Cheek cell phospholipids in human infants: a marker of docosahexaenoic and arachidonic acids in the diet, plasma, and red blood cells[1–3]

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ABSTRACT

Background: Assessment of essential fatty acid status requires collection of blood or adipose tissue samples. However, these invasive techniques cannot always be used in studies involving infants, young children, or subjects from whom it is difficult to obtain blood. A body tissue that is easy to access is the buccal mucosa (cheek cells).

Objective: The objective was to investigate the degree to which fatty acids of cheek cells reflect the fatty acid content of plasma, red blood cells, and the diet.

Design: Thirty-one infants aged 12 mo were enrolled. Five infants were fed human milk and 26 infants received formulas that provided a wide range of arachidonic acid and docosahexaenoic acid (DHA) intakes. Cheek cells were collected on a small piece of gauze by gently swabbing the inside of the cheek 3 times. Lipids were extracted from the gauze and the phospholipid fatty acid content of the cheek cells was determined.

Results: Cheek cell DHA and arachidonic acid in phospholipids were significantly correlated with DHA and arachidonic acid in plasma [$r = 0.61$ ($P < 0.001$) and $r = 0.37$ ($P < 0.05$), respectively], red blood cells [$r = 0.58$ ($P < 0.001$) and $r = 0.37$ ($P < 0.05$), respectively], and the diet [$r = 0.65$ ($P < 0.001$) and $r = 0.51$ ($P < 0.01$), respectively].

Conclusions: Given these correlations and the ease and noninvasive nature of this technique, cheek cell fatty acids may serve as a marker of the essential fatty acid content, especially of DHA and arachidonic acid, in plasma, red blood cells, and the diet. *Am J Clin Nutr* 2000;71:21–7.

KEY WORDS Infants, cheek cells, buccal mucosa, fatty acids, diet, plasma, red blood cells, arachidonic acid, docosahexaenoic acid

INTRODUCTION

The fatty acid content of the diet may determine the risk of many chronic diseases, including atherosclerosis, coronary heart disease, and breast and colon cancers as well as the risk of essential fatty acid deficiency (1–3). Most studies rely on current or retrospective reports of dietary intake for determining dietary fatty acid intake. The accuracy of such data may well be questioned. Objective biochemical measures of fatty acid intakes are useful for validating the dietary intakes of subjects in epidemiologic and interventional studies and in evaluating essential fatty acid deficiency (4). Fatty acids in adipose tissue (5–8), plasma (6), and red blood cells (9, 10) have been suggested as markers to confirm dietary fatty acid intakes; however; the collection of these markers requires invasive techniques for sample collection and may not be acceptable in studies involving infants or young children. Additionally, these invasive techniques may not be acceptable to older children and adults resistant to having their blood drawn or who have inaccessible veins. These problems are exacerbated in studies requiring multiple blood specimens to monitor changing fatty acid intakes and in studies of populations resistant to the idea of removing a vital substance such as blood.

A tissue that can be acquired much less invasively would be valuable for the validation of fatty acid intakes. It has been suggested that a tissue with a rapid turnover time (≈5 d) (11), such as the cheek cells of the mouth, could reflect the fatty acid content of the diet (12). Even though one study showed no significant correlation between the ratio of polyunsaturated to saturated fatty acids in the cheek cells and the diet (13), a second study by Hoffman et al (14) showed excellent correlation of cheek cell phospholipid docosahexaenoic acid (DHA; 22:6n−3) with dietary 22:6n−3. In such studies of cheek cell fatty acids, care has to be taken to avoid contamination with the fatty acids of recently ingested food and microorganisms (13). Because dietary fat is predominantly triacylglycerol, contamination would be minimized by analyzing cheek cell phospholipids.

Therefore, it was of interest to investigate whether cheek cell fatty acids could reflect dietary fatty acid intakes as well as mirror the fatty acid composition of plasma and red blood cells in human infants. In the study to be described, we used cheek cell membrane phospholipids to avoid possible triacylglycerol contamination of the specimens by recently consumed food.

