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# ESTIMATION AND GAS CHROMATOGRAPHIC ANALYSIS OF THE RATIO OF OMEGA-6 TO OMEGA-3 FATTY ACIDS IN BLOOD SERUM

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## ABSTRACT

Omega (n)-6 and omega (n)-3 are long-chain polyunsaturated fatty acids (LCPUSFA) derived from the 18-carbon essential dietary precursors linoleic acid (LA) and alpha-linolenic acid (ALA), respectively. These two families of fatty acids are metabolically and functionally distinct and often have important opposing physiological functions. Their balance is important for homeostasis, normal development and may affect susceptibility to different diseases. Fatty acid concentrations were determined by using Gas Chromatography (GC). The subjects were divided into two groups, younger and older, with 40 years old being the dividing age. The concentration of fatty acids and the ratio of n-6:n-3 were analyzed among both groups in relation to age, body mass index (BMI) and lipid profiles. Regarding the fatty acid concentrations, the analysis showed that most of n-6 fatty acids (LA, GLA and AA) and EPA were positively correlated with age. The data also showed no correlation between fatty acid concentrations and BMI in all subjects.

**Keywords:** Omega-6, Omega-3, Fatty acid and Gas Chromatographic analysis.

## INTRODUCTION

The two classes (n-6 and n-3) are derived from the essential fatty acids linoleic acid (LA; 18:2, $\Delta$ 9,12; n-6) and $\alpha$-linolenic acid (ALA; 18:3, $\Delta$ 9,12,15; n-3) respectively (Gibson and Makriddes, 1998). LA is plentiful in nature and mostly found in the seeds of most plants except for coconut, cocoa and palm (Simopoulos, 1991). It has several different functions, known to be important for growth, dermal integrity, wound healing, liver, kidney and protection against infection (Carlson, 1997).

Formation of AA, the most important fatty acids in the n-6 family, is accomplished by the conversion of LA through a desaturation and elongation series. The pathway is first started by dehydrogenation of Co-A ester through GLA, followed by the addition of a two-carbon unit, by the elongase enzyme, via malonyl-CoA in the microsomal system for chain elongation, to produce DHGLA. The latter forms AA by a further dehydrogenation step (Murray *et al.,*1996; Voet and Voet, 1995).

In studies of learning ability, it was suggested that there might be a direct connection between the amount of n-3 in the diet, and the ability to learn. It is well established that DHA is required for brain development and function and that the incorporation and selectivity of biomembranes for DHA are extremely efficient (Galli and Simopoulos, 1989; Cooper, 1998).

Otherwise, several studies show that DHA is the preferred fatty acid for use in the retina of human being, where it is found in the photoreceptor cells, especially the outer segments of the rods, and it may make up as much as half of the total fatty acid presents (Cooper, 1998).

Like AA, DHA is critically important for growth and central nervous system development in fetal and infant stages. Various studies show that it is extremely important for a developing baby (both in the womb and in early infancy) to have adequate levels of DHA for optimal mental and vision function. The principal source of PUSFA, DHA and EPA, for infant is mother's milk, where the amount of these substances mainly depends on the mother's diet during pregnancy and lactation (Uauy and Mena, 2001). Since the greatest need for DHA is in the first months of life, the authors go on to make a recommendation concerning the inclusion of DHA in infant formulas (Cooper, 1998).

As expected, fatty acid analysis of the transplanted tumors reflects the specific composition of the dietary fat ingested by the host. Furthermore, these studies indicate that the composition of dietary lipids modifies lipids metabolism and that high dietary intake of n-3 fatty acids can prevent or delay the expression of these neoplasm. In other studies involving human breast-cancer cells in nude mice, the mice fed n-3 fatty acids had fewer pulmonary metastases, decreased serum estrogen and prolactin concentrations in the tumor-tissue cells. The opposite occurred in the corn oil-fed mice (Simopoulos *et al.,* 1991). The present study was carried out to investigate serum levels of LA, gamma linoleic (GLA), dihommo-gamma-linolenic (DHGLA), arachadonic (AA), ALA, eicosapentanoic (EPA) and Docosahexanoic (DHA) acids in 48 healthy women aged between 20-70 years and to define the ratio of n-6: n-3. Fatty acid concentrations were determined by using Gas Chromatography (GC). The subjects were divided into two groups, younger and older, with 40 years old being the dividing age. The concentration of fatty acids and the ratio of n-6:n-3 were analyzed among both groups in relation to age, body mass index (BMI) and lipid profiles.

