Current Journal of Applied Science and Technology

32(1): 1-7, 2019; Article no.CJAST.45960
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Association between Antioxidant Content in Mature Human Breast Milk and Diet in Abidjan, Côte d'Ivoire

Matogoma Digbé Ble[1,2*], Assi Roméo Boni[1], Sadikou Touré[4], Michèle Aké[2,3] and Jean David N'Guessan[1]

[1]Laboratory of Biochemical Pharmacodynamy, Félix Houphouët-Boigny University of Abidjan (UFHB), 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Laboratory of Nutrition, National Institute of Public Health (INSP) of Adjamé, BP V 47 Abidjan 01, Côte d'Ivoire.
[3]Laboratory of Analytical Chemistry, UFR of Pharmaceutical Sciences, Félix Houphouët-Boigny University of Abidjan (UFHB), 22 BP 582 Abidjan 22, Côte d'Ivoire.
[4]Swiss Centre for Scientific Research (CSRS), Abidjan, 01 BP 1303 Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Author MDB designed the study, performed the technical aspect and wrote the first draft of the manuscript. Author ARB performed the statistical analysis, managed the literature searches and corrected the manuscript. Author ST contributed to the technical aspect. Authors JDN and MA supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2019/45960
Editor(s):
(1) Dr. Yahya Elshimali, Professor, Department of Internal Medicine, Charles Drew University of Medicine and Science, USA.
Reviewers:
(1) Stephen Olorunfemi, Southern Africa.
(2) Maria Cristina Gonzalez-Torres, Universidad Autonoma Metropolitana-Iztapalapa, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/27977

Original Research Article

Received 21 September 2018
Accepted 16 December 2018
Published 26 December 2018

ABSTRACT

Background: Cell damage due to oxidative stress during the neonatal period has been associated with diseases. Human breast milk is an important source of antioxidants which protect newborn against oxidative stress damages. However, the dietary habits of lactating mothers may influence the nutritional value of milk during lactation period.
Aims: The principal aim of this study was to compare the antioxidant content of mature human milk during two periods of lactation in Ivorian women who consumed different diets.
Methodology: Samples of mature human milk were collected from healthy breastfeeding women on the 45th and 105th day postpartum. Food intake frequencies of mothers were obtained using

**Corresponding author: E-mail: digbematogoma@yahoo.fr;*

questionnaire and infants were also weighted. The Total antioxidant capacity (TAC) of mature milk samples were determined using FRAP (Ferric Reducing Antioxidant Power) assay and free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

Results: In this study, a final sample size of 75 breastfeeding women was retained. Two predominant basic diets which are cooked rice with oil palm seed juice sauce (R-SG) and cooked pasty mixture of plantain and cassava accompanied with eggplant sauce (F-SAU), involving, respectively, 50 and 25 lactating women, were determined. Regarding R-SG and F-SAU diets, a significant increase (P < .05) in level of TAC was observed between milk collected on 45th and 105th day postpartum, while no significant difference was found concerning percentage of inhibition of DPPH radical at the same periods. Also, TAC and antiradical activity in breast milk of women who received R-SG diet and F-SAU diet as well as day 45 and day 105 postpartum were statistically comparable (P > .05). Otherwise, TAC and antiradical activity of women's breast milk following these diets were correlated with weight of the newborns on 105th day postpartum.

Conclusion: Based on our results, it is concluded that antioxidant activities in breast milk of women from R-SG diet and F-SAU diet were comparable.

Keywords: Antioxidant; breast milk; diet; Côte d'Ivoire.

1. INTRODUCTION

Oxygen is essential to the life of all aerobic cells because they use it to produce energy. During this process of oxidative respiration, free radicals are created following the production of adenosine triphosphate (ATP) by mitochondria. These free radicals are generally Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) [1,2]. These ROS or RNS are normally produced in living organisms at low but measurable concentrations, and may be beneficial or even crucial in processes such as intracellular signaling and defense against microorganisms. Also, ROS are involved in the cell growth, differentiation, progression, and death [3]. On the other hand, when excessively produced, they induce oxidative stress, which is responsible for cell and tissue injury [4].

