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Long-term personalized high-protein, high-fat diet in pediatric patients with glycogen storage disease type IIIa: Evaluation of myopathy, metabolic control, physical activity, growth, and dietary compliance

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Abstract

Dietary lipid manipulation has recently been proposed for managing glycogen storage disease (GSD) type IIIa. This study aimed to evaluate the myopathic, cardiac, and metabolic status, physical activity, growth, and dietary compliance of a personalized diet high in protein and fat for 24 months. Of 31 patients with type IIIa GSD, 12 met the inclusion criteria. Of these, 10 patients (mean age 11.2 ± 7.4 years) completed the study. Patients were prescribed a personalized high-protein, high-fat diet, comprising 3.0–3.5 g/kg/day of protein and 3.0–4.5 g/kg/day of fat, constituting 18.5%–28% and 70.5%–75.7% of daily energy, respectively. Dietary compliance was ensured and assessed via the regular administration of questionnaires. Our results revealed consistent and significant decreases of 22%, 54%, and 30% in the creatinine kinase, creatine kinase–myocardial band, and lactate dehydrogenase levels, respectively. Echocardiography revealed improvements in the Z-scores of the left ventricular mass and interventricular septum thickness. A significant increase in body muscle mass was observed, and a higher score was achieved using the Daily Activity Questionnaire. Growth monitoring revealed an arrest in the height-SDS at the 6th and 12th months, followed by subsequent improvement at the end of the second year. A gradual and persistent decline in the periods of hypo- and hyperglycemia has been reported. Biotinidase activity decreased, whereas hepatosteatosis increased and then decreased by the end of the study. Implementing a high-protein, high-fat diet and monitoring key parameters in patients with type IIIa GSD can lead to myopathic and cardiac improvements and increased physical activity.

KEYWORDS
cardiomyopathy, efficacy, glycogen storage disease type IIIa, high-protein high-fat nutrition, myopathy, safety

1 | INTRODUCTION

Glycogen storage disease (GSD) type III (OMIM #232400) is caused by biallelic variants in the *AGL* gene, resulting in a deficiency in the debranching enzyme amylo-1,-6-glucosidase. GSD type III is classified into two distinct clinical types: type IIIa, which affects the liver and skeletal and cardiac muscles, and type IIIb, which primarily affects the liver. In the first year of life, common clinical presentations of GSD type IIIa include fasting hypoglycemia, poor growth, delayed motor milestones, hepatomegaly, elevated liver transaminases, hypertrophic cardiomyopathy, and hyperlipidemia.[1] Additionally, most patients exhibit myopathy and elevated creatine kinase (CK) levels, which tend to become more pronounced over time. Furthermore, complications, such as cirrhosis and progressive fibrosis, may occur during long-term follow-up.[2]

Currently, complex carbohydrates and protein enrichment are the cornerstones of dietary management in hepatic GSD.[3] One of the first reports described a case with the successful treatment of severe cardiomyopathy in a patient with GSD type IIIa using synthetic ketone bodies (D, L-3-hydroxybutyrate) and a ketogenic and high-protein diet in the ratio 2:1.[1,4]

A ketogenic diet (KD), comprising low carbohydrate, high fat, and adequate protein, was initially developed for treating epilepsy and has since demonstrated effectiveness in other chronic diseases. Four major types of ketogenic diets exist. The first two are known as the "classic" long-chain triglyceride KD and the medium-chain triglyceride (MCT) diets, and the other two are termed the modified Atkins diet (MAD) and low glycemic index treatment. The classic KD was calculated as the ratio of fat (g), protein, and carbohydrates (g). The most common ratio is 4 g of fat to 1 g of protein plus carbohydrate (described as "4:1"). A ratio of 3:1 or lower can be used to increase the protein or carbohydrate intake. Various commercial products are available for formula-based KD that provide higher compliance. However, the KD may not be easily tolerated, and calculating the ratio of fat-to-protein and carbohydrate intakes can be challenging for a few households.[5,6]

MAD is a high-fat, low-carbohydrate diet similar to the classic KD in terms of food choices. However, there are no limitations on proteins, fluids, or calories, which makes meal planning easier. MAD provides an approximately 1:1–1.5:1 ketogenic ratio, but no set ratio is mandated, and a few children can achieve a high ratio. Carbohydrate intake is limited to 10–20 g/day in children and 15–20 g/day in adults. Detailed calculations are not required, which may be ideal for households with working parents or those who have limited literacy and struggle with the weighing process.[5,6]

Different types of ketogenic diets without supplementation with synthetic ketone bodies were used in patients with GSD type IIIa, resulting in significant improvements in cardiomyopathy ([$n = 2$, classic KD][7]; [$n = 2$, MAD][8]; [$n = 1$, MAD][9]; [$n = 6$, MAD][10]).

Specific prospective studies, particularly qualitative ones, examining the clinical, body composition, and performance outcomes of a high-protein ketogenic diet in patients with GSD type IIIa are scarce.

Based on the notion of reprogramming the metabolic pathway in the muscles of patients with GSD type IIIa, we hypothesized that a high-protein, high-fat diet would improve the physical activity of patients, which may enhance their compliance. Therefore, as a primary endpoint, the efficacy (improvement in myopathic parameters, cardiac and muscle activity, and physical activity) and safety (metabolic parameters and growth) of a high-protein, high-fat diet in patients with GSD type IIIa were evaluated for 2 years. The secondary endpoints were the evaluation of dietary compliance and the assessment of body composition changes during the intake of a high-protein, high-fat diet.

2 | MATERIALS AND METHODS

2.1 | Study design

This prospective, 2-year, open-label, single-arm, before-and-after comparison pilot study was conducted at the Ege University Children's Hospital, Division of Nutrition and Metabolic Disorders.

2.2 | Patients

A total of 31 patients with GSD type IIIa were admitted to our facility. The patients were recruited for the study

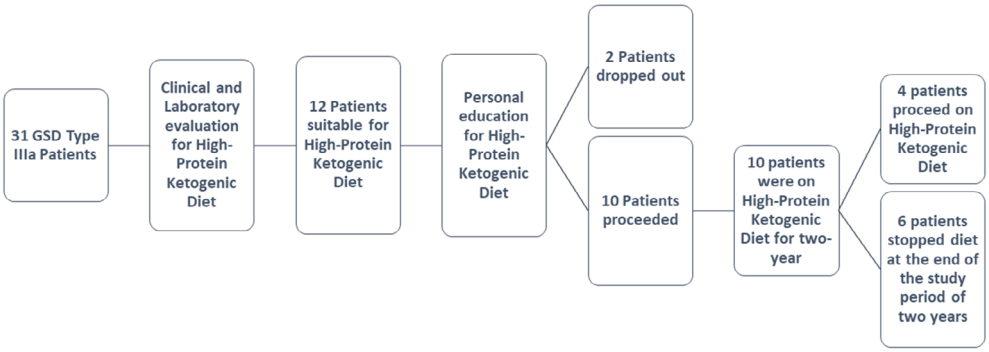

FIGURE 1 Flowchart of study design illustrated selection and categorization of the patients. KD, Ketogenic diet.

based on the following inclusion criteria: genetically diagnosed patients with GSD type IIIa (pathogenic or likely pathogenic variations in the *AGL* gene) with hepatic (hepatomegaly, elevated transaminase levels, hypoglycemia), myopathy elevated CK (>250 U/L), skeletal myopathy, and/or cardiac involvement (detected echocardiographically), and willingness to participate. Patients with GSD type IIIa with normal muscle enzyme levels (<250 U/L), severe hepatic dysfunction (AST >1000 U/L, ALT >750 U/L), elevated alpha-fetoprotein levels (above normal reference values for age), renal dysfunction (urea, creatinine, and tubular function tests above the normal references for age), or liver transplantation were excluded from the study (Figure 1).

