

# Assessments of energy, macro and micronutrient intakes in children and adolescents with type 1 diabetes mellitus

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## ABSTRACT

**Objective:** This study aims at examining dietary intake in children and adolescents with type 1 diabetes mellitus (DM) and comparing the results with national dietary intake recommendations.

**Patients and Methods:** One hundred fifty children and adolescents (52.7% female) with an average age of 12.2±3.1 years and with type 1 DM who were followed by the Pediatric Endocrinology Polyclinic participated in the study. Three-day food intake records and clinical information regarding the type 1 DM condition of the participants were obtained.

**Results:** No gender-related significant difference was found among the participants regarding food intake. The percentage of energy derived from fat (average 39.6%) and saturated fat (16.1%) were higher than the recommended levels in both gender groups. The percentage of energy derived from carbohydrates (female 44.1±5.7%, male 43.0±6.8%) was below the recommended levels. The dietary fiber intake in children aged 6-10 years with type 1 DM met recommendations, whereas, it was below the recommended levels in other age groups. Micronutrient inadequacy was common in children and adolescents with type 1 DM.

**Conclusions:** The authors believe that guidelines and programs are needed for children and adolescents with type 1 DM to reduce total fat and saturated fat intake, increase carbohydrate and dietary fiber intake up to the recommended levels, and prevent multiple micronutrient inadequacies.

**Keywords:** Type 1 Diabetes, Children, Adolescents, Nutritional Status, Dietary Intake, Macronutrient Distribution

## 1. INTRODUCTION

Type 1 diabetes mellitus (type 1 DM) is an autoimmune illness that results from insulin deficiency due to the autoimmune destruction of insulin-producing pancreatic  $\beta$ -cells characterized by hyperglycemia [1]. Some factors such as modern insulin infusions, advanced medical care, strict glycemic control, insulin types, insulin application frequency, the amount of insulin applied, bolus and basal insulin rates, the frequency of hyperglycemia-hypoglycemia and its treatment, more flexible mealtimes, more frequent snacking, and changes in the lifestyle can lead to excessive weight gain in patients with type 1 DM [2,3]. The increase in Body Mass Index (BMI) is associated with poor metabolic control and increased risk for comorbidity [4]. Controlling the amount of energy and macronutrient intake before leading to hypoglycemia is recommended for the treatment of this condition [5].

The main aim of DM treatment is to maintain glucose levels within a normal range. Metabolic control of type 1 DM depends on an effective plan of intensive insulin therapy, follow-up, and lifestyle changes. A child or person with type 1 diabetes needs to take insulin regularly and adjust their food intake and exercise according to the action and dose of insulin. Although, there are significant improvements in the treatment and technology, Medical Nutrition Therapy (MNT) is still an essential part of controlling diabetes [5,6]. This treatment's primary components are to follow the carbohydrate intake at each meal and adjust the insulin dose [7]. Effective dietary intervention may help obtain better clinical and metabolic results in children and adolescents with type 1 DM [8]. While applying the MNT, energy, and macronutrient intakes should be planned according to age, growth rate, and physical activity and these intakes should be sufficient to ensure optimal growth and maintain ideal body

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weight [5,8]. Dietary recommendations for children with diabetes are based on those recommended for healthy children. Dietary recommendations should be planned considering the child's cultural, ethnic, customary, and psychosocial needs [5]. Current nutritional recommendations include limiting intakes of simple carbohydrates, saturated fatty acids, trans-fatty acids, and salt and increasing intakes of complex carbohydrates and unsaturated fatty acids [7]. However, previous studies pointed out that the carbohydrate intake was below and saturated fat intake was above the recommended levels in children and adolescents with type 1 DM [9].

Therefore, the current paper aims to determine the energy and nutrition intakes of children and adolescents with type 1 DM and compare the results with the national dietary intake recommendations.

## 2. PATIENTS and METHODS

This study was carried out between December 2019-June 2020 on 150 children and adolescents aged 6-18 years with type 1 DM who were followed up at Bursa Uludağ University Health Application and Research Center Pediatric Endocrinology Polyclinic. The sample of this study was selected among the patients who were followed up in the pediatric endocrinology outpatient clinic and voluntarily agreed to participate in this study using the simple random sampling method. The ethics committee approval (no. 2018-14/38) was obtained from Bursa Uludağ Ethics Committee. And permission to conduct the study (no. 73115338-819/38160) was received from Bursa Uludağ University Directorate for Health Application and Research Center. All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patients have given their informed consent for participation in the study.

