

## Effects of dairy-products' consumption on sebum lipids and fatty acids

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### ABSTRACT

**Objectives:** To investigate the effect of intake of dairy-products on the sebum lipid components and fatty-acid composition.

**Patients and Methods:** Sebaceous cysts were obtained, by simple surgery, from the scalp, face and neck of 21 men (aged 25-50 y) and divided into two groups. The first group included 11 cysts for subjects consuming dairy products and the second group included 10 cysts for subjects not consuming dairy products. Simple lipids, phospholipids (PLs), triglycerides (TGs), cholesterol (C), cholesterol ester (CE) and fatty acids (FAs) were removed by organic solvents extraction. The sebum lipid components, TGs and C, were determined enzymatically and PLs were determined using a colorimetric method based on the formation of a phosphomolybdate complex. Lipid components were separated by TLC. The separated components were hydrolyzed and their FAs were esterified by super dried-acidified methanol. Fatty-acid methyl esters were identified by capillary gas chromatography.

**Results:** Compared to the non-consumers group, the consumers group exhibited a significant increase in lipid contents, viz, PLs and C. Concerning the FA composition of CE, PLs and TGs,  $n_6$ -polyunsaturated FAs showed a higher percentage, viz.  $C_{18:2} n_6$  and  $C_{9,t11}$  CLA, while  $n_3$ -polyunsaturated FAs showed a significant decrease, viz.  $C_{20:5} n_3$ .

**Conclusion:** Consumption of dairy products affects the lipid content and the FA composition of sebum and  $C_{9,t11}$  Conjugated Linoleic Acid could be used as a marker for intake of dairy-product lipid.

### الخلاصة

**أهداف البحث:** دراسة تأثير تناول الحليب ومشتقاته على المواد الدهنية وتركيبية الأحماض الدهنية لمحتويات الأكياس الدهنية.

**العينات وطرائق العمل:** تم الحصول على أكياس دهنية استئصلت بجراحة بسيطة من مناطق مختلفة من الجسم (الرأس، الوجه والعنق) من ٢١ من الذكور تراوحت أعمارهم بين ٢٥ - ٥٠ سنة. تم تقسيم العينات الى مجموعتين، الأولى شملت ١١ كيسا دهنيا تم الحصول عليها من أشخاص يتناولون منتجات الحليب بصورة مستمرة في حين شملت المجموعة الثانية ١٠ أكياس دهنية تم الحصول عليها من أشخاص لا يتناولون منتجات الحليب. تم قياس المكونات الدهنية لمحتويات الأكياس (الكليسيريدات الثلاثية بطريقة انزيمية، الدهون المفسفرة بطريقة لونية، الكولستيرول الكلي والكولستيرول المؤسטר بطريقة انزيمية)، ثم تم تحليل الحوامض الدهنية لكل من هذه المكونات باستثناء الكولستيرول الحر، بعد فصلها بواسطة كروماتوغرافيا الشرائح الرقيقة، بطريقة كروماتوغرافيا الغاز السائل الشعري.

**النتائج:** بينت النتائج ان النماذج المأخوذة من أشخاص يتناولون منتجات الحليب تحتوي على نسب أعلى لجميع المكونات الدهنية. أما الحوامض الدهنية فقد بينت النتائج ان نماذج المجموعة المستهله لمنتجات الحليب كانت تحتوي على نسب أعلى من الحوامض الدهنية المتعددة الأواصر المزوجة.

**الاستنتاج:** ان تناول منتجات الحليب يؤثر على المحتويات الدهنية وتركيب الأحماض الدهنية للأكياس الدهنية ويعتبر حامض اللينوليك المزوج مؤشرا على ذلك .

The sebaceous follicle, in which the sebaceous gland is greatly enlarged, is filled with keratinous material, sebum, bacteria and fungi. The sebum secreted from sebaceous glands keeps the skin of the human body soft and oily<sup>(1,2)</sup>. Sebum is a complex lipid mixture and its complete chemical nature has not been fully elucidated and it varies widely from species to species<sup>(2,3)</sup>. Free fatty acids (FAs), triglycerides (TGs), cholesterol (C), cholesterol ester (CE), phospholipids (PLs), squalene, wax esters and paraffins have all been identified in sebum<sup>(4)</sup>.

