Effect of oral contraceptives on composition and volume of breast milk[1-3]

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ABSTRACT Nitrogen and protein composition was determined in milk from women using oral contraceptives during lactation. Total nitrogen and nonprotein nitrogen as well as lactose and the individual milk proteins lactoferrin, α-lactalbumin, and serum albumin were analyzed before introduction of oral contraceptives and thereafter throughout the lactation period. Twenty-four hour milk volumes were registered by weighing the infant before and after each feeding. The four oral contraceptives used consisted of different combinations of *d*-norgestrel, megestrolacetate, and ethinylestradiol. Significant changes were observed between groups and controls for all parameters studied. However, the changes observed were generally within the physiological variation of normal breast milk. It is suggested that the use of oral contraceptives during lactation should be limited. *Am. J. Clin. Nutr.* 33: 816–824, 1980.

A number of studies have so far been performed in order to evaluate the effect of oral contraceptives (OC) on the metabolism and function of various nutrients (1–5). Several biochemical changes indicate increased requirement of vitamin B_6 but there are also reports of changes in the plasma levels of other vitamins (folacin, vitamins A, C, and B_{12}) (6) as well as of some minerals (Fe, Cu) (3, 7, 8). The effect of sex hormones on protein synthesis and metabolism is well documented (3) and the plasma levels of amino acids may alter secondarily to OC administration (3). Of special interest is, however, to further elucidate the effect of OC on lactation performance as well as on the composition of milk. This is of considerable importance with regard to the nutrition situation of developing countries for several reasons. First, the majority of women in these countries still nurse their infants for more than 9 months and breast-feeding is a question of life for the infant. Second, although breast-feeding seems to have a contraceptive effect there is still an urgent need for the mother to get effective means for child spacing. Third, there are great international campaigns to promote breast-feeding in order to counteract the observed decline in breast-feeding in developing countries. There is no alternative to breast-feeding that has the same security for the newborn as breast-feeding from the nutritional as well as infection preventive points of view.

So far several studies have shown that OC interfere with lactation mainly by decreasing the milk volume but also on milk composition (4, 9). It is often recommended that OC should not be used until breast-feeding is well established, i.e., a few months after delivery or even better when supplementary feeding has started. However, this means that the contraceptive effect of lactation is already reduced and the mother has to rely on other contraceptive methods. It is thus of utmost interest to study further the effect of OC on the breast milk composition. Several specific proteins of physiological importance are synthesized in the mammary gland. It was thus regarded to be of interest to study the specific effect on the contents of the various protein components in breast milk. The present article will report the results obtained from a longitudinal study of Swedish well-nourished

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mothers who were given four different OC. The effect on the protein composition as well as on lactose content and milk volume will be reported in this paper. The excretion of the hormones in the same milk specimens are reported elsewhere (10, 11).

Materials

OC

Four different compositions of the pills were used (Table 1). One of the OC, type II, is a progestin, while the others are combinations of progestins and an estrogen. Type III was withdrawn from the Swedish market during the experimental period because of a sudden report on carcinogenic effects on dogs[6] and the mothers taking this pill were excluded from the study from that day.

Mothers

Women in the maternity ward who previously stated that they intended to breast-feed their children and at the same time use OC were asked whether they were willing to participate in the study. If interested, they were asked once more 6 weeks after delivery. At this time they were visited in their homes and supplied with the equipment needed for the experiment (a balance, a pump, containers for storage of milk). The sampling procedure was discussed in detail with them and they also received some information on nursing technique and lactation physiology. A close contact was kept with the mothers throughout the complete experimental period by the nurse who regularly visited the mothers to take blood samples and collect milk samples and by ourselves who had continuous telephone contact with all mothers. Further details about the mothers are given by Nilsson et al. (10, 11). This project has been reviewed and approved by an ethic committee at the University of Uppsala.

Milk samples

Samples were taken during two to seven 5-day periods. The first period (period A) when the mothers were not on OC, started 8 to 12 weeks postpartum. OC were introduced and taken regularly, according to the instructions given for the particular pills, the day after period A was finished. Period B_1 started when OC had been taken for 9 days. After this 23 days elapsed before the next sampling period started, period B_2. The consecutive periods, B_2 to B_6, followed with 23 day intervals, as long as the mother was willing to participate or as long as breast-feeding, complete or partial, continued.

