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DIETARY AND PLASMA RETINOL AND BETA-CAROTENE RELATIONSHIPS IN FILIPINOS, NON-ABORIGINAL AND ABORIGINAL AUSTRALIANS

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Apparently healthy young adult Filipinos (Fil) (resident in the Philippines) non-Aboriginal (non-AA) (resident in Geelong) and Aboriginal Australians (AA) (resident in the North West of Western Australia), 23 subjects in all, had food intake recorded and plasma retinol and β-carotene measured at weekly intervals for four weeks. This enabled the relationships between nutrient intakes and plasma concentrations to be examined in each of the 3 food cultures. Retinol intake was highest in Fil [Fil women 826, men 910; non-AA women 383, men 495 and AA women 450 μg per day; $p = 0.05$ (women) and < 0.05 (men)] whilst β-carotene intake was highest in non-AA [Fil women 1377, men 801, non-AA women 3278, men 7914; AA women 524 μg per day; $p < 0.001$ (women and men)]. These differences were largely accounted for by liver intake in Fil and β-carotene-rich fruits and vegetable intakes in non-AA. Plasma retinol was highest in non-AA [Fil women 33, men 46; non-AA women 51, men 53; AA women 41 μg per 100 ml; $p < 0.001$ (women) and < 0.05 (men)], despite their relatively lower retinol intake; and this indicates that factors other than retinol intake affect plasma retinol within the physiological range above 30 μg per 100 ml. When β-carotene intake was highest in non-AA plasma β-carotene was also high (Fil women 35, men 5; non-AA women 71, men 68; AA women 24 μg per 100 ml; $p < 0.001$ for women and men). Thus, β-carotene or its covariates are likely to contribute to the achievement of a particular physiological plasma retinol concentration. From the dietary data available in these subjects, candidates for such covariates are higher fat intake and lower carbohydrate intake.

This study indicates that plasma vitamin A level within the physiological range is influenced by dietary variables other than the vitamin A active components of the diet. Recognition of this fact is likely to be particularly relevant to the interpretation of cross-cultural differences in plasma vitamin A levels.

KEY WORDS: Vitamin A, retinol, Beta-carotene, diet, nutritional status, Filipino, Australian, Aboriginal Australian, energy intake, fat, carbohydrate

INTRODUCTION

Dietary vitamin A intake in apparently healthy individuals does not appear to affect the plasma retinol concentration (Peto *et al.* 1981). Nevertheless, wide variations in the plasma retinol of individuals are observed, and the effects of dietary factors on these variations remain unclear. Consideration of this aspect is important since both low intakes and blood levels of carotene and retinol have been associated with an increased risk of cancer at various body sites (Ong and Chytil 1984).

This study was conducted to investigate the possibility that dietary factors other than vitamin A, influence the plasma retinol and β-carotene level in apparently healthy adult individuals consuming their usual diet. To obtain a wide range of dietary intakes, particularly of retinol and carotene, three groups of individuals, Filipinos, non-Aboriginal and Aboriginal Australians were included in the study.

Although the subjects chosen were apparently in good health, adolescents from one of these food cultures, the Filipinos, are still prone to vitamin A deficiency with a nationwide prevalence of Bitot's spots accompanied by conjunctival xerosis as high as 0.6% and nightblindness of 1.0% (FNRI 1987).

The present study compares the dietary patterns, nutrient intakes and plasma concentrations of these three groups for retinol and β-carotene; and considers how well the dietary nutrient intakes predict the plasma concentrations.

METHODS

Subjects

The healthy subjects included in the study were:

a) Six (three males and three females) non-Aboriginal Australian staff members and postgraduate students at Deakin University (aged 25–38 years).

b) Five female Aboriginal Australians living near Fitzroy Crossing, Western Australia (aged 18–32). For cultural reasons it was not possible to include any Aboriginal males in the 4 weeks of observations. The Aboriginal community at Fitzroy Crossing has some access to traditional hunter-gatherer foods, but in general prefers to use foods which can be purchased through the community store.

c) Twelve (eight females and four males) Filipino staff members of the Institute of Public Health, Manila, Philippines (aged 24–38 years).

All subjects gave their informed consent to participate in the study. None of the women were pregnant or taking oral contraceptive pills during the study period.

Dietary Data

A detailed 28-day record of all food and drink consumed was maintained by each subject. Amounts were recorded in household measures and portion sizes which were later converted to weights using either data from weighed items or relevant tables for Filipinos and Australians, respectively (FNRI 1977, Thomas and Corden 1977). In some instances for bought cooked foods, the investigator also bought samples of these foods and actually weighed them.

To aid respondents in estimating the size of portions of food in terms of length, width, diameter and thickness, a size chart consisting of a 12-cm square subdivided into 1-cm squares and thicknesses from 0.1 to 0.9 cm was provided with each food diary booklet.

In regard to compliance and maintaining accurate records, the investigator checked the records with the respondents as often as possible (at least 3 times a week) and even daily in the case of the Aboriginal Australians.

Non-compliance in recording food intake in part explains the small sample size obtained. Of the three groups, close supervision was provided for the Aborigines who were not familiar with recording. On the other hand, Filipinos and non Aboriginal Australians were familiar with recording and required less supervision.

Plasma Retinol and β-carotene

A fasting blood sample was obtained between 8.00 and 8.30 am on the same day at the end of each week and related to the food record of the preceding week.

