INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-1, Jan-Mar-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 5th Nov-2015 *Revised: 20th Nov -2015* *Accepted: 24th Nov -2015*

Research article

MATERNO-FETAL PLASMA PHOSPHATIDYL FATTY ACIDS GRADIENTS IN SUDANESE POPULATION

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ABSTRACT

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 their neonates. 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 and the maternal side of the cord for neonates samples, in Khartoum state hospitals. Full and detailed history and examinations were performed for the study groups. Plasma lipids were extracted by Folch method and separated by Gas Liquid Chromatography (GLC).

Results: Regarding saturated fatty acids there were significantly higher level ($p \leq 0.001$) of stearic acid (18:00) and arachidic acid (20:00) in neonates, while myristic (14:00) and palmitic acid (16:00) were significantly higher among pregnant women ($p \leq 0.001$).

Most of the omega-6 fatty acids (DHGLA, Aracidonic acid and Adrenic acid) were significantly higher among neonates ($p \leq 0.001$), except Linoleic acid which was higher in pregnant women ($p \leq 0.001$).

Regarding omega-3 fatty acids, ALA and DPA were higher in pregnant women, except DHA which was higher in neonates ($p \leq 0.001$).

Total omega-6 , omega-3 and the omega-3/omega-6 ratio were significantly higher in neonates than pregnant women ($p \leq 0.001$).

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 others. There were different in concentration of essential fatty acids between pregnant and their neonates. 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.

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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 acids deficiency (Bezard, 1994). 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 (Fan YY and Chapkin RS, 2008). These fatty acids are required for the formation of the foetal vascular and central 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 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 (Innis SM, 2007). Although consumption of 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 (Hornstra G, 2001). 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, 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 third trimester when the demand is highest. 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 functional status of both AA and DHA. This observation is supported by the higher level of DHA insufficiency marker –Osbond acid, and Mead acid, a general marker for PUFA deficiency (Al MD, 1995). These findings have been confirmed under different environmental and cultural conditions (Otto SJ, 1997) and do not appear to be related to dietary intake as most changes in LCPUFA start in early pregnancy. Hornstra (Hornstra G, 2001) suggest that the cause of increase level of LCPUFA in pregnancy could be due to: 1) enhanced enzymatic conversion of LA and ALA precursors 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.
- Still births.
- 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 Ministry of the Health in Sudan) was taken from each participant after explaining the aims of the research.

- Personal, medical and obstetrical history was taken by using standard questionnaire.
- Socioeconomic status, classified according to annually income (CCWB-2012).
- Determination of essential fatty acids intake was roughly calculated by taking nutritional information of the popular foods which contain omega 3 and omega 6 fatty acids, using 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 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.
5.Clinical examination of the baby demonstrate the general examination and addition to weight, height and head circumference.

Five ml venous blood was taken from pregnant women, another 5 ml from the control after fulfilling the criteria. The samples were collected in tubes containing anti coagulants (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 ºC.The initial process of separation and measurements of cholesterol and glucose was conducted in biochemistry lab Faculty of Medicine Al Neelain University. The second step of investigation were 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 addition of 1 ml H_2O. 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 (Thornburg KL et al 2000).

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 are composed of 198 candidates, 66 of them were controls non-pregnant females, another 66 were pregnant women with their 66 neonates. 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 intern national bank classification having monthly income between (200-700SD), and most of them were university educated. 38.7% has more than eight members in their family and most of them were originally from northern Sudan. No significant differences were found between them concerning age, residence, socio economic status, education, number of family and ethnic group ($p \geq 0.05$).

Table (2): shows the anthropometric measurements (weight, height, BMI and MUAC) of control and pregnant women. Mean Weight in pregnant women was (73 Kg) ranging between (59-89 Kg), while in non 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 was significantly higher than the non pregnant ladies ($p \leq 0.05$). The biochemical measurements included random blood glucose and cholesterol. The mean random blood glucose level of pregnant women was 105 mg/dl ranging between (70-140) which was significantly higher than the non pregnant 95 mg/dl ranging between (72-140). Both mean levels were less than the international level (108-140),(80-140)respectively(9). The mean cholesterol level of pregnant women was 169.4 mg/dl (Range103-260 mg/dl) which was significantly higher than the control group 156.8 mg/dl. Both values are less than the mean international level of cholesterol (200- 240 mg/dl) (Qureshi IA et al, 1999).