Milk samples were taken at midmorning feeding (9:00 to 11:00 AM), if possible 10 ml before and 10 ml after nursing. This sampling technique has been proved to be adequate for water-soluble components such as total protein, nonprotein nitrogen, individual proteins, and lactose (12). A manual pump (Egnell, Trollhättan, Sweden) was used for collection of milk specimens. During 1 day of each sampling period samples were taken before and after each feeding (5 + 5 ml) throughout 24 hr. The samples were stored at −20 C and at the time of analysis equal amounts of fore and hind milk were sometimes pooled if the sample volume was insufficient. If sufficient amounts were present these samples were analysed separately and the mean value was calculated. All analyses were performed on defatted milk.

Blood samples

On the 1st and last day of each period blood samples were taken from a peripheral vein in heparinized tubes when the milk samples were collected. The blood samples were taken to the institute on the same day, centrifuged, and the plasma obtained was stored at −20 C.

Control subjects

Healthy mothers with uncomplicated deliveries were asked in the maternity ward if they were willing to participate as volunteers in the collection of breast milk and the determination of breast milk quantity. Mothers who accepted were provided with a balance, a pump for collection of milk, and suitable plastic containers for storage of milk. They measured their milk production in their homes by weighing the infant before and after each feeding throughout 24 hr, once a week. Milk was collected on the same day, 5–10 ml, before and after each feeding. Furthermore, another control material of healthy mothers in full lactation is also used. Details of these data have been published earlier (13).

Methods

Measurements of milk volume

During two of each 5-day period 24-hr milk production was estimated by weighing the infant before and after each feeding by the mother herself. The mother was also told to note such things as leaking breasts, vomiting by the infant, common colds, introduction of supplementary foods, etc.

Total nitrogen (total N) and nonprotein nitrogen (NPN) were determined by a modified Kjeldahl method as described earlier (14). For the determination of NPN the proteins were precipitated by trichloroacetic acid (12% w/v) (15).

Lactoferrin and serum albumin were determined by immunoelectrophoresis according to Laurell (16). Lactoferrin for standards was isolated according to Johansson (17).

α-Lactalbumin was determined by means of the Mancini technique (18). Antibodies against serum albumin were obtained from Boehringerwerke (Mannheim, West Germany) and against lactoferrin and α-lactalbumin from Dakopatts (Copenhagen, Denmark).

Evaluation of the mothers' personal opinions regarding OC during lactation

After termination of the lactation period the mothers were asked to complete a form with a few questions concerning their personal opinion regarding the influence of the OC on their own lactation performance.

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[6] "Committee on Safety of Medicines". Meeting in London, December 4–5, 1975.

Statistical methods

Students *t* test (19) was used to compare the effect of different pills on breast milk composition to the corresponding values of control subjects.

Results

Concentrations of total-N, NPN, α-lactalbumin, lactoferrin, and serum albumin of the control subjects were not significantly different from earlier reported values for healthy Swedish mothers in full lactation (13).

Concentration in midmorning feeding of total-N, NPN, α-lactalbumin, lactoferrin, serum albumin, and lactose as well as the volume of breast milk secreted during 24 hr at different periods postpartum are shown in Table 2. The values given are represent mean values and SD of all mothers taking the same OC. Values of each individual mother are shown in Figure 1 to 4. In these figures, the regression lines and tolerance intervals for the healthy mothers in full lactation referred to above are shown for comparison. It is seen that, for total-N, NPN, α-lactalbumin, and serum albumin, with a few exceptions, all estimations of milk from mothers taking OC fall within the tolerance limits of well-nourished Swedish mothers, before as well as after the introduction of the pills. Lactoferrin showed a somewhat different pattern with values outside the tolerance limits before as well as after the introduction of OC.

