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The Effect of Breastfeeding Duration on Executive and Cognitive Functions at Healthy School Age Children

Anne Sütü Alım Süresinin Okul Çağı Çocuklarında Yürütücü İşlevlere ve Bilişsel Düzeye Etkisi

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Abstract

Breast milk is a natural food and contains all the nutrients the baby needs for healthy growth. The last trimester of the intrauterine period and the first 2 years of life are the periods which brain development peaks. Docosahexaenoic Acid (DHA) and Arachidonic Acid (AA) accumulate in the membranes of brain cells during these periods when brain development peaks. DHA is particularly involved in the myelination process of the frontal lobe. In addition to DHA and AA, breast milk also contains cholesterol, sphingomyelin, phosphoditylcholine, which are necessary for myelin synthesis. The aim of this study is to evaluate the effect of duration of exclusively breastfeeding on cognitive level and executive functions in school-aged children with no psychiatric complaint. A total of 71 children aged 8-13 years without any psychiatric diagnosis constituted the study group. According to the duration of receiving only breast milk, they were separated into 2 groups as those who receive only breast milk for less than 6 months and those who receive breast milk for 6 months or more. The children's executive functions were assessed with Stroop Test and Wisconsin Card Sorting Test (WCST) respectively. After the executive function tests, Verbal subtests of the Wechsler Intelligence Scale for Children-Revised (WISC-R) including General Knowledge and Vocabulary and Performance subtests of the WISC-R including Picture Completion, Picture Arrangement were applied to the children. In our study, the parent form of the Strengths and Difficulties Questionnaire (SDQ) was used to screen the behavioral and emotional problems of children. Standard scores of WISC-R (total and subtests), Stroop Test and WCST were similar for both groups (p>0.05). In our study, we found that the duration of exclusively breastfeeding did not have an effect on the cognitive level and executive functions of the child.

Keywords: Breastfeeding, Executive Functions, Cognition, Intelligence, Child and Adolescent.

Özet

Anne sütü bebeğin sağlıklı büyümesi için ihtiyaç duyduğu tüm besinleri içeren doğal bir besindir. İntrauterin son üç ay ve yaşamın ilk 2 yılı beyin gelişiminin zirve yaptığı dönemlerdir. Dokosaheksaenoik Asit (DHA) ve Araşidonik Asit (AA) beyin gelişiminin zirve yaptığı bu dönemlerde beyin hücrelerinin zarlarında birikir. DHA, özellikle frontal lobun miyelinasyon sürecinde yer alır. Anne sütünde DHA ve AA'nın yanı sıra miyelin sentezi için gerekli olan kolesterol, sfingomyelin, fosfoditilkolin de bulunmaktadır. Bu çalışmanın amacı, psikiyatrik yakınması olmayan okul çağındaki çocuklarda sadece anne sütü ile beslenme süresinin bilişsel düzey ve yürütücü işlevler üzerine etkisini değerlendirmektir. Çalışma grubunu 8-13 yaşları arasında herhangi bir psikiyatrik tanısı olmayan toplam 71 çocuk oluşturdu. Anne sütü alma sürelerine göre 6 aydan az sadece anne sütü alanlar ile 6 ay ve üzerinde anne sütü alanlar olarak 2 gruba ayrıldı. Çocukların yürütücü işlevleri sırasıyla Stroop Testi ve Wisconsin Kart Eşleme Testi (WKET) ile değerlendirildi. Yürütücü işlev testleri sonrasında çocuklara WISC-R'nin Genel Bilgi ve Sözcük dağarcığını içeren Sözel alt testleri ve WISC-R'nin Resim Tamamlama, Resim Düzenleme gibi Performans alt testleri uygulanmıştır. Çalışmamızda çocukların duygusal ve davranışsal problemlerini taramak için Güçler ve Güçlükler Anketi'nin (GGA) ebeveyn formu kullanılmıştır. Stroop testi, WKET ve WISC-R(toplam ve alt testler) standart puanları her iki grup için benzerdi (p>0.05). Çalışmamızda sadece anne sütü ile beslenme süresinin çocuğun bilişsel düzeyi ve yürütücü işlevleri üzerinde etkisi olmadığını saptadık.

Anahtar Kelimeler: Anne Sütü, Yürütücü İşlevler, Biliş, Zekâ, Çocuk ve Ergen.

