

THE FATTY ACIDS OF HUMAN MILK. II. ALTERATIONS PRODUCED BY MANIPULATION OF CALORIC BALANCE AND EXCHANGE OF DIETARY FATS* †

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The observation that human serum lipid concentrations may be altered by diet and the concept that this may be due to the nature of the dietary fatty acids (1) has intensified interest in the study of the metabolism of individual fatty acids. Detailed studies of natural lipid mixtures and their fatty acid components are now made possible on a microscale by recently developed methods of lipid analysis (2, 3). However, in clinical studies many organs of prime metabolic interest are not accessible for examination. In this circumstance the study of human milk fat provides unique advantages since it is a metabolic end-product which may be sampled frequently without risk or discomfort to the patient. It is a simple mixture of lipids composed almost entirely of triglycerides; however, we have noted at least forty different fatty acids in milks of mothers on *ad libitum* diets (4).

Purposeful alterations of human milk fat by dietary means was first attempted by Thiemich in 1898 (5); he showed marked changes in the iodine number of the milk fat after feeding mothers unsaturated fat. Recent studies by Söderhjelm (6) have shown an increased content of diene fatty acids following feedings of corn oil and of higher polyene acids following feedings of fish oil.

The present report describes specific alterations of the fatty acid pattern of human milk produced by rigidly controlled variations in the maternal diet. By observing the effects of dietary conditions which might favor *de novo* synthesis of fatty acids in the mammary gland or, alternatively, transfer from storage depots or from dietary fat,

it was hoped that some insight might be gained into fundamental mechanisms of fat metabolism in organs other than the breast. These objectives were partially achieved, but certain data indicated that fatty acid synthesis in the human breast is somewhat specialized.

METHODS

Nomenclature. The very large number of acids of different chain lengths and double-bond contents which are now demonstrable by gas-liquid chromatography necessitates a shorthand designation. In this report fatty acids are numbered according to chain length (number before colon) and double-bond content (number after colon). Thus, 18:0 refers to the normal saturated C₁₈ acid, stearic; 18:2 = C₁₈ diene, and so forth.¹ Wherever definitive information on double-bond structures is available, it would be more appropriate to specify the exact structure, *i.e.*, octadeca-9,12-dienoic acid (or linoleic acid), but when this is lacking, acids are called dienes, trienes, and so on. The weight percentage compositions of fatty acid mixtures are called *fatty acid patterns*.

Metabolic ward procedure. The subject of this study was a 23 year old white para III whose pregnancy was normal with spontaneous delivery at term.² She had mild chronic spastic colitis limited to the rectosigmoid colon for one year. The patient and her normal newborn daughter were admitted to this hospital on the fifth postpartum day and remained in the rooming-in unit of a metabolic ward for seven weeks' study. This metabolic ward has been described in detail elsewhere (1).

The mother was ambulatory but sedentary. The baby was exclusively breast fed, six feedings per day at four hour intervals. Both were weighed daily under standard conditions. Feedings were successively initiated at alternate breasts, and both were offered at each nursing. The mother was nourished solely by an orally administered liquid formula, the total daily intake being divided into five equal portions, supplemented with vitamins and minerals as previously described (7). Water was offered *ad libitum*. The mother's intakes exceeded

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¹ This system of designation was evolved in discussions with Dr. V. P. Dole.

² The patient was referred through the cooperation of the Department of Obstetrics, Lincoln Hospital, Bronx, N. Y.

recommended standards for nursing mothers (8). This regimen has proven adequate for growth, maintenance and physical activity in long-term clinical feeding studies carried out over the last five years (1).

Maternal milk output was determined by weighing the baby immediately before and after each nursing. At the end of each dietary period, milk was collected and sampled during a 24 hour period as previously described (4), 90 per cent of the milk being fed to the infant by bottle. Additional samples of four hour production were obtained every other day in similar manner. Milks were refrigerated immediately after collection and samples were frozen for storage. Fasting blood specimens were taken during each 24 hour milk collection.

