

# Association of Pregnant Sudanese Women Plasma Essential Fatty Acids Levels and Their Neonates Head Circumference

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Essential fatty acids are polyunsaturated fatty acids (PUFA) which contain more than one double bond. The purpose of this study was to investigate the level of fatty acid composition in plasma of neonates and compare them to their head circumference. There were no records about the levels of these fatty acids in Sudanese people. Blood samples were obtained from the maternal side of the cord during delivery in Khartoum state hospitals. Full and detailed history and examinations were performed in the study groups. Plasma lipids were extracted by Folch method and separated by Gas Liquid Chromatography (GLC). These results were expressed as mean and standard deviations. The differences in mean were compared by Mann-Whitney U test using SPSS for windows version 15. The mean head circumference of our neonates was (29±0.6 cm) range (28-35 cm) which was lower than the standard value (35±1.7 cm). Our study demonstrated a negative correlation between head circumference of neonates with different saturated fatty acids and linoleic acid ( $r=-0.36, -0.34$ ) ( $p \leq 0.05$ ), and positively with other omega-6 and omega-3 fatty acids of neonates ( $r=0.53$ ) ( $p \leq 0.05$ ). Low level of neonatal DHA and high level of LA interfere with our neonatal head circumference, which was lower than the international level.

**Key words:** DHA Docosahexaenoic Acid, LCPUFA Long Chain Polyunsaturated Acid, H.C Head Circumference, E.F.A Essential Fatty Acids, AA Arachidonic Acid, LA Linoleic Acid.

## INTRODUCTION

Higher consumption of fish or fish oil has been shown to increase gestation significantly. In a series of studies evaluating effects of DHA-rich fish oil on gestation, Williams *et al.*, have consistently shown that women who consume diets rich in n-3 fatty acids have significantly longer gestation and higher birth weights than those consuming diets low in n-3 fatty acids, and lower risk of preterm delivery<sup>[1]</sup>. Although birth weight, length and head circumference were found to increase compared with the non-supplemented group. Preterm birth remains the leading cause of neonatal morbidity and mortality in the United States, with the pathophysiology largely unknown. Modifiable risk factors such as maternal diet remain an area of research. A high ratio of omega-6 to omega-3 fatty acids will result in increased proinflammatory eicosanoid production (ie, prostaglandin E<sub>2</sub> [PGE<sub>2</sub>] and prostaglandin F<sub>2α</sub> [PGF<sub>2α</sub>]). These metabolites have been associated with the initiation of labor and preterm labor. Including more EPA (Eicosapentaenoic acid) in the diet may lead to a reduction in

the production of proinflammatory eicosanoids and increased production of prostacyclin (PGI<sub>2</sub>), which may promote myometrial relaxation. Omega-3 fatty acids down regulate the production of prostaglandins PGE<sub>2</sub> and PGF<sub>2α</sub>, and may thereby inhibit the parturition process<sup>[1]</sup>. The dominance of DHA and AA in the infant brain and neural tissues suggests that they play an important role in the optimal development of mental function during pregnancy and early postnatal life. Several trials and observational studies have evaluated the role of LCPUFA on cognitive development of both preterm and term infants<sup>[1]</sup>. Greater evidence seems to suggest that breast milk feeding or consumption of formula milk supplemented with LCPUFA is associated with higher cognitive ability in childhood. In a randomized double blind trial by Helland *et al.*,<sup>[3]</sup> where pregnant women were supplemented with either cod liver oil or corn oil until three months after birth, it was found that supplementation led to a substantial increase in the DHA level in breast milk. In addition, the children born to those

mothers who had supplemented their diets with DHA scored higher in IQ tests at the age of four years when compared with children whose mothers took placebo. The investigators concluded that 'maternal intake of LCPUFA during pregnancy and lactation may be favorable for later mental development of children'. In another study by Willatts *et al.*, [2], it was also shown that infants fed formula milk supplemented with DHA and AA, from birth to four months, had a significantly better problem-solving ability at 10 months when compared to similar children who received a standard infant milk formula without LCPUFA.