## EXPERIMENTAL

The experimental work of the present study was conducted at the King Abdulaziz University Hospital and King Fahad research center, Jeddah, Saudi Arabia.

### Subjects

Forty-eight healthy female volunteers aged 20-70 years were included in this study. All volunteers were free of any medical illness including diabetes and hypertension and they had not been taking any medication. Blood samples were collected after an overnight fast. At the time of the collection, information recorded for all subjects, including weight, height, smoking and physical fitness.

### Reagents and standards

**Triglycerides GPO- PAP Kit Reagents**:

Triglycerides Reagents were obtained from Boehringer Mannheim GmbH (Mannheim, Germany)

R1: Buffer/ 4- chlorophenol/ enzymes.

One bottle. Ready to use.

**HDL-Cholesterol Plus reagents:**

HDL-Cholesterol reagents were obtained from Boehringer Mannheim GmbH (Mannheim, Germany)

R1 $\alpha$-Cyclodextrin/buffer. Ready to use.

R2 PEG-enzymes/4-aminophenazone/ buffer. Ready to use.

### Biochemical Analysis:

**Determination of Lipid Profiles:**

Plasma Cholesterol, High-density lipoproteins (HDL), Low-density lipoprotein (LDL) (Friedewald *et al.,*1972), and triglycerides were analyzed by enzymatic methods on a Hitachi 917 blood chemistry analyzer (Tokyo, Japan).

**Determination of Total Fatty Acids levels using GC:**

The first step toward determination of total fatty acid levels was done by using one-step transmethylation reaction based on the method of Lepage and Roy (Lepage and Roy, 1986).

**Extraction and methylation method:**

1. 2 ml of methanol-toluene (4:1, v/v) was added to 100-µl serum in a screw-capped glass tube.
2. A small magnetic bar was placed in the tube, and while stirring, 100-µl acetyl chloride was added slowly for one minute.
3. The tube was tightly closed and subjected to methanolysis at 100°C for 60 minute on the heating/stirring module.
4. The tube cooled in water (25°C).
5. 5 ml of 6% aqueous potassium carbonate solution was slowly added to stop the reaction and neutralize the mixture.
6. The tube was then shacked and centrifuged (1800g ) for 10 minute.
7. 40-µl of the toluene upper phases with fatty acids-methyl esters was collected in new epandrof tube and kept at -5°C.

At the same day of preparation, 1-µl from the extracted sample was directly injected into the GC, a mixture of fatty acid.

**Gas Chromatography (GC)-apparatus:**

The GC separation was performed on a model 8700 gas chromatograph (Perkin-Elmer, U.K.) equipped with a flame ionization detector. Helium was used as a carrier gas (17 PSIG) at split ratio of 7:1. The column was a 60-m fused silica with an internal diameter of 0.32mm and wall-coated with 0.20 ìm SP-2331 (25% bonded phase). After an initial isothermal period (5 min) at 150º C, the temperature was programmed to reach 165º C by raising 6.5º C/min. The temperature was kept at 165º C for 7 min. The second increment was

