The Effects of Low-Fat Diets Rich in Arachidonic Acid on the Composition of Plasma Fatty Acids and Bleeding Time in Australian Aborigines

Kerin O’DEA,1 and Andrew J. SINCLAIR2

1 University of Melbourne Department of Medicine, Royal Melbourne Hospital, Victoria 3050, Australia
2 Department of Agriculture, Victoria, Veterinary Research Institute, Park Drive, Parkville 3052, Australia

(Received October 1, 1984)

Summary In the present study we measured the bleeding times in fourteen Aborigines (10 diabetic, 4 non-diabetic) before and after 2 weeks on a diet of tropical seafood (rich in both arachidonic acid and the ω3 PUFA), followed by 3 weeks on a diet in which kangaroo and freshwater fish (linoleic and arachidonic acid-rich) were the major fat sources. Both diets were very low in fat. Bleeding times increased in all subjects after the 2 weeks of tropical seafood and continued to rise on the mixed diet. The increase over 5 weeks from 4.1±0.4 to 5.9±0.4 min was highly significant (p<0.01).

Due to the extreme isolation of the study location it was only possible to measure the plasma fatty acid composition at the beginning and end of the study. The concentration of arachidonic acid in the plasma lipids doubled whereas that of linoleic acid was almost halved, despite the fact that the diet in the second part of the study contained considerably more linoleic than arachidonic acid. That there appeared to be preferential incorporation of arachidonic acid into the plasma lipids is further supported by the observation that the rise in arachidonic acid in the cholesterol ester and phospholipid fractions was almost exactly counter-balanced by the fall in linoleic acid. In conclusion, the present study demonstrated a rise in bleeding time associated with an increased concentration of arachidonic acid and decreased concentration of linoleic acid in plasma lipids, and suggests that the mechanism by which diet modulates haemostatic function may be more complex than currently assumed.

Key Words low-fat diets, polyunsaturated fatty acids, plasma fatty acids, arachidonic acid, bleeding time, Australian Aborigines, kangaroo, tropical seafood
We recently observed that the proportion of arachidonic acid in the plasma fatty acids of a group of healthy young Aborigines tripled after 2 weeks on a diet derived almost exclusively from tropical seafood (1). Apart from this unexpected observation, all other plasma lipid changes in response to the diet (fall in triglycerides, fall in linoleic acid, rises in the $\omega_3$ PUFA 20:5, 22:6) were consistent with findings in other fish feeding studies including the extensive work done on Eskimos (2–6). Analysis of the tropical seafood eaten in this study revealed an arachidonic acid content ranging from 5 to 14% of the total fatty acids (1, 7). This contrasts with most other available data on fish fatty acid composition in which arachidonic acid content does not exceed 5% (8). In order to investigate this unexpected finding in more detail, we measured the bleeding times in a group of normal and diabetic Aborigines before and after 2 weeks on a diet rich in tropical seafood, followed by 3 weeks on a non-seafood diet containing a variety of traditional animal and vegetable foods. This was part of a larger study of the effect of temporary lifestyle change on diabetes in Aborigines (9).

**METHODS**

**Subjects.** Ten diabetic (5 women, 5 men) and four non-diabetic (2 women, 2 men) full-blood Aborigines from the Mowanjum Community (Derby, Western Australia) participated in this study. The mean age of the group was 53.4±1.7 years. All subjects were weight stable before the study. Their initial mean body weight was 80.4±2.6 kg, equivalent to a body mass index of 26.7±0.8 kg/m². Five of the diabetics and three of the non-diabetics were heavy drinkers in the urban setting. Painkilling preparations such as aspirin which are known to prolong bleeding time were widely consumed in the urban setting.

**Urban diet.** The main dietary components were flour, sugar, rice, carbonated drinks, alcoholic beverages (beer and port), powdered milk, cheap fatty meat, potatoes, onions and variable contributions of other fresh fruit and vegetables. At the time of the study the composition of the diet was estimated to be: carbohydrate 50%, fat 40%, protein 10%. There was considerable variation within the group depending on the contribution of alcohol to the diet. The non-drinkers were more concerned about their diet in the urban environment and tended to eat more fresh fruit and vegetables and wholemeal bread.

