Long-Term Effects of a Classic Ketogenic Diet on Ghrelin and Leptin Concentration: A 12-Month Prospective Study in a Cohort of Italian Children and Adults with GLUT1-Deficiency Syndrome and Drug Resistant Epilepsy

Ramona De Amicis 1,*, Alessandro Leone 1, Chiara Lessa 1, Andrea Foppiani 1, Simone Ravella 1, Stefano Rasasenghi 1, Claudia Trentani 2, Cinzia Ferraris 2,†, Pierangelo Veggiotti 3,4, Valentina De Giorgis 5,†, Anna Tagliabue 2,†, Alberto Battezzati 1 and Simona Bertoli 1

1 International Center for the Assessment of Nutritional Status (ICANS), Department of Food Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Sandro Boticelli 21, 20133 Milan, Italy
2 Human Nutrition and Eating Disorder Research Center, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Via Agostino Bassi 21, 27100 Pavia, Italy
3 Pediatric Neurology Unit, Vittore Buzzi Hospital, Via Lodovico Castelvetro 32, 20154 Milan, Italy
4 Biomedical and Clinical Sciences Department, Luigi Sacco Hospital, University of Milan, via G. B. Grassi 74, 20157 Milan, Italy
5 Department of Child Neurology and Psychiatry, IRCCS Mondino Foundation, Via Mondino 2, 27100 Pavia, Italy

* Correspondence: ramona.deamicis@unimi.it; Tel.: +39-02-5031-6652
† Member of ERN-Epicare.

Received: 3 June 2019; Accepted: 23 July 2019; Published: 25 July 2019

Abstract: The classical ketogenic diet (cKD) is an isocaloric, high-fat, very low-carbohydrate diet that induces ketosis, strongly influencing leptin and ghrelin regulation. However, not enough is known about the impact of a long-term cKD. This study evaluated the effects of a 12-month cKD on ghrelin and leptin concentrations in children, adolescents and adults affected by the GLUT1-Deficiency Syndrome or drug resistant epilepsy (DRE). We also investigated the relationship between the nutritional status, body composition and ghrelin and leptin variations. We carried out a longitudinal study on 30 patients: Twenty-five children and adolescents (15 females, 8 ± 4 years), and five adults (two females, 34 ± 16 years). After 12-months cKD, there were no significant changes in ghrelin and leptin, or in the nutritional status, body fat, glucose and lipid profiles. However, a slight height z-score reduction (from −0.603 ± 1.178 to −2.343 ± 1.354, p ≤ 0.001) and a drop in fasting insulin occurred. We found no correlations between ghrelin changes and nutritional status and body composition, whereas leptin changes correlated positively with variations in the weight z-score and body fat (\( \rho = 0.4534, \ p = 0.0341; \rho = 0.5901, \ p = 0.0135; \) respectively). These results suggest that a long-term cKD does not change ghrelin and leptin concentrations independently of age and neurological condition.

Keywords: drug-resistant epilepsy; GLUT1-Deficiency Syndrome; ketogenic diet; leptin; ghrelin

1. Introduction

The classic ketogenic diet (cKD) is an isocaloric, high-fat, very low-carbohydrate and normal-protein diet. It requires all foods and beverages to be carefully calculated and precisely weighed on a gram scale in order to obtain a specific ratio between fats (gr) and carbohydrates (gr)
plus proteins (gr), generally equal to 3:1 or 4:1 [1]. It has been used safely and effectively for decades as a recognized treatment for drug-resistant epilepsy (DRE) [2–4], in GLUT1-Deficiency Syndrome (GLUT1-DS) [5,6] and in pyruvate dehydrogenase complex deficiency (PDCD) [7,8]. While in DRE it remains currently unclear how the cKD works despite a much better understanding of anticonvulsant mechanisms [4], in the GLUT1-DS and PDCD it is the treatment of choice in order to switch brain metabolism from glucose to ketone bodies (KBs) and leading to a powerful improvement in neurologic symptoms. In fact, GLUT1-DS is a rare disease caused by mutations in the SLC2A1 gene that encodes the glucose carrier protein type 1 (GLUT1), the main carrier of glucose across the blood-brain barrier, which is characterized by early-onset seizures, developmental delay, and a complex movement disorder [9]. cKD has also been explored in other neurological and neurodegenerative diseases, such as Alzheimer’s, Parkinson’s, amyotrophic lateral sclerosis, spinal cord injury, glycogenosis [10,11], and more recently in metabolic syndrome and type 2 diabetes [12,13].

The cKD produces the highest ketogenic effect among the various ketogenic diets (KDs). It induces a constant production of KBs, called ketosis, aimed at mimicking the starvation state while providing adequate calories to support growth and energy needs in childhood and adult age, respectively [2,14].

KBs, such as acetone, acetoacetate and β-hydroxybutyrate, are involved in several mechanisms [15]. Firstly, they have a “neuroketotherapeutic” effect [16], modulating the release of inhibitory neurotransmitters, reducing neuronal excitation and seizure activity. They also have an antioxidant effect, reducing the production of reactive oxygen species (ROS) and damage caused by them, and increasing mitochondrial biogenesis and function [16]. In the GLUT1-DS and pyruvate dehydrogenase complex deficiency, KBs are the main neuronal energy source [17]. Finally, KBs also appear to decrease the pro-inflammatory cytokines that have an anti-inflammatory effect [15].

cKD may possibly also influence the modulation of multifunctional hormones such as ghrelin and leptin [18–21]. These hormones are involved in food intake, glucose metabolism, neuronal activity and also the maintenance of nutritional status [22,23]. Specifically, KBs may be implicated in the reduction of food intake and consequent weight loss in adults [24] or in the potential adverse effect of growth retardation in children during a long-term cKD [25–27].

Ghrelin is the only gastrointestinal peripheral peptide with orexigenic properties, whose circulating plasma levels are increased by fasting and decreased by feeding [22]. Ghrelin’s concentration is also inversely correlated with body weight and age [22,28]. It has been found to be an endogenous ligand for the growth hormone secretagogue receptor (GHSR 1a), which can stimulate the release of growth hormones (GH) from the anterior pituitary gland [29], inducing a broad spectrum of functions in relation to food intake, adiposity and glucose metabolism [22]. In addition, it has anti-seizure effects, which stimulate the release of protective neurotransmitters, which help to improve the survival and proliferation of neurons [18,30]. Previous studies have investigated the short-term changes of ghrelin during the cKD with contrasting results [18]. In fact, in children with DRE, decreased levels of ghrelin were found after 3-months of cKD [19], whereas in adults the level increased [18,31].

Leptin, on the other hand, is an anorexigenic hormone, which is produced in small amounts at a central level and widely released by a white adipose tissue into the circulatory system in order to suppress hunger and, consequently, food intake [28]. Its blood concentration is directly proportional to the amount of body fat mass, which is why its plasma levels increase in obese subjects [28]. The most significant roles of leptin include the regulation of energy homeostasis, the neuroendocrine function and metabolism [32], as it interacts with other hormonal mediators and regulators of energy status and metabolism such as insulin, glucagon, insulin-like growth factors and growth hormones [23]. Some studies have hypothesized an increase in leptin in humans on the cKD, as shown also in rodents placed on a cKD [33,34]. However, most human studies have shown that leptin decreases during KD, probably due to the concomitant reduction of the adipose tissue [18,20,35].

Although ghrelin and leptin changes during cKD are of great interest due to their impact on the nutritional and metabolic outcomes of cKD and their potential effects as therapeutic targets in neurological diseases [30], to the best of our knowledge they have been analysed several times in DRE.
and GLUT1-DS [19–21], where the cKD must be followed for long term, but it is difficult to draw substantial conclusions due to short follow-up examinations or incomplete data.