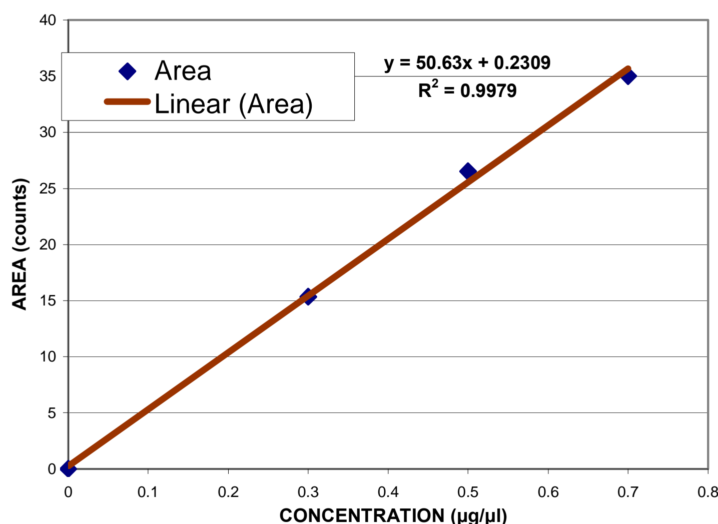


**Fig. - 1.1: Linoleic acid calibration curve**

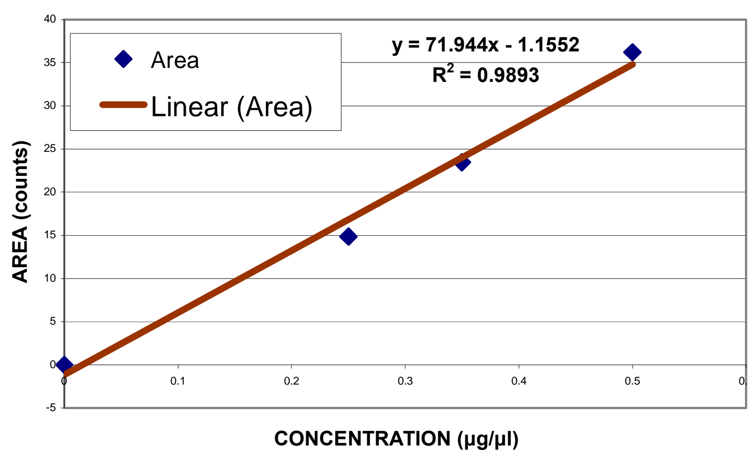


**Fig. - 1.2: Gamma linolenic acid calibration curve**

1.6º C/min to reach 200º C and hold at that temperature for 5 min. The third increment was 0.3º C/min to reach 204º C. After 1 min, the temperature increased to 220º C with increments of 10º C/min and held at that temperature for 5 min. Injector and detector temperatures were 200º C and 250º C, respectively.

**Fatty acids standards and calibration curves:**

Calibration curves were established to determine the concentration of fatty acids in the samples, with series of appropriate standards. Then the peak areas in counts were plotted against concentrations in µg/µl for each fatty acid (Liebich *et al.,* 1991).

By using the definition, minimum detectable peak height equal twice the recorder trace noise level, the detection limit for the fatty acids (LA, GLA, DHGLA, AA, ALA, EPA, and DHA) was almost 0.001 µg/µl. This was done to confirm the sensitivity of GC instrument.

**Statistical analysis**

Statistical analysis of the data was performed using computer program package (SPSS, Version 10). All data were expressed as mean with their standard deviation. The t-test was employed to test the differences between the two groups. Differences were considered significant at $p< 0.05$, and highly significant at $p< 0.01$. Correlations were studied by Pearson's method.

Correlation analyses with a p-value < 0.05 were considered to be significant.

## RESULTS AND DISCUSSIONS

### Determination of the fatty acid

Separation of fatty acids of representative sample by Gas Chromatography (GC) takes place. Their concentrations were calculated by using the linear equation from the calibration curve for each fatty acid; LA, GLA, ALA, DHGLA, AA, EPA and DHA, respectively Fig.1 (1-4).

The forty-eight women of ages between 20-70 years old were divided into two groups according to their age. First group included 25 women younger than 40-years and the second group included 23 women older than 40-years old.

The physical and biochemical characteristics for all subjects and for both groups are summarized in Table (1) The analysis of the data by using t-test revealed that there are significant differences between the two groups. The group of women that is older than 40-years old are significantly higher in BMI (25.65±4.70) than the younger group (29.20±4.55).

