show values of plasma tocopherols/total lipids more than 0.6 mg/g, the level proposed to be adequate by Farrell et al (3) for the assessment of vitamin E nutriture.

2) The Brazilian infants, artificially fed on cows' milk during the 1st yr of early life, show higher percentages of plasma tocopherol values less than 0.6 mg/g of total lipids. This trend corrects itself toward adequate levels of vitamin E as age progresses and with the supplementation of an adult diet.

3) A positive relationship was observed between the differences in the hematological indices and the differences in plasma vitamin E levels of breast-fed and cows' milk-fed infants. However, there may be other factors in addition to vitamin E which may also be involved.

4) Since the infants who were nutritionally inadequate as far as vitamin E is concerned did not present growth abnormalities and other clinical repercussions, the significance of vitamin E deficiency in cows' milk-fed infants cannot be stipulated at this time.

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Original Research Communications—method

Dietary-induced changes in the fatty acid composition of human cheek cell phospholipids: correlation with changes in the dietary polyunsaturated/saturated fat ratio[1,2]

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ABSTRACT Healthy normotensive volunteers aged 20 to 59 yr were randomly allocated either to a control group or to one of two experimental groups. The control group ate a low P/S ratio diet for 12 wk while the first experimental group ate a high P/S ratio diet for 6 wk followed by a low P/S ratio diet for the next 6 wk. The second experimental group ate a low P/S ratio diet in the first 6 wk followed by a high P/S ratio diet for the next 6 wk. Dietary P/S ratio, plasma linoleic acid (18:2), and cheek cell phospholipid 18:2 levels were compared in each dietary group at the end of the 1st and 2nd 6 wk. On change from a low to a high P/S ratio diet, there was a 36% increase in the proportion of 18:2 in the cheek cell phospholipids in comparison with the proportion existing before the change. This was associated with an increase in the proportion of 18:2 in the plasma lipids of this group. No reduction in the proportion of 18:2 in the cheek cell phospholipids was apparent in the control group or the group which changed from a high to a low P/S ratio diet, although in the latter group there was a reduction in the proportion of 18:2 in the plasma lipids. As the phospholipid fatty acid composition of human cheek cells reflects dietary lipid status under certain conditions, this observation may be useful in dietary and nutritional studies, particularly as human cheek cells can be obtained in a noninvasive manner. *Am J Clin Nutr* 1984;39:975–980

KEY WORDS Cheek cell fatty acids, dietary lipids, plasma fatty acids

Introduction

Coronary heart disease, colorectal cancer, and a number of other chronic diseases are thought to be related etiologically to the intake and composition of dietary lipids (1, 2). Studies of the dietary etiology of these diseases in humans have depended on estimates of dietary lipid intake obtained from diet records or recall and food composition tables (3). These estimates are imprecise and often inaccurate (3) and therefore the credibility of dietary studies in humans can be

lipid intake, is measurement of the fatty acid composition of lipids in tissues and body

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fluids, eg, plasma, erythrocyte membranes, platelets, and adipose tissues (4–9). The fatty acid composition of these lipids does correlate with the fatty acid composition of dietary lipid and its measurement is considered to provide a valid index of this aspect of habitual diet (7, 8).

We have developed a method for studying the fatty acid composition of phospholipids in human cheek (buccal epithelial) cells (10). It has the advantage of being completely noninvasive and has proven to be a sensitive indicator of differences in the composition of lipids eaten by vegetarians and nonvegetarians (10). Herein, we describe the effects of controlled changes in the ratio of dietary P/S on the fatty acid composition of cheek cells.

Materials and methods

Experimental design

Details of sample selection and experimental design have been presented elsewhere (11). In summary, 54 volunteers from nonmedical staff at the Royal Perth Hospital were randomly allocated in strata of age (5-yr groups) and sex to a control group or one of two experimental groups. They completed a 2-wk period in which their usual diet was monitored and then a 14-wk period in which they were asked to follow a carefully defined diet. The latter period was divided into a 2-wk familiarization period and two 6-wk experimental periods (periods I and II). The control group ate a low P/S ratio diet throughout. Experimental group A ate a high P/S ratio diet in period I and a low P/S ratio diet in period II. Experimental group B ate a low P/S ratio diet in period I and a high P/S ratio diet in period II. The protocol used in these experiments was approved by the human ethics committees of the CSIRO Division of Human Nutrition, The Queen Elizabeth II Medical Centre and the Department of Medicine, Royal Perth Hospital.