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INTRODUCTION

The World Health Organization suggest that only breastfed for the first 6 months from birth, start complementary foods after 6 months, and continue breastfeeding until the age of 2 years (1). The positive effects of breastfeeding on maternal and infant health are well known. While breastfeeding protects the mother against breast cancer, it also protects the baby against the diseases. Breast milk is a natural food and contains all the nutrients the baby needs for healthy growth (2). Polyunsaturated fatty acids such as Docosahexaenoic Acid (DHA) and Arachidonic Acid (AA) in breast milk are a significant effect on the neurodevelopmental process. The last trimester of the intrauterine period and the first 2 years of life are the periods which brain development peaks (3). DHA and AA accumulate in the membranes of brain cells during these periods when brain development peaks (4). DHA is particularly involved in the myelination process of the frontal lobe. Disruption of myelenization because of consuming nutrition deficient in DHA causes deterioration of cognitive functions (5). In addition to DHA and AA, breast milk also contains cholesterol, sphingomyelin, phosphoditylcholine, which are necessary for myelin synthesis (5). The increase in white matter volume in adolescents who were breastfed as infants is evidence that the content of breast milk is important in the formation and development of myelin (6,7). Exclusively breastfeeding is associated with earlier development of the frontal and temporal lobe white matter, corticospinal tract, periphery of the internal capsule, superior longitudinal fascicle, and superior occipito-frontal fascicle (8). These regions and pathways are related to higher-order cognition such as language, executive functions, planning, social emotional functioning, and language domains (9,10). Skin-to-skin contact, verbal communication, and stimuli are more common in breastfed babies compared to bottle feeding. It has been reported that the contribution of breastfeeding to the cognitive development of the child is not only related to breastfeeding, but also with other positive parameters such as the intelligence level, education, socioeconomic level of the mother and father (11).

Although many benefits of breast milk during infancy are known, its effects in late childhood are not well known. While the effect of breastfeeding on Intelligence Quotient (IQ) has been examined in many studies, studies investigating the effect on executive functions are limited. Executive functions affect academic achievement and social functioning in later ages.

The purpose of our study is to assess the effect of exclusive breastfeeding duration on cognitive level and executive functions in school-age children without psychiatric complaints.

METHODS

Participants

The ethics committee approval of the study was given by the ethics committee of Marmara University with the protocol number of 09.2014.0190. After obtaining the approval of the ethics committee, with the permission of the Provincial Directorate of National Education the 5th, 6th and 7th grades of the school were determined by random sampling. A total of 300 children in these classes were screened with the Child Behavior Checklist (CBCL). Children with low symptom severity on the CBCL scale and their parents were invited to Marmara University Pendik Training and Research Hospital. The children were assessed clinically by child and adolescent psychiatrist. A total of 71 children aged 8-13 years without any diagnosis constituted the study group. Children with an intelligence level of less than 80 (WISC -R verbal, performance and/or total score<80), any psychiatric disorder, a history of Autism Spectrum Disorders, chronic and serious medical illness and seizure-like neurological disorder were excluded from the study.

In the sociodemographic information form we asked the families that duration of exclusive breastfeeding without feeding additional food or formula. The study group divided into two according to the information received from the families. The first group consisted of children who were exclusively breastfed for 6 months or more. The second group consisted of children who were exclusively breastfed for less than 6 months.

Measurements

The children's executive functions were assessed with Stroop Test and Wisconsin Card Sorting Test (WCST) respectively. After the executive function tests, Verbal subtests of the WISC-R including General Knowledge and Vocabulary and Performance subtests of the WISC-R including Picture Completion, Picture Arrangement were applied to the children. In our study, the parent form of the Strengths and Difficulties Questionnaire (SDQ) was used to screen the behavioral and emotional problems of children. After the tests applied to the child and the clinical interview were completed, the forms filled by the parents were reviewed together with the child and adolescent psychiatrist and the parts that the parents could not understand were evaluated. Clinical Interviews lasted approximately 1.5 hours for each participant and were completed in a single session. The tools we used in our study were briefly explained below.

Stroop Test

The Stroop test was first developed by Stroop in 1935 (12). The adaptation study of the test to the 6-11 age group was carried out by Kılıç and Koçkar in 2002, and the validity and reliability study for adults in our country was carried out by Karakaş in 1999(13,14). The Stroop test measures ability to change response, information processing speed, and especially selective attention.