The data also showed that there are significant differences between the two groups in the level of lipid profiles. The older group has significantly higher levels than the younger group

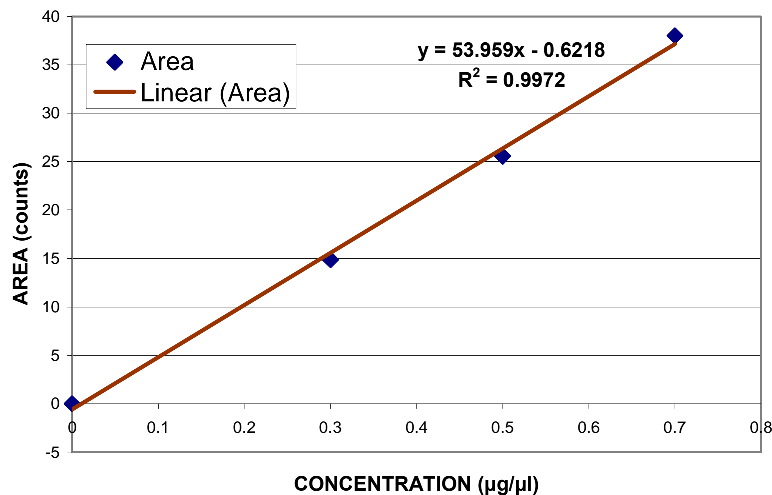


**Fig. - 1.3: Alpha linolenic acid calibration curve**

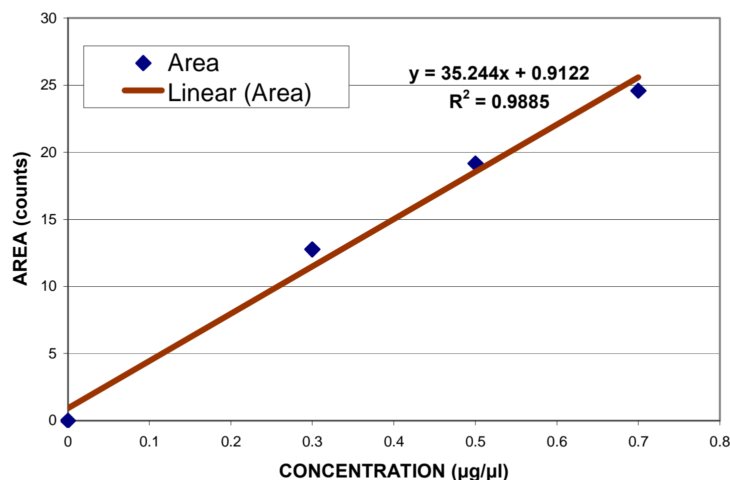


**Fig. - 1.4: Dihommo gamma linolenic acid calibration curve**

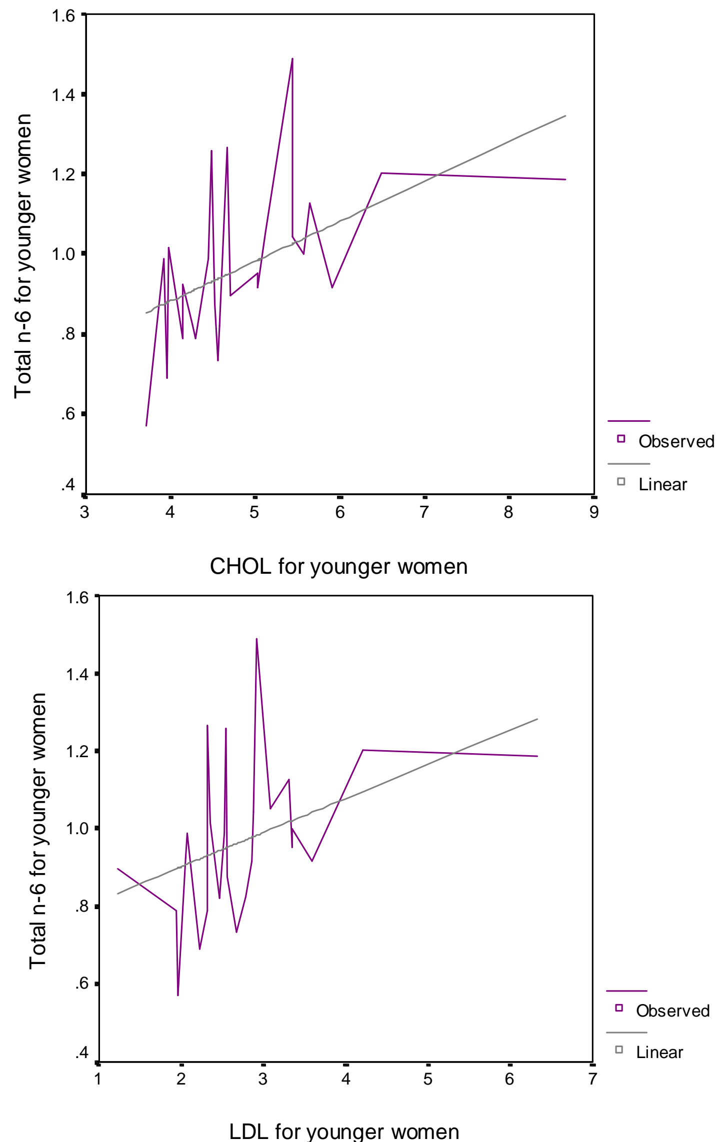


**Fig. - 2: Correlation between total n-6(µg/µl) and lipid profiles (mmol/l) in the younger group**

**Abbreviations:** CHOL=cholesterol; LDL=low-density lipoprotein

of TG (1.52±0.67 vs 1.11±0.66 mmol/l; p<0.05), CHOL (6.08±1.36 vs 4.90±1.05 mmol/l; p<0.01), and LDL (3.59±1.30 vs 2.79±097; p<0.05).

Regarding the fatty acids, the older group also have significantly higher level than the younger group of LA (0.90±0.21 vs 0.65±0.15 mmol/l; p<0.01), GLA (0.03±0.01 vs 0.02±0.01 mmol/l; p<0.01), AA (0.38±0.17 vs 0.28±0.08 mmol/l; p<0.05), total n-6 (0.1.34±0.37 vs 0.98±0.21 mmol/l; p<0.01) and EPA (0.03±0.01 vs 0.02±0.01 mmol/l; p<0.01).

**Correlation between fatty acids levels and other parameters**

**Correlations with age**

The analysis of the results by Pearson correlation showed that age correlate strongly (Pd"0.01) with LA (r=0.56), GLA (r=0.64), total n-6 (r=0.50), and EPA (r=0.40) and positively (Pd"0.05; r= 0.30) with AA. Fig. 2 shows the comparison between fatty acid levels among younger and older groups. The other fatty acids, DHGLA, ALA, DHA and total n-3 failed to show any significant correlation with age.

AA in the younger group failed to show any significant correlation with lipid profiles. In the older group, AA correlate significantly (p<0.01) with TG (r=0.75), CHOL (r=0.61), LDL (r=0.58) and negatively with HDL (p<0.05; r= -0.50).

Regarding the total n-6, there were significant correlations with lipid profiles in the younger and the older group. The correlation with CHOL showed a value of (p<0.01; r=0.51) and a value of (p<0.05; r=0.41) with LDL in the younger group (figure3). In the older group, the total n-6 showed a positive correlation (p<0.01) with TG (r=0.75), CHOL (r=0.69), LDL (r=0.72) and there were negative correlation with HDL (p<0.05; r= -0.50) (Fig. 3).