All subjects were asked to avoid intake of visible fat, such as meat, skin of chicken, roasts, cream, desserts, butter, margarine, cooking oils and fats, chocolate, and mayonnaise. The visible fat removed was replaced with unlabeled margarine (either polyunsaturated or saturated) and specially prepared biscuits made with polyunsaturated margarine and walnuts (polyunsaturated fat) or saturated fat and hazelnuts (saturated fat). The intakes of margarine and biscuits were individually tailored to maintain preexperimental fat and total energy intakes and were formulated so as to provide a dietary lipid intake typical of either an omnivorus diet, with a P/S fatty acid ratio of about 0.4, or a vegetarian diet with a P/S ratio of about 1.0. Data presented in

Determination of dietary intake

During each of the two 6-wk experimental periods, dietary intakes were assessed by seven 24-h estimated diet records. Subjects were randomly allocated days on which to keep records such that each day of the week was surveyed. Nutrient intakes were estimated using McCance and Widdowson's Composition of Food Tables (12).

Determination of plasma fatty acid composition

At the end of each experimental period blood was taken from fasted subjects for determination of the fatty acid composition of total plasma lipids. After removal of the red blood cells by centrifugation, lipids were extracted by the method of Bligh and Dyer (13). Total plasma fatty acid methyl esters were prepared by transmethylation in 14% boron trifluoride in methanol and then analyzed by gas-liquid chromatography on a column of 10% DEGS on 80/100 mesh chromosorb (Allteck Associates, Summer Hill, NSW, Australia) operated isothermally at 185°C.

Collection of cheek epithelial cells

Cheek cells were collected at the end of each experimental period at the time of blood sampling. Subjects were asked under supervision to clean their mouths by rinsing with glass distilled water (GDW). Cheek cells were then collected by having the subject rinse the mouth vigorously with 30 to 35 ml of GDW and expectorate the fluid into a container. Usually three of these collecting cycles were performed after the initial cleansing rinse, with the final volume collected being about 40 ml. The sample was then centrifuged at 6000 × *g* for 10 min. After discarding the supernatant, the pellet of cells was resuspended in GDW and washed once by centrifugation as above. The cells were finally resuspended in 1 ml of GDW and stored at −20°C until lipid analysis was performed about 2 wk after the end of period II. On thawing, and just before lipid analysis, cells were examined by phase contrast microscopy to estimate the yield of cells and to determine whether bacterial or other foreign matter was present in the preparation.

Fatty acid analysis of cheek cell phospholipids

The lipids were extracted from the cheek cells according to the method of Bligh and Dyer (13). One volume of cheek cells suspended in GDW was extracted with 20 volumes of chloroform/methanol (2:1) in the presence of the antioxidant butylatedhydroxytoluene. The phospholipids were separated from the other lipid classes by thin-layer chromatography on silica gel H plates, using petroleum ether and acetone (3:1) containing butylatedhydroxytoluene. The phospholipids remaining at the origin were eluted from the silica gel and fatty acid methyl esters were prepared by methylation in 1% (v/v) H_2SO_4 in methanol by heating for 3 h at 70°C as described by McMurchie et al (14). Fatty acid methyl esters were extracted using petroleum ether and analyzed by gas-liquid chromatography by the method

programmed from 125 to 225°C at 4°C/min. The total proportions of the six major fatty acids of the cheek cell phospholipids were normalized to a value of 100%. For the control group, these fatty acids represented 72.9 ± 2.1% of the total fatty acids (mean ± SE for samplings at the end of periods I and II); for group A they were 74.1 ± 1.7% and for group B, 68.3 ± 2.6%.

Statistical significance

Statistical analyses were performed by use of statistical package for the social sciences (16) with paired *t*-tests (two-tailed p values) unless otherwise stated.

Results

The estimated dietary P/S ratio measured during periods I and II and the plasma and cheek cell linoleic acid (18:2) content, expressed as percentage of total fatty acids measured at the end of periods I and II, are shown in **Table 1.** There were no significant changes in any of the above variables from periods I to II in the control group. There were statistically significant increases in estimated dietary P/S ratio and in the proportions of linoleic acid in plasma and cheek cells on changing from the low to the high P/S ratio diet (group B). The experimental group returning from the high P/S ratio diet to the low P/S diet in period II (group A) showed a statistically significant fall in estimated dietary P/S ratio and plasma linoleic acid, and a small reduction in cheek cell linoleic acid which was not statistically significant.

Expressing the change in cheek cell linoleic acid from periods I to II as a percentage of the initial proportion, the rise in linoleic acid in group B was about 36% whereas on return from the high P/S ratio diet to the low P/S ratio diet (group A), the fall was about 7%. This fall was similar to that observed in the control group, which is about 5% (Table 1).