We therefore carried out a longitudinal study to investigate the effects of a 12-months cKD on ghrelin and leptin concentrations in children, adolescents and adult patients affected by GLUT1-DS or DRE. We also explored the relationship between the nutritional status, body composition and ghrelin and leptin concentrations at the baseline as well as at 12 months.

2. Materials and Methods

2.1. Study Design

This was a 12-month, prospective, multi-centre study in patients with GLUT1-DS and DRE treated with isocaloric cKD, according to the following criteria:

- age > five months;
- absence of absolute contraindications, such as carnitine deficiency, β-oxidation defects, pyruvate carboxylase deficiency, and porphyria, as stated in the latest consensus [8];
- parents’ or caregivers’ compliance ensured by the physician who well explained to caregivers their critical role in the administration of cKD to their children or parents, including the involvement of time in the preparation of meals for the child who will require meals other than the rest of the family, the cost of food, the avoidance of carbohydrates, additional supplementation and potential side effects [8].

The main outcome measures were the changes in ghrelin and leptin from the baseline. The assessment included neurological examinations; ghrelin and leptin dosage, glucose, insulin, and lipid profile (triglycerides [TGs], total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]); nutritional status evaluation by anthropometry and abdominal body fat distribution by ultrasonography. In children and adolescents, we conducted the assessment at the baseline and six and 12 months after cKD intervention, while in adults the assessment was conducted at the baseline and after 12 months.

The study was approved by the ethical committee of the Fondazione IRCCS Policlinico San Matteo of Pavia (reference number 20180083746) and complied with all tenets of the Helsinki declaration. All the children’s and adolescents’ caregivers and adult patients provided written informed consent before the beginning of the study.

2.2. Settings

Patients were recruited at the Department of Child Neurology and Psychiatry, Fondazione IRCCS Istituto Neurologico C.Mondino in Pavia, and at the Pediatric Neurology Unit, “V. Buzzi” Hospital in Milan, Italy from October 2010 to February 2018.

KD treatment was implemented at the Human Nutrition and Eating Disorders Research Centre in Pavia, Italy.

Biochemical and anthropometric measurements were performed at the International Center for the Assessment of Nutritional Status (ICANS) of the University of Milan, Italy.

2.3. Patients

We prospectively enrolled 30 patients: Twenty-five children and adolescents (15 females and 10 males, mean age 8 ± 4 years), of which 19 were affected by GLUT1-DS, and five adults (two females and three males, mean age 34 ± 16 years), were all diagnosed with GLUT1-DS.

All the patients met the clinical criteria for the DRE and GLUT1-DS diagnosis:

- concerning GLUT1DS, all patients underwent a lumbar puncture in the fasting state (after 5–6 h of fasting); a blood sample for glucose measurement was obtained immediately before the procedure
to avoid stress-related hyperglycaemia [9]. A cerebrospinal fluid-to-blood glucose rate of <0.6 was considered suspicious for GLUT1 DS. Subsequently, for a definitive confirmation, all the patients were submitted to the SLC2A1 mutation analysis;

- DRE was defined as “failure of adequate trials of two tolerated and appropriately chosen and used antiepileptic drugs schedules to achieve sustained seizure freedom” according to the Current International League Against Epilepsy (ILAE) Consensus [36]. It was diagnosed with the Lennox Gastaut Syndrome (LGS), Electrical Status Epilepticus during Sleep (ESES), and epileptic encephalopathy. All patients underwent magnetic resonance imaging (MRI) in order to classify the cortical malformation. All patients were treated with specific pharmacotherapy. Seizure types and epilepsy syndromes were classified according to the criteria proposed by the International League Against Epilepsy (ILAE) [36].

2.4. Ketogenic Diet

A non-fasting dietary protocol with an at-home gradual increase of the ketogenic ratio was implemented at the Human Nutrition Research Centre outpatient clinic according to a standardized protocol [37].

At the baseline, we evaluated the usual caloric intake and food intolerances and preferences for each patient using seven-day weighted food diaries analysed by a dietitian using the WinFood version 3.0. The initial calorie prescription was based on age-related energy requirements considering weight and height (both current and recent trends), and physical activity levels. The macronutrient composition included a minimum of 0.8 g–1 g of protein from animal sources per kilogram of body weight (e.g., eggs, milk, meat, poultry and fish).

All patients and caregivers received pre-diet counselling in order to ensure understanding regarding the meal preparation, cooking strategy, importance of avoiding carbohydrates, additional supplementation, and potential side effects.

All patients started a 1:1 cKD at home and gradually proceed to 2:1, 3:1 or 4:1 ketogenic ratio in order to obtain blood values of beta-hydroxybutyrate >2.0 mmmol/L. All children’s and adolescents’ caregivers and adults were instructed to check capillary ketonemia and ketonuria on a daily basis during the first month and then twice a week, and to report the values in a specific format. To measure the dispersion of the ketonemia during cKD, for each patient we calculated the coefficient of variation (CV) at the baseline and at 12 months as the ratio of the standard deviation to the mean of the reported KBs. Ketonemia at the baseline was considered as the average of the daily KBs of the first two weeks after the induction phase, while the final as the average of the last two weeks before the evaluation at 12 months.

24-h dietary recalls were collected during each follow-up examination to evaluate the compliance.

2.5. Main Outcome Measurements

2.5.1. Neurological Assessment

In accordance with the 2011 Italian consensus on KD therapy [38], neurologic evaluations and electroencephalography (EEG) were performed after one, six and twelve months. The following neurological symptoms were monitored: Paroxysmal dyskinesia, dysarthria, ataxia, spasticity dystonia, muscle strength, as well as seizure types and frequency. Children’s and adolescents’ caregivers and adult patients completed a daily record regarding alertness, activity and seizure occurrence.

2.5.2. Anthropometric Measurements

Anthropometric measurements were taken by the same trained dietician in accordance with conventional criteria and measuring procedures [39].

Body weight (BW, kg) and body height (BH, cm) were measured to the nearest 100 g and 0.5 cm, respectively. The body mass index (BMI) was calculated using the formula: BW (kg)/BH^2 (m^2).
For children and adolescents, sex-specific BMI-for-age Z scores were calculated from the growth charts of 2000 Centers for Disease Control and Prevention (CDC) [40]. In accordance with CDC guidelines, a z-score of ≤−2 was considered as severely underweight, a score between −2 and −1 was considered underweight, between −1 and +1 was considered as normal weight, +1 and +2 was considered overweight, and >+2 was considered as obese. For adults, the BMI was classified into four categories: Underweight (BMI <18.5 kg/m²), normal weight (BMI 18.5 kg/m²–24.9 kg/m²), overweight (BMI 25.0 kg/m²–29.9 kg/m²), and obese (BMI >30.0 kg/m²) [41].

Skinfold thickness was measured as proposed in a previous work [36] by a Holtain LTD caliper, at the triceps, biceps, subscapular, and supra-iliac sites on the non-dominant side of the body. All measurements were taken in triplicates for all sites, and the average of the three values was calculated. The intra-observer variation for the skinfold measurement ranged between 2.5% and 2.9%. The body density and fat mass (FM, kg) were calculated for children and for adolescents and adults by the Brook method [42] and the Brozek Formula [43], and by the Durnin and Womersley method [44] and the Siri formula [45], respectively. We calculated the fat mass index (FMI, kg/m²) in children and adolescents by dividing FM by the squared height.

### 2.5.3. Abdominal Fat Distribution

Abdominal subcutaneous fat (SAT) and abdominal visceral fat (VAT) were measured on fasting patients by the same operator using a Logiq 3 Pro Ultrasonography equipped with a 3.5 MHz convex-array probe and with a 7.5 MHz linear probe (GE Healthcare, Milwaukee, WI, USA) following a validated standardized protocol [46]. Specifically, SAT was measured as the distance between the epidermis and the external face of the rectus abdominis muscle. VAT was measured as the distance between the anterior wall of the aorta and the posterior surface of the rectus abdominis muscle measured at the level of the xipho-umbilical line or linea alba. The within-day intra-operator coefficient of variation for repeated measures of VAT and SAT in our laboratory is 0.8%.