2.3 | Anthropometric evaluation

Weight and height were measured in the morning after fasting. Weight was measured to the nearest 0.1 kg using an electronic scale (SECA762; Vogel & Hakle, Humburg, Germany). Height was measured using a stadiometer in a vertical position with the feet and ankles parallel and the shoulders and buttocks touching the wall. The BMI (kg/m^2) was calculated as the body weight ratio to the square of body height.

2.4 | Body composition

The body composition was evaluated using a bioelectrical impedance device, the Tanita SC-240OMA. The analyzer was certified according to the 93/42 EEC (EU standard for medical devices). After manually entering the data on the patient's height, sex, and age, measurements were collected at 50 Hz using a standard setting. During the measurements, the patient was barefoot and wore minimal clothing. All patients were instructed to stand still with their feet touching all four metal plates. Fat mass (FM), muscle mass (MM), body fat (BF%), and body muscle mass (BM%) were measured.

2.5 | Diet

Participants reported data on all consumed food and beverages, including dressings, along with serving sizes for 3 days every 4 weeks (2 days a week, one on a weekend). Three-day food records were obtained using a standardized Daily Consumption Records Form (DCRF). Before entering the data into the nutrient analysis program, the same dietician reviewed all completed diet records and requested supplementary information. Energy intake and nutrient composition were calculated using the Turkish version of EbisPro for Windows (BeBiS 8.2) (Stuttgart, Germany). Universally accepted equations were used to determine energy requirements based on age and sex.[11]

Personal dietary preferences were assessed using Food Consumption Frequency Records (FCFR). Dietary interventions were standardized and offered to each patient during face-to-face meetings, primarily based on family preferences. Following acceptance, the patient and family received instructions regarding the ketogenic diet, and a personalized fresh diet was prepared following FCFR. Standardized ketogenic diet education, including specific information on hypoglycemia, extreme ketosis, and possible gastrointestinal symptoms, was provided to each patient, caregiver, and family member. In this study, country-specific recipes were developed and adapted for a high-protein ketogenic diet. The same dieticians guided a high-protein ketogenic diet from the beginning to the end of the study period. Phone calls were scheduled weekly.

Dietary compliance was evaluated using questionnaires completed by the parents/caregivers (Dietary Compliance Questionnaire-Parents [DCQ-P]) (Supplementary file S1) and dietitians or physicians (Dietary Compliance Questionnaire-Dietitians [DCQ-D]) (Supplementary file S1). The evaluation of the patient's diet from the patient's perspective was provided by the Patient/Parents Diet Assessment Questionnaire (PAQ) (Supplementary file S1). Reasons for noncompliance were reported in line with the DCRF, DCQ, and PAQ. The diet was rearranged as

required. In contrast, the Assessment of the Mother/Feeding Caregivers by Simple Simulation Test (M/FC- SST) (Supplementary file S1) was performed to evaluate dietary compliance. Assessments were conducted during clinical visits via email exchanges (involving written texts or videos) and Zoom meetings throughout the study.

A high-protein, high-fat diet was personalized based on the patient's age, metabolic control, nutritional habits, and social factors. The protein quantities in the diet were maintained at high levels in line with the elevated levels recommended by monitoring protocols for GSD type IIIa.[12,13]

In this study, dietary adjustments were aimed at a standardized daily energy intake with a fat content between 60% and 75% (approximately 6 g/kg/day), as suggested by Rossi et al.[3] A moderate level of ketosis was targeted. MCT supplementation is recommended for patients with high serum triglyceride levels (>250 mg/dL). Multivitamins containing minerals (including trace minerals, especially selenium), calcium, and vitamin D (meeting the Recommended Dietary Allowance) were introduced to each patient. Uncooked corn starch was gradually tapered off upon the initiation of the ketogenic diet.

2.6 | Laboratory assessment

Blood samples were collected under consistent conditions (overnight fasting at the initiation, first week, and second week of the diet, and then monthly for the initial 3 months, followed by quarterly intervals). The samples included metabolic control parameters, liver function, and neuromuscular and cardiac assessments. Metabolic control parameters included glucose homeostasis (blood glucose, insulin, HbA1c, biotinidase, and ketones), lactate, uric acid, triglycerides, total cholesterol, and serum aminotransferases. Liver function studies included activated partial thromboplastin time (APTT), albumin, ammonia, total bilirubin, direct bilirubin, and gamma-glutamyl transferase (GGT). Furthermore, we assessed the calcium, phosphorus, alkaline phosphatase (ALP), and 25-hydroxy vitamin D levels in each patient. Neuromuscular and cardiac parameters included CK, creatine kinase–myocardial band (CK-MB), N-terminal prohormone, and brain natriuretic peptide (NT-proBNP).

Echocardiography (ECHO) and abdominal ultrasonography were performed annually. To evaluate bone mineral density (L1–L4 vertebrae), we conducted annual imaging using dual-emission X-ray absorptiometry (DEXA). The recommendations of Binkovitz et al. were followed with special consideration of height. We used CHILDMETRIX and referenced Supplementary file S2.[14]

During the high-protein ketogenic diet education, patients were advised to monitor capillary blood glucose and ketone levels using glucometers and ketometers, respectively. For the first 4 weeks, blood glucose levels were analyzed seven times daily: 30 min before meals, 2 h after meals, and after sleeping (4.00 a.m.); the number of measurements was reduced throughout the study. Ketone and capillary blood glucose levels were measured daily.

All patients were instructed to measure their daily urinary ketone (acetoacetate) levels using self-testing strips (Ketostix; Bayer Vital GmbH, Leverkusen, Germany).

2.7 | Continuous glucose monitoring system (CGMS)

Patients and their parents/caregivers attended 2 or 3 h training sessions in a metabolic education room. Annual weeklong continuous glucose monitoring was conducted using Medtronic iPro®2 professional CGM system (MiniMed Medtronic, Northridge, USA) CGM sensors. An experienced nurse performed the sensor placement following the calibration protocol outlined in the MiniMed CGM manual. The participants were instructed to measure their blood glucose levels using at least four fingersticks per day and to record values, meals, and exercise periods using an AccuCheck Performa Nano glucometer (Roche Diagnostics, Germany).

2.8 | Physical activity/exercise test

Exercise tolerance was assessed using the six-minute walk distance (SMWD), walking without feeling fatigue/distance (W-FT), time (min), running without feeling fatigue/distance (R-FT), and three-minute climbing test (TMCT) without fatigue or myalgia onset. These tests were conducted before the dietary intervention and repeated throughout the 2-year follow-up period. The physical activity of each participant was evaluated 2 h after breakfast. Furthermore, patients/parents/caregivers maintained a daily activity log ("activity diary"), providing information about physical activity at school and home. The strength and duration of activities, as assessed by the patients, were collected using a Daily Activity Questionnaire (DAQ) (Supplementary file S1). The questions were designed for use in daily activities involving the proximal and trunk muscles and the intrinsic muscles of the hands. This questionnaire consists of five questions, each offering three answer options, with each option receiving up to three points. An assessment was conducted over time based on these changes. Weekdays on which the children were active were considered for the evaluation.

TABLE 1 Demographic, genetic and clinical data about patients enrolled in the study.