**Field study.** The field study was carried out at Pantijan, the Mowanjum Community’s cattle station and traditional country of many of the Aborigines now resident at Mowanjum. It is an extremely isolated location north of Derby, 1 1/2 days travel by 4-wheel drive vehicle or an hour by light plane. The Aborigines had no access to store foods or beverages from the time they left Derby until when they returned 7 weeks later. One of us (K.O'D.) was present throughout the study to ensure strict compliance with the experimental diet. The only food eaten after leaving Derby was that hunted or collected by the participants. They travelled from Derby to Pantijan by vehicle. The 7-week period was spent as follows: en route to

*J. Nutr. Sci. Vitaminol.*
Pantijan 1 1/2 weeks; at the coastal location 2 weeks; inland 3 1/2 weeks.

**Experimental diet.** During the ten-day trip from Derby to the coastal location, the major dietary component was locally killed beef (meat and fat) which was estimated to account for 75% of energy intake. The remaining 25% was made up from wild foods (Table 1). No further beef was consumed in the study after arriving at the coastal location.

During the 2-week period spent on the coast the diet was derived predominantly from seafood (barramundi, catfish, trevally, stingray, mullet) with supplements of birds (duck, geese, turkey), kangaroo and crocodile.

The lack of vegetable food in this area eventually precipitated a move inland to a river where the diet was much more varied: kangaroo, freshwater fish (bream), turtle, crocodile, shellfish, birds (turkey, brolga), yams, figs, bush honey. The final 3 1/2 weeks of the study was spent in this area. A detailed analysis of the food intake was conducted over a 2-week period (weeks 5 and 6) and the results are reported in Table 1. All food obtained each day was weighed and samples were collected and stored frozen in liquid nitrogen before being flown to Melbourne where they were analysed for available carbohydrate, fat content and fatty acid composition. The average energy intake over the 2-week period was 1,200 kcal/person/day. The major sources of energy were kangaroo (36%), freshwater bream (19%) and yams (28%). Animal foods accounted for 64% of total energy with vegetable foods making up the remaining 36%.

**Bleeding time measurements.** Due to the high frequency of aspirin ingestion by the participants when resident in the urban setting (Derby), bleeding-time measurements were not made before leaving this environment. No aspirin or related pain-killing preparations were ingested by the participants in this study from the time they left Derby until they returned 7 weeks later. The first bleeding-time measurements were made 10 days after leaving Derby, which should have been long enough for any effects of aspirin to have disappeared (10). The second measurements were made 3 1/2 weeks into the study after 2 weeks on a diet derived almost exclusively from seafood. The final measurements were made 6 1/2 weeks into the study after 3 weeks on a mixed traditional diet described in detail above.

Ivy bleeding times (10) were performed using a disposable Simplate II device (General Diagnostics). The upper, lateral surface of the forearm was shaved if necessary, lightly cleaned with an alcohol sponge and allowed to dry. A standard sphygmomanometer cuff was applied to the upper arm and inflated to 40 mmHg. With the arm placed horizontally, two incisions were made with the Simplate device and blood was absorbed by capillary attraction every 30 s using the edge of torn strips of filter paper, taking care not to disturb the edges of the wound. The time was recorded when blood had completely stopped flowing from each site.

**Plasma lipid analyses.** Fasting triglyceride concentrations were determined enzymatically after enzymatic hydrolysis using a Technicon autoanalyser. The normal range for triglyceride concentrations in fasting plasma is 0.5–2 mmol/liter. Total cholesterol concentration in fasting plasma was measured colorimetrically
after reaction with acetic anhydride and concentrated sulphuric acid using a commercially available kit (Boehringer). The normal range for cholesterol concentration in fasting plasma from Caucasoids is 3.5–6.5 mmol/liter.

**Plasma fatty acid analysis.** Fasting blood samples were collected in heparinized tubes before the subjects left the urban setting and immediately upon returning from the bush study 7 weeks later. Due to the extreme isolation of the experimental locations and lack of availability of a power supply, refrigerator or freezer, blood samples could not be taken for plasma fatty acid analysis at the same time as bleeding time measurements were performed during the field study. Blood sampling was possible only at the beginning and end of the study. The drinkers had abstained from alcohol for at least 24 h before the baseline sample was taken. The samples were placed on ice, centrifuged and the plasma frozen until analysed for fatty acid composition.