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. lower middle	56.1%	62.1%	p≥0.05
3.upper middle/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
Number of family			
1. two	5.9%	7.5%	p≥0.05
2 .two-five	20.5%	20%	p≥0.05
3.six to eight	34.9%	33.4%	p≥0.05
4.more than eight	38.7%	39.1%	p≥0.05
Ethnic group			
1.Northern	30%	31.3%	p≥0.05
2.Souhern	20%	19.4%	p≥0.05
3.Estern	19.9%	21.7%	p≥0.05
4.Western	30.1%	28.6%	p≥0.05

Table 2: Anthropometric and biochemical measurements of the pregnant and non pregnant 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
Glucose level mg/dl	95.8±31.9	105±24.3	p≤0.05
Cholesterol level mgldl	156.8±34.5	169.4±33.0	p≤0.05

Table 3 shows the obstetrical history of pregnant women: 97.1% of our pregnant women were married, 42.3% married below 20 years, and 39.9 % having first child below 20 years.72% were multigravida, 30% having three pregnancies including this one.38.7% spacing 1-2 years between this pregnancy and the last one, and 71.2% had no multiple pregnancy.

Table-3: Obstetrical history of pregnant women.

1.Marital status	Percentage
Single	0%
Married	97.1%
Divorced &Widow	2.9%
2.Age of Marriage	
Below 20 years	42.3%
Between 20 -30 years	37.7%
Above 30 years	20%
3.Age of having first child	
Below 20 years	39.9%
Between 20 -30 years	34.1%
Above 30 years	26%
4.Gravida	
Primary	27.5%
Multi	72.5%
5.Numbers of pregnancies including this	
one	27.5%
Two	20%
Three	30%
Four and more	22.5%
6.Spacing of Pregnancies	
Less than one year	27.4%
1-2 years	38.7%
2-3 years	20.5%
More than three years	13.4%
7.Number of multiple pregnancies	
None	71.2%
One	15.1%
Two	10.4%
Three and more	3.3%

Table 4: Shows the Anthropometric and biochemical measurement of the neonates is shown in Sixty six neonates were included in our study, 34 were girls and 32 were boys. ($p \geq 0.05$).

No significant differences ($p \geq 0.05$) was 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 (Range2.6 - 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) (Field, T, 2002). 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) (Hadlock FP et al, 1992). No significant difference ($p \geq 0.05$) 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 (13). Regarding the biochemical measurements the mean random blood glucose level of girls baby was 84 mg/dl (Range 56-123 mg/dl) and for the boys babies was 84 mg/dl (Range 66-123), both of them were lower than the international random glucose level of full term babies (70-120) mg/dl (Adamkin DJ, 2011). The mean cholesterol of girls babies was 116 mg/dl (Range 89-154) and for the boys babies was 115 mg/dl (Range 99-154), both of them were lower than the international cholesterol level of full term baby 158 mg/dl (Juárez IE et al, 2012). From this table we can conclude that gender has no significant effect regarding the anthropometric and biochemical measures, and all parameters are less than the international values.

Table 4: Anthropometric and biochemical 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
Glucose level mg/dl	84.8±13.1	83.8±12.1	p≥0.05
Cholesterol level mg/dl	115.7±13.4	116.8±14.8	p≥0.05

Table 5 shows the mean (weight %) plasma level of fatty acids among pregnant and neonates. Regarding saturated fatty acids there were significantly higher level (p≤0.001) of stearic acid (18:00) and arachidic acid (20:00) in neonates, while myristic (14:00) and palmitic acid (16:00) were significantly higher among pregnant women (p≤0.001). Most of the omega-6 fatty acids (DHGLA, Aracidonic acid and Adrenic acid) were significantly higher among neonates (p≤0.001), except Linoleic acid which was higher in pregnant women (p≤0.001). Regarding omega-3 fatty acids, ALA and DPA were higher in pregnant women, except DHA which was higher in neonates (p≤0.001). Total omega-6, omega-3 and the omega-3/omega-6 ratio were significantly higher in neonates than pregnant women (p≤0.001).

