Fatty acid composition of human milk in Kuwaiti mothers

L. Hayat, M.A. Al-Sughayer, M. Afzal *

Department of Biological Sciences, Faculty of Science, Kuwait University, Kuwait, Kuwait

Received 2 September 1998; received in revised form 13 June 1999; accepted 25 June 1999

Abstract

Kuwaiti diet is exceptionally rich in fat, carbohydrates and proteins. In addition, subjects in Kuwait are exposed to extreme heat and sun light. Fatty acid profiles of human milk obtained from 19 full breast feeding Kuwaiti mothers were analyzed. Dietary patterns for individual mothers were determined by 24 h dietary recall and food frequency questionnaire. The fatty acid content of human milk was affected by the diet consumed by the lactating mother. The content of long chain polyunsaturated fatty acids (LCP) in human milk lipids did not correlate with their parent fatty acids like linoleic and α-linolenic acids. However the human milk LCP were related to the content of LCP in the maternal diet. Mothers reporting a high fish consumption showed significant amounts of C22:6, α3 and C20:5, α3 fatty acids. As a general conclusion, breast milk produced by a well nourished mother is better suited to meet the lipid requirements of infants. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Arachidonic; Decosahexaenoic; Ecosapentaenoic; Geographic distribution; Mother milk; Trans-fatty acids

1. Introduction

During pregnancy, the fetus receives a continuous intravenous infusion of high-carbohydrate and low-fat diet from its mother. After birth, this is replaced by a discontinuous eternal high-fat, low-carbohydrate breast milk. During the last trimester of fetal development, α3 and α6 fatty acids are the essential components of structural lipids (e.g. brain). The major long chain polyunsaturated fatty acid (LCP) components in the brain are of the α3- and α6-class [4].

The fetus receives its required fatty acids through placental transfer, while the breast-fed neonate receives the same through mammary glands. The human placenta is permeable to free fatty acids (FFA) and these substrates do not contribute to fetal oxidative metabolism, since fetal tissues have a very low capacity for FFA oxidation [11]. Accordingly, they are stored in the adipose tissue and the liver [12].

At birth, a great metabolic adaptation is observed. Gluconeogenesis and ketogenesis which are absent or very low in fetal liver, emerge after birth to reach adult values after only 24 h. The supply of gluconeogenic substrates and of FFA is of crucial importance to support a high rate of gluconeogenesis and to maintain normoglycemia in the new-born [10].

There are three main sources of fatty acids present in a mothers’ milk: diet, biosynthesis in the mammary gland, and mobilization from other sites, like adipose tissue. The fatty acids in adipose tissue, in lactating women, are a mixture from the diet and endogenous synthesis which is dependent on the caloric balance and the type of diet [14].

The fatty acid composition of human milk shows considerable variation with maternal diet. Thus geographic, cultural traditions, socioeconomic status, and maternal metabolism play an important role [7,19,25]. The fatty acid content of breast milk is changed as lactation proceeds and presumably as the requirement of the infant changes, so the mothers’ diet is important. The energy content of the mothers’ diet is obviously important since a shortfall in calories may restrict endogenous fatty acid synthesis by the mother and/or fetus such that the final fatty acid deficiency may involve all types of fatty acids.

Many studies have demonstrated the relationship between maternal dietary intake and milk fatty acid composition and have shown a dietary effect on both

* Corresponding author. Tel.: +965-484-8437; fax: +965-482-0794.
E-mail address: afzal@kuc01.kuniv.edu.kw (M. Afzal)

0305-0491/99/$ - see front matter © 1999 Elsevier Science Inc. All rights reserved.
PII: S0305-0491(99)00112-1
mobilization of fats from body stores and from endog-
eneous synthesis [13]. Human milk lipids are affected by
maternal diet: when on a high fat diet, the women
under study produced milk, high in triacylglycerols of
LCP, reflecting the fatty acid composition of the dietary
fat and low endogenous fatty acid synthesis [13]. While
on a low fat, calorie-restricted diet, the milk predomi-
nates in triacylglycerols of LCP derived from body fat
sources.

