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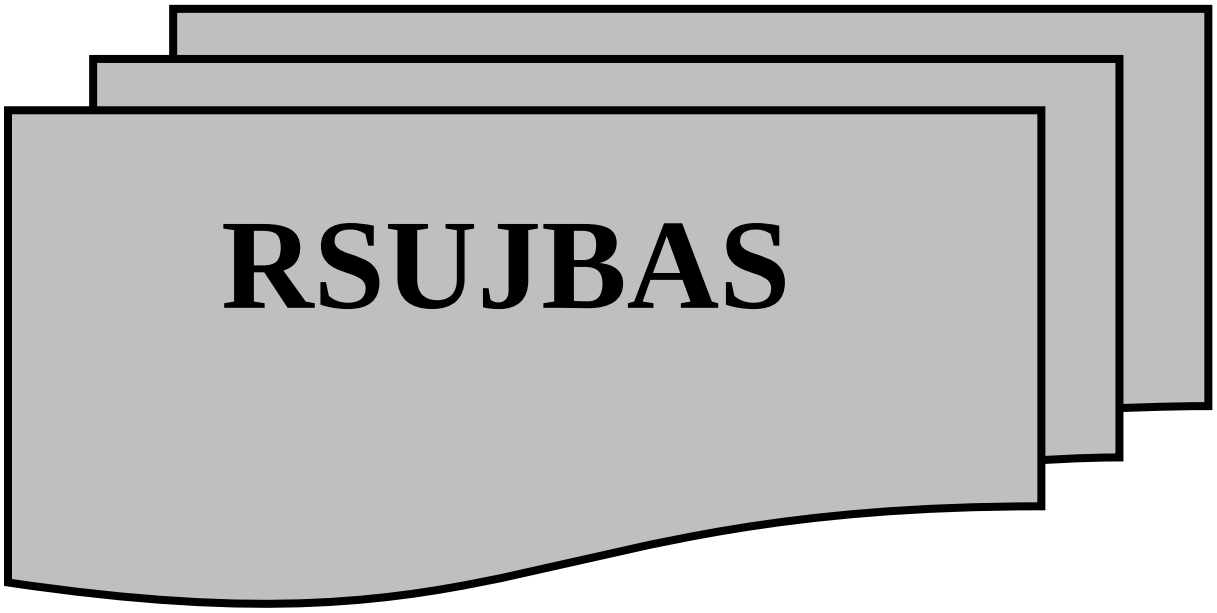

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The acknowledgment of people, grants or funds should be brief.

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CARBOHYDRATE AND AMINO ACIDS COMPOSITION IN BREAST MILK OF LACTATING MOTHERS FROM RUMUOLUMENI HEALTH CENTER, RIVERS STATE.

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ABSTRACT

Breastmilk is a complex fluid, rich in nutrients and in non-nutritional bioactive components. This study investigated the amino acid and carbohydrate compositions in breast milk of mothers of different age groups from Rumuolumeni health center. Eighteen breast milk samples were collected from lactating mothers aged 16-45 years old. Amino acid content was analyzed with amino acid analyzer, while the carbohydrate content was analyzed with HPLC and data generated was analyzed using one-way ANOVA and presented as mean and standard deviation. Results showed that rabinose, maltose, and HMF levels for mothers of age 16-25 were significantly higher when compared with values for mothers of age 26-35 years. Raminose and glucose levels for mothers of age 36-45 were significantly higher ($P \leq 0.05$) when compared with values for mothers of other age groups. Young mothers had galactose value of 1.29 ppm while the values for middle and older aged mothers were 1.43 ppm and 1.39 ppm respectively. Values for the young mothers showed a significant decrease when compared with the other age groups. Results of amino acid composition showed that alanine, serine, proline, valine, threonine, lysine, glutamate, phenylalanine, histidine, arginine and tyrosine values for mothers of age 26-35 were higher than those of mothers of 16-25 and 36-45 years. Value for threonine (3.54 mg/100g), isoleucine (4.50 mg/100g), and lysine (4.54 mg/100g) for mothers of 36-45 years were significantly higher when compared with the values for mothers of age 16-25. The amino acid compositions of some were below the standard recommended by WHO/FAO in all age grades. While methionine and isoleucine values for age 36-45 years, cysteine, histidine and methionine values for mothers of 26-35 years and cysteine values for age 16-25 years were above the recommended standard.