Patients	*AGL* gene				Gender	Clincal findings			
	Genotype								
	Allele 1	Allele 2	Protein	ACMG		Age at diagnosis (months)	Current age (years)	Hepatic involve ment	Myopa thic involve ment
1	c.1083-2A > G	c.1083-2A > G	- (IVS8-2A > G)	Pathogenic	F	72	8.8	+	+
2	c.1999C > T	c.1999C > T	p.Gln667Ter	Likely Pathogenic	F	60	16.2	+	+
3	c.3538 del	c.3538 del	p.Ser1180ProfsTer47	Pathogenic	M	24	5.2	+	+
4	c.1020del	c.1020del	p.Glu340AspfsTer9	Pathogenic	M	64	13.6	+	+
5	c.753_756del	c.753_756del	p.Asp251GlufsTer23	Pathogenic	M	72	6.6	+	+
6	c.1858delC	c.1858delC	p.Leu620CysfsTer44	Pathogenic	F	108	11.1	+	+
7	c.1020del	c.1020del	p.Glu340AspfsTer9	Pathogenic	M	168	15.5	+	+
8	c.3652C > T	c.3652C > T	p.Arg1218Ter	Pathogenic	M	6	14.6	+	+
9	c.364_365dup	c.364_365dup	p.Pro123TyrfsTer12	Likely Pathogenic	M	3	2.4	+	+
10	c.753_756del	c.753_756del	p.Asp251GlufsTer23	Pathogenic	F	12	2.8	+	+

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AGL, Amylo-Alpha-1,6- glucosidase, 4-alpha-glucanotransferase; F, Female; M, Male; Hepatic involvement, Hepatomegaly, Elevated transaminase levels, Hypoglycemia; Myopathic involvement, Skeletal muscle weakness, Elevated muscle enzymes, Hypertrophic cardiomyopathy, Left ventricular hypertophy.

2.9 | Statistical analyses

Statistical analyses were performed using Statistical Package for the SPSS version 28 software (IBM SPSS Inc., Armonk, NY, USA). The level of significance was set at $p < 0.05$. Categorical variables were represented as counts and percentages. Quantitative variables were tested for normal distribution. Continuous variables with normal or skewed distributions were represented as mean (standard deviation) or median (interquartile range, min–max). Differences between repeated measurements were examined using Friedman and Wilcoxon signed-rank tests.

2.10 | Ethics

This study was approved by the Medical Ethics Committee of Ege University (70198063-050.06.04, approval number 18-7/33) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the participants and their parents.

3 | RESULTS

3.1 | Patients

3.1.1 | Demographic data

A total of 10 patients (60% male) were enrolled in the study. The mean age of the patients was 11.2 ± 7.4 years. The median number of family members was 4 (3–7, IQR: 3). The patient demographic data is shown in Table 1.

Dietary manipulations were performed in 12 patients with GSD IIIa. Given that 12 patients met the study's inclusion criteria, all received extended invitations to participate. Two patients, although initially expressing willingness to participate, withdrew from the study because of family-related challenges before being able to adapt and comply fully. The remaining 10 patients completed the study. Moreover, four patients, despite completing the 2-year duration, continued a high-protein ketogenic diet.

3.1.2 | Anthropometric evaluation

The differences in median weight, height, and BMI SDS during the study period based on the measurements at each visit are shown in Table 2. Statistically significant differences in height SDS were observed at the 6th ($p = 0.0440$) and 12th months ($p = 0.025$) compared to that in baseline measurements. However, these

TABLE 2 The table containing data on anthropometric, biochemical, imaging and body composition obtained during the 2-year follow-up of 10 patients with GSD type IIIa participating in the study.

Evaluated parameter (Reference values)	Basal Med (IQR) (Min–Max) ($n = 10$)	6. Month Med (IQR) (Min–Max) ($n = 10$)	12. Month Med (IQR) (Min–Max) ($n = 10$)	18. Month Med (IQR) (Min–Max) ($n = 10$)	24. Month Med (IQR) (Min–Max) ($n = 10$)	*p*
Antropometric data						
Height-for-age (SDS)	−0.81 (1.03) (−2.0–0.3)	−1.3 (1.53)* (−2.3–0.0)	−0.4 (1.92) * (−1.8–1.0)	−0.68 (2.09) (−1.3–1.0)	−0.66 (2.03) (−1.1–1.2)	0.141
Weight-for-age (SDS)	0.21 (1.24) (−1.4–1.1)	−0.14 (1.17) (−1.3–1.1)	−0.46 (2.01) (−1.3–2.0)	−0.23 (1.62) (−1.2–1.8)	0.09 (2.42) (−0.9–2.0)	0.589
BMI (SDS)	0.85 (1.03) (−1.0–2.0)	0.64 (2.14) (0.9–1.9)	0.7 (1.78) (−1.0–2.0)	0.87 (2.0) (0.0–2.2)	0.86 (1.62) (0.0–2.0)	0.437
Biochemical markers						
(1) Blood glucose homeostasis						
Glucose mg/dL (60–110)	72.5 (41.0) (35–107)	73.0 (10.0) (48–87)	63.5 (21.0) (52–98)	67.0 (15.0) (56–119)	69.5 (21.0) (54–100)	0.756
Number of hypoglycemia measured by fingerprint blood (*n*)	0.5 (2.0) (0.0–2.0)	0.0 (1.0) (0.0–2.0)	0.0 (0.0) (0.0–0.0)	0.0 (0.0) (0.0–0.0)	0.0 (1.0) (0.0–1.0)	0.223
Percentage (%) of the day the target range:70–140 mg/dL (GCMS values)	Median 86, Min–max 64–96, IQR: 14	Median 90, Min–max 67–99, IQR: 11		Median 95, Min–max 72–99, IQR: 16		0.049
Percentage (%) of the day [glucose] <70 mg/dL	Median 10, Min–max 0–38, IQR: 18	Median 8, Min–max 0–33, IQR: 14		Median 4.5, Min–max 0–26, IQR: 12		0.050
Percentage (%) of the day [glucose] >140 mg/dL	Median 4, Min–max 0–40, IQR: 4	Median 2, Min–max 0–33, IQR: 3		Median 0.5, Min–max 0–18, IQR: 1		0.018
Morning ketone concentrations (mmol/L) (0.5–3)	0.0.5 (0.7) (0.0–1.0)	1.5 (1) (0.0–3.1)	1.6 (1.1) (0.0–3.5)	1.5 (1.1) (0.0–3.0)	1.5 (1.2) (0.0–3.2)	0.051
Biotinidase (%)	112.0 (53.0) (82.0–164.0)	101.5. (38.0) (11.0–145.0)	95.0 (18.0) (74.0–110.0)	94.0 (5.0) (78.0–97.0)	88.5 (16.0) (68.0–128.0)	0.008
Insulin mU/L (2.6–10)	5.0 (11.0) (0.0–67.0)	3.5 (2.0) (0.0–5.0)	4.0 (3.0) (0.0–23.0)	4.0 (2.0) (2.0–5.0)	3.0 (4.0) (1.0–16.0)	0.376
HOMA- IR	1.0 (3.0) (0.0–18.0)	1.0 (2.0) (0.0–3.0)	1.0 (1.0) (0.0–5.0)	1.0 (0.0) (0.0–5.0)	1.0 (1.0) (0.0–3.0)	0.395
(2) Laboratory analytes						
AST U/L (<35)	285.5 (330.0) (62.0–854.0)	197.0 (324.0) (91.0–535.0)	158.0 (277.0) (80.0–748.0)	151.0 (93.0) (95.0–415.0)	171.0 (87.0) (100.0–245.0)	0.832
ALT U/L (<45)	250.0 (395.0) (44.0–639.0)	190.0 (328.0) (55.0–524.0)	147.0 (195.0) (48.0–645.0)	147.0 (136.0) (48.0–645.0)	158.0 (59.0) (48.0–645.0)	0.252

TABLE 2 (Continued)