Table 5: Mean (weight %) of fatty acids among pregnant women and neonates

Fatty acid	Pregnant (66) Mean ±SD	Neonate (66) Mean ±SD	95 C.I	P value
14:00 myristic	0.25±0.02	0.16±0.03	0.08-0.10	p≤0.001
16:00 palmitic	31.78±0.58	28.12±0.69	3.44-3.88	p≤0.001
18:00 stearic	9.83±0.66	14.37±0.66	-4.76- -4.31	p≤0.001
20:00 arachidic	0.03±0.01	0.22±0.04	-0.20- -0.18	p≤0.001
18:2n-6 linoleic	19.70±0.58	12.29±0.93	7.14-7.68	p≤0.001
20:3n-6 DHGLA	3.70±0.41	4.83±0.60	-1.31- -0.96	p≤0.001
20:4n-6 arachidonic	6.52±0.45	13.74±0.99	-7.49- -6.96	p≤0.001
22:4n-6 adrenic	0.37±0.06	0.89±0.11	-0.56- -0.49	p≤0.001
18:3n-3 ALA	0.17±0.04	0.01±0.01	0.14- 0.16	p≤0.001
22:5n-3 DPA	0.52±0.03	0.25±0.01	0.25-0.28	p≤0.001
22:6n-3 DHA	1.36±0.40	3.86±0.86	-2.73- -2.27	p≤0.001
20:3n-9 mead	0.28±0.05	0.81±0.11	-0.56- -0.51	p≤0.001
Total w-3	2.06	4.12	0.25-0.28	p≤0.001
Total w-6	30.28	31.76	-2.73- -2.27	p≤0.001
w-3/w-6	0.07	0.13	-0.56- -0.51	p≤0.001

DISCUSSION

In our study when comparing mothers and the term neonates at time of birth consistently report low concentration of LA and ALA; and higher concentration of LCPUFA, particularly DHA and AA in cord than in maternal phospholipids. The same result was obtained by (Berghaus TM, 2000). Different results were obtained by Al, M.D., et al who stated that the absolute amounts of the fatty acids in umbilical plasma were significantly lower compared with maternal values, because the total phospholipid concentration was significantly lower in cord blood than in maternal blood at delivery (Al MD, 1995).

The differences in fatty acids levels between pregnant and neonates has been interpreted as evident of preferential transport of the LCPUFA by the placenta to the fetus (Crawford M, 2000), to participate in development of fetal membranes and neurovisual tissues (Gerard Hornstra, 2000). The majority of PUFA in the maternal circulation exists as TG, PL and CE of lipoprotein particles.

