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Original Research Article

Lipids Contents Differences in Plasma Phosphatidylcholine of Pregnant And Control Healthy Sudanese Women

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Background: 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 phosphatidylcholine of pregnant Sudanese women and healthy control. There were no records about the levels of these fatty acids in Sudanese people.

Material and Methods: Blood samples were obtained from the pregnant women at delivery time 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).

Results: These results were expressed as mean and standard deviations. Regarding saturated fatty acids (palmitic acid)was higher among pregnant ladieswhile (staeric, myristic and arachidic) were higher among the control group ($p \leq 0.001$).There were significantly higher level of Docosapentaenoic acid (DPA), Docosahexaenoic acid (DHA), linoleic acid (L.A), Arachidonic acid (A.A), total omega-3 (w-3), total omega-6 (w-6) and total w-3/total w-6 ratio (w-3/w6) ratio in control ($p \leq 0.001$), whereas significantly higher level of alpha linolenic acid (ALA), Di homogamma linoleic acid (DHGLA) and Adrenic Acid in pregnant female ($p \leq 0.001$).

Conclusion: 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 and lower in comparison to theirs. There were differences in concentration of essential fatty acids between pregnant and non pregnant females. The DHA level of the pregnant women was the lowest level being measured which affects the neonatal DHA 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 Linoliec Acid.

INTRODUCTION

The PUFA is composed of four families of fatty acids,which are either essential or non-essential. The palmitoleic (n-7) and oleic (n-9) families are regarded as nonessential because they can be synthesized by almost all cells de novo from acetyl CoA. In contrast, the other two families of linoleic (n-6) and α-linolenic (n-3) acids cannot be synthesized de novo by mammals due to lack of Δ^{12} and Δ^{15} desaturase enzymes, which are necessary for insertion of a double bond at the n-6 and n-3 positions respectively, counting from the methyl end. Hence, they must be provided in the diet. Oleic acid is first desaturated by Δ^{6} desaturase particularly in the absence of linoleic acids and other PUFA and immediately followed by elongation step and further desaturation by Δ^{5} desaturase and chain elongation to form the n-9 family of PUFA. Desaturation of 20:2n-9 by Δ^{5} desaturase results in formation of eicosatrienoic acid (20:3n-9 - mead acid), which is found normally in trace amounts in human tissues. The accumulation of this acid is now considered as a marker for essential fatty acid deficiency [1].The Δ^{6} desaturase inserts a double bond at the 6-7 position and Δ^{5} desaturase at the 5-6 position of the fatty acid chain. Palmitoleic acid (16:1n-7) is converted in a similar fashion to form members of the n-7 PUFA family.

Pregnancy and lactation are associated with modest increases in maternal energy needs and high demand for LCPUFAs, particularly AA and DHA [2]. These fatty acids are required for the formation of the fetal vascular and central

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nervous systems. DHA accounts to about one third of total fatty acids in cerebral grey matter and approximately 60% of the photoreceptor outer segment. In the last trimester of pregnancy, the fetal brain grows rapidly increasing in weight almost five fold. It is therefore critical that an adequate supply of these acids, particularly DHA, is optimal during pregnancy as insufficiency may result in irreversible and harmful consequences for postnatal growth and function [3]. Although consumption of a balanced diet can to some extent meet some of the demand, it is generally believed that consumption of n-3 PUFA, particularly DHA, is often insufficient during pregnancy to cover the increased demand [4]. The absolute amount (mg/l) of plasma fatty acid increases significantly during pregnancy. Al and co-workers found that the normal level of AA 110mg and DHA 40 mg increased 23% and 52% respectively at week 40. They also observed similar increases in the total sum of n-3 (41%) and n-6 (44%) PUFA, SFA (57%) and MUFA (65%). However, when the values for AA and DHA were expressed as relative amounts of total fatty acids, a different pattern emerges. An initial increase in the level of DHA and other n-3 LCPUFA is observed in the first trimester, which significantly decreases thereafter, especially during the third trimester when the demand is highest. A similar decline in the level of AA and other n-6 PUFA were also noticed, but the declines were steady. It therefore appeared that pregnancy is associated with a reduction in the functional status of both AA and DHA. This observation is supported by the highest level of DHA insufficiency marker–Osbond acid, and Mead acid, a general marker for PUFA deficiency [5].These findings have been confirmed under different environmental and cultural conditions [6] and do not appear to be related to dietary intake as most changes in LCPUFA start in early pregnancy. Hornstra [4] suggest that the cause of the increase level of LCPUFA in pregnancy could be due to: (1) enhanced enzymatic conversion of LA and ALA precursor fatty acids, (2) mobilization of maternal stores or (3) metabolic LCPUFA shift from energy production to structural use.