Medium-chain fatty acids (MCFA) are not affected
directly by the mothers diet because they are synthe-
sized in the mammary gland. Parturition, irrespective of
length of pregnancy, triggers the synthesis of MCFA
which is specific to the mammary gland, because only
this tissue contains a specific thioesterase II, that termin-
nates chain elongation at 6–14 carbon atoms [26].

The LCP content of human milk is less affected by
variations in maternal diet. The balanced ratio of ω6-
and ω3-LCP in milk may reflect a protective metabolic
mechanism for breast-fed infants against alterations in
maternal diet [5]. The dietary intake of fish and marine
products containing abundant ω3 such as ecosapen-
taenoic acids (EPA) and docosahexaenoic acid (DHA)
in lactating mothers is reported to elevate the dietary
ω3/ω6 ratio [27].

The trans-fatty acid content in human milk depends
mainly on the mothers’ recent dietary intake, including
hydrogenated vegetable oils, margarine, shortenings,
and processed food. The dietary intake of trans-fatty
acids correlate well with their concentration in human
milk [1]. Mothers, with a preference for margarine, are
known to have significantly higher milk levels of trans-
fatty acids than those who preferred butter [7].

The previous investigators have also made an impor-
tant observation that the rate of post-pregnancy weight
loss significantly affects the level of elaidic acid (C18:1t)
in milk and this may have a large influence on milk
trans-fatty acid levels relative to the maternal diet. For
example, mothers losing 0–2 kg by week 5 postpartum,
have a milk elaidic acid concentration of 1.5–2.0%,
while mothers losing 4–7 kg by week 5 had a concen-
tration of 2.8–3.5%. The higher levels of C18:1t in milk
from mothers who lose weight more rapidly can be
explained by mobilization of fatty acids from adipose
tissue [1,7]. Since no study has reported the effect of
diet on the fatty acid pattern in mothers living in the
Middle East, a comparative study of Kuwaiti mothers
milk is the objective of the present effort. Kuwaiti
subjects consume diets rich in fat, carbohydrates, and
proteins. Since geographic, cultural traditions, socioe-
omic status have a profound effect on the mothers’
milk composition, the purpose of this study was to
examine any possible differences in Kuwaiti mothers’
milk composition who consumed Kuwaiti diet, rich in
fat and proteins and carbohydrates, and were exposed
to extreme temperature and sun light. The fatty acid
pattern was examined by gas–liquid chromatography
to quantify these compounds. The fatty acid pattern
was examined in Kuwaiti mothers with traditional di-
etary habits and compared to that of mothers from
other geographic origin. These studies provide evidence
of the qualitative and quantitative differences in fatty
acids of Kuwait mothers’ milk.

2. Materials and methods

2.1. Mothers’ milk sample collection and preparation

The collection procedure was approved by the
Kuwait University Medical School Human Subjects
Committee. Breast milk samples were obtained from 19
healthy Kuwaiti mothers at 20–30 years of age and at
6–14 weeks postpartum. All subjects were in good
health and delivered healthy full term infants. Samples
were collected in screw cap vials, between 10:00–12:00
h from both breasts by the aid of a battery operated
pump expression and pooled. Samples were placed on
ice, and transferred to the laboratory for immediate
processing. Volume and weight for each sample were
measured. Milk samples were frosted for 1 h in a freeze
shell bath containing methanol and then lyophilized.
Weights of the lyophilized samples were taken and
stored at 4°C under nitrogen in tightly closed screw cap
bottles. The method was standardized by storing the
samples with a known amount of C21:2 as an internal
standard.

All mothers were individually interviewed at their
homes and their age, self-reported weight and height,
their infant’s sex and date of birth were recorded and
body mass index (BMI; weight height $^{-2}$) of the mother
was calculated. For specified mothers, age range, over-
weight margin was considered when BMI > 25, while
obesity margin was considered when BMI > 30 [22].