These are hydrolyzed by endothelial bound lipases releasing free fatty acids, which can be taken up by the placenta by passive diffusion or protein-mediated mechanism facilitated by placental plasma membrane fatty acid-binding protein (p-FABPpm), fatty acid translocase (FAT) and fatty acid transport protein (FATP) (Dutta-Roy AK, 2000). Placental lipoprotein lipase preferentially hydrolyses fatty acids in the sn-2 position of glycerol backbone, which often contained more unsaturated fatty acid than the sn-1, or sn-3 positions (De Vriese RS, 2003). In our study, the pregnant women had significantly higher level of saturated fatty acids myristic (14:00), Palmitic acid (16:00), ALA 18:3n-3, DPA 22:5n-3, linoleic acid (18:2n-6), low total n-3, low total n-6 and low n-3/n-6 ratio. In contrast, the neonates has higher Stearic acid (18:00), Arachidic acid (20:00), total saturated fatty acids, higher DHA (22:6n-3), DHGLA (20:3n-6), adrenic (22:4n-6), arachidonic acid AA (20; 4n-6), high total w-3, high w-6 and high w-3/w-6 ratio. So pregnant ladies had significantly higher short chain saturated fatty acids, n-3 fatty acids except DHA, and lower n-6 fatty acids except L.A. while neonates had higher long chain saturated fatty acids, higher n-6 fatty acids except L.A and lower n-3 fatty acids except DHA. High level of total saturated fatty acids in neonates (42.8 % ±3.0) compared to mothers (41.8 % ±3.2) was explained by the ability of the fetus to synthesize extra saturated fatty acids apart from that which pass through the placenta from the mother. The neonates uses saturated fatty acids to synthesize branched chain fatty acids which participate in formation of the gut, and this was explained by the presence of branched chain fatty acids in the vernix and meconium of the fetus. The selected saturated fatty acids which were high in neonates was Stearic (18:00) acids and arachidic (20:00) acid. The same result was obtained by De Vriese and Yoeju Min), and was explained by the relation between the length of the saturated fatty acids and total high unsaturated fatty acids (HUFA) as stated above by De Vriese (24). These observations support the hypothesis of placental selectivity for transport of certain FA (25, 26). Asim K Dutta-Roy explained that the selectivity of placenta towards some essential fatty acids (DHA and AA), assisted by certain membrane transporter which enable essential fatty acids transferal to fetal circulation and incorporated into fetal phospholipid (PL) ,in contrast to small amount of L.A and ALA. Low essential fatty acids intake by nutritional questionnaire or pregnancy-induced physiological response may provide an explanation for the differences in the levels of the n-6 and n-3 fatty acids between mothers and neonates. Our study has found that 42% of women were married before the age of 20 and that 72% of Sudanese women were multigravida, 52% had three and more pregnancies. A number of studies have demonstrated that pregnancy and lactation can deplete maternal tissues of DHA and that this effect increases with multi-parity (27). In this study, the population of women had high parity; it is therefore highly likely that these factors could have significantly influence on the maternal DHA status and that of their neonates. Omega-3 mainly DHA and omega-6 mainly (AA) are vital structural and functional components of cell membranes. Unlike AA, which is distributed widely in all tissues, DHA is selectively incorporated into brain and retinal tissues (19). There is evidence that high levels of maternal and infants' DHA status or levels of DHA in milk are positively correlated to infant's cognitive development (28) and maturity of visual system (29). Poor or suboptimal maternal supply of DHA in utero via placenta and post-natal through milk during the critical periods of brain and retinal development may result in adverse neuro-visual development in the neonates and infants. The proportions of the n-3 metabolites DPA (0.25%±0.01) was consistently lower in our neonates in comparison to Belgium and U.K which was (0.48%±0.17, 0.7%±0.5) respectively. The level of our neonates DPA was significantly lower than the level in pregnant women (0.52%±0.03) $p \leq 0.001$. The same result was obtained by De Vreise (0.59%±0.2) (121). Nevertheless, the higher levels of DPA in the mothers indicate that these precursors of DHA may not be favourably transferred to the foetus. It is conceivable that the synthesis of DHA from DPA may be inefficient in the foetus. This would necessitate the retention of DPA in the maternal circulation for DHA synthesis and subsequent transfer to the foetus (Hornstra, G, 1995).On the other hand, the differences in levels of unsaturated fatty acids among pregnant and neonates provide an indication that the placenta and/ or the fetus may handle the n-6 and n-3 fatty acids differently. We found a significantly higher value of mead acid in the plasma CPG of neonates (mean of mead acid of neonate was 0.81±0.11, of pregnant was 0.26±0.04), which is a strong indicator of Docosahexaenoic acid (DHA) deficiency. In general, if essential PUFAs are not available to meet PUFA requirements, the body starts to synthesize certain fatty acids that are hardly present if the EFA and PUFA status is adequate. Therefore, these fatty acids can be essential PUFA status markers. The best known marker is Mead acid (20:3n−9). The synthesis of this fatty acid is promoted if there are insufficient concentrations of LA and ALA to meet the need for LCPs. Because LCPs inhibit Mead acid synthesis, the presence of Mead acid which may be transferred to the foetus, indicates a general shortage of all essential PUFAs (De Vriese RS, 2003).The same results were obtained by Min, Y. and Otto, S.J.

CONCLUSION

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 health should deserves more attention, by improving and replenishing their nutritional status throughout the child bearing 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.

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ISSN : 0976-4550

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