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FILE	HERS_WITH_CHRONIC_ENERGY_DEFICIENCY__CED__AND_NORMAL_S TATUS.PDF (375.62K)	WORD COUNT	5628
TIME SUBMITTED	27-NOV-2020 04:55PM (UTC+0700)	CHARACTER COUNT	29195
SUBMISSION ID	1458044609		

Differences of Oleic Acid Levels in Breast Milk of Lactating Mothers with Chronic Energy Deficiency (CED) and Normal Status

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Article Info

Article history¹:

Received: 18 October 2020

Revised: 14 November 2020

Accepted: 15 November 2020

Keywords:

Matured Breast Milk,
Oleic Acid,
Nutritional Status,
Complementary Foods to
Breastfeeding

Paper Type :

Research Article

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Abstract²⁶

Purpose: This study aims to determine the oleic acid levels of mature breast milk (6-12 months) and to analyze the differences in oleic acid levels in mature breastfeeding mothers with chronic energy deficiency (CED) and normal nutritional status.

Methods: This research was conducted in July - September 2020 in the work area of the Sudiang and Sudiang Raya puskesmas, Makassar City and the research laboratory of the State University Hospital (RSPTN) Unhas. The type of research used is analytic observation with a cross-sectional study approach. The population in the study amounted to 406 mothers. The sample in this study was breastfeeding mothers with 6-12 months of lactation. The sample size was determined by using the Dahlan formula with a total sample size of 38 participants consisting of 19 Chronic Energy Deficient breastfeeding mothers and 19 normal breastfeeding mothers. The sampling technique used was purposive sampling method. Data were analyzed using the Manwithney test.

Results: The average levels of oleic acid in breastfeeding mothers with nutritional status of chronic energy deficiency were 1.00 ± 0.37 and breastfeeding mothers with normal nutritional status was 0.95 ± 0.36 . The results of statistical tests found no significant differences ($p > 0.05$) between the oleic acid levels of breastfeeding mothers in chronic energy deficiency and normal nutritional status. Most of the found levels of oleic acid in the low category (reference 1.5) are namely 94.7% in normal nutritional status and 78.9% in nutritional status of women with chronic energy deficiency.

Conclusion: The average oleic acid levels of breast milk in chronic energy deficiency and normal breastfeeding mothers were still low compared to the standard. There was no difference in oleic acid levels in breastfeeding mothers and breastfeeding mothers with normal nutritional status. A further qualitative research is needed in nursing mothers whose oleic acid levels are equal or exceed the reference.

1. Introduction

⁴The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend that children initiate breastfeeding within the first hour of birth and be exclusively breastfed for the first six months of life – meaning no other food⁵ or liquids are provided, including water (Unicef, 2019). It is also recommended that and the introduction of adequate and safe complementary nutrition (solid) food at 6 months along with continued breastfeeding for up to 2 years⁵ or more are required (Unicef, 2019). Despite all these recommendations, many babies and children do not receive optimal feeding, where only about 36% of infants aged 0 to 6 months worldwide are exclusively breastfed during the

period from 2007 to 2014 (Unicef, 2019).

The results of the National Socio-Economic Survey (Susenas) from BPS (2017) in Indonesia show that the coverage of exclusive breastfeeding for infants aged <6 months is 55.96%, with the type of area in rural areas the exclusive breastfeeding coverage is 57.22%, while in the urban areas it amounted to 54.77%. Based on the 2018 Indonesian Health Profile report, it shows that nationally the coverage of exclusive breastfeeding in Indonesia is 65.16%. Meanwhile, based on the 2018 Basic Health Research report, it shows that nationally the coverage of exclusive breastfeeding for babies is 37.8%. For provinces in South Sulawesi, it is above the national average but it has not reached the national target (Ministry of Health, 2018).

The low level of breastfeeding is a threat to child development which will affect the growth and development of the quality of human resources in general. Breast milk can reduce the risk of being overweight, preventing infections such as rotavirus diarrhea and hand, foot and mouth disease in infants (Didehbani et al., 2016). In addition to infectious diseases, long-term breastfeeding reduces the risk of obesity, diabetes and cardiovascular disease (Leonnerdal, 2016). Research in Chile in 2018 suggested that breastfeeding emerged as a protective factor. The group that was breastfed from 3 to 6 months had a lower prevalence of obesity and a component of the metabolic syndrome than the 0 to 3 months group.

