

The Relationship between Motor Levels of Children with Cerebral Palsy and Their Eating and Drinking Skills and Energy Intakes

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ABSTRACT

Aim: Cerebral Palsy (CP) is defined as a musculoskeletal system disorder characterized by a non-progressive but the most important symptom of the disease is loss of motor function. In addition to difficulty in movement, as the severity increases, various problems are seen depending on where the brain damage occurred and the extent of the damage. Among these problems, nutritional problems have an important share. This study was conducted to evaluate the relationship of eating and drinking skills with the intake of energy and some nutrients according to gross motor skills of children with CP.

Study Design: The research was conducted on 74 volunteer children (52.7% male; 8.7±3.32 years) and their parents. For the evaluation of the nutritional status of the children, a 3-day food intake record and Eating and Drinking Ability Classification System (EDACS) was used.

Results: In all age groups, the daily energy, carbohydrate and fiber intake of children in the GMFCS IV-V group is less than those in the GMFCS I-II-III group. There was a statistically significant positive strong relationship between GMFCS and EDACS, however there was a low negative correlation between GMFCS and average daily carbohydrate intakes.

Conclusions: Results are supported by this study and EDACS can be an easy and practical method in classifying children's eating and drinking skills in order to create a common language in the literature.

Keywords: Nutrition, Cerebral Palsy, Children

Serebral Palsili Çocukların Motor Düzeyleri ile Yeme-İçme Becerileri ve Enerji Alımları Arasındaki İlişki

ÖZET

Amaç: Serebral Palsi (SP), ilerleyici olmayan motor fonksiyon kaybı ile karakterize kas-iskelet sistemi bozukluğu olarak tanımlanmaktadır. Hareket zorluğunun yanı sıra, beyin hasarının meydana geldiği bölge ve hasarın boyutuna bağlı olarak çeşitli problemler görülmektedir. Beslenme sorunları da bu problemler arasında yer almaktadır. Bu çalışma, SP'li çocukların kaba motor becerilerine göre yeme ve içme becerileri ile enerji ve bazı besin ögesi alım düzeyleri arasındaki ilişkiyi değerlendirmek amacıyla yapılmıştır.

Yöntem: Bu çalışma, gönüllü 74 çocuk (%52,7 erkek; 8,7±3,32 yıl) ve ebeveynleri üzerinde gerçekleştirilmiştir. Çocukların beslenme durumlarının değerlendirilmesi için 3 günlük besin tüketim kaydı ile Yeme ve İçme Becerisi Sınıflandırma Sistemi (EDACS) kullanılmıştır.

Bulgular: Tüm yaş gruplarında GMFCS IV-V grubundaki çocukların günlük enerji, karbonhidrat ve lif alımı GMFCS I-II-III grubundaki çocuklara göre daha azdır. GMFCS ve EDACS arasında istatistiksel olarak anlamlı, pozitif ve güçlü bir ilişki varken, GMFCS ve ortalama günlük karbonhidrat alım düzeyleri arasında düşük negatif korelasyon vardır.

Sonuç: Literatürde ortak bir dil oluşturmak için EDACS çocukların yeme içme becerilerinin sınıflandırılmasında kolay ve pratik bir yöntem olabilir ve sonuçlar bu çalışma ile desteklenmektedir.

Anahtar sözcükler: Beslenme, Serebral Palsi, Çocuk

Cerebral Palsy (CP) is defined as a musculoskeletal system disorder characterized by a non-progressive but permanent abnormal posture and movement disorder that occurs due to a brain defect in the developing fetal period or infant brain (1). In community-based studies, it is reported that global incidence rate of CP is 1.5-4 per 1000 live births (2-4). In Turkey, 4.4/1000 live births were reported in children of 2-16 years (5).

In recent years, individuals have been widely classified according to motor impairment, limb involvement, and the gross motor function classification system (GMFCS) (6,7). The GMFCS classifies children from level I to level V based on their age-specific gross motor activity. As the level increases, the child's care and dependence status increases (7).

Although the most important symptom of the disease is loss of motor function, its severity is also important. In addition to difficulty in movement, as the severity increases, various problems are seen depending on where the brain damage occurred and the extent of the damage (1,8). Among these problems, nutritional problems have an important share. Some behaviors such as gastroesophageal reflux (GER), constipation, refusal of food, and problems with swallowing cause nutritional difficulties (9). Energy and nutrient intake of children with nutritional problems/difficulties are also affected (10). As CP severity increases, the problems as well as the difficulty in feeding increase. For this reason, the eating and drinking skill classification system, which was developed by taking into account CP severity of the children, aims to classify, using meaningful distinctions, how individuals with CP practically eat and drink in life (11).