At birth, the newborn is exposed to a relatively hyperoxic extra uterine environment caused by an increased oxygen bioavailability which greatly enhanced generation of ROS. As a result, human infant is under oxidative stress due to the difficulty of adapting to ambient oxygen, especially because of antioxidant defense mechanisms which are poorly developed in the neonatal period. It is believed that oxidative stress is involved in the pathogenesis of numerous neonatal diseases such as necrotizing entercolitis, bronchopulmonary dysplasia, kidney failure, retinopathy of prematurity and intraventricular hemorrhage [5-7]. In response, mammalian cells have developed antioxidant defense mechanisms that prevent ROS- and RNS-induced damage.

Human milk is regarded as the ideal nutrient source for the growth and development of the infant, with unique composition characteristics distinct from both bovine milk an infant formula [8,9]. The complete list of active antioxidant components in human milk is not known, but carotenoids, cysteine, coenzyme Q, lactoferrin and vitamins A, E and C have been found together with antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase [10-12]. So, recent years have seen a growing appreciation of the importance of promoting and supporting human milk feeding for optimizing beneficial effects in newborn. Thus, a preliminary report of Ministry of Health of Cote d'Ivoire showed that the exclusive breastfeeding rate from 0 to 6 months increased from 4% in 2006 to 12% in 2012 [13,14].

The composition of human milk may be influenced by different variables, such as genetic characteristics, socioeconomic state of the mother, duration of lactation and dietary habits [15, 16]. Diet contains secondary plant metabolites commonly referred to as phytochemicals including polyphenols, one of the largest groups. Polyphenols have received significant attention in recent years due to their proposed nutritional and health promoting functions in humans. For mothers, phytochemicals intake occurs through the consumption of foods such as fruits, vegetables, and grains. Research has shown that flavonoids, phenolic acids and carotenoids are present in the mother's milk and can contribute to its oxidative stability [17,18].

This study was conducted to compare the antioxidant capacity of mature human milk during two periods of lactation in Ivorian women who consumed different diets.

2. MATERIALS AND METHODS

2.1 Study Subjects

This is a prospective study that was conducted from January 24, 2017 to June 28, 2017, in Maternal and Child Health Services (SMI) of the National Institute of Public Health (INSP) of Adjamé and General Hospital of Yopougon Attié, in District of Abidjan (Côte d'Ivoire).

Eligibility criteria included women giving birth to a single full-term gestation, practicing exclusive breastfeeding and neonates in good general status with normal birth weight (2.5 kg-4.5 kg).

The exclusion criteria included smoking women, acute and chronic disorders (diabetes, arterial hypertension), treatment with antibiotics or any drugs that potentially pass in human milk. Also, women following a vegan diet, with significant food allergies or intolerances that restricted the intake of food groups.

Women gave their consent to participate in the study after explanation of the procedures. All procedures were duly approved by the Hospital Ethical Committee.

2.2 Study Design

2.2.1 Dietary data and sample collections

Prior to sampling, lactating women were interviewed by a trained investigator, using a structured questionnaire. The questionnaire requested information on age, geographical localization, marital status, educational level, professional activity as well as diet/dietary intake assessment of the participants. Also, the body weight of newborns was measured with a mechanical scale.

2.2.1.1 Dietary intake assessment

Dietary survey was conducted using a food frequency questionnaire used around the world to evaluate food intake [19]. In this study, lactating women will be requested to provide complete basic diet descriptions including frequency of consumption, food preparation (ingredients) and cooking method (such as frying, boiling, grilling and steaming) as detail as possible in a week. Thus, a basic diet was retained on the fact that it was consumed at least three to four times in the week and repeated throughout the study.

2.2.1.2 Milk samples

Mature milk samples were collected the morning from breastfeeding women at 45^{th} and 105^{th} day postpartum, corresponding respectively to appointment dates of the first and third newborns vaccination sessions, using breast pump. A 20 to 35 mL sample was collected from each participant into sterile glass container. Immediately after collection, each sample was devised in aliquots of 5 mL in test tubes, protected at all times from light and then transported on ice to the laboratory where they were stored at -20°C until analysis.