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TABLE 1
Fatty acid composition of human milk and the study formulas[1]

Fatty acid	Human milk[2]	Formula[3]			
		DHA+AA	DHA	Control	Commercial[4]
	% of total fatty acids	*% of total fatty acids*			
12:0	3.9 ± 2.3	11.1	14.1	12.9	17.5
14:0	5.9 ± 2.1	4.5	5.7	5.1	7.2
16:0	19.8 ± 2.2	9.0	7.9	7.7	10.2
18:0	7.4 ± 1.3	4.0	3.2	3.1	4.2
18:1n−9 + 18:1n−7	38.3 ± 3.7	42.1	40.2	39.5	16.1
18:2n−6	15.3 ± 3.6	21.7	20.7	21.9	34.2
20:4n−6	0.5 ± 0.1	0.4	0.0	0.0	0.0
22:5n−6	0.1 ± 0.02	0.0	0.0	0.0	0.0
Total n−6	16.9 ± 3.7	22.1	20.7	21.9	34.2
18:3n−3	1.3 ± 0.6	1.9	1.9	2.2	4.8
20:5n−3	0.1 ± 0.03	0.0	0.0	0.0	0.0
22:5n−3	0.2 ± 0.04	0.0	0.0	0.0	0.0
22:6n−3	0.2 ± 0.1	0.1	0.2	0.0	0.0
Total n−3	1.8 ± 0.6	2.0	2.2	2.2	4.8
Ratio of n−6 to n−3	9.6 ± 1.9	11.1	9.4	10.0	7.1
Ratio of 20:4n−6 to 22:6n−3	3.0 ± 1.1	3.6	NA	NA	NA

[1] $\bar{x}$ ± SD. NA, not applicable; DHA, docosahexaenoic acid; AA, arachidonic acid.
[2] Values are based on 140 samples from 30 women lactating from 2 to 12 mo analyzed at Oregon Health Sciences University.
[3] Values first published in reference 15. Composition provided by Ross Products Division, Abbott Laboratories, Chicago.
[4] Infants in this group were originally breast-fed but had received only commercial formula for 7-9 mo before the cheek cell and blood collections.

SUBJECTS AND METHODS

Subjects

The subjects were selected from a group of infants who were participants in a 1-y feeding study (15) or in a maternal-infant diet study. Twenty-six infants were receiving 1 of 4 formulas and 5 were breast-fed. All infants were born full term (38–42 wk gestation) with weight appropriate for gestational age, 5-min Apgar scores ≥6, and no known physical or metabolic disease, intravenous lipid infusion, or blood transfusion. Infants born to mothers with a history of diabetes, tuberculosis, hyperlipidemia, or perinatal infection with known adverse effects on the fetus were not eligible for participation. Approval for these studies was obtained from the Oregon Health Sciences University's human ethics committee and informed consent was obtained from the parents of all infants before participation.

Diets

Liquid, ready-to-feed formulas were provided by Ross Products Division, Abbott Laboratories (Chicago), and differed only in the addition of arachidonic acid (AA; 20:4n−6) or 22:6n−3. The fatty acid composition of human milk, which contained both 22:6n−3 and 20:4n−6, and of each formula are given in **Table 1**. These formulas provided a wide range of 22:6n−3 and 20:4n−6 intakes. Five infants were fed human milk containing 0.5% (by wt) 20:4n−6 and 0.2% 22:6n−3 (human milk group) and 26 infants were fed 1 of 4 formulas: the DHA+AA formula (0.4% 20:4n−6 and 0.1% 22:6n−3; n = 10), the DHA formula (no 20:4n−6 and 0.2% 22:6n−3; n = 6), the control formula (no 20:4n−6 and no 22:6n−3; n = 8), or the commercial formula (no 20:4n−6 and no 22:6n−3; n = 2). The commercial formula was Similac with iron (Ross Products Division, Abbott Laboratories, Columbus, OH). All formulas provided 2791 kJ/L (20 kcal/fluid oz) and met or exceeded the nutrient recommendations of the American Academy of Pediatrics (16).