Evaluated parameter (Reference values)	Basal Med (IQR) (Min–Max) ($n = 10$)	6. Month Med (IQR) (Min–Max) ($n = 10$)	12. Month Med (IQR) (Min–Max) ($n = 10$)	18. Month Med (IQR) (Min–Max) ($n = 10$)	24. Month Med (IQR) (Min–Max) ($n = 10$)	*p*
GGT U/L (<55)	52.0 (60.0) (13.0–163.0)	45.5 (49.0) (14.0–208.0)	41.0 (47.0) (12.0–155.0)	34.0 (45.0) (16.0–121.0)	42.5 (52.0) (16.0–222.0)	0.550
Total Protein g/dL (6.4–8.3)	7.0 (0.0) (6.0–8.0)	7.0 (1.0) (7.0–8.0)	7.0 (0.0) (6.0–8.0)	7.5 (1.0) (6.0–8.0)	7.0 (1.0) (7.0–9.0)	0.111
Albumin g/L (35–52)	4.0 (0.0) (4.0–5.0)	5.0 (0.0) (4.0–5.0)	4.0 (0.0) (4.0–5.0)	4.5 (1.0) (4.0–5.0)	4.5 (1.0) (4.0–5.0)	0.451
T-Chol mg/dL (<200)	204.0 (84.0) (104.0–273.0)	205.0 (119.0) (142.0–327.0)	194.0 (101.0) (100.0–299.0)	216.0 (84.0) (109.0–310.0)	211.5 (44.0) (117.0–266.0)	0.428
HDL-Chol mg/dL (>40)	26.5 (27.0) (21.0–76.0)	28.0 (19.0) (18.0–51.0)	27.5 (28.0) (14.0–147.0)	27.0 (26.0) (21.0–52.0)	35.0 (19.0) (21.0–62.0)	0.137
LDL-Chol mg/dL (<130)	133.0 (89.0) (50.0–188.0)	131.0 (73.0) (44.0–224.0)	118.5 (92.0) (43.0–221.0)	145.0 (54.0) (17–232)	143.0 (45.0) (56.0–190.0)	0.699
TG mg/dL (<150)	216.5 (197.0) (98.0–536.0)	239.0 (164.0) (120.0–690.0)	272.5 (224.0) (144.0–447.0)	208.5 (54.0) (110–368)	150.0 (45.0) (88.0–300.0)	0.014
Lactate mg/dL (<20)	8.5 (10.0) (4.0–46.0)	8.0 (6.0) (3.0–20.0)	7.0 (5.0) (4.0–14.0)	7.0 (3.0) (4–12.0)	8.0 (3.0) (4.0–10.0)	0.431
Uric acid mg/dL (3.5–7.2)	3.6 (2.5) (3.0–8.0)	5.0 (3.0) (3.0–35.0)	5.0 (3.0) (4.0–8.0)	5.5 (3.0) (4.0–36.0)	5.5 (3.0) (3.0–32.0)	0.395
(3) Blood muscle markers						
CK U/L (<150)	802.0 (1208.0) (250–3040)	628.0 (1391.0) (115–2277)	562.0 (1194.0) (128–2348)	744.5 (1012.0) (70–1356)	618.5 (846.0) (64–1428)	0.039
CPK-MB U/L (<25)	23.0 (30.0) (3–53)	13.0 (17.0) (4–44)	14.0 (3.0) (4–48)	17.5 (20.0) (2–34)	10.5 (20.0) (2–33)	0.028
LDH U/L (135–225)	378.5 (160.0) (185–446)	311.5 (111.0) (191–436)	294.0 (91.0) (171–397)	296.5 (123.0) (174–349)	264.0 (96.0) (203–354)	0.054
NT-pro-BNP Ng/L	78.5 (88.0) (10–206)	35.0 (41.0) (20–170)	56.0 (55.0) (9–120)	28.0 (68.0) (5–100)	63.0 (52.0) (10–86)	0.904
(4) Blood bone markers						
25 Hydroxy Vitamin D ng/mL (30–100)	30.0 (16.0) (5–32)	32.0 (11.0) (20–37)	33.0 (21.0) (14–48)	35.0 (9.0) (17–42)	34.5 (8.0) (21–45)	0.144
Calcium, mg/dL (8.6–10.2)	10.0 (0.0) (9–10)	10.0 (1.0) (9–11)	10.0 (1.0) (9–10)	10.0 (1.0) (9–10)	10.0 (1.0) (9–10)	0.844
Phosphorus, mg/dL (2.3–4.5)	5.0 (0) (4–5)	5.0 (1.0) (3–5)	5.0 (0.0) (4–6)	5.0 (2.0) (3–6)	5.0 (1.0) (4–5)	0.585
Alkaline phosphatase, U/L (40–129)	273.5 (162.0) (58–392)	299.5 (123.0) (57–442)	280.0 (147.0) (54–435)	240.0 (199.0) (55–535)	232.0 (261.0) (62–479)	0.592

(Continues)

TABLE 2 (Continued)

Evaluated parameter (Reference values)	Basal Med (IQR) (Min–Max) ($n = 10$)	6. Month Med (IQR) (Min–Max) ($n = 10$)	12. Month Med (IQR) (Min–Max) ($n = 10$)	18. Month Med (IQR) (Min–Max) ($n = 10$)	24. Month Med (IQR) (Min–Max) ($n = 10$)	p
Images						
(1) ECHO						
LVM (Z)	1.5 (2.0) (0.6–2.4)	1.3 (1.8) (0.6–2.2)		1.1 (1.1) (0.5–2.1)		0.038
IVSd (Z)	4.8 (3.1) (2.4–7.3)	3.84 (2.5) (2.2–6.2)		3.2 (2.0) (2.0–5.2)		0.030
LVPWd (Z)	3.9 (1.1) (3.6–4.4)	3.5 (1.1) (3.0–4.1)		2.9 (1.0) (2.6–3.9)		0.036
LVEF%	75 (4.0) (70–85)	75 (6.0) (74–88)		75 (5.0) (73–88)		0.475
(2) Liver USG						
Liver longitudinal diameter (cm)	16.3 (4.3) (12.2–20.0)	15.3 (3.5) (12.1–18.2)		15.0 (4.6) (11.1–17.8)		0.590
Hepatomegaly (%)	100%	90%		80%		
Hepatosteatosis (%)	80%	90%		70%		
Parenchymal coarse echotexture (%)	80%	90%		80%		
Parenchymal lobulation (%)	20%	30%		20%		
Splenomegaly	50%	50%		50%		
(3) DEXA						
Z-Score (L1–L4) (g/cm^2)	−1.17 (1.4) ((−1.4) – (1.1))	−1.18 (1.6) ((−1.3) – (1.3))		−1.19 (1.5) ((−1.3) – (1.4))		0.374
Body composition						
Fat mass	9.4 (8.3) (5.2–13.3)	9.8 (8.1) (5.7–14.1)		10.2 (8.2) (6.7–14.6)		0.068
Body fatness (%)	20.8 (13.6) (15.5–25.3)	21.4 (14.9) (15.7–26.1)		21.7 (15.9) (15.8–26.2)		0.064
Muscle mass	35.6 (28.4) (21.8–44.1)	35.9 (31.0) (22.8–46.1)		36.2 (31.2) (24.0–48.0)		0.066
Body muscle mass (%)	74.9 (12.4) (66.2–79.1)	75.1 (13.8) (67.8–80.2)		76.1 (14.9) (70.1–80.6)		0.044

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; CK, Creatinine Kinase; GGT, Gamma Glutamyl Transferase; HDL-Chol, High Density Lipoproteins – Cholesterol; IVSd, Interventricular Septum Diameter; LDH, Lactate Dehydrogenase; LDL-Chol, Low Density Lipoproteins – cholesterol; LVEF%, Left Ventricular Ejection Fraction; LVM, Left Ventricle Mass; LVPWd, Left Ventricular Posterior Wall Thickness Diameter; NT-pro-BNP, N-terminal pro-hormone Brain Natriuretic Peptide; SDS, Standard Deviation Score; T-Chol, Total Cholesterol; TG, Triglyceride; Z, Z-Score.

*$p < 0.05$.

differences were not significant by the end of the 2-year dietary intervention period. No changes were observed in the percentile channels for each patient. No statistically significant differences in weight and BMI SDS were observed between each visit and the basal/last visit evaluations.

