



## Fatty Acid Composition of Mature Human Milk in Mothers Who Referring to the Health Care Centers of South of Tehran

\* Sedigheh Soleimani \*\* Shahnaz Khaghani \*\*\* Narjes Ardestani  
 \*\*\*\* Parastoo Asa \*\*\*\*\* Azar Shams \*\*\*\*\* Hoda Boushehri  
 \*\*\*\*\* Hossein Mirmiranpour

\* \*\*, \*\*\*\*, \*\*\*\*\* Department of Biochemistry, Tehran University of Medical Sciences, Tehran, Iran.

\*\*\* Mofatteh Hospital, Shahid Beheshti University, Tehran, Iran.

\*\*\*\*\* Faculty of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran.

### ABSTRACT

**Objective:** Human milk contains large quantity of fatty acids that they prevent and suppress autoimmune diseases, Asthma, type I and type II diabetes, metabolic syndrome x, cardiovascular disease, lymphoma, leukemia and other cancers, schizophrenia, depression, autoimmune diseases and some light inflammation. Due to the important role of fatty acids in human milk assessing their quantities is essential and helps to improve when they are insufficient.

**Methods:** 43 mothers referring to the health care centers of south of Tehran were examined. Samples were collected at a midmorning feeding and stored at -20°C. Fatty acids were measured by Gas Chromatography.

**Findings:** Milk samples were thawed at room temperature (25°C). First Supelco standards with 0.2, 0.3, 1, 3, 5 and 10 mg/mL concentrations and 1 mg/mL internal standard (ethyl ester margaric acid) were prepared.

**Conclusion:** According to results the highest rate of fatty acids in human milk were related to saturated acids like C16:0 (212.7±96.1 mg/mL) and C18:0 (77.3±19.1 mg/mL). In this study high rate of C16:0 (palmitic acid) and C18:0 (Stearic acid). The most concentration of human milk fatty acids were C16:0 and C18:0 saturated FAs.

**Keywords :** Human milk; Infant feeding; Fatty acid

### Introduction

Human milk as first choice is recommended as the only source of nourishment for healthy infants during the first 4-6 months of life<sup>[1]</sup>. Mature milk generally contains from 3.5% to 4.5% lipid, mostly triacylglycerols<sup>[1]</sup>. Milk fatty acids (FAs) are either synthesized in the mammary gland or are derived from plasma FAs that originate from the diet synthesis in other tissues or lipid stores<sup>[2,3]</sup>. Human milk contains a large quantity of fatty acids that prevent and suppress autoimmune disease. It is shown that in newborns they have an important role in the prevention of asthma, type I and type II diabetes, metabolic syndrome x and cardiovascular disease<sup>[1]</sup>.

Some of these lipids play a role in the brain function as they form 50-60 % of brain net weight in the evolution. Half parts are unsaturated fatty acids<sup>[2]</sup>. Brain, retina and other nerve cells are rich in long-chain polyunsaturated fatty acids (LCPU-FAs)<sup>[1]</sup>.

Researchers have shown the conservative effects of exclusive lactation against atopic eczema, bronchial asthma, allergic rhinitis, asthma, and dietary allergy<sup>[4]</sup>.

Undurti and their coworkers showed that gamma-linolenic acid (GLA) prevents genetic injuries of gamma rays and some medicines. EPA, DHA, GLA and AA have selective tumoricidal function<sup>[4]</sup>.

Okolo SN et al showed 43 mothers' BMI was 16-38 kg/m<sup>2</sup> and the quarter of milk was saturated C10-14 FAs<sup>[5]</sup>. Vander Jagt and his colleagues showed that the comprehensive analysis of breast-milk DHA and AA indicates a broad range

of these nutrients worldwide and serves as a guide for infant feeding<sup>[6]</sup>.

### Subjects and Methods

#### Subjects

43 mothers who referring to the health care centers of south of Tehran were put under studied. The milk was collected at 6-8 weeks after delivery. All subjects were in good health. The mean ages and BMI of mothers were 26.78 ± 3.5 years and 41 ± 0.0 Kg/m<sup>2</sup>. The protocol was approved by the committee of Human Ethics, The Tehran University of Medical Sciences and signed consent was obtained from each mother. Ethical concentrations were goal research explanation for researches unit, authorize to researches unit for participation reject or accept in researches, ensure information security to researches unit and ensure clinic manager that they can access to the information whenever they want.

#### Milk collection

Mothers were collected the milk samples (10-15 mL) at a midmorning feeding each in a bottle washed previously with deionized water and were stored immediately at -20° C.

#### Chemical analysis of human milk

Total lipids were extracted from 20 ml of milk with methanol-chloroform (2:1, v/v) according to Folch et al.<sup>[5]</sup>. Measuring of fatty acids was performed by Gas Chromatography (GC) [7, 8, 9, 10, and 11].

