scientific reports

OPEN

Association of healthy eating index (HEI), alternative healthy eating index (AHEI) with antioxidant capacity of maternal breast milk and infant's urine: a cross-sectional study

Zahra Asadi[1,8], Afsane Bahrami[2,3,8], Asghar Zarban[4,5], Amir Hassan Asadian[6], Gordon A. Ferns[7] & Samira Karbasi[4]✉

Maternal dietary quality may alter the nutrient content of breast milk. In this study, we aimed to investigate the relationship between the healthy eating index (HEI) and alternative healthy eating index (AHEI) of a breastfeeding mother's diet with the antioxidant profile of her breast milk and her infant's urine. This study included 300 healthy mother-infant pairs. The participants' dietary intake was estimated using a validated semi-quantitative food frequency questionnaire. The diet quality of participants was assessed using the HEI and AHEI. The total antioxidant content of the breast milk and infant's urine was evaluated using ferric reducing antioxidant power (FRAP), 2, 2′-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid reactive substances (TBARs), and Thiol quantification assays. After adjusting for confounding factors, the odds of a low malondialdehyde (MDA) content of breast milk were significantly higher in the highest quartile of HEI than in the lowest quartile. The odds of low DPPH and FRAP in infant urine decreased in the highest quartile of HEI compared to the lowest quartile. No significant relationship was found between AHEI and antioxidant levels of breast milk and the infant's urine. Our findings demonstrate that a high quality diet of breastfeeding mothers, identified by a higher HEI, can affect the oxidant-antioxidant balance of a mother's breast milk and her infant's urine.

Keywords HEI, AHEI, Oxidant-antioxidant balance, Breast milk, Urine, Breastfeeding mothers

The optimal food for infants is human breast milk (BM), which contains all the essential nutrients, fluid, and energy required for infants that is necessary for their physical and psychological growth[1]. While formula milk has a standard content of nutrients, the composition of BM varies with the mothers' dietary habits, ethnicity, and the time of the day that she breastfeeds her infant[2]. BM contains unique antioxidants that can improve an infant's immune function and protect against some diseases[3,4].

Infants are at high risk for generating excess reactive oxygen species (ROS) because of the rapid transfer from the womb to an environment with higher oxygen concentrations[5]. Infants who receive BM containing high levels of antioxidants have lower oxidative stress than infants fed by formula[6]. Oxidative injury in infants may be implicated in causing many disorders, including respiratory diseases, necrotizing enterocolitis, and other

[1]Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. [2]Clinical Research Development Unit, Faculty of Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. [3]Clinical Research Development Unit of Akbar Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. [4]Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran. [5]Clinical Biochemistry Department, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran. [6]Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Birjand, Iran. [7]Brighton and Sussex Medical School, Division of Medical Education, Falmer, Sussex, Brighton BN1 9PH, UK. [8]Zahra Asadi and Afsane Bahrami contributed equally to this work. ✉email: s.karbasi@bums.ac.ir

chronic diseases[7,8]. Prematurity is also associated with oxidative stress because of the deficiency in antioxidant systems, and BM provides a complete antioxidant profile advantageous for premature neonates[9].

A mother's diet can affect the levels of vitamins in BM, including A, C, B6, B12, and other nutrients such as fatty acids[10]. A comparison of dietary patterns among mothers who breastfed their infants showed that different dietary patterns resulted in different levels of antioxidants in BM[11]. A healthy diet, rich in fruits, vegetables, grains, and nuts during lactation can increase the antioxidants in BM. Higher levels of some antioxidants such as 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) and thiol were found in the milk of mothers who adhere to a healthy dietary pattern. In comparison, adherence to an unhealthy dietary pattern led to lower levels of thiol in the BM[12].

With the increasing interest in evaluating diet quality in diverse populations, several methods have been developed to assess health outcomes. The Healthy eating index (HEI) is a measure of the quality of the diet in terms of compliance with the Dietary Guidelines for Americans, which is the basis of nutrition decisions for the United States government[13]. The HEI-2010 consists of twelve components including nine adequacy (total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein, seafood and plant protein, and fatty acids) as well as three moderation (refined grains, sodium, and empty calories) and uses minimum restriction standards to score each component[14]. The Alternative Healthy Eating Index (AHEI) was created in 2002 as a revision of the HEI, and is based on foods that can predict the risk of chronic diseases[15]. The AHEI-2010 contains 11 different food groups. Six dietary elements are recommended for highest intake including vegetables, fruit, whole grains, nuts and legumes, long-chain omega-3 fatty acids and polyunsaturated fatty acids (PUFA); one component for which moderate intake is ideally advocated (alcohol); and ascertain four dietary elements for to avoid or limit including sugar-sweetened beverages and fruit juices, red and processed meat, trans fatty acids and sodium[16].

HEI is a standard nutritional index that can be used for lactating mothers[17]. Freitas et al.. found that the total HEI score was inversely related with the margaric and linoleic acids of mother's BM[17]. Moreover, a higher intake of food groups such as vegetables, fruits and meats was directly correlated with specific BM oligosaccharides and adiponectin[18,19]. Previous studies have shown that a high maternal AHEI scores during pregnancy significantly improves anthropometric outcomes in newborn infants[20–22]. Few studies have focused on food intake during human milk feeding and its effect on the antioxidant content of BM and infant's urine. We have previously investigated the relationship of quality indicators of BM and infant urine with different dietary plans such as Mediterranean diet (MedDiet), dietary approaches to stop hypertension (DASH), low carbohydrate diet (LCD), and food quality score (FQS)[23–26]. In this study, we aimed to determine the HEI and AHEI score of a lactating mother's diet and then evaluate the association of these nutritional indicators with the antioxidant panel of BM and the infant's urine.

Methods

Study population

In this cross-sectional study, 350 healthy mother-infant pairs were recruited from 4 health centers in Birjand, South Khorasan, Iran, in February 2021. Inclusion criteria were healthy breatfeeding mothers aged 20–35 years who had given birth 1–6-months previously. Mothers with acute or chronic diseases, incomplete data as well as low/high enery intake (< 800 kcal and > 4200 kcal, respectively) were excluded. Before the study started, all of the participants were asked to provide written informed consent. The Birjand University of Medical Sciences Medical Ethics Committee approved the study (IR.BUMS.REC.1400.379), which was carried out following the Helsinki Declaration on human subjects' studies.