### 2.5.4. Biochemical Parameters

Fasting blood samples were taken by venepuncture of the antecubital vein in either the sitting or lying position, using vacuum tubes. After centrifugation (800×g 10 min at 5 °C), aliquots of samples were stored at 80 °C until further analysis.

Ghrelin and leptin (pg/mL) were measured using an enzymatic immunoassay kit (R & D Systems; Wiesbaden, Germany). Interassay precision (CV) was 3.3%, and 3.4%, respectively.

We used an autoanalyzer (Cobas Integra 400 plus, Roche Diagnostics, Mannheim) to determine the serum glucose, TC, HDL-C, LDL-C, TG concentrations. Circulating insulin was measured in duplicate by an autoanalyzer (Cobas e411 Hitachi, Roche Diagnostics). The homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as [fasting glucose (mg/dL) × fasting insulin (mU/L)/405] [47].

High glucose was defined as glucose ≥100 mg/dL [48,49], high insulin as >23 uU/mL, and high HOMA-IR as ≥3.16 for children or ≥2.5 for adults [47,50]. High TC was defined as ≥200 mg/dL, high LDL-C as ≥130 mg/dL, and high TG as >150, while low HDL as < 40 mg/dL for male and < 50 mg/dL for females [48,49].

Capillary ketonemia was measured with an in vitro diagnostic medical device for β-ketone self-testing (GlucoMen LX PLUS, Menarini Diagnostics, test range 0.1 mmol/L–8.0 mmol/L). Ketonuria was measured by a urine ketone stick test (Ketostix®, Bayer Diabetes, Berkshire, UK).

### 2.6. Statistical Analysis

Continuous variables are presented as a mean ± standard deviation. Leptin, insulin and HOMA-IR were not normally distributed and were normalized using log-transformation. Similarly, ghrelin was normalized using a square root transformation. An independent T-test was used to compare the means of nutritional and biochemical variables between GLUT1-DS and DRE children. A one-way
repeated measures ANOVA with Bonferroni’s test for comparison was run to determine if there were differences in the variables of interest during the 12-month ketogenic dietary treatment. The Spearman correlation was used to investigate the association of ghrelin and leptin concentrations and changes in the nutritional status and body composition. All P-values were two-tailed, and \( p < 0.05 \) was considered significant. Statistical analysis was performed using Stata 12.1 (Stata Corporation, College Station, TX, USA).

3. Results

3.1. Pre-Intervention

Table 1 shows the characteristics of the patients at recruitment.

| Table 1. Characteristics of the patients at recruitment.                     | Children and Adolescents | Adults            |
|--------------------------------------------------------------------------------|--------------------------|-------------------|
|                                                                                  | Total        | GLUT1-DS          | DRE          | GLUT1-DS          |
|                                                                                  | (n = 25)     | (n = 19)          | (n = 6)      | (n = 5)           |
| Age (years)                                                                       | 8 ± 4        | 8 ± 4             | 6 ± 5        | 0.487 ± 34 ± 16   |
| Nutritional status and body composition                                         |             |                   |              |                   |
| Weight (kg)                                                                       | 27.5 ± 20.4  | 28.2 ± 19.0       | 25.4 ± 24.5  | 0.573 ± 63.5 ± 9.1|
| Weight z-score                                                                    | −0.365 ± 1.830| −0.340 ± 1.868    | 0.454 ± 1.888| 0.761 ± -        |
| Height (cm)                                                                       | 120.0 ± 29.1 | 122.5 ± 27.8      | 111.9 ± 34.3 | 0.751 ± 168.4 ± 6.9|
| Height z-score                                                                    | −0.603 ± 1.177| −0.508 ± 1.162    | −0.825 ± 1.294| 0.424 ± -        |
| BMI (kg/m²)                                                                       | 16.5 ± 4.3   | 16.5 ± 4.3        | 16.4 ± 4.9   | 0.745 ± 22.6 ± 4.9|
| BMI z-score                                                                       | −0.587 ± 1.839| −0.423 ± 1.353    | −1.141 ± 2.685| 0.283 ± -        |
| Body fat (%)                                                                      | 20.8 ± 7.0   | 21.0 ± 6.2        | 34.4 ± 11.6  | 0.773 ± 27.2 ± 12.8|
| FMI (kg/m²)                                                                       | 5.4 ± 2.6    | 5.2 ± 2.5         | 5.9 ± 3.1    | 0.874 ± -        |
| SAT (cm)                                                                           | 0.8 ± 0.7    | 0.8 ± 0.8         | 0.7 ± 0.7    | 0.561 ± 5.4 ± 6.9|
| VAT (cm)                                                                           | 2.9 ± 1.6    | 2.7 ± 1.7         | 3.5 ± 1.1    | 0.307 ± 9.65 ± 11.2|
| Biochemical parameters                                                             |             |                   |              |                   |
| Serum glucose (mg/dL)                                                             | 83 ± 11      | 82 ± 12           | 89 ± 8       | 0.255 ± 90 ± 6    |
| Log-Insulin (mU/L)                                                                | 1.34 ± 1.19  | 1.33 ± 1.27       | 1.40 ± 0.94  | 0.483 ± 2.14 ± 0.43|
| Log-HOMA-IR                                                                       | 0.24 ± 0.27  | 0.27 ± 1.35       | 0.64 ± 0.11  | 0.473 ± 0.63 ± 0.45|
| TC (mg/dL)                                                                         | 170 ± 57     | 174 ± 39          | 154 ± 20     | 0.255 ± 201 ± 14  |
| HDL-C (mg/dL)                                                                     | 58 ± 14      | 60 ± 15           | 49 ± 9       | 0.631 ± 68 ± 8    |
| LDL-C (mg/dL)                                                                     | 99 ± 33      | 100 ± 36          | 98 ± 16      | 0.269 ± 121 ± 12  |
| TG (mg/dL)                                                                         | 61 ± 18      | 60 ± 17           | 66 ± 25      | 0.743 ± 57 ± 14   |
| √ghrelin (pg/mL)                                                                  | 19.74 ± 6.44 | 17.98 ± 5.91      | 25.3 ± 4.930 | 0.895 ± 17.84 ± 2.83|
| Log-leptin (pg/mL)                                                                | 8.58 ± 1.16  | 8.60 ± 1.20       | 8.49 ± 1.12  | 0.796 ± 9.26 ± 1.30|
| KBS (mmol/L)                                                                       | 0.06 ± 0.05  | 0.06 ± 0.04       | 0.06 ± 0.05  | 0.708 ± 0.05 ± 0.05|

BMI = body mass index; FMI = fat mass index; SAT = abdominal subcutaneous fat; VAT= abdominal visceral fat; HOMA-IR = homeostatic model assessment-insulin resistance; TC = total cholesterol, LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; KBS = ketone bodies.