The FA components have carbon chains up to C<sub>25</sub> and they are both saturated and unsaturated, the latter being essential and non-essential<sup>(3,5)</sup>. The characteristic features of hyperkeratosis and decreased barrier function in essential FA-deficiency can lead to plugging the follicle and it becomes distended by sebum, leading to the formation of sebaceous cysts<sup>(6,7)</sup>. Sebaceous cysts occurs most frequently on the scalp, face and neck, though they appear on any part of the body<sup>(8,9)</sup>.

The components of sebum can be influenced by diet and hormones<sup>(1,4)</sup>. Dairy products contain a considerable amount of fat; more than 57% of its FAs are saturated, which is considered to be one of the common hypercholesterolemic factors<sup>(10,11)</sup>. Dairy-product fat and meat from ruminants contain conjugated linoleic acid (CLA). The Cis<sub>9</sub>, trans<sub>11</sub> (c<sub>9</sub>,t<sub>11</sub>) is the major CLA isomer in dairy products<sup>(11,12)</sup>. It is formed as a result of biohydrogenation reactions carried out by bacteria in the rumen, producing the precursor trans-11 octadecanoic acid (trans-vaccenic acid, tVA) and by Δ9 desaturase, that converts tVA to c<sub>9</sub>,t<sub>11</sub> CLA, primarily in the mammary gland<sup>(13,14)</sup>. Studies in animal models in which CLA intakes were increased showed anti-tumorogenic activity<sup>(15,16)</sup>, decreased

atherogenesis<sup>(17)</sup>, decreased adiposity and increased lean body mass<sup>(18)</sup>.

Concerning n<sub>3</sub>-polyunsaturated fatty acids (PUFAs), their percent in dairy-product fats are almost absent<sup>(19)</sup>.

In this study, we describe the effect of consuming large amounts of dairy products on the lipid content and on incorporation of c<sub>9</sub>,t<sub>11</sub> CLA into sebum lipids of sebaceous cysts obtained from healthy men who were regular dairy-product consumers.

## Materials and methods

The study was conducted on free-living subjects and was not strictly controlled for nutrient and energy intake but, in general, the subjects were divided into two groups. The first group (the consumers group) included subjects who usually consumed not less than 250 g/day of milk, yogurt or other dairy products. The second group (the non-consumers group) included subjects who consumed less than 250 g/week of dairy products. It is worth noting that meat, the second source of CLA, was taken a few times a week by most of the Iraqi population owing to its high cost and money was scarce in the period of growth of the sebaceous cysts and doing this study (2002) as a result of the economic sanctions imposed on Iraq (1990-2003).

Sebaceous cysts were obtained by simple surgery (in Al-Jumhuri Hospital, Mosul) from the scalp, face and neck of 21 men, 25-50 year old, who were non-smokers and free from any apparent metabolic disorders. The samples were divided into two groups. The first group included 11 cysts for consumers and the second group included 10 cysts for non-consumers. The cysts were immediately put into sterile screw-capped vials containing 3 ml of methanol to limit the lipolytic and

oxidative degradation of sebum-lipids before extraction<sup>(20)</sup>.

### Extraction of sebum lipids

Based upon their solubility characteristics, two types of sebum lipid were described: simple lipids which could be removed with organic solvents, and complex lipids that required more drastic extraction procedures. The former contained PLs, free FAs, free sterols, hydrocarbons, sterol esters and TGs<sup>5</sup>. In the present research, 0.5 g was weighed from each sebaceous cyst and then the simple lipids were extracted by a chloroform / methanol (2:1, V/V) mixture to a final dilution 1:20 W/V. The insoluble compounds in the homogenate were then removed by filtration<sup>(21)</sup>. The extract was mixed with 2 ml of distilled water and the mixture was allowed to separate into two phases, by standing in a separating funnel<sup>(22)</sup>. The lipid extract was evaporated (under nitrogen) to dryness, then the lipid was redissolved in 1 ml of methanol.