Results of the statistical treatment are shown in Table 3. In Table 3, the changes in concentration from period A to the B periods for the parameters studied in milk of the mothers taking OC are compared to the corresponding changes in milk of the control mothers. For total-N the statistical analysis showed variations in concentrations signifi-

TABLE 1
Composition of OC studied

OC		
I	250 μg *d*-norgestrol	50 μg ethinyloestradiol
II	30 μg *d*-norgestrol	
III	4000 μg megestrolacetate	50 μg ethinylestradiol
IV	150 μg *d*-norgestrol	30 μg ethinylestradiol

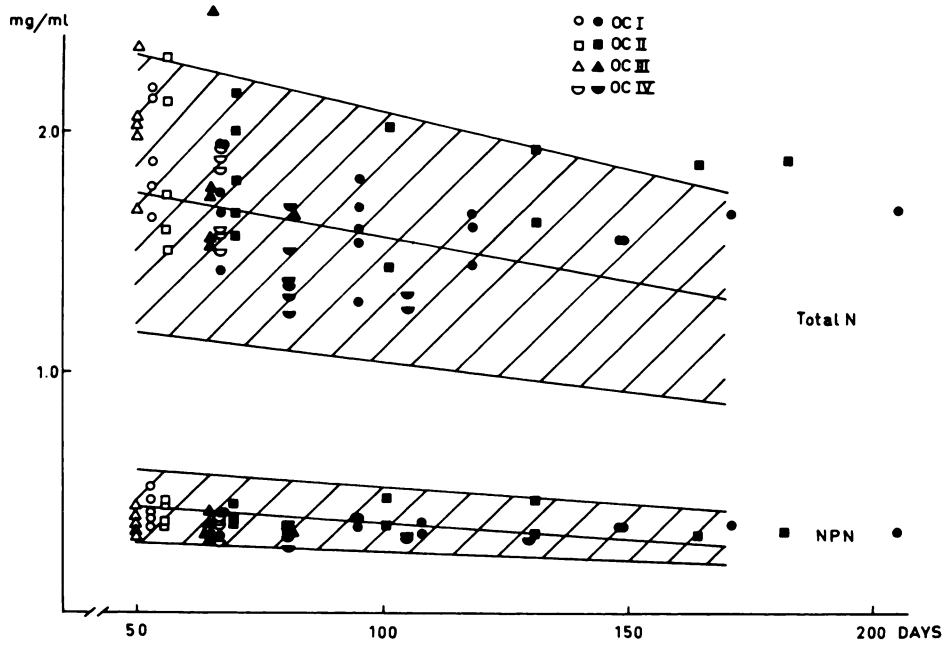

FIG. 1. Total-N and NPN of breast milk from mothers taking four different OC (I to IV). *Unfilled* and *filled symbols* represent values obtained before and after introduction of OC.

TABLE 2
Concentrations of total-N, NPN, α-lactalbumin, lactoferrin, human serum albumin, and lactose in breast milk as well as the milk volume for 24 hr (values for the different pills I to IV and for controls (C) are given for different weeks postpartum