Wisconsin Card Sorting Test (WCST)

It was developed by Berg in 1948 in order to evaluate abstraction and conceptualization skills (15). In our country, standardization studies were carried out by Karakaş (16). WCST is associated with features such as attention, feature identification, perseveration, working memory, executive functions, conceptualization, and abstract thinking.

Wechsler Intelligence Scale for Children-Revised (WISC-R)

It is a scale developed by David Wechsler to evaluate the general mental capacity of children aged 5-15 years (17). Standardization studies in our country were made by Savaşır and Şahin and WISC-R scale was adapted to Turkish culture (18).

Strengths and Difficulties Questionnaire (SDQ)

SDQ was developed by Robert Goodman in 1997 (19). The Turkish validity and reliability study was conducted by Güvenir et al. in 2008 (20). SDQ is used to screen for the behavioral and emotional problems in children and young people.

RESULTS

A total of 71 children between 8 and 13 year ages were evaluated in our study. All of the cases participating in our study consisted of healthy children who did not have any psychiatric

complaints and were not diagnosed with any disorder with the semi-structured interview. Our groups were divided into two based on the retrospective information given by the mother in terms of duration of exclusive breastfeeding. 51% (n= 36) of the cases were exclusively breastfed for 6 months or more, and 49% (n=35) of cases were exclusively breastfed for less than 6 months. Mean age and gender distributions of two the groups were similar. There was no statistically significant difference between the groups in the term of developmental stages such as saying the first words, making the first sentence, walking and starting toilet training.

The duration of bottle use in exclusively breastfed less than 6 months was longer than the other group and there was a statistically limited difference (p=0.05). Duration of pacifier use were similar in both groups.

There was no statistically significant difference between the two groups in terms of medical and mental problems of the mothers during pregnancy, drug using and/or smoking, exposure to radioactive rays and trauma in perinatal period. Parents' ages, education levels and socioeconomic levels were similar in both groups.

Attention deficit and hyperactivity, behavioral problems, emotional problems, peer problems, social behavior subtest and total difficulty scores of the SDQ were compared separately, the mean score of those who received only breastmilk for less than 6 months was higher than the other group, but it did not reach the level of statistical significance (p > 0.05). (Table 1)

Table 1. Strengths and Difficulties Questionnaire (SDQ) Parent Form, Comparative Subtest Scores

SDQ Subscales		eastfed for less	Exclusively breastfed for 6 months or more				
	tnan o	months					
	Mean±sd	Median	Mean±sd	Median	z and p values		
		(min-max)		(min-max)			
Hyperactivity/	4.23±1.88	4 (0-9)	3.94±1.51	4 (1-7)	z=-0.598		
Inattention					p=0.550		
Conduct Problems	1.43±1.79	1 (0-9)	1.11±1.14	1 (0-5)	z=-0.462		
					p=0.644		
Emotional	2.26±2.44	1 (0-8)	1.67±1.64	1 (0-7)	z=-0.559		
Problems							
Peer Relationship	3.09±2.23	3 (0-8)	2.72±1.5	3 (0-6)	z=-0.432		
Problems					p=0.665		
Prosocial	8.29±1.47	8 (4-10)	8.14±2.29	8 (4-15)	z=-0.231		
Behaviour					p=0.818		
Total Difficulty	10.49±6.43	9 (0-35)	9.44±3.81	9 (2-16)	z=-0.345		
Points					p=0.568		

Mann-Whitney U Test.

Standard scores of Verbal subtests of the WISC-R including General Knowledge and Vocabulary and Performance subtests of the WISC-R including Picture Completion, Picture Arrangement were similar for both groups (p>0.05). (Table 2)

Table 2. WISC-R Subtest Standard Scores

	Exclusively bre	astfed for less	Exclusively breastfed for 6 months or more				
	than 6 n	nonths					
	Mean±sd	Median	Mean±sd	Median	z and p values		
		(min-max)		(min-max)			
General Knowledge	10.37±2.51	10 (4-15)	9.89±2.14	10 (6-15)	z=-1.131		
					p=0.258		
Vocabulary	11.77±1.88	12 (9-17)	11.28±1.88	11 (7-14)	z=-0.706		
					p=0.480		
Picture Completion	12.03±2.06	12 (9-16)	12.08±1.93	12 (7-16)	z=-0.408		
					p=0.683		
Picture Arrangement	10.65±1.68	11 (7-15)	10.75±2.75	11 (7-20)	z=-0.169		
					p=0.866		

Mann-Whitney U Test.