2.2. 24 h dietary recall

The calculation of energy and nutrient supply of the
mothers’ diet was made by ‘the recall method’. Mothers
were asked to list everything they had ingested during
the last 24 h. Measuring cups and spoons were used as
standard measurement units. Nutrient intake was
analyzed by using the ‘N-Squared’s Nutritionist III’
computerized dietary analysis package and food-
composition tables, and compared to recommended
dietary allowance (RDA) values for lactating mothers
in the first six month period.

2.3. Food frequency questionnaire

The food frequency questionnaire collected informa-
tion on the intake of specific groups of foods on a daily,
weekly and monthly basis. The questionnaire was of a selective type including only three categories: cholesterol, saturated fat and unsaturated fat. Mothers were asked about the frequent intake of some common foods related to these groups. Food frequency was analyzed statistically as described for recall.

2.4. Fatty acid determination

The lypholized milk samples were extracted by the Folch method [8] and the total lipid content was gravimetrically measured. Fat samples were stored in screw cap glass vials at \(-20^\circ\text{C}\), in the presence of a minimal amount of organic solvent containing the antioxidant butylated hydroxytoluene.

Modification of the transesterification reaction was made by using benzene as a solvent to dissolve fat. Thus 10–20 µg of extracted lipid was dissolved in 1 ml of sodium dried benzene prior to the addition of methanolic boron trifluoride BF3 (0.5 ml) and the tightly closed vials left in an oven at 60°C for 30 min. No lipid globules were observed in the reaction vial indicating a complete suspension of fat in the mixture leading to complete transesterification. The reaction mixture was cooled, 1 ml of water added and then extracted with hexane (3 \times 1 ml). The hexane extract was collected and dried over anhydrous sodium sulfate. The solution was filtered through a 10 ml glass syringe fitted with a micro-filtration tip and the filtrate evaporated to dryness under nitrogen gas at 35°C to give a mixture of fatty acid methyl esters (FAME).

2.5. Gas chromatography

Methyl esters of fatty acids were determined by using a split-injection capillary gas-chromatographic-profiling method. FAMEs were separated on an auto-system gas chromatograph (Perkin–Elmer Corporation), equipped with a WCOT (50 m) fused silica capillary column (0.25 mm internal diameter) coated with CP-Sil 88 for FAME analysis (Chrompack), using a flame ionization detector (FID). Nitrogen was used as carrier gas at an inlet pressure of 22.0 psi. The injector temperature was 270°C while the detector temperature was fixed at 300°C. A programmed operating temperature was applied for the oven. The initial oven temperature was 80°C (0 min) and was programmed to increase to 160°C at 10°C min\(^{-1}\), then heated to 210°C at 5°C min\(^{-1}\) for 5 min, followed by 220°C at 2°C min\(^{-1}\) for 4 min. Total run time was 32 min. Samples were dissolved in chromatographically pure dichloromethane before injection. Samples (0.3 µl) were injected and the data were analyzed with Omega-2 software (version 5.0, Perkin–Elmer). Results are expressed as relative amounts (% wt/wt of total fatty acids).

2.6. Standard fatty acid methyl esters

Transmethylated fatty acids were identified from their relative retention times when compared with an authentic FAME mixture (\# 189-19/Sigma Chemical, St Louis, MO) containing 37 fatty acid methyl esters (C4:0–C22:6). Standards were analyzed under the same conditions as the samples. The relative amounts of the fatty acids were achieved from their peak areas.