Initially, 350 breastfeeding women were recruited in the first stage. After the interview, we excluded participants with more than 10% incomplete food frequency questionnaire (FFQ) items ($n = 19$), an energy intake of < 800 kcal ($n = 18$) or > 4200 kcal ($n = 11$), and missing data on other variables ($n = 2$) from data analysis. As a result, our final statistical analysis was conducted on 300 breastfeeding women.

Demographic and anthropometric assessment

Demographic, anthropometric, and socio-economic data of the study participants was gathered by a trained nurse. A standard questionnaire was used to collect information on demographic status including the mother's age, type of delivery (natural/cesarean), history of chronic diseases (Yes/No), infant age, as well as infant sex. In terms of socio-economic variables, the educational attainment of mothers was evaluated separately, categorized into three distinct response options: 'elementary (9 years),' 'intermediate (10–12 years),' and 'university (13 years or more)'. Mothers were asked to report their perception of their economic status by selecting one of three descriptive choices: less than enough, enough, or more than enough. Each subject's height and weight were measured using standard method and then, BMI [weight (kg)/height (m^2)] was calculated. Furthermore, the infant's height and head circumference were recorded using precise tape measurements to the nearest millimeter. Weight assessments were made using electronic scales, ensuring precision to the nearest 0.1 kg. For maternal blood pressure assessment, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured multiple times using a mercury sphygmomanometer after the patient had a period of sitting and resting. This method enhances the accuracy of blood pressure readings, and the average of these measurements over a 25-minute period was documented.

Milk and urine sampling

BM samples were manually collected from mothers 1 to 6 months postpartum in the early morning between 7 and 10 am. Each mother provided 20 ml samples, that were collected into sterile tubes, and transferred to the laboratory on dry ice. On the same day, a urine specimen of 10 ml was gathered from each baby in a urine bag. The samples were freeze-dried and stored at -80 °C until analysis.

Oxidant-antioxidant status assessment

The test panel including four analytical tests to measure the total antioxidant status of each BM and urine sample, which is briefly described as follows:

Ferric reducing, antioxidant power (FRAP) assay

The FRAP assay was carried out using the methodology established by Benzie and Strain[27], as described previously[12]. This assay reduces a Fe3 + tripyridyltriazine complex to its ferrous form in the presence of antioxidants within the samples. By combining the test sample with the FRAP reagent and measuring the absorbance at 593 nm, the antioxidant capacity of the samples could be quantified in terms of µmol/L.

DPPH assay

The DPPH method was employed to quantify the free radical scavenging activity of the samples, as described formerly[12]. Based on the Brand-Williams protocol with modifications[28], this method involved incubating the samples with DPPH solution and measuring the absorbance at 517 nm. The percentage of antiradical efficiency was calculated to determine the scavenging capacity of the milk samples, expressed in µmol Trolox equivalent/L.

Thiobarbituric acid reactive substances (TBARs) assay

The TBARs assay was used to assess the level of malonyldialdehyde (MDA), a byproduct of lipid peroxidation[29]. Mixing the samples with TBARs reagent and measuring the fluorescence spectrum at specific wavelengths, the concentration of TBARs adducts was determined and compared to a standard curve for quantification in µmol TBARs/L[26].

Ellman's assay

The Ellman assay measures the total thiol concentration or sulfhydryl groups (T-SH) within the samples[26]. Following the technique initially described by Ellman and modified by Hu[30], this assay relies on the interaction of TS-H with DTNB to produce a colored anion, with absorbance measured at 412 nm. The concentration of sulfhydryl groups in the human milk samples could be accurately determined by comparing the results to a T-SH group standard and presenting the data in µmol/L.

Nutritional assessment

The study participants' food intake was assessed using a semi-quantitative FFQ, which comprised 65 food items and demonstrated good reliability and validity for an Iranian population[31]. The intake of each food group was determined by the portion size and frequency of use (per day, week, month, rarely, and never). Finally, the amount of each food item intake was calculated (based on grams) and inverted to daily intake using household scales. We used Nutritionist IV software (version 7.0; N-Squared Computing, Salem, OR) rectified for Iranian food ingredients for nutrient and energy intake assessment. Based on this software, the intake of energy and all macronutrients including carbohydrate, non-starch polysaccharide, protein, fat, saturated fat, monounsaturated fat, PUFA, trans fat, cholesterol, starch, total sugar (glucose, fructose, sucrose, maltose, and lactose), and fiber, and also micronutrients (vitamins and minerals) including sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, chloride, manganese, selenium, iodine, retinol, carotene, vitamin D, vitamin E, thiamin, riboflavin, niacin, vitamin B6, vitamin B12, folate, pantothenate, biotin, and vitamin C were determined for all participants.

Each participant was given a specific score for each component of HEI-2010 and AHEI-2010 according to their daily food group intake[14,16]. Each nutritional index has several components, reflecting an essential aspect of diet quality. The HEI and AHEI components and scoring methods are shown in the Tables 1 and 2. For calculating HEI score, 12 components were used for all study population (Table 1). Participants with higher intake of healthy items of this nutritional index including whole fruit, total fruit, dark green vegetables, total vegetables, whole grains, dairy products, total protein foods, seafood and plant protein and lower intake of

Component	Maximum score	Standard for maximum score	Standard for zero score
Whole fruit	5	≥ 0.8 serving / 1000 kcal	Without whole fruit
Total fruit	5	≥ 0.4 serving / 1000 kcal	Without total fruit
Dark green vegetables	5	≥ 0.2 serving / 1000 kcal	Without dark green vegetables
Total vegetables	5	≥ 1.1 serving / 1000 kcal	Without total vegetables
Whole grains	10	≥ 1.5 ounce / 1000 kcal	Without whole grains
Dairy products	10	≥ 1.3 cups / 1000 kcal	Without dairy product
Total protein foods	5	≥ 2.5 ounce / 1000 kcal	Without total protein foods
Seafood and plant protein	5	≥ 0.8 ounce / 1000 kcal	Without seafood and plant proteins
Fatty acids	10	(PUFA + MUFA) / SFA ≥ 2.5	(PUFA + MUFA) / SFA ≤ 1.2
Refined grains	10	≤ 1.8 ounce / 1000 kcal	≥ 4.3 ounce / 1000 kcal
Sodium	10	≤ 1.1 g / 1000 kcal	≥ 2 g / 1000 kcal
Empty calories	20	≤ 19% of total energy intake	≥ 50% of total energy intake

Table 1. Distribution of healthy eating index-2010 (HEI-2010) components and standards for scoring.