Breast milk (ASI) is rich in nutrients, and it has a complex composition. It is the key to the health of the baby. As it contains carbohydrates (lactose), protein in the form of alpha-lactalbumin, fat in the form of fatty acids and oleic acid, vitamins and minerals, breast milk provides all the nutrients for babies to live in the first 6 months (Butts et al., 2018). Fat is one of the macronutrients that contain sufficient fatty acids. The fat content of human milk is characterized by high concentrations of palmitic acid and oleic acid (Ballard et al., 2013). One of the important functions of monounsaturated fatty acids is for the development of the central nervous system in early life (Wu et al., 2010).

In infants and children, the content of fatty acids in breast milk is very important, especially oleic acid because oleic acid functions for the formation and brain development, transportation, and metabolism. Hence, maternal food intake is very important so that breastfeeding in lactating mothers increases (Arsic et al., 2017).

Other functions of omega-9 fatty acids (Oleic Acid) have various health benefits such as lowering cholesterol, reducing arthritis symptoms, strengthening the immune system, and stopping hair loss. Arsic et al. (2017) reported that oleic acid is associated with a reduced risk of coronary heart disease, cardiometabolic risk, type 2 diabetes and hypertension. Based on the findings of previous studies, it is suggested that oleic acid has a potential protective effect on the development of several types of cancer including breast and colorectal cancer.

Oleic acid functions in brain development so that food intake in nursing mothers will increase (Arsic et al., 2017). The nutritional content of protein in breast milk in the form of alpha-lactalbumin and oleic acid is a component of Human α -lactalbumin *nascent* lethal to tumor cells (HAMLET). Mossberg et al. (2010) pointed out that HAMLET is a complex consisting of alpha-lactalbumin and oleic acid which has anti-tumor activity that is naturally found in breast milk. Some studies on oleic acid are still minimal, whereas in Indonesia the research on oleic acid has not been reported.

Research conducted in New Zealand by Butss et al. (2018) found oleic acid levels for several ethnicities, including Asian, European and New Zealand ethnicities. Their analysis for fatty acids using gas chromatography indicated that oleic acid levels were found in Asian ethnic breastfeeding mothers of 1.5 g / L, 1.2 g / L of Maori and Pacific island ethnicities, and 1.2 European ethnic New Zealand.

The quality and composition of breast milk is influenced by the condition of the mother's nutritional status. The duration of exclusive breastfeeding, the growth of the baby, and the nutritional status of the mother after breastfeeding are indicators of the success of breastfeeding where the nutritional status of the breastfeeding mother plays a very important role (WHO, 2002). As quoted by Fikawati et al. (2015), the optimal duration of exclusive breastfeeding for 6 months can be achieved if the nutritional status of breastfeeding is good.

The amount of breast milk that comes from mothers with poor nutritional status can decrease to the amount of only 100-200 ml per day. The breasts of women who are affected by pregnancy hormones, and especially women who have breastfeeding, will be able to produce sufficient breast milk when the woman is re-lactating (Roos, 2013). Research conducted in Kenya explains that the volume of breast milk consumed by infants is significantly affected by the LILA (arm circumference) of breastfeeding mothers during pregnancy (Ettyang, 2005). Efforts to improve nutrition in infants can be done through improving maternal nutrition, where breastfeeding mothers must have a good nutritional status so that they can produce optimal breast milk and can meet the nutritional needs of the baby (Jafri, 2012).

The report on the results of the implementation of nutritional status assessment (PSG) in 2016 states that the prevalence of Chronic Low Energy (CED) is 16.2% and in 2017 it decreased to 14.8%. For the province of South Sulawesi, it is still below the national average, namely 15.9% in 2016 and 13.1% in 2017 (Ministry of Health, 2017). The South Sulawesi Provincial Health Office report states that out of 24 districts/cities in South Sulawesi, Makassar City has the highest number of CED cases with 3,373 cases in 2018, and it was also reported that out of 10 Puskesmas in Makassar City, Sudiang Raya Puskesmas had the number of cases. Most of the Chronic Energy Deficiency cases are at a level of 16.1%.