When all of the participants (n=71) were evaluated together without being divided into two groups, it was determined that total and exclusive breastfeeding duration were not related to the intelligence test results. (Table 3)

Table 3. The Relationship Between Total And Exclusive Breastfeeding Duration And WISC-R Test Results

		Exclusively Breastfeeding	Total Breastfeeding
WISC-R Verbal	r	-0.044	-0.14
	p	0.714	0.244
	n	71	71
WISC-R	r	-0.121	-0.137
Performance	p	0.313	0.253
	n	71	71
WISC-R Total	r	-0.091	-0.176
	p	0.448	0.143
	n	71	71

Spearman's rho correlation analysis

When the Stroop test results were compared, no statistically significant difference was found between the two groups. (Table 4)

Table 4. Stroop Test TBAG Version Comparative Results

Stroop Test TBAG Form		Exclusively l less than			oreastfed for 6 for more		
		Mean±sd	Median (min-max)	Mean±sd	Median (min-max)	z and p values	
Section 1	Time	10±2.5	10 (6-19)	10.64±2.81	10 (7-18)	z=-0.890 p=0.374	
	Fault	0.00	0 (0-0)	0.03±0.17	0 (0-1)	z=-0.986 p=0.324	
	Correction	0.11±0.4	0 (0-0)	0.00	0 (0-0)	z=-1.782 p=0.075	
Section 2	Time	10.74±5.68	10 (2-40)	11.58±3.33	10,5 (8-20)	z=-1.671 p=0.095	
	Fault	0.06±0.34	0 (0-2)	0.00	0 (0-0)	z=-1.014 p=0.310	
	Correction	0.03±0.17	0 (0-1)	0.08±0.28	0 (0-1)	z=-0.993 p=0.320	
Section 3	Time	15.34±4.43	15 (8-28)	17.67±6.52	16 (11-41)	z=-1.495 p=0.135	
	Fault	0.09±0.37	0 (0-2)	0.08±0.28	0 (0-1)	z=-0.389 p=0.679	
	Correction	0.17±0.38	0 (0-1)	0.42±0.69	0 (0-2)	z=-1.491 p=0.136	
Section 4	Time	23.14±8.14	23 (8-45)	26.06±12.65	24 (13-82)	z=-0.835 p=0.404	
	Fault	0.14±0.69	0 (0-4)	0.06±0.23	0 (0-1)	z=-0.058 p=0.954	
	Correction	0.74±0.82	1 (0-3)	1.11±1.33	1 (0-6)	z=-0.982 p=0.326	
Section 5	Time	34.03±10.57	34 (12-75)	36.56±18.08	31 (17-115)	z=-0.086 p=0.931	
	Fault	0.17±0.45	0 (0-2)	0.47±0.84	0 (0-3)	z=-1.541 p=0.123	
	Correction	1.63±1.29	2 (0-4)	2±1.62	2 (0-7)	z=-0.838 p=0.402	

Mann-Whitney U Test.

When the WCST results of the 2 groups were compared, we could not find a statistically significant difference. (p>0.05). (Table-5)

Table 5. Wisconsin Card Sorting Test Comparative Results

Wisconsin Card Sorting Test (WCST)	Exclusively bless than	oreastfed for		oreastfed for 6 or more		
	Mean±sd	Median (min-max)	Mean±sd	Median (min-max)	z and p values	
WCST2	37.57±19.45	30 (15-94)	41.17±19.28	35 (16-90)	z=-1.358 p=0.174	
WCST3	90.43±19.45	98 (34-113)	86.83±19.28	93 (38-112)	z=-1.358 p=0.174	
WCST4	5.46±2.41	6 (0-10)	5.03±2.42	5 (1-9)	z=-0.620 p=0.535	
WCST5	23.91±22.97	16 (7-113)	25.61±19.77	18.5 (7-106)	z=-1.502 p=0.133	
WCST6	20.91±17.05	15 (6-84)	22.67±15.05	17.5 (7-81)	z=-1.497 p=0.134	
WCST7	16.66±8.78	15 (5-45)	18.5±9.94	17 (5-52)	z=-1.054 p=0.292	
WCST8	16.32±13.32	11.72 (4.66-65.63)	17.71±11.76	13.67 (5.47-63.28)	z=-1.520 p=0.128	
WCST9	15.18±6.53	13 (10-32)	15.47±8.81	12 (10-54)	z=-0.873 p=0.382	
WCST11	62.812±19.96 70.31 (7.03-85.		58.85±20.23 66.8 (12.5-87.5)		z=-1.236 p=0.216	
WCST12	2.03±1.69	1 (0-5)	1.94±1.33	2 (0-6)	z=-0.235 p=0.814	