Formula was provided ad libitum as the sole source of nutrition for ≥4 mo in the formula groups. Human milk was provided exclusively for a minimum of 3 mo in the human milk group and, thereafter, supplementation with commercial formula was permitted. Breast-feeding was encouraged, however, and the 5 infants in the human milk group had received no formula supplementation at the time of this study. Two infants, who were initially breast-fed, had been fed commercial formula for 7–9 mo. Because plasma fatty acids turn over in a few days and red blood cell fatty acids turn over in 3 mo, the values from these subjects would be expected to be solely determined by the commercial formula. Beginning at 4 mo, solid food supplementation was permitted for all infants. All 31 infants were 12 mo old at the time of the study.

Collection and analysis of cheek cells

In previous studies in adults, the mouth was rinsed 3 times with 40-mL glass-distilled water. The water was collected, pooled, and centrifuged at 6000 × g for 10 min at room temperature and the cell pellet was analyzed for fatty acids (12, 13, 17). This method of collection was not appropriate for infants, so a technique developed by Hoffman et al (14) was used.

Cheek cells were collected at a visit when blood was also obtained. The inside of the baby's mouth was first rinsed with ≈3–5 mL water. A small piece of sterile gauze (Johnson & Johnson Medical, Inc, Arlington, TX) that had been determined to be free of fatty acids was then rubbed gently over the inside of the cheek 3 times. The gauze was then placed in a clean plastic container and taken to the laboratory and frozen for ≥30 min because freezing and thawing disrupts the cell membrane and facilitates subsequent lipid extraction. The lipids in the gauze were then extracted in 5 mL chloroform:methanol (2:1) by grinding the gauze gently in a mortar and pestle for 30 s; the extract was then filtered. The gauze was extracted one more time and the total extracts were then dried under nitrogen at room temperature. The

TABLE 2
Fatty acid composition of cheek cell phospholipids in infants fed human milk or formula[1]

Fatty acid	Human milk (n = 5)	Formula		
		DHA+AA (n = 10)	DHA (n = 6)	Control (n = 8)
	% of total fatty acids		*% of total fatty acids*	
12:0	1.9 ± 4.2	0.8 ± 1.6	0.6 ± 1.1	0.1 ± 0.1
14:0	1.5 ± 0.3	1.8 ± 0.3	1.6 ± 0.5	2.0 ± 0.8
16:0	14.0 ± 2.0	15.9 ± 2.1	14.8 ± 2.1	14.5 ± 2.5
18:0	11.5 ± 2.5	12.0 ± 2.1	11.8 ± 1.9	12.3 ± 2.4
Total saturated	33.8 ± 6.3	36.6 ± 5.6	33.2 ± 3.0	32.3 ± 3.4
16:1n−7	5.7 ± 1.9	4.6 ± 1.6	3.6 ± 0.8	5.5 ± 1.6
18:1n−9 + 18:1n−7	25.2 ± 1.7[a,b]	23.3 ± 5.4[a]	31.2 ± 6.5[b]	25.8 ± 5.0[a,b]
Total monounsaturated	32.0 ± 1.2	29.2 ± 4.8	35.8 ± 6.6	32.4 ± 4.2
18:2n−6	14.7 ± 4.6	14.6 ± 3.9	17.1 ± 3.3	16.5 ± 2.7
20:4n−6	2.9 ± 0.9[a]	2.6 ± 1.2[a]	1.4 ± 0.6[b]	2.0 ± 0.5[a,b]
22:5n−6	0.6 ± 0.7	0.5 ± 0.2	0.3 ± 0.2	1.0 ± 0.9
Total n−6	19.5 ± 4.6	20.4 ± 3.6	19.9 ± 3.4	20.9 ± 3.5
18:3n−3	0.3 ± 0.2	0.5 ± 0.4	0.5 ± 0.3	0.4 ± 0.3
20:5n−3	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:5n−3	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1
22:6n−3	0.9 ± 0.3[a]	0.7 ± 0.4[a,b]	0.8 ± 0.2[a]	0.4 ± 0.1[b]
Total n−3	1.7 ± 0.5[a]	1.7 ± 0.7[a]	1.6 ± 0.2[a,b]	1.2 ± 0.3[c]
Ratio of n−6 to n−3	12.3 ± 5.7	13.3 ± 4.1	12.6 ± 3.6	18.8 ± 6.2
Ratio of 20:4n−6 to 22:6n−3	3.4 ± 1.1[a]	3.3 ± 1.1[a]	1.8 ± 0.4[b]	4.0 ± 1.2[a]

