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Amino acids and carbohydrates composition in breast milk of lactating mothers of different age group from Aleto Health Center, Rivers State

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Breast milk is the white liquid produced by women to breast-feed their babies. It has many benefits for infants, including reduced risk of gastroenteritis and respiratory infection. This study investigated the amino acids and carbohydrates composition in breast milk of lactating mothers. Eighteen (18) lactating mothers between the ages of 16-45 years participated in this study. Amino acid content was analysed with amino acid analyser, while the carbohydrate content was analysed with high performance liquid chromatography (HPLC) and generated data was analysed using one-way analysis of variance (ANOVA). The result showed that values of ribose, arabinose, fructose, sorbitol and mannitol were statistically ($p<0.05$) different in younger mothers when compared with middle and older mothers. Values for HMF, glucose and maltose were statistically different than that of other age groups. Also, sucrose level for older mothers was 2.65 ppm, which was statistically different when compared with the values for other age groups. The amino acid composition showed that younger mothers had the highest concentration of aspartate (2.43 mg/100 g) though not statistically significant with the values of other age groups. Proline value (4.20 mg/100 g) was statistically different in middle aged mothers when compared with other age groups. Values of valine, alanine, arginine and serine were statistically higher when comparing older mothers to other age groups. The varying concentrations of both amino acid and carbohydrate composition in these mothers might be due to the nutritional status of the lactating mothers.

Key words: Breast milk, amino acid, carbohydrate, lactating mothers.

INTRODUCTION

Breastfeeding, also called nursing, is the process of feeding a mother's breast milk to her infant, either directly from the breast or by expressing (pumping out) the milk from the breast and bottle-feeding it to the infant (Raj et al., 2020). The milk is produced by the gland located in the breasts of females which is responsible for lactation or the production of milk. Both males and females have glandular tissue within the breasts, however, females glandular tissues begin to develop after puberty in response to oestrogen release (Collins et al., 2016; Yu et

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al., 2019). Mammary glands produce milk after childbirth, during pregnancy progesterone and prolactin are released. The progesterone interferes with prolactin, preventing the mammary glands from lactating. During this period, small amounts of a pre-milk substance called colostrum are produced (Collins et al., 2016; Yu et al., 2019). This liquid is rich in antibodies and nutrients to sustain an infant during the first few days of life. After childbirth, progesterone levels decrease and the levels of prolactin remain high. This signals the mammary glands to begin lactating. Each time a baby is breastfed, the milk is emptied from the breast and immediately the mammary glands are signalled to continue producing milk. As a woman approaches menopause, the tissues of the ductile system become fibrous and degenerate. This causes shrinkage of the mammary gland, the gland then loses the ability to produce milk (Collins et al., 2016; Yu et al., 2019).

During the first few weeks of life, babies may nurse roughly every two to three hours, and the duration of a feeding is usually ten to fifteen minutes on each breast while older children feed less often (Victora et al., 2016). Breastfeeding has a number of benefits to both mother and baby, mothers may pump milk so that it can be used later when direct breastfeeding is not possible (Ip et al., 2009). The benefits of human milk include reduced risk of neonatal necrotizing, enterocolitis, gastroenteritis, respiratory infection and improved later cognitive development (Schanler and Atkinson, 1999). Human milk provides non-immune protection and the bioactive substances play important roles in the non-nutritional effects of human milk on the development of the infant (Lönnerdal, 2003).

Newborn gastrointestinal tract undergoes maturational changes in the first weeks after birth and human milk has been shown to stimulate gastrointestinal mucosal proliferation and maturation in animal models and is thought to protect the neonatal infant from harmful environmental factors by affecting and promoting the mucosal barrier (Takeda et al., 2004). The growth factors in human milk [epidermal growth factor (EGF), transforming growth factor alpha (TGF) and insulin-like growth factors (IGFs)], stimulate the proliferation of intestinal cells and the formation of the mucosal barrier (Wagner and Forsythe, 2000). EGF is thought to have the most significant effect on the proliferation of cells that line the intestine and the promotion of the covering mucosal layer (Wagner and Forsythe, 2000).