Among the children and adolescents, five patients were underweight (BMI z-score < −1.9) according to the CDC BMI standards, one adult was underweight (BMI < 18.5), none were overweight or obese. None of the children, adolescents or adults showed fasting glucose >100 mg/dL. Three children and one adult had a high HOMA-IR index value (>2.5); three children and one adult had both TC and LDL-C levels above the cut off; three children had low HDL-C levels, and none of them showed triglyceride levels above the cut off. Both GLUT1-DS and DRE children and adolescents reported ghrelin and leptin values in the normal range (395.19 ± 204.34 pg/mL; 6.82 ± 2.70 ng/mL; respectively) [51,52]. No differences in the nutritional status, body composition and biochemical parameters were found between GLUT1-DS and DRE children and adolescents.
3.2. Intervention

Table 2 shows the macronutrient composition and ketogenic ratio of the cKD implemented by the dietitian.

|                      | Children and Adolescents \((n = 25)\) | Adults \((n = 5)\) |
|----------------------|----------------------------------------|------------------|
|                      | Total \((n = 25)\)                     | GLUT1-DS \((n = 19)\) | DRE \((n = 6)\) | GLUT1-DS \((n = 5)\) |
| Energy intake \(\text{kcal/day}\) | Mean 1373 Sd 518 | Mean 1433 Sd 535 | Mean 1132 Sd 401 | Mean 413 Sd 325 |
| Energy intake \(\text{kcal/kg} \text{BW}\) | Mean 61.5 Sd 21.3 | Mean 59.5 Sd 17.5 | Mean 69.2 Sd 34.2 | Mean 31.6 Sd 9.5 |
| Protein \(\text{g/day}\) | Mean 26.4 Sd 12.4 | Mean 27.1 Sd 13.5 | Mean 23.9 Sd 7.2 | Mean 57.9 Sd 16.9 |
| Protein \(\text{g/kg} \text{BW}\) | Mean 1.2 Sd 0.5 | Mean 1.1 Sd 0.3 | Mean 1.5 Sd 0.9 | Mean 1.3 Sd 0.7 |
| Protein \(\%\) | Mean 8 Sd 2 | Mean 7 Sd 1 | Mean 8 Sd 2 | Mean 13 Sd 5 |
| Fat \(\text{g/day}\) | Mean 131.3 Sd 51.0 | Mean 137.4 Sd 51.8 | Mean 107.0 Sd 43.6 | Mean 316 Sd 43.5 |
| Fat \(\text{g/kg} \text{BW}\) | Mean 5.0 Sd 2.0 | Mean 5.7 Sd 1.7 | Mean 6.4 Sd 3.2 | Mean 753 Sd 29.1 |
| Fat \(\%\) | Mean 86 Sd 5 | Mean 86 Sd 3 | Mean 85 Sd 8 | Mean 697 Sd 82 |
| SFA \(\text{g/day}\) | Mean 47.4 Sd 19.0 | Mean 47.1 Sd 20.7 | Mean 39.0 Sd 17.5 | Mean 371 Sd 40.3 |
| SFA \(\text{g/kg} \text{BW}\) | Mean 2.0 Sd 0.7 | Mean 1.7 Sd 0.8 | Mean 1.7 Sd 0.8 | Mean 407 Sd 0.6 |
| SFA \(\%\) | Mean 28 Sd 8 | Mean 28 Sd 7 | Mean 28 Sd 8 | Mean 796 Sd 19 |
| Carbohydrate \(\text{g/day}\) | Mean 21.6 Sd 12.6 | Mean 22.4 Sd 11.7 | Mean 18.6 Sd 17.1 | Mean 641 Sd 20.1 |
| Carbohydrate \(\text{g/kg} \text{BW}\) | Mean 1.1 Sd 0.9 | Mean 1.0 Sd 0.7 | Mean 1.4 Sd 1.6 | Mean 680 Sd 0.4 |
| Carbohydrates \(\%\) | Mean 7 Sd 4 | Mean 7 Sd 3 | Mean 7 Sd 6 | Mean 873 Sd 4 |
| Ketogenic ratio | Mean 2.9 Sd 0.8 | Mean 2.9 Sd 0.7 | Mean 2.8 Sd 1.1 | Mean 1000 Sd 2.5 |

\(\text{BW} = \text{body weight (kg)}; \text{SFA} = \text{saturated fatty acids.}\)

There were no differences in the diet composition between GLUT1-DS and DRE children and adolescents. All patients completed the 12-month protocol.

3.3. Post-Intervention

Children, adolescents and adults reached the therapeutic range of KBs (beta-hydroxybutyrate >2.0 mmol/L) and tolerated the diet well. Table S1 shows changes of the diet composition at six and 12 months.

Neurological data on the effect of cKD have already been published in two papers by our research group [53,54]. Table 3 shows changes at six and 12 months from the beginning of the cKD.

After 6-months of cKD, KBs increased significantly both in children and adolescents and in adults (from 0.06 ± 0.05 to 2.78 ± 0.64 mmol/L, \(p\)-value = < 0.0001; from 0.05 ± 0.05 to 2.35 ± 1.36 mmol/L, \(p\)-value = 0.022, respectively), and remained stable at 12-months, in line with the diet. At the end of the induction phase, KBs were 2.99 ± 0.78 mmol/L with a CV equal to 19.3 ± 8.6%. At 12 months KBs were 2.89 ± 0.66 mmol/L with a CV equal to 20.4 ± 10.0% (\(p\)-value = 0.904).

Concerning children and adolescents, in the first six months, no significant differences were found in the BMI z-score and the amount of body fat and its abdominal distribution. However, we found a slight reduction of height z-score after 12 months of cKD (from −0.603 ± 1.178 to −0.953 ± 1.354, \(p\)-value = <0.001). Regarding the biochemical parameters, although serum glucose did not change, fasting insulin decreased after cKD, and HOMA-IR was significantly modified. Neither the lipid nor leptin and ghrelin profiles changed significantly. None of these variables changed even at 12 months.
Table 3. Time course of nutritional status, body composition and biochemical parameters during 12-month classical ketogenic diet (cKD).

| Nutritional status and body composition | Children and Adolescents (n = 25) | Adults (n = 5) |
|----------------------------------------|----------------------------------|----------------|
| **Baseline** Mean | **6 Months** Mean | **12 Months** Mean | **Baseline** Mean | **12 Months** Mean |
| Weight (kg) | 27.5 | 29.4 | 29.0 | 63.5 | 9.1 | 62.4 | 5.0 |
| Weight z-score | -0.365 | -0.605 | -0.953 | <0.001 | 168.4 | 6.9 | 170.2 | 7.3 |
| Height (cm) | 120.0 | 124.0 | 125.2 | 167.4 | 6.9 | 170.2 | 7.3 |
| Height z-score | -0.605 | -0.505 | 0.04 | -0.953 | 3.5 | <0.001 | - | - |
| BMI (kg/m²) | 16.5 | 17.1 | 16.7 | 22.6 | 4.9 | 21.7 | 3.4 |
| BMI z-score | -0.587 | -0.228 | -0.953 | <0.001 | 22.6 | 4.9 | 21.7 | 3.4 |
| Body fat (%) | 20.8 | 21.7 | 21.2 | 21.2 | 5.8 | 21.7 | 3.4 |
| FMI (kg/m²) | 5.4 | 6.01 | 6.35 | 0.207 | - | - | - |
| SAT (cm) | 0.8 | 0.7 | 0.8 | 2.6 | 1.2 | 0.921 | - |
| VAT (cm) | 2.9 | 3.0 | 2.6 | 6.01 | 2.6 | 0.207 | - |