2.3 DPPH Radical Scavenging Activity

The analytical procedure was performed using modified method proposed by Brand-Williams et al. [20]. Briefly, 100µL of mature milk were added to 2 mL of DPPH in ethanol solution (100 µM) in a test tube. After incubation at 37°C for 30 minutes in darkness, 1 mL of chloroform was added followed by centrifugation at 3000 g for 5 minutes, the absorbance of supernatant was measured at 517 nm using a spectrophotometer UV-visible SCHIMADZU UV 3600. An ethanolic solution of DPPH was used as control and the percentage of DPPH radical scavenging activity was calculated according to the following equation:

Scavenging activity (%) = [(Absorbance control – Absorbance sample) / Absorbance control] x 100

2.4 Total Antioxidant Capacity (TAC) by FRAP Assay

The Ferric Reducing Antioxidant Potential (FRAP) assay, developed by Benzie and Strain [21], was adopted in this study. At low pH, reduction of ferric 2,4,6-tripyridyl-s-triazyne [Fe^{3+}-TPTZ] complex to the ferrous 2,4,6-tripyridyl-s-triazyne [Fe^{2+}-TPTZ] complex, which has an intense blue color, in the presence of antioxidants, can be monitored by measuring the change in absorption at 593 nm.

Practically, the FRAP reagent was prepared fresh prior to the experiments with 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1. In this experiment, 50 µL of each sample of mature breast milk was mixed with 1.5 mL of FRAP reagent and 1.5 mL of distilled water. The absorbance of the mixture was measured spectrophotometrically at 593 nm against FRAP solution as blank, after incubation at 37°C for 10 min. Aqueous solutions of $FeSO_4$, $7H_2O$ (125 - 1000 µM) were used for the calibration and the results were expressed as µmol/L of Fe^{2+} equivalent of the samples.

2.5 Statistical Analysis

The results were expressed as mean ± standard error of means (SEM). Data were analyzed using Student's t-test. The paired t-test compared the variations in antioxidant and antiradical activities between two sampling periods within a group while the unpaired *t*-tests compared these activities inter-group following a particular sampling period. Correlation between antioxidant and antiradical activities and infant weight was determined by Pearson correlation analysis. The differences at the 5% level of probability were considered significant. Graph Pad Prism 5.0 (San Diego, USA) was used for all analyses.

3. RESULTS

3.1 Nutritional Profile and Demographic Characteristics of Lactating Women

One hundred and ten lactating women gave their consent to participate in the study after explanation of the procedures, but only 87 completed the study. Twelve participants belonging to several basic diet groups whose sample size are not large (less than 5 participants for each diet group), were later removed from the analyzes, in order not to interfere on statistical analyzes. Therefore, the study was restricted to final sample size of 75 breastfeeding women. Data of 75 lactating women were grouped according two predominant basic diets: rice with oil palm seed juice sauce (R-SG, n = 50) and cooked pasty mixture of plantain and cassava accompanied with eggplant sauce (F-SAU, n = 25). It is also important to mention that all dishes are consumed with meat and/or fresh or smoked fish into the sauces. These sauces contain mostly three to four vegetables per day (onion, pepper, tomato). Moreover, lactating women consumed less than 2 to 3 fruits in week whatever the diet.

The demographic data of the selected mothers are summarized in Table 1. The age of the women that entered the study ranged between 20 and 38 years old, with an average of 27.09 ± 5.51 years and 62.67% over 25 years. Thirty women or 40% of the sample were housewives while 43 lactating women (57%) had the primary level. In addition, 60 women who participated in this study lived with their partners (80%).