3.2 | Dietary composition and compliance

3.2.1 | Dietary composition

The protein, carbohydrate, and fat ranges per month are shown in Table 3. The protein content ranged from 3.0 to 3.5 g/kg/day (18.5%–28% of daily energy); carbohydrate intake was 0.5–1.0 g/kg/day (4.0–5.5% of daily energy); and fat consumption varied from 3.0 to 4.5 g/kg/day (70.5%–75.7% of daily energy). MCT was incorporated into the diets of four patients (36%). In these patients, fat accounted for 76% (69%–82%, IQR: 12%) of the total energy, and MCT accounted for 15.5% (13%–35%, IQR: 17%) of the fat energy.

3.2.2 | Diet diversity and exchanges

Of the 10 patients, five (50%) followed the MAD diet. In contrast, the remaining followed the ketogenic diet (with ratios ranging from 1:1 to 2:1). Daily diets were adjusted based on the patients' metabolic control and compliance with the diet (for more detailed patient-specific information, please refer to Supplementary file S3). Global changes in the distribution of dietary styles throughout the study period are described in Table 4. Although the median protein content remained stable, the median carbohydrate content decreased from 49.5% to 5% at baseline, and the median fat content increased from 28.5% to 70.5%.

3.2.3 | Dietary compliance

Compliance with diet, which was assessed four times using questionnaires administered by dietitians (DCQ-D, $p = 0.397$) and patients (DCQ-P, $p = 0.561$), showed no significant differences. Patient satisfaction with diet, evaluated using the PAQ ($p = 0.764$), demonstrated no significant changes during the follow-up period. However, a significant difference ($p = 0.001$) was observed in the M/FC-SST scores, indicating changes in awareness and implementation of the diet between the onset and end of the study (Figure 2).

3.2.4 | Diet support

The total duration of the diet was 24 months. The median number of personal interviews (phone calls, telemedicine, online meetings, and WhatsApp calls) was 64 (17–117, IQR: 34). Of these, 88% (0.00–96.88, IQR:85.60) were positive. "Non-compliance" interviews reflected approximately 1.5 months on the diet (0.5–12, IQR: 10.25).

TABLE 3 Contents of the macronutrients during the two-year follow up of 10 patients.

Macronutrients		Basal Med (IQR) (Min–Max) ($n = 10$)	6. Month Med (IQR) (Min–Max) ($n = 10$)	12. Month Med (IQR) (Min–Max) ($n = 10$)	18. Month Med (IQR) (Min–Max) ($n = 10$)	24. Month Med (IQR) (Min–Max) ($n = 10$)	p
Fat	g/kg/day	1.5 (2.0) (0.0–3.0)	4.5 (4.0) (2.0–8.0)	4.0 (4.0) (2.0–8.0)	4.0 (4.0) (2.0–7.0)	3.5 (4.0) (1.0–7.0)	<0.001
	% of daily energy	28.5 (6.0) (16.0–32.0)	75.7 (14.0) (58.0–81.0)	75.5 (14.0) (52.0–81.0)	74.0 (12.0) (62.0–81.0)	70.5 (20.0) (52.0–81.0)	<0.001
Carbohydrate	g/kg/day	6.5 (6.0) (3.0–12.0)	1.0 (1.0) (0.0–3.0)	0.5 (1.0) (0.0–3.0)	0.5 (1.0) (0.0–3.0)	0.5 (1.0) (0.0–3.0)	<0.001
	% of daily energy	49.5 (5.0) (47.0–53.0)	4.5 (4.0) (2.0–15.0)	4.0 (4.0) (2.0–15.0)	4.0 (2.0) (2.0–15.0)	5.0 (4.0) (3.0–13.0)	<0.001
Protein Protein	g/kg/day	1.0 (1.0) (2.0–4.0)	2.0 (1.0) (2.0–3.0)	3.5 (0.0) (2.0–3.5)	3.5 (1.0) (2.0–3.7)	3.0 (1.0) (2.0–3.0)	0.437
	% of daily energy	23.0 (10.0) (15.0–34.0)	20.0 (11.0) (13.0–40.0)	18.5 (13.0) (14.0–46.0)	25.0 (22.0) (15.0–34.0)	22.0 (21.0) (12.0–45.0)	0.736

TABLE 4 Dietary intervention changes performed during the two-year follow-up period.

Patients	Diet type Fat:Prot:CHO	Time periods			
		First (0–6 months)	Second (7–12 months)	Third (13–18 months)	Forth (19–24 months)
1	Diet type	1.5:1	1.8:1	1:1	1:1
	Fat:Prot:CHO	1.5:1.1:0.4	1.8:0.9:0.1	1:0.55:0.45	1:0.55:0.45
2	Diet type	MAD 10 g CHO	MAD 15 g CHO	MAD 15 g CHO	MAD 20 g CHO
	Fat:Prot:CHO	1:0.9:0.1	1:0.9:0.1	1:0.9:0.1	1:0.9:0.1
3	Diet type	MAD 15 g CHO	MAD 15 g CHO	MAD 15 g CHO	MAD 20 g CHO
	Fat:Prot:CHO	2:0.8:0.2	2:0.8:0.2	2:0.8:0.2	2:0.8:0.2
4	Diet type	MAD 15 g CHO	1:1	MAD 20 g CHO	MAD 20 g CHO
	Fat:Prot:CHO	1:0.7:0.3	1:0.7:0.3	1:0.7:0.3	MAD 1:0.7:0.3
5	Diet type	2:1	2:1	2:1	2:1
	Fat:Prot:CHO	2:0.5:0.5	2:0.55:0.45	2:0.7:0.3	2:0.7:0.3
6	Diet type	1.5:1	1.5:1	1.5:1	1.5:1
	Fat:Prot:CHO	1.5:0.8:0.2	1.5:0.8:0.2	1.5:0.8:0.2	1.5:0.8:0.2
7	Diet type	1.5:1	1.5:1	1.5:1	MAD 25 g CHO
	Fat:Prot:CHO	1.5:0.9:0.1	1.5:0.9:0.1	1.5:0.9:0.1	1.2:0.9:0.1
8	Diet type	MAD 20 g CHO	MAD 20 g CHO	MAD 20 g CHO	MAD 20 g CHO
	Fat:Prot:CHO	1:0.6:0.4	1:0.75:0.25	1:0.8:0.2	1:0.9:1
9	Diet type	1:1	1:1	1:1	1:1
	Fat:Prot:CHO	1:0.5:0.5	1:0.55:0.45	1:0.55:0.45	1:0.55:0.45
10	Diet type	MAD 15 g CHO	MAD 20 g CHO	MAD 20 g CHO	MAD 20 g CHO
	Fat:Prot:CHO	2:0.7:0.3	1.6:0.75:0.25	1.6:0.75:0.25	1.6:0.7:0.3

Note: Diet type ratios (1:1; 1.5:1; 1.8:1; 2:1): In the context of a ketogenic diet the term "ratio 1:1" refers to the ratio of the fat to combined protein and carbohydrates or for every 1 g of combined protein and carbohydrates 1 g of fat should be consumed. Time periods (1st, 2nd, 3rd, 4th): Each period spanned approximately 6 months.

Abbreviations: Fat:Prot:CHO, The ratio of Fat:Protein:Carbohydrates; MAD, Modified Atkins Diet.

3.3 | Metabolic control

3.3.1 | Blood glucose homeostasis

No significant differences were observed in the mean glucose levels assessed using the handheld device or in the number of hypoglycemic episodes during the dietary intervention period (Table 2). All the patients were monitored annually using the C-GMS system (Table 2). The mean percentage of days in the target range was 90% (67–99%)/95% (72–99%) ($p = 0.04$), hypoglycemia 8% (0–33%)/4.5% (0–26%) ($p = 0.05$), and hyperglycemia 2% (0–33%)/0.5% (0–18%) for the first and second years, respectively ($p = 0.01$).