One hundred fifty children and adolescents aged 6-18 who attended the Pediatric Endocrinology Polyclinic and were diagnosed with type 1 DM (diagnosed at least 1 year ago) were included in the study. Those with chronic diseases (thyroid, celiac, etc.) and using obesity-related drugs were excluded from the study.

The children's body weight and height percentile values were calculated using the World Health Organization (WHO) AnthroPlus software. The obtained percentile values were evaluated using the WHO 2007 BMI-for-age (5-19 years) growth curves [10,11]. Body weight was measured without shoes using a Seca-813 (Kimeks, Istanbul, Turkey) professional weighing scale with a resolution of 0.1 kg. Mesilife-13539 (Mesitaş, Istanbul, Turkey) portable stadiometer with a resolution of 1 mm was used for height measurements. BMI was used to evaluate body weight according to height. BMI values were calculated using the following formula:  $BMI = \text{Body weight (kg)}/\text{height (meter)}^2$  [12].

## Nutrient Analysis

To determine the daily energy and nutrition intakes of the children and adolescents with type 1 DM, the full version of the Nutrition Information System 8.2 (BeBiS 8.2), which was developed on a computer and adapted for Turkey, was used [13]. The nutrition intake data of the children and adolescents with type 1 DM were recorded on three consecutive days (two weekdays and a weekend day). To increase the accuracy of nutrition intake data, training was given to the participants by an expert dietitian. The dietitian reviewed all nutrition intake records. The energy, carbohydrate, protein, fat, and micronutrient contents were calculated using Nutrition Information System 8.2 (BeBiS 8.2). The data obtained from the nutrition intake records were compared with the "Turkey Dietary Guidelines 2015 (TDG)" [13]. While evaluating the daily energy and nutrition intakes,  $\leq 66\%$  was considered poor,  $67\%-133\%$  was adequate, and  $>133\%$  was high [14].

## Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 23. The Kolmogorov-Smirnov test was used to evaluate the normality of data. Independent Samples t-test was used to compare two independent and normally distributed populations. One-way analysis of variance (ANOVA) was used to compare more than two independent populations. The obtained data are presented as mean  $\pm$  standard deviation ( $\bar{x} \pm ss$ ). Mann-Whitney U test was used for the data that are not normally distributed and the Kruskal-Wallis H test was used for three or more independent populations and the results are presented as median (minimum-maximum) values. Pearson's chi-squared test, Fisher's exact test, and Fisher-Freeman-Halton test were used to compare categorical variables and the results are presented as frequency (n, %) values. A significance level of  $\alpha = 0.05$  was adopted in this study [15].

## 3. RESULTS

The baseline characteristics of the participants are summarized in Table I. A total of 150 children and adolescents with an average age of  $12.2 \pm 3.1$  years with type 1 DM were included in the study. A total of 79 participants were female (52.7%) and 71 were male (47.3%), the average type 1 DM diagnosis age was  $6.9 \pm 3.3$  years, and the duration of DM was  $5.3 \pm 3.3$  years. Average body weight, height, and BMI were  $46.2 \pm 15.1$  kg,  $148.4 \pm 16.6$  cm, and  $20.3 \pm 3.4$  kg/m<sup>2</sup>, respectively. The mean HbA1c value of the participants was calculated as  $9.5\% \pm 1.8$ . Regarding insulin therapy, 30 of the participants (20%) were using insulin pump therapy (IPT) and 120 (80%) were multiple daily injections (MDI).

The energy, macronutrients, and dietary fiber intakes by gender are presented in Table II. Daily energy, carbohydrate (g), protein (g), fat (g), cholesterol, and omega-3 intakes in boys with type 1 DM were higher than in girls ( $p < 0.05$ ). The percentages of daily energy derived from fat were similar among genders.