After exclusive breastfeeding for the first 6 months, then breastfeeding is followed by complementary feeding. The Academy of Nutrition and Dietetics confirms that exclusive breastfeeding provides optimal nutrition and health protection during the first six months of life, and that breastfeeding with complementary foods from six months to at least 12 months of age is the ideal feeding (Martin et al., 2016). Usually, after exclusive breastfeeding in the first 6 months, many people no longer provide breastfeeding to their babies. The 2017 Indonesian Health Demographic Survey (IDHS) data shows that 41.6% of babies aged 6-11 months are not breastfed and only 9% of babies of that age are breastfed.

In infants aged 6-12 months, the need for various nutrients is increasing and can no longer be fulfilled by breast milk alone. In order to achieve balanced nutrition, it is necessary to supplement with complementary foods (complementary foods), while breast milk is still given until the baby is 2 years old. At the age of 6 months, babies are introduced to other foods, first in the form of crushed, soft food and then switch to family food when the baby starts to be 1 year old. Various studies conducted stated that nutritional problems in infants and children were caused by inappropriate breastfeeding and complementary feeding habits, both in terms

of quantity and quality. Another factor that affects complementary feeding is that mothers are less aware that since the baby is 6 months old, they need complementary foods in good quantity and quality (Hermina, 2010).

It is suspected that the provision of food other than breast milk is one of the factors affecting the composition of breast milk. A study conducted by Verd et al. (2018) states that the composition of breast milk changes during infancy given complementary foods. Several recent studies have shown that changes in the composition of breast milk are greatly influenced by weaning. It was further noted that the volume of milk decreased to 66% of the volume of the initial breastfeeding when the baby started weaning. In addition, the overall protein concentration increased by 1.6 times in weaned subjects, whereas in non-weaned subjects the protein concentration in milk was 2.8 times. Some sources emphasize that changes in the composition of breast milk in breastfeeding mothers are greatly influenced by the weaning process. Other factors that affect the variability of the composition of breast milk include parity, food intake, sex and maternal health status (Verd et al., 2018).

2. Methods and Procedures

This type of research is analytic observational with a cross-sectional study approach. This research was conducted in the working area of the Sudiang and Sudiang Raya puskesmas, Makassar City and the Hasanuddin University Hospital research laboratory. The population in this study was all breast milk of breastfeeding mothers in the working area of Sudiang and Sudiang Raya puskesmas with a total of 406 mothers. The sample in this study was breast milk for mature breastfeeding mothers (6-12 months) using purposive sampling technique with a sample size of 19 breastfeeding mothers. CED and 19 samples of normal breastfeeding mothers with a total sample size of 38 samples.

At the beginning of the planning, the ASI sampling was carried out only in one health center, namely the Sudiang and Sudiang Raya puskesmas which were in one sub-district. Data on respondent characteristics including maternal age, occupational education and maternal parity were obtained through direct interviews. The maternal nutritional status (CED and normal) was obtained by measuring the mother's upper arm level (LILA). ASI samples were taken at the same time, namely at 09-11.00 WITA. Breast milk is obtained using an electric Real bube brand ASI pump with serial number RBX-8023S-2 and then transferred to a Gea Baby brand ASI bag and tightly closed. It is then stored in a cool box brand gren lay. From these breast milk samples, the researchers took as many breast milk samples as the mothers produced and then brought them to the laboratory to be temporarily stored in the Thermo brand of breast milk refrigerator at a temperature of -20oC. Breastfeeding examination is done by only taking a sample of 5-10 ml of breast milk, to see the oleic acid level of breast milk using the Enzyme-Linked Immunosorbent Assay (ELISA) method. To see the differences in oleic acid levels of mature breast milk in breastfeeding mothers with CED nutritional status and normal nutritional status were analyzed using the Mann Withney test and processed using the Windows version of the SPSS 24 program.

All ethical considerations were taken when conducting this study. The researchers obtained prior consent from all the participants.