Median insulin levels showed a decreasing trend with the dietary intervention; however, the difference was not statistically significant. In contrast, biotinidase activity was significantly decreased ($p < 0.05$) (Table 2).

The median value of the morning or daily average ketone concentration was 1.5 mmol/L (0.5–3.5, IQR: 1.1).

3.3.2 | Biochemical outcomes

The results of blood biomarker measurements obtained under similar conditions are discussed in Table 2. The

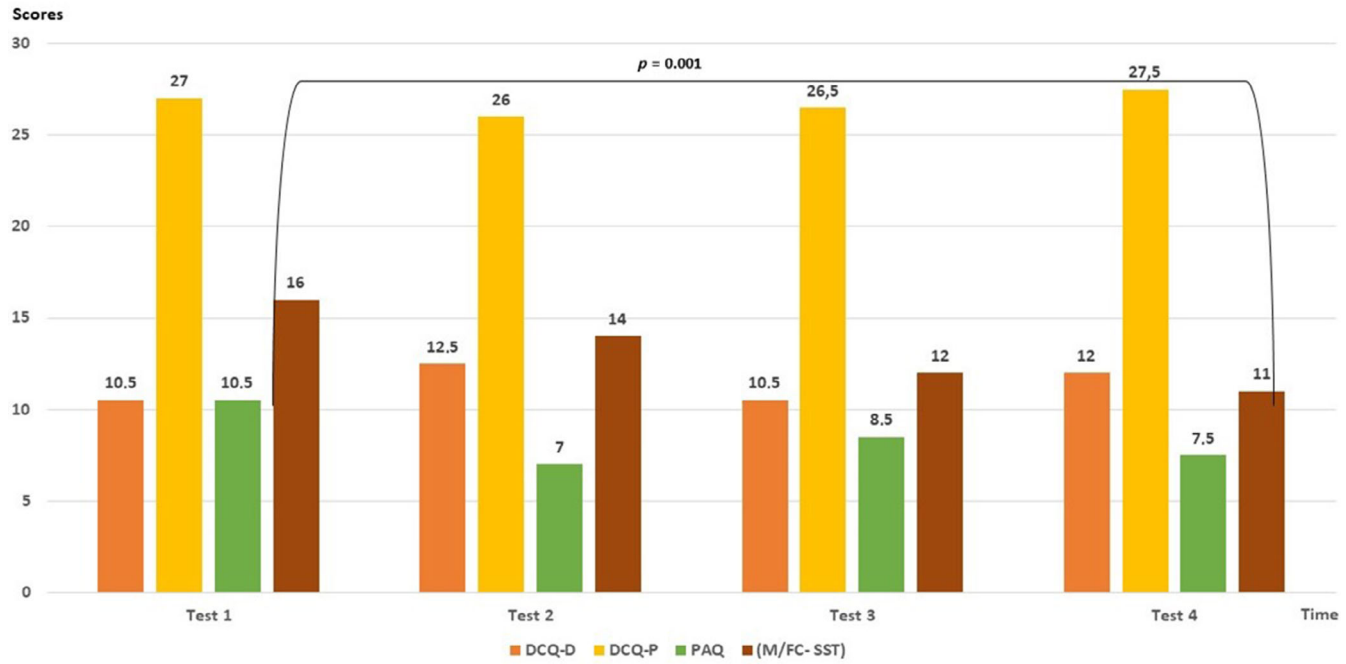

FIGURE 2 This figure displays the high-protein ketogenic diet compliance evaluation results conducted at the end of every sixth month. Adherence to the diet was assessed using four questionnaires: DCQ-P (Dietary Compliance Questionnaire-Parents), DCQ-D (Dietary Compliance Questionnaire-Dietitians), PAQ (Patient/Parents Diet Assessment Questionnaire), and M/FC-SST (Assessment of the Mother/Feeding Caregivers by Simple Simulation Test).

median levels of transaminase and gamma-glutamyl transferase decreased; however, these changes were not statistically significant. Median total protein and albumin levels remained stable and showed no significant changes during the study period.

The median triglyceride level increased by the end of 12 months from 216.5 (98–536, IQR: 197.0) to 272.5 (144–447, IQR: 224.0) mg/dL and at the second year decreased to 150.0 (88–300, IQR: 45.0) ($p = 0.015$).

The main improvement measurements demonstrated a permanent 22% decline in CK levels, from 802.0 (250–3040, IQR:1208.0) to 618.5 (64–1428, IQR:846.0) ($p = 0.03$). Additionally, the CK-MB decreased by 54%, from 23.0 (3–53, IQR: 30.0) to 10.5 (2–33, IQR:20.0) ($p = 0.02$), and lactate dehydrogenase decreased by 30%, from 378.5 (185–446, IQR:160.0) to 264.0 (203–354, IQR: 96.0) ($p = 0.05$).

3.3.3 | Liver ultrasound

Liver ultrasonography revealed persistent hepatomegaly, hepatosteatosis, and coarse parenchymal echotexture. Splenomegaly was detected in half of the patients. Ultrasound findings during the follow-up period are shown in Table 2.

3.3.4 | Echocardiographic findings

Systolic myocardial function was assessed by measuring ejection fraction (EF%), which remained stable throughout the study. Significant decreases were observed in the Z-scores of the end-diastolic interventricular septum (IVSd), left ventricular posterior wall thickness diameter (LVPWd), and left ventricular mass (LVM) ($p < 0.05$) (Table 2).

3.3.5 | Bone mineral density (L1–L4 vetrebrae–DEXA)

The *Z*-scores of the patients were −1.17 (−1.4 to 1.1, IQR: 1.4), −1.18 (−1.3 to 1.3, IQR: 1.6), and −1.19 (−1.3 to 1.4, IQR: 1.5) before, at the end of the first year, and at the end of the second year of dietary intervention, respectively ($p = 0.374$) (Table 2).

Furthermore, no fractures were reported before or during the study.

3.4 | Body composition

Body composition evaluated using a bioelectrical impedance device revealed a slight increase in FM and BF%,