		Period A		Period B_1		Period B_2		Period B_3		Period B_4		Period B_5		Period B_6	
		MV ± SD	n	MV ± SD	n	MV ± SD	n	MV ± SD	n	MV ± SD	n	MV ± SD	n	MV ± SD	n
Total N (mg/mg)	I	1.91 ± 0.10	5	1.75 ± 0.11	4	1.55 ± 0.10	3	1.56 ± 0.07	3	1.55 ± 0.05	2	1.66	1	1.68	1
	II	1.84 ± 0.15	5	1.83 ± 0.07	5	1.73 ± 0.14	2	1.92	1	1.86	1	1.88	1		
	III	2.00 ± 0.10	5	1.84 ± 0.21	4	1.67	1								
	IV	1.67 ± 0.09	6	1.42 ± 0.07	6	1.29 ± 0.03	2	1.29	1						
	C	1.68 ± 0.33	9	1.52 ± 0.26	8	1.63 ± 0.26	8	1.81 ± 0.49	6	1.76 ± 0.26	4	1.66	2		
NPN (mg/ml)	I	0.43 ± 0.02	5	0.37	1	0.38	2	0.36 ± 0.02	2	0.36 ± 0.02	2	0.37	1	0.34	1
	II	0.42 ± 0.02	4	0.41 ± 0.02	3	0.42 ± 0.04	2	0.33	1	0.32	1	0.34	1		
	III	0.40 ± 0.03	4	0.36 ± 0.02	4	0.34	1								
	IV	0.37 ± 0.02	6	0.33 ± 0.02	6	0.31 ± 0.01	2	0.30	1						
	C	0.40 ± 0.04	9	0.40 ± 0.07	8	0.39 ± 0.07	8	0.38 ± 0.05	6	0.39 ± 0.06	5	0.39 ± 0.08	3		
α-Lactalbumin (mg/ml)	I	3.09 ± 0.22	5	2.47 ± 0.21	4	2.36 ± 0.26	4	2.49 ± 0.11	3	2.57 ± 0.07	2	2.45	1	2.63	1
	II	2.98 ± 0.24	5	2.67 ± 0.23	5	2.85 ± 0.22	2	3.13	1	2.80	1	2.73	1		
	III	3.25 ± 0.18	5	2.25 ± 0.14	5	2.10	1								
	IV	2.92 ± 0.21	6	2.42 ± 0.18	6	2.09 ± 0.14	2	1.74	1						
	C	2.42 ± 0.33	9	2.28 ± 0.49	8	2.31 ± 0.41	8	2.45 ± 0.61	5	1.95 ± 0.14	4	1.54 ± 0.40	4		
Lactoferrin (mg/ml)	I	2.42 ± 0.22	5	2.86 ± 0.31	4	2.00 ± 0.19	4	1.58 ± 0.24	3	1.88 ± 0.07	2	1.77	1	1.69	1
	II	2.55 ± 0.35	5	2.67 ± 0.32	5	3.00	2	3.20	2	2.99	1	2.83	1		
	III	2.79 ± 0.45	5	3.23 ± 0.35	4	3.28	1								
	IV	1.62 ± 0.13	6	1.57 ± 0.21	6	1.22 ± 0.21	2	1.48	1						
	C	1.75 ± 0.60	9	1.61 ± 0.60	8	1.54 ± 0.35	8	1.32 ± 0.37	5	1.35 ± 0.62	4	1.82 ± 1.07	4		
Human serum albumin (mg/ml)	I	0.42 ± 0.04	5	0.45 ± 0.04	4	0.41 ± 0.02	4	0.36 ± 0.01	3	0.35 ± 0.02	2	0.37	1	0.35	1
	II	0.41 ± 0.07	5	0.43 ± 0.06	5	0.44 ± 0.02	2	0.42	1	0.47	1	0.45	1		
	III	0.48 ± 0.03	5	0.48 ± 0.06	4	0.36	1								
	IV	0.53 ± 0.06	6	0.52 ± 0.06	6	0.38 ± 0.01	2	0.37	1						
	C	0.40 ± 0.19	9	0.38 ± 0.13	8	0.40 ± 0.10	8	0.44 ± 0.10	6	0.50 ± 0.13	5	0.54 ± 0.09	3		
Lactose (g/100 ml)	I	5.97 ± 0.49	5	5.75 ± 0.58	4	5.93 ± 0.61	3	6.75 ± 0.79	2	5.79 ± 1.00	2	5.20	1		
	II	6.03 ± 0.39	5	6.15 ± 0.31	5	6.61 ± 0.22	2	6.59 ± 0.49	2	6.59	1	6.70	1		
	III	5.70 ± 0.51	5	5.73 ± 1.16	4										
	IV	6.25 ± 0.60	6	6.23 ± 0.58	6	6.29 ± 0.74	2	6.83	1						
	C	6.58 ± 1.11	8	6.41 ± 0.86	8	6.38 ± 0.55	8	6.78 ± 0.75	5	6.46 ± 0.22	4	7.04 ± 0.77	4		
Volume (ml/24 hr)	I	803 ± 107	5	752 ± 92	5	717 ± 252	5	755 ± 178	3	750 ± 410	2	825	1	675	1
	II	686 ± 153	5	581 ± 250	5	820 ± 156	2	930	1	865	1	635	1		
	III	807 ± 134	5	541 ± 207	5										
	IV	726 ± 113	6	632 ± 134	6	677 ± 225	3	705	1						
	C	759 ± 166	9	802 ± 152	9	865 ± 120	9	844 ± 152	7	747 ± 282	6	474 ± 283	5		

