

Saturated Fatty Acid Composition of Human Milk in Israel: A Comparison between Jewish and Bedouin Women

Tali Silberstein MD¹, Ariela Burg PhD², Jeanine Blumenfeld PhD², Boaz Sheizaf MD¹, Tamar Tzur MD¹ and Oshra Saphier PhD²

¹Department of Obstetrics and Gynecology, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

²Department of Chemical Engineering, Shamoon College of Engineering, Beer Sheva, Israel

ABSTRACT: **Background:** Breast milk is well established as the ideal source of nutrition for infants. Mature human breast milk generally contains 3.5–4.5% lipids comprising mostly triacylglycerols. In general, the fat composition of maternal human milk in developing countries shows higher levels of saturated fats, reflecting diets rich in carbohydrates.

Objectives: To determine the profile of unsaturated fatty acids in the breast milk of two populations in southern Israel – urban Jewish and rural tent-dwelling Bedouin women.

Methods: This study involved 48 lactating Israeli mothers, 29 Jewish and 19 Bedouin (16–20 weeks postpartum), whose full-term infants were fed exclusively with breast milk. Total milk lipid extracts were transmethylated and analyzed by using an improved gas chromatographic method.

Results: The breast milk of the Bedouin women contained significantly higher levels of total major saturated fatty acids, lauric acid and palmitic acid ($45.2 \pm 4.7\%$ vs. $41.0 \pm 5.6\%$, $P = 0.005$; 5.2 ± 2.1 vs. $6.8 \pm 2.0\%$, $P = 0.03$; and 22.7 ± 2.4 vs. $20.6 \pm 3.8\%$, $P = 0.02$) respectively. No difference was found in the myristic acid level between the groups. The level of stearic acid was significantly higher in the Jewish group compared to the Bedouin group (5.7 ± 1.1 vs. $5.1 \pm 1.1\%$, $P = 0.04$). There was a linear correlation between the levels of C14:0 and C12:0 in the Bedouin and Jewish groups respectively ($R = 0.87$, $R = 0.82$, $P < 0.001$).

Conclusions: Higher levels of saturated fatty acids were measured in the breast milk of Bedouin women, an economically weaker population. The results emphasize the importance of diet among lactating women and its influence on milk quality.

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KEY WORDS: human milk, fatty acids, saturated fatty acids (SFA), nutrition, Jewish mothers, Bedouin mothers

fat-soluble vitamins and the n-6 and n-3 polyunsaturated fatty acids necessary for brain development [2]. Human milk fats vary widely around the world, depending on maternal dietary habits and socioeconomic conditions [3–5].

Cholesterol is an essential lipid component in cell membranes and is a precursor of bioactive lipids such as bile acids and steroid hormones. Cholesterol is a lipid component that is an essential for human survival from earliest life [6]. Cholesterol is synthesized in the body or absorbed from ingested food in the gastrointestinal tract.

Two types of dietary fatty acids raise plasma cholesterol: saturated fatty acids (mainly palmitic acid, C16:0) and trans-fatty acids [7]. After absorption from ingested milk, palmitic acid (C16:0) – the predominant dairy SFA – but also myristic acid (C14:0) and lauric acid (C12:0) are preferentially directed to triglyceride formation rather than to phospholipid acylation. These three long chain fatty acids also raise total cholesterol, but their effect on the ratio of low density lipoprotein to high density lipoprotein is different. Palmitic acid raises LDL-cholesterol more than it raises HDL-cholesterol [8]. Myristic acid increases total cholesterol as much as palmitic acid but does not affect the total cholesterol:HDL ratio [9,10]. Lauric acid (C12:0) is the most potent fatty acid in raising plasma total cholesterol [10]. Stearic acid (C18:0) improves the plasma cholesterol profile by decreasing the total:HDL-cholesterol ratio compared to other SFAs [11].