Biochemical parameters

| Serum glucose (mg/dL) | Log-Insulin (mU/L) | Log-HOMA-IR | TC (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | TG (mg/dL) | Vghrelin (pg/mL) | Log-leptin (pg/mL) | KBs (mmol/L) |
|-----------------------|-------------------|-------------|------------|------------|------------|------------|------------------|-------------------|--------------|
| 83 | 11 | 78 | 8 | 79 | 12 | 0.094 | 90 | 6 | 96 | 10 | 0.354 |
| 1.34 | 1.19 | 0.89 | 0.83 | 1.10 | 0.93 | <0.001 | 2.14 | 0.43 | 1.66 | 0.37 | <0.001 |
| 0.24 | 1.25 | -0.78 | 0.89 | -0.54 | 1.01 | <0.001 | 0.63 | 0.45 | 0.21 | 0.40 | 0.034 |
| 170 | 37 | 178 | 39 | 174 | 40 | 0.688 | 201 | 14 | 188 | 34 | 0.348 |
| 58 | 14 | 61 | 16 | 62 | 19 | 0.207 | 68 | 8 | 66 | 11 | 0.135 |
| 99 | 33 | 110 | 32 | 102 | 32 | 0.546 | 121 | 12 | 123 | 31 | 0.963 |
| 61 | 18 | 70 | 33 | 67 | 29 | 0.294 | 57 | 14 | 57 | 25 | 0.990 |
| 19.74 | 6.44 | 18.77 | 6.23 | 18.90 | 6.17 | 0.693 | 17.84 | 2.83 | 15.06 | 3.43 | 0.102 |
| 8.58 | 1.16 | 8.57 | 1.02 | 8.68 | 0.90 | 0.808 | 9.26 | 1.30 | 9.13 | 1.01 | 0.898 |
| 0.06 | 0.05 | 2.78 | 0.64 | 2.45 | 1.77 | <0.001 | 0.05 | 0.05 | 2.65 | 0.82 | 0.022 |

Similarly, in adults after 12-month cKD, no significant changes were observed in the body composition and abdominal fat amount (SAT and VAT). Maintenance of serum glucose occurred, while insulin and HOMA-IR changed significantly. Neither ghrelin nor leptin and nor TC/LDL/HDL/TG changed significantly.

3.4. Associations between Nutritional Status, Body Composition, Ghrelin and Leptin

Tables 4 and 5 show the associations between the nutritional status and body composition with concentrations and changes in ghrelin and leptin pre-intervention and post-intervention, both in children and adults, respectively.

At pre-intervention, in children and adolescents, the ghrelin concentration correlated negatively with the age and BMI z-score (\( \rho = -0.5180, p = 0.0080; \rho = -0.4939, p = 0.0410; \) respectively) and as expected, the leptin concentration correlated positively with the weight z-score, BMI z-score and body fat amount (\( \rho = 0.4707, p = 0.0234; \rho = 0.6351, p = 0.0020; \rho = -0.5896, p = 0.0101; \) respectively). Insulin correlated negatively with ghrelin concentration (\( \rho = 0.5134, p = 0.0210; \) respectively).

At post-intervention, no correlations were found between \( \Delta \)ghrelin concentration and changes in the nutritional status and body composition variables, whereas the \( \Delta \)leptin concentration correlated positively with variations in the weight z-score and body fat (\( \rho = 0.4534, p = 0.0341; \rho = 0.5901, p = 0.0135; \) respectively).

In adults, no correlations were found between the nutritional status, body composition, leptin, and ghrelin either in pre-intervention or in post intervention.
Table 4. Associations between nutritional status, body composition, ghrelin and leptin at the baseline and 12-months in children and adolescents.

|                | Pre-Intervention | Post-Intervention | p-value | p-value |
|----------------|------------------|------------------|---------|---------|
| Age            | 1.0000           |                  |         |         |
| Weight z-score | 0.2170           |                  | 0.1261  | 0.0420  |
| BMI z-score    | 0.3199           |                  | 0.6500  | 0.7020  |
| Height z-score | 0.3113           |                  | 0.6856  | 0.2350  |
| Body fat       | 0.1816           |                  | 0.0012  | 0.0950  |
| ghrelin        | 0.5076           |                  | 0.8784  | 0.4246  |
| Log-Leptin     | 0.0186           |                  | 0.0000  | 0.0260  |
| Log-Insulin    | 0.5412           |                  | 0.7368  | 0.3167  |

BMI = Body mass index. Age (years); Body fat (%); ghrelin (pg/mL); Log-Leptin (pg/mL); Log-Insulin (mU/L).

Table 5. Associations between nutritional status, body composition, ghrelin and leptin at the baseline and 12-months in adults.

|                | Pre-Intervention | Post-Intervention | p-value | p-value |
|----------------|------------------|------------------|---------|---------|
| Age            | 1.0000           |                  |         |         |
| Weight z-score | -0.1010          |                  | 0.7370  | 0.6060  |
| BMI z-score    | -0.1900          |                  | 0.0190  | 0.8850  |
| Height z-score | -0.9710          |                  | 0.9654  | 0.5390  |
| Body fat       | -0.0370          |                  | 0.0630  | 0.3940  |
| ghrelin        | 0.5012           |                  | 0.5960  | 0.1820  |
| Log-Leptin     | 0.1017           |                  | 0.4040  | 0.8340  |
| Log-Insulin    | 0.0490           |                  | 0.5060  | 0.8920  |

BMI = Body mass index. Age (years); Body fat (%); ghrelin (pg/mL); Log-Leptin (pg/mL); Log-Insulin (mU/L).
4. Discussion

The main aim of our longitudinal study was to investigate the effects of a 12-month cKD on ghrelin and leptin concentrations in children, adolescents and adult patients affected by GLUT1-DS or DRE. To the best of our knowledge, this is the first that it has been demonstrated that in the long-term, cKD did not change ghrelin and leptin concentrations independently of age and the neurological condition for which the cKD was prescribed.

These results are in contrast with those obtained by Marchiò et al. [19]. These authors studied only children affected by DRE and found a significant reduction in both ghrelin and des-acyl ghrelin plasma levels during a cKD. However, their study was short-term with a follow-up of only three months, and the differences in ghrelin concentrations are probably due to an initial metabolic adaptation to the high fat content of the cKD. Other authors, such as Sumithran [55] and Nymo et al. [56], examined changes in all appetite-regulating peripheral hormones and found a significant increase in ghrelin associated with a high change in body composition after a KD. However, they examined the effects of a very low energy diet (VLED) on overweight and obese adults with an only 8–10-week follow-up. VLEDs are also called “starvation diets” due to their extremely low daily amount of energy, equal to about 800 kcal/day, one third of the average energy requirement for a man (2500 kcal) and half for a woman (2000 kcal). VLEDs are usually undertaken by overweight and obese patients for rapid weight loss and, due to the strong reduction in food calories that VLED requires, cannot be followed for long periods. Compared to cKD, VLEDs provide a higher protein content (1.5 g/kg/day), in order to accelerate fat oxidation and KB production and better control hunger and satiety.

Regarding leptin, these results confirm our previous ones in a smaller sample (n = 10) and with a shorter follow-up (3-months) [34] but are in contrast with the results of Lambrechts et al. [20]. These authors found a leptin decrease after a 12-month KD in patients affected by DRE aged between one and 40 years, following various KDs, such as cKD and medium chain triglyceride (MCT) KD which uses a fat supplement consisting only of MCT fats that produce ketones more easily than the long-chain ones used in the cKD. However, their KD was different from the cKD, because they gradually added five-gram steps to the diet with a maximum of 20 g/day of carbohydrates when adequate ketosis was not reached or to prevent weight loss. Rauchenzauner et al. [21] also reported decreased leptin levels in children affected by GLUT-1 DS and treated with a cKD for at least six months: this change was not dependent on weight loss.

The different types of KDs examined, the various study-designs and the different ages and diseases of the examined patients, could explain these contrasting results.

Our study showed stable ghrelin and leptin concentrations, probably due to the isocaloric cKD, which permitted a stable KB production without impairing the nutritional status. Indeed, the BMI did not change significantly, and the amount and distribution of body fat also remained stable, corroborating our previously published data on the short-term effects of cKD on the nutritional status and body composition in children affected by GLUT1-DS [28]. We found, as expected [23,32], that the leptin concentration changes were positively associated with the weight gain and the increase in body fat amount ($\rho = -0.4534, p = 0.0344$; $\rho = 0.5901, p = 0.0135$; respectively). Some authors have hypothesized a downregulated concentration in ghrelin and, in contrast, an increase in leptin levels during KDs ad libitum due to the increase in dietary fat intake and, consequently, a higher body fat mass [18,33]. However, the strict and constant evaluation of both the caloric and fatty acid intakes, probably led to the maintenance of the nutritional status, lipid profile and both ghrelin and leptin levels in the 12-month cKD. These data, taken together, suggest that, weight loss, probably including both fat mass and fat free mass loss, as occurred during the VLED, rather than KBs, affects the ghrelin and leptin concentration, acting on hungry and satiety control. During a normocaloric cKD, KBs do not seem to directly regulate body weight and body composition.