## MATERIALS AND METHODS

### Study Area

This research was conducted in Khartoum state hospitals; includes Omdurman Maternity hospital, Khartoum teaching hospital and Bahri teaching hospital. The control groups were selected from healthy different populations.

### Study Duration

From January 2010 to Feb 2014.

### Study Subjects and Population

Samples were taken from 3rd trimester ladies (35-40 weeks), before delivery, and neonatal samples from the umbilical cord after delivery.

### Exclusion criteria

- Pregnant women < 35weeks.
- Lactating non pregnant females.
- Stillbirths.
- Pregnant women suffering from any chronic diseases (hypertension, diabetes mellitus and hyperlipidemia).

### Data Collection and Design

An oral & written consent (according to the Ministry of Health in Sudan) was taken from each participant after explaining the aims of the research. A clinical examination had been conducted by an expert obstetrical & pediatric medical registrar, concentrating on the following parameters:

1. Gestational age (in weeks) clinically by measuring the fundal level.
2. Weight in (Kg) using glass smart weighing machine scale (RS 6006) from Shenzhen Resse Technology, China.
3. Height in (metre) by using a mechanical height scale, from Xiamen Kuanyl Electronic Technology. China.

4. Clinical examination of the baby demonstrates the general examination and addition to weight, height and head circumference. Five ml venous blood was taken from cord blood at delivery after fulfilling the criteria. The samples were collected in tubes containing anticoagulants (lithium heparin) in its wall. Blood was rolled against the wall to be mixed with the heparin. Plasma was separated from RBC by centrifugation at 2000 rpm for 15 minutes. Four ml plasma was divided into two tubes 1 ml for estimation of cholesterol and glucose, and 3 ml for fatty acids measurement. The samples were stored at -20 ° C. The initial process of separation and measurements of cholesterol and glucose was conducted in the biochemistry lab, Faculty of Medicine Al Neelain University. The second step of investigation conducted in the research lab, Faculty of Science, University of Khartoum (extraction, lipids separation and methylation).

An aliquot of cell pellet or tissue homogenate (<50 µl) in a glass methylation tube was mixed with 1 ml of hexane and 1 ml of 14% BF<sub>3</sub>/MeOH reagent. After being blanketed with nitrogen, the mixture was heated at 100°C for 1 hour, cooled to room temperature and methyl esters extracted in the hexane phase following the addition of 1 ml H<sub>2</sub>O. The samples were centrifuged for 1 minute, and then the upper hexane layer was removed and concentrated under nitrogen. Fatty acid methyl esters were analyzed by gas chromatography [4].

### Data System

Peak area was quantified by a computer chromatography data system (EZ Chrom chromatography data system, scientific software Inc. San Ramon, CA).

## RESULTS AND DISCUSSION

Table 1 Shows the Anthropometric of the neonates is shown in Sixty six neonates were included in our study, 34 were girls and 32 were boys (p≥0.05).

No significant differences (p≥0.05) were found between the mean weight of the girls 3.4 Kg (range 2.5 -4.5 Kg) and the mean weight for boys 3.3 Kg (Range 2.6 - 4.6 Kg).

The mean weight of the full term Sudanese newborns was 3.34 Kg which was less than the international weight for full term babies (4.0) (5).

The mean Height of girls and boys was 0.5 meter ranging between (0.3-0.6m). The height was nearly similar to the international height for full term babies (0.51m) (6).

No significant difference (p≥0.05) was found between the two groups regarding the mean head circumferences 29.8 cm ranging between (27-31), both of them were less than the international head circumference for full term babies 35 cm (7).