Component		Maximum score	Standard for maximum score	Standard for zero score
Vegetables		10	≥ 5 serving/ day	0 serving/ day
Fruit		10	≥ 4 serving/day	0 serving/ day
Whole grains	Males	10	90 gram/ day	0 gram/ day
	Females		75 gram/ day	
Sugar sweetened beverages		10	≤ 1 serving / day	≥ 1 serving/ day
Nuts and beans		10	≥ 1 serving/ day	0 serving/ day
Red meat/processed meat		10	≤ 1 serving / day	≥ 1.5 serving/ day
Trans fatty acids		10	≤ 0.5% of energy/day	≥ 4% of energy/day
n-3 fatty acids		10	≥ 250 mg/ day	0 mg/ day
PUFA		10	≥ 10% of energy intake/ day	≤ 2% of energy intake/day
Sodium			The lowest decimal/ mg a day	The highest decimal/ mg a day
Alcohol	Males	10	0.5–2 liquor / day	≥ 3.5 liquor/ day
	Females		0.5–1.5 liquor/ day	≥ 2.5 liquor/ day

Table 2. Distribution of alternative healthy eating index-2010 (AHEI-2010) components and standards for scoring.

unhealthy items including fatty acids, refined grains, sodium and empty calories (including snacks and sweets) got the maximum score (score = 5) for each item and those with low intake of healthy items and high intake of unhealthy items got the minimum score (score = 0). In order to calculate the AHEI score, 11 components were used for all study population (Table 2). Participants with a higher intake of healthy items of this nutritional index including vegetables, fruit, whole grains, nuts and beans, n-3 fatty acids, and PUFA and low intake of unhealthy items including sugar sweetened beverages, red meat/processed meat, trans fatty acids, sodium and alcohol got the maximum score (score = 10) for each item and those with low intake of healthy items and high intake of unhealthy items got the minimum score (score = 0). Finally, the scores of all components were added together and recorded as the final score. The total score is 100 for HEI and 110 for AHEI; higher scores indicate better diet quality.

Statistical analysis

SPSS software version 16 was used to analyze the study data. A Kolmogorov–Smirnov test was applied to judge whether the data were normally distributed. Data presented as Mean ± SD/median (interquartile range) or percent (number) for continuous or categorical variables, respectively. Normally-distributed continuous compared between four quartiles by using one-way ANOVA. We used linear regression to calculate adjusted β estimates for assessment of the association between HEI and AHEI components with the antioxidant concentration of breast milk (BM) and the infant's urine. Multivariate binary logistic regression was used to examine the odds of low antioxidant concentration of BM and infant's urine across quartiles of HEI and AHEI. The lowest HEI and AHEI quartile was considered the reference category for analysis of the odds ratios (ORs) and their 95% confidence intervals (CIs) for the other quartiles. The first model was adjusted for the mother's age and energy intake for BM samples and the infant's age and sex for the infant's urine samples. The second model was adjusted for the related samples' mother's BMI, infant weight, and head circumference. p value < 0.05 was considered statistically significant.

Results

The baseline characteristics of the study participants ($n = 300$) are shown in Table 3. The average age of breastfeeding mothers was 29.5 ± 5.9 years, and the mean BMI was 24.8 ± 4.3 kg/m^2. The average HEI and AHEI for the participants were 70.1 ± 11.1 and 55.8 ± 7.3, respectively. The mean total energy intake of mothers was 2102 ± 796.5 Kcal/day, and carbohydrate, protein, fat and fiber intake were 184.7 ± 41.0, 45.7 ± 11.2, 117.3 ± 19.9, and 22.2 ± 5.4 gr/day, respectively.

The average age of the infants was 106.38 ± 57.36 days, and their average weight and head circumference were 5.9 ± 1.5 kg and 39.6 ± 4.6 cm, respectively.

The mean concentration of the BM and infant's urine antioxidants across different quartiles of HEI is shown in Table 4. The mean concentration of infant's urine DPPH was significantly different between HEI quartiles and it was highest in the last quartile compared to the first quartile (12.6 ± 8.6 vs. 9.2 ± 7.1, $p = 0.028$). However, we found no significant difference in the BM or infant's urine concentration of other antioxidants between HEI quartiles.

The mean concentration of the BM and infant's urine antioxidants were not associated with AHEI quartiles (Table 5).

Linear regression analysis of HEI and AHEI components and antioxidant concentration of BM and infant's urine is indicated in Table 6. There was a direct association between whole fruit score and the DPPH level of BM ($p < 0.05$). Also, there was a direct relationship between the dark green vegetable score and the level of the infant's urine DPPH, FRAP, and MDA ($p < 0.01$). However, there was an inverse association between this component

Variables	
Mother	
Mother's age (year)	29.5 ± 5.9
Mother Height (cm)	159.6 ± 11.7
Mother Weight (kg)	63.7 ± 12.05
Mother BMI (kg/m2)	24.8 ± 4.3
Mother SBP (mmHg)	104.2 ± 10.5
Mother DBP (mmHg)	72.1 ± 1.4
Mother's education, n(%)	
10–12 y	71 (23.5%)
0–9 y	102 (34%)
> 13y	127 (42.5%)
Income status, n (%)	
Less than enough	84 (28%)
Enough	207 (69%)
To the extent of savings	9 (3%)
Type of delivery (natural)	183 (61.0%)
Mother's chronic disease history, n (%)	
Yes	33 (11.0%)
No	267 (89.0%)
Mother HEI score	70.1 ± 11.11
Mother AHEI score	55.8 ± 7.3
Total energy intake (kcal/day)	2102 ± 796.5
Carbohydrate (g/day)	184.7 ± 41.0
Protein (g/day)	45.7 ± 11.2
Fat (g/day)	117.32 ± 19.90
Fiber (g/day)	22.2 ± 5.4
Fruit (g/day)	194.4(120.8, 299.0)
Vegetables (g/day)	160.4 (107.3, 221.0)
Legumes (g/day)	21.1(14.9, 27.4)
Nuts (g/day)	9.7 (3.04, 20.7)
Whole grains (g/day)	111.1 (81.4, 149.9)
Dairy products (g/day)	139.62 (70.2, 239.5)
Sea foods (g/day)	5.88 (2.4, 12.5)
n-3 fatty acids (mg/day)	18.97 (1.9, 54.1)
Infant	
Infant age (day)	106.4 ± 57.4
Infant sex, % (n)	
Boy	162 (54.0%)
Girl	138 (46.0%)
Infant weight (kg)	5.9 ± 1.5
Infant height (cm)	58.7 ± 8.1
Infant head circumference (cm)	39.6 ± 4.6