2.7. Statistical analyses

The statistical analyses were based on the assumption of normal distribution of variables. All data were expressed as mean \(\pm\) standard deviation (S.D.). For individual fatty acids, mean and S.D. were calculated for \(n\) readings for each group. For total fatty acid categories, weighted mean and weighted S.D. were calculated by multiplying each total value by \(n\) readings prior to the calculation of weighted mean and S.D. Pearson’s correlation coefficients were applied to examine relationships between dietary intake of mothers and fatty acid content of their breast milk. A two-tailed test of significance was selected and positive and negative correlations were considered significant at \(p\) values of \(< 0.05\) and \(< 0.01\). All analyses were carried out using SPSS for Windows (version 6.1.3, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Characteristics of milk donating mothers

Nineteen mothers, 20–30 years of age, out of twenty-nine lactating women (65.5%), were enrolled in this study. Two hundred and seventeen Kuwaiti mothers were interviewed to determine their babies feeding pattern. It was found that only 13.4% breast feed their babies for a minimum of 100 days postpartum, while others were either incomplete breast feeders, or they had introduced formulas at early stages of infant life as shown in Table 1. Mothers who introduced mixed type feedings (breast milk and infant formula) for their infants were not included in this study.

Table 2, represents the main characteristics of milk donating mothers and their milk. The mean value of BMI was relatively high (29.31 kg m\(^{-2}\)). Four mothers were classified as overweight (BMI > 25) and eleven were classified obese (BMI > 30). A high percentage of obesity was observed among milk donating...
milk fat content (g dl$^{-1}$) and extracted human milk fat was 4.69 g dl$^{-1}$ (37% of milk composition). As the samples in this study were collected between 10:00–12:00 h they do not allow for diurnal variation. The fat content found in this study was similar to reported values for fat in human milk during the first three months of lactation [6].

A positive correlation was found between milk fat content (g dl$^{-1}$) and dried milk weight (g dl$^{-1}$) ($r = 0.9307$, $p < 0.01$) and a negative correlation between milk fat content (g dl$^{-1}$) and liquid milk density (g ml$^{-1}$) ($r = -0.5373$, $p < 0.05$). These results suggest an increase in dried milk weight with an increase in total fat content. Since fat density is lower than water, this explains the negative correlation between milk fat content and liquid milk density. A high fat content is known to decrease the milk density. These results also suggest that an increase in human milk fat content and as a consequence an elevated milk solute weight could lead to changes that keep milk density in the acceptable range. Whether the milk

Table 1
Type of infant feedings during 100 day postpartum for all interviewed Kuwaiti mothers

<table>
<thead>
<tr>
<th>Type of feeding</th>
<th>No. of mothers</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast feeding</td>
<td>29</td>
<td>13.4</td>
</tr>
<tr>
<td>Formula feeding</td>
<td>42</td>
<td>19.4</td>
</tr>
<tr>
<td>Mixed feeding</td>
<td>20</td>
<td>9.2</td>
</tr>
<tr>
<td>Breast feeding ≤ 30 days then mixed feeding</td>
<td>15</td>
<td>6.9</td>
</tr>
<tr>
<td>Breast feeding ≤ 60 days then mixed feeding</td>
<td>26</td>
<td>12.0</td>
</tr>
<tr>
<td>Breast feeding ≤ 30 days then formula feeding</td>
<td>24</td>
<td>11.1</td>
</tr>
<tr>
<td>Breast feeding ≤ 60 days then formula feeding</td>
<td>13</td>
<td>6.0</td>
</tr>
<tr>
<td>Mixed feeding ≤ 30 days then formula feeding</td>
<td>22</td>
<td>10.1</td>
</tr>
<tr>
<td>Mixed feeding ≤ 60 days then formula feeding</td>
<td>26</td>
<td>12.0</td>
</tr>
<tr>
<td>Total</td>
<td>217</td>
<td>100</td>
</tr>
</tbody>
</table>

mothers (58%). Dried milk weight averaged 12.7 g dl$^{-1}$ and extracted human milk fat was 4.69 g dl$^{-1}$ (37% of milk composition). As the samples in this study were collected between 10:00–12:00 h they do not allow for diurnal variation. The fat content found in this study was similar to reported values for fat in human milk during the first three months of lactation [6].