3. Results and Discussion

Sample Characteristics

Table .1 Characteristics of breastfeeding mothers respondents

Characteristics of Breastfeeding Mothers	Mothers who lack chronic energy (n=19)		Normal Mothers (n=19)		Total (n=38)	
	n	%	N	%	n	%
Age						
≤ 19 Years	0	0	0	0	0	0
20 – 35 Years	15	53,5	13	46,5	28	100
≥ 35 Years	4	40	6	60	10	100
Education						
Elementary School	4	50	4	50	8	100
Junior School	3	37,5	5	62,5	8	100
High School	10	71,4	4	28,6	14	100
Diploma/Bachelor	2	25	6	75	8	100
Parity						
≤ 2	11	55	9	45	20	100
> 2	8	44,4	10	55,6	18	100
Employment						
Civil Servant	1	100	0	0	1	100
House Wife	18	48,6	19	51,4	37	100

Source: Primary data processed 2020

The results of this study indicate that the age of breastfeeding mothers in the group of mothers with chronic energy deficiency (CED) nutritional status and mothers with normal nutritional status are mostly in the age range of 20-35 years with a percentage of (53.5%) and (46, respectively). 5%). The level of maternal education shows that breastfeeding mothers with nutritional status of CED are more dominated at the SMA (High School)/equivalent level (71.4 %) and in mothers with normal nutritional status (75%), the education level of breastfeeding mothers is of Diploma / Bachelor degrees. Parity of breastfeeding mothers with nutritional status CED 55% had parity ≤ 2, whereas in the group of breastfeeding mothers with normal nutritional status the parity is > 2 (55.6%) (Table 1).

Oleic acid levels in breastfeeding mothers with chronic energy deficiency (CED) nutritional status and normal nutritional status

Table 2. Differences in oleic acid levels in breastfeeding mothers with less chronic energy (CED) and normal breastfeeding mothers

Nutritional Status of Breastfeeding Mothers	Breast Milk Oleic Acid (g/L)		p value*
	n	Mean ± SD	
CED	19	1,00±0.37	0.917*

Normal 19 0,95±0.36

*Mann whitney test

Source: Primary data processed 2020

28 Table 3 The relationship between nutritional status of breastfeeding mothers and levels of Oleic Acid in mature breast milk

Nutritional Status	Oleic Acid Levels				Total		p value*
	Low		High		n	%	
	n	%	n	%			
CED	15	78,9	4	21,1	19	100	0.340*
Normal	18	94,7	1	5,3	19	100	

*Fisher's Exact Test

Source: Primary data processed 2020

3 The results of this study indicate that the oleic acid levels of breast milk in nursing mothers with chronic nutritional deficiency (CED) are in the range of 0.6 g / L to 1.8 g / L with an average level of oleic acid is 1.0 g / L (standard 1.5 g / L), whereas in breastfeeding mothers with normal nutritional status, the oleic acid level of breast milk is in the range of 0.6 g / L to 2.3 g / L with an average level of oleic acid in breast milk is 0,95 g / L (standard 1.5 g / L). As indicated in data analysis, 5 breastfeeding mothers had oleic acid levels above the standard from the group of breastfeeding mothers with CED nutritional status. The analysis also shows that 4 respondents are with the highest levels of 1.8 g / L and 1 from the breastfeeding mothers with normal nutritional status is with levels of 2.3 g / L.

This study revealed that oleic acid levels in mature breast milk in breastfeeding mothers with normal nutritional status were slightly lower than oleic acid levels in lactating mothers with CED nutritional status. The difference was not statistically significant ($p > 0.05$) (Table 2). Most of the breast milk oleic acid levels found in the nutritional status of KEK and normal nutritional status were low when compared to the standard levels (1.5 g / L), namely (94.7% and 78.3%).

The results of this study indicate that the oleic acid level of the mother's milk is almost the same as the average value of 1.0 g / L in breastfeeding mothers with KEK nutritional status and 0.95 g / L in breastfeeding mothers with normal nutritional status. Nevertheless, these results are still lower than the standard levels of Asian ethnicity (standard 1.5 g / L) and even with the average level of oleic acid of European and New Zealand ethnicities. The research study conducted by Butts et al. (2018) examined the composition of breastfeeding for breastfeeding mothers of all nutrients including oleic acid in New Zealand for Asian ethnicity, the Maori Islands and the Pacific Island as well as the European ethnicity in New Zealand. The study found out that Asian ethnicity had the most oleic acid levels; 1.5 g / L, followed by European ethnicity 1.3 g / L, and ethnic Maori and Pacific at 1.2 g / L.