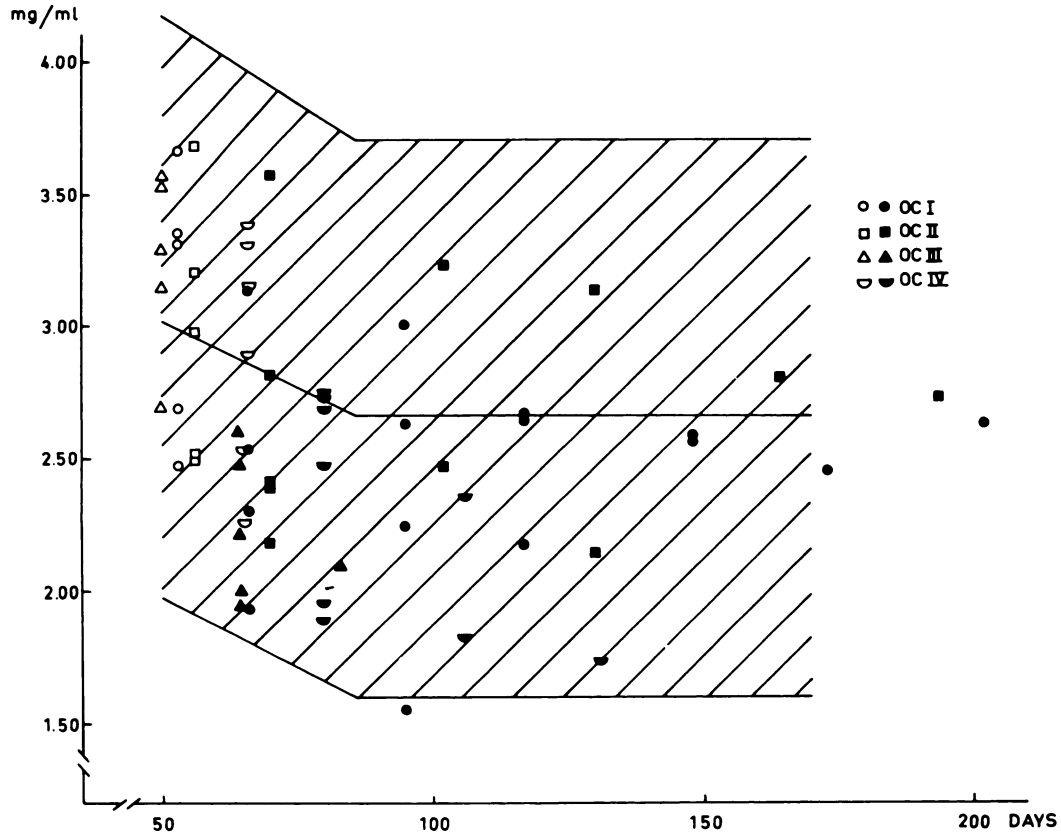

FIG. 2. α-Lactalbumin of breast milk from mothers taking four different OC (I to IV). *Unfilled* and *filled symbols* represent values obtained before and after introduction of OC.

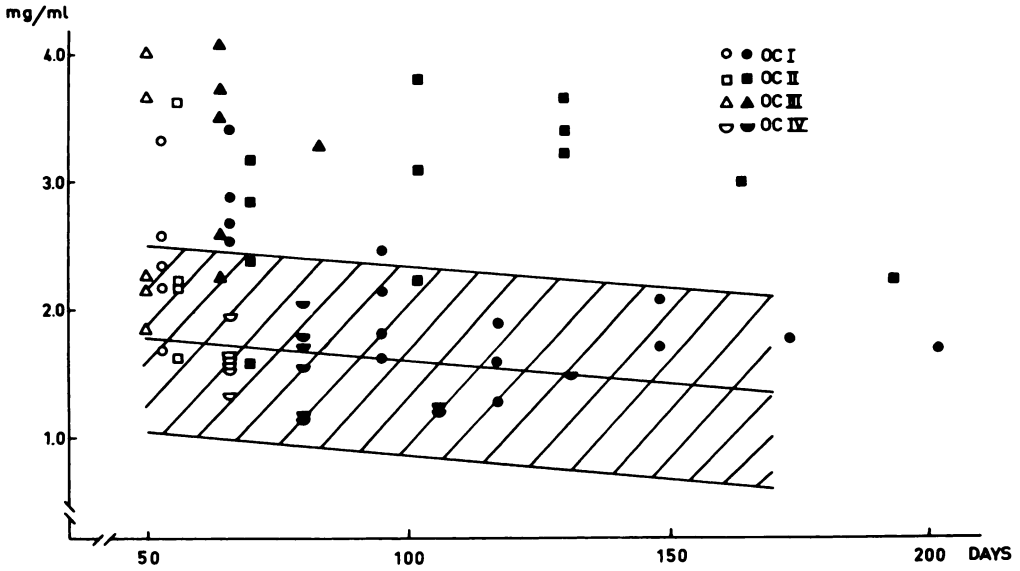

FIG. 3. Lactoferrin of breast milk from mothers taking four different OC (I to IV). *Unfilled* and *filled symbols* represent values obtained before and after introduction of OC.