Key Words: Breast milk, amino acid, carbohydrates

INTRODUCTION

Breastmilk is a complex fluid, rich in nutrients and in non-nutritional bioactive components. Knowledge of the composition of human milk and the factors that influence it has increased considerably over the past two decades (Raj *et al.*, 2020). Breast milk has long been recognized as the optimal food for infants and young children (Girerd-Barclay, 2013; Raj *et al.*, 2020). The practice of breastfeeding babies from birth until about six months of age without additional supplements, also known as exclusive breastfeeding (EB), and of continued breastfeeding for two years or more, combined with appropriate, nutritious complementary food, has been acknowledged globally as optimal feeding for all young children, regardless of their origin (Girerd-Barclay, 2013).

Human milk is the first and the best feeding option for growth and healthy development of the newborns and infants (Brodribb, 2015). Human milk contains numerous components (proteins, carbohydrates, and inorganic elements) which provide basic nutrients for infants during the first period of their lives. The qualitative composition of milk components from healthy mothers maybe similar, but their levels change during lactation stages (Rasmussen & McGuire, 1996). Colostrum which is the fluid secreted during the first days postpartum by mammary epithelial cells is replaced by transitional milk during 5–15 days postpartum and from 15 days postpartum, mature milk is produced (Ballard & Morrow, 2013). Human milk, apart from the nutritional components, is a source of biologically active molecules such as immunoglobulins, growth factors, hormones, antiviral, and antibacterial proteins. These bioactive molecules present in the milk support the immature immune system of the new born and also protect against the development of infection (Ballard & Morrow, 2013).

Human milk composition varies considerably within and between mothers and even within a single milk expression. This multidimensional variation in composition is believed to be an adaptation to the infants' changing needs, geographical region and food supply (Hinde & German, 2012). The variations in human milk composition between individual woman and populations have been reported to be in response to cultural differences such as diet and other lifestyle factors, environmental factors, such as mineral content of the soil that is then reflected in the mineral density of the foods grown there and human genetic differences (Zachara and Pilecki, 2000). However, human milk composition data has not been collected from all world regions, populations and among different age groups. Therefore, studies of human milk composition in other regions and populations are important, particularly with regard to specific carbohydrates and amino acids, where a large variability has been noted from existing studies (Yang *et al*., 2018). This work examines the compositions of carbohydrates and amino acids in breast milk of lactating mothers and possible variations among different age groups.

MATERIALS AND METHODS

Inclusion Criteria

The criteria for defining lactating women were if they were apparently healthy and reported breast feeding at least 3 times a day and are within 16-45 years old. The purpose of the study was explained to the lactating mothers and their consent was obtained prior to commencement of sample collection. This study was approved by the ethics committee of the Rivers State Hospital Management Board, Port Harcourt.

Collection of samples

Breast milk samples (10ml) were collected from eighteen (18) breast feeding mothers from Rumuolumeni Health Center, Rivers State, Nigeria. The subjects were categorized into young mothers (16-25), middle aged mothers (26-35), and older mothers (36-45) years old. The milk was expressed with a manual pump into sterile containers. The samples were placed in ice-packed container and transported to the laboratory.

ANALYSIS OF CARBOHYDRATE WITH HPLC (APHA, 1998)

Hydrolysis of the sample: ten milligrams (10mg) of breast milk was dissolved in 1ml of 3M trifluroacetic acid (TFA) in a 5ml ampole. The breast milk was incubated at 130^0C for 2hours, further centrifuged at 200 rpm for 5 minutes and evaporated to dryness under reduced pressure to remove TFA.the hydrolysed and dried samples were dissolved in 1ml of distilled water.

Derivatization of hydrolysed sample: Thirty microliters (30) µL of NaOH (0.3M) was added to the breast milk. Fructose was added as an internal standard to each sample, the breast milk was incubated at 70 ^{0}C for 60 minutes cooled to room temperature and neutralised with 30 µl of HCl. One milliliter (1 ml) of trichloromethane was added to the breast milk and vigorously shaken. The breast milk sample passed through 0.45µm syringe and filtered before HPLC analysis.

AMINO ACID ANALYSIS [Modified method of Elkin and Griffith (1985)].

Preparation of sample and standards:

Zero point one gram (0.1g) of breast milk was weighed into a 16 X 125mml screw cap pyrex. Fifteen micromole (15mml) of 6N hydrochloric acid was added to the breast milk, and the tube was thoroughly flushed with N_2. The breast milk was placed in an oven at 110^oC for 24hrs. After hydrolysis, the breast milk was filtered to remove solid and a standard solution containing 125 µm/mL of each amino acid in 0.1N hydrochloric acid was created.