Lipid extracts were prepared from plasma by chloroform/methanol extraction (11). Heptadecanoic acid (Nu-Chek-Prep, Elysian, MN) was added to each plasma sample as an internal standard. The fatty acid methyl esters of the total lipids were prepared as described previously (12). The esters were separated using a 50 × 0.5 mm ID wall-coated open tubular glass capillary column coated with OV-275 (Chromalytic Technology, Melbourne, Australia). The chromatograph was equipped with a flame ionization detector. Separation was achieved by temperature programming from 160 to 220°C at 2°C/min with a nitrogen carrier gas flow rate of 20 cm/s. Standard methyl esters (Nu-Chek-Prep) were routinely chromatographed to determine the response of the detector to different methyl esters and to establish the identity of the fatty acid methyl esters in the plasma and food samples. Quantitative response factors were determined and used in the calculation of the data. The percentage fatty acid compositions were provided by an integrator (Hewlett Packard, Melbourne, Australia). This column separated 20:3 ω3 from 20:4 ω6. Triglyceride, phospholipids, and cholesterol esters from the lipid extracts were separated by thin layer chromatography (12) and the fatty acid methyl esters of these fractions were also separated by capillary gas liquid chromatography (GLC).

**Analysis of experimental diet.** Samples of food eaten were collected for analysis. They were frozen as soon as possible after being caught or collected and stored in liquid nitrogen until analysis in Melbourne. The lipid content was measured gravimetrically after homogenizing a 5 g sample in chloroform/methanol (11) and weighing an aliquot of the dried lipid extract. The fatty acid composition of the total lipid extract was determined after preparation of the methyl esters and then separation of these by capillary GLC as described above for the plasma samples. Available carbohydrate in frozen samples of vegetable food was analysed after gelatinization and enzymic hydrolysis by standard techniques as previously described (13, 14).

**Statistical analysis.** Paired data (initial and final) were compared using the paired t-test. Bleeding-time data were analysed using analysis of variance.
RESULTS

The overall design of the study, the main foods eaten and the approximate composition of the diets during the three phases of the lifestyle-change study are reported in Table 1. In terms of its fat composition, phase 1 resembled the habitual urban diet of these people, being relatively high in total fat with a high proportion of saturated and mono-unsaturated fat and a low proportion of PUFA. The striking feature of the diets in both phase 2 and 3 of the study was that the total fat content was low and contained a high proportion of PUFA.

The fatty acid composition of the meat and fat from the major animal foods eaten during each phase of the study is given in Table 2. The wild animals eaten in this study had very little obvious carcass fat and their meats had low fat contents and a high proportion of PUFA. Although tropical seafood (phase 2) contained an unusually high proportion of arachidonic acid (1, 7), the long chain ω3 PUFA clearly predominated over the ω6 PUFA. Freshwater bream on the other hand

| Phase of study | 1 Travelling | 2 Coast | 3 Inland |
|----------------|-------------|---------|---------|
| Main foods (as % total calories) | Beef 75% | Fish 80% | Kangaroo 36% |
| | Kangaroo | Birds | Freshwater fish(bream)19% |
| | Turtle | Kangaroo 20% | Yams 28% |
| | Bream | Crocodile | Honey, figs |
| | 25% | | birds, crocodiles 17% |
| | Yams | | turtle, yabbies |
| | Honey | | |
| Composition of diet | Estimate only | Estimate only | Measured over 2-week period |
| Carbohydrate | 10% | <5% | 33% |
| Protein | 50% | 80% | 54% |
| Fat | 40% | 20% | 13% |
| Saturated fat (% total energy) | 22 | 8 | 4 |
| Monounsaturated | 15 | 4 | 5 |
| Polyunsaturated | 3 | 7 | 4 |
| Time in weeks | 0 Bleeding time | 1 Bleeding time | 2 Bleeding time | 3 Bleeding time | 4 Bleeding time | 5 Bleeding time | 6 Bleeding time | 7 Bleeding time |