In general, the composition of maternal human milk fat in developing countries shows higher levels of saturated and monounsaturated fats, reflecting diets high in carbohydrates [12]. The higher quality diets of mothers in wealthier settings are reflected in the correspondingly higher levels of polyunsaturated fats contained in maternal milk [5]. Both total and LDL-cholesterol concentrations are higher in breast-fed babies than formula-fed babies [13,14]. Because of the importance of the composition of saturated fatty acids in infant nutrition, we sought to determine the profile of unsaturated fatty acids in breast milk of two populations in southern Israel: Jewish and rural Bedouin women. The two populations live in different

SFA = saturated fatty acid
LDL = low density lipoprotein
HDL = high density lipoprotein

It is well established that breast milk is the ideal source of nutrition for infants. Mature human breast milk generally contains 3.5% to 4.5% lipids, which comprise mostly triacylglycerols [1]. Human milk fats are the main source of energy for infants, providing 40–50% of their total energy intake. Additionally, it provides essential nutrients such as

settings and have different cultures. According to a report by Abu-Bader and Gottlieb [15], the Bedouin population lives in extremely poor socioeconomic conditions compared to the Jewish study population that lives in urban areas. All the Bedouin women who participated in the study were living in tents with no flowing water, garbage disposal or road system. The electricity they used was produced by a generator. The Jewish women had at least 12 years of schooling; the Bedouin women had 8 years or less.

The aim of this study was to analyze the saturated fatty acid composition of the mature breast milk of Israeli Jewish and Bedouin women, using gas chromatography. The study assumption was that an inverse relationship exists between socioeconomic status and content of saturated fat in breast milk.

SUBJECTS AND METHODS

This study involved 48 lactating Israeli mothers (16–20 weeks postpartum) from southern Israel whose full-term infants (born at 37–41 weeks gestation) were fed exclusively with breast milk. There were 29 Jewish women (age 31 ± 6 years, Group 1) and 19 Bedouin women (age 29 ± 5 years, Group 2). All the women consumed self-selected diets and were healthy. The study was approved by the institutional ethics committee of Soroka Medical Center, Ben-Gurion University of the Negev. Informed consent was obtained from all participants.

The mothers were interviewed by a medical doctor regarding their dietary habits in general. The purpose of this interview was to exclude from the study those women who had a special diet (for example, vegetarian), those taking supplements, or those suffering from any disease that might influence their fatty acids profile.

HUMAN MILK SAMPLES

Samples (60–100 ml) of mature breast milk were collected by the mother using a breast pump or expressing milk manually after washing the nipple with sterilized distilled water. The samples were transported on ice to the laboratory and immediately frozen and stored at -80°C until analysis. All samples were lyophilized (freeze-drying) to a dry powder by Lyophilizer (Laboratory Freeze Dryer, Millrock Technology Inc, NY, USA)

MATERNAL MILK LIPID PROFILE

The SFA profile of the human milk was analyzed using the following procedure, as described in the literature [14]. Fatty acid methyl esters were prepared using a simplified and efficient method for FAME analysis suitable for large clinical studies, as described by Masood et al. [15]. Lipids were extracted from freeze-dried milk by a mixture of

dichloromethane and methanol (2:1 v/v) after protein precipitation with a solution of 0.05 M CaCl_2 . The procedure was conducted in the dark and was followed by the addition of 0.005% butylated hydroxytoluene to prevent oxidation of PUFA and the spontaneous cis-trans isomerization of fatty acids. The methyl esters were prepared by KOH catalyzed trans-esterification in methanol.

FAME ANALYSIS

A sample of extracted fat was dissolved in hexane followed by the addition of a 2 M solution of KOH at room temperature. The mixture was vortexed for 10–15 min. After methanolysis occurred (based on thin layer chromatography analysis), the alkaline solution was neutralized with two to three drops of concentrated acetic acid. Solution pH was verified by pH paper. After neutralization, distilled water (2–3 ml) was added to the solution that was slowly mixed, and the hexane layer was separated. FAME in the hexane layer were analyzed by gas chromatography (Agilent 6820 GC System, Agilent Technologies, USA) at 260°C .

GAS CHROMATOGRAPHY ANALYSIS

A 1 μl sample from the upper hexane layer was directly injected into a gas chromatograph equipped with a flame ionization detector. A fused-silica SPTM-2560 capillary column (100 m \times 0.25 mm inner diameter, 0.20 μm film) was used under the following conditions: temperature was programmed from 140°C (5 min) to 240°C at a rate of $4^{\circ}\text{C}/\text{min}$, detector temperature was 260°C , and helium was used as a carrier gas with a flow of 20 cm/sec and a split ratio of 100:1. All peaks were identified by comparing their retention times relative to a standard based on known FAME (this fatty acids standard was purchased together with the capillary column from Eldan, Israel). Fatty acid quantification was calculated by internal normalization. Results were expressed as the weight percentage (% wt/wt) of all SFAs detected with chain lengths of 8–22 carbon atoms. Fatty acid quantification was determined with high precision.