The lactation period is a major source of concern in developing countries because of its positive impact on the health and nutrition of infants. Lactating women from developing countries are considered nutritionally vulnerable groups because this period places a high nutritional demand on the mother. Inadequate maternal diet during this period will lead to poor secretion of nutrients in breast milk and this can have long term impact on the child's health (Jones et al., 2010). Some studies have looked at correlations between diets, baby's age and genetic factor and nutritional composition of maternal breast milk. However, there is paucity of studies on correlation of maternal age and nutrients composition in breast milk, this research examines the composition of amino acids and carbohydrates in breast milk of lactating mothers of different age group and possible variations among different age groups.

MATERIALS AND METHODS

Collection of breast milk samples

Breast milk samples (10 ml) each were collected from eighteen (18) lactating mothers aged 16-45 years from Aleto Health Centre, Rivers State, Nigeria. The subjects, six in each group were categorised into different age groups younger mothers (16-25) years, middle mothers (26-35) years, and older mothers (36-45) years. The samples were expressed with a manual pump into sterile containers and placed on ice and transported to the laboratory at 4°C.

Amino acid analysis

Hydrolysis

0.5 g of lyophilized breast milk was weighed into a test tube and 15 ml of 6N HCl was added. The tube was flushed with N_2, capped and placed in an oven at 110°C for 24 h. The tube was then brought out and the content filtered. The filtrate was made up to 25 ml with water. An aliquot of this solution was further filtered with 0.50 µm pore-size membrane and a standard solution containing 1.25 µmol/ml of each amino acid in 0.1 N HCl was made (Elkin et al., 1985).

Derivatization

A standard solution of the sample (20 µL) was pipette into a tube and dried in vacuum at 65°C. 30 µL of methanol-water-phenylisothiocianate (2:2:1 (v/v)) was added to the formed residue, the tube was agitated and allow to stand at room temperature for 20 min.

The solvents were then removed under a nitrogen stream, and the tube was sealed and stored at 4°C (Elkin et al., 1985).

Chromatographic procedure for amino acid analysis

Prior to injection, 150 µL of diluent (5 mM sodium phosphate with 5% acetonitrile) was added to derivatized sample.

Analysis of carbohydrate with HPLC

Hydrolysis/Derivatization

10 mg of lyophilized sample was weighed into a tube and dissolved in 1 mL of 3 M triflouroacetic acid (TFA) in a 5 ml ampoule, the mixture was then incubated at 130°C for 2 h. The mixture was cooled and centrifuge at 2000 rpm for 5 min and evaporated to dryness under reduced pressure to allow removal of TFA. The hydrolyzed and dried sample was re-dissolved in 1 ml of water.

Thirty microliters of NaOH (0.3 M) trichloromethane were added to the sample before derivatization and the mixture was incubated at 70°C for 60 min, cooled at room temperature and further neutralized with 30 µL of HCl (0.3 M). 1 ml of trichloromethane was added and mixed properly, the organic phase was carefully removed and discarded. The aqueous phase was filtered before injection (APHA, 1998; Kuanget al., 2011).

Statistical analysis

The statistical analysis for this research was done with the aid of Statistical Package for Social Sciences (SPSS) for windows (SPSS Inc., Chicago, Standard version 21.0), it was used to determine difference between means using ANOVA. Data was reported as mean ± standard error of mean (SEM) and the level of significance were set at P< 0.05.

RESULTS

The result in Table 1 showed that the compositions of glutamate and leucine were more when compared with the compositions of all the other amino acids. The values of glutamate, leucine and phenylalanine showed no significant difference in all the age groups. The values of phenylalanine in all the groups were different than the value of the FAO/WHO standard. The values of lysine for mothers of 26-35 and 36-45 differ significantly when compared with the value for mother of 16-25 years old.

The result in Table 2 showed that the compositions of glucose, maltose and fructose were more than the other saccharides present; maltose is a disaccharide of two glucose units. Glucose and fructose are both dietary monosaccharides used for generation of energy (ATP) and other high energy yielding compound (NADH). The maltose and glucose values for mothers of 26-35 years old were significantly different when compared with the values for mothers of 16-25 and 36-45 years old. Also the value of fructose for mothers of 16-25 years old was significantly different when compared with the values for mothers of 26-35 and 36-45 years old.