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 healthy mature female (14-40years) as control.

Exclusion criteria

- Pregnant women < 35weeks.
- Lactating non pregnant females.
- Stillbirths.
- Pregnant women or control 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.

- Personal, medical and obstetrical history was taken by using a standard questionnaire.
- .Socioeconomic status, classified according to annual income [7].
- Determination of essential fatty acid intake was roughly calculated by taking nutritional information of the popular foods which contain omega 3 and omega 6 fatty acids, using the standard questionnaire form.
- A clinical examination had been conducted by an expert obstetrical, 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. Body mass index: by calculating wt(kg)/(ht in metre) 2.
5. Blood pressure using manual blood pressure mercury from Ningbo Tianjin international trading. China.

Five ml venous blood was taken by pregnant women, another 5 ml from the control 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 º 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 the investigation was conducted in the research lab, Faculty of Science, University of Khartoum (extraction, lipid 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% BF3/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 H2O. 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 [8].

Data system

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

RESULTS

Table (1) shows the personal criteria of our study groups that were 66 controls non pregnant females, another 66 were pregnant women in the third trimester.

Table 1: Personal criteria of the study groups

Criteria	Control (66)	Pregnant (66)	P value
Age Mean ±SD	24.9±7.3	26.8±4.5	p≥0.05
Residence %			
1.khartoum	28.8%	33.3%	p≥0.05
2. Omdurman	39.4%	37.9%	p≥0.05
3. Bahri	31.8%	28.8%	p≥0.05
Economical status			
1.low	13.6%	18.2%	p≥0.05
2. middle low	56.1%	62.1%	p≥0.05
3. moderate/high	30.3%	19.7%	p≥0.05
Education %			
1. illiterate	0%	0%	p≥0.05
2. primary	10.6%	6.1%	p≥0.05
3. secondary	25.8%	45.5%	p≥0.05
4. university	47.0%	40.9%	p≥0.05
5. post university	16.7%	7.6%	p≥0.05

The mean age of the controls was (24.9) years, ranging between (18-44) and mean age of pregnant ladies was (26,8) years ranging between (21-37). They were middle low socioeconomic status according to an international bank classification having annual income between($1,026 to $4,035), and most of them were university educated. No significant differences were found between them concerning age, residence, socioeconomic status and education (p≥0.05).

Table (2) shows the anthropometric measurements (weight, height, BMI) of control and pregnant women. Mean Weight in pregnant women was (73 Kg) ranging between (59-89 Kg), while in no pregnant the mean value was (61) Kg with a range of (40-86 Kg). BMI in pregnant women was 29 ranging between (20-43) whereas in non pregnant was (24) with a range of (14 – 39).Weight and BMI of pregnant women were significantly higher than the non pregnant ladies (p≤0.05).

Table 2: Anthropometric and biochemical measurement of the pregnant and nonpregnant groups

Criteria	Control (66) Mean ±SD	Pregnant (66) Mean ±SD	P value
Weight(kg)	61.4±10.7	73.1±8.4	p≤0.05
Height (meter)	1.6±0.1	1.6±0.1	p≤0.05
BMI	24.00±5.7	29.00±7.7	p≤0.05

Table (3) displays the Mean plasma level of fatty acids among pregnant and nonpregnant (control) women. Regarding saturated fatty acids (palmitic acid) was higher among pregnant ladies while (staeric, myristic and arachidic) were higher among the control group (p≤0.001).Linoleic Acid, Arachidonic acid of omega 6 fatty acids were significantly higher among the control, while DHGLA and adrenic were higher among the pregnant women (p≤0.001).The overall total omega-6 was significantly higher among the control group(p≤0.001).DPA and DHAof omega-3 fatty acids were significantly higher (p≤0.001) among the controls, while the ALA was higher among pregnant. The overall total omega 3 was higher among the controls (p≤0.001).Omega 3 omega 6 ratio was higher among the controls (0.11) than the pregnant (0.07) (p≤0.001).

Table 3: Mean level of fatty acids among pregnant women and controls

Fatty acid	Control(66) Mean ±SD	Pregnant(66) Mean ±SD	P value
14:00 myristic	0.36±0.02	0.25±0.02	p≤0.001
16:00 palmitic	27.73±0.54	31.78±0.58	p≤0.001
18:00 stearic	12.58±1.21	9.83±0.66	p≤0.001
20:00 arachidic	0.06±0.008	0.034±0.004	p≤0.001
18:2n-6 linoleic	22.39±0.32	19.70±0.58	p≤0.001
20:3n-6 DHGLA	2.68±0.20	3.69±0.41	p≤0.001
20:4n-6 arachidonic	8.63±0.43	6.52±0.45	p≤0.001
22:4n-6 adrenic	0.24±0.04	0.37±0.06	p≤0.001
18:3n-3 ALA	0.08±0.01	0.17±0.04	p≤0.001
22:5n-3 DPA	0.69±0.06	0.52±0.03	p≤0.001
22:6n-3 DHA	2.94±0.20	1.37±0.40	p≤0.001
20:3n-9 mead	0.13±0.02	0.28±0.04	p≤0.001
Total w-3	3.71	2.06	p≤0.001
Total w-6	33.94	30.28	p≤0.001
w-3/w-6 ratio	0.11	0.07	p≤0.001