Derivatization procedure: The procedure used was a modified method of Elkin and Griffith (1985) in which 5,10,15, 20 and 50ul of breast milk was pipetted into a 10 X 5mm tube and dried at 65^{o}C. 30 µL of methanol water-phenylisothiocianate (2:2:1 (v/v)) was added to each tube containing the breast milk and then removed in vacuo at 65^{o}C. Then 30 µL of methanol water-Phenylisothiocianate (7:1:1:1 (w/v)) was added, and the tube was agitated and left to stand at room temperature for 20min.Finally, the solvent where removed under a nitrogen stream, and the tube was sealed and stored at 4^OC, pending analysis. Prior to injection, 150 µL of diluent consisting of 5Mm sodium phosphate with 5% acetonitrile was added to each tube.

RESULTS AND DISCUSSION

S/N	CHO	16-25 (yrs) (ppm)	26-35 (yrs) (ppm)	36-45 (yrs) (ppm)
1	HMF	0.72±0.21[bd]	0.28±0.09*[ac]	0.28±0.9*[ac]
2	Xylose	3.86±1.77[d]	2.45±1.62	0.00±0.00*[c]
3	Arabinose	3.71±0.00[b]	0.00±0.00*[a]	2.35±1.29[b]
4	Raminose	0.55±a0.00[ad]	0.57±0.09[ad]	0.82±0.00*[bc]
5	Fructose	3.16±0.12	2.82±0.57	3.14±0.02
6	Rabinose	1.26±0.05[bd]	0.01±0.01*[ac]	0.01±0.01*[ac]
7	Maltose	12.47±0.01b[d]	4.43±0.01*[ac]	4.58±0.27*[ac]
8	Galactose	1.29±0.00[bd]	1.43±0.07*[ac]	1.39±0.00*[ac]
9	Glucose	19.77±0.89[ad]	24.5±0.10[ad]	32.0±4.25*[bc]

Table 1: Carbohydrate content in breast milk of mothers of different age groups.

Table 2: Amino acid content of breast milk of mothers of different age groups.

S/N	Amino acid	16-25 (yrs) mg/100g)	26-35 (yrs) (mg/100g)	36-45 (yrs) (mg/100g)	WHO/FAO Standard
1	Lysine	2.91±1.62[b]	6.83±2.72[a]	4.54±0.12*[a]	11.1
2	Methionine	1.35±0.24	3.78±4.07	4.44±0.06	1.3
3	Tryptophan	0.92±0.84	1.27±0.34	1.55±0.79	-
4	Phenylalanine	2.54±1.61[ac]	3.55±1.87[ad]	0.55±0.16[bc]	3.2
5	Valine	1.92±0.72	2.46±1.83	0.76±0.12	9.4
6	Threonine	1.70±0.14[bd]	3.94±0.29*[ac]	3.54±0.14*[ac]	8.6
7	Iso leucine	2.60±0.24[d]	3.70±1.00	4.50±0.38*[c]	4.0
8	Leucine	6.59±0.09[ad]	6.17±1.34[ad]	1.63±0.30*[bc]	8.9
9	Histidine	2.26±0.96[ad]	3.13±0.44[ad]	0.61±0.33*[bc]	2.6
10	Glycine	3.01±0.64	2.44±0.28	1.67±0.15	-
11	Alanine	2.37±0.64	3.14±2.20	2.69±0.36	4.2
12	Serine	1.88±0.07	3.11±1.29	1.52±0.17	3.2
13	Glutamate	11.30±0.44	16.43±4.34	15.63±1.11	-

14	Proline	2.49±0.71	3.32±2.00	1.36±0.10	10.2
15	Arginine	2.44±0.92^{bc}	5.12±1.36^{ad}	2.01±0.69*^{bc}	3.9
16	Tyrosine	2.29±1.19^{ac}	3.16±0.71^{ad}	0.92±0.58b^{c}	2.6
17	Aspartate	4.27±4.85	2.27±1.11	1.66±0.02	6.8
18	Cysteine	2.13±0.94	1.41±0.10	0.91±0.78	1.2

Values for both tables are expressed as mean ± standard error of mean (SEM) for n=6 at 95% confidence level. Values with super script * differ significantly when comparing age range 16-25years with others. Values with different superscript ab differ significantly when comparing age rang 26-35 years with other ages. Values with superscript cd differ significantly when comparing age range 36-45 years with other ages.