Regarding the levels of n-6 fatty acids in the two groups, the present data showed that the levels of all except DHGLA were significantly higher in the older group than in the younger group. However, the n-3 fatty acids were broadly similar in both groups with the exception of EPA, which was significantly higher in the older group. Although very few data have been published regarding the relationship between fatty acids and age, Okita *et al.,* (1994) have carried out research comparing middle-aged women living in rural areas and middle-aged women living in urban areas of Okagama, Japan. They showed that the levels of n-6 and n-3 failed to show any significant differences except for LA in rural subjects.

n-3 fatty acids, ALA, EPA, and DHA, have been part of the human diet throughout ages. Modern agriculture and aquaculture have led to change in the production of both plants and animals and to marked change in the composition of food supply. As a consequence, an increase in the amount of n-6 and a decrease in the amount of n-3 affected the ratio of n-6 to n-3 fatty acids.

Our study showed that the ratio of n-6: n-3 was 2.84:1 and 3.82:1 for the younger and the older groups respectively. This result indicate that the Saudi diet is still traditional and has been minimally affected by the modern diet of decreased the n-3 (Simopoulos, 2000).

In comparing the present study of n-6: n-3 ratio with those of others, European and United States as example reported a ratio that ranged between 10-20:1 (Simoupolos, 1999). Study by Dewailly *et al.* (2001) on 1460 Quebecers/ Canada population aged between 18-74 years showed that Quebecers consume smaller quantities of fish and have substantially lower concentrations of EPA and

**Table - 1: Physical and biochemical parameters of study subjects.**

| Parameters | All | Young d≤ 40 | Old > 40 |
|---|---|---|---|
| Age (year) | 40.44±12.81 | 29.71±5.25 | 51.47±8.26 |
| BMI (Kg/m$^2$) | 27.42±4.87 | 25.65±4.70 | 29.20±4.55** |
| **LIPID PROFILE:** | | | |
| Triglyceride (mmol/l) | 1.30± 0.70 | 1.11± 0.66 | 1.52± 0.67* |
| Cholesterol (mmol/l) | 5.50± 1.33 | 4.90± 1.05 | 6.08± 1.36** |
| HDL(mmol/l) | 1.21± 0.25 | 1.22± 0.22 | 1.21± 0.28 |
| LDL(mmol/l) | 3.20± 1.20 | 2.79± 0.97 | 3.59± 1.30* |
| **FATTY ACIDS:** | | | |
| Linoleic acid (µg/µl) | 0.76± 0.22 | 0.65± 0.15 | 0.90± 0.21** |
| Gammalinoleic acid (µg/µl) | 0.02± 0.01 | 0.02± 0.01 | 0.03± 0.01** |
| Dihomogamalinolenic acid (µg/µl) | 0.03± 0.03 | 0.03± 0.02 | 0.03± 0.03 |
| Archadonic acid (µg/µl) | 0.32± 0.14 | 0.28± 0.08 | 0.38± 0.17* |
| Total omega-6 (µg/µl) | 1.14± 0.35 | 0.98± 0.21 | 1.34± 0.37** |
| Alpha linolenic acid (µg/µl) | 0.10± 0.02 | 0.10± 0.02 | 0.10± 0.02 |
| Eicosapentaeonic acid (µg/µl) | 0.02± 0.01 | 0.02± 0.01 | 0.03± 0.01** |
| Docosahexaenoic acid (µg/µl) | 0.23± 0.04 | 0.23± 0.04 | 0.22± 0.06 |
| Total omega-3 (µg/µl) | 0.35± 0.06 | 0.35± 0.05 | 0.34± 0.08 |

(Values are mean ± s.d.)

* Significant at p<0.05,

** Significant at p<0.01 compared with lean groups.

Abbreviations: BMI =body mass index; WHR=ratio of waist-to-hip circumferences;
HDL= high-density lipoprotein; LDL= low-density lipoprotein.