Mann-Whitney U Test.

When the relationship between total or exclusively breastfeeding duration and executive functions was evaluated; In the Stroop test, a positive correlation was found between the

duration of exclusive breastfeeding and the time score of the 2nd Section, the total duration of breastfeeding and the time scores of the 1st, 2nd and 4th Sections. (Table 6)

Table 6. The Relationship Between Total And Exclusive Breastfeeding Duration And Stroop Test

STROOP TES	STROOP TEST		Stage 1 Time Stage 2 Time Stage 3 Time		Stage 4 Time	Stage 5 Time	
Exclusively	-		0.261	0.204	0.107	0.073	
Breastfeeding	p	0.402	0.028	0.088	0.376	0.546	
	n	71	71	71	71	71	
Total			0.279	0.226	0.327	0.208	
Breastfeeding	p	0.009	0.018	0.058	0.005	0.081	
	n	71	71	71	71	71	

Spearman's rho correlation analysis.

In the WCST, no correlation was found between the duration of exclusive breastfeeding in WCST (p>0.05). Positive relationship between total breastfeeding time and WCST 2, WCST 5, WCST 6, and WCST 8 results; A negative correlation was found with WCST3 and WCST 11 (p<0.05).(Table 7)

Table 7. The Relationship Between Total And Exclusive Breastfeeding Durations And WCST Results

		WCST 2	WCST 3	WCST 4	WCST 5	WCST 6	WCST 7	WCST 8	WCST 9	WCST 11	WCST 12
Exclusively Breastfeeding	r	-0.191	0.191	-0.119	0.223	0.215	0.125	0.217	-0.098	-0.175	0.057
	p	0.11	0.11	0.324	0.062	0.073	0.297	0.069	0.42	0.144	0.635
	n	71	71	71	71	71	71	71	70	71	71
Total Breastfeeding	r	0.328	-0.328	-0.19	0.415	0.41	0.137	0.41	0.023	-0.297	-0.159
	p	0.005	0.005	0.111	0.001	0.001	0.255	0.001	0.85	0.012	0.184
	n	71	71	71	71	71	71	71	70	71	71

Spearman's rho correlation analysis.

DISCUSSION

In our study, 97% of the cases were breastfed for varying periods of time. Breastfeeding is very common in our country. According to Turkey Demographic and Health Survey 2018 data, 98% of all children have been breastfed for a while and 41% of infants younger than 6 months were exclusively breastfed (21). Breastfeeding duration data of our study is compatible with 2018 Turkey data.

In our study, developmental stages of the children were similar regardless of the duration of breastfeeding. In a study investigating the impact of breastfeeding duration on mental development between the ages of 2-3, it was shown that breastfed children were better at developmental stages including language, communication, fine and gross motor compared to non-breastfeed children. In the same study, it was stated that there was a meaningful positive relation between the duration of breastfeeding and motor development (22). The reason why the developmental stages did not differ in our study may be due to the low number of children (n=2) who were not breastfeed at all in who take only breast milk for less than 6 months.

In our study, the SDQ was used to examine the effect of exclusively breastfeeding duration on emotional and behavioral problems in children. There was no statistically significant difference between subtest and total difficulty scores of SDQ in both groups. In a comprehensive randomized controlled study in which 13889 children were followed for 6.5 years, it was determined that there was no risk or benefit on the behavior of the child, according to the SDQ filled by both parents and teachers, however, it was stated that these results cannot be generalized on behavioral outcomes that may occur in adolescence and adulthood (23).

We applied the WISC-R test with 4 sub-test. The WISC-R total, performance and verbal scores and the standard scores of the general knowledge, vocabulary, picture completion and picture editing subtests of the two groups were compared to examine the effect of breastfeeding duration on intelligence. In our study, we did not detect a statistically significant difference between the groups.