This study also shows that there are 4 respondents with nutritional status of KEK who have levels above the average standard (1.5 g / L), namely, 1.5, 1.6, 1.6 and, 1.8 g / L. If we look at the intake of 4 respondents who have high levels of oleic acid compared to the standard, it shows that the whole energy intake is classified as less, while for the intake of 2 respondents who have oleic acid levels of 1.6 g / L and 1.8 g / L intake, it is considered as sufficient with a percentage of 84.6% and 91.8%. In normal nutritional status, there is 1 respondent who has oleic acid levels above the standard, namely 2.3 g / L. When viewed from the energy intake and fat intake, it is classified as sufficient with the percentage of intake of 80.2% for energy

and 89.9% for fat intake. The source of fat intake from respondents who have high levels of oleic acid comes from consumed beef.

The composition of breast milk was found to vary widely among breastfeeding mothers. Based on the study conducted by Butts et al. (2018), the variations in the composition of breast milk between mothers on cultural differences such as diet and lifestyle factors as well as environmental factors such as soil mineral content are then reflected in the mineral density of the food grown there, as well as the presence of human genetic differences. In addition, low levels of oleic acid in breast milk in nursing mothers with chronic energy deficiency (CED) and normal nutritional status are most likely caused by the weaning process in infants. A study conducted by Verd et al. (2018) states that the composition of breast milk changes during infancy given complementary foods.

The results of this study also found that high levels of oleic acid in breastfeeding mothers with normal nutritional status were 2.3 g / L, while in the nutritional status of CED, high levels of oleic acid were 1.8 g / L and 1.6 g / L. This shows that the level of oleic acid in mother's milk is not influenced by the nutritional status of the mother. This can explain the importance of breastfeeding as the main source of food even in conditions of poor nutritional status (Quinn et al., 2012). In addition, Kominiarek & Rajan (2016) explained that the production, quality and quantity of breast milk is not affected by weight loss during breastfeeding, BMI, body fat percentage, and weight gain during pregnancy.

Apart from nutritional factors, another factor that can affect oleic acid levels is the intake of breastfeeding mothers. Based on a research report by Citrakesumasari et al. (2020), it is revealed that the nutritional status of breastfeeding mothers in mothers with CED and normal nutritional status is different except for fat intake. Another study conducted in Ethiopia explained that food intake per day is one of the factors related to nutritional problems, namely CED against breastfeeding mothers and this study also shows that the average nutritional intake of the respondents does not reach the standard of sufficiency number (Sitotaw, 2017). In the same vein, another study conducted by Kim et al. (2017) on the fatty acid composition of breast milk and fatty acid intake of breastfeeding mothers in South Korea showed that the nutritional status of breastfeeding mothers did not significantly affect breast milk fatty acid levels. The results of this study indicate that the food intake of breastfeeding mothers at normal nutritional status for energy and carbohydrate intake is classified as less with an average percentage of 79% and 77%, but their fat intake is classified as sufficient (96%). For breastfeeding mothers with nutritional status, the intake of food sources of energy and fat classified as less with the percentage of intake of 54% for energy and 50% for fat intake. The results of statistical analysis showed no significant relationship between food intake and levels of oleic acid in breast milk. The results of this study are in line with the research study conducted by Nakul K, et al. (2017) who found that the composition of breast milk is not influenced by the mother's food intake. Another study conducted by Kurniati et al. (2016) found that there was no correlation between breast milk fat content and energy, fat, carbohydrate and protein intake. Studies conducted by (Quinn et al, 2012; Soliman et al, 2014; Innis, 2014) also revealed that although determinants of breastfeeding mothers such as intake and good nutritional status have an influence on the composition of breast milk. Accordingly, most of the relevant studies have found a weak or no effect on the nutritional composition of breast milk.

In this study, high levels of oleic acid in breast milk were found in breastfeeding mothers who had low nutrient intake. This occurs in energy nutrients, carbohydrates and protein, while the fat category is sufficient. The results of this study indicate that adequate fat intake may be related to the quality of oleic acid levels in mother's milk. The results of statistical analysis show that there is no relationship between fat intake and oleic acid levels. A study conducted by Antonakou et al. (2013) found that there was no significant correlation between the group of women who consumed high fat intake and breast milk fatty acid levels.