DISCUSSION

In our study groups the mean age of the pregnant women was 24 years±7.3, their mean gestational age was 38 weeks± 2.0, and 56% of them were found to be classified as lower middle income socioeconomic status according to the international banks list of economy [7]. Regarding the educational level nearly half of them (47%) were university graduated, none of them were illiterate, 38.7% of their family have more than eight members, and most of them were originally from northern Sudan. There was no significant difference between pregnancy and controls regarding age, residence, socioeconomic status, educational level, number of members and ethnic groups (p≥0.05).For the anthropometric measurements (weight, height and BMI) only there was a significant increases in the mean weight 73.07Kg ±8.4,(Range 50-90Kg) and mean BMI 29.00 ±7.7,(Range 22-38 kg) of pregnant ladies (p≤0.05) compared to non pregnant, as their mean weight & BMI was 61.4 Kg ± 10.7 and 22.4± 5.7 respectively, and this difference may be due to increasing mass of the mothers because of the physiological changes attributed to pregnancy [8]. Garaulet *et al.,* stated that n-3 and n-6 fatty acids are related to a reduced adipocyte size. In contrast, adipose tissue and dietary Saturated Fatty Acids (SFAs) significantly correlated with an increase in fat cell size and number. No significant associations were found between n-9 acids content and adipocytes size. However, n-9 adipose tissue fatty acids content was associated with an increase in fat cell number showing that this type of fatty acid could limit hyperplasia in obese populations [9]. From this theory we can deduce that there would be another explanation for high BMI in pregnant women, which may be due to increase the size rather than the number of fat cells due to low n-3, n-6 fatty acids and high n-9 (mead acid) with increase total SFA in circulation of pregnancy compared to non pregnant control women.

Nutritional history taken from pregnant women and controls demonstrated that their intake of omega-3 rich diets were so poor, and this was found neither related to educational level nor to socioeconomic status, but might be related to Sudanese eating habits [10].The mean level of essential fatty acids in our study groups was lower in comparison to other countries, except for the linoleic acid. The mean percentage weight of LA in our pregnant women was (19.7 % ±0.5) found slightly higher in comparison to some countries like Belgium (19.5%±3.1), but lower than Taiwan and UK (27.7%±2.3 and 22.7%±2.7), and this depends mainly on the type of diets preferred in these regions [11-13]. Since both essential fatty acids; linoleic acid and alpha linolenic acid can only be obtained from diet, it can be deduced from our nutritional history and plasma FA profiles that the intake of alpha linolenic acid (ALA) was low and that of linoleic acid (LA) was sufficient. As Sudanese women in this region relied largely on cereal or seed based foods which are linoleic acid (LA) rich and contain little alpha linolenic acid (ALA), in addition to that, they also consume less amount of marine foods which contain n-3 fatty acids like tuna, and sea fish [14]. The mean percentage weight of DHA in our pregnant women was (1.37% ±0.4) which was the lowest level being measured in comparison to other countries like Belgium, Taiwan, UK and Nigeria (4.73%±1.3, 5.16 %±1.2, 5.2 %±1.6 and around 8.1%±1.5) respectively. The present plasma fatty acid data of pregnant and nonpregnant women provided comprehensive information on the changes in fatty acid metabolism which may occur as a consequence of pregnancy. Significant higher values were found in the levels of selected fatty acids among the saturated and polyunsaturated fatty acids. It has been found that the total saturated fatty acids among pregnant and non pregnant women were 41.8%±3.1 and 40.6%±3.5 respectively, which was lower than the pregnant of other countries like Belgium(43.04%±4.5) and Taiwan(41.94%±4.1) (11,13). Higher concentration of estrogen and insulin resistance is thought to be responsible for the hypertriglyceridemia of pregnancy [15]. Fatty acids are used for placental oxidation and membrane formation [15].