The number of studies evaluating the energy and nutrient intake of children with CP and associating them with gross motor activities is limited (9,10). This study was conducted to evaluate the relationship of eating and drinking skills with the intake of energy and some nutrients according to gross motor skills of children with CP.

METHODS

Study Design

This is a descriptive cross-sectional study. In the study, 'survey method' was used as the data collection tool, and data collection was carried out via face-to-face interviews. The patients who visited the Pediatrics Unit were interviewed twice during the research. During the first interview, the survey questionnaire was administered by the

researcher in the mothers of the children or in those who provided care in cases where the mother could not be reached. For the evaluation of the nutritional status of the children, a 3-day food intake record was used. Training on how to fill the 3-day food intake record was provided by the researcher to the caregivers and they were asked to fill it up before the next meeting. In addition, during the first interview, the Eating and Drinking Ability Classification System (EDACS) was filled via face-to-face interviews with the caregivers.

In the second meeting, the 3-day food intake record requested from the caregivers was examined. The caregivers were asked to complete the missing parts that were detected by the researcher.

The child's gross motor function status was evaluated using the GMFCS and grouped at five different levels. Then, the participants were pooled in two groups: GMFCS I-II-III as "good and moderate", and GMFCS IV-V as "severe" (7).

Participants

When the test power was 80% and 90% and at the 5% significance level, sample values were calculated as 52 and 68, respectively. Therefore, the study was carried out with 74 children (52.7% male/47.3% female) between the ages of 5 and 13 years, and who were diagnosed with CP and referred to the Pediatric Rehabilitation Unit of the Physiotherapy and Rehabilitation Department of the Faculty of Health Sciences, Gazi University.

Those who have been diagnosed with any other genetic and/or neurological disease other than CP, children and adolescents whose caregivers could not be reached, and those with a contracture to prevent height measurement were not included in the study. Children and adolescents 5-13 years with a diagnosis of CP were included in the study. Approval of the Ethics Commission of Gazi University was obtained (# 77082166-604.01.02). An Informed Voluntary Consent Form was read and signed by the parents of all children participating in the study.

Data Collection

The reported amount of food and/or drink consumed daily by the children was quantified. The amount of the nutrients included in the meals consumed by the children was calculated using the books titled: *Standard Meal Tariffs* (12) or *Samples from Turkish Cuisine* (13) Daily dietary energy and nutrients were analyzed using the

Nutrition Package Information Systems Program (BEBIS). The daily energy and nutrient status of the children calculated through the data obtained from the 3-day food intake record forms were compared with the Daily Reference Intakes (DRI) data based age and gender, and intake ratios were calculated (14).

Eating and Drinking Ability Classification System (EDACS)

The children's eating and drinking abilities were assessed using EDACS (11). The EDACS is a classification system used to determine the level of eating skills in children with CP and ranges from level I to level V.

Data Analysis

SPSS for Windows 22.0 statistical package program was used to evaluate the data (15). Whether the data were normally distributed according to the groups was examined via the Shapiro-Wilk test and appropriate graphical methods. The categorical data obtained are expressed in numbers and percentage (%) values. Average (X), standard deviation (SD), minimum and maximum values were used as descriptive statistics for numerical variables. In numerical variables, the Independent Samples T-Test was used to compare two normally distributed independent groups, and the Mann-Whitney U test was used to compare two independent groups in non-normally distributed variables. The Pearson Test was used to determine the correlation coefficients and statistical significance of numerical variables with normal distribution. The Spearman's Test was used for variables, of which at least one does not exhibit normal distribution, or for ordinal variables. In defining the correlation coefficient (r) value:0.05-0.30: low/insignificant correlation;0.30-0.40: low/moderate correlation,0.40-0.60: moderate correlation;0.60-0.70: good correlation;0.70-0.75 very good correlation;0.75-1.00: perfect correlation (16). In evaluating the significance levels (p values) of the data, $p < 0.05$ was considered as significant.

RESULTS

According to GMFCS, 69% of the children were in the GMFCS I-II-III group, while 31% were in the GMFCS IV-V group. Of the children, 66.7% of the GMFCS I-II-III group and 65.2% of the GMFCS IV-V group were in the 5-8 age group.