Plasma fatty acid analysis

Vol. 31, No. 4, 1985
Table 2. Fatty acid composition of meat and fat from the major animal foods eaten during the study (% of total fatty acids).

| Major foods       | n  | Lipid content | Total saturated | Total monounsaturated | 18:2 ω6 | 20:4 ω6 | Total ω6<sup>a</sup> | 18:3 ω3 | 20:5 ω3 | 22:6 ω3 | Total ω3<sup>b</sup> |
|-------------------|----|---------------|----------------|----------------------|---------|---------|----------------------|---------|---------|---------|----------------------|
| Beef              |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (2) | 2.6           | 36.5           | 42.4                 | 9.9     | 4.4     | 15.5                 | 1.7     | 1.5     | 0.2     | 5.5                  |
| Fat               | (2) | 91            | 62.1           | 35.3                 | 2.1     | n.d.    | 2.1                  | 0.4     | n.d.    | n.d.    | 0.4                  |
| Kangaroo          |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (4) | 1.1           | 29.8           | 26.2                 | 22.7    | 7.7     | 32.0                 | 6.0     | 1.9     | 0.9     | 12.0                 |
| Liver             | (4) | 4.0           | 34.9           | 35.6                 | 13.5    | 6.9     | 22.6                 | 3.3     | 0.7     | 1.3     | 6.8                  |
| Fat               | (2) | 90            | 45.2           | 50.3                 | 3.0     | —       | 3.0                  | 1.5     | n.d.    | n.d.    | 1.5                  |
| Freshwater bream  |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (6) | 1.6           | 40.4           | 29.0                 | 6.6     | 5.3     | 16.3                 | 4.2     | 2.5     | 5.6     | 14.3                 |
| Fat               | (2) | 91            | 39.3           | 41.3                 | 8.6     | 2.0     | 14.1                 | 3.0     | n.d.    | 1.5     | 5.3                  |
| Minor foods       |    |               |                |                      |         |         |                      |         |         |         |                      |
| Turtle            |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (4) | 0.5           | 31.8           | 16.2                 | 11.2    | 20.9    | 36.8                 | 1.1     | 2.1     | 11.1    | 15.2                 |
| Fat               | (4) | 80            | 36.0           | 36.4                 | 10.5    | 3.9     | 17.4                 | 5.8     | 1.0     | 2.5     | 10.2                 |
| Duck              |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (1) | 1.9           | 31.0           | 21.6                 | 20.5    | 14.9    | 36.9                 | 6.2     | n.d.    | 3.1     | 10.5                 |
| Fat               | (1) | 91            | 29.0           | 32.7                 | 24.7    | 2.8     | 29.4                 | 8.7     | n.d.    | 0.1     | 8.9                  |
| Crocodile         |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (3) | 0.8           | 30.8           | 13.0                 | 10.5    | 24.4    | 37.8                 | 0.7     | 1.7     | 13.1    | 18.4                 |
| Yabbie            |    |               |                |                      |         |         |                      |         |         |         |                      |
| Meat              | (2) | 1.4           | 33.0           | 31.8                 | 12.5    | 13.5    | 26.6                 | 1.4     | 5.5     | 1.6     | 8.6                  |

<sup>a</sup>Includes 18:2, 20:2, 20:3, 20:4, 22:4, and 22:5 ω6. <sup>b</sup>Includes 18:3, 20:3, 20:5, 22:5, and 22:6 ω3. n.d. = not detectable.
PLASMA FATTY ACIDS AND BLEEDING TIME

contained similar amounts of ω3 and ω6 PUFA, whereas the wild meats contained 2–3 times more ω6 PUFA than ω3 PUFA. Some (kangaroo, duck) contained more linoleic acid than arachidonic acid, while in others (turtle, crocodile) this ratio was reversed. Some depot fats (turtle, duck and bream) contained much more PUFA than others (beef, kangaroo). Because food intake was only measured accurately over a single 2-week period during phase 3, precise dietary intakes throughout the study could not be calculated. However, the three dietary phases had quite different overall compositional characteristics:

Phase 1 high fat, lowest in PUFA, particularly the ω3 fatty acids.
Phase 2 low fat, highest in PUFA, particularly arachidonic acid and the long-chain ω3 fatty acids
Phase 3 low fat, intermediate PUFA, more linoleic than arachidonic and more linolenic than EPA. Arachidonic and ω3 PUFA levels greater than in phase 1.