[1] $\bar{x}$ ± SD. All fatty acids were statistically analyzed. Values with different superscript letters are significantly different, $P < 0.05$. DHA, docosahexaenoic acid; AA, arachidonic acid.

residue was redissolved in 0.2 mL chloroform:methanol (2:1). This extract was subjected to thin-layer chromatography. Five major lipid classes (phospholipids, cholesterol, fatty acids, triacylglycerol, and cholesteryl esters) were separated on silica gel G plates (500 μm; Analtech, Newark, DE). The R_f value (the migration of the peak of interest divided by the migration of the solvent front) for each lipid class was 0 for phospholipids, 0.070 for cholesterol, 0.298 for fatty acids, 0.509 for triacylglycerol, and 0.703 for cholesterol esters. The solvent system was hexane:chloroform:ethyl ether:acetic acid (80:10:10:1.5). The phospholipid band was extracted with chloroform:methanol (2:1) and the extract dried under nitrogen and saponified with alcoholic potassium hydroxide. The phospholipid fatty acids were recovered by acidifying the aqueous phase and extracting with hexane. Fatty acids were then methylated with boron trifluoride and their methyl esters were analyzed by gas-liquid chromatography with an instrument equipped with a hydrogen flame ionization detector (Auto System gas chromatograph; Perkin-Elmer, Norwalk, CT) and a 30-m SP-2330 fused silica capillary column (Supelco, Bellefonte, PA). Temperatures of the column, detector, and injection port were 184, 250, and 250°C, respectively. Helium was used as the carrier gas. A computer program (Turbochrom; Perkin-Elmer) measured the retention time and area of each peak. A mixture of fatty acid standards was run daily (18).

Blood collection and analysis

A 1–2-mL blood sample was collected from each infant by venipuncture into tubes containing disodium EDTA. Plasma and red blood cells were separated by centrifugation at 800 × g for 10 min at room temperature and the red blood cell pellet was washed 3 times with saline solution. The lipids of the red blood cells were extracted by the procedure of Rose and Oklander (19) with chloroform and isopropanol. Butylated hydroxytoluene (0.05 g/L) was added as an antioxidant (20). Plasma lipids were extracted by the procedure of Bligh and Dyer (21). The lipid extracts of plasma and red blood cells were dried under nitrogen and saponified with alcoholic potassium hydroxide. Fatty acids were recovered by acidifying the aqueous phase and then extracted with hexane and methylated (22). Methyl esters of fatty acids were analyzed by gas-liquid chromatography with the same instrument used for cheek cell fatty acid determination.

Statistical analysis

Statistical analysis was performed by using analysis of variance (ANOVA), with the Bonferonni inequality used for post hoc detection of pairwise differences (23). The data that failed a normality test were either log transformed before ANOVA or a Kruskal-Wallis one-way ANOVA on ranks was used. Because of the small sample size, the data for the commercial-formula group were excluded from the analyses of differences across groups. However, data from all 31 subjects were used to determine correlation coefficients with the Spearman rank-correlation test.