Table 3. Characteristics of the study participants. Data presented as Mean ± SD /median (interquartile range) or number (%) as appropriate. *BMI* Body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HEI* healthy eating index, *AHEI* alternative healthy eating index.

and the thiol level of BM ($p < 0.01$). The total vegetable score indicated a direct association with the FRAP of BM, the infant's urine FRAP, and DPPH ($p < 0.05$). There was an inverse association between seafood and plant protein and also beans and nuts with the MDA of BM ($p < 0.05$). HEI was directly associated with the DPPH of BM, the infant's urine DPPH, and FRAP ($p < 0.05$). However, AHEI was not significantly associated with any BM or infant urine antioxidant concentration.

Odds ratios for the low antioxidant concentration of BM and infant's urine across quartiles of HEI are assessed using crude and adjusted models (Table 7).

The odds of low levels of BM MDA increased in the last quartile of HEI compared to the first quartile (OR = 2.03, 95% CI: 1.02–4.01; $p < 0.05$). However, the odds of low levels of infant's urine DPPH (OR = 0.33, 95% CI: 0.17–0.66; $p < 0.01$) and FRAP (OR = 0.41, 95% CI = 0.20–0.83; $p < 0.05$) decreased in the last quartile compared to the first quartile.

Variables	**HEI quartiles**				***p* value**
	Q1	**Q2**	**Q3**	**Q4**	
Milk DPPH (μmol Trolox equivalent /L)	308.2 ± 74.2	307.1 ± 77.6	315.7 ± 82.4	334.3 ± 121.4	0.25
Milk FRAP (μmol /L)	536.6 ± 127.6	540.1 ± 153.5	543.4 ± 137.7	551.1 ± 175.6	0.95
Milk MDA (μmol TBARs/L)	0.12 ± 0.06	0.11 ± 0.06	0.11 ± 0.09	0.11 ± 0.08	0.86
Milk thiol (μmol /L)	81.0 ± 23.6	76.0 ± 19.5	82.3 ± 21.4	79.5 ± 18.5	0.32
Infant's urine DPPH (μmol Trolox equivalent /L)	9.2 ± 7.1	9.3 ± 8.2	9.7 ± 7.4	12.6 ± 8.6	**0.028**
Infant's urine FRAP (μmol /L)	19.1 ± 13.1	20.8 ± 15.8	22.1 ± 17.2	25.8 ± 17.8	0.07
Infant's urine MDA (μmol Trolox equivalent /L)	1.8 ± 1.7	1.8 ± 1.7	1.8 ± 1.4	1.9 ± 1.8	0.97

Table 4. The comparison of anti-oxidant concentration of breast milk and the infant's urine between quartiles of HEI. Significant value are in bold. *DPPH* Diphenylpicrylhydrazyl, *FRAP* Ferric reducing ability of plasma, *MDA* Malondialdehyde, *HEI* healthy eating index. p value obtained from one-way ANOVA.

Variables	**AHEI quartiles**				***p* value**
	Q1	**Q2**	**Q3**	**Q4**	
Milk DPPH (μmol Trolox equivalent /L)	310.0 ± 79.3	320.9 ± 90.8	311.5 ± 80.8	323.2 ± 98.8	0.60
Milk FRAP (μmol /L)	515.4 ± 117.7	547.2 ± 104.3	549.2 ± 132.2	552.5 ± 114.3	0.43
Milk MDA (μmol TBARs/L)	0.18 ± 0.10	0.17 ± 0.12	0.17 ± 0.07	0.16 ± 0.07	0.82
Milk thiol (μmol /L)	77.6 ± 17.2	79.0 ± 16.0	78.4 ± 12.5	77.9 ± 16.3	0.95
Infant's urine DPPH (μmol Trolox equivalent /L)	9.5 ± 7.6	10.1± 7.3	9.8 ± 6.7	11.41 ± 9.62	0.45
Infant's urine FRAP (μmol /L)	20.5 ± 15.7	24.5 ± 16.8	20.9 ± 14.0	23.4 ± 18.73	0.36
Infant's urine MDA (μmol Trolox equivalent /L)	1.8 ± 1.6	1.7 ± 1.2	1.7 ± 1.3	2.05 ± 1.91	0.57

Table 5. The comparison of anti-oxidant concentration of breast milk and the infant's urine between quartiles of AHEI. *DPPH* Diphenylpicrylhydrazyl, *FRAP* Ferric reducing ability of plasma, *MDA* Malondialdehyde, *AHEI* alternative healthy eating index. p value obtained from one-way ANOVA.

Odds ratios for the low antioxidant concentration of BM and infant's urine across quartiles of AHEI are assessed using crude and adjusted models (Table 8). In all models, we found no significant association between AHEI quartiles and antioxidant concentration of BM and infant's urine.

Discussion

In this cross-sectional study, we found lower breast milk MDA and higher infant urine DDPH and FRAP levels among mothers with higher HEI scores. However, there was no significant association between AHEI with the antioxidant content of BM, and the infant's urine.

The health of infants is directly associated not only with the nutritional elements and quality of the BM but also with its antioxidant content. Our findings suggest that breastfeeding mothers who adhere to a healthier diet pattern are more likely to provide BM rich in antioxidants, which may protect cells from oxidative stress and maintain overall health. Oxidative stress occurs when an imbalance exists between oxidants, such as free radicals, and antioxidants in the body. Free radicals are unstable molecules potentially damage cells and promote susceptibility to different health conditions, including inflammation, cardiovascular disease, and cancer. Antioxidants are compounds that combat free radicals and help prevent oxidative damage. BM contains a broad spectrum of antioxidants, including vitamins C and E, β-carotene, and selenium, essential for infant growth and development[32]. Our research shows that mothers with a higher HEI score tend to have BM with lower concentrations of MDA, a marker of oxidative stress. So, mothers with lower HEI may likely produce higher ROS levels through mammary gland lactocytes. Maternal diet quality directly impacts the oxidant-antioxidant balance in BM, which may, in turn, influence the health and development of breastfed infants. Because of these effects, we found that infants of mothers with higher HEI scores had higher levels of DDPH and FRAP in their urine, further supporting the link between maternal diet quality and infant antioxidants.