Palmitic (16:0) and stearic (18:0) acids were the major saturated fatty acids of the cheek cell phospholipids in each dietary group, while oleic acid (18:1) was the major unsaturated fatty acid (**Table 2**). The only significant changes observed between the ends of periods I and II in the proportions of the individual fatty acids were in the proportion of linoleic acid in group B and the proportion of oleic acid in both the control group and group B. As a result of these minor changes in individual fatty acids, little overall change was observed in the proportions of total unsaturated and monounsaturated fatty acids. In group B, however, there was a trend toward elevation of the proportion of polyunsaturated fatty acids at the end of period II relative to period

TABLE 1
Dietary P/S ratios and linoleic acid (18:2) in plasma lipids and cheek cell phospholipids at the end of periods I and II

Group	Period I			Period II			% Change in cheek cell 18:2‡
	Dietary P/S	Plasma* 18:2	Cheek cell† 18:2	Dietary P/S	Plasma 18:2	Cheek cell 18:2	
Control	0.31 ± 0.02 (16)	28.2 ± 1.2 (16)	8.43 ± 0.56 (14)	0.38 ± 0.07 (14)	27.1 ± 1.65 (15)	7.98 ± 0.42 (14)	−5.3
Group A (HL)	1.04 ± 0.05 (20)	36.5 ± 1.18 (18)	8.42 ± 0.82 (17)	0.33 ± 0.04§‖ (19)	31.0 ± 0.92¶ (19)	7.81 ± 0.66 (17)	−7.2
Group B (LH)	0.27 ± 0.03 (18)	30.1 ± 0.98 (16)	7.63 ± 0.46 (16)	1.06 ± 0.06‖ (16)	32.8 ± 1.38** (16)	10.41 ± 0.91¶ (16)	+36.4

Data are presented as the mean ± SE with the number of respondents indicated in parentheses. Groups are defined as control, no change in diet from period I to period II; group A, change from a high P/S to a low P/S diet from period I to period II (HL); group B, change from a low P/S to a high P/S diet from period I to period II (LH).

* Plasma 18:2 represents the mean percentage of linoleic acid in the total plasma fatty acids.

† Cheek cell 18:2 represents the mean percentage of linoleic acid in the major fatty acids of the cheek cell phospholipids.

TABLE 2
Fatty acid composition of cheek cell phospholipids after changes in dietary lipid (P/S) intake

	Group Control (n = 14)		Group A (HL; n = 17)		Group B (LH; n = 16)	
	Period I	II	I	II	I	II
Fatty acid*						
16:0	23.4 ± 0.64	23.3 ± 0.67	23.3 ± 0.78	23.6 ± 0.65	23.1 ± 0.63	23.0 ± 0.63
18:0	20.4 ± 0.61	21.1 ± 0.67	22.4 ± 0.65	22.2 ± 0.78	22.1 ± 0.65	21.4 ± 0.98
16:1	6.7 ± 0.61	5.9 ± 0.53	6.7 ± 0.61	6.3 ± 0.61	5.9 ± 0.60	6.5 ± 0.53
18:1	37.1 ± 0.64	38.3 ± 0.56†	36.6 ± 0.51	37.1 ± 0.56	37.8 ± 0.58	36.0 ± 0.55†
18:2	8.4 ± 0.56	8.0 ± 0.43	8.4 ± 0.82	7.8 ± 0.65	7.6 ± 0.45	10.4 ± 0.90‡
20:4	3.8 ± 0.64	3.3 ± 0.29	2.5 ± 0.56	2.9 ± 0.44	3.4 ± 0.70	2.8 ± 0.25
Σ saturated (S)	43.8 ± 0.75	44.4 ± 0.60	45.7 ± 1.00	45.8 ± 1.10	45.2 ± 0.70	44.4 ± 1.00
Σ unsaturated (U)	55.9 ± 0.72	55.5 ± 0.61	54.1 ± 0.91	54.1 ± 1.13	54.7 ± 0.76	55.8 ± 0.96
Σ monounsaturated	43.7 ± 0.78	44.2 ± 0.36	43.2 ± 0.73	43.4 ± 0.73	43.7 ± 0.59	42.6 ± 0.58
Σ polyunsaturated (P)	12.2 ± 0.81	11.3 ± 0.44	10.9 ± 1.10	10.7 ± 1.00	11.0 ± 0.70	13.2 ± 1.00
P/S	0.28 ± 0.02	0.26 ± 0.01	0.25 ± 0.03	0.24 ± 0.02	0.25 ± 0.02	0.31 ± 0.03†
U/S§	1.28 ± 0.04	1.25 ± 0.03	1.20 ± 0.05	1.20 ± 0.05	1.22 ± 0.04	1.27 ± 0.05
Unsaturation index	75.7 ± 2.20	73.5 ± 1.30	69.9 ± 2.70	70.7 ± 2.80	72.5 ± 2.40	74.7 ± 2.30