RESULTS

The fatty acid composition of cheek cell phospholipids isolated from the infants is given in **Table 2**. This study focused primarily on the fatty acids that were intentionally varied (22:6n−3 and 20:4n−6) and the 2 polyunsaturated fatty acids that are precursors of 22:6n−3 and 20:4n−6, namely linolenic acid (18:3n−3) and linoleic acid (18:2n−6), respectively. The 22:6n−3 content of the cheek cell phospholipids was 0.9% in the human milk group, 0.7% in the DHA+AA group, 0.8% in the DHA group, and 0.4% in the control group. The 22:6n−3 con-

TABLE 3
Selected polyunsaturated fatty acid content of red blood cells and plasma in infants fed human milk or study formulas[1]

Fatty acid	Human milk (n = 5)	Formula: DHA+AA (n = 10)	Formula: DHA (n = 6)	Formula: Control (n = 8)
	% of total fatty acids		*% of total fatty acids*	
Red blood cells				
18:2n−6	13.7 ± 1.9[a]	13.7 ± 1.5 [a]	15.3 ± 1.3[a,b]	16.0 ± 1.2 [b]
20:4n−6	13.9 ±1.4[a,c]	14.2 ± 1.0[a]	11.4 ± 1.4[b]	12.5 ± 1.3[b,c]
18:3n−3	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
22:6n−3	3.8 ± 0.5[a]	3.5 ± 0.4[a]	5.4 ± 0.4[b]	1.8 ± 0.3[c]
Ratio of n−6 to n−3	5.6 ± 0.4[a]	7.5 ± 1.2[b]	4.3 ± 0.4[c]	9.4 ± 0.8[d]
Ratio of 20:4n−6 to 22:6n−3	3.7 ± 0.4[a]	4.5 ± 1.1[a]	2.1 ± 0.5[b]	7.2 ± 0.7[c]
Plasma				
18:2n−6	29.7 ± 5.6	31.8 ± 2.6	33.1 ± 2.4	32.9 ± 1.6
20:4n−6	6.2 ± 0.9[a]	6.7 ± 1.3[a]	3.6 ± 0.8[b]	4.6 ± 1.0[b]
18:3n−3	0.7 ± 0.3	0.7 ± 0.2	0.9 ± 0.6	0.9 ± 0.2
22:6n−3	1.8 ± 0.4[a,b]	1.5 ± 0.3[a]	1.9 ± 0.4[b]	0.7 ± 0.2[c]
Ratio of n−6 to n−3	11.9 ± 1.4[a]	16.0 ± 1.5[b]	11.4 ± 2.1[a]	18.1 ± 1.8[c]
Ratio of 20:4n−6 to 22:6n−3	3.5 ± 0.5[a]	4.7 ± 0.7[b]	1.9 ± 0.5[c]	6.5 ± 0.7[d]

[1] $\bar{x}$ ± SD. Values with different superscript letters are significantly different, $P < 0.05$. DHA, docosahexaenoic acid; AA, arachidonic acid.

tent of cheek cell phospholipid in the human milk group and of total n−3 fatty acids in all the 22:6n−3-containing diet groups were significantly higher than those in the control group. There were no significant differences in the cheek cell phospholipid 18:3n−3 content (the precursor to 22:6n−3) among the groups.

The 20:4n−6 content of the cheek cell phospholipids in the human milk group (2.9%) and the DHA+AA group (2.6%) differed significantly from that in the DHA group (1.4%). Accordingly, the ratio of 20:4n−6 to 22:6n−3 was significantly lower in the DHA group. There were no significant differences in the 18:2n−6 content (the precursor to 20:4n−6) among groups.