The fasting plasma triglycerides in the diabetic subjects fell from 4.02 ± 0.46 mM before the study to 1.15 ± 0.10 mM after it (p < 0.001), and in the non-diabetics from 1.57 ± 0.15 mM before to 0.84 ± 0.13 mM after (p < 0.025). Fasting plasma cholesterol was 5.65 ± 0.23 mM in the diabetics before the study and 4.98 ± 0.34 mM at the end. In the non-diabetics, fasting plasma cholesterol concentration was low before the study (4.1 ± 0.1 mM) and did not change over the 7-week period. The subjects lost an average of 8 kg on the diet (range 3–12 kg), weight loss being most pronounced in the most obese. Details of the changes in carbohydrate and lipid metabolism in the diabetics and non-diabetics have been published elsewhere (9).

The fatty acid composition of the plasma lipids in the fourteen subjects before and after the 7-week study is given in Table 3. The results for the 10 diabetic and 4 non-diabetic subjects have been combined since preliminary data analysis indicated that there were no significant differences between the two groups. The concentration of arachidonic acid in the plasma lipids rose markedly in all subjects while that of linoleic acid fell in all subjects. Increases in the concentrations of the more highly polyunsaturated ω3 PUFA (20:5 and 22:6) were variable (6/14 and 10/14 respectively). There were consistent falls in the concentrations of the saturated and monounsaturated fatty acids in all subjects, reflecting the consistent decreases in plasma triglyceride concentrations.

Thin layer chromatographic separation of plasma lipids from four of the subjects into their major lipid classes indicated that the proportion of arachidonic acid increased in all three major lipid classes (Table 4). In contrast, the proportion of linoleic acid in the phospholipid and cholesterol ester fractions fell significantly, while it rose in the triglyceride fraction. The proportion of EPA was low in all lipid fractions and did not increase significantly in either phospholipids or cholesterol esters. The proportion of docosahexaenoic acid was also low but rose significantly in phospholipids and triglycerides at the end of the study.

The baseline bleeding-time measurements were made after 10 days away from aspirin and alcohol (phase 1) immediately prior to beginning the bush foods diet.
Table 3. Concentration of plasma fatty acids in 14 Aborigines before and after 7 weeks traditional lifestyle (mg/100 ml plasma, mean ± SEM).

| Fatty acids | Before | After |
|-------------|--------|-------|
| 16:0        | 154±12 | 79±5  |
| 16:1        | 31±5   | 13±1  |
| 18:0        | 34±2   | 17±1  |
| 18:1        | 158±13 | 76±4  |
| 18:2ω6      | 77±5   | 45±3  |
| 20:3ω6      | 5±0.4  | 3±0.2 |
| 20:4ω6      | 17±1   | 34±2  |
| 20:5ω3      | 3±0.3  | 3±0.2 |
| 22:5ω3      | 3±0.3  | 3±0.3 |
| 22:6ω3      | 6±0.4  | 9±1   |

*p<0.001. †p<0.02.

Table 4. The proportion of arachidonic and linoleic acid in plasma lipid fractions before and after 7 weeks on a traditional diet (g/100 g total fatty acids, mean ± SEM, n=4).

|                  | Total lipids | Cholesterol esters | Triglycerides | Phospholipids |
|------------------|--------------|--------------------|---------------|---------------|
| Linoleic acid    | pre 14.3±1.5 | 40.2±1.9          | 8.0±1.7       | 17.1±0.8      |
|                  | post 16.1±2.1| 30.4±2.3*         | 11.0±2.3      | 10.7±1.3*     |
| Arachidonic acid | pre 3.3±0.3  | 5.6±0.4           | 0.7±0.2       | 7.4±0.5       |
|                  | post 10.2±0.1*| 15.2±0.7*         | 2.4±0.3*      | 16.0±0.7*     |
| Eicosapentaenoic | pre 0.4±0.1  | 1.1±0.2           | n.d.          | 1.1±0.2       |
| acid             | post 0.9±0.1+ | 1.5±0.3           | 0.4±0.2†      | 1.4±0.2       |
| Docosahexaenoic  | pre 0.8±0.3  | 0.7±0.2           | 0.4±0.03      | 3.0±0.6       |
| acid             | post 2.2±0.4† | 1.4±0.2           | 1.1±0.3†      | 5.0±1.0†      |