Chemical methods. Milk lipids were quantitatively extracted with 95 per cent ethanol-ethyl ether (3:1), and the solvent-free residue of the extract was dissolved in petrol ether (30 to 60°) to obtain a lipid extract free of nonlipid contaminants. This method has been validated elsewhere (4). More than 98 per cent of the milk lipid was triglyceride, with only small amounts of free fatty acids and traces of cholesterol and phospholipids. Specific analyses of short-chain acids (C_{1-6}) have not been carried out; however, other data (4) indicate that in human milk these acids must comprise less than 4 per cent of total acids. For present purposes the milk lipid was considered to be entirely triglyceride and was not fractionated. Methyl esters of the fatty acids were formed as described previously (4).

Analyses of the composition of the complex mixture of fatty acids (fatty acid patterns) were made by two independent but complementary methods, gas-liquid chromatography and ultraviolet spectrophotometry after isomerization with alkali. The latter method (9) offered a quantitative picture of the five polyene groups (diene through hexene) without regard to fatty acid chain length, and was based on use of constants derived from study of certain pure C_{18-22} acids. As applied in this report, gas-liquid chromatography (2) partially described and measured acids of chain length C_{6-18} , in a few cases through C_{20} . The eight major acids, comprising more than 95 per cent of total C_{6-18} acids, are named with considerable assurance, and all except the linoleate and linolenate pair (18:2+3) were clearly separated. For resolution of this pair, the data obtained by ultra-violet spectroscopy have proven to be completely reliable (4).

Gas-liquid chromatography of the mixed methyl esters

was carried out at 197° with Apiezon M® as the stationary phase and nitrogen as the mobile phase. The gas-density balance was used as detector (10), and records were analyzed by triangle mensuration with the calibration factors of James and Wheatley (11). The column efficiency was 2,900 theoretical plates, calculated at methyl stearate. With the separation factor of stearate/oleate = 1.18, these esters were 99 per cent separated. In another report describing these techniques in detail (12) we have shown the remarkable stability of highly unsaturated long-chain fatty acid esters on such columns.

Our most recent studies have shown the presence in human milk of a wide array of C_{18-22} acids, many as yet unidentified (4). However, these acids comprise only about 4 per cent of the total acids, and the conclusions to be drawn from the present data, which are restricted to consideration of the C_{6-18} acids, seem in no way weakened by the omission of $C_{>18}$ data.

Serum lipid classes (triglycerides, free and total cholesterol, and phospholipids) were analyzed as described elsewhere (13). Cholesterol esters, triglycerides and total phospholipids were isolated by chromatography on silicic acid (3). After preparation of the methyl esters (14) analyses of the fatty acids in each of these ester groups were made by gas-liquid chromatography as described above.

Experimental design. In this study the mammary gland is regarded as a metabolic unit from which milk is derived as the net result of the gland's metabolic activity; possible differences in function of the anatomic divisions of the gland are disregarded. Three possible sources of milk lipids were considered: synthesis *in situ*, transfer via the blood stream from fat depots and other extramammary sites of fat synthesis, and transfer of dietary fat. It seemed reasonable to postulate that the interplay of forces acting to favor one or another source of milk fat would be influenced by the total energy balance of the mother and also by the proportion of fat and non-fat calories in her diet. By defining the milk fatty acid pattern when these experimental variables were rigidly controlled, it was hoped that the source of the milk fat could be identified.

The experimental study was divided into seven consecutive feeding periods (Table I). Each period was at least four days in duration, a time sufficient for the dietary fat to exert a maximal effect on the milk fat (5, 6).

TABLE I
Experimental design *

	Dietary period						
	I	II	III	IV	V	VI	VII
Caloric intake	<i>ad lib.</i>	2,750	3,750	1,838	3,750	2,938	2,938
Fat calories, % of total		40%	~0%	~0%	70%	70%	40%
Fat intake, Gm./day		123	3.0	1.4	292	228	131
Type of fat		Lard	Cow's milk	Cow's milk	Corn oil	Corn oil	Corn oil
Days postpartum	5-11	12-18	19-26	27-33	34-43	44-48	49-52

* All illustrations show this scheme in abbreviated form.

In Period I the patient was fed a regular hospital diet *ad libitum* until lactation was established. Thereafter, liquid formula feeding was used exclusively. The entire protein intake was derived from cow's milk protein at levels of 15 per cent of total calories in all six formula periods. Changes in the proportion of fat calories were effected by reciprocal changes in carbohydrate calories, and, in addition, two dietary fats were interchanged which were readily recognizable by their fatty acid compositions (lard and corn oil).