Selected polyunsaturated fatty acid contents of plasma and red blood cells are given in **Table 3**. The major differences among them were in the 22:6n−3 and 20:4n−6 contents and the ratios that included these fatty acids. In red blood cells, the 22:6n−3 contents of the 22:6n−3–containing diet groups were significantly higher than those in the control group. The ratio of 20:4n−6 to 22:6n−3 in red blood cells was significantly lower in the DHA group than in all other groups. The mean red blood cell 20:4n−6 content ranged from 11.4% to 14.2%, the content being significantly higher in the human milk group (13.9%) and the DHA+AA group (14.2%) than in the DHA group (11.4%). The ratios of n−6 to n−3 fatty acids were significantly different among all groups, with the lowest ratio occurring in the DHA group and the highest ratio in the control group. There were only slight differences in the 18:2n−6 and 18:3n−3 contents of the red blood cells.

Plasma 22:6n−3 was significantly higher in all of the 22:6n−3–containing diet groups than in the control group. The 20:4n−6 content was 6.2% in the human milk group and 6.7% in the DHA+AA group, both of which were significantly higher than that in the DHA group (3.6%) and the control group (4.6%). The ratios of 20:4n−6 to 22:6n−3 were significantly different among all groups, with the DHA group having the lowest ratio and the control group the highest ratio, as in the red blood cells. The ratio of n−6 to n−3 fatty acids was significantly lower in the human milk group (11.9%) and the DHA group (11.4%) than in the DHA+AA group (16.0%) and the control group (18.1%).

The ratio of 20:4n−6 to 22:6n−3 was similar across tissues for each of the groups. For the human milk group, the ratio of 20:4n−6 to 22:6n−3 was 3.0 for human milk, 3.4 for cheek cells, 3.7 for red blood cells, and 3.5 for plasma. For the DHA+AA group, the ratio of 20:4n−6 to 22:6n−3 was 3.6 for formula, 3.3

TABLE 4
Correlation coefficients (r) between diet, cheek cells, plasma, and red blood cell fatty acids[1]

	Fatty acid: 22:6n−3[1]	Fatty acid: 20:4n−6[1]	Fatty acid: Ratio of 20:4n−6 to 22:6n−3[2]	Fatty acid: Ratio of n−6 to n−3 fatty acids[1]
Correlation of diet with				
Cheek cells	0.65[3]	0.51[4]	0.29	0.19
Plasma	0.84[3]	0.69[3]	0.82[3]	0.61[3]
Red blood cells	0.89[3]	0.57[3]	0.81[3]	0.45[5]
Correlation of cheek cells with				
Plasma	0.61[3]	0.37[5]	0.81[3]	0.45[5]
Red blood cells	0.58[3]	0.37[5]	0.79[3]	0.47[4]

[1] Data from all 5 groups (human milk and 4 formulas) were combined for this analysis (n = 31).
[2] Only data from the human milk and DHA+AA (formula containing both docosahexaenoic and arachidonic acids) groups were used.
[3] $P < 0.001$.
[4] $P < 0.01$.
[5] $P < 0.05$.