*p<0.01. †p<0.02. ‡p<0.05. n.d. = not detected.

Subsequent bleeding time measurements were made after 2 weeks on a diet derived predominantly from tropical seafood (phase 2) followed by 3 weeks on a diet containing a variety of traditional animal and vegetable foods (phase 3). Individual and mean bleeding time data for the fourteen subjects are presented in Fig. 1. As with the plasma fatty acid data, preliminary analysis indicated that there was no difference in bleeding times between men and women or between diabetic and non-diabetic subjects, allowing the data to be combined. Two weeks after the Aborigines changed from a beef-based diet to a seafood-based diet, bleeding time rose in all
Fig. 1. Bleeding times of male (■) and female (□) diabetic and male (●) and female (○) non-diabetic Aborigines after 1.5 weeks on a mainly beef diet (A), followed by 2 weeks on a mainly tropical seafood diet (B) followed by 3 weeks on a diet of wild animal, freshwater fish and vegetables (C). Mean bleeding times: A, 4.1 ± 0.4 min; B, 5.3 ± 0.4 min; C, 5.9 ± 0.4 min. Significant increase over the experimental period (p < 0.01, analysis of variance).

subjects, the mean increase being 29%. When the Aborigines switched to a mixed traditional diet the mean bleeding time increased a further 15%. However, the changes were less consistent in this final phase: 10 of the 14 subjects showed a further increase beyond that occurring in response to seafood, one subject showed no increase, and 3 subjects showed a decrease (1 back to baseline and 2 remaining above baseline). The increase in bleeding time over the entire 5-week period was
highly significant statistically ($p < 0.01$).

**DISCUSSION**

The major finding in this study in a group of Aborigines over a 5-week period in which they were consuming a low-fat diet derived from wild foods was the association between increased bleeding time and the following changes in plasma lipid fatty acid concentrations: decreased linoleic acid, increased arachidonic acid and a modest increase in docosahexaenoic acid. These results raise questions on the inter-relationship between dietary fatty acids, plasma fatty acid composition and the regulation of thrombosis.

The first increase in bleeding time followed 2 weeks on a diet derived predominantly from tropical seafood. Due to the extreme geographical isolation of the study location we were unable to take blood samples for fatty acid analysis at the same time as bleeding-time measurements were made. However, in a previous study in the same region we measured the plasma fatty acid composition in a group of Aborigines before and after 2 weeks on an essentially identical diet of tropical seafood and found that the proportion of arachidonic acid rose from 4 to 11% of total fatty acids, linoleic acid fell from 14.9 to 9.4% and the $\omega 3$ PUFA (20:5 and 22:6) rose from 1.5 to 3.4% and 1.2 to 1.9% respectively (1). These changes reflected the fatty acid composition of the tropical seafood consumed which was rich in arachidonic acid and the $\omega 3$ PUFA 20:5 and 22:6, but relatively poor in linoleic acid. Due to the close similarities in the two studies (including remarkably close agreement in baseline plasma fatty acid composition), it is not unreasonable to suggest that similar changes in fatty acid composition would have occurred in the present study in association with the first increase in bleeding time.

The second increase in bleeding time (a further 15%) followed 3 weeks on a mixed wild-food diet which contained no seafood. This diet was rich in both linoleic and arachidonic acids, but contained less of the $\omega 3$ PUFA than the diet consumed in the previous period, and the plasma fatty acid composition at the end of the study would have been a reflection of this diet. This second increase in bleeding time was not as consistent as the first rise after 2 weeks on the seafood diet. Bleeding time increased in all subjects during phase 2 but in only 10 out of 14 in phase 3. This could possibly have been related to the order of the diets or to the lower level of the $\omega 3$ PUFA present in the diet in the third phase. Further studies are needed to clarify this point.