After 12 months of cKD, in our sample of children and adolescents, we found a statistically significant reduction in the height $z$-score, with a magnitude of variation of very low clinical impact ($-0.19 \pm 0.15$ height $z$-score). A decline in height [27] or weight and height [57–61] with the KD has
been previously described in epileptic patients on the diet for more than six months; and the percentage of affected patients varies between 6% and 30% in recent long-term studies [62–64].

Data on factors that may affect the growth of children treated with the KD (e.g., ketosis or nutrient adequacy) remain scarce and contrasting [26,65]. However, a reduction in both ghrelin and insulin, both of which are hormones stimulating GH concentration, has been hypothesized [29,66]. Our study does not suggest the potential role of ghrelin on the delay of growth, because we did not find either a significant change in ghrelin over the 12 months ($p = 0.693$), or an association with the ghrelin and height $z$-score changes ($p = 0.0019, p = 0.9963$). As for insulin, although we found a significant difference between the baseline and 12 months ($p < 0.001$), insulin was not significantly associated with the height $z$-score changes ($p = -0.1182, p = 0.6751$). However, we cannot rule out that insulin changes may have had an effect during cKD and led to growth failure. This is because from a metabolic point of view not only is the fasting concentration of insulin relevant but also the after-meal concentration. The chronic very low amount of carbohydrates of the cKD could significantly impair insulin secretion after a meal. Further studies on the effects of the ketogenic diet on insulin secretion are needed to clarify this issue.

Regarding the biochemical parameters, children, adolescents and adults reported unchanged fasting glucose, while there was a drop in the fasting insulin and a relative improvement in HOMA-IR, as previously demonstrated [34]. The three children and one adult (13%) with high TC and LDL at the baseline, reported a rise (+25%) in both levels, while triglycerides remained in the normal range. In the other patients, the lipid profile did not change, despite the daily high-fat dietary consumption, in contrast with a previous study [67] that found a substantial increase in TC, HDL, LDL and TG at six months. Our results agree with a previous study that found no significant changes in TC or LDL-C in 10 prepubertal children affected by GLUT1-DS after 10 years of a cKD and did not identify any cardiovascular risks [6].

One of the strengths of the study is the quality of data collected: All the biochemical measurements and dosages were collected in the same centre, thus guaranteeing less variability. It is also the first time that ghrelin, leptin, insulin and nutritional status parameters have been measured all together and both in DRE and, above all, in GLUT1-DS. Finally, our study involved what we believe to be the longest follow-up ever reported in the literature. However, a number of potential limitations need to be addressed. A control group was not included in the study, since this is difficult to achieve especially because the GLUT1-DS management guidelines require the use of KD from the first day of diagnosis [5]. The sample size is also globally small, especially in adults, and thus more studies are needed.

5. Conclusions

The present study is, to the best of our knowledge, the first to investigate the effects of a 12-month cKD on ghrelin and leptin concentrations in children, adolescents and adults affected by GLUT1-DS or DRE. We found no significant changes and associations between the ghrelin and leptin and nutritional status changes, suggesting that these appetite-regulating peripheral hormones are not downregulated by chronic ketosis or by a very high fat diet. Our results contribute to the growing literature on changes during cKD of these two peripheral hormones implicated in several metabolic outcomes, and thus to a better understanding of the long-term effects of a cKD and how it could be implemented in other diseases.

**Supplementary Materials:** The following are available online at [http://www.mdpi.com/2072-6643/11/8/1716/s1](http://www.mdpi.com/2072-6643/11/8/1716/s1), Table S1: Time course of the diet composition.

**Author Contributions:** Conceptualization, S.B. A.B. and A.T.; Methodology, S.B. and A.B.; Formal analysis, A.L. and R.D.; Investigation, S.B., R.D., C.L., C.T., C.F., A.F., S.R., P.V., V.D., A.T.; Data curation, R.D., A.L., and A.F.; Writing—original draft preparation, R.D.; Writing—review and editing, S.B., A.L., A.B., A.T., P.V.; Supervision, S.B., A.T.; Project administration, A.T., S.B.

**Funding:** This research received no external funding and the ICANS internal funds funded the APC.
Acknowledgments: We would like to thank Adrian Wallwork for editing the English and proofreading the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Leone, A.; De Amicis, R.; Lessa, C.; Tagliabue, A.; Trentani, C.; Ferraris, C.; Battezzati, A.; Veggiotti, P.; Foppiani, A.; Ravella, S.; et al. Food and Food Products on the Italian Market for Ketogenic Dietary Treatment of Neurological Diseases. *Nutrients* 2019, 11. [CrossRef] [PubMed]
2. van der Louw, E.; van den Hurk, D.; Neal, E.; Leindecker, B.; Fitzsimmon, G.; Dority, L.; Thompson, L.; Marchió, M.; Dudzińska, M.; Dressler, A.; et al. Ketogenic diet guidelines for infants with refractory epilepsy. *Eur. J. Paediatr. Neurol.* 2016, 20, 798–809. [CrossRef] [PubMed]
3. deCampo, D.M.; Kossoff, E.H. Ketogenic dietary therapies for epilepsy and beyond. *Curr. Opin. Clin. Nutr. Metab. Care* 2019, 1. [CrossRef] [PubMed]
4. D’Andrea Meira, I.; Romão, T.T.; Do Prado, H.J.P.; Krüger, L.T.; Pires, M.E.P.; Da Conceição, P.O. Ketogenic diet and epilepsy: What we know so far. *Front. Neurosci.* 2019, 13, 5. [CrossRef] [PubMed]
5. Klepper, J. Glucose transporter deficiency syndrome (GLUT1DS) and the ketogenic diet. *Epilepsia* 2008, 49, 46–49. [CrossRef] [PubMed]
6. Heussinger, N.; Della Marina, A.; Beyerlein, A.; Leindecker, B.; Hermann-Alves, S.; Dalla Pozza, R.; Klepper, J. 10 patients, 10 years-Long term follow-up of cardiovascular risk factors in Glut1 deficiency treated with ketogenic diet therapies: A prospective, multicenter case series. *Clin. Nutr.* 2018, 37, 2246–2251. [CrossRef]
7. Sofou, K.; Dahlin, M.; Hallböök, T.; Lindefeldt, M.; Viggledal, G.; Darin, N. Ketogenic diet in pyruvate dehydrogenase complex deficiency: Short-and long-term outcomes. *J. Inherit. Metab. Dis.* 2017, 40, 237–245. [CrossRef]
8. Kossoff, E.H.; Zupec-Kania, B.A.; Auvin, S.; Ballaban-Gil, K.R.; Christina Bergqvist, A.G.; Blackford, R.; Buchhalter, J.R.; Caraballo, R.H.; Cross, J.H.; Dahlin, M.G.; 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, 175–192. [CrossRef]
9. De Giorgis, V.; Veggiotti, P. GLUT1 deficiency syndrome 2013: Current state of the art. *Seizure* 2013, 22, 803–811. [CrossRef]
10. McDonald, T.J.W.; Cervenka, M.C. The expanding role of Ketogenic diets in adult neurological disorders. *Brain Sci.* 2018, 8. [CrossRef]
11. Włodarek, D. Role of Ketogenic Diets in Neurodegenerative Diseases (Alzheimer’s Disease and Parkinson’s Disease). *Nutrients* 2019, 11. [CrossRef] [PubMed]
12. Paoli, A.; Rubini, A.; Volek, J.S.; Grimaldi, K.A. Beyond weight loss: A review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *Eur. J. Clin. Nutr.* 2013, 67, 789–796. [CrossRef] [PubMed]
13. Westman, E.C.; Tondt, J.; Maguire, E.; Yancy, W.S.J. Implementing a low-carbohydrate, ketogenic diet to manage type 2 diabetes mellitus. *Expert Rev. Endocrinol. Metab.* 2018, 13, 263–272. [CrossRef] [PubMed]
14. McDonald, T.J.W.; Cervenka, M.C. Ketogenic diets for adults with highly refractory epilepsy. *Epilepsy Curr.* 2017, 17, 346–350. [CrossRef]
15. Veech, R.L. The therapeutic implications of ketone bodies: The effects of ketone bodies in pathological conditions: Ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot. Essent. Fat. Acids* 2004, 70, 309–319. [CrossRef] [PubMed]
16. Koppel, S.J.; Swerdlow, R.H. Neuroketotherapeutics: A Modern Review of a Century-Old Therapy; Elsevier Ltd.: Amsterdam, The Netherlands, 2018; Volume 117.
17. Veyrat-Durebex, C.; Reynier, P.; Procaccio, V.; Hergesheimer, R.; Corcia, P.; Andres, C.R.; Blasco, H. How Can a Ketogenic Diet Improve Motor Function? *Front. Mol. Neurosci.* 2018, 11. [CrossRef] [PubMed]
18. Giordano, C.; Marchió, M.; Timofeeva, E.; Biagini, G. Neuroactive peptides as putative mediators of antiepileptic ketogenic diets. *Front. Neurol.* 2014, 5, 63. [CrossRef]
19. Marchió, M.; Roli, L.; Giordano, C.; Trenti, T.; Guerra, A.; Biagini, G. Decreased ghrelin and des-acyl ghrelin plasma levels in patients affected by pharmaco-resistant epilepsy and maintained on the ketogenic diet. *Clin. Nutr.* 2018, 38, 954–957. [CrossRef]
20. Lambrechts, D.A.J.E.; Brandt-Wouters, E.; Verschuure, P.; Vles, H.S.H.; Majoie, M.J.M. A prospective study on changes in blood levels of cholecystokinin-8 and leptin in patients with refractory epilepsy treated with the ketogenic diet. *Epilepsy Res.* 2016, 127, 87–92. [CrossRef]