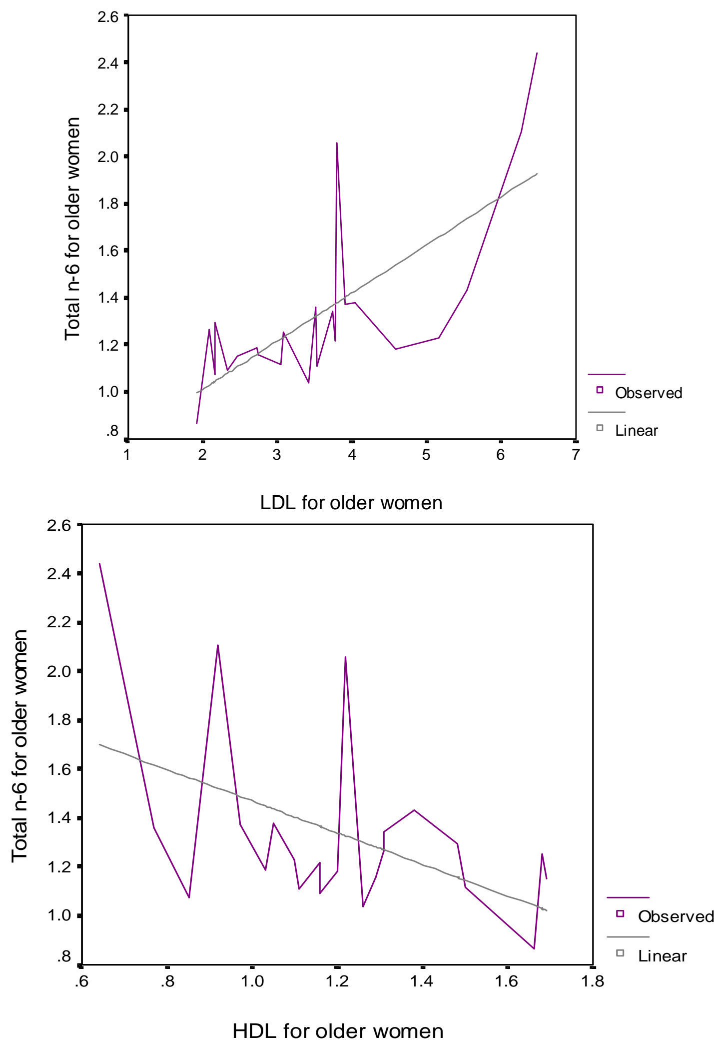


**Fig. - 3: Correlation between total n-6 (µg/µl) and lipid profiles (mmol/l) in the older group**

**Abbreviations:** TG=triglycerides; CHOL=cholesterol; LDL=low-density lipoprotein; HDL=hogh-density lipoprotein.

DHA than do the Japanese and Inuit, but they have similar concentration to those of United States populations (Blanchet *et al.,* 2000; Kris-Etherton *et al.,* 2000; Sugano and Hirahara, 2000; although analysis of a food type frequency questionnaire indicated that the intake of fish in the diet of Saudi women was much less than that of European and United States women, the ratio of n-6: n-3 is much lower than the reported ratio for Europe and USA.

The mean ratio of n-6 to n-3 fatty acids in both groups in our study is within the recommended value. Unfortunately, the ratio of n-6: n-3 has not been investigated previously in Saudi population. Therefore, the ratio of our study which was higher than1.0. The recommended ratio for P:S is 1.0. The nutritional significance of these results can be understood considering the general agreement among nutritionists that a high-fat diet and low ratio of (P:S), raises the serum cholesterol level and promotes the development of atherosclerosis. In comparing the results to other studies, they showed that AA in the food samples has been too high that led to an overestimation of AA intake (Al-Khalifa and Al-Othman, 1999). The aim of this study was to determine the ratio of n-6: n-3 fatty acids from a representative sample of healthy Saudi women aged between 20-70 years old, living in the western

region of the Kingdom of Saudi Arabia. To our knowledge, the ratio of these two classes of unsaturated fatty acids has not been investigated amongst the Saudi population. It is evident from this study that most of the Saudi diet contains high levels of n-6 and that contributes to the ratio of n-6: n-3 in favor of n-6 fatty acids. Although the ratio is within the acceptable range, a modification in the diet to reach a balance of ratio of at least 2:1 is ideal for health benefit. Our recommendation is to reduce the ratio of n-6: n-3 in the Saudi diet. In conclusion, we believe that there is no level of awareness of health benefits of n-3 fatty acids in KSA and the specialists have to educate the population about the benefits of n-3 fatty acids and their balance with n-6 fatty acids.