In a meta-analysis study, a positive correlation was found between breastfeeding and performance intelligence test. Also, the same relationship was shown when the maternal IQ level controlled (24). Mothers who are conscious about breastfeeding create a more stimulating and suitable environment for the cognitive development of their children. The effect of breastfeeding on cognitive functions may be caused not only by nutritional effect but also by the supporting family environment

In a large sample cluster-randomized study followed 13.889 children at age 6.5. Clinicians applied to children IQ test included 4 subtests (vocabulary, similarities, block designs, and matrices). The researchers found that in breastfed group verbal IQ was 7.5 points higher, the performance IQ also 2.9 points and full-scale IQ was 5.9 points higher at the age of 6.5 (25). In the randomized cluster study, which is a 16-year follow-up study examining whether the neurocognitive effect continues in the long term, higher scores were found in the verbal domains in exclusively breastfed (more than 3 months) group but the same effect was not detected in other domains (26). In another a comprehensive prospective study investigating the effect of breastfeeding on intelligence in children by controlling the effect of maternal intelligence and other confounding factors, the intelligence level of children was evaluated at the age of 5 and 14 years, the researchers found that breastfeeding had little or no effect on the child's intelligence. In that study, it was stated that the mother's intelligence level had a greater effect on breastfeeding than the mother's race, education level, age, economic status, smoking, living environment, birth weight of the child. It has been emphasized that the children of mothers with high IQ levels also have a genetically better intelligence level, and there is a positive relationship between the mother's IQ level and breastfeeding initiation and breastfeeding duration (27). The reason of the different results in the studies investigating the effect of breastfeeding on the intelligence of the child may be due to the methodological differences of the studies.

In the literature, while the effect of breastfeeding on intelligence has been investigated in many studies, studies investigating the effect on executive functions are limited. We applied Stroop Test and WCST to children in our study to understand the effect of breastfeeding on executive functions. When we compared the results of Stroop and WCST between the two groups, there was no statistically significant difference. In a large sample study investigating the relation between breastfeeding duration and cognitive performance at the age of 9-10 years showed that breastfeeding duration is not associated with executive functions and memory. In the same study General Ability performance was higher in those breastfed for more than 12 months compared to those who never breastfed (28). In a comprehensive study

examining the effect of breastfeeding duration on the neuropsychological development of healthy children aged 10-12, the neuropsychological performances of less than 6 months breastfed children and 6 months or more breastfed children were compared. It was found that there was no significant or clinically significant relationship between the duration of breastfeeding and neuropsychological test result. It was noted that they performed better only in the language domain (29). In the results of studies, the causal relationship between breastfeeding and neurocognitive functions are uncertain. There is a need for more comprehensive studies to be conducted with different tests and methods investigating the effect of exclusive breastfeeding duration on executive functions.

Limitations

In our study, the duration of breastfeeding was obtained from the retrospective information given by the parents. The fact that the children included in the study were between the ages of 8-13 and this condition raises the possibility of misremembering the information given by the parents about the duration of breastfeeding. Presence of health records regarding the duration of breastfeeding and the prospective design of the study would have eliminated the bias towards recall.

The small sample size of our study makes it difficult to generalize the results. The fact that the cases in our study were taken from a single school and reflect only a part of the society is one of the important limitations. There is a need for comprehensive community-based studies on this subject.

In our study, the number of patients who did not receive any breast milk was only 2. In order to better investigate the effect of breastfeeding on cognitive level and executive functions, a longitudinal evaluations of more non-breastfed cases are required.

The development of executive functions continues throughout childhood and adolescence and varies according to age. In our study, the subjects were in a similar age range, but they were not in the same age range. Evaluation of children in the same age group could provide more specific results.

In our study, the mother's intelligence, which is the most important confounding factor that may have an effect on the child's intelligence and executive functions, was not evaluated. The mother's IQ level may affect genetic transmission to their children's IQ and duration of breastfeeding.

CONCLUSION

There are limited number of studies in the literature investigating the effect of breastfeeding on executive functions in healthy children. In our study, we found that the duration of breastfeeding had no effect on executive functions and cognitive levels. As a result, prospective studies with large samples are needed to evaluate participants who never breastfed.

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