**Table 1:** Anthropometric measurement of the neonates

Criteria	girls (34)	boys (32)	P value
Weight(kg)	3.4±0.52	3.3±0.5	p≥0.05
Height(meter)	0.5±0.1	0.5±0.1	p≥0.05
Head circumference(cm)	29.8±0.7	29.7±0.6	p≥0.05

**Table 2:** Correlation between neonatal plasma fatty acids levels and their head circumference

Fatty acids	Pearson correlation to head circumference	P value
14:00	-0.319	p≤0.05
16:00 palmitic	-0.364	p≤0.05
18:00 stearic	-0.422	p≤0.05
20:00 arachidic	-0.596	p≤0.05
18:2n-6 linoleic	-0.341	p≤0.05
20:3n-6 DHGLA	0.484	p≤0.05
20:4n-6 arachidonic	0.599	p≤0.05
22:4n-6 adrenic	0.467	p≤0.05
18:3n-3 ALA	0.337	p≤0.05
22:5n-3 DPA	0.428	p≤0.05
22:6n-3 DHA	0.533	p≤0.05
20:3n-9 mead	0.368	p≤0.05

Table 2 showed that our study demonstrated a negative correlation between head circumference of neonates with different saturated fatty acids and linoleic acid ( $r=-0.36,-0.34$ ) ( $p\leq 0.05$ ), and positive with other omega -6 and omega-3 fatty acids of neonates ( $r=0.53$ ) ( $p\leq 0.05$ ). The same result was obtained by Gerard Hornstra<sup>[8]</sup>. In contrast to our study, Sandra *et al.*,<sup>[9]</sup> stated that there was no association between the infant plasma concentrations of trans linoleic acid and birth weight, birth length, or head circumference, as in spite of the negative effect of trans linoleic acid on the desaturation of LA and ALA to AA and DHA, this effect need higher amount of trans LA relative to LA, which are not relevant in usual human diets containing >2% of dietary energy as LA<sup>[9]</sup>. It was found that high amount of saturated fatty acids interferes with brain growth and function as it interrupts the signaling of the brain causing memory deficiency, and reducing the brain nutrients by blood vessels<sup>[10]</sup>. Like other studies which showed a significant, negative relationship between head circumference and fetal linoleate of n-6 series<sup>[11]</sup>. Two reasons appeared to explain the negative relation between LA and head circumferences. Firstly, evidence from in vitro studies showed that increased intake of LA leads to increased oxidative stress, which may be associated with endothelial damage, that

interferes with brain supply and maturation<sup>[12]</sup>. Secondly, high level of LA decreases the fetal amounts of n-3 LCPs (DHA) which is the major polyunsaturated building bricks of the brain. LA is widely available in the diet and its conversion to AA and other n-6 metabolites are very efficient. However, the human ability to convert ALA to EPA and particularly to DHA appears to be inefficient. It has been demonstrated that increased dietary intakes of ALA results in an increase in the level of EPA and docosapentaenoic acid (clupanodonic-DPA n-3) but no great change in the level of DHA in plasma or tissues<sup>[13,14]</sup>. Also, alteration in the LA/ALA ratio in the diet affects the metabolism of these acids since they compete for the same desaturation enzymes<sup>[15]</sup>, and in this regard various recommendations have been made, for example FAO/WHO recommend a ratio of between 5:2 and 10:1<sup>[16]</sup>. The high level of LA in typical western diets, led a group of experts to suggest a reduction of n-6 PUFA and increase of n-3 PUFA in the diet in order to reduce adverse effects of too much AA and its eicosanoids<sup>[17]</sup>.

The mean level of linoleic acid (LA) was higher among Sudanese neonates compared with neonates of other countries<sup>[18]</sup>, as the LA content of the fetus depends on the maternal diet, which has a higher LA source as, seeds and

cereal based food, and on the levels of n-3 and n-6 which compete each other on the same desaturase enzyme. This may explain the small head circumference of Sudanese neonates regard to the neonates of other countries. So the above data explain that the interference of LA with the maturation and function of the brain, and lower n3/n6 fatty acid ratio in the maternal diet is associated with a low head circumference, but we cannot claim that to neurodevelopment of the baby as appeared by some Meta analysis studies. These data argue in favor of the promotion of breastfeeding and the limitation of intakes of n6 (LC) PUFAs during pregnancy. Omega-3 and omega-6 fatty acids affect the brain through, DHA and AA which were found at very high concentrations in the cell membranes of the retina and the phospholipids of the brain's gray matter, changes in DHA and AA content of neuronal cell membranes could alter the function of ion channels or membrane associated receptors, as well as the availability of neurotransmitters<sup>[19]</sup>.