**Table I.** Demographic and Diabetes-Related Characteristics of Children and Adolescents with Type 1 Diabetes

Characteristic	Amount represented
<b>Demographics</b>	$\bar{x}\pm SD$
Age (y)	12.2±3.1
<b>Sex</b>	<b>n (%)</b>
Female	79 (52.7)
Male	71 (47.3)
<b>Mother's Education</b>	
Secondary school and below	98 (65.7)
High school	36 (24.2)
College degree and higher	15 (10.1)
<b>Father's Education</b>	
Secondary school and below	73 (49.7)
High school	51 (34.7)
College degree and higher	23 (15.6)
<b>Income (TL)</b>	
< 2500	36 (24.0)
2501-5000	73 (48.7)
>5001	41 (27.3)
<b>Diabetes and health-related characteristics</b>	
	$\bar{x}\pm SD$
Height (cm)	148.4±16.6
Body Weight (kg)	46.2±15.1
BMI (kg/m <sup>2</sup> )	20.3±3.4
BMI Z-score	0.41±0.92
Type 1 DM diagnosis age (years)	6.9±3.3
Duration of Type 1 DM (years)	5.3±3.3
Total insulin dose (U/day)	42.8±18.0
Insulin dose (U/kg/day)	0.92±0.21
HbA1c (%)	9.5±1.8
<b>Insulin regimen</b>	<b>n (%)</b>
Multiple daily injections	120 (80.0)
Pump	30 (20.0)

Mean ± standard deviation ( $\bar{x}\pm SD$ )

**Table II.** Daily Energy and Nutrition Intakes of Children and Adolescents with Type 1 DM By Gender

	Girls	Boys
Nutrients	$\bar{x}\pm SD$	$\bar{x}\pm SD$
Energy (kcal)**	1376.2±328.1	1546.5±360.3
Carbohydrate (g)*	147.8±38.9	162.5±48.6
Carbohydrate (%)	44.1±5.7	43.0±6.8
Protein (g)**	55.3±14.8	65.1±19.2
Protein (%)	16.5±2.1	17.2±2.8
Fat (g)**	61.1±17.5	68.9±18.2
Fat (%)	39.4±5.2	39.8±5.7
Cholesterol (mg)*	267.8±100.4	307.6±130.3
Saturated fat, % of energy	15.9±3.2	16.4±2.8
Monounsaturated fat, % of energy	13.7±2.2	13.9±2.7
Polyunsaturated fat, % of energy	7.4±2.1	7.3±2.2
Omega 3*	1.2±0.4	1.4±0.6
Omega 6	9.5±3.7	10.4±4.2
Dietary fiber (g)	15.4±4.8	16.9±5.6

Mean ± standard deviation ( $\bar{x}\pm SD$ ). Mann-Whitney U test, \*p<0.05, \*\*p<0.01

In relation to whether the nutrition recommendations provided in the TDG were met or not, the protein intakes were found to be above recommended amounts for 60.8% of the girls and 73.2% of the boys (Table III). Similarly, daily fat intakes were above recommended amounts in almost half of the boys and girls. Regarding dietary fiber, the ratio of girls with inadequate dietary fiber intake (24.1%) was higher than boys (15.5%). Furthermore, the ratio of girls with inadequate daily thiamine, vitamin B6, calcium, and iron intake was higher than boys (p<0.05). Moreover, inadequate daily iodine intake was higher in girls compared to boys (p<0.05).

Daily nutrition intakes in children and adolescents with type 1 DM and TDG's recommended amounts according to age are presented in Table IV. Daily energy intake was below recommended levels in all age groups. The carbohydrate intakes in adolescents aged 15 years and over with type 1 DM were within the recommended amounts, whereas, the carbohydrate intakes in other age groups were below the recommended doses. Fat intake was above recommended values in all age groups. The cholesterol intake was above recommended values in children aged 6–10 years, while it was within recommended levels in other age groups. On the other hand, the fiber intake was above recommended values in children aged 6–10 years, whereas it was below recommended levels in other age groups.

The daily vitamins A and K, niacin, biotin, sodium, and phosphor intakes of children and adolescents in all age groups were above recommended amounts, while vitamin D, potassium, iron, and selenium intakes were below recommended values (Table V).