In Period II lard contributed 40 per cent of total calories. Since pig depot fat is very similar in fatty acid composition to the fat mixture in an average American diet (1, 15), this period served to mimic Period I. The total daily caloric requirement for maintenance of body weight was established at 2,750 calories. In Period III a "fat-free" diet was fed. In order to promote fat synthesis from carbohydrate at all sites (including the mammary gland) and to minimize the transport of fat out of storage, excess calories were fed (1,000 calories above the maintenance requirement) in the form of carbohydrate and protein. Traces of dietary fat shown in Table I were carried into the formula in the cow's milk protein. In Period IV, in order to stimulate mobilization of depot fat and its utilization by the breast, 1,000 calories less than the maintenance requirement were fed. Again, the "fat-free" formula was fed in order that the depot fat pattern might be identified in the milk fatty acids.

In Period V a corn oil formula was fed. To favor transfer of this diet fat directly into milk fat and to suppress synthesis and transport of endogenous fat, 1,000 excess calories were fed with 70 per cent of total calories derived from corn oil. During Periods VI and VII the total caloric intake was reduced to that required to maintain body weight constant (2,938 calories per day at this time), and the proportion of corn oil calories was successively 70 and 40 per cent. These two final periods were designed to determine the effect of fat to carbohydrate ratios on the transport of dietary fat into milk fat, as well as for sake of comparison with Period II.

To summarize the experimental design, endogenous synthesis of milk fat from carbohydrate was encouraged by feeding excess calories and no fat (Period III); transfer of depot fat was promoted by feeding deficient calories (Period IV); and transfer of dietary fat by feeding excess calories as corn oil (Period V). Effects of substituting one fat for another were compared in Periods II (lard) and VII (corn oil).

RESULTS

Lactation

Lactation was readily established by the ninth postpartum day, with a daily milk production thereafter averaging 723 ± 65 Gm. during Periods II through VII (Figure 1). More milk was regularly obtained when 24 hour collections were made by mechanical and manual expression than

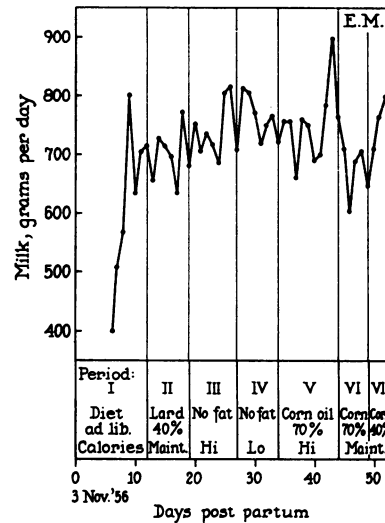


FIG. 1. DAILY MILK PRODUCTION

Total daily milk production was not significantly changed during the various dietary periods.

when the breasts were nursed by the baby. The concentration of milk fat in the 24 hour collections averaged 3.5 Gm. per 100 ml. milk to give a total fat production of 24 to 30 Gm. per day (Figure 2). The two breasts produced almost equal amounts of milk fat after lactation was well established. Neither the daily milk volume nor

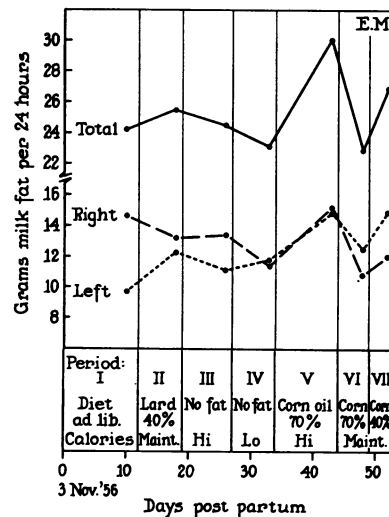


FIG. 2. DAILY MILK FAT PRODUCTION

Total daily milk fat production was highest on a diet high in both calories and fat. There was no significant difference in the milk fat production of the two sides during the experimental period.