Table 1. Demographic data of women breastfeeding (n = 75)

Parameters	Number (%)
Age (years)	
< 25	28 (37.33)
> 25	47 (62 .67)
Mean (27.09 ± 5.51)	
Geographical localisation	
Adjamé	30 (40.50)
Yopougon	45 (59.50)
Professional activity	
Homewives	30 (40)
Traders	6 (8)
Students	13 (17,33)
Officials	8 (10,67)
Informal sector	18 (24)
Educational level	
Primary	43 (57)
High school	11 (15)
Graduate level	21 (28)
Marietal status	
Married/couple	60 (80)
Single	15 (20)

3.2 Total Antioxidant Capacity and Radical Scavenging Activity

The total antioxidant capacity (TAC) and radical scavenging activity of women breast milk at Day 45 and day 105 postpartum were summarized in Table 2.

The means values of TAC in breast milk of R-SG diet and F-SAU diet increased significantly ($P < .05$) from day 45 to day 105 postpartum, respectively from 961.10 ± 60.31 µmol/L of Fe^{2+} equivalent to 1031 ± 62.62 µmol/L of Fe^{2+} equivalent and 1114 ± 65.09 µmol/L of Fe^{2+} equivalent to 1208 ± 70.98 µmol/L of Fe^{2+} equivalent. However, there are no significant difference ($P > .05$) between TAC in breast milk of women who received R-SG diet and F-SAU diet as well as day 45 and day 105 postpartum.

Regarding antiradical activity, percentage of DPPH radical scavenging activity in breast milk of R-SG diet and F-SAU diet ranged respectively from 35.16 ± 1.49% to 35.98 ± 1.55% and 35.50 ± 2.07% to 36.79 ± 2.11%, and this, no significantly ($P > .05$). Also, between R-SG and F-SAU diets, antiradical activities of human milk were statistically comparable ($P > .05$) to the two sampling periods.

3.3 Relationship between Antioxidant Activities of Human Milk and Infant Weight

Table 3 presented correlation between antioxidant activities of human milk and weight of infants collected at 45th and 105th day postpartum. Concerning R-SG diet, TAC and DPPH radical scavenging activity of breast milk were correlated with infant weight at 45th and 105th day postpartum whereas for women who followed F-SAU diet, correlation was found between these activities and newborns weight only at 105th day postpartum.

4. DISCUSSION

Breastfeeding remains an essential tool to help in the protection against free radicals, oxygen reactive species and oxidative stress. The present study has examined antioxidant content of mature breast milk of 75 Ivorian mothers, according to their diet. After dietary intake of R-SG and F-SAU diets, widely consumed in Abidjan, there was, usually, an increase in TAC of women's breast milk with values varying from 961.10 ± 60.31 µM to 1031 ± 62.62 µM and 1114 ± 65.09 µM to 1208 ± 70.98 µM, respectively for RSG and FSAU diets, at Day 45 and Day 105 postpartum. A study conducted on mature breast milk of 115 Iranian mothers with total antioxidant capacities of 816.3 ± 379.4 µM at 30 ± 3 days and 862.7 ± 457.7 µM at 90 ± 7 days postpartum [22]. Another study was performed by Ezaki et al. [23], on 56 samples of breast milk collected from Japanese mothers and showed an average of total antioxidant activity of 3807 ± 103.5 µM. Ezaki et al. used the biological antioxidant potential (BAP) test for measuring of TAC. The difference between levels of women's breast milk TAC in these studies is probably due to ethnic or geographical particularities. It is important to also mention that heterogeneity of the study methodologies used (including sample collection protocol, sample size, study parameters, measurement units used, etc) makes it difficult to establish comparisons among the different studies.