**Table III.** Information Regarding Meeting the Daily Nutrition Recommendations in Children and Adolescents with Type 1 DM By Gender

Nutrients	Girls		Boys	
	Inadequate (≤66%) n (%)	Excess (>133%) n (%)	Inadequate (≤66%) n (%)	Excess (>133%) n (%)
Carbohydrates	1 (1.3)	20 (25.3)	-	23 (32.4)
Protein	3 (3.8)	48 (60.8)	-	52 (73.2)
Fat	-	36 (45.6)	-	38 (53.5)
Dietary fiber	19 (24.1)	5 (6.3)	11 (15.5)	11 (15.5)
Vitamin A	-	75 (94.9)	-	68 (95.8)
Vitamin E	35 (44.3)	2 (2.5)	30 (42.3)	6 (8.5)
Vitamin C	15 (19.0)	34 (43.0)	12 (16.9)	27 (38.0)
Thiamin	*29 (36.7)	7 (8.9)	*13 (18.3)	10 (14.1)
Riboflavin	13 (16.5)	29 (36.7)	5 (7.0)	37 (52.1)
Vitamin B <sub>6</sub>	*28 (35.4)	16 (20.3)	*11 (15.5)	20 (28.2)
Vitamin B <sub>12</sub>	18 (22.8)	23 (29.1)	10 (14.1)	29 (40.8)
Niacin	7 (8.9)	40 (50.6)	4 (5.6)	48 (67.6)
Folate	28 (35.4)	8 (10.1)	17 (23.9)	11 (15.5)
Calcium	*45 (57.0)	6 (7.6)	*26 (36.6)	3 (4.2)
Iron	*49 (62.0)	-	*27 (38.0)	3 (4.2)
Zinc	37 (46.8)	4 (5.1)	23 (32.4)	8 (11.3)
Selenium	70 (88.6)	-	64 (90.1)	2 (2.8)
Iodine	*10 (12.7)	**28 (35.4)	*1 (1.4)	**41 (57.7)

Inadequate (≤66%), Excess (>133%). chi-squared test, \*p<0.05, \*\*p<0.01

**Table IV.** Daily Energy and Macronutrients Intake of Children and Adolescents with Type 1 DM by Age

	6-10 years		11-14 years		15 years and older	
	Recommended	Intake	Recommended	Intake	Recommended	Intake
Energy (Kkal)	1576	1473.8±292.4	1851	1420.3±339.8	2619	1503.6±454.5
Carbohydrate (g)	130	150.1±31.1	130	148.3±39.9	130	174.9±61.1
Carbohydrate (%)	45-60	40.9±5.2	45-60	41.9±5.9	45-60	46.3±6.3
Protein (g)	18.2-28.4	61.7±15.0	31.5-45.0	58.0±17.0	49.8-53.0	61.0±22.5
Protein (%)	5-20	16.7±2.1	8-20	16.3±2.4	9-20	16.1±2.9
Fat (%)	20-35	41.2±5.4	20-35	40.8±5.1	20-35	36.4±5.1
Cholesterol (mg)	<300	305.0±100.0	300	269.9±103.6	300	292.0±158.1
Omega 3 (g)	0.6-1.2	1.3±0.4	0.6-1.2	1.40±0.6	0.6-1.2	1.2±0.5
Omega 6 (g)	5-10	9.8±4.4	5-10	10.0±3.6	5-10	10.1±3.8
Dietary fiber (g)	16	17.5±5.1	19	15.0±4.7	21	16.2±6.1