It could possibly be argued that the fall in plasma triglycerides, the reductions in body weights or the improvement in hyperglycemia associated with this dietary change could also have contributed to the increase in bleeding time. However, in view of the baseline bleeding times being in the normal range rather than below it (10), the similarities in responses of diabetic and non-diabetic subjects, and the increase in bleeding time being similar to that in other fish feeding studies (2, 3), these alternative explanations are unlikely.

*J. Nutr. Sci. Vitaminol.*
The two diet periods (phases 2 and 3) had notable common features as well as notable differences. They were both low in fat with a high proportion of PUFA and both resulted in increased bleeding times and increased concentration of arachidonic acid in the plasma lipids. On the other hand, while both diets contained arachidonic acid, the tropical seafood diet contained more ω3 PUFA with very little linoleic acid, whereas the mixed traditional diet contained predominantly ω6 PUFA, especially linoleic acid. Thus, although linoleic acid was the predominant PUFA in the diet in phase 3, the proportion of this fatty acid in phospholipids and cholesterol esters actually fell, while the proportion of the less abundant arachidonic acid rose. These observations strongly suggest that when arachidonic acid is present in the diet, it is preferentially incorporated into the structural lipids (15). This data is also consistent with previous observations that the conversion of dietary linoleic acid to arachidonic acid is an inefficient process in man and animals compared with the direct incorporation of dietary arachidonate into tissue arachidonic acid (15–17).

Most of the previous studies carried out on the relationship between dietary fat and the regulation of thrombosis have concentrated on linoleic acid, eicosapentaenoic acid and more recently docosahexaenoic acid (2–5, 18–24). These studies have generally reported increased bleeding times in association with reduced concentrations of arachidonic acid in plasma lipids. The results of the present study indicate a comparable increase in bleeding time on a diet which elevated plasma arachidonic acid concentration rather than depressing it. While it is not being suggested that the increases in plasma arachidonic acid and bleeding time are necessary causally related, it is clear from these results that they are not mutually exclusive. Furthermore, the relatively minor changes in the plasma levels of the long chain ω3 PUFA (20:5, 22:6) in both the present study and our previous one (1) are not consistent with the bleeding-time changes being directly attributable to increases in these PUFA. We plan to investigate these findings in more detail in carefully controlled dietary studies in both humans and animals with particular emphasis on the two arachidonic acid-derived prostanoids (prostacyclin and thromboxane) which have been implicated in the regulation of haemostatic function (25–27).

This work was supported by grants to Kerin O'Dea from the Australian Institute of Aboriginal Studies and the National Health and Medical Research Council of Australia.

We are very grateful to the people from the Mowanjum Aboriginal Community, Derby, W. A., who participated in this study either directly or indirectly. Without their good-humoured help and co-operation, the study could never have been carried out. we would also like to thank Dr R. M. Spargo and the staff at Kimberley health, Derby (Western Australia) who assisted in the smooth running of the field study, Joan Naughton who assisted with the fatty acid analyses in Melbourne, and Professors T. J. Martin and R. G. Lakins for their helpful and critical discussions during the preparation of this manuscript.

REFERENCES

1) O'Dea, K., and Sinclair, A. J. (1982): Increased proportion of arachidonic acid in Vol. 31, No. 4, 1985
plasma lipids after two weeks on a diet of tropical seafood. Am. J. Clin. Nutr., 36, 868–872.

2) Sanders, T. A. B., Naismith, D. J., Haines, A. P., and Vickers, M. (1980): Cod-liver oil, platelet fatty acids, and bleeding time. Lancet, 1, 1189.

3) Goodnight, S. H., Harris, W. S., and Connor, W. E. (1981): The effects of dietary \( \omega3 \) fatty acids on platelet composition and function in man: a prospective controlled study. Biol., 58, 880–885.

4) Seiss, W., Roth, P., Scherer, B., Kurzmann, I., Bouhlig, B., and Weber, P. C. (1980): Platelet membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. Lancet, 1, 441–444.

