with a deficient adequacy of 26.6% and 59.9%, respectively. These results are in line with previous studies that have reported plasma selenium and magnesium deficiency in patients with classic KD without supplementation<sup>12,27</sup>. Arslan, in his most recent study, found that serum selenium levels decreased after 6 and 12 months of KD initiation, and 49% of patients had selenium deficiency without clinical findings and with normal electrocardiogram<sup>28</sup>. Other studies identified a link with cardiomyopathy<sup>13</sup>. A similar situation occurs with zinc intake, where classic 3:1 and 4:1 KD without formula showed an adequacy level of 33.3% and 33.8%, re-

spectively, compared with classic KD with formula (75%). Christodoulides reported a decrease in plasma selenium, zinc, and magnesium levels in children at 12 months of treatment with the classic and the MCT KD not supplemented<sup>12,22,27</sup>. However, Hayashi describes a decrease in plasma levels of selenium, zinc, and copper in patients with DRE after 6 months of initiation KD with a special formula (Ketonformula)<sup>29</sup>, suggesting an increased risk of long-term deficits if not supplemented.

The level of iron adequacy we obtained was very low in all KD modalities except for MAD with formula, however, the literature does not report anemia or iron deficiency in patients with KD<sup>12,22</sup> but there is a paper that found lower plasma ferritin levels in supplemented patients who received classic KD and KD with MCT<sup>26</sup>.

Vitamin D showed median adequacy of intake with RDI > 100% in both the classic KD and MAD with formula, understanding that this complements the intake of micronutrients. In the case of MAD without formula, the intake mainly came from foods such as salmon, tuna, egg, and egg yolks (100 g = 9 µg, 6.7 µg, 2.5 µg, 5.4 µg, respectively). This can be explained when compared to the RDI by age, which is 5 µg/d<sup>14</sup>, but it should be evaluated with a measurement of 25-OHD, as this constitutes a group of special patients with a higher requirement of this vitamin<sup>30</sup>.

Currently, there are ketogenic formulas on the market to be prepared in liquid form, which are enriched with vitamins and minerals, indicated for children under 6 months of age as the only source of ketogenic food or as a complement to the classic KD and MAD in children older than 6 months. However, it is important to determine the intake of micronutrients and vitamins to cover the RDIs by age and sex, in case of the need to use additional pharmacological supplementation of vitamins and minerals.

Limitations of this study include the small sample size to determine micronutrient intake and the lack of plasma or urinary micronutrient markers to confirm deficits associated with our patients. However, one of the strengths of this work is the comparison of multiple modalities regarding KD micronutrient intake in a multidisciplinary KD program.

## Conclusions

Our research showed that in classic 4:1 and 3:1 with and without formula, as well as MAD without formula, there is a deficit in most of the micronutrients, confirming the imperative need to calculate micronutrient intake and supplement to achieve the RDIs.

Although the MAD with formula met most of the



RDI, it is imperative to quantify the micronutrient intake and adjust and supplement, if necessary.

It is thus determined that the strict follow-up of the micronutrient intake of KD and its supplementation with special formula and/or pharmacological supplementation should be monitored regularly with the measurement of blood levels of these micronutrients and prevent potential nutritional deficiencies.

## Ethical Responsibilities

**Human Beings and animals protection:** Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

**Data confidentiality:** The authors state that they have

followed the protocols of their Center and Local regulations on the publication of patient data.

**Rights to privacy and informed consent:** This study was approved by the respective Research Ethics Committee, which, according to the study's characteristics, has accepted the non-use of Informed Consent.

## Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

## Financial Disclosure

Authors state that no economic support has been associated with the present study.