A positive correlation was found between milk fat content (g dl$^{-1}$) and dried milk weight (g dl$^{-1}$) ($r = 0.9307$, $p < 0.01$) and a negative correlation between milk fat content (g dl$^{-1}$) and liquid milk density (g ml$^{-1}$) ($r = -0.5373$, $p < 0.05$). These results suggest an increase in dried milk weight with an increase in total fat content. Since fat density is lower than water, this explains the negative correlation between milk fat content and liquid milk density. A high fat content is known to decrease the milk density. These results also suggest that an increase in human milk fat content and as a consequence an elevated milk solute weight could lead to changes that keep milk density in the acceptable range. Whether the milk

Table 2
Characteristics of mothers donating milk in the present study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Range</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20–27</td>
<td>24.42 ± 2.04</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49–105</td>
<td>76.29 ± 16.94</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>152–173</td>
<td>160.92 ± 5.67</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>18.2–37.6</td>
<td>29.31 ± 5.42</td>
</tr>
<tr>
<td>Day after postpartum</td>
<td>47–99</td>
<td>71.95 ± 16.7</td>
</tr>
<tr>
<td>Milk density (g ml$^{-1}$)</td>
<td>1–1.03</td>
<td>1.01 ± 0.01</td>
</tr>
<tr>
<td>Dried milk weight (g dl$^{-1}$)</td>
<td>10.2–16.5</td>
<td>12.70 ± 1.84</td>
</tr>
<tr>
<td>Milk fat content (g dl$^{-1}$)</td>
<td>1.77–8.01</td>
<td>4.69 ± 1.71</td>
</tr>
</tbody>
</table>

Table 3
Fatty acid composition (% wt/wt)$^a$ of Kuwaiti mother milk fat

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Human milk (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturates</td>
<td></td>
</tr>
<tr>
<td>8:0</td>
<td>0.15 ± 0.03 (1)$^b$</td>
</tr>
<tr>
<td>10:0</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>12:0</td>
<td>6.01 ± 1.79</td>
</tr>
<tr>
<td>14:0</td>
<td>6.41 ± 1.97</td>
</tr>
<tr>
<td>15:0</td>
<td>0.41 ± 0.17 (2)</td>
</tr>
<tr>
<td>16:0</td>
<td>21.79 ± 3.09</td>
</tr>
<tr>
<td>17:0</td>
<td>0.37 ± 0.09 (24)</td>
</tr>
<tr>
<td>18:0</td>
<td>6.53 ± 0.98</td>
</tr>
<tr>
<td>20:0</td>
<td>0.39 ± 0.16 (27)</td>
</tr>
<tr>
<td>Total</td>
<td>42.85 ± 5.18</td>
</tr>
<tr>
<td>Monounsaturates</td>
<td></td>
</tr>
<tr>
<td>14:1t</td>
<td>1.8 (27)$^b$</td>
</tr>
<tr>
<td>14:1</td>
<td>0.36 ± 0.13 (18)</td>
</tr>
<tr>
<td>16:1 (PI)</td>
<td>0.31 (27)</td>
</tr>
<tr>
<td>16:1t</td>
<td>0.50 ± 0.12 (1)</td>
</tr>
<tr>
<td>16:1</td>
<td>2.94 ± 0.84</td>
</tr>
<tr>
<td>17:1t</td>
<td>0.25 (26)</td>
</tr>
<tr>
<td>17:1</td>
<td>0.31 ± 0.08 (29)</td>
</tr>
<tr>
<td>18:1t</td>
<td>2.75 (26)</td>
</tr>
<tr>
<td>18:1</td>
<td>32.49 ± 3.68</td>
</tr>
<tr>
<td>20:1t</td>
<td>0.46 ± 0.13 (27)</td>
</tr>
<tr>
<td>20:1</td>
<td>0.93 ± 0.32</td>
</tr>
<tr>
<td>24:1</td>
<td>0.54 (27)</td>
</tr>
<tr>
<td>Total 06 LCP</td>
<td>2.02 (11)</td>
</tr>
<tr>
<td>Total 06 series</td>
<td>19.64 ± 4.88</td>
</tr>
<tr>
<td>03 series</td>
<td></td>
</tr>
<tr>
<td>18:3</td>
<td>0.42 ± 0.14</td>
</tr>
<tr>
<td>20:5</td>
<td>0.67 ± 0.23 (28)</td>
</tr>
<tr>
<td>22:5</td>
<td>0.49 ± 0.03 (29)</td>
</tr>
<tr>
<td>22:6</td>
<td>0.60 ± 0.24 (1)</td>
</tr>
<tr>
<td>Total 03 LCP</td>
<td>1.76 (21)</td>
</tr>
<tr>
<td>Total 03 series</td>
<td>1.02 ± 0.71</td>
</tr>
<tr>
<td>Total polyunsaturates</td>
<td>20.27 ± 4.72</td>
</tr>
<tr>
<td>Total trans-fatty acids</td>
<td>2.80 ± 1.75 (30)$^b$</td>
</tr>
<tr>
<td>Total cis-fatty acids</td>
<td>56.23 ± 5.34</td>
</tr>
<tr>
<td>trans/cis-fatty acids</td>
<td>0.052 ± 0.034</td>
</tr>
<tr>
<td>18:2 06/18:3 03</td>
<td>46.83 ± 22.96</td>
</tr>
<tr>
<td>06PUFA/03 PUFA</td>
<td>45.76 ± 24.61</td>
</tr>
<tr>
<td>06 LCP/03 LCP</td>
<td>1.15</td>
</tr>
<tr>
<td>Polyunsaturated/saturated</td>
<td>0.49 ± 0.16</td>
</tr>
</tbody>
</table>