Also, some components of HEI showed a relationship with the antioxidant content of BM and the infant's urine. High whole and total fruit intake indicated a higher DPPH and thiol content in BM, respectively. Dark green vegetable intake directly affected all antioxidant concentrations in the infant's urine, including DPPH, thiol, and MDA, but had an inverse effect on the amount of thiol in the BM. It has been shown that non-heme iron dietary nutrients such as fruits and vegetables could beneficially affect oxidative stress. In contrast, heme-iron dietary nutrients, including meat groups, especially red meat, worsen this situation throughout the body[33].

HEI component	Breast milk								Infant's urine					
	DPPH		FRAP		MDA		Thiol		DPPH		FRAP		MDA	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Whole fruit	7.97	**0.022**	-0.83	0.86	-0.004	0.36	-1.11	0.08	-0.16	0.59	0.23	0.70	0.031	0.62
Total fruit	4.22	0.36	-3.60	0.58	-0.008	0.10	-1.81	**0.034**	0.15	0.70	0.98	0.23	0.026	0.76
Dark green vegetables	3.60	0.24	3.63	0.37	0	0.91	-1.42	**0.009**	0.98	**< 0.001**	1.77	**0.001**	0.17	**0.002**
Total vegetables	2.28	0.16	4.25	**0.046**	-0.001	0.50	-0.26	0.37	0.35	**0.011**	0.69	**0.015**	0.04	0.16
Whole grains	-2.18	0.30	-5.04	0.07	-0.002	0.41	0.53	0.16	0.24	0.18	0.16	0.66	-0.04	0.34
Dairy products	-0.74	0.75	-1.71	0.58	0	0.86	0.14	0.73	0.37	0.06	0.77	0.06	0.006	0.88
Total protein foods	0.40	0.93	-6.51	0.32	-0.007	0.19	-0.21	0.81	0.45	0.29	1.10	0.20	0.018	0.84
Seafood and plant protein	1.42	0.73	-3.28	0.55	-0.011	**0.018**	-0.34	0.64	0.56	0.12	0.58	0.43	0.090	0.23
Nuts and beans	1.69	0.34	-1.56	0.51	-0.005	**0.010**	-0.12	0.69	0.055	0.68	-0.09	0.75	0.014	0.61
Fatty acids (MUFA + PUFA/SFA)	1.77	0.31	-2.07	0.38	0.0001	0.89	0.22	0.48	0.082	0.58	0.075	0.81	-0.016	0.61
Trans fatty acid	2.93	0.71	1.90	0.86	-0.011	0.19	2.01	0.16	0.60	0.38	0.72	0.61	0.084	0.56
n-3 fatty acid	1.18	0.60	-0.26	0.93	-0.002	0.41	-0.73	0.07	0.09	0.62	0.07	0.85	0.012	0.76
PUFA	1.72	0.40	1.12	0.68	-0.001	0.74	-0.073	0.84	0.23	0.20	0.36	0.39	0.023	0.54
Refined grains	0.59	0.78	-0.63	0.83	0.001	0.79	0.52	0.17	0.003	0.99	0.27	0.47	0.035	0.37
Sodium	0.89	0.882	12.89	0.09	0.002	0.80	-0.36	0.73	-0.54	0.26	-0.57	0.56	-0.025	0.81
Empty calories	1.18	0.16	-0.03	0.98	-0.001	0.42	-0.005	0.97	-0.021	0.75	-0.044	0.74	-0.02	0.14
Sugar-sweetened beverages	-0.74	0.81	-0.59	0.90	-0.002	0.54	0.64	0.29	-0.06	0.83	0.074	0.89	-0.053	0.36
Red meat and processed meat	-0.21	0.879	0.101	0.96	0.002	0.23	0.39	0.11	0.07	0.51	0.054	0.79	-0.021	0.33
HEI	0.97	**0.049**	-0.14	0.83	-0.001	0.15	-0.033	0.71	0.09	**0.031**	0.17	**0.035**	0.001	0.93
AHEI	0.86	0.25	-0.08	0.94	0.0001	0.58	0.094	0.483	0.11	0.11	0.13	0.30	0.010	0.43

Table 6. Multivariate linear regression between each component of the HEI and AHEI and anti-oxidant concentration of breast milk and infant's urine. Breast milk adjusted for mother's age and energy intake and mother's BMI Infant's urine adjusted for infant age and sex, weight, and head circumference Significance of bold values are $p < 0.05$.

	Crude	Model 1	Model 2
Breast milk			
DPPH			
Q1	1	1	1
Q2	1.32 (0.68–2.56)	1.34 (0.69–2.63)	1.37 (0.69–2.70)
Q3	0.74 (0.39–1.41)	0.75 (0.39–1.45)	0.74 (0.38–1.45)
Q4	0.83 (0.43–1.58)	0.92 (0.47–1.80)	0.97 (0.49–1.94)
FRAP			
Q1	1	1	1
Q2	0.94 (0.48–1.82)	0.94 (0.48–1.83)	0.97 (0.49–1.93)
Q3	1.24 (0.65–2.38)	1.21 (0.63–2.35)	1.22 (0.62–2.39)
Q4	1.25 (0.65–2.40)	1.26 (0.64–2.50)	1.39 (0.69–2.79)
MDA			
Q1	1	1	1
Q2	1.23 (0.63–2.39)	1.28 (0.65–2.50)	1.30 (0.66–2.56)
Q3	1.78 (0.92–3.42)	1.93 (0.990–3.77)	2.03 (1.02–4.01)*
Q4	1.35 (0.70–2.60)	1.44 (0.73–2.85)	1.37 (0.68–2.74)
Thiol			
Q1	1	1	1
Q2	1.61 (0.82–3.14)	1.59 (0.81–3.14)	1.54 (0.77–3.07)
Q3	0.95 (0.50–1.82)	0.94 (0.48–1.82)	0.98 (0.50–1.93)
Q4	0.89 (0.46–1.72)	0.97 (0.49–1.92)	0.99 (0.49–1.99)
Infant’s urine			
DPPH			
Q1	1	1	1
Q2	0.85 (0.43–1.69)	0.84 (0.43–1.67)	0.84 (0.42–1.68)
Q3	0.58 (0.30–1.14)	0.55 (0.29–1.08)	0.59 (0.30–1.16)
Q4	0.32 (0.16–0.64)**	0.27 (0.13–0.54)**	0.33 (0.17–0.66)**
FRAP			
Q1	1	1	1
Q2	0.60 (0.30–1.22)	0.61 (0.31–1.20)	0.58 (0.29–1.19)
Q3	0.51 (0.26–1.02)	0.51 (0.26–0.98)*	0.51 (0.25–1.02)
Q4	0.43 (0.21–0.85)	0.42 (0.21–0.83)*	0.41 (0.20–0.83)*
MDA			
Q1	1	1	1
Q2	0.87 (0.43–1.73)	0.90 (0.46–1.75)	0.86 (0.43–1.73)
Q3	0.65 (0.33–1.27)	0.65 (0.33–1.25)	0.66 (0.33–1.32)
Q4	0.83 (0.43–1.63)	0.70 (0.36–1.37)	0.83 (0.42–1.63)