* Fatty acids are presented as the mean percentage by weight of the total major fatty acids ± SE for the indicated number of respondents (n) in each group. Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The unsaturation index is $\sum[(a)(b)]$ where a is the relative percentage of each unsaturated fatty acid and b is the number of double bonds for that particular fatty acid. Differences between periods I and II were significant as follows, † $p < 0.05$, ‡ $p < 0.01$.

§ U/S = ratio of unsaturated to saturated fatty acids. Variance of ratios was determined according to the method of Armitage (18).

I. The P/S ratio in group B was significantly increased at the end of period II, compared with the end of period I.

Discussion

Cheek cells can be obtained by a convenient noninvasive method and their phospholipid fatty acid composition responds to changes in dietary lipid intake (10). Sufficient cheek cells, free from bacterial and other contamination, can be obtained in one sampling for fatty acid analysis. The phospholipid fatty acids reflect the fatty acids associated with the cheek cell membranes.

In this study, the proportion of linoleic acid (18:2) in cheek cell phospholipids was significantly increased by increasing the dietary P/S ratio. The change in the dietary P/S ratio was achieved mainly by substitution of saturated and monounsaturated fatty acids with linoleic acid and was associated with an increase in plasma linoleic acid which occurred within 6 wk of the introduction of the high P/S ratio diet portion of linoleic acid in cheek cells, suggesting that an increase in phospholipid linoleic acid may have occurred more rapidly than a decrease in this tissue at least. This may mean that there is a minimum level of linoleic acid required for cheek cell phospholipids. Alternatively, it may simply reflect differences in relative rates of incorporation and removal of linoleic acid from phospholipid.

There are other interesting aspects of these data which raise additional possibilities. There was no difference in the proportion of linoleic acid in the cheek cell phospholipids between group A (high P/S ratio diet) and the control group (low P/S ratio diet) at the end of period I. Moreover, comparison of group A at the end of period I with group B at the end of period II (both after 6 wk on the high P/S ratio diet), suggests that the proportion of linoleic acid in cheek cells of group A at the end of period I was unexpectedly low. This may represent chance variation in the data. Alternatively, variation in total fat intake could have influenced cell

ever, few appreciable differences in total fat intake between groups or within groups over time (11). Indeed, the study was designed to hold total fat intake constant.

The profile of human cheek cells phospholipid fatty acids has previously been observed to differ between vegetarians and nonvegetarians (10). In that study the differences in dietary fat intake were closely reflected in the cheek cell phospholipid fatty acid profile. The vegetarians exhibited a significantly lower saturated fat intake than the nonvegetarians and this was associated with a relatively lower proportion of saturated fatty acids in their cheek cell phospholipids. There were no differences between vegetarians and nonvegetarians in the proportions of linoleic acid in the cheek cells or in the estimated dietary intakes of linoleic acid (10). This suggests that cheek cell lipids are sensitive to chronic differences in dietary lipid but does not help to resolve the inconsistencies in the present data with respect to short-term changes in dietary linoleic acid.

The time scale of changes in cheek cell lipids in response to changes in dietary lipids is not accurately known. However, with a reported turnover time for human cheek (buccal epithelial) cells of 5 days (17), the 6-wk experimental period used in this study should have been adequate to allow for a maximum diet-induced change to occur. It is interesting to note that both the control and the high to low P/S ratio groups exhibited similar downward trends in the proportion of linoleic acid over the complete time course of the experiment (periods I and II). Thus the 36% increase in cheek cell linoleic acid observed in the group going from the low to high P/S ratio diet could be of greater magnitude because of this opposing shift in the proportion of linoleic acid in the other two groups.

The use of cheek cells in the analysis of tissue lipid profiles is of potential value in dietary and nutritional studies on human subjects. As cheek cells can be obtained in a completely noninvasive manner, they could extend the pool from which samples can be taken. For example, the fact that we have useful for the lipid analysis of tissues from children in epidemiological surveys. Moreover, this method offers an alternative to the sampling of blood or adipose tissue for the validation of measurements of dietary lipid intake and for specific study of incorporation of dietary lipids into tissues. The use of this method could extend to the measurement of other components such as trace elements and vitamins in human epidemiological studies.

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