## References

- Alberti MJ, Agostinho A, Argumedo L, et al. Recommendations for the clinical management of children with refractory epilepsy receiving the ketogenic diet. *Arch Argent Pediatr*. 2016;114(1):56-63. English, Spanish. doi: 10.5546/aap.20
- Becker F, Schubert J, Weckhuysen S, et al. Do Glut1 (glucose transporter type 1) defects exist in epilepsy patients responding to a ketogenic diet? *Epilepsy Res*. 2015;114:47-51. doi: 10.1016/j.epilepsyres.2015.04.012. Epub 2015 May 1. PMID: 26088884.
- Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia*. 2007;48(1):43-58. doi: 10.1111/j.1528-1167.2007.00915.x. PMID: 17241207.
- Zhang Y, Xu J, Zhang K, et al. The Anticonvulsant Effects of Ketogenic Diet on Epileptic Seizures and Potential Mechanisms. *Curr Neuropharmacol*. 2018;16(1):66-70. doi:10.2174/1570159X15666170517153509. PMID:28521671; PMCID: PMC5771386.
- Kossoff EH, Zupec-Kania BA, Auvin S, et al. Optimal clinical management of children receiving dietary therapies for epilepsy: Updated recommendations of the International Ketogenic Diet Study Group. *Epilepsia Open*. 2018;3(2):175-92. doi: 10.1002/epi4.12225. PMID: 29881797; PMCID: PMC5983110.
- Ulamiek-Kozioł M, Czuczwar SJ, Januszewski S, et al. Ketogenic Diet and Epilepsy. *Nutrients*. 2019;11(10):2510. doi: 10.3390/nu11102510. PMID: 31635247; PMCID: PMC6836058.
- Goswami JN, Sharma S. Current Perspectives On The Role Of The Ketogenic Diet In Epilepsy Management. *Neuropsychiatr Dis Treat*. 2019;15:3273-85. doi: 10.2147/NDT.S201862. PMID: 31819454; PMCID: PMC6883945.
- D'Andrea Meira I, Romão TT, Pires do Prado HJ, et al. Ketogenic Diet and Epilepsy: What We Know So Far. *Front Neurosci*. 2019;13:5. doi: 10.3389/fnins.2019.00005. PMID: 30760973; PMCID: PMC6361831.
- Hauptman JS. From the bench to the bedside: Breaking down the blood-brain barrier, decoding the habenula, understanding hand choice, and the role of ketone bodies in epilepsy. *Surg Neurol Int*. 2010;1:86. doi: 10.4103/2152-7806.74143. PMID: 21206538; PMCID: PMC3011105.
- McNally MA, Hartman AL. Ketone bodies in epilepsy. *J Neurochem*. 2012;121(1):28-35. doi: 10.1111/j.1471-4159.2012.07670.x. Epub 2012 Feb 7. PMID: 22268909; PMCID: PMC3969728.
- Veggiotti P, Burlina A, Coppola G, et al. The ketogenic diet for Dravet syndrome and other epileptic encephalopathies: an Italian consensus. *Epilepsia*. 2011;52 Suppl 2:83-9. doi: 10.1111/j.1528-1167.2011.03010.x. PMID: 21463288.
- Verrotti A, Iapadre G, Di Francesco L, et al. Diet in the Treatment of Epilepsy: What We Know So Far. *Nutrients*. 2020;12(9):2645. doi: 10.3390/nu12092645. PMID: 32872661; PMCID: PMC7551815.
- Bergqvist AG, Chee CM, Lutchka L, et al. Selenium deficiency associated with cardiomyopathy: a complication of the ketogenic diet. *Epilepsia*. 2003;44(4):618-20. doi: 10.1046/j.1528-1157.2003.26102.x. PMID: 12681013
- Institute of Medicine FaNB. Dietary Reference intakes. Recommended Intakes for Individuals. National Academies Press. 2000. <https://nap.nationalacademies.org>
- Stevenson RD. Use of segmental measures to estimate stature in children with cerebral palsy. *Arch Pediatr Adolesc Med*. 1995;149(6):658-62. doi: 10.1001/archpedi.1995.02170190068012. PMID: 7767422.
- Patrones de crecimiento de escolares y adolescentes entre 5 años 1 mes y 19 años World Health Organization. 2007. <https://www.who.int/tools/growth-reference-data-for-5to19-years>
- De Onis M, Garza C, Victora CG, et al. The WHO Multicentre Growth Reference Study: planning, study design, and methodology. *Food Nutr Bull*. 2004;25(1 Suppl):S15-26. doi: 10.1177/15648265040251S103. PMID: 15069916.
- Patrones de crecimiento. Para la evaluación nutricional de niños, niñas y adolescentes, desde el nacimiento hasta los 19 años de edad. Ministerio de Salud Subsecretaría de Salud Pública División Políticas Públicas Saludables y Promoción Departamento de Nutrición y Alimentos 2018. <https://diprece.minsal.cl>
- Zemel BS, Pipan M, Stallings VA, et al. Growth Charts for Children With Down Syndrome in the United States. *Pediatrics*. 2015;136(5):e1204-11. doi: 10.1542/peds.2015-1652. Erratum in: *Pediatrics*. 2022;150(5): PMID: 26504127; PMCID: PMC5451269.