Table 7. Multivariate-adjusted odds ratios β (95% CIs) for low anti-oxidant concentration of breast milk and infant’s urine across quartiles of HEI. *DPPH* Diphenylpicrylhydrazyl, *FRAP* Ferric reducing ability of plasma, *MDA* Malondialdehyde. Odds ratios with 95% CI obtained from binary logistic regression tests. Breast milk model 1 adjusted for the mother’s age and energy intake; and model 2 additionally adjusted for the mother’s BMI. Infant’s urine model 1 adjusted for infant’s age and sex; and model 2 additionally adjusted for infant’s weight and head circumference. $^{*}p < 0.05$. $^{**}p < 0.01$. $^{**}p < 0.001$.

Several studies have investigated the relationship between breastfeeding mothers’ diet quality and the BM’s antioxidant content and infant’s urine[12,23,26]. Higher levels of DPPH and thiol were reported in the BM of lactating mothers who adhered to a healthy dietary pattern characterized by high consumption of fruits, vegetables, grains, and nuts. Debski et al.. reported that the activity of glutathione peroxidase was considerably higher in milk specimens of vegetarian versus non-vegetarian mothers[34].

It has been reported that following a four-week MedDiet style significantly promoted HEI scores (+27 units) among breastfeeding obese women[35]. In another study, the commitment of mothers to Polish-adapted MedDiet meal was a remarkable predictor of BM mineral composition in the 1st month of lactation[36].

We have previously demonstrated that adherence to healthy dietary patterns such as MedDiet or DASH styles was associated with higher BM and infant urine DDPH and FRAP levels[23,26]. However, following an unhealthy dietary pattern resulted in a decrease in thiol levels[12]. A high score for MedDiet indicated a higher consumption of fruit, vegetables, legumes, grains, nuts, and olive oil; a moderate intake of poultry, eggs, seafood, and dairy products; and a low intake of red and processed meats[37]. Similarly, a high HEI score correlates with

	Crude	Model 1	Model 2
Breast milk			
DPPH			
Q1	1	1	1
Q2	0.60 (0.31–1.14)	0.61 (0.32–1.17)	0.70 (0.36–1.35)
Q3	1.21 (0.63–2.32)	1.25 (0.65–2.41)	1.29 (0.65–2.53)
Q4	0.85 (0.45–1.62)	0.89 (0.46–1.71)	1.02 (0.52–1.98)
FRAP			
Q1	1	1	1
Q2	0.50 (0.26–0.97)	0.51 (0.26–0.99)	0.57 (0.29–1.12)
Q3	0.54 (0.28–1.05)	0.55 (0.28–1.07)	0.54 (0.27–1.09)
Q4	0.57 (0.30–1.11)	0.58 (0.30–1.13)	0.65 (0.33–1.27)
MDA			
Q1	1	1	1
Q2	1.18 (0.62–2.25)	1.16 (0.60–2.22)	1.16 (0.60–2.25)
Q3	1.11 (0.58–2.13)	1.12 (0.58–2.17)	1.20 (0.61–2.36)
Q4	1.42 (0.74–2.73)	1.44 (0.75–2.77)	1.38 (0.71–2.68)
Thiol			
Q1	1	1	1
Q2	0.83 (0.43–1.58)	0.60 (0.44–1.61)	0.82 (0.42–1.59)
Q3	0.68 (0.53–1.31)	0.66 (0.34–1.28)	0.62 (0.31–1.23)
Q4	1.12 (0.58–2.14)	1.11 (0.58–2.16)	1.03 (0.53–2.02)
Infant's urine			
DPPH			
Q1	1	1	1
Q2	1.03 (0.54–1.96)	0.98 (0.51–1.91)	1.02 (0.52–2)
Q3	0.97 (0.51–1.84)	0.98 (0.51–1.90)	1 (0.51–1.93)
Q4	0.79 (0.41–1.49)	0.75 (0.39–1.46)	0.72 (0.37–1.41)
FRAP			
Q1	1	1	1
Q2	0.71 (0.37–1.35)	0.54 (0.27–1.07)	0.58 (0.29–1.18)
Q3	0.67 (0.35–1.27)	0.60 (0.30–1.18)	0.63 (0.32–1.26)
Q4	0.71 (0.37–1.34)	0.62 (0.31–1.23)	0.62 (0.31–1.24)
MDA			
Q1	1	1	1
Q2	1.03 (0.54–1.95)	0.95 (0.48–1.87)	1.03 (0.52–2.04)
Q3	1.03 (0.54–1.94)	1.04 (0.53–2.03)	1.11 (0.56–2.18)
Q4	0.97 (0.51–1.84)	0.92 (0.47–1.80)	0.92 (0.47–1.82)

Table 8. Multivariate-adjusted odds ratios (95% CIs) for low anti-oxidant concentration of breast milk and infant's urine across quartiles of AHEI. *DPPH* Diphenylpicrylhydrazyl, *FRAP* Ferric reducing ability of plasma, *MDA* Malondialdehyde. Odds ratios with 95% CI obtained from binary logistic regression tests. Breast milk model 1 adjusted for the mother's age and energy intake, and model 2 additionally adjusted for the mother's BMI. Infant's urine model 1 adjusted for infant's age and sex, model 2 additionally adjusted for infant's weight and head circumference.