VARIAN-CP-3800 GC device with VARIAN CP 8410 auto sampler was used for measuring of fatty acids concentration in human milk. Milk samples were thawed at room temperature

(25°C). Milk fatty acid methyl esters (FAME) were prepared directly by the trans esterification method of homogenizing milk with a 2:1 chloroform-methanol mixture and washing the extract by addition to it of 0.2 its volume of either water or an appropriate salt solution. The resulting mixture separates into two phases. The lower phase is the total pure lipid extract [6]. The esters were separated and quantitated with a silar 10C (Alltech, IL) capillary column and flame ionization detector.

### Statistical analysis

Descriptive data were presented as a mean values  $\pm$  standard deviation. One way Anova test was used for further statistical analyses. All data are presented as the mean  $\pm$  1SD.

### Findings

Each concentration three times was injected to the GC device (each time 10 $\mu$ l). First Supelco standards with 0.2, 0.3, 1, 3, 5 and 10 mg/mL concentrations and 1 mg/mL internal standard (ethyl ester margaric acid) were prepared. Fig 1 shows the chromatogram of Supelco standards for fatty acids. The concentration rate of butyric acid (C4), caproic acid (C6), undecanoic acid (C11) with this method and standard was not measurable. Table 1 shows fatty acid composition of human milk from 43 milk samples expressed as% wt/wt.

### Discussion

Human milk Fatty acids are the result of dietary intake, mobilization from fat depots and endogenous synthesis by the mammary gland which are capable of synthesizing saturated fatty acid (SFA), primarily 10 to 14 carbons in length. Fatty acids of chain lengths >16 carbons are not synthesized in the mammary gland and must be obtained either from the diet or mobilized from fat depots [6]. The fatty acid composition of human milk does not change during nursing [12 and 13]. FAs in human milk are in four types saturated, mono unsaturated, 3-n poly unsaturated and 6-n poly unsaturated [7, 8 and 9], we can conclude that the quality and quantity of FAs in human milk is related to their quantity in mother's dietary which in literature showed that the FAs composition of human milk can be influenced by diet [11, 12 and 13].

In our study the mean age was 26.78 $\pm$ 3.5 and the mean BMI of mothers was 41 $\pm$ 0.0 Kg/m<sup>2</sup>, so they were obese which affected on breast milk FAs profiles. Okolo SN et al showed 43 mothers' BMI was 16-38 kg/m<sup>2</sup> and the quarter of milk was saturated C10-14 FAs [5]. Dorothy and his colleagues studied the relation between human milk and blood fatty acids in mothers and their breast-fed infants [6]. All the mothers in the reproductive age (15-45 years) having 1-10 delivery records. BMI of mothers were 16-24 Kg/m<sup>2</sup>. Blood fatty acids in mothers were reported: docosahexaenoid acid (0.20g/100), arachidonic acid (0.51g/100), linolenic acid (13.5g/100) and alpha-linolenic acid (0.66g/100).

Breast milk fatty acids were docosahexaenoid acid (0.197g/100), alpha-linolenic acid (0.2g/100), arachidonic acid (9/79g/100) and linolenic acid (21.4 g/100).

There was a strong positive relation between these compounds in breast milk and mother's serum. These rates in infant's serum were docosahexaenoid acid (3.4g/100), alpha-linolenic acid (0.13g/100), and arachidonic acid (12.8g/100), linolenic acid (21.4g/100). DHA in infant's serum was reported 73% more than mother's serum [6].

Okolo SN and et al showed 43 mothers' BMI was 16-38 kg/m<sup>2</sup> and the quarter of milk were saturated C10-14 FAs [5].

C18:2 was 0.5  $\pm$  1.6 mg/mL, C18:3n6 was 0.9  $\pm$  1.6 mg/mL, C18:3n3 was 9.1  $\pm$  3.0 mg/mL, C20:1 was 5.3  $\pm$  2.7 mg/mL, C20:2 was 3.2  $\pm$  1.2 mg/mL and C20:4n6 was 3.2  $\pm$  1.2 mg/mL ( $\omega$ -3 and  $\omega$ -6 FAs) that existed in breast milk. Many published studies of breast milk FAs composition are limited to populations from one or two countries. Yuhas examined the degree to which FAs composition vary across a number of diverse populations. Because diet and maternal adipose stores influence breast milk FAs composition, differences in FAs

composition between groups most likely reflect habitual dietary differences. Approximately 50 breast milk samples (full breast expression) were collected from women in Australia, Canada, Chile, China, Japan, Mexico, Philippines, the United Kingdom, and the United States. The proportion of saturated FAs were relatively constant among countries with the exception of the Philippines where levels of lauric and myristic acids were elevated (Means greater than two times the mean of most other countries). Monounsaturated FAs also varied little with the exception of low levels of oleic acid in the Philippines and high levels of erucic acid in China. Although arachidonic acid (C20:4n-6) levels were similar among all countries (Means ranging from 0.36 wt % to 0.49 wt %), mean DHA (C22:6n-3) levels ranged from 0.17 to 0.99 wt % with the highest levels in Japanese milk and the lowest levels in Canadian and U.S. samples [15].