STATISTICAL ANALYSIS

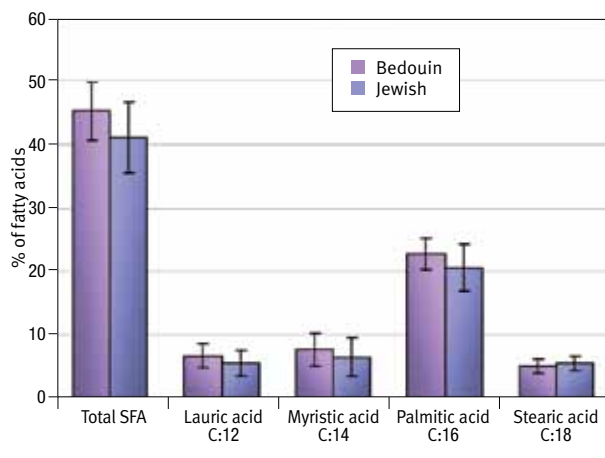
All data were analyzed using the statistical data analysis package provided with Microsoft Excel 97. Results are given as means \pm standard deviation. Correlations between the percentage (w/w) of the SFAs were examined using Pearson correlation coefficients. Relations were declared significant at $P < 0.05$.

RESULTS

This work focused on the saturated fatty acid content in the breast milk of two Israeli populations. The method used

FAME = fatty acid methyl esters

PUFA = polyunsaturated fatty acids

Figure 1. Distribution of total and major saturated fatty acids

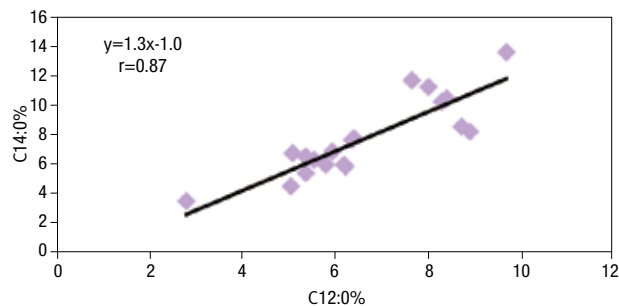
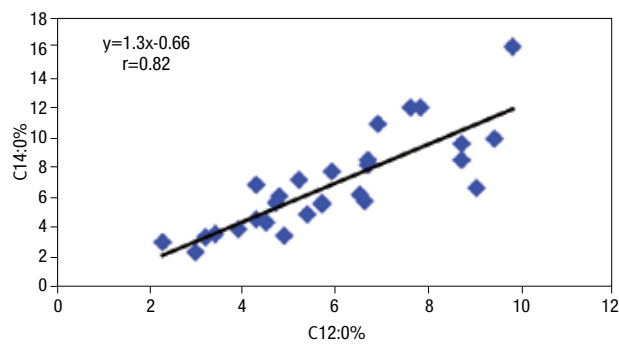
allowed high quality and accurate separation of fatty acids. Figure 1 shows the distribution of the total saturated fatty acids and the major saturated fatty acids (lauric, miristic, palmitic and stearic acids) in the two study populations. The results are presented as an average \pm SD.

The level of total major SFAs in breast milk in the Bedouin group was significantly higher than in the Jewish group ($45.2 \pm 4.7\%$ vs. $41.0 \pm 5.6\%$ respectively, $P = 0.005$). The level of lauric acid C12:0 was significantly higher in the Bedouin group compared to the Jewish group ($6.8 \pm 2.0\%$ vs. $5.2 \pm 2.1\%$ respectively, $P = 0.03$). There was also a significant difference in the levels of palmitic acid C16:0 between the Bedouin and Jewish mothers ($22.7 \pm 2.4\%$ vs. $20.6 \pm 3.8\%$ respectively, $P = 0.02$). The level of myristic acid C14:0 in breast milk was higher in Bedouin compared to Jewish mothers, but the difference was not statistically significant ($7.7 \pm 2.6\%$ vs. $6.6 \pm 3.1\%$ respectively, $P = 0.1$). The level of stearic acid C18:0 was the only SFA that was higher in the breast milk of the Jewish group compared to the Bedouin group ($5.7 \pm 1.1\%$ vs. $5.1 \pm 1.1\%$ respectively, $P = 0.04$).