Table 1 shows the dependency of the children during eating according to the GMFCS. While 74.5% of the children at GMFCS I-II-III were independent while eating, 60.9% of the children at GMFCS IV-V were dependent during

eating. All of the children at GMFCS I-II-III and 60.9% of the children at GMFCS IV-V were in EDACS I-II-III.

The percentages of the energy and the macro and micronutrients consumed by the children and adolescents with the daily diet according to the gross motor function level and the recommended amounts according to the age groups are given in Table 2. The difference between the other parameters except carbohydrate (9-13 years; GMFCS I-II-III $183.2 \pm 89.27\%$ and GMFCS IV-V meet $116.9 \pm 46.75\%$) and sodium intake (9-13 years; GMFCS I-II-III $128.9 \pm 44.37\%$ and GMFCS IV-V meet $80.0 \pm 31.96\%$) according to GMFCS was not statistically significant for both age groups ($p > 0.05$) (Table 2).

Table 1: Addictive situations in eating of the children according to the Gross Motor Function Classification System (GMFCS)

	GMFCS I-II-III (n=51)		GMFCS IV-V (n=23)	
	n	%	n	%
Status of Dependence during Eating				
Independent	38	74.5	2	8.7
Slightly Dependent	6	11.8	3	13.0
Moderately Dependent	7	13.7	4	17.4
Dependent	-	-	14	60.9
EDACS				
I-II-III	51	100	14	60.9
IV-V	-	-	9	39.1
Age, mean (SD) range, year	8.8±3.33		8.5±3.34	
EDACS, Eating and Drinking Ability Classification System				

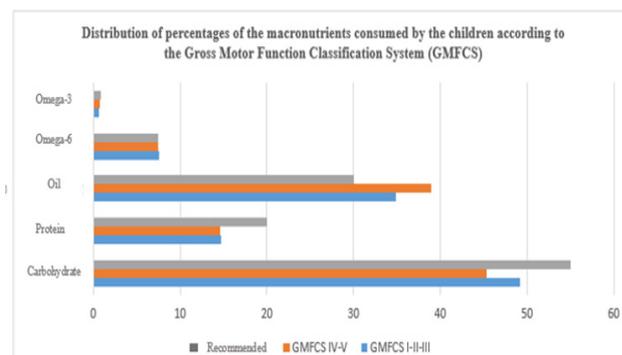


Figure 1: The macro nutrients and fatty acid distribution of the energy to be taken daily according to the DRI recommendations

Table 2: Children's dietary intake of energy and macronutrient and the percentage of meeting the recommended amounts according to Gross Motor Function Classification System (GMFCS)

	Age: 5-8 years (n=49)				p	Age: 9-13 years (n=25)				p
	GMFCS I-II-III (n=34)		GMFCS IV-V (n=15)			GMFCS I-II-III (n=17)		GMFCS IV-V (n=8)		
	$\bar{X} \pm SD$	$\bar{X} \pm SD$ (%)*	$\bar{X} \pm SD$	$\bar{X} \pm SD$ (%)*		$\bar{X} \pm SD$	$\bar{X} \pm SD$ (%)*	$\bar{X} \pm SD$	$\bar{X} \pm SD$ (%)*	
Energy (kcal)	1294.2±279.17	76.3±16.81	1255.9±292.28	73.9±16.75	.712	1799.5±667.89	83.4±30.90	1315.3±421.61	59.5±18.06	.081
Energy (kcal/kg)	65.15±19.40	-	76.3±21.87	-	.140	51.4±12.15	-	59.2±15.57	-	.315
Protein (g/kg)	2.5±0.83	326.1±108.59	2.6±1.07	343.9±141.09	.099	1.8±0.71	234.6±93.69	2.3±0.76	306.3±99.43	.071
Carbohydrate (g/day)	154.8±40.82	119.0±31.40	146.2±63.28	112.4±48.67	.488	238.1±116.05	183.2±89.27	151.9±60.77	116.9±46.75	.036
Fiber (g)	15.9±6.04	63.7±24.15	11.8±6.53	47.1±26.13	.051	20.5±14.39	73.5±54.18	15.2±8.60	51.8±27.62	.351
Vitamin C (mg)	113.5±85.91	453.9±343.65	83.8±61.65	335.2±246.58	.515	97.6±81.84	216.8±181.86	82.8±69.40	183.9±154.23	.683
Vitamin B12 (mcg)	3.8±2.01	316.0±167.69	3.1±2.36	256.6±196.39	.273	3.6±2.59	201.5±143.74	4.5±3.35	252.2±185.92	.449
Thiamin (mg)	0.7±0.22	123.7±36.11	0.6±0.21	106.2±35.09	.103	0.9±0.48	96.7±52.86	0.7±0.23	79.9±31.03	.502
Riboflavin (mg)	1.1±0.39	184.4±66.18	1.1±0.41	175.8±67.64	.625	1.1±0.46	126.9±50.62	1.1±0.32	127.4±36.02	.727
Niacin (mg)	9.2±5.89	114.7±73.71	8.1±6.39	100.7±79.91	.250	11.7±6.96	97.1±57.99	9.5±6.26	79.1±52.16	.485
Calcium (mg)	586.0±278.68	58.6±27.87	589.3±279.62	58.9±27.96	.983	546.3±278.68	42.0±21.44	541.5±217.49	41.7±16.73	.727
Iron (mg)	8.1±2.88	81.4±28.89	6.7±2.38	67.0±23.80	.118	10.9±6.43	136.6±80.33	8.7±3.74	108.4±46.76	.294
Zinc (mg)	6.6±1.85	131.2±36.91	5.4±2.20	108.7±44.06	.068	9.0±3.91	112.7±48.90	6.8±2.93	85.3±36.62	.145
Sodium (g)	1.1±0.70	76.5±50.20	1.1±0.57	78.6±40.63	.712	1.8±0.62	128.9±44.37	1.1±0.45	80.0±31.96	.009
Potassium (g)	2.0±0.61	53.3±16.15	1.9±0.66	48.8±17.31	.398	2.0±1.01	44.9±22.53	1.9±0.70	42.6±15.65	.954