**Table V.** Daily Micronutrients Intake of Children and Adolescents with Type 1 DM by Age

	6-10 years		11-14 years		15 years and older	
	Recommended	Intake	Recommended	Intake	Recommended	Intake
<b>Vitamins</b>						
Vitamin A (mcg)	400	1155.4±1698.6	600	957.7±787.1	750	829.5±499.4
Vitamin D (mcg)	15	14.8±10.6	15	6.6±6.5	15	7.4±8.9
Vitamin E (mg)	9	12.0±5.5	13	10.8±3.8	13	11.5±4.1
Vitamin K (mcg)	55	106.0±79.2	60	85.3±63.5	75	92.1±76.9
B <sub>1</sub> (mg)	0.6	0.80±0.24	0.9	0.69±0.2	1.2	0.7±0.2
B <sub>2</sub> (mg)	0.6	1.45±0.41	0.9	1.1±0.3	1.3	1.0±0.4
Niacin (mg)	10	10.4±4.4	10	10.1±04.4	10	10.4±5.4
pantothenic acid (mg)	4	4.8±1.2	5	4.1±1.1	5	4.2±1.5
B <sub>6</sub> (mg)	0.6	1.0±0.3	1	0.90±0.2	1.3	0.8±0.3
Biotin (mcg)	25	50.1±17.5	35	37.4±12.6	35	36.6±16.4
Folic acid (mcg)	200	242.5±71.8	270	211.4±61.3	330	217.9±71.7
B <sub>12</sub> (mcg)	2.5	4.24±1.89	3.5	3.9±2.6	4	3.3±1.7
Vitamin C(mg)	45	103.2±45.1	70	77.4±39.3	100	74.9±37.1
<b>Minerals</b>						
Sodium (mg)	1200	3118.9±818.4	1500	3219.1±1040.7	1500	3411.6±1213.6
Potassium (mg)	3800	2390.5±633.9	4500	1944.3±545.1	4700	1929.2±582.5
Calcium (mg)	800	863.2±258.9	1150	714.6±259.2	1150	660.3±257.2
Magnesium (mg)	230	238.0±59.1	300	208.6±59.4	300	215.5±69.9
Phosphor (mg)	440	1074.4±259.2	640	930.9±258.2	640	937.0±339.4
Iron (mg)	11	7.8±2.0	11	7.4±2.2	11	7.7±2.5
Zinc (mg)	7.4	8.4±2.2	10.7	8.0±2.4	14.2	7.9±2.9
Selenium (mcg)	35	18.5±8.9	55	15.2±12.1	70	15.5±10.3

#### 4. DISCUSSION

Only a few studies have examined the nutrient intake of children and adolescents with type 1 DM in Turkey. Accordingly, the present study aimed to compare the energy and nutrient intakes in children and adolescents with type 1 DM in Turkey with the national nutrition guide (TUBER) [14]. The findings showed that the energy intake in all participants was lower than the recommended amount and the percentage of energy from fat was higher than the recommendation. An examination of

dietary fiber intake indicated that only the participants in the 6–10-year-old group took more dietary fiber than recommended. Multiple micronutrient intake deficiencies were observed in all participants. This was particularly pronounced in participants aged 15 years and over.

Reducing carbohydrate intake is used to arrange insulin dosing and balance postprandial glucose fluctuations [9,15]. TDG recommended that the percentage of energy derived from



carbohydrates should be at least 45%. According to the commonly used carbohydrate counting model for calculating insulin dose, the percentage of energy derived from carbohydrates is recommended to be 50%. Our findings indicated that the carbohydrate intake in children and adolescents was below recommended levels. Similar results were reported in previous studies [9,16]. Although, carbohydrate intake (g) in boys was higher than in girls, the contribution of carbohydrates to energy was lower in boys. The analysis of macronutrient intake by age revealed that the lowest carbohydrate intake was found in children aged 6–10 years ( $40.9 \pm 5.2\%$ ), whereas, the contribution of carbohydrates to energy was increased at older ages (15 years and over;  $46.3 \pm 6.3\%$ ).

According to the TDG and The International Society for Pediatric and Adolescent Diabetes (ISPAD), the percentage of energy derived from fat and saturated fat should be 30-35% and <10%, respectively [5,14]. Although, carbohydrate intake was below recommended values, fat (g) intake in the diet and the percentage of energy derived from fat ( $39.6 \pm 5.4\%$ ) and saturated fat ( $16.1 \pm 3.0\%$ ) were above recommended levels in our participants. According to the previous studies that examined the nutrition status of children and adolescents with type 1 DM, the percentage of energy derived from fat and saturated fat was found to be above recommended levels [9,16,17]. Seckold et al., examined nutritional status of children aged <7 years with type 1 DM and found that the energy derived from fat was within recommended amounts ( $33 \pm 5\%$ ), whereas, the energy derived from saturated fat was above the recommended amount ( $15 \pm 3\%$ ) [18]. Our results indicated that fat intake in 45.6% of the girls and 53.5% of the boys were above recommended levels. Among different age groups, while the highest fat intake was found in children aged 6-10 years ( $41.2 \pm 5.4\%$ ), the lowest fat intake was observed in the participants aged 15 years and over ( $36.4 \pm 5.1\%$ ). These results suggest that children's eating habits change and they consume more high-carbohydrate foods as they grow older. Mackey et al., stated that a higher proportion of energy derived from fat is associated with poor glycemic control [16]. Moreover, according to the Diabetes Control and Complications Trial, a diet high in total and saturated fat is associated with the worst HbA1c values [19].