Discussion

Carbohydrates are poly hydroxyl aldehydes or ketones and could be classified into monosaccharide, oligosaccharide and polysaccharide. The result in table 1, showed that HMF, xylose, arabinose, fructose, rabinose and maltose values for mothers of age 16-25 years old were higher than that for mothers of 26-35 and 36-45 years old. Raminose and glucose levels for mothers of age 36-45 years old were significantly high ($p<0.05$) when compared with the values from mothers of age 26-35 years old. HMF, xylose, fructose, rabinose, and maltose values for mothers of age 16-25 years were significantly higher when compared with values for mothers of age 36-45 years old. Young mothers had galactose value lower than the values for middle and older mothers. The values for the young mothers showed a statistical decrease when compared with the other age groups. Carbohydrates generally constitute the major source of energy in human diet. The basic raw materials for energy production are these simple sugars. This research showed high glucose level in breast milk especially in mothers of age 26-35 and 36-45 years old.

The result in table 2, showed that alanine, serine, proline, valine, threonine, lysine, glutamate, phenylalanine, histidine, arginine and tyrosine values for mothers of age 26-35 were higher than that for mothers of age 16-25 and 36-45. Phenylalanine is a primary amino acid that is abundant in dietary protein. Its main metabolic pathway yields the amino acid tyrosine, which is involved in the production of melanin pigments (Sterkel and Oliveira, 2017). Tyrosine contains hydroxyl and aromatic group, it is an essential component for the production of several important brain chemicals called neurotransmitters, including epinephrine, norepinephrine, and dopamine and contributes to the absorptivity of protein molecules (Sterkel and Oliveira, 2017).

Histidine is required for synthesis of proteins. It plays important roles in the active site of enzymes, such as serine proteases (trypsin) where it is a member of the catalytic triad. Excess histidine may be converted to trans-urocanate by histidine ammonia lyase (histidase) in liver and skin (Brosnan and Brosnan, 2020). Middle aged mothers had the highest histidine value and were significantly higher when compared with the value for older mothers, also the

histidine value for middle aged mothers was higher than the WHO/FAO/UNU (1985) recommended value.

Threonine, isoleucine and lysine levels for mothers of 36-45 years were significantly higher when compared with the values for mothers of age 16-25. Also, Leucine, phenylalanine and arginine values for mothers of age 26-35 were significantly higher when compared with the values for mothers of age 36-45. Arginine plays an important role in cell division, wound healing, immune function, and the release of hormones. It is a precursor for the synthesis of nitric oxide (NO), making it important in the regulation of blood pressure (Scibior and Czeczot, 2004).

Glycine, aspartate and cysteine values for mothers of age 16-25 were the highest when compared with the values for mothers of age 26-35 and 36-45 though not statistically significant.

From the results of this study, it was observed that most of the amino acids in the breast milk of mothers of age 16-25 were lower than standard except for methionine and cysteine which were higher than the reported standard by WHO/FAO/UNU (1985). Methionine and cysteine are sulphur containing compounds that plays unique role in epigenetic regulation by affecting DNA methylation (Colovic *et al.*, 2018). Linbald and Rahimtoola (1974) reported that poorly nourished mothers in Pakistan secreted milk low in methionine and cysteine and attributed it to poor protein quality in the diet of the mothers studied. Methionine values for mothers of age 26-35 and 36-45 in this study agrees with that reported by Ukegbu and Ijeh (2013), they reported methionine value in their study to be higher than WHO/FAO/UNU 1985 recommended value. The limiting amino acids (lysine, threonine and valine) may be explained by low consumption of animal protein and soy which are good sources of the essential amino acid lysine. Erdman Jr and Fordycee (1989) Reported that soy is inexpensive and contains adequate quantities of essential amino acids. Inadequate protein intake of the mothers could have also contributed to the limiting amino acids observed.

Conclusion

The study revealed the carbohydrates and amino acids composition in the breast milk of mothers of different age group. Mothers of age 16-26 years old have the highest carbohydrate composition than the other age groups. The amino acids composition in the breast milk of the three age groups were below the recommended standard except for the values of methionine for mothers of 16-45 years old, cysteine value for mothers of 16-25 and 26-35 years old, phenylalanine, histidine and arginine for age 26-25 years old and isoleucine for age 36-45 years old which were higher than the recommended standard. The amino acid composition was higher in mothers of age 36-45 years old.

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