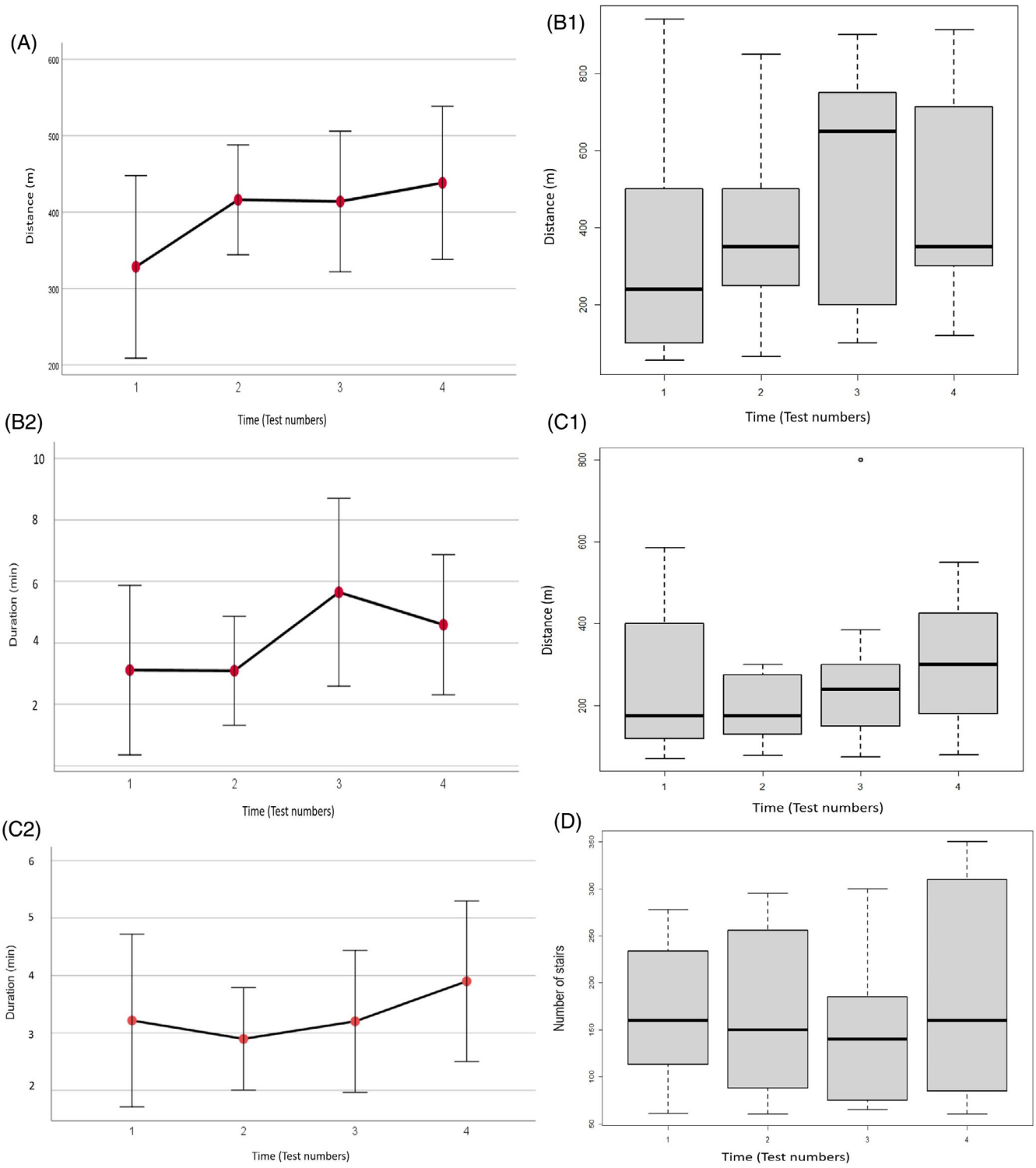

FIGURE 3 Physical activity has been assessed through functional tests: (A) Six-minute walking test: distance (m), (B) Walk without the feeling of fatigue: distance (m) (B1) and time (min) (B2), (C) Running without the feeling of fatigue: distance (m) (C1) and time (min) (C2), (D) Three-minute climbing test: number of stairs. Each functional test was conducted four times at eight-month intervals, represented on the X-axis of the figures as time (test numbers).

which was not statistically significant. MM and BM% increased by the end of the second year, with only the increase in BM% being statistically significant ($p < 0.05$) (Table 2).

3.5 | Physical activity/exercise test

The exercise tolerance evaluated using SMWD was 328.33 ± 155.48 m at the initial evaluation (before

starting the high-protein, high-fat diet), which increased by 33% (438.33 ± 130.43 m) ($p = 0.089$) at the end of the second year of dietary intervention (Figure 3A). The mean measurement of W-FT reached the peak value of 510 ± 305.41 m in the second part of the second year with a high-protein, high-fat diet (Figure 3B1), and the maximal duration of walking was 7.65 ± 3.98 min (Figure 3B2). R-FT demonstrated a sustained increase in the covered distances (from 261.11 ± 204.57 to 318.44 ± 165.84 m) (Figure 3C1) and duration (from 3.22 ± 1.96 to 3.9 ± 1.82 min) (Figure 3C2). According to the TMCT, the mean number of steps taken by the patients at the onset of the high-protein ketogenic diet was 168.89 ± 78.15 (Figure 3D). By the end of the second year, it had increased to 199.22 ± 116.93 steps (Figure 3D). The measurement data are represented in supplementary file S4.

DAQ scores significantly differed ($p = 0.001$) between the initial self-evaluation and the end of the second year, as assessed using the Friedman test. Additionally, the Wilcoxon signed-rank test revealed significant differences among the first ($p = 0.007$), second ($p = 0.007$), third ($p = 0.017$), and fourth ($p = 0.011$) self-evaluations.

4 | DISCUSSION

This was a prospective, pilot, long-term, open-label, monocentric cohort study of 10 patients with GSD type IIIa on a high-protein, high-fat diet. This study reported significant reductions in blood CK concentrations and IVS-LVM measurements, along with improvements in the physical activity of the patients.

Treating GSD type IIIa traditionally emphasizes a diet rich in complex carbohydrates and proteins. However, recently published case reports[4,9] and small retrospective series (involving up to six patients)[7,8,10] have explored high-fat dietary interventions. Nevertheless, this literature is challenging because of the number of cases and significant variability in patient ages (2 months to 47 years), follow-up durations (4 months to 5 years), types of nutritional interventions, and clinical results. Therefore, to clarify further, our study presents the outcomes of a high-protein, high-fat diet, with protein intake ranging from 3.0 to 3.5 g/kg/day (18.5%–28% of daily energy) and fat intake ranging from 3.0 to 4.5 g/kg/day (70.5%–75.7% of daily energy) implemented for 24 months.

From a clinical perspective, our results revealed a sustained decrease in CK levels of 22%, which aligns with the recommendations for the follow-up of dietary lipid manipulations in patients with type III GSD published by Rossi et al,[3] wherein improvement was defined as a reduction of at least 10% in CK levels. This conclusion was based on reduced CK concentrations in 89% of patients with GSD III in the previously published reports.[3] The data, combined with the existing literature for a decrease of CK with MAD reported by Mayorandan et al[8] (in 2 patients), Francini Pesenti et al[9] (in 2 patients), and Olgaç et al[10] (in 5 from 6 patients), with KD by Marusic et al[15] (in 1 patient), with high-protein, high-fat by Kumru Akin et al[16] (in 1 patient) and Massimino et al[17] (in 1 patient), suggest that the positive effect of a ketogenic or high-fat diet on CK concentrations results from reduced carbohydrate intake, minimizing abnormal glycogen accumulation in muscle tissue while providing an alternative energy substrate (ketone bodies). Additionally, the observed positive effect on CK levels may be attributed, in part, to the protein-rich dietary composition of all patients in this study.

The main results of our study revealed a significant, gradual, and sustained reduction in the IVSd, LVPWd, and LVM measurements ($p < 0.05$). Increased fat and protein intake may encourage the heart to use fatty and amino acids for energy production, potentially reducing the need for glucose derived from glycogen. Thus, initiating an early high-fat, high-protein diet could potentially reverse or reduce cardiac glycogen storage. Ketone bodies serve as alternative energy sources for the brain, cardiac and skeletal muscles during metabolic stress. In addition to their role as energy sources, they have the potential to suppress proteolysis in muscles, mitigate glucose depletion, and stimulate muscle regeneration or remodeling by enhancing satellite cell activation and differentiation.[18] Our data, along with the findings from a previous case series, support this hypothesis.[3,4,7,8,10] For example, Massimo et al[17] reported a decrease in IVS diameter (mm) from 15 to 14 and LV mass index (g/m^2) from 65 to 58 in a 24-year-old patient followed up for 2 years. Kumru Akın et al[16] reported a decrease in IVS diameter in a 9-year-old patient who was followed up for 18 months.

Existing publications indicate diminished physical performance in adults[19] and pediatric patients[20] diagnosed with GSD type IIIa. Paschall et al[20] demonstrated that children with Type III GSD performed SMWD below the age-adjusted predicted score for their non-diseased peers (67.57 [IQR: 14.97]). Based on an objective analysis of our exercise tests (SMWD, W-FT, R-FT, and TMCT), DAQ, and cardiac assessments, we inferred that a high-protein, high-fat diet contributes to enhanced exercise capacity. As a plausible explanation for this, the apparent cause seems to be the muscle glycogenolytic block; consequently, the provision of an alternative energy source through a ketogenic diet, along with increased protein intake, might offer a potential explanation for heightened daily activity.