a high consumption of fruits, vegetables, grains, dairy products, seafood, and plant protein[13]. Tsopmo et al.. have reported that a high intake of fruits, vegetables, and grains during lactation can improve the antioxidant phytochemicals of the BM[38]. Fruits and vegetables are rich food sources of phytonutrients, which can improve our body's antioxidant defense[38,39]. The mother's nutritional habit also affects the antioxidant capacity of BM. No similar investigation was found to compare our results about the HEI/AHEI and total antioxidant capacity (TAC) of BM. However, an elevated intake of dairy products, fruits/vegetables, cereals, and nuts enhanced the TAC of human milk[40]. Antioxidant compounds of fruits and vegetables such as α-Tocopherol, ascorbic acid, and β-Carotene can enhance TAC of blood plasma[41]. TAC is representative of all body antioxidants that can be used to evaluate the quality of one's diet[42]. Moreover, dietary TAC is another potential indicator for assessing diet quality, and it reveals a positive correlation with several nutritional indexes such as HEI[43]. Based on this correlation and direct relation between dietary TAC and plasma TAC status[44], HEI score can be positively associated with dietary TAC and serum TAC levels.

To the best of our knowledge, this is the first study reporting a comprehensive analysis of the oxidant-antioxidant status through specialized assays such as the FRAP, DPPH, TBARs, and Ellman assays, exemplified a thorough and systematic approach to understanding the nutritional composition and antioxidant properties of human milk. Regarding limitations, notably, our study is observational, and further research is needed to establish causality. Additionally, oxidative status was measured in the infant only by indices in the urine; we cannot necessarily extrapolate this to their serum status. BMI is not an appropriate indicator for assessing the nutritional status of women after childbirth. However, our findings provide valuable insights into the potential benefits of a healthy diet for breastfeeding mothers and their infants.

Conclusion

Our study demonstrates that a higher quality of diet in breastfeeding mothers, as measured by the HEI, can positively impact the oxidant-antioxidant balance of BM and the infant's urine. A healthier diet pattern is associated with lower oxidative stress markers in BM and infant urine levels, indicating greater antioxidant activity. The advantages of BM against oxidative stress were shown through the lower levels of urine markers in BM-feeding infants versus those fed with formula due to the higher and more diverse presence of antioxidants in BM[45]. These findings suggested the critical role of maternal nutrition in supporting the health and development of breastfed infants and emphasized the need for further investigation in this area. By promoting healthy eating habits among breastfeeding mothers, health authorities can help ensure future generations' optimal growth and development.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Received: 13 June 2024; Accepted: 16 September 2024

References

1. Landrigan, P. J., Sonawane, B., Mattison, D., McCally, M. & Garg, A. Chemical contaminants in breast milk and their impacts on children's health: An overview. *Environ. Health Perspect.* **110** (6), A313–A5 (2002).
2. Saarela, T., Kokkonen, J. & Koivisto, M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatr.* **94**(9), 1176–1181 (2005).
3. Matos, C., Ribeiro, M., Guerra, A. & Breastfeeding Antioxidative properties of breast milk. *J. Appl. Biomed.* **13**(3), 169–180 (2015).
4. Verd, S. et al. Human milk feeding is Associated with decreased incidence of moderate-severe bronchopulmonary dysplasia in extremely Preterm infants. *Children*. **10** (7), 1267 (2023).
5. Kavurt, S. et al. The effect of galactagogue herbal tea on oxidant and anti-oxidant status of human milk. *J. Maternal-Fetal Neonatal Med.* **26**(10), 1048–1051 (2013).
6. Hernández-Aguilar, M. T., de la Torre, M. J. L., Borja-Herrero, C., Lasarte-Velillas, J-J. & Martorell-Juan, L. Antioxidant properties of human milk. *J. Pediatr. Biochem.* **3**(03), 161–167 (2013).
7. Ozsurekci, Y. & Aykac, K. Oxidative stress related diseases in newborns. *Oxidative Med. Cell. Longev.* **2016**(1), 2768365 (2016).
8. Peila, C. et al. The gestational pathologies Effect on the Human Milk Redox Homeostasis: A First Step towards its definition. *Nutrients* **15**(21), 4546 (2023).
9. Ramiro-Cortijo, D. et al. Maternal and neonatal factors modulating breast milk cytokines in the first month of lactation. *Antioxidants* **12**(5), 996 (2023).
10. Zielinska-Pukos, M. A., Kopiasz, Ł. & Hamulka, J. The effect of maternal Overweight/Obesity on serum and Breastmilk Leptin, and its associations with body composition, Cardiometabolic Health Indices, and maternal Diet: the BLOOM Study. *Metabolites* **14**(4), 221 (2024).
11. Codini, M. et al. Relationship between fatty acids composition/antioxidant potential of breast milk and maternal diet: Comparison with infant formulas. *Molecules* **25**(12), 2910 (2020).
12. Karbasi, S. et al. The association of maternal dietary quality and the antioxidant-proxidant balance of human milk. *Int. Breastfeed. J.* **17**(1), 56 (2022).
13. Ohls, T. K. E. N. N. E. D. Y. E., Carlson, J. & Fleming, S. The healthy eating index: design and applications. *J. Am. Diet. Assoc.* **95**(10), 1103–1108 (1995).
14. Guenther, P. M. et al. Update of the healthy eating index: HEI-2010. *J. Acad. Nutr. Dietetics* **113**(4), 569–580 (2013).
15. McCullough, M. L. et al. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. *Am. J. Clin. Nutr.* **76**(6), 1261–1271 (2002).
16. Chiuve, S. E. et al. Alternative dietary indices both strongly predict risk of chronic disease. *J. Nutr.* **142**(6), 1009–1018 (2012).
17. Freitas, R. F., Macedo, M. S., Lessa, A. C., Pinto, N. A. V. D. & Teixeira, R. A. Relationship between the diet quality index in nursing mothers and the fatty acid profile of mature breast milk. *Revista Paulista De Pediatria* **39**, e2019089 (2020).
18. Urrutia-Baca, V. H. et al. Exploring the impact of maternal factors and dietary habits on human milk oligosaccharide composition in early breastfeeding among Mexican women. *Sci. Rep.* **14**(1), 14685 (2024).
19. Essa, A. R. et al. Dietary intervention to increase fruit and vegetable consumption in breastfeeding women: A pilot randomized trial measuring inflammatory markers in breast milk. *J. Acad. Nutr. Dietetics* **118**(12), 2287–2295 (2018).
20. Roumi, Z., Djazayery, A. & Keshavarz, S. A. Association between infants anthropometric outcomes with maternal AHEI-P and DII scores. *Clin. Nutr. Res.* **12**(2), 116 (2023).
21. Rodríguez-Bernal, C. L. et al. Diet quality in early pregnancy and its effects on fetal growth outcomes: The Infancia Y Medio Ambiente (Childhood and Environment) Mother and Child Cohort Study in Spain. *Am. J. Clin. Nutr.* **91**(6), 1659–1666 (2010).
22. Gonzalez-Nahm, S. et al. Associations of maternal diet with infant adiposity at birth, 6 months and 12 months. *BMJ Open* **9**(9), e030186 (2019).
23. Karbasi, S. et al. A Mediterranean diet is associated with improved total antioxidant content of human breast milk and infant urine. *Nutr. J.* **22**(1), 11 (2023).
24. Moradi-binabaj, M., Khorasanchi, Z., Karbasi, S., Ferns, G. A. & Bahrami, A. Investigating the Relationship between a low Carbohydrate Diet score and inflammatory and oxidative stress biomarkers in female students. *Endocrinol. Res. Pract.* **27**(3). (2023).
25. Karbasi, S. et al. The association of maternal food quality score (FQS) with breast milk nutrient content and antioxidant content of infant urine: A cross-sectional study. *BMC Pregnancy Childbirth* **23** (1), 126 (2023).