The level of antioxidant activities of Ivoirian women's breast milk in this study is due to antioxidant compounds present in different diets consumed. Indeed, Franke et al. [18] demonstrated that levels of daidzein and genistein, two flavonoids in human milk, increased significantly, after the consumption of soy-rich diets, suggesting that these flavonoids are likely absorbed and transferred to human milk from the mother's diet. In our study, palm oil, ingredient food of R-SG diet is rich in phytosterols, vitamin E, carotenoids and especially tocotrienols which have antioxidant properties [24]. Also, eggplant from F-SAU diet is one of the vegetable species that has high superoxide radical scavenging activity and hydroxyl radical inhibition by chelating ferrous

Table 2. Total antioxidant capacity and DPPH radical scavenging of mature human milk collected at Day 45 and Day 105 postpartum

	Total antioxidant capacity (µmol/L of Fe^{2+} equivalent)		DPPH radical scavenging activity (%)	
Diet	**Day 45**	**Day 105**	**Day 45**	**Day 105**
R-SG (n= 50)	961.10 ± 60.31^a	1031 ± 62.62^b	35.16 ± 1.49^a	35.98 ± 1.55^a
F-SAU (n = 25)	1114 ± 65.09^a	1208 ± 70.98^b	35.50 ± 2.07^a	36.79 ± 211^a

Values are expressed as means ± standard error mean (SEM).
Means with different superscript (a–b) letters within same line are significantly different (P < .05) from each other.
Within same column, means followed by the symbol (#) are significantly different (P < .05) from each other.

Table 3. Relationship between antioxidant activities of human milk and infant's weight collected at 45th and 105th day postpartum

		TAC		DPPH	
		p-value	**r**	**p-value**	**r**
Day 45 postpartum	R-SG	0.002	0.412	0,001	0.429
	F-SAU	0.970	-0.007	0.405	0.174
Day 105 postpartum	R-SG	0.001	0.440	0,001	0.430
	F-SAU	0.006	0.528	0,010	0.504

TAC: Total Antioxidant Capacity; DPPH: DPPH radical scavenging activity.
P-values are considered significantly when P < .05; r: Pearson correlation

ions [25]. In addition, tomatoes, meanwhile, present in two basic diets contain β-carotene, lutein and lycopene were the most abundant carotenoids in human milk [26]. All these antioxidant compounds may help the infant cope with oxidative stress. In other hand, infant weight was correlated with antioxidant capacity of breast milk at day 105 postpartum. This result may be suggested as a consequence of nutritional and food intake status by mothers. Ezaki et al. [23] were showed a relationship between TAC and other anthropometric parameters such as infant's age, height and head circumference. Thus, antioxidants contained in Ivorian women breast milk play an important role in the growth and development infant's life.

5. CONCLUSION

This study is the first such performed in our country exploring antioxidant activity in human milk. In our study, antioxidant activities of women's breast milk who consumed respectively R-SG diet and F-SAU diet are the same, at two sampling periods. However, a much larger prospective study, recruiting a large number of participants should be conducted, which would allow to evaluate the effects of several diets on the antioxidant capacity of breast milk of Ivorian women. Otherwise, this study may encourage breastfeeding women to adopt healthy nutritional habits, in order to optimize the antioxidant content of breast milk.