### Lipid analysis

Sebum lipid components, TGs and C, were determined enzymatically using kits obtained from bioMerieux, France. The PLs were determined by a colorimetric method based on the formation of a phosphomolybdate complex<sup>(23)</sup>. Thin layer chromatography was applied to separate the lipid components, TGs, PLs and CEs using Merck silica gel G 0.25 mm and hexane: diethyl ether: formic acid mixture (80:20:2 by volume). After drying, the plates were sprayed with 1% alcoholic solution of 2,7-dichlorofluorescin (a non-destructive agent) to visualize each band under UV-light. The separated components were scraped into separate vials and stored at -20 °C, then their FAs moiety was converted to fatty acid methyl esters (FA-MEs) by super dried-acidified methanol at 90-95 °C for 2 h<sup>(20)</sup>. The proportional composition (%) of methylated fatty acids was determined by capillary gas chromatography. The samples were analyzed in the National Centre for Scientific Research (CNRS), the Institute of Chemistry of Natural Substances (ICSN), France. The capillary gas chromatograph was CP-3800, Varian, USA. The capillary column was CP-Sil 8 CB, 0.25

mm LD × 30 m with film thickness: 0.25 mm and helium as carrier gas. The programme used a temperature gradient from 80 to 220 °C, 5 °C for each min. The identity of 14 individual FA-peaks was ascertained by comparing each peak's retention time relative to the retention times of FAs in synthetic standards. The relative amount of each FA (% of total FAs) was quantified by integrating the area under the peak and dividing the result by the total area for all FAs. To minimize transcription errors, the data from the gas chromatogram were electronically transferred to a computer for analysis.

### Statistical Analysis

The t-test was used to compare the means for the two groups in terms of the lipid components and FA composition of the sebum of sebaceous cysts. All the data were expressed as mean ± standard deviation. P-values ≤ 0.05 were considered significant.

## Results

### a. Sebum lipid components:

Figure (1) shows the relation between the amount of general classes of simple lipids (C, PLs and TGs) in the sebum of sebaceous cysts of two groups of subjects, dairy-product consumers and non-consumers. The average amounts of C, PLs and TGs were 18.96 and 26.17 mg/g, 11.61 and 15.29 mg/g and 4.35 and 4.44 mg/g of cysts, respectively. These results indicate that the consumers group showed a significantly higher level of C and PLs in contrast to the non-consumers group, but there was no significant difference in the amount of TGs.

### b. Sebum fatty-acid composition:

Fatty acid composition of sebum-lipid components (CE, PLs and TGs) for the two groups of subjects were shown in Tables a,b,c:

#### i. Sebum-CE fatty acids:

The consumers group, compared with the non-consumers group, (Table a) showed a significant decrease in saturated fatty acids (SFAs). Both groups, however, showed a low level of medium-chain fatty acids (MCFAs), C<sub>10</sub>-C<sub>14</sub>, and a high level of C<sub>16</sub> and C<sub>18</sub>. Concerning monounsaturated fatty acids

(MUFAs),  $C_{16:1}$  and  $C_{18:1}$ , there was a significant decrease in the consumers group. As regards the polyunsaturated fatty acids (PUFAs),  $C_{18:2}$   $n_6$  and  $c_{9,t11}$  CLA showed higher percentages for the consumers group, but the other PUFAs exhibited lower percentages. The Table showed a significant decrease in  $n_3$ -FAs for the consumers group.

### ii. Sebum-PL fatty acid:

As shown in Table (b), PLs exhibited a non-significant decrease in SFAs for the consumers group. The relative amount of  $C_{18:1}$  for the consumers group was significantly lower than that for the non-consumers group, while  $C_{16:1}$  was nearly absent in both groups. As regards PUFAs,  $C_{18:2}$   $n_6$  and  $c_{9,t11}$  CLA showed higher percentages for the consumers group but the other PUFAs showed lower percentages. There was a significant decrease in  $n_3$ -FAs for the consumers group compared with the non-consumers group.