*Calculated based on Daily Reference Intakes (DRI); Mann Whitney U testi; p<0.05.

In Figure 1, the macro nutrients and fatty acid distribution of the energy to be taken daily according to the DRI recommendations are shown according to the groups of gross motor function levels. The difference between groups was not statistically significant (p>0.05).

Table 3 presents the relationship between children's GMFCS levels and energy and some macro-micro nutrient intakes. There was a statistically very good correlation between GMFCS and EDACS (r = 0.736, p = 0,000). There was a negative low correlation between GMFCS levels and average daily carbohydrate (r = -0.326; p = 0.005), fiber (r = -0.348; p = 0.002), thiamine (r = -0.243; p = 0.037), iron (r = -0.241; p = 0.039 with GMFCS), zinc (r = -0.246; p = 0.034), and sodium (r = -0.264; p = 0.023) intake amounts.

Table 3: Relationship between children's gross motor function levels (GMFCS) and Eating and Drinking Skill Classification System (EDACS), energy, and some nutrients intakes

	GMFCS	
	r [†]	p
EDACS	0.736	.000
Energy (kcal)	-0.207	.076
Energy (kcal/kg)	0.194	.098
Protein (g/kg)	0.171	.144
Carbohydrate (g/day)	-0.326	.005
Fiber (g)	-0.348	.002
Vitamin C (mg)	-0.108	.359
Vitamin B12 (mcg)	0.006	.962
Thiamin (mg)	-0.243	.037
Riboflavin (mg) †	-0.008	.948
Niacin (mg)	-0.123	.296
Calcium (mg) †	0.043	.719
Iron (mg)	-0.241	.039
Zinc (mg)	-0.247	.034
Sodium (mg)	-0.264	.023
Potassium (mg)	-0.076	.520

†Spearman Correlation Analysis; †Pearson Correlation Analysis;
*p<0.05 **p<0.001

DISCUSSION

In 74 children aged 5-13 years with a CP diagnosis, in order to determine nutritional status and eating-drinking skills based on GMFCS, general information, their food consumption records, eating and drinking skills, anthropometric measurements, and gross motor function levels were inquired and assessed. One of the important factors affecting the growth of children with CP is the nutritional status. Nutritional problems in children with CP make it difficult to provide care for these children and their struggles with the disease (17,18). By following closely the dietary habits of these children, the chance of early intervention can be obtained (19).

With the increase in motor impairment in children with CP, nutritional problems and dependence while eating increase (19). Dahlseng et al. (20) reported that 19.9% of the children were completely dependent while eating. They showed that children's dependence during eating and their status of joining family meals are affected by the gross motor function level. It is also observed in this study that children and adolescents with severe CP are more dependent during eating, they have a lower rate of joining family meals, and they start joining the family meal at a later age than those who are mildly/moderately involved ($p < 0.05$).