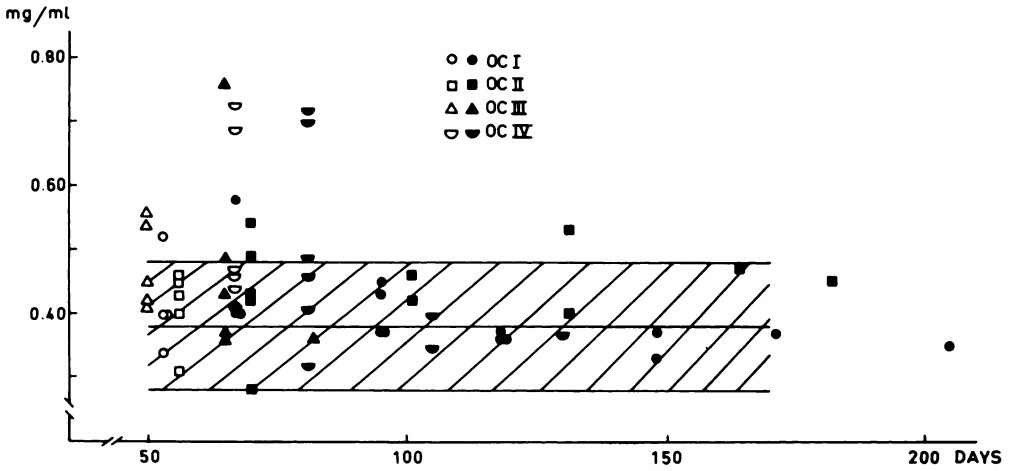

FIG. 4. Human serum albumin in plasma and milk from mothers taking four different OC (I to IV). *Unfilled* and *filled symbols* represent values obtained before and after introduction of OC.

TABLE 3
Levels of significance for differences in changes of milk concentrations of total-N, NPN, α-lactalbumin, lactoferrin, serum albumin, and lactose between two periods (each test group is compared to controls)

Comparison of controls with OC	Level of significance of differences between periods			
	$A-B_1$	$A-B_2$	$A-B_3$	$A-B_4$
I Total-N	$0.05 > P > 0.01$	$P \leq 0.001$	$P \leq 0.001$	
NPN	$P \leq 0.001$		$P \leq 0.001$	$P \leq 0.001$
α-Lactalbumin	$P \leq 0.001$	$P \leq 0.001$		
Lactoferrin	$P \leq 0.001$	$0.01 > P > 0.001$	$0.05 > P > 0.01$	
Serum albumin	$P \leq 0.001$			$P \leq 0.001$
Lactose				
II Total-N	$P \leq 0.001$	$0.01 > P > 0.001$		
NPN	$0.05 > P > 0.01$			
α-Lactalbumin	$P \leq 0.001$	$P \leq 0.001$		
Lactoferrin	$P \leq 0.001$	$0.05 > P > 0.01$		
Serum albumin	$P \leq 0.001$			
Lactose				
III Total-N				
NPN	$P \leq 0.001$			
α-Lactalbumin	$P \leq 0.001$			
Lactoferrin	$P \leq 0.001$			
Serum albumin	$P \leq 0.001$			
Lactose				
IV Total-N	$P \leq 0.001$	$0.01 > P > 0.001$		
NPN	$P \leq 0.001$	$P \leq 0.001$		
α-Lactalbumin	$P \leq 0.001$	$P \leq 0.001$	$0.01 > P > 0.001$	
Lactoferrin	$P \leq 0.001$			
Serum albumin	$0.01 > P > 0.001$			
Lactose				

cantly different from controls for OC I, II, and IV after the B_1 as well as the B_2 period. These changes were in some cases positive and in some cases negative. Similar results were obtained with NPN. For α-lactalbumin, changes in the concentration of this protein occurred after introduction of OC and these changes were significantly different from controls for all OC in the B_1 period and for OC I, II, and IV in the B_2 period. Thus, the small

decrease in the α-lactalbumin content of breast milk with increasing length of lactation was greater for women taking OC than for controls.