### iii. Sebum-TGs fatty acids:

Table (c) showed no significant difference in the percentages of SFAs and MUFAs for the two studied groups. As regards PUFAs, the consumers group exhibited a decrease in their level except for  $c_{9,t11}$  CLA and, to some extent,  $C_{18:2}$   $n_6$ . As in the case of CE and PLs, TGs showed a significant decrease in  $n_3$ -FAs for the consumers group compared with the non-consumers group.

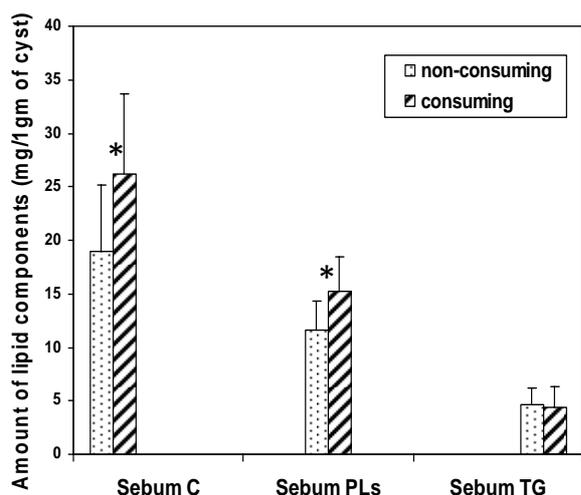


Fig (1): Comparison between the amount of sebum lipid components of sebaceous cysts of

subjects non-consuming and consuming dairy products

C = cholesterol, PLs = phospholipids, TGs = triglycerides

\* Significant difference at  $p \leq 0.05$

Table: Sebum-lipid fatty acid composition of subjects consuming and non-consuming dairy products

(a) Sebum CE-fatty acid composition (wt%)

Fatty acid	Mean $\pm$ SD	
	Non-consumer	Consumer
Saturated		
10:0	0.06 $\pm$ 0.03	0.05 $\pm$ 0.03
12:0	1.02 $\pm$ 0.01	1.14 $\pm$ 0.29
14:0	1.56 $\pm$ 0.40	1.50 $\pm$ 0.33
16:0	12.42 $\pm$ 0.77	12.13 $\pm$ 1.13
18:0	6.99 $\pm$ 1.05	5.67 $\pm$ 1.06*
Total	22.05 $\pm$ 1.07	20.49 $\pm$ 1.43*
Monounsaturated		
16:1	1.12 $\pm$ 0.15	0.82 $\pm$ 0.11*
18:1	20.95 $\pm$ 0.66	18.11 $\pm$ 1.39*
Total	22.07 $\pm$ 0.63	18.94 $\pm$ 1.40*
Polyunsaturated		
18:2 $n_6$	45.5 $\pm$ 4.13	52.81 $\pm$ 4.41*
18:3 $n_6$	1.45 $\pm$ 0.39	0.74 $\pm$ 0.06*
18:3 $n_3$	0.46 $\pm$ 0.06	0.36 $\pm$ 0.13*
20:4 $n_6$	6.79 $\pm$ 0.77	5.29 $\pm$ 0.78*
20:5 $n_3$	0.62 $\pm$ 0.07	0.33 $\pm$ 0.06*
22:6 $n_3$	0.62 $\pm$ 0.07	0.42 $\pm$ 0.09*
18:2 $C_{9,t11}$	0.44 $\pm$ 0.02	0.62 $\pm$ 0.04*
$n_3$	1.70 $\pm$ 0.13	1.12 $\pm$ 0.13*
$n_6$	53.74 $\pm$ 4.50	58.84 $\pm$ 4.91

\*  $p \leq 0.05$  = significant difference

(b) Sebum PL-fatty acid composition (wt%)