## CONCLUSIONS AND RECOMMENDATIONS

In conclusion to our study, we found that the nutritional behavior of our study groups deficient in n-3 and has adequate n-6 sources. The levels of essential fatty acids in our groups were lower than the international levels, except for LA which was higher in comparison to some countries. Low level of neonatal DHA and high level of LA interfere with our neonatal head circumference, which was lower than the international level. Reproduction that results in healthy, well developed children is vital for society and the economy. So we recommend implicate the essential fatty acids as compulsory supplements during pregnancy and encourage child bearing females before conception, and school pupils to increase balance essential fatty acids food or supplements.

## REFERENCES

- [1] Williams C. Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. *Am J Clin Nutr.* 2001; 73(2):316-22.
- [2] Willatts P. Influence of long-chain polyunsaturated fatty acids on infant cognitive function. *Lipids.* 1998; 33(10):973-80.
- [3] Helland IB. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics.* 2003; 111(1): 39-44.
- [4] Pingoud A, Urbanke C, Hoggett J and Jeltsch A. *Biochemical methods: a concise guide for students and researchers.* Weinheim: Wiley-VCH Verlag GmbH; 2002. P.360.
- [5] Field, T. Infants' Need for Touch. *Human Development.* 2002;45(2): 100-103.
- [6] Hadlock FP, Shah YP, Kanon DJ. Fetal crown rump length: Reevaluation of relation to menstrual age with high resolution real-time US *Radiology.* 182:501, 1992.
- [7] New birth body length and head circumference charts for the British and Columbian, 1995-2000.
- [8] Gerard Hornstra. Essential fatty acids in mothers and their neonates. *American Journal of Clinical Nutrition.* 2000;60(3):3-18.
- [9] Hornstra, G., et al., Essential fatty acids in pregnancy and early human development. *Eur J Obstet Gynecol Reprod Biol.* 1995. 61(1): p. 57-62.
- [10] Patricia E. Wainwright . Dietary essential fatty acids and brain function: A developmental perspective on mechanisms. *Proceedings of the Nutrition Society.* 2002;56(16):98-109
- [11] Crawford MA, Hassam AG, and Williams G. Essential fatty acids and fetal brain growth. *Lancet.* 2006;1(7957): 452-3.
- [12] Michal Toborek , Yong Woo Lee , Rosario Garrido, Simone Kaiser, and Bernhard Hennig. Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells 1,2,3.A. *J of Nutr.* 2002 ;45(15):43-56
- [13] Food and Nutrition Board, Institute of Medicine. *Dietary Fats: Total Fat and Fatty Acids. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, D.C.: National Academies Press; 2002.
- [14] Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids.* 2003;38(4):323-341.
- [15] Smit EN, Muskiet FA, and Boersma ER. The possible role of essential fatty acids in the pathophysiology of malnutrition. *Prostaglandins Leukot Essent Fatty Acids.* 2004; 71(4): 241-50.
- [16] FAO, *Fats and Oils in Human Nutrition: Report of the joint expert consultation organised by Food and Agriculture Organisation and the World Health Organisation, Rome, 19-26 October 1993, in FOA Food and Nutrition Paper 57.* 1994: Rome. 147.
- [17] Plourde M. and Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Appl Physiol Nutr Metab.* 2007; 32(4):619-34.
- [18] Qureshi IA, Xi XR, Limbu YR, Bin HY, Chen MI. Hyperlipidaemia during normal pregnancy, parturition and lactation. *Ann Acad Med Singapore.* 1999;28(2): 217-21.
- [19] SanGiovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res.* 2005;24(1):87-138.