26. Karbasi, S. et al. Maternal adherence to a Dietary approaches to stop hypertension (DASH) Dietary Pattern and the relationship to breast milk nutrient content. *Matern. Child Health J.* **27**(2), 385–394 (2023).
27. Benzie, I. F. & Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.* **239**(1), 70–76 (1996).
28. Bondet, V., Brand-Williams, W. & Berset, C. Kinetics and mechanisms of antioxidant activity using the DPPH. Free radical method. *LWT-Food Sci. Technol.* **30**(6), 609–615 (1997).
29. Papastergiadis, A., Mubiru, E., Van Langenhove, H. & De Meulenaer, B. Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods. *J. Agric. Food Chem.* **60**(38), 9589–9594 (2012).
30. Hu, M-L. [41] measurement of protein thiol groups and glutathione in plasma. Methods in Enzymology. 233: Elsevier; 380–385. (1994).
31. Ahmadnezhad, M. et al. Validation of a short semi-quantitative food frequency questionnaire for adults: A pilot study. *J. Nutritional Sci. Dietetics* 49–55. (2017).
32. Ramiro-Cortijo, D. et al. Influence of neonatal sex on breast milk protein and antioxidant content in Spanish women in the first month of lactation. *Antioxidants* **11**(8), 1472 (2022).
33. Romeu, M. et al. Diet, iron biomarkers and oxidative stress in a representative sample of Mediterranean population. *Nutr. J.* **12**, 1–9 (2013).
34. Debski, B., Finley, D. A., Picciano, M. F., Lönnerdal, B. & Milner, J. Selenium content and glutathione peroxidase activity of milk from vegetarian and nonvegetarian women. *J. Nutr.* **119**(2), 215–220 (1989).
35. Sims, C. R. et al. A Mediterranean diet plan in lactating women with obesity reduces maternal energy intake and modulates human milk composition–a feasibility study. *Front. Nutr.* **11**, 1303822 (2024).
36. Zielinska-Pukos, M. A. et al. Breastmilk mineral composition among well-educated mothers from Central Poland–associations with maternal dietary intake, dietary patterns and infant psychomotor development. *J. Trace Elem. Med Biol.* **83**, 127393 (2024).
37. Martínez-González, M. A. et al. Benefits of the Mediterranean diet: Insights from the PREDIMED study. *Prog. Cardiovasc. Dis.* **58**(1), 50–60 (2015).
38. Tsopmo, A. Phytochemicals in human milk and their potential antioxidative protection. *Antioxidants* **7**(2), 32 (2018).
39. Bacchetti, T., Turco, I., Urbano, A., Morresi, C. & Ferretti, G. Relationship of fruit and vegetable intake to dietary antioxidant capacity and markers of oxidative stress: A sex-related study. *Nutrition* **61**, 164–172 (2019).
40. Oveisi, M. R. et al. Human breast milk provides better antioxidant capacity than infant formula. *Iran. J. Pharm. Research: IJPR* **9**(4), 445 (2010).
41. Harasym, J. & Oledzki, R. Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. *Nutrition* **30**(5), 511–517 (2014).
42. Górska, P., Górna, I. & Przysławski, J. Mediterranean diet and oxidative stress. *Nutr. Food Sci.* **51**(4), 677–689 (2020).
43. Puchau, B., Zulet, M. A., de Echávarri, A. G., Hermsdorff, H. H. M. & Martínez, J. A. Dietary total antioxidant capacity: a novel indicator of diet quality in healthy young adults. *J. Am. Coll. Nutr.* **28**(6), 648–656 (2009).
44. Wang, Y. et al. Dietary total antioxidant capacity is associated with diet and plasma antioxidant status in healthy young adults. *J. Acad. Nutr. Dietetics* **112**(10), 1626–1635 (2012).
45. Shoji, H. & Shimizu, T. Effect of human breast milk on biological metabolism in infants. *Pediatr. Int.* **61**(1), 6–15 (2019).

Acknowledgements

This research was supported by Birjand University of Medical Sciences (grant No.5629). We would like to thank the Clinical Research Development Unit, Imam Reza Hospital, Mashhad University of Medical Sciences, for their assistance in this manuscript.

Author contributions

SK AB and AZ Conceptualization, methodology, and design of the study; ZA and AHA They are writing original drafts and data curation. GF review and editing; and All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

The study was approved by the Ethical approval was obtained from the Birjand University of Medical Sciences (IR.BUMS.REC.1400.379), which was carried out following the Helsinki Declaration on human subjects' studies. Participation in the study was voluntary and with full consent of respondents. All participants provided written informed consent.

Additional information

Correspondence and requests for materials should be addressed to S.K.