ETHICAL APPROVAL

The ethical clearance was taken from the National Committee on Ethics and Research of Côte d'Ivoire (1127/2014/MSLS/DGS/eah).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford University Press, Oxford, United Kingdom; 2007.
2. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science. 2008;4(2):89-96.
3. Matés JM, Pérez-Gómez C, Núñez de Castro I. Antioxidant enzymes and human diseases. Clinical Biochemistry. 1999; 32(8):595-603.
4. Djordjević VB, Zvezdanović L, Cosic V. Oxidative stress in human diseases. Srpski Arhiv za Celokupno Lekarstvo. 2008; 136(2):158-165.
5. Friel JK, Friesen RW, Harding SV, Roberts LJ. Evidence of oxidative stress in full-term healthy infants. Pediatric Research. 2004; 56(6):878-882.
6. Yzydorczyka C, Mitanchezc D, Buffate C, Ligi I, Grandvuillemin I, Boubred F, Simeoni U. Stress oxydant chez l'enfant prématuré: causes, biomarqueurs et possibilités thérapeutiques. Archives de Pédiatrie. 2015;22(10):1047-1055. French.
7. Schaller B. Prospects for the future, the role of free radicals in the treatment of stroke. Free Radical Biology and Medicine. 2005;38(4):411-425.
8. Picciano MF. Human milk: Nutritional aspects of a dynamic food. Biology of Neonate. 1998;74(2):84-93.
9. Hartman HM, Dryden LP. The vitamins in milk and milk products. In: Webb BH, Johnson AH, Alford JA, editors. Fundamentals of dairy chemistry, 2nd ed. Westport Connecticut: AVI Publication Corporation; 1974.
10. Gossage CP, Deyhim M, Yamini S, Douglass LW, Moser-Veillon PB. Carotenoid composition of human milk during the first month postpartum and the response to b-carotene supplementation. American Journal of Clinical Nutrition. 2002;76(1):193-197.
11. Friel JK, Martin SM, Langdon M, Herzberg GR, Buettner GR. Milk from mothers of both premature and full term infants provides better antioxidant protection than does infant formula. Pediatric Research. 2002;51(1):612-618.
12. L'Abbé M, Friel JK. Superoxide dismutase and glutathione peroxidase content of human milk from mothers of premature and full term infants during the first 3 months of lactation. Journal of Pediatric Gastroenterology and Nutrition. 2000; 31(3):270-274.
13. World Health Organization (WHO). Promoting appropriate feeding for infants and young children. In: Global strategy for infant and young child feeding. World Health Organization, Geneva, Switzerland; 2003.
14. République de Côte d'Ivoire, Ministère de la santé et de la lutte contre le sida.

Enquête démographique et de santé à indicateurs multiples, Rapport préliminaire de Juillet 2012; 2014. French.

15. Rueda R, Ramirez M, Garcia-Salmeron JL, Maldonado J, Gil A. Gestational age and origin of human milk influence total lipid and fatty acid contents. Annals of Nutrition and Metabolism. 1998;42(1):12-22.
16. Al-Tamer YY, Mahmood AA. Fatty-acid composition of the colostrum and serum of fullterm and preterm delivering iraqui mothers. European Journal of Clinical Nutrition. 2004;58(8):1119-1124.
17. Jewell VC, Mayes CB, Tubman TR, Northrop-Clewes CA, Thurnham DI. A comparison of lutein and zeaxanthin concentrations in formula and human milk samples from Northern Ireland mothers. European Journal of Clinical Nutrition. 2004;58(1):90-97.
18. Franke AA, Custer LJ. Daidzein and genistein concentrations in human milk after soy consumption. Clinical Chemistry. 1996;42(6):955-964.
19. Johnson RK, Driscoll P, Goran MI. Comparison of multiple-pass 24-hour recall estimates of energy intake with total energy expenditure determined by the doubly labeled water method in young children. Journal of the American Dietetic Association. 1996;96(11):1140-1144.
20. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxydant activity. Lebensmittel-Wissenschaft und Technologie. 1995;28:25-30.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. Analytical Biochemistry. 1996;239(1):70-76.
22. Zarban A, Taheri F, Taiebeh C, Gholamreza S, Mohsen K. Antioxidant and radical scavenging activity of human colostrums, transitional and mature milk. Journal of Clinical Biochemistry and Nutrition Transitional and Mature Milk. 2009;45(2):150-154.
23. Ezaki S, Tomoo I, Keiji S, Masanori T. Association between total antioxidant capacity in breast milk and postnatal age in days in premature infants. Journal of Clinical Biochemistry and Nutrition. 2008;42(2):133-137.
24. Monde AA, Michel F, Carbonneau MA, Tiahou G, Vernet MH, Duvernay-Eymard S, et al. Teneur en acides gras et en antioxydants de l’huile de palme en Côte d’Ivoire. Pharmacopée et Médecine Traditionnelle Africaines. 2008;15:11-17. French.
25. Nisha P, Abdul NP, Jayamurthy P. A comparative study on activities of different varieties of *Solanum melongena*. Food and Chemical Toxicology. 2009;47(10):2640-2644.
26. Song BJ, Jouni ZE, Ferruzzi MG. Assessment of phytochemical content in human milk during different stages of lactation. Nutrition. 2013;29(1):195-202.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27977