$^a$ Mean ± S.D.  
$^b$ n detectable samples.
3.2. Fatty acid content in human milk

Saturated fatty acids constituted 43.0% of the total fatty acids in mature breast milk (Table 3). In a European study, the average saturated fatty acid content of mature human milk has been reported to be 44.9% [17]. Palmitic acid accounted for half of the saturated fatty acid content (50.8%), followed by stearic (6.5%), myristic (6.4%) and lauric acids (6.0%). The high percentage of palmitic acid in human milk, with a high absorption efficiency, is known to be a potential source of energy [2]. These fatty acids can either be synthesized de novo in mammary tissue or derived from the mothers’ diet [15].

Unsaturated fatty acids accounted for 57.5% of the total fatty acids in mature breast milk, of which 37.3% were monounsaturated fatty acids. The major fatty acid fraction in human milk was represented as oleic acid (32.5%), an important source of energy, which was consistent with the reported values [18]. Palmitoleic acid and eicosanoic acid were detected in all mother milk samples. Close results for monounsaturates in mother milk (38.0%), with major proportion contributed by oleic acid have also been reported [17].

The essential fatty acid, linoleic acid (ω6) content averaged 17.4%, and ranged from 11.0–26.5%. In the present study linoleic acid level was found to be higher than reported human milk values in Spain (12.02%) [27], Germany (10.8%) [18] and Australia (11%) [9]. The mean for ω3-linolenic acid (ω3) content was 0.42% in Arab mothers’ milk on the lower side of the mean average (0.3–2.4%) reported for other studies which amounted to 7.2 and 4.4%, respectively [3,18]. This again reflects the regional differences of these fatty acids in human milk, which is in agreement with the earlier reports [18]. Thus, the LCP content of human milk appeared not to be closely related to the maternal intake of their parent fatty acids. This finding suggested interindividual and/or regional differences in the capacity of desaturating and chain-elongating pathways.