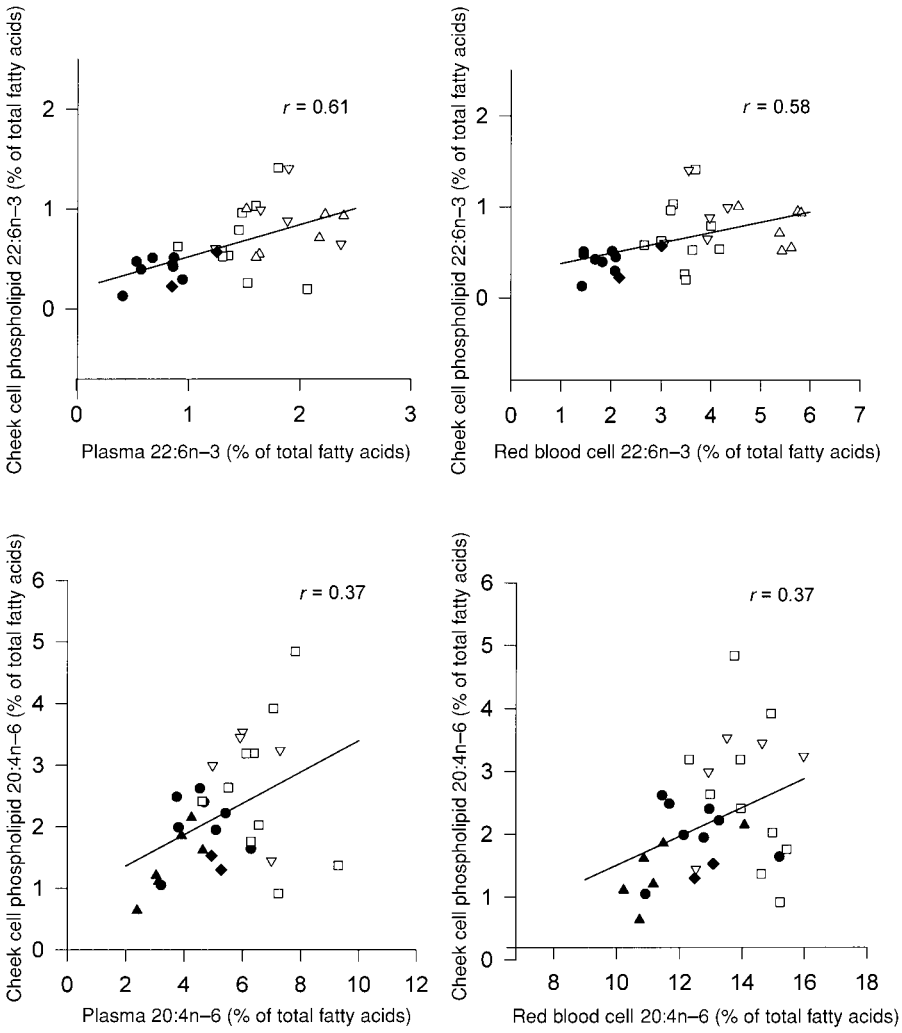

FIGURE 1. Scatter diagrams of comparisons between cheek cell phospholipid docosahexaenoic acid (DHA; 22:6n−3) and arachidonic acid (AA; 20:4n−6) and plasma and red blood cell 22:6n−3 and 20:4n−6 (n = 31). For 22:6n−3, filled symbols represent the formulas that did not contain 22:6n−3: control (●) and commercial (◆); open symbols represent the formulas and human milk that contained 22:6n−3: DHA (△), DHA+AA (□), and human milk (▽). For 20:4n−6, filled symbols represent the formulas that did not contain 20:4n−6: control (●), commercial (◆), and DHA (▲); open symbols represent the formula and human milk that contained 20:4n−6: DHA+AA (□) and human milk (▽).

for cheek cells, 4.5 for red blood cells, and 4.7 for plasma. The ratio of 20:4n−6 to 22:6n−3 for the DHA group was 1.8 for cheek cells, 2.1 for red blood cells, and 1.9 for plasma. The ratio of 20:4n−6 to 22:6n−3 for the control group was 4.0 for cheek cells, 7.2 for red blood cells, and 6.5 for plasma.

Correlations between diet, cheek cell phospholipid fatty acids, plasma, and red blood cells for all 31 subjects are given in **Table 4** and **Figure 1**. Dietary 22:6n−3 was significantly correlated with cheek cell phospholipid 22:6n−3, plasma 22:6n−3, and red blood cell 22:6n−3. Dietary 20:4n−6 was significantly correlated with cheek cell phospholipid 20:4n−6, plasma 20:4n−6, and red blood cell 20:4n−6. The fatty acid ratios of the diet were significantly correlated with those in plasma and red blood cells, but not with those in cheek cells.

Cheek cell phospholipid 22:6n−3 was significantly correlated with plasma and red blood cell 22:6n−3. Cheek cell phospholipid 20:4n−6 was significantly correlated with plasma and red blood cell 20:4n−6. Scatter diagrams indicated that the relation was linear throughout the range of plasma and red blood cell 22:6n−3 contents.