Our findings indicated that daily protein intake (g) in boys was higher than in girls. The difference between boys and girls was statistically significant ( $p < 0.05$ ). For all participants, the percentage of energy derived from protein ( $16.8 \pm 2.5\%$ ) was within the recommended levels (15-20%) reported by both TDG and ISPAD [5,13]. Similarly, previous studies reported that the protein intake in children and adolescents was within recommended levels [17,20,21].

Our results showed that the mean fiber intake was  $16.1 \pm 5.3$  g/day. Although the fiber intake in girls was lower than in boys, the difference was not statistically significant. While the fiber intake in 6-10-year-old children was above recommended amounts, it was below in other age groups. Similarly, Stechova et al., found that fiber intake in participants with type 1 DM was below recommended amounts (median: 42% of recommended intake) [22]. Lamichhane et al., stated that the fiber intake of

908 children and adolescents with type 1 DM was below the amount recommended by the American Diabetes Association (14g/1000kcal/day). Fiber intake is associated with improved daily blood glucose concentration and glycemic control development in the long term [23]. However, Thomson et al., reported that 84% of 8-18-year-old Australian children with type 1 diabetes met their recommended fiber intake [24]. Higher fiber intake reduces inflammation and lowers mortality risk in adults with type 1 DM [16]. Therefore consumption of fresh vegetables and fruits and unprocessed foods with high fiber among children and adolescents with type 1 DM should be encouraged.

According to the analysis of daily micronutrient intake, micronutrient intake in children aged 6-10 years was within recommended levels except for iron and selenium. However, vitamin E, thiamine, vitamin B<sub>6</sub>, folic acid, pantothenic acid, iron, zinc, calcium, and selenium intakes (the required amounts increase as children grow older) in adolescents aged 11-14 years and 15 years and above with type 1 DM were below recommended levels. Parthasarathy et al., found that mean riboflavin,  $\beta$ -carotene, zinc, and iron intakes in 70 Indian children and adolescents with type 1 DM were below 50%, and thiamine and calcium intakes in the same population were below 60% of the recommended values. They stated that children and adolescents with type 1 DM may be at risk of inadequate intakes of multiple micronutrients [25]. Koç et al., conducted a study with 52 Turkish individuals aged 2-18 years with type 1 DM and determined that intakes of vitamin A in 71.2% of the participants, vitamin E in 86.5%, folate in 76.9%, vitamin C in 32%, and potassium in 100% of the participants were below the recommended levels [26]. In the present study, intakes of multiple micronutrients in children and adolescents with type 1 DM were found to be inadequate as reported in previous studies [25,27]. Inadequate intakes of thiamine, vitamin B<sub>6</sub>, calcium, iron, and iodine were higher in girls than boys and the difference was statistically significant. Calcium intake in 57% of the girls and iron intake in 62% were found to be inadequate. These results suggest that girls with Type 1 DM consume fewer foods rich in vitamins and minerals than boys and therefore, are more vulnerable to multiple micronutrient inadequacy.

It is known that there is no specific nutrition guidance for children and adolescents with type 1 DM and their needs are similar to healthy peers. Nutrition suggestions for type 1 DM focus on optimal glycemic control and ensuring growth and development. Our findings indicate that the diets of children and adolescents with type 1 DM include an unbalanced macronutrient proportion and some inadequate micronutrient intakes. Poor adherence to medical nutrition therapy may lead to health complications in the long term. Therefore, the authors suggest that all healthcare professionals involved in the treatment and follow-up of type 1 DM should encourage patients to perform medical nutrition therapy.

The study's cross-sectional design and the random sampling of the participants may have weakened the representativeness of the sample and the generalizability of the findings.

Selecting participants from a single clinic may reduce the representativeness of the results on nutritional status. Therefore, comprehensive studies are needed to evaluate the nutritional intake of children and adolescents with type 1 Diabetes and to establish effective policies.

### Compliance with Ethical Standards

**Ethical Approval:** Ethics committee approval was received before conducting the study (No: 2018-14/38). This study was conducted in accordance with the Declaration of Helsinki. Informed consents were obtained from both children and their parents. All patients provided written informed consent.

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