The concentration of lactoferrin was significantly changed after introduction of OC as compared to controls (Table 3). However, these changes were in some cases positive and in some cases negative and were not consistent with time. For example, pill I gives rise to an increase in lactoferrin in the B_1 period and a decrease in the B_2 period, both changes being statistically different from controls. Pill II, however, increased during the B_1 as well as the B_2 period.

Introduction of OC appears to influence significantly the concentrations of human serum albumin in milk as compared to controls (Table 3). The plasma levels of human serum albumin were within normal physiological limits for all mothers taking OC before as well as after the introduction of OC. No significant effect of the concentration of lactose in breast milk was observed after introduction of any of the OC.

The mean milk volumes of the mothers of all groups and controls were during the A period within normal limits for Swedish mothers in full lactation, although in some cases, infant formulas were used along with breast milk. After introduction of OC a decrease in milk volume for all treatments was observed which was most pronounced for OC III. This decrease in addition to the lower concentrations of some parameters in the milk of mothers taking OC resulted in a totally decreased 24 hr output of the milk components studied but these differences were not significant. During the corresponding time of lactation, however, no decrease in milk production was observed in the control group.

It was of interest to note that the mothers' opinions of the influence of OC on their own lactation performance were very divergent. Three of the 21 mothers attributed a positive effect of OC on the milk yield while 12 thought that OC suppressed lactation. The remaining six mothers found that OC did not influence lactation performance in any direction. The three different opinions were evenly distributed between mothers taking OC I, II, and IV, respectively. For OC III, three of five mothers noted decreased lactation performance, while the other two noted no influence on lactation by the OC.

Discussion

The statistical evaluation of the results obtained in this study require some further comments. It has earlier been found (14) that the degree of biological variation of all parameters studied is high, i.e., the variation between individuals can be considerable. However, the breast milk of each individual mother shows a very slight variation in concentration of the actual components after the colostrum period. This means that a very large number of observations would be required to confirm an effect of a treatment on the concentration of a certain component in breast milk if simply the test population is compared to the control population. However, the effect of OC on the concentration of a certain component in breast milk can be estimated if the concentration before medication is compared to the concentration after a certain period of medication. The change in concentration thus obtained for a certain treatment can be statistically compared to the corresponding change in concentration for a control group. This will enable the detection of statistically significant differences by comparatively few mothers in each group.

Significant changes in concentration of total protein, NPN, and individual milk proteins were observed. The magnitude of these changes varied between the components investigated as well as between the pills. It should also be noted that one of the pills is a progestin (type II) while the others are mixtures of progestins and an estrogen. Differences in plasma high density lipoproteins have been observed among these medications (20) and this might partly explain the differential effects on protein synthesis. Of special interest were the changes in concentration observed for the iron-binding protein lactoferrin. In another study (21) we have reported high lactoferrin concentrations in milk samples from mothers with very high iron intakes and good iron status. The iron status of these mothers was not investigated, but hemoglobin values were normal. As increased concentrations of both copper and the copper-bind-

ing protein, ceruloplasmin (22), as well as serum iron and total iron binding capacity have been observed in plasma during the use of OC (23), the possible interrelationship between serum and milk iron and copper should be investigated further.

Changes in daily milk volumes were also observed after introduction of OC. There seems to be an initial decrease which is more pronounced than the long time decrease in milk production. However, this is probably related to the fact that mothers with low milk yields tended to lactate only during the first few months postpartum. Thus values obtained after 4 to 6 months postpartum almost entirely consist of mothers with very good lactation capacity.

In conclusion, the magnitude of the changes observed were still within the normal ranges and consequently they are not likely to be of nutritional importance. It should, however, be noted that even if no major effects were observed for these well-nourished mothers, the effects on marginally or severely malnourished mothers are still not known. It is also possible that the concentrations of other nutrients such as vitamins and minerals may be affected. Furthermore, hormone analyses of the milk samples (10, 11) showed significant transfer of some hormones in physiologically active doses. These effects, however, were related to dosage and hormonal combination. Thus we suggest that the use of OC during lactation should be carefully considered and if OC during lactation is chosen the lowest possible dose should be given.

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