In late pregnancy, production of human chorionic somatomammotropin (HCS) promotes lipolysis and fat mobilization. The increase in plasma fatty acid and glycerol concentrations is consistent with mobilization of lipid stores; this shift from an anabolic to a catabolic state promotes the use of lipids as a maternal energy source while preserving glucose and amino acids for the fetus [15]. Among pregnant women with higher levels of short chain saturated fatty acids (Palmitic acid 16:00) was found with concomitant low levels of long chain saturated fatty acids (Stearic18:00 and Arachidic 20:00) compared to controls. Total Highly Unsaturated Fatty Acids (HUFA) (20 and more carbons with three and more double bonds) (Di-homo-gamma-linoleic acid DHGLA, Adrenic Acid, Arachidonic acid AA, Docosa pentaenoic acid DPA and Docosahexaenoic acid DHA) decreases in the maternal plasma during pregnancy, but selected HUFA was found higher (DHGLA and Adrenic acid) in pregnant women. The same result was obtained by Ghebremeskel K and Suzie J Otto [16,17]. This difference can be explained in two ways; De Vriese *et al.,* [11] proposed that in maternal plasma PL the decrease of total HUFA during gestation is accompanied by increase synthesis of short chain of saturated fatty acid palmitic acid (16:00), and the high total of HUFA in umbilical plasma PL is associated with a significantly longer chain fatty acids stearic 18:00 and arachidic 20:00), and this may be due to the changes in SFA of plasma phospholipid in a way to counteract changes in the mean chain length (MCL) and consequently in mean melting point (MMP) induced by changes in HUFA composition during pregnancy. Low total HUFA in pregnancy lead to increase MMP, which is decreased by concomitant increased synthesis of short chain saturated fatty acids 16:00 which had low MMP, and high total HUFA in non pregnant and also in neonates, accompanied by synthesis of long chain saturated fatty acids 18:00 and 20:00 which has a high MMP [18].This counteract phenomenon was demonstrated firstly in pathological conditions like preeclampsia and multiple sclerosis [18], but De Vriese *et al.,* [11] proof that the MMP counteract process could occur during pregnancy and neonatal life due to hormonal changes and fetal demand for special fatty acids. The striking differences in fatty acids composition of pregnant women was also explained by changes in the hormonal level mainly oestrogen during pregnancy which affect alternative pathway of Phosphatidylcholine (PC) synthesis (Greenberg pathway) that use (stearic18:00) and (Arachidonic acid A.A), but in fact the levels of those fatty acids were found low in our pregnant plasma, so this may be due to synthesizing PC by the major pathway (Kennedy pathway which uses (palmitic 16:00) in PC synthesis, in spite of the high estrogens level. Skryten *et al.,* [19] also suggested subclinical cholestatic changes in the liver during normal pregnancy to explain the use of Kennedy pathway during pregnancy rather than Greenberg pathway. The LA level was found low in pregnant ladies (19.7±0.5) compared to controls (22.3±0.3), the same result was obtained by Suzie J Otto [17], but Ghebremeskel K *et al.,* postulates that there were no significant changes in LA level between pregnancies and control [20]. High levels of DHGLA and Adrenic in pregnant women might be explained by the placental selectivity theory.

The role of placenta in providing the fetus with LCPUFA during pregnancy has been studied extensively [21]. Essentially, it mobilizes the maternal adipose tissues, concentrate and channel the important n-3 and n-6 fatty acids to the fetus via multiple mechanisms. This process is thought to involve the selective uptake and translocation that is facilitated by several membrane-associated and cytoplasmic fatty acid binding proteins, which favors n-3 and n-6 fatty acids over non-essential fatty acids; and DHA and AA over LA, ALA, DHGLA and Adrenic [22,23]. Another explanation for the selectivity of placenta was proposed by Elvira Larqué *et al.,* [23] who stated the compartmentalization theory which demonstrate the esterification of fatty acids into different lipids fractions of maternal blood, like saturated fatty acids participate in Triacylglycerol formation, whereas LCPUFA participate in TG and PL fraction of the mother.PL of the mother accumulates in placental tissues rather than TG, so this explains the preferentiality of LCPUFA among saturated fatty acids on placental selectivity [24].

CONCLUSION AND RECOMMENDATION

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 and lower in comparison to others. The DHA level of the pregnant women was the lowest level being measured which affects the neonatal DHA level. Women's health should deserve more attention, by improving and replenishing their nutritional status throughout the childbearing years, this includes the time before, during and between pregnancies. Optimal dietary intake of the essential fatty acids and their long chain polyunsaturated fatty acids should be maintained as successful reproduction and lactation. Reproduction that results in healthy, well developed children is vital for society and the economy. So our recommendations are:

a. Implicate the essential fatty acids as compulsory supplements during pregnancy.
b. Provide and encourage child bearing females before conception, and school pupils increase balance essential fatty acids food or supplement.
c. Educate the population about the benefit and usefulness of essential fatty acids.

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