Gangil et al. (21) conducted a study in 100 children with CP aged 1-9 years old, and reported that all children had oral motor dysfunction, and especially children with spastic quadriplegia and hypotonic children had poorer feeding ability. In addition, these researchers found that 16% of the children participating in the study could not feed themselves, 19% had swallowing problems, and 20% could not control their saliva (21).

Many factors that are not found in healthy children can affect the energy needs of children with CP. The brain damage that exist in children with CP determines the motor type and disease severity of each child, and thus, determines their mobility level and muscle functions. Therefore, the resulting clinical picture can change their total energy expenditure and needs (22). Some studies argue that children with CP require less energy than healthy children (23,24) while some others argue that involuntary movements (such as, dyskinetic subtypes) require more energy (25,26). In this study, the 3-day food intake records of the children were evaluated, and the daily energy and nutrient consumption levels were examined without including vitamin and mineral supplements.

Lopes et al. (27) evaluated the energy intake and nutrient consumption of 90 children with chronic encephalopathy aged 2-13 years. When energy intakes were compared with the DRI, it was observed that the energy intakes of the children in the 2-3 years age group were parallel to the recommended levels, while children in the 4-6 age range who were hemiparetic and tetraparetic remained below the recommended levels. Boys and girls aged 9-13 years were also found to have energy intakes below the DRI. Sullivan et al. (28) reported that 71% of the tetraparetic children had low energy intake, which was regardless of gender. Thommessen et al. (29) found that children of 1-16 years of age with nutritional problems and disabilities had lower energy intakes than those with disabilities, but not suffering nutritional problems. It has been reported that energy intakes in children with CP is 71%-96% of the recommended values for healthy children in the same age group (23,28). In this study, based on the 3-day food intake records of the children, the daily energy intakes of children in the 5-8 age group at GMFCS I-II-III meets $76.3 \pm 16.81\%$ of the DRI, and in the 9-13 age group, it was observed that it meets $83.4 \pm 30.90\%$. It was found that the rates of daily energy intakes of the children and adolescents aged 5-8 years and 9-13 years at GMFCS IV-V in terms of meeting the DRI recommendations were $73.9 \pm 16.75\%$ and $59.5 \pm 18.06\%$, respectively (Table 2). Although there was a difference between the GMFCS groups, there was no significant relationship between GMFCS and energy intake levels (Table 3). This result contradicts studies supporting the decrease in energy intake as the severity of influence increases (22,30,31). This may be due to the fact that the present study did not include many severely affected children or adolescents, and children and adolescents fed enterally or parenterally were not included in the study.

Balancing macronutrients is as important as energy intake (32) Walker et al. (22) assessed the protein intake in 73 children with CP in an average age of 2.6 ± 0.8 years, and found that the daily protein intake per kilogram in children at GMFCS I-II-III was 3.1 ± 0.7 , and those at GMFCS IV-V was 2.7 ± 1.2 . The ratio of energy from fat was found to be $33.7 \pm 7.3\%$ in children at GMFCS I-II-III, and $34.5 \pm 7.1\%$ in children at GMFCS IV-V. In addition, the distribution of macronutrients in the diet, regardless of energy intake, was found balanced and similar in all children.

Kilpinen-Loisa et al. (31) evaluated the nutritional intake of 54 children with motor impairment (59% children with CP, median age 10.9 years) with the 3-day food intake record and found that 17% of total energy was from protein, 32% from fat, and 50% from carbohydrates. Sabuncular (33) found that 55% of energy was provided from carbohydrate, 12.8% from protein, and 32.1% from fat. Sangermano et al. (34) evaluated the daily energy intakes of 30 children with neurological problems in an age range of 2-15 years using the 3-day food intake record. It was found that there was an increase in fat and protein intakes (37% of total energy from fat, 17% from protein), and a decrease in carbohydrate intake (46% of total energy from carbohydrate).

The amount of protein intake per kilogram in children and adolescents at GMFCS I-II-III and GMFCS IV-V was well above the recommended percentage, and the rate of energy from fat was high (34.0-38.5%) and from carbohydrates (45.4-50.1%) was low in all age groups. The amount of fiber intake was well below the recommended level for all age groups and both genders (Table 2). Balancing macronutrients is as important as energy intake. In this context, it is seen that the study group displays an unbalanced diet modeling by taking high fat and low carbohydrates, which is in line with some studies (27,34). These may be the reasons for the unbalanced diet pattern revealed by the study: tendency of school-age children to consume fast food and similar foods during school time, fatty foods are more delicious, some children and adolescents' dependence on feeding.