Experiment has shown that FAs in mother's dietary affect different kinds of physiologic processes so it has an important effect on child health [17, 18, 19, 20, 21 and 22]. Therefore developing consumption pattern of fatty acids in mothers and its effect on the breast milk fatty acids composition can have important role in future health of infants and some human milk fatty acids seemed to be influenced by diet. Current evidence indicates that infant demand is the major determinant of the quantity of milk transferred to the nursing infant. Human milk is remarkable for its variability, and ranges of intakes of milk constituents are comparable with normal patterns of infant growth and development. Lipids are by far the most variable constituents in human milk with both long-term maternal nutrition states and daily intake capable of exerting an influence [13]. The most important changes were observed in 18:2 n-6, 18:1 n-9/n-7, n-6 LCP, and n-3 LCP content due to diets rich in olive or seed oils, eggs, and fish, respectively [18 and 23]. Regional differences seemed also due to dietary habits. The results of this study demonstrate that the proportion of saturated and monounsaturated FAs are relatively constant across a large number of countries, whereas the level of some of the PUFA, especially DHA, are highly variable and the percentage values of major and minor human milk fatty acids do not depend on the total lipids content of the milk [21 and 24].

In summary, some human milk fatty acids seemed to be influenced by diet. The most important changes were observed in C16:0 (Palmitic acid) and C18:0 (Stearic acid) which fatty acids of chain lengths >16 carbons are not synthesized in the mammary gland and must be obtained either from the diet or mobilized from fat depots [6].

### Conclusion

The most concentration rate of human milk fatty acids were C16:0 and C18:0 which were saturated fatty acids.

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### Conflict of interest:

The authors declare no conflict of interest.

### Abbreviations:

FA, fatty acid; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LCP, long chain polyunsaturated fatty acid; LNA,  $\alpha$ -linolenic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; GC, gas chromatography; GLA, gamma-linolenic acid

**Table 1: Fatty acid composition of human milk from 43 mothers (% wt/wt)**

Fatty acids	Mean $\pm$ SD
C12:0	43.9 $\pm$ 7.5
C13:0	0.5 $\pm$ 0.2
C14:0	59.8 $\pm$ 13.7

C14:1	1.6±0.9
C15:0	4.9±1.8
C16:0	212. ±96.1
C16:1	4.2± (1.7
C17:0	5.5±2.7
C18:0	77.3±19.1
C18:1	31.1±13.9
C18:2	1.6±0.5
C18:3n6	1.6±0.9
C18:3n3	9.1±3.0
C20:0	2.0±0.9
C20:1	5.3± 2.7
C20:2	3.2±1.2
C20:4n6	3.3±1.7
C24:0	0.2±0.1

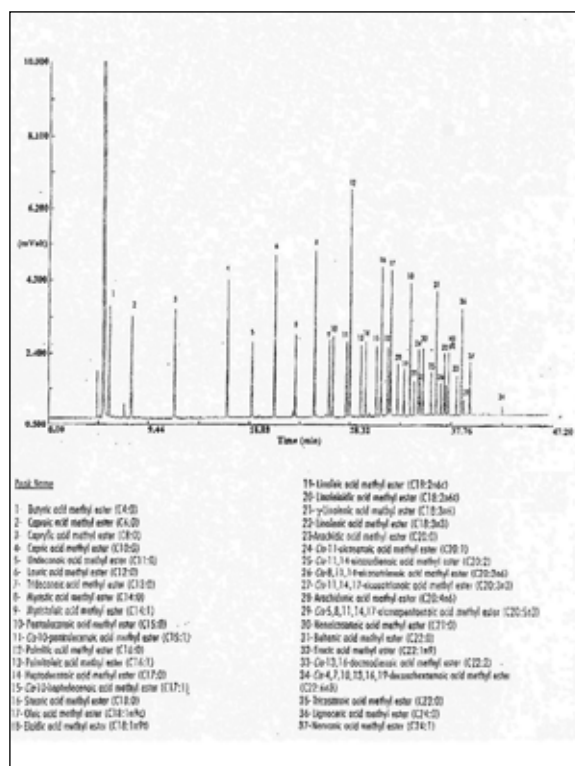


Fig 1. Chromatogram of Supelco standards for fatty acids

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