DISCUSSION

The fatty acids 22:6n−3 and 20:4n−6 are important because they are the major polyunsaturated fatty acids of cellular membranes. Although these fatty acids are particularly rich in the brain, retina, and sperm, both are present in all cells of the body (24, 25). This study showed that 22:6n−3 and 20:4n−6 in the cheek cell phospholipids of human infants correlated well with the 22:6n−3 and 20:4n−6 contents in the diet (in human milk and various formulas). These data confirm the previous observation that the 22:6n−3 content of cheek cell phospholipids reflects the content of 22:6n−3 in the diet and in red blood cells (14).

The fatty acids 22:6n−3 and 20:4n−6 and the fatty acid ratios that included 22:6n−3 and 20:4n−6 of the cheek cells also correlated well with these fatty acids in the plasma and red blood cells of human infants who had consumed various quantities of these fatty acids in the diet. These infants had been given for 12 mo formulas, human milk, or both that contained a wide range of 22:6n−3 and 20:4n−6. Additionally, cheek cell fatty acids were significantly correlated with the fatty acids in the diet, plasma, and red blood cells. Of importance was the high degree of correlation between the ratio of 20:4n−6 to 22:6n−3 in the cheek cells and that in the plasma (r = 0.81) and red blood cells (r = 0.79).

The 22:6n−3 and 20:4n−6 contents of cheek cell phospholipids were approximately one-fifth of their contents in red blood cells and about two-fifths of their contents in plasma. Furthermore, in each of the feeding groups, the ratio of 20:4n−6 to 22:6n−3 was almost identical for cheek cells, plasma, and red blood cells. This close similarity lends credence to the strong relations between cheek cell fatty acids and the fatty acids of the red blood cells and plasma. The origin of 22:6n−3 and 20:4n−6 in the cheek cell phospholipids was most likely from the plasma fatty acids bound to albumin and from the fatty acids of lipoproteins that are incorporated into the developing mucosal cells of the mouth. Thus, similar amounts of these fatty acids in cheek cells and the blood are to be expected.

A test for the possible contamination of the cheek cell specimens with residual food indicated the validity of the cheek cell phospholipid analysis because of the virtual absence of lauric acid (12:0) in the cheek cell phospholipid preparation; 12:0 represented 4–14% of total fatty acids in the various diets, but typically <0.2% in the phospholipids of the cheek scrapings. Further validity of the cheek cell phospholipid analysis was indicated by the presence of several polyunsaturated fatty acids in the cheek cells of infants in all 4 groups despite the absence of the polyunsaturated fatty acids eicosapentaenoic acid, docosapentaenoic acid, and 22:5n−6 in the infants' diet. These are synthesized in the liver from n−3 and n−6 fatty acid precursors. Thus, contamination of the final cheek cell phospholipid fraction by food was minimal.

This study showed that the 22:6n−3 and 20:4n−6 contents of cheek cell phospholipids might provide an indication of their contents in the diet, plasma, and red blood cells. Other than differences in the amounts of 22:6n−3 and 20:4n−6, the formulas fed to the various infant groups were designed to have similar contents of all other fatty acids, except that 18:2n−6 and 18:3n−3 were higher in the commercial formula. Therefore, on the basis of the data from this study, one might also expect to find similar relations between other phospholipid fatty acids in cheek cells and those in the diet, plasma, and red blood cells whenever there would be a sufficient range of these fatty acids in the diet.

From a practical point of view, the content of cheek cell fatty acids would be useful as an indicator of the fatty acid status of infants or children with essential fatty acid deficiency. The rapid 5-d turnover of cheek cells would greatly facilitate the follow-up of essential fatty acid–deficient infants. By serving as a marker of fatty acids in the diet, plasma, and red blood cells, cheek cell phospholipid fatty acids might be useful in clinical practice and in epidemiologic and metabolic studies, in which it is difficult or not feasible to obtain blood or other tissue samples.

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