The current study showed a high mean linoleic acid to ω3-linolenic acid ratio (ω6/ω3) of 46.83 for the 19 milk samples. This value is extremely high as compared to other studies which were reported to be in the range of 6.3–19.7, except for two African studies showing higher values, 40.5 and 157.0 [16] where subjects are exposed to higher temperature and light intensities. The dietary linoleic to ω3-linolenic acid ratio is more important than the absolute intake of each of these essential fatty acids due to the competition for the same enzyme for further desaturation and chain-elongation to biologically active LCPs.

In the present study total trans-fatty acids (C14:1t, C16:1t, C17:1t, C18:1t, C20:1t and C18:2t) were found to be 2.8% in mothers’ milk samples. This value for trans-fatty acids is low, as compared to Canadian and German women which amounted to 7.2 and 4.4%, respectively [3,18]. This again reflects on the regional differences of these fatty acids in mothers’ milk. Trans-isomers detected in human milk also reflect the mothers’ dietary intake of trans-fatty acids [1,7] and higher levels alter the metabolism of essential fatty acids and early growth [16].

Table 4
Kuwaiti mother diet pattern with reference to RDA values for lactating women — 1st 6 months

<table>
<thead>
<tr>
<th>Component</th>
<th>RDA lactating-1st 6 months</th>
<th>RDA% Kuwaiti mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>10</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>50</td>
<td>54 ± 11</td>
</tr>
<tr>
<td>Fat</td>
<td>30</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>Kcal</td>
<td>2700</td>
<td>94 ± 33</td>
</tr>
<tr>
<td>Protein</td>
<td>65 g</td>
<td>146 ± 48</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>338 mg</td>
<td>108 ± 46</td>
</tr>
<tr>
<td>Fat</td>
<td>90 mg</td>
<td>98 ± 45</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>300 mg</td>
<td>106 ± 89</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>30 mg</td>
<td>48 ± 43</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>30 mg</td>
<td>44 ± 42</td>
</tr>
<tr>
<td>Polyunsaturated fat</td>
<td>30 mg</td>
<td>17 ± 14</td>
</tr>
<tr>
<td>PUFA/SFA*</td>
<td></td>
<td>57 ± 49</td>
</tr>
</tbody>
</table>

* PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
3.3. The effect of maternal diet on fatty acid content of breast milk

Table 4 presents the diet pattern of Kuwaiti mothers who took part in this study. According to published RDA (recommended daily allowance) tables for nutrient intake of lactating women at the first six months, proteins account for 10% of total calories, carbohydrates 50% and fats 30%. Kuwaiti mothers calories intake showed a higher calorie percentage yield from proteins and carbohydrates (14 and 55%), as compared to daily recommended intake. However energy from fat consumption was within the recommended limits (31%).

High standard deviations were observed in most of the nutrients intake. Carbohydrates, fat and cholesterol consumption were all close to the recommended values of daily intake but consumption of protein was high (146% of RDA). The daily consumption of saturated, monounsaturated and polyunsaturated fatty acids was very low and could reflect on the fatty acid pattern in breast milk.

Correlation analysis between fatty acids in Kuwaiti mothers’ milk and the dietary intake parameters of these mothers were tested. As reported earlier, [21] no correlation was observed between total calorie intake and the dried milk weight, or fat content. The general lack of effect of diet on total milk fat could arise from the use of depot fat to synthesize milk fat. However a high degree of variation in milk fat among individuals, during each nursing and during the day has been observed [20], which makes it difficult to detect significant differences. Thus, a 24 h milk collection may represent the ideal breast milk sample rather than one nursing sample.

Body mass index (BMI), was positively correlated to breast milk content of C14:0 (r = 0.5075, p < 0.05), and also correlated to total saturated fatty acid content (r = 0.5734, p < 0.05). In addition C12:0 and C14:0 were not affected by the mothers’ diet; indicating that these breast milk fatty acids (medium-chain fatty acids) were not from dietary sources, but synthesized in the body by the mammary gland. Furthermore, BMI negatively correlated with C18:1 (r = −0.5356, p < 0.05) and total cis-fatty acids (r = −0.5984, p < 0.05). Thus, milk produced by obese mothers had low C18:1 and high C14:0.