20. Brooks J, Day S, Shavelle R, et al. Low weight, morbidity, and mortality in children with cerebral palsy: new clinical growth charts. *Pediatrics*. 2011;128(2):e299-307. doi: 10.1542/peds.2010-2801. Epub 2011 Jul 18. PMID: 21768315.
21. Zacarías I, Barrios L, González CG, et al. Tabla de composición de alimentos 2018. Instituto de Nutrición y tecnología de los Alimentos INTA, Universidad de Chile. ISBN: 9789561910898
22. Christodoulides SS, Neal EG, Fitzsimmons G, et al. The effect of the classical and medium chain triglyceride ketogenic diet on vitamin and mineral levels. *J Hum Nutr Diet*. 2012;25(1):16-26. doi: 10.1111/j.1365-277X.2011.01172.x. Epub 2011 May 27. PMID: 21615805.
23. Van der Louw E, van den Hurk D, Neal E, et al. Ketogenic diet guidelines for infants with refractory epilepsy. *Eur J Paediatr Neurol*. 2016;20(6):798-809. doi: 10.1016/j.ejpn.2016.07.009. Epub 2016 Jul 17. PMID: 27470655
24. Kossoff EH, Zupec-Kania BA, Amark PE, et al; Charlie Foundation, Practice Committee of the Child Neurology Society; Practice Committee of the Child Neurology Society; Optimal clinical management of children receiving the ketogenic diet: recommendations of the International Ketogenic Diet Study Group. *Epilepsia*. 2009;50(2):304-17. doi: 10.1111/j.1528-1167.2008.01765.x. Epub 2008. PMID: 18823325.
25. Olivares C, Sonia BZN. Consumo de verduras y frutas en grupos específicos de consumidores chilenos: elementos a considerar en su promoción. *Rev Chil Nutr*. 2006 33:260-4.
26. Liu YM, Williams S, Basualdo-Hammond C, et al. A prospective study: growth and nutritional status of children treated with the ketogenic diet. *J J Am Diet Assoc*. 2003;103(6):707-12. doi: 10.1053/jada.2003.50136. PMID: 12778041.
27. Armeno M, Araujo C, Sotomontesano B, et al. [Update on the adverse effects during therapy with a ketogenic diet in paediatric refractory epilepsy]. *Rev Neurol*. 2018;66(6):193-200. Spanish. PMID: 29537059.
28. Arslan N, Kose E, Guzel O. The effect of ketogenic diet on serum selenium levels in patients with intractable epilepsy. *Biol Trace Elem Res*. 2017;178(1):1-6. doi: 10.1007/s12011-016-0897-7. Epub 2016 Nov 21. PMID: 27873289.
29. Hayashi A, Kumada T, Nozaki F, et al. [Changes in serum levels of selenium, zinc and copper in patients on a ketogenic diet using Ketonformula]. *No To Hattatsu*. 2013;45(4):288-93. Japanese. PMID: 23951940.
30. Ozel S, Switzer L, Macintosh A, et al. Informing evidence-based clinical practice guidelines for children with cerebral palsy at risk of osteoporosis: an update. *Dev Med Child Neurol*. 2016;58(9):918-23. doi: 10.1111/dmcn.13196. Epub 2016 Jul 20. PMID: 27435427.