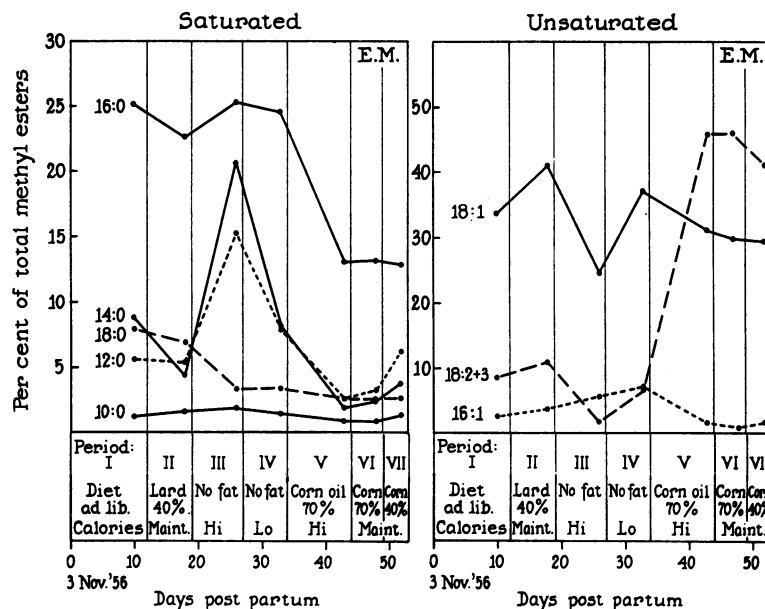


FIG. 3. THE PROPORTION OF MAJOR FATTY ACIDS IN MILK FROM THE LEFT BREAST PER 24 HOURS, AS DETERMINED BY GAS-LIQUID CHROMATOGRAPHY

There are striking changes in both saturated and unsaturated acids during various feeding periods.

daily fat production appeared to be influenced by changes in the mother's diet. The baby remained healthy and gained weight constantly at an average rate of 28.3 Gm. per day during the period of hospitalization, and on this basis lactation was judged to be normal. The mother's sense of well being was unimpaired throughout, and complaints attributed to mild chronic colitis remained unchanged.

Milk fatty acid patterns

The proportions of the major fatty acids in the 24 hour milks from the left breast are shown in Figure 3. Milk samples obtained simultaneously from right and left breasts uniformly had almost identical patterns, whether analyzed by gas-liquid chromatography or by ultraviolet spectroscopy after alkali isomerization. Moreover, in each feeding period analyses of terminal 24 hour col-

TABLE II
Comparison of fatty acid patterns in dietary fat and milk lipids *

Diets.....	Period II		Period IV		Period V		Period VII	
	Lard (40% of total calories), maintenance calories		"Fat-free" deficient calories		Corn oil (70% of total calories), excess calories		Corn oil (40% of total calories), maintenance calories	
Fatty acids	Lard	Milk	Depot fat†	Milk	Corn oil	Milk	Corn oil	Milk
12:0	0.2	4.6	0.1	7.9	0.1	3.0	0.1	4.9
14:0	1.7	9.3	2.7	9.0	0.3	2.3	0.3	4.3
16:0	22.3	23.8	24.0	23.5	12.6	13.1	12.6	12.7
16:1	2.7	3.1	5.0	6.8	0	1.4	0	1.5
18:0	9.3	7.8	8.4	3.2	2.7	3.0	2.7	2.9
18:1	44.9	42.6	46.9	36.9	30.4	31.4	30.4	28.7
18:2+3	13.5	10.3	10.2	7.3	52.9	43.0	52.9	42.0
% of total acids	94.6	96.5	97.3	94.6	99.0	97.2	99.0	97.0

* Data calculated as weight percentages of total methyl esters.

† Fifty-three year old female, Cramer and Brown (16).

lections were closely mimicked by those of milks obtained one or two days previously, indicating that by two days after each dietary change the maximal dietary effect had been obtained.

Figure 3 demonstrates that palmitic (16:0) and oleic (18:1) acids predominated in milk fat during Periods I and II, when fat calories were 40 per cent, total calories adequate to maintain body weight, and the dietary fat pattern that of lard or the average American diet (15). The proportions of all acids in the milk samples during these two periods closely resembled those seen in the milk of mothers on *ad libitum* diets (4).