DISCUSSION

Living things require food for existence, food provide macronutrients such as proteins and carbohydrates. These nutrients serve as cellular fuel, support growth, repair of tissues and other metabolic activities. Breast milk protein is a key nutrient which supports cellular growth and organ development during the first few months of life. The function of a protein is dependent on its amino acids composition. The result in Table 1, showed the values of amino acids component in breast milk of mothers of different age groups. The result showed that glycine, alanine, serine, valine, lysine, glutamate, phenylalanine, histidine, and arginine values for mothers of age 36-45 years were different than that for 16-25 and 26-35 years old. Valine, arginine and serine, values for this group (36-45 years) old were significantly higher when compared with values for other groups. Also glycine and alanine values for mothers of 36-45 years old had a significantly increase when compared with the values for middle age mothers. Aspartate value for mothers of age 16-25 years differ when compared with its values for other groups. Middle aged mothers had higher values of cysteine and proline than that for other groups. Methionine, cysteine, tyrosine, and phenylalanine values in all the groups were different than the recommended standard (WHO/FAO/UNU, 1985), while the values for proline, valine, threonine, isoleucine, leucine, aspartate and lysine, irrespective of the age group were less than the standard recommended by WHO/FAO/UNU (1985). Alanine and serine values for mothers of age 36-45 were different than the recommended standard (WHO/FAO/UNU, 1985).

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. It also contributes to the inherent fluorescence of proteins (Lakowicz, 1999). This study showed that the breast milk of these mothers were rich in tyrosine. The values were higher than the recommended. Cysteine is a component of glutathione (an antioxidant) that function in reducing oxidative stress (Larsson et al., 2015); the concentration of cysteine was above the WHO/FAO/UNU standard for all the groups. The values of proline for mothers of 36-45 years old, lysine for mothers of 26-35 and 36-45 years old, Methionine for mothers of 26-35 years old and phenylalanine and arginine for mothers of 16-25 years old were similar to that reported by Ogechi and Irene (2013). Methionine plays an important role in the immune system as its catabolism leads to an increase in the production of glutathione. Also, studies have shown that methionine can chelate lead and remove it from tissues, thus decreasing oxidative stress (Martínez et al., 2017). Furthermore like tyrosine, phenylalanine also contributes to the inherent fluorescence of proteins (Lakowicz, 1999) and this study showed that the samples were rich in phenylalanine.

Carbohydrates are biological molecules which consist of poly hydroxyl aldehyde and ketone; it is one of the nutrients required by the body in large amount and constitutes the major source of energy in human diet. The result in Table 2 showed that the values of ribose, arabinose, rhaminose, fructose, galactose, sorbitol, and mannitol, for mothers of age 16-25 were above the values for ages 26-35 and 36-45. HMF, ribose, arabinose, fructose, maltose, glucose, sorbitol, and mannitol values for mothers of age 26-35 were significantly increased when compared with the values for mothers of age 36-45. Ribose, fructose, galactose, sorbitol, mannitol, and sucrose values for mothers of age 16-25 were significantly increased when compared with values for mothers of age 26-35. Ribose, arabinose, fructose, maltose, galactose, glucose, sorbitol and

Table 1. Amino acid composition in the breast milk of lactating mothers from Aleto Health Center.

S/N	Amino acid (mg/100 g)	16-25 years (group 1)	26-35 years (group 2)	36-45years (group 3)	FAO/WHO Standard
1	Histidine	0.88±0.13^{d}	1.11±0.42^{c}	2.42±1.25^{c}	2.6
2	Tryptophan	0.89±0.87	1.13±0.57	1.00±0.13	-
3	Phenylalanine	4.38±1.42	4.16±0.87	4.77±0.74	3.2
4	Methionine	2.58±2.16	2.90±2.38	1.31±0.21	1.3
5	Valine	1.70±0.17^{ad}	1.41±0.30^{ad}	4.71±0.17*^{bc}	9.4
6	Threonine	3.24±1.36	4.13±0.51	4.13±0.29	8.6
7	Isoleucine	3.94±1.13^{bc}	1.73±0.21*^{ad}	3.89±0.83^{bc}	4.0
8	Leucine	7.17±0.50	7.73±0.10	7.25±2.00	8.9
9	Lysine	3.37±1.99	6.01±1.03	6.50±3.14	11.1
10	Glycine	3.63±0.23^{bc}	1.36±0.12*^{ad}	3.78±0.42^{bc}	-
11	Alanine	3.52±0.78^{ac}	1.73±0.21^{ad}	4.73±1.36^{bc}	4.2
12	Glutamate	13.35±1.36	14.33±0.52	14.95±0.84	-
13	Aspartate	2.43±1.86	1.62±0.23	0.79±0.31	6.8
14	Cysteine	1.64±0.83	2.66±1.60	1.75±0.74	1.2
15	Arginine	3.89±1.07^{ad}	2.59±1.39^{ad}	6.20±1.09^{bc}	3.9
16	Tyrosine	3.02±0.27	3.02±0.27	3.08±0.19	2.6
17	Serine	1.51±0.37^{ad}	1.57±0.18^{ad}	4.32±0.16*^{bc}	3.2
18	Proline	1.36±0.33^{bd}	4.20±0.55*^{ac}	3.79±0.66*^{ac}	10.2