There was a linear correlation [Figures 2 and 3] between the levels of C14:0 and C12:0 in the Bedouin and Jewish groups ($r = 0.87$, $r = 0.82$ respectively, $P < 0.001$). No correlation was found between levels of C14:0 vs. C16:0 ($r = -0.0034$) and C16:0 vs. C18:0 ($r = 0.12$) in both populations.

DISCUSSION

The purpose of this study was to compare the levels of saturated fatty acids in the breast milk of Jewish and Bedouin women. The results showed that by far the milk of Bedouin mothers contained higher levels of saturated fatty acids C12:0 and C14:0. These two SFAs are known to be harmful at high levels since they cause an elevation in the triglyceride and

Figure 2. Linear correlation between C14:0 and C12:0 in breast milk of Bedouin mothers**Figure 3.** Linear correlation between C14:0 and C12:0 in breast milk of Jewish mothers

cholesterol levels in human plasma. On the other hand, the levels of stearic acid C18:0 were significantly higher in the breast milk of the Jewish mothers. Stearic acid is the only saturated fatty acid that improves plasma cholesterol profile by decreasing total/HDL cholesterol ratio [11].

The linear correlation obtained between C14:0 and C12:0 in both populations, with r close to 0.8 ($P < 0.001$) indicates that about 80% of these fatty acids are synthesized de novo in the mammary glands. The fact that the slopes of the linear graphs were similar in both populations together with the similar regression coefficients (0.82, 0.87) indicate that about 20% of the fatty acid content in breast milk is the result of diet. We can assume that the significant differences between the two populations in C12:0 and C14:0 are probably not genetic but may be due to differences in diet, with the Bedouin population consuming a diet rich in carbohydrates and low in fats compared to the Jewish population [16]. These results reinforce previously published results on the endogenous synthesis of fatty acids in the mammary gland [17].

The lack of correlation between C14:0 vs. C16:0 and between C16:0 vs. C18:0 in both populations supports the fact that these fatty acids are not formed in the mammary glands and are the result of diet alone [17]. Therefore, it is

more likely that a healthy diet will reduce fatty acids C14:0 and C16:0 and will increase C18:0. These data are also supported by previous work [5,17,18]. In addition to the importance of diet, it was shown in a recent study that the time period of breastfeeding also has an impact on milk quality. Women who lactated for more than 1 year had higher C12 and C14 fatty acid percentages in their milk than women who lactated for 2–6 months [19].

In conclusion, this study confirmed our initial hypothesis that the levels of saturated fatty acids in breast milk are higher in women from an economically weak population, in this case the Bedouin. We attribute this mainly to the high consumption of flour products (carbohydrates) among Bedouin. The saturated fatty acids C12:0 and C14:0 are known to promote hypercholesterolemia. Furthermore, DHA (omega 3), present in human milk, is essential for the central nervous system since early deficiencies in key nutrients have the potential to exert a long-term adverse effect on cognitive and behavioral functions [20]. The study findings emphasize the influence of low socioeconomic state – and the associated poor diet – on milk quality among lactating women, and demonstrate the need for intervention. This intervention mandates the improvement of nutrition by creating nutritional guidelines for breastfeeding woman, such as reducing the consumption of carbohydrate and saturated fats and raising the consumption of unsaturated vegetable fats.

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Corresponding author:

Dr. O. Saphier
 Dept. of Chemical Engineering, Shamoan College of Engineering, Beer Sheva 84100, Israel
Fax: (972-8) 647-5636
email: oshras@sce.ac.il

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“I shall live badly if I do not write, and I shall write badly if I do not live”

Francoise Sagan (1935-2004), French playwright, novelist and screenwriter

“We are like tenant farmers chopping down the fence around our house for fuel when we should be using Nature’s inexhaustible sources of energy -- sun, wind and tide. ... I’d put my money on the sun and solar energy. What a source of power! I hope we don’t have to wait until oil and coal run out before we tackle that”

Thomas Edison (1847-1931), American inventor who developed many devices that greatly influenced life around the world, including the phonograph, the motion picture camera, and a long-lasting practical electric light bulb. He was one of the first inventors to apply the principles of mass production and large-scale teamwork to the process of invention, and is thus often credited with the creation of the first industrial research laboratory. He holds 1093 U.S. patents