Fatty acid	Mean $\pm$ SD	
	Non-consumer	Consumer
Saturated		
10:0	0.10 $\pm$ 0.04	0.13 $\pm$ 0.05
12:0	1.64 $\pm$ 0.35	0.98 $\pm$ 0.37*
14:0	1.38 $\pm$ 0.44	1.31 $\pm$ 0.38
16:0	25.91 $\pm$ 4.59	23.24 $\pm$ 4.45
18:0	14.95 $\pm$ 1.87	14.83 $\pm$ 2.54
Total	43.97 $\pm$ 5.89	40.50 $\pm$ 6.19
Monounsaturated		
16:1	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02
18:1	9.31 $\pm$ 1.15	7.54 $\pm$ 1.37*
Total	9.33 $\pm$ 1.14	7.56 $\pm$ 1.37*
Polyunsaturated		
18:2 n6	27.44 $\pm$ 4.18	36.02 $\pm$ 4.42*
18:3 n6	0.18 $\pm$ 0.04	0.18 $\pm$ 0.06
18:3 n3	0.17 $\pm$ 0.05	0.21 $\pm$ 0.06
20:4 n6	11.43 $\pm$ 3.34	9.23 $\pm$ 4.04
20:5 n3	0.86 $\pm$ 0.16	0.45 $\pm$ 0.14*
22:6 n3	6.11 $\pm$ 0.97	5.16 $\pm$ 1.3
18:2 C <sub>9</sub> ,t <sub>11</sub>	0.51 $\pm$ 0.03	0.69 $\pm$ 0.04*
n3	7.13 $\pm$ 0.95	5.82 $\pm$ 1.31*
n6	30.63 $\pm$ 6.47	38.81 $\pm$ 6.10*

\* p  $\leq$  0.05 = significant difference

(c) Sebum TG-fatty acid composition (wt%)

Fatty acid	Mean $\pm$ SD	
	Non-consumer	Consumer
Saturated		
10:0	0.29 $\pm$ 0.04	0.38 $\pm$ 0.09*
12:0	0.19 $\pm$ 0.03	0.38 $\pm$ 0.05*
14:0	2.22 $\pm$ 0.61	2.25 $\pm$ 0.5
16:0	24.46 $\pm$ 4.86	21.96 $\pm$ 4.04
18:0	4.26 $\pm$ 0.51	7.01 $\pm$ 0.72*
Total	31.42 $\pm$ 5.11	31.98 $\pm$ 4.32
Monounsaturated		
16:1	2.38 $\pm$ 0.48	2.35 $\pm$ 0.52
18:1	25.26 $\pm$ 5.59	23.12 $\pm$ 4.46
Total	27.64 $\pm$ 5.61	25.47 $\pm$ 4.59
Polyunsaturated		
18:2 n6	26.01 $\pm$ 5.25	30.73 $\pm$ 3.89
18:3 n6	0.71 $\pm$ 0.13	0.68 $\pm$ 0.08
18:3 n3	0.91 $\pm$ 0.42	1.09 $\pm$ 0.33
20:4 n6	7.32 $\pm$ 1.29	5.53 $\pm$ 1.22*
20:5 n3	1.14 $\pm$ 0.29	0.59 $\pm$ 0.15*
22:6 n3	4.64 $\pm$ 1.46	3.60 $\pm$ 1.35
18:2 C <sub>9</sub> ,t <sub>11</sub>	0.21 $\pm$ 0.04	0.33 $\pm$ 0.04*
n3	6.69 $\pm$ 1.38	5.28 $\pm$ 1.28*
n6	34.04 $\pm$ 5.32	36.94 $\pm$ 7.76

\* p  $\leq$  0.05 = significant difference

## Discussion

### a. Sebum lipid components:

As shown in the figure (1), the level of C was higher in the consumers-group. The increase in the level of sebum C might be affected by the increase of its level in serum since the habit of consuming large quantities of dairy products leads to increased serum-C level. The dairy-product lipids include the main cholesterolemic SFAs; C<sub>12</sub>, C<sub>14</sub> and C<sub>16</sub> <sup>(24)</sup>, and the stratum basale is equipped with the LDL-receptors that allow the potential uptake of cholesterol. On the other hand, essential-fatty acid (EFAs) deficiency leads to increase cholesterol synthesis by affecting the activity of HMG-CoA reductase (cholesterol rate-limiting enzyme)<sup>(5,19)</sup>.