Moving from the main muscle benefits to the concerns related to the diet, we need to start with chronic liver disease, which is a significant disease-related concern among patients with GSD type IIIa.[2,21] In our cohort, similarly to Mayorandan et al,[8] Olgaç et al,[10] Massimino et al,[17] we reported that ALT, AST, and GGT levels did not exhibit statistically significant changes. Theoretically, a reduction in carbohydrate intake through dietary intervention results in reduced glycogen synthesis and accumulation in the liver. However, increased fat content in the diet may lead to hepatosteatosis.[22] We observed that hepatosteatosis was present in 80% of our patients before the dietary intervention, which increased to 90% during the first year of the intervention and then decreased to 70% by the end of the second year. Therefore, special attention and close monitoring of hepatic steatosis are required in patients with glycogen storage diseases on a ketogenic diet. Since ketones are primarily synthesized in the liver, their utilization through dietary modulation raises the question of whether ketones place an additional burden on the liver. Patients with GSD type IIIa are at an increased risk of chronic liver disease, fibrosis, and hepatocellular carcinoma, making it essential to pay particular attention to dietary management.[2,3,21] Therefore, the oral administration of synthetic ketones, as suggested by Valayannopoulos et al,[4] and MCT oil as a booster of ketosis warrant further investigation through extensive clinical studies.

In patients with GSD type IIIa, a negative correlation between biotinidase activity and age and a positive correlation between biotinidase activity and triglyceride levels have been well described in the literature,[23] which has been attributed to advanced liver involvement and fibrosis. As per classical knowledge for GSD type IIIa, the metabolic phenotype and liver enzymes appear to "deceivingly improve" with age, despite worsening muscle and liver disease observed through imaging and biopsy. In our study, a significant decrease in biotinidase levels was observed within 2 years, accompanied by a significant decrease in triglyceride levels. However, no significant changes were observed in other metabolic and imaging parameters of the liver. This suggests a decrease in biotinidase levels may indicate existing or potential liver fibrosis that conventional ultrasound may not detect. Therefore, monitoring patients with GSD type IIIa using FibroScan or liver ultrasound with elastography is recommended.

Hypoglycemia, a side effect of a ketogenic diet or an intrinsic symptom of poor metabolic control in GSD type III, was reported in 10% of the cases before the study, 8% in the first year, and 4.5% in the second year, as determined by CGMS measurements in our cohort. Conversely, the median percentage of days spent within the target range (70–140 mg/dL) significantly increased ($p < 0.05$), and the duration of hyperglycemia significantly decreased from 4% before the study to 2% in the first year and 0.5% in the second year during the dietary intervention period. Similarly, Massimino et al[17] reported a decrease in the average duration of hypoglycemia and an increase in time in the range (70–140 mg/dL) in a single patient with type III GSD who transitioned to a high-protein and high-fat diet. This is an important achievement because it occurs through the elimination of uncooked corn starch and the reduction of carbohydrates in the diet of the patients. Considering the increased incidence of insulin resistance and type 2 diabetes mellitus in aging patients with GSD type III,[21,24,25] maintaining euglycemia with a low-carbohydrate diet is an important achievement for the future.

This study provides insights into how a low-carbohydrate, high-protein, and high-fat diet affects growth in patients with type 3 glycogen storage disease, although it may not fully answer all questions. As it was reported by Gumuş et al,[12] patients with GSD type III and failure to thrive often catch up in height in adulthood. Subsequently, Senter et al[21] described that in 24% of patients reaching adulthood, the BMI was above 25 kg/m^2. Carbohydrate overtreatment was avoided by decreasing the daily intake of major macronutrient carbohydrates and inhibiting the consumption of uncooked corn starch. Monitoring the patient's growth is crucial. In our study, growth arrest and decline were observed in approximately the 6th to 12th months of the diet, which were overcome by the second part of the second year.

The findings of the body composition evaluation using a bioelectrical impedance device suggested that dietary intervention and other factors implemented during the first year might have had a substantial impact on MM compared with that in FM and BF%. Currently, substantial gaps exist in the literature in this domain. In a heterogeneous group of patients with hepatic glycogenosis, Dos Santos et al[26] demonstrated that conventional treatment was associated with elevated body fat content. The statistically significant increase in BM% observed in our study indicates a notable change in the proportion of muscle mass relative to the overall body composition. We consider that the significant changes in the DAQ may reflect better physical activity and potentially demonstrate the possibility of incorporating everyday life activities to achieve better outcomes and remodel body composition.

Owing to the association of long-term KD and GSD type III with an elevated risk of reduced bone mineral density and osteoporosis,[27,28] L1–L4 Z scores, vitamin D levels, bone basic parameters (calcium, phosphorus, and ALP), and DAQ were carefully monitored. Although

there was a gradual decline in Z-scores over the years due to the ketogenic diet and the underlying primary diagnosis of GSD, it was not statistically significant, likely attributable to increased DAQ scores, high protein intake,[29,30] and the regular use of vitamin D supplementation.

The hallmark of the dietary intervention in this study was the initiation, modification, and sustained use of a high-protein, high-fat diet tailored to individual needs and lifestyles. Given the tendency for decreased KD compliance over time, as suggested by Kossoff et al,[5] O'Neill B and Raggi P,[31] regular questionnaires were used to assess patient adherence and satisfaction. However, no significant difference was observed in compliance (DCQ-D [$p = 0.397$] and DCQ-P [$p = 0.561$]) or satisfaction (PAQ [$p = 0.764$]). The patients had the opportunity for retraining, which could potentially influence their compliance. Supporting evidence demonstrated a significant improvement in the awareness levels of the mothers/caregivers and the implementation of the ketogenic diet, as indicated by the M/FC-SST scores ($p = 0.001$) from the onset of the diet to the conclusion of the study. Additionally, positive outcomes in 88% of the interviews underscored the trust and collaboration established between the dietitian and the patient.

When interpreting our study, it is important to consider the potential benefits of a more compliant dietary regimen during dietary intervention and the wide age range of the participants. Furthermore, as this study was a small, nonrandomized observational investigation involving a specific patient cohort that adhered to the prescribed dietary intervention, the alterations in metabolic parameters observed during the study period might have been influenced by factors other than diet alone. Additionally, the long-term sustainability of this diet and its potential metabolic consequences remain unknown. The lack of a thorough evaluation of trace element levels and muscle involvement, including muscle ultrasound and heart and muscle MRI, could be other limitations. However, this study provides real-life data on the efficacy and safety of a 2-year administration of a high-protein, high-fat diet, raising concerns about potential side effects, particularly for long-term use, necessitating further investigation to determine the duration of safe usage.

5 | CONCLUSIONS

This pilot study provides real-life data on the efficacy and safety of a 2-year high-protein, high-fat diet in patients with GSD type IIIa. By employing practical and accurate methods to ensure compliance with a personalized high-protein, high-fat diet, along with regular monitoring of metabolic parameters (including biotinidase activity), liver function, deep morphology, growth (with a particular focus on height), and body composition, sustainable reductions in CK levels and IVS-LVM measurements and increased physical activity can be achieved.

AUTHOR CONTRIBUTIONS

SKU and MÇ conceived the study. SKU, YAA, YM, EC, HY, MYÇ, FE, AY, and ZU did the patient management and supplied patient data. YAA performed the statistical analysis. SKU wrote most of the content in the manuscript. All authors provided input and reviewed the manuscript. SKU was responsible for the final text of this paper.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon request from the corresponding authors.

ETHICS STATEMENT

The human studies described here were conducted with the approval of the Ege University Medical Ethics Committee (70198063-050.06.04, approval number: 18-7/33).

INFORMED CONSENT

All volunteers who participated in this study provided appropriate informed consent under Ege University Medical Ethics Committee protocol (70198063-050.06.04) and adhered to the tenets of the Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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