21. Rauchenzauner, M.; Klepper, J.; Leindecker, B.; Luef, G.; Rostasy, K.; Ebenbichler, C. The Ketogenic Diet in Children with Glut1 Deficiency Syndrome and Epilepsy. *J. Pediatr.* 2008, 153, 716–718. [CrossRef]

22. Müller, T.D.; Nogueiras, R.; Andermann, M.L.; Andrews, Z.B.; Anker, S.D.; Argente, J.; Batterham, R.L.; Benoit, S.C.; Bowers, C.Y.; Broglio, F.; et al. Ghrelin. *Mol. Metab.* 2015, 4, 437–460. [CrossRef] [PubMed]

23. Margetic, S.; Gazzola, C.; Pegg, G.G.; Hill, R.A. Leptin: A review of its peripheral actions and interactions. *Int. J. Obes.* 2002, 26, 1407–1433. [CrossRef] [PubMed]

24. Paoli, A.; Bosco, G.; Camporesi, E.M.; Mangar, D. Ketosis, ketogenic diet and food intake control: A complex relationship. *Front. Psychol.* 2015, 6, 27. [CrossRef] [PubMed]

25. Vining, E.P.; Pyzik, P.; McGrogan, J.; Hladky, H.; Anand, A.; Kriegler, S.; Freeman, J.M. Growth of children on the ketogenic diet. *Dev. Med. Child Neurol.* 2002, 44, 796–802. [CrossRef] [PubMed]

26. Nation, J.; Humphrey, M.; MacKay, M.; Boneh, A. Linear growth of children on a ketogenic diet: Does the protein-to-energy ratio matter? *J. Child Neurol.* 2014, 29, 1496–1501. [CrossRef] [PubMed]

27. Groleau, V.; Schall, J.I.; Stallings, V.A.; Bergqvist, C.A. Long-term impact of the ketogenic diet on growth and resting energy expenditure in children with intractable epilepsy. *Dev. Med. Child Neurol.* 2014, 56, 898–904. [CrossRef] [PubMed]

28. Klok, M.D.; Jakobsdottir, S.; Drent, M.L. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obes. Rev.* 2007, 8, 21–34. [CrossRef]

29. Dimaraki, E.V.; Jaffe, C.A. Role of endogenous ghrelin in growth hormone secretion, appetite regulation and metabolism. *Rev. Endocr. Metab. Disord.* 2006, 7, 237–249. [CrossRef]

30. Ge, T.; Yang, W.; Fan, J.; Li, B. Preclinical evidence of ghrelin as a therapeutic target in epilepsy. *Oncotarget* 2017, 8, 59929–59939. [CrossRef]

31. Berilgen, M.S.; Mungen, B.; Ustundag, B.; Demir, C. Serum ghrelin levels are enhanced in patients with epilepsy. *Seizure* 2006, 15, 106–111. [CrossRef]

32. Kelesidis, T.; Kelesidis, I.; Chou, S.; Mantzoros, C.S. The Role of Leptin in human Physiology NIH Public Access. *Ann. Intern. Med.* 2011, 152, 93–100. [CrossRef] [PubMed]

33. Thio, L.L. Hypothalamic hormones and metabolism. *Epilepsy Res.* 2012, 100, 245–251. [CrossRef] [PubMed]

34. Rho, J.M.; Stafstrom, C.E. The ketogenic diet: What has science taught us? *Epilepsy Res.* 2012, 100, 210–217. [CrossRef] [PubMed]

35. Bertoli, S.; Neri, I.G.; Trentani, C.; Ferraris, C.; De Amicis, R.; Battezzati, A.; Veggiotti, P.; De Giorgis, V.; Tagliabue, A. Short-term effects of ketogenic diet on anthropometric parameters, body fat distribution, and inflammatory cytokine production in GLUT1 deficiency syndrome. *Nutrition* 2015, 31. [CrossRef] [PubMed]

36. Kwan, P.; Arzimanoglou, A.; Berg, A.T.; Brodie, M.J.; Allen Hauser, W.; Mathern, G.; Moshe, S.L.; Perucca, E.; Wiebe, S.; French, J. Definition of drug resistant epilepsy: Consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 2010, 51, 1069–1077. [CrossRef] [PubMed]

37. Tagliabue, A.; Bertoli, S.; Trentani, C.; Borrelli, P.; Veggiotti, P. Effects of the ketogenic diet on nutritional status, resting energy expenditure, and substrate oxidation in patients with medically refractory epilepsy: A 6-month prospective observational study. *Clin. Nutr.* 2012, 31, 246–249. [CrossRef]

38. Veggiotti, P.; Burlina, A.; Coppola, G.; Casmair, R.; De Giorgis, V.; Guerrini, R.; Tagliabue, A.; Dalla Bernardina, B. The ketogenic diet for Dravet syndrome and other epileptic encephalopathies: An Italian consensus. *Epilepsia* 2011, 52, 83–89. [CrossRef]

39. Lohman, T.; Roche, A.; Martorell, R. *Anthropometric Standardization Reference Manual*; Human Kinetics Books: Champaign, IL, USA, 1988.

40. Centers for Disease Control and Prevention, National Center for Health Statistics. CDC Growth Charts: United States. Available online: http://www.cdc.gov/growthcharts/ (accessed on 9 September 2010).

41. WHO Obesity and overweight. Body Mass Index (BMI) Classifications. Available online: http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed on 28 February 2019).