Concerning the sebum PLs, they exhibited a higher level in the consumer group, which reflects the fact that high-fat diets, including dairy products, tend to increase PLs<sup>(23,24)</sup>. This seems to be in harmony with what Mahmood (2002) states about the serum PLs of subjects who consume dairy products.

As figure (1) shows, there is no significant difference in the level of sebum TGs between the two groups. This indicates that the effect of dairy-product consumption on the level of sebum TGs was almost absent. Although about 99 wt% of human adipose tissue-lipids consisted of TGs<sup>(22)</sup>, the percentage of sebum TGs was much lower in this and other studies<sup>(4)</sup>.

### b. Sebum fatty-acid composition:

The SFAs of CE of dairy-product consumers exhibited a decrease in their amount compared with that of the non-consumers group as shown in Table (a). This indicates that dairy-product SFAs do not affect the sebum SFAs since they are synthesized in situ<sup>19,25</sup>. The results obtained for the SFAs of PLs and TGs, when there was no significant difference in their percentage for both groups, seem to agree with this explanation. The Table shows a lower percentage of MUFAs for CE, PLs and, to some extent, TGs although dairy-product lipid contains a high proportion of oleic acid<sup>(26,27)</sup>. The negative correlation may be attributed to either the consumption of other sources of the diet by the two groups or to the

enzymatic competition for desaturases and elongases during the endogenous synthesis<sup>(24)</sup>. Generally, when analyzing the FA composition of tissue samples to estimate the FA composition of the diet, strong correlations have been found between EFAs and the estimated dietary intake. Correlations for nonessential unsaturated and saturated FAs are weaker<sup>(9,28)</sup>.

Consumption of dairy products increases the level of C<sub>18:2</sub> n<sub>6</sub> in the body<sup>(19,29)</sup> and this mimics the result listed in the table. In addition, there was a negative relationship between the amount of MUFAs (C<sub>16:1</sub> and C<sub>18:1</sub>) and the amount of C<sub>18:2</sub> of sebum<sup>(5)</sup>.

The consumers group showed a significant decrease in C<sub>20:5</sub> n<sub>3</sub> and C<sub>22:6</sub> n<sub>3</sub>. This might be due to the high level of C<sub>18:2</sub> n<sub>6</sub> of this group which modulates the action of 5-desaturase under the control of hormonal factors. Furthermore, C<sub>18:3</sub> n<sub>6</sub> and C<sub>20:4</sub> were also lower in the consumers group probably due to the same effect of the high level of linoleic acid<sup>(25,30,31)</sup>. Dairy-product lipid seems to decrease n<sub>3</sub>-FAs and increase n<sub>6</sub>-FAs in sebum lipid as shown in the Table, and it is worth mentioning that the balance between the level of n<sub>3</sub> and n<sub>6</sub>-FAs is important because of the competitive nature of their different biological roles<sup>(5,32,33)</sup>.

As regards CLA, the concentration of c<sub>9,t11</sub> which is the major CLA isomer in the diet<sup>(13)</sup>, probably reflects habitual intakes<sup>(12)</sup>. The present study showed that consuming dairy products naturally enriched in CLA, especially c<sub>9,t11</sub> CLA, increases its concentration in all the studied sebum components. The positive association in the concentration of c<sub>9,t11</sub> CLA between CE and PL might reflect the synthesis of CE from PL, particularly phosphatidylcholine by lecithin: cholesterol acyl transferase activity<sup>(12,19)</sup>. The FA composition of human adipose tissues reflects, to a great extent, the average distribution of the dietary FAs over a period of 2-3 y<sup>(22)</sup> and it has been found that CLA is present principally in ruminant-animal food products' mainly dairy products<sup>(14)</sup>.

In conclusion, the high consumption of dairy products affects sebum lipid contents and fatty

acid composition mainly  $c_9,t_{11}$  CLA so it can be used as a marker for the intake of dairy-product lipid. These findings are in agreement with a recent report<sup>(34)</sup>, which showed a dietary influence on the sebaceous lipogenesis.

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