Maternal nutrient intake during lactation has a very ⁸ important role in improving the quality of breast milk. In this study, high levels of oleic acid in breast milk were found in women who are breastfeeding SEZs and who consume less nutritional intake in energy, low carbohydrate intake. For fat and protein, it was sufficient indicating high levels of oleic acid in mothers with sufficient fat intake. This can be directly related to the quality of oleic acid levels in mother's milk. The low intake of macro nutrients in some breastfeeding mothers, especially in some developing countries, reflects an indication about the condition of the mother's nutritional metabolism. The RDA in Indonesia states that breastfeeding mothers with the second 6 months of age require an additional 400 kcal of energy. The results of previous studies show that breastfeeding mothers consume at least two times the additional food per day during breastfeeding (Gautam, 2018).

Previous studies have also indicated that developing countries energy needs during lactation are associated with conditions of household food security, and breast feeding women cannot increase their energy intake (Vinoy, 2000). For developed countries, the nutritional intake of pregnant and lactating women is often inadequate and very low from the recommended daily intake (RDI) which has been determined by the Nutrition Foundation of Italy. In a research conducted by Marangoni et al. ¹⁷2016) in Italy, it is revealed that lifestyle and dietary habits (adequate micro nutrient intake) during pregnancy and breastfeeding greatly affect the health of women and their offspring. Even in the most developed countries, the nutritional ³ intake of pregnant and lactating women is often inadequate.

The results of this study indicate that the average food intake of breastfeeding mothers in the group with nutritional status as well as breastfeeding mothers with normal nutritional status is still low with a range of 50-79% of the Nutritional Adequacy Rate. The RDA set by Indonesia is the addition of 400 kcal of energy during the second 6 months of breastfeeding. For the group of women aged 19-29 years, it shows that the intake requirement for the Indonesian RDA is much higher, namely 2250 kcal and for 30-49 years old, 2150 kcal compared to what has been determined by ESFA, namely 1886 kcal.

Another study conducted by Butts (2018) in New Zealand for various ethnicities in New Zealand, namely Asia, the Pacific and European ethnic groups native to New Zealand, revealed that the energy and protein intake of New Zealand's European ethnicities was higher than the Asian and Pacific ethnic groups. The fat intake is higher in Asian ethnicities than other ethnicities. The recommended daily intake (RDI) for each shows that Asian ethnicity has a high enough RDI in energy intake (107%) and protein (145%) when compared to the standard intake category issued in 2003 Indonesian WNPG, namely 80-110% (Butts, 2018).

The addition of nutritional intake in breastfeeding women is done so that the mother gets extra calories during the lactation process needed for milk production. However, an imbalance of both excess and deficiency will both have an impact on the health of the mother's body. This can be a material consideration for Indonesia in determining the intake ¹² of breastfeeding mothers (Marangoni et al., 2016; Kominiarek & ¹ajan, 2016). Another study conducted by Czosnykowska-Łukacka et al. (2018) revealed that lactation is a dynamic process and the variability of the macronutrient content in breast milk is very large and is influenced by many factors, one of which is the mother's daily food intake but it has very little effect on quality and quantity of Breast milk.

4. Conclusion

The mean oleic acid level was still low when compared to the standard (1.5) for both breastfeeding mothers with CED nutritional status and normal nutritional status. However, there was no statistically significant difference ($p > 0.05$). The results of this study indicate that the oleic acid levels of breast milk in nursing mothers with chronic nutritional deficiency (CED) are in the range of 0.6 g / L to 1.8 g / L with an average level of oleic acid at 1.0 g / L (standard 1.5 g / L), whereas in breastfeeding mothers with normal nutritional status, the oleic acid level of breast milk is in the range of 0.6 g / L to 2.3 g / L with an average level of oleic acid in breast milk at 0.95 g / L (standard 1.5 g / L). In addition, the average food intake of breastfeeding mothers in the group with nutritional status as well as breastfeeding mothers with normal nutritional status is still low with a range of 50-79% of the Nutritional Adequacy Rate.

11 **Conflict of Interest**

The authors of the article declare no conflict of interest.

Funding:

The authors received no funding for conducting this study.

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