In Period III the weight percentages of 12:0 and 14:0 doubled at the expense of 18:0, 18:1 and 18:2 + 3. On a *molar* basis 12:0 plus 14:0 made up 39 per cent of the total acids. The proportions of 16:0 and 16:1 remained essentially unchanged. Thus, when endogenous synthesis of fatty acids from carbohydrate precursors was promoted by feeding excess calories and a "fat-free" diet, saturated acids of intermediate chain length appeared in the milk in striking proportions.

In Period IV the fatty acid pattern of the milk fat came to resemble that found when the 40 per

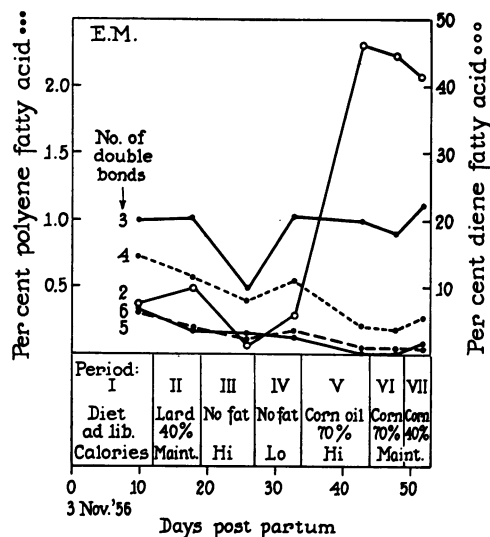


FIG. 4. THE POLYENE FATTY ACIDS IN 24 HOUR MILK COLLECTIONS FROM THE LEFT BREAST

Note that the diene scale (on the right) is 20 times that of the other polyenes.

cent lard diet was fed (Period II) or during *ad libitum* feeding (Period I), and furthermore it resembled that reported (16) for human depot fat (Table II). Thus, when deficient calories of

TABLE III
Polyene contents of dietary and milk fats expressed as percentages of total fatty acids by weight

Period	Breast	Dienes		Trienes		Tetraenes		Pentaenes		Hexaenes	
		Diet	Milk	Diet	Milk	Diet	Milk	Diet	Milk	Diet	Milk
I <i>Ad lib.</i>	L*		7.6		1.0		0.7		0.3		0.3
	R†		7.8		1.0		0.7		0.3		0.4
II 40% lard	L	12.0	9.9	0.8	1.0	0.3	0.6	0.1	0.2	tr	0.2
	R	12.0	11.8	0.8	1.4	0.3	0.6	0.1	0.2	tr	0.2
III Fat-free, excess calories	L		1.2		0.5		0.4		0.1		0.1
	R		1.5		0.5		0.4		0.1		0.1
IV Fat-free, deficient calories	L		5.9		1.0		0.6		0.2		0.1
	R		6.4		1.1		0.6		0.2		0.1
V 70% corn, excess calories	L	50.9	46.1	1.6	1.0	0.7	0.2	tr‡	tr	tr	tr
	R	50.9	43.3	1.6	0.9	0.7	0.2	tr	tr	tr	tr
VI 70% corn, maint. calories	L	50.9	44.5	1.6	0.9	0.7	0.2	tr	tr	tr	tr
	R	50.9	44.7	1.6	0.9	0.7	0.3	tr	tr	tr	tr
VII 40% corn, maint. calories	L	50.9	41.4	1.6	1.2	0.7	0.3	tr	tr	tr	tr
	R	50.9	41.4	1.6	1.1	0.7	0.3	tr	tr	tr	tr

* Left breast.
† Right breast.
‡ Tr = <0.1 per cent.

a "fat-free" formula were fed, it appeared that depot fat was mobilized and transferred into the milk.

In Periods V through VII when corn oil was the sole dietary fat, the content of 18:2 + 3 increased more than fivefold over all previous levels. This increase occurred mainly at the expense of the saturated acids. Thus, the major acid of corn oil (18:2) appeared in breast milk in large amounts, and the pattern of milk acids approached that of corn oil itself (Table II). In Periods VI and VII, when the percentage of corn oil calories was reduced from 70 to 40 per cent of total calories, 18:2 + 3 decreased slightly, while 12:0 and 14:0 increased.

Table II demonstrates that milk fat is distinctive in having a higher content of 12:0 and 14:0 acids than the dietary fat in all feeding periods. In fact, these contents of lauric and myristic acids, noted also in the milks of mothers on *ad libitum* diets (4), are higher than those encountered in most other animal fats except butter oil.