The analysis of milk donated by mothers who consumed a high calorie percentage of proteins showed high C16:0 (r = 0.5658, p < 0.05) and low C18:2 (r = −0.5384, p < 0.05) content. Low Ω6-PUFA (r = −0.4857, p < 0.05) and correspondingly low total polyunsaturated fatty acids (PUFA) (r = −0.4643, p < 0.05) were observed in the milk of these mothers. There was a significant correlation between Ω3-PUFA concentration and calorie percentage from carbohydrates (r = −0.7120, p < 0.01) and fat (r = 0.0324, p < 0.01). Therefore a balanced diet is important for the mother to achieve the best milk fat profile.

Dietary fat consumed by the mother influences Ω3-PUFA concentration in her breast milk (r = 0.4939, p < 0.05) which is also affected by the consumption of carbohydrates (r = −0.4570, p < 0.05). Therefore we expected the mothers’ high carbohydrate intake should raise the Ω6/Ω3 in her milk (r = 0.5299, p < 0.05). However, no correlation was found between the concentration of medium chain fatty acids and the level of carbohydrate intake as reported in earlier studies [13], suggesting an increase in endogenous synthesis of these fatty acids with high maternal carbohydrate intake. There was a significant effect of protein intake on the milk fatty acid pattern. In addition a positive correlation between cholesterol intake and linoleic acid content (r = 0.4867, p < 0.05) was observed. The low intake of mono-unsaturated fatty acid in the mothers’ diet was reflected in a high level of palmitic acid (r = −0.4795, p < 0.05) and correlated positively with the Ω6/Ω3 (r = 0.5042, p < 0.05) ratio.

PUFA in the mothers’ diet affected many fatty acid profiles. High dietary intake resulted in milk with high linoleic acid (r = 0.6561, p < 0.01), Ω6-PUFA (r = 0.6419, p < 0.01), total PUFA (r = 0.6338, p < 0.01), Ω6/Ω3 (r = 0.6341, p < 0.01), Ω6/Ω3 (r = 0.5990, p < 0.01) and linoleic/α-linolenic acids (r = 0.6317, p < 0.01) but showed low palmitic concentration (r = −0.5138, p < 0.05). No correlation was found between dietary linoleic acid intake and milk Ω6-LCP and Ω3-LCP. This observation suggested either an inefficient biosynthesis of LCP from parent essential fatty acid, or weak transfer of these fatty acids into the mammary gland. Only C18:0 in milk fat was found to be affected by the mother dietary consumption of saturated fatty acids (r = 0.4586, p < 0.05).

In only two milk samples were Ω3-LCPs (C20:5, C22:5 and C22:6) detected. These milk samples were collected from mothers who consumed about 300 gm fish in their diet 20 h before milk collection. Similar observations have been reported by other workers [7]. The level of docosahexaenoic acid DHA in breast milk is reported to be directly proportional to DHA in the maternal diet and women with high fish diets had elevated breast milk DHA [23].

Diets with high PUFA/SFA ratios resulted in high total trans-fatty acids (r = 0.7447, p < 0.01) and as a consequence, a high trans/cis fatty acid ratio in human milk (r = 0.7413, p < 0.01). Lowering trans-isomer fatty acids in the diet is therefore recommended. Also, linoleic acid concentration and PUFA/SFA ratio in milk were well correlated (r = 0.9521, p < 0.01) as reported by Lonnerdal [21]. These results show regional differences in human milk and its compositional dependency on the type of the diet intake and also exposure to heat and sun light.
Acknowledgements

M.A.S. gratefully acknowledges research grant from School of Graduate Studies, University of Kuwait, to carry out this study.

References