Values 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 range 26-35 years with other ages. Values with superscript cd differ significantly when comparing age range 36-45 years with other ages.

Table 2. Carbohydrate composition in the breast milk of lactating mothers from Aleto health center.

S/N	Carbohydrates (ppm)	16-25 years (group 1)	26-35 years (group 2)	36-45 years (group 3)
1	HMF	0.35±0.11^{b}	0.853±0.25*^{a}	0.537±0.10^{a}
2	Ribose	6.10±0.01^{bd}	2.68±0.16*^{ad}	1.77±0.19*^{bc}
3	Arabinose	8.55±4.08^{ad}	4.193±1.50^{ca}	2.157±0.18*^{ac}
4	Raminose	1.39±1.20	0.55±0.00	0.99±0.01
5	Fructose	10.23±0.01^{bd}	6.54±0.18*^{ad}	3.37±0.01*^{bc}
6	Maltose	7.62±0.44b^{bd}	12.01±0.21*^{ad}	5.15±0.01*^{bc}
7	Galactose	2.41±0.08^{bd}	1.27±0.08*^{ac}	1.23±0.06*^{ac}
8	Glucose	17.51±0.327^{bd}	21.78±2.16*^{ad}	8.21±2.01*^{bc}
9	Sorbitol	6.44±0.53^{bd}	3.73±0.19*^{ad}	0.00±0.00*^{bc}
10	Mannitol	7.85±0.46^{bd}	4.27±0.15*^{ac}	3.57±1.37*^{ac}
11	Sucrose	1.89±0.01^{bd}	1.20±0.01*^{ad}	2.55±0.04*^{bc}

Values 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 16-25 years with others. Values with different superscript ab differ significantly when comparing age range 26-35 years with other ages. Values with superscript cd differ significantly when comparing age range 36-45 years with other ages.

mannitol values of mothers of age 16-25 had significant increase when compared with values for mothers of age 36-45. Carbohydrates assist in the complete metabolism of fats and emptying of bowel. Pyruvate which is the end product of glycolysis is usually carboxylated to yield oxaloacetate when there is a drop in its cellular level, this reaction ensures continuous oxidation of acetylCoA generated during fatty acid oxidation. Some studies have shown that constituents of breast milk could reflect the diet state of the individual, Garcia-Rodenas et al. (2016) noted that nutrients in breast milk is an evolutionary conserved trait largely independent of geographical, ethnical or dietary factors. However, similar work by Nwachoko et al. (2021) noted that breast milk of younger mothers (16-26) years old contain high carbohydrates content than that in the breast milk of older mothers. Fields et al. (2017) stated that carbohydrates are of major importance, particularly with regard to oligosaccharides. In animals, oligosaccharides have been reported to influence brain function through different mechanisms such as sialylation of oligosaccharides which contribute sialic acid to gangliosides for brain signaling, and greater abundance of sialylated gangliosides enhances learning ability (Gridneva et al., 2019). According to Berger et al. (2020), there is a growing body of evidence showing breast milk composition is affected by genetic factors, with the greatest impact on oligosaccharides. The profile of oligosaccharides in breast milk is largely determined by maternal blood antigen (secretor status) which is genetically determined.

Conclusion

This work showed that the breast milk of these mothers were rich in phenylalanine, tyrosine, cysteine and arginine. Also, mothers of age 36-45 years had the highest total concentration of amino acids while mothers of age 16-25 had the highest total carbohydrate content. Older mothers (36-45 years) had the highest concentration of both essential amino acids (35.98 mg/100 g) and non-essential amino acid (43.39 mg/100 g). This research showed that there could be maternal age influence on the amino acids and carbohydrate content in breast milk.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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