5) Dyerberg, J., and Bang, H. O. (1979): Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet, 2, 433–435.

6) Bang, H. O., and Dyerberg, J. (1972): Plasma lipids and lipoproteins in Greenland West Coast Eskimos. Acta Med. Scand., 192, 85–94.

7) Sinclair, A. J., O’Dea, K., and Naughton, J. M. (1983): Elevated levels of arachidonic acid in fish from northern Australian coastal waters. Lipids, 18, 877–881.

8) Stansby, M. E. (1969): Nutritional properties of fish oils. World Rev. Nutr. Diet., 11, 46–195.

9) O’Dea, K. (1984): Marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines following temporary reversion to traditional lifestyle. Diabetes, 33, 596–603.

10) Mielke, C. H., Kaneshiro, M. M., Maher, L. A., Weiner, J. M., and Rapaport, S. I. (1969): The standardized normal Ivy bleeding time and its prolongation by aspirin. Blood, 34, 204–215.

11) Folch, J., Lees, M., and Sloane-Stanley, G. H. (1959): A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497–509.

12) Sinclair, A. J., McLean, J. G., and Monger, E. A. (1979): Metabolism of linoleic acid in the cat. Lipids, 14, 932–936.

13) Hudson, G. J., John, P. M. V., Bailey, B. S., and Southgate, D. A. T. (1976): The automated determination of carbohydrate. Development of a method for available carbohydrate and its application to foodstuffs. J. Sci. Food Agric., 27, 681–687.

14) Snow, P., and O’Dea, K. (1981): Factors affecting the rate of hydrolysis of starch in food. Am. J. Clin. Nutr., 34, 2721–2727.

15) Sinclair, A. J. (1975): Incorporation of radioactive polyunsaturated fatty acids into liver and brain of the developing rat. Lipids, 10, 175–184.

16) Seyberth, A. W., Oelz, O., Kennedy, B. S., Sweetman, B. J., Danon, A., Frolich, J. C., Heimberg, M., and Oates, J. A. (1975): Increased arachidonate in lipids after administration to man: effects on prostaglandin biosynthesis. Clin. Pharmacol. Therap., 18, 521–529.

17) Sinclair, A. J. (1974): Fatty acid composition of liver lipids during development of the rat. Lipids, 9, 809–818.

18) Simpson, H. C. R., Barker, K., Carter, R. D., and Mann, J. I. (1982): Low dietary intake of linoleic acid predisposes to myocardial infarction. Brit. Med. J., 285, 683–684.

19) Sanders, T. A. B., and Roshanai, F. (1983): The influence of different types of \( \omega3 \) polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. J. Nutr. Sci. Vitaminol., 34, 343–350.
(1980): Eicosapentaenoic acid and platelet function in Japanese. *Lancet*, **22**, 1132–1133.

22) Kagawa, Y., Nishizawa, M., Suzuki, M., Miyatake, T., Hamamoto, T., Goto, K., Motonaga, E., Izumikawa, H., Hirata, H., and Ebihara, A. (1982): Eicosapolyenoic acids of serum lipids of Japanese Islanders with low incidence of cardiovascular disease. *J. Nutr. Sci. Vitaminol.*, **28**, 441–453.

23) Renaud, S., Dumont, E., Godsey, F., Suplisson, A., and Thevenon, C. (1978): Platelet functions in relation to dietary fats in farmers from two regions in France. *Thrombos. Haemostas.*, **40**, 518–531.

24) Renaud, S., Dumont, E., Godsey, F., Morazain, R., Thevenon, C., and Ortchanian, E. (1980): Dietary fats and platelet function in French and Scottish farmers. *Nutr. Metab.*, **24** (Suppl. 1), 90–104.

25) Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S., and Vane, J. R. (1978): Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet*, **2**, 117–119.

26) Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A., and Sprecher, H. (1979): Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 944–948.

27) Morita, I., Saito, Y., Change, W. C., and Murota, S. (1983): Effects of purified eicosapentaenoic acid on arachidonic acid metabolism in cultural murine aortic smooth muscle cells, vessel walls and platelets. *Lipids*, **18**, 42–49.