42. Brook, C.G.D. Determination of body composition of children from skinfold measurements. *Arch. Dis. Child.* 1971, 46, 182–184. [CrossRef]

43. Brožek, J.; Grande, F.; Anderson, J.T.; Keys, A. Densitometric Analysis of Body Composition: Revision of Some Quantitative Assumptions. *Ann. N. Y. Acad. Sci.* 1963, 110, 113–140. [CrossRef]
44. Durnin, B.Y.J.V.G.A.; Womersley, J. Body fat assessed from total body density and its estimation from skinfold thickness: Measurements on 481 men and women aged from 16 to 72 Years. Br. J. Nutr. 1974, 32, 77–97. [CrossRef]

45. Siri, W.E. Body composition from fluid spaces and density: Analysis of methods. Nutrition 1956, 9, 480–491.

46. Armellini, F.; Zamboni, M.; Rigo, L.; Todesco, T.; Bergamo-Andreis, I.A.; Procacci, C.; Bosello, O. The contribution of sonography to the measurement of intra-abdominal fat. J. Clin. Ultrasound 1990, 18, 563–567. [CrossRef] [PubMed]

47. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985, 28, 412–419. [CrossRef] [PubMed]

48. Alberti KG, E.R.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.-C.; James, W.P.T.; Loria, C.M.; Smith, S.C. Harmonizing the Metabolic Syndrome. Circulation 2009, 120, 1640–1645. [CrossRef] [PubMed]

49. Al-Hamad, D.; Raman, V. Metabolic syndrome in children and adolescents. Transl. Pediatr. 2017, 6, 397–407. [CrossRef] [PubMed]

50. Keskin, M.; Kurtoglu, S.; Kendirci, M.; Atabek, M.E.; Yazici, C. Homeostasis Model Assessment Is More Reliable Than the Fasting Glucose/Insulin Ratio and Quantitative Insulin Sensitivity Check Index for Assessing Insulin Resistance Among Obese Children and Adolescents. Pediatrics 2005, 115, e500–e503. [CrossRef] [PubMed]

51. Whatmore, A.J.; Hall, C.M.; Jones, J.; Westwood, M.; Clayton, P.E. Ghrelin concentrations in healthy children and adolescents. Clin. Endocrinol. 2003, 59, 649–654. [CrossRef]

52. Erhardt, E.; Foraita, R.; Pigeot, I.; Barba, G.; Veidebaum, T.; Tornaritis, M.; Michels, N.; Eiben, G.; Ahrens, W.; Moreno, L.A.; et al. Reference values for leptin and adiponectin in children below the age of 10 based on the IDEFICS cohort. Int. J. Obes. 2014, 38, S32–S38. [CrossRef]

53. Pasca, L.; Caraballo, R.H.; De Giorgis, V.; Reyes, J.G.; Macasaet, J.A.; Masnada, S.; Armeno, M.; Musico, M.; Tagliabue, A.; Veggio, P. Ketogenic diet use in children with intractable epilepsy secondary to malformations of cortical development: A two-centre experience. Seizure 2018, 57, 34–37. [CrossRef]

54. De Giorgis, V.; Masnada, S.; Varesio, C.; Chiappedi, M.A.; Zanaboni, M.; Pasca, L.; Filippini, M.; Macasaet, J.A.; Valente, M.; Ferraris, C.; et al. Overall cognitive profiles in patients with GLUT1 Deficiency Syndrome. Brain Behav. 2019, 9, e01224. [CrossRef]

55. Sumithran, P.; Prendergast, L.A.; Delbridge, E.; Purcell, K.; Shulkes, A.; Kriketos, A.; Proietto, J. Ketosis and appetite-mediating nutrients and hormones after weight loss. Eur. J. Clin. Nutr. 2013, 67, 759–764. [CrossRef] [PubMed]

56. Nymo, S.; Coutinho, S.R.; Jørgensen, J.; Rehfeld, J.F.; Truby, H.; Kulseng, B.; Martins, C. Timeline of changes in appetite during weight loss with a ketogenic diet. Int. J. Obes. 2017, 41, 1224–1231. [CrossRef] [PubMed]

57. Williams, S.; Basualdo-Hammond, C.; Curtis, R.; Schullier, R. Growth retardation in children with epilepsy on the ketogenic diet: A retrospective chart review. J. Am. Diet. Assoc. 2002, 102, 405–407. [CrossRef]

58. Peterson, S.J.; Tangney, C.C.; Pimentel-Zablh, E.M.; Hjelmgren, B.; Booth, G.; Berry-Kravis, E. Changes in growth and seizure reduction in children on the ketogenic diet as a treatment for intractable epilepsy. J. Am. Diet. Assoc. 2005, 105, 718–725. [CrossRef] [PubMed]

59. Neal, E.G.; Chaffe, H.M.; Edwards, N.; Lawson, M.S.; Schwartz, R.H.; Cross, J.H. Growth of children on classical and medium-chain triglyceride ketogenic diets. Pediatr. Clin. North Am. 2008, 55, 1343–1359. [CrossRef] [PubMed]

60. Spulber, G.; Spulber, S.; Hagenas, L.; Amark, P.; Dahlin, M. Growth dependence on insulin-like growth factor-1 during the ketogenic diet. Epilepsia 2009, 50, 297–303. [CrossRef] [PubMed]

61. Kim, J.T.; Kang, H.-C.; Song, J.-E.; Lee, M.J.; Lee, Y.J.; Lee, E.J.; Lee, J.S.; Kim, H.D. Catch-up growth after long-term implementation and weaning from ketogenic diet in pediatric epileptic patients. Clin. Nutr. 2013, 32, 98–103. [CrossRef]

62. Dressler, A.; Stocklin, B.; Reithofer, E.; Benninger, F.; Freilinger, M.; Hauser, E.; Reiter-Fink, E.; Seidl, R.; Trimmel-Schwahofer, P.; Feucht, M. Long-term outcome and tolerability of the ketogenic diet in drug-resistant childhood epilepsy—The Austrian experience. Seizure 2010, 19, 404–408. [CrossRef]

63. Wibisono, C.; Rowe, N.; Beavis, E.; Kepreotes, H.; Mackie, F.E.; Lawson, J.A.; Cardamone, M. Ten-year single-center experience of the ketogenic diet: Factors influencing efficacy, tolerability, and compliance. J. Pediatr. 2015, 166, 1030–1036. [CrossRef]
64. Lambrechts, D.A.J.E.; de Kinderen, R.J.A.; Vles, H.S.H.; de Louw, A.J.; Aldenkamp, A.P.; Majoie, M.J.M. The MCT-ketogenic diet as a treatment option in refractory childhood epilepsy: A prospective study with 2-year follow-up. *Epilepsy Behav.* 2015, 51, 261–266. [CrossRef]

65. Ferraris, C.; Guglielmetti, M.; Pasca, L.; De Giorgis, V.; Ferraro, O.E.; Brambilla, I.; Leone, A.; De Amicis, R.; Bertoli, S.; Veggiotti, P.; et al. Impact of the Ketogenic Diet on Linear Growth in Children: A Single-Center Retrospective Analysis of 34 Cases. *Nutrients* 2019, 11. [CrossRef] [PubMed]

66. Gat-Yablonski, G.; De Luca, F. Effect of Nutrition on Statural Growth. *Horm. Res. Paediatr.* 2017, 88, 46–62. [CrossRef] [PubMed]

67. Azevedo de Lima, P.; Baldini Prudência, M.; Murakami, D.K.; Pereira de Brito Sampaio, L.; Figueiredo Neto, A.M.; Teixeira Damasceno, N.R. Effect of classic ketogenic diet treatment on lipoprotein subfractions in children and adolescents with refractory epilepsy. *Nutrition* 2017, 33, 271–277. [CrossRef] [PubMed]