Not shown in tabular form, the odd-numbered acids, C_{7-17} , occurred in amounts less than 1 per cent and were depressed during Periods II, V and VI. C_{13} and C_{15} acids were not affected in Period III when 12:0 and 14:0 increased so strikingly.

The polyene classes measured by ultraviolet spectroscopy are shown in Figure 4. The diene curve clearly resembles that of 18:2 + 3 in Figure 3, indicating that the 18:2 + 3 of Figure 3 and Table II refer almost entirely to 18:2. This is borne out by the data in Table III, which indicates that trienes at no time constituted more than 1.4 per cent of total fatty acids. In addition, the ultraviolet data indicated that the tri- to hexaenoic acids never exceeded 2.5 per cent of total acids. Thus, it is clear that the very highly unsaturated C_{19-22} acids which were *not* analyzed in these chromatographic runs were low in concentration.

Figure 4 shows also that all polyenes decreased in Period III when 12:0 and 14:0 increased strikingly. In Periods V through VII, when dienes were highest, tetraenes did not increase.

Serum lipids and fatty acid patterns

The trends in concentrations of the mother's serum lipids were as expected (Figure 5). Cholesterol and phospholipid concentrations were

lowest when corn oil was fed and were somewhat higher on the lard and "fat-free" diets. The triglycerides increased sharply when the "fat-free" diet in caloric excess was fed in Period III. However, all feeding periods were too short to permit establishment of the full effect of each diet on serum lipid concentrations. Previous long-term studies (1) have indicated that two or more weeks are usually required before a new equilibrium is established.

The fatty acids of the major ester groups in the serum of the mother were determined on three occasions at times of 24 hour milk collections: in Periods II, III and V and VI (pooled). The data in Periods II and V and VI indicated that the dietary fatty acid pattern was most closely simulated by the pattern of the triglyceride fatty acids. In addition, the cholesterol esters showed considerable avidity for the 18:2 of corn oil. The

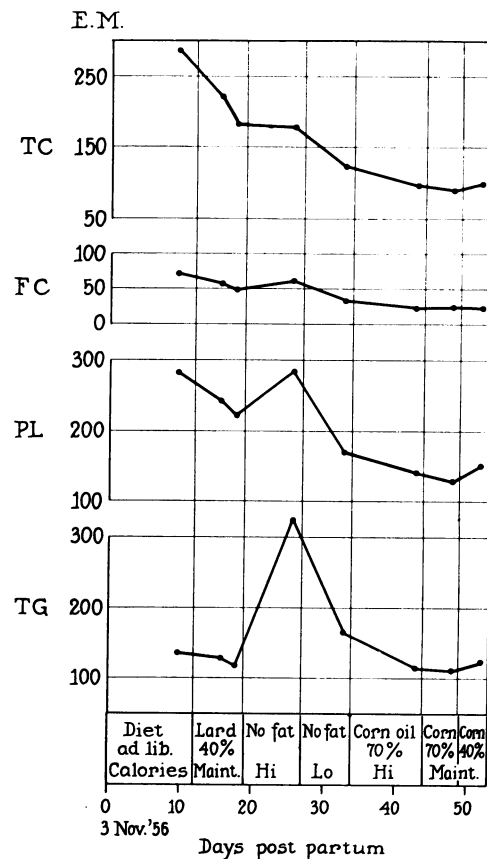


FIG. 5. SERUM LIPID CONCENTRATIONS DURING EACH FEEDING PERIOD

From top to bottom: total cholesterol, free cholesterol, phospholipids and triglycerides, all given in mg. per cent.

phospholipids were least sensitive to shifts in dietary fats, and in this group saturated acids comprised 55 to 70 per cent of total acids with a 16:0 per 18:0 ratio of nearly two. There was some variation in content of 18:1 and 18:2 + 3 in the phospholipids, but these changes were less marked than in the other two ester groups.

The main usefulness of these analyses was shown in Period III, when the milk lipids contained a marked increase in 12:0 and 14:0 acids. By contrast, none of the serum ester groups contained larger than usual amounts of these two acids. This disparity in serum and milk contents of 12:0 and 14:0 has been seen subsequently in three other patients ingesting fat-free diets.