## REFERENCES


1. Al-khalifa, A.S. and Al-Othman A.A. Fatty acid composition and arachidonic acid intake of selected Saudi foods. *Int J Food Sci Nutr.* **50,** 255-63 (1999)
2. Appel L.F., Miller E.D., Seidler A.J., and Whelton P.K. Does-supplementation of diet with fish oils reduce blood pressure? A meta-analysis. *Ann Intern Med.* 120 (suppl): **9** (1993)
3. Appel L.F., Miller E.D., Seidler A.J., and Whelton P.K. Diet supplementation with fish oils and blood pressure reduction. A meta-analysis of controlled clinical trials. *Arch Intern Med.* **153,** 1429-38 (1994)
4. Blanchet C., Dewailly E., Ayotte P., Bruneau S., Receveur O., and Holub B.J. Contribution of selected traditional and market food to Nunavik Inuit women diet. *Can J Diet Pract Res.* **61,** 50-9 (2000)
5. Carlson S. E. Functional effects of increasing omega-3 fatty acid intake*. The Journal Of Pediatrics.* **131,**173-5 (1997)
6. Cooper R. DHA: The essential omega-3 fatty acid. Woodland. Pleasant Grove, UT. 5-26 (1998)
7. Dewailly E., Blanchet C., Gingras S., Lemieux S., Sauve L., Bergeron J., and Holoub B.J. Relations between n-3 fatty acid status and cardiovascular disease risk factors among Qubecers (2001)
8. Friedewald W. T., Levery R.J., and Fredrickson D.S. Estimation of the concentration of low-density lipoprotein cholesterol. *Clin Chem.* **18,** 499-502 (1972)
9. Gibson R.A., and Makriddes M. The role of long chain polyunsaturated fatty acids (LCPUFA) in neonatal nutrition. *Acta paediatr.* **87,** 1017-22 (1998)
10. Kris-Etherton P.M., Taylor D.S.,Yu-Poth S., Huth P., Moritarty K., Fishell V., Hargrove R.L., Zhao G., and Etherton T.D. Polyunsaturated fatty acids in the food chain in the united states. *Am J Clin Nutr.* **71**(suppl), 179S-88S (2000)
11. Lepage G., and Roy C.C. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res.* **27,** 114-20 (1986)
12. Liebich H.M., Wirth C., and Jakober B. Analysis of polyunsaturated fatty acids in blood serum after fish oil administration. *Journal of Chromatography.* **572,** 1-9 (1991)
13. Murray R., Granner D.K., Mayes P.A., and Rodwell V.W. (Harper's Biochemistry. (24th ed). Appleton and Lange. Stamford, Connecticut. 236-244 1996)
14. Okita M., Yoshida S., Yamamoto J., Suzuki K., Kaneyuki T., Kubota M., and Sasagawa T. N-3 and n-6 fatty acid intake and serum phospholipid fatty acid composition in middle-aged women living in rural and urban areas in Okayama prefecture. *J Nutr Sci Vitaminol.* **41,** 313-23 (1994)
15. Simopoulos A.P.Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr.* **54,** 438-63 (1991)
16. Simopoulos A.P. Human requirement for n-3 polyunsaturated fatty acids. *Poult Sci.* **79,** 961-70 (2000)
17. Simopoulos A.P., Leaf A., and Salem N. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann Nutr Metab.* **43,** 127-30 (1999)
18. Sugano M., and Hirahara F. Polyunsaturated fatty acids in the food chain in Japan. *Am J Clin Nutr.* 71 (suppl), 189S-96S (2000)
19. Voet D., and Voet J. D., Biochemistry. (2nd ed). John Wiley &Sons. New York. 272-3; 639-41; 658-665 (1995)