It is stated that chewing and swallowing problems, inability to feed on their own, and long and stressful meal times may cause inadequate macro and micronutrients in children with CP (35). Micronutrients have important roles in many metabolic pathways and needed for metabolism to work in an order (36). Insufficiency of one or a few micronutrients can cause symptoms, and in children with neurological problems, such deficiencies can be difficult to be distinguished. Low energy intake can also result in inadequate micronutrient intake (37).

Grammatikopoulou et al. (38) found that children with CP had low intake of vitamin A, biotin, folate, vitamin K, and copper, and also observed that other micronutrients were in line with the recommended rates or above. Calis et al. (30) evaluated the 7-day food intake record of 176 children with CP, and the daily average calcium intake was found to meet 87% of the recommended values, 77% for vitamin A, 13% for vitamin B6, and 78% for folate. Kalra et

al. (39) evaluated 50 children with CP, and argued that there is a difference between GMFCS levels and nutrient intakes, and the amount of intake decreases as the severity of the involvement increases. However, when they compared the nutritional consumption of children with CP and healthy children, they found that children with CP had lower intake of copper, iron, and magnesium. The Oxford Nutrition Study II (28) reported that children with CP met 77% of calcium, 96% of phosphorus, and 89% of riboflavin compared to the DRI. Contrary to these studies, Baglam (40) found in 96 neurological children that the children's levels of vitamin A, vitamin E, vitamin B1, vitamin B2, niacin, vitamin B6, and vitamin B12 were well above the recommendations, and argued that this was due to the vitamin and mineral supplements used. Patrick and Gisel (37) argue that nutritional problems in children with neurological problems are due to inadequate energy intake rather than micronutrient deficiencies.

In this study, children and adolescents at GMFCS I-II-III and in age groups of 5-8 and 9-13 met $199.8 \pm 179.19\%$ and $112.7 \pm 73.26\%$ of the average daily vitamin A needs, respectively, and the difference between age groups was statistically significant ($p < 0.05$). Mildly involved children's rate of meeting calcium (42.0%-58.6%) and potassium (44.9%-54.2%) DRI were low in all groups ($p > 0.05$). When the micronutrient intake levels of the severely affected children were evaluated, the average daily calcium intake of children aged 5-8 years at GMFCS IV-V was 589.3 ± 279.62 mg, and the average daily calcium intake of children in the 9-13 age group was 541.5 ± 217.49 mg. The amount of magnesium intake decreased as the age increased ($p < 0.05$). However, there was no relationship between the severity of the involvement and the intake levels of micronutrients. While the results of this study contradict with some studies (30,39) in the literature, they also coincide with some other studies (37,40). The reason for this outcome may be that the percentage of children with less severe involvement in this study is low, and that the diet they consume is insufficient in volume but high quality.

Although a significant amount of time is allocated for feeding children with CP, it is observed that children and adolescents are malnourished especially in terms of energy, fiber, and calcium. Though there is no statistical difference between GMFCS levels and energy and nutrients, it is known that malnutrition is more evident in children with moderate and severe involvement (27,41). In addition, the reason for the lack of insufficiency in energy and macro and micronutrients in this study is that the rate of children

with chewing and swallowing problems was low in the study.

The EDACS was used to classify how children with CP eat and drink in daily life using meaningful distinctions. A very good correlation was found between GMFCS and EDACS ($r = 0.736$, $p = 0.000$). Sellers et al. (11) found that there was a good positive relationship between EDACS and GMFCS, and that there were more limitations in the eating and drinking skills of children and adolescents whose gross motor functions were more affected. The EDACS can be an easy and practical method to classify children's eating and drinking skills in order to create a common language in the literature.

CONCLUSIONS

The nutritional status of children with CP is of great importance to closely follow their growth and development. EDACS can be a good tool for evaluating nutritional habits, as it is a practical and easy tool besides food consumption records.

DECLARATIONS

Conflict of Interest

Authors declare that there is no conflict of interest.

Ethical Approval

The study procedures were approved by the Ethics Commission of GAZİ University (Date: 20.01.2016, Number:77082166-604.01.02-). Informed consent was obtained from all individual participants included in the study.

Human and Animal Rights Statement

All procedures performed in studies involving human participants were 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.

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