The possibility remains that comparison of patterns of fatty acids in the serum ester groups with that in the milk can indicate which ester group in the serum most effectively transfers its fatty acids into the milk. The present data suggest that the triglycerides serve this function, but this aspect of the problem remains to be explored in greater detail.

DISCUSSION

The experimental results confirm our working hypothesis that the composition of milk fat can be altered by changes in total caloric intake as well as by changes in types of dietary fats. In agreement with previous workers (5, 6) the data indicate clearly that dietary fatty acids are readily transported into milk fat. The marked changes in milk fatty acid patterns which were produced did not affect total daily milk or milk fat output.

The importance of positive and negative energy balance states in determining the character of milk fat has not been noted previously in animals or in man. With deficient caloric intake, milk fat closely resembled human depot fat (and indeed lard also), suggesting a transfer process. On the other hand, when excess calories of a "fat-free" diet were fed, we expected carbohydrate to be converted primarily into oleic, palmitic and stearic acids in the various sites of synthesis (including the breast), since the fats of animals grown on fat-poor diets (17) are composed mainly of these three acids (75 per cent). However, in this dietary situation 60 per cent of the fatty acids in breast milk were lauric, myristic and palmitic acids, while stearic, oleic and linoleic acids actually decreased. Since none of the serum ester groups

showed high concentrations of lauric and myristic acids, there was no evidence that these acids, if synthesized in an extramammary site, were transported to the breast via the blood.

While it is possible that these acids may find their way into breast milk by selective rapid transfer from the small amounts present in one of the serum ester groups or from the nonesterified fatty acid fraction in serum (18, 19) we are tempted to suggest two other mechanisms. Figure 3 shows that the major unsaturated acids decreased in Period III during positive energy balance on a fat-free diet. If the mammary gland had a low capacity for synthesis of unsaturated acids, the mixture of saturated acids formed from carbohydrate would have an exceedingly high melting point, *unless* a considerable amount of intermediate chain-length acids was present. When hogs are raised on low-fat rations, their depot fat contains a high concentration of oleic acid as well as palmitic and stearic acids. The net effect is a triglyceride which melts below body temperature. However, if the human breast differed from adipose tissue in having a deficiency of enzymes which synthesize unsaturated acids, the milk glycerides would have a high melting point if 12:0 and 14:0 were not present in significant concentrations. Thus, the increased amounts of these acids in the milk in Period III may represent an adaptive response to an unusual dietary challenge.

Alternatively, the human breast may be deficient in the enzymes which add acetate fragments after fatty acids have reached C_{14} and C_{16} in chain length, since Figure 3 shows that all C_{18} acids decreased in Period III. In their studies of lactation, Folley and French (20) demonstrated that ruminants synthesize fatty acids from acetate while non-ruminants utilize glucose. Popjak, French, Hunter and Martin (21) extended these findings in goats to show a remarkable cut-off of synthesis beyond C_{16} . However, the postulate that the human breast lacks the enzymes which carry synthesis past C_{14} seems weakened by the continually high concentration of 16:0 in Period III.

A definitive choice between the two hypotheses cannot be made on the basis of present data. Indeed, it cannot be forgotten that some transfer of acids from the blood may be taking place and may contribute to the content of 18:1 and 16:0 in the milk during Period III. Even though

none of these questions can be resolved with currently available facts, it seems apparent that fatty acid metabolism in the human mammary gland is not identical to that in adipose tissue.

CONCLUSIONS

The fatty acid composition of human breast milk can be radically altered without affecting milk volume or milk fat output. During energy equilibrium milk fat closely resembles dietary fat, but, when deficient calories are fed, milk fat approaches the composition of human depot fat. These findings suggest that, under either of these nutritional conditions, transfer of dietary or depot fat dictates the fatty acid pattern of human breast milk.

However, when excess nonfat calories are fed to the mother, the milk shows a striking increase in lauric and myristic acids and a marked decline of all polyenoic acids. Yet, none of the serum ester groups (cholesterol esters, triglycerides and phospholipids) show a parallel rise in these acids. Thus, synthesis of fatty acids in the breast appears to be promoted when excess calories are fed, and the accumulation of C_{12} and C_{14} acids in the milk suggests that human mammary fatty acid synthesis differs in important respects from that in extramammary depots.

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