Nutrient Analysis

Nutrient intakes were analyzed utilizing McCance and Widdowson's 'The Composition of Foods' (Paul and Southgate 1978) for the Australian subjects and the Table of Food Composition for use in the Philippines (FNRI 1980) for the Filipinos. Energy, macronutrients (protein, fat, carbohydrate and alcohol), retinol, β-carotene and total vitamin A, dietary fibre and zinc intakes were estimated. The latter two nutrients have each been reported to affect vitamin A status, by absorption (dietary fibre) (Kasper *et al.* 1979) or turnover effects (zinc) (Smith *et al.* 1976). Unfortunately, food composition data on dietary fibre and zinc content were not available for Filipino foods.

Blood Analysis

Plasma retinol (Flint *et al.* 1980) and β-carotene (Briggs *et al.* 1983) were analyzed by high performance liquid chromatography.

Carotenoids other than β-carotene such as (α-carotene, lycopene, lutein and phytoene) are also present in the plasma. However, quantitation of these carotenoids was not possible at the time of the study.

Retinol in 200 μl plasma was extracted with ethanol and hexane containing an internal standard (IS), n-octyl-alpha-naphthyl urethane. Separation of retinol was accomplished on a reverse phase HPLC column with a mobile phase, 95% methanol in water at 1.0 ml/min flow rate. Retinol was detected with an ultraviolet detector at 318 nm suitable for both retinol and the IS. Retinol was determined by calculating peak height ratios relative to the IS. A standard curve was prepared by addition of known amounts of retinol to a plasma pool and analyzed as for the sample. Intra-assay and inter-assay reproducibility ranged from 2.9–3.0% and 5.1–8.0% respectively. Extraction recovery ranged from 98–100%.

Similar extraction procedures were used for beta carotene using β-apo-8'-carotenal as the internal standard. The mobile phase was acetonitrile:methanol:chloroform (46:46:8) pumped at a flow rate of 2.0 ml/min. β-carotene was detected at 436 nm. Peak height ratios relative to IS were calculated and concentration was obtained from the standard curve prepared by addition of standard β-carotene to a plasma pool and analysed as the sample. Intra-assay and inter-assay variation were from 3.2% and 11.3% respectively. Results have been expressed in μg/100 ml. Conversion factors to SI units in μmol/L are 0.01862 for retinol and 0.03496 for β-carotene respectively.

Statistical Analysis

One way analysis of variance (ANOVA) was used to compare the three groups. When the F-ratios obtained differed significantly Duncan's multiple range test was used to further test the differences. When only two groups were compared, the Student's t-test was employed (Snedecor and Cochran 1967).

RESULTS

Dietary Patterns

Table I shows the dietary patterns of the three groups studied. Clear differences in dietary patterns existed between three groups. Firstly, there were differences in the

TABLE I
Dietary patterns of Filipinos (n = 12), non-Aboriginal (n = 6) and Aboriginal (n = 5) Australians over 4 weeks

	Filipinos	Non-Aboriginal Australians	Aboriginal Australians
Breakfast	Bread Rice w/ meat/fish Coffee w/ milk and sugar	Cereals Bread, spreads, jams	Bread Jam or butter Tea, sugar, milk
Lunch	Rice, meat or fish, vegetables, fruits or sweets	Pastie, sandwich, coffee or juice, fresh fruit	Bread, butter, jam, meat, fish, tinned foods
Evening meal	Rice, meat or fish, liver (chicken, beef or pig), vegetables (mainly cabbage, gourds, egg plant, and stalks of kang-kong), fresh fruit or sweets (candies)	Meat (beef and lamb), potato, bread, green and yellow vegetables, fruit juice, coffee, fresh fruits	Bread, butter, jam, meat (beef, kangaroo), fish, tinned foods (such as pork and beans, ham, corned beef, frankfurters or sausages)
Mid-morning, afternoon snacks	Sandwich, bakery products or native cakes	Coffee, biscuits or bakery products	Coffee, bread

range and variety of foods included in the diet. The non-Aboriginal Australians had the widest range and variety of foods (20 food items/day), followed by the Filipinos (13 food items/day) and the Aborigines the least (4 items/day). Secondly, the amounts of food consumed varied for similar items. For example, the Aborigines consumed more meat (beef) than either the Filipinos or the non-Aboriginal Australians. Thirdly, differences in the methods of cooking and preparation were also seen. For example, the non-Aboriginal Australians usually consumed boiled vegetables while the Filipinos most often had theirs stir-fried. Fourthly, there were some distinctive differences in food sources of pro- and pre-formed vitamin A. For example, Filipinos regularly ate liver, and vegetables like cabbage, gourds, egg plant and stalks of kangkong which are not as good sources of β-carotene as the vegetable sources eaten by Australians.

Nutrient Intakes

Figures 1a–1c show the average daily nutrient intakes of the Filipinos, non-Aboriginal and Aboriginal Australians. The differences in nutrient intake between the food cultures were most evident for women (Table II). However, the average daily intakes of energy did not differ despite differences in body size. The Filipinos had a significantly higher carbohydrate intake than the non-Aboriginal ($P < 0.01$) and Aboriginal Australians ($P < 0.005$) due to a high consumption of rice, native cakes, bread and bakery products. There were considerable differences in alcohol intake between individuals within each culture. The most consistent pattern was observed for the Filipino men who drank beer (mean ± SEM alcohol intake for Fil women 0, men 20.9 ± 5.3; non AA women 23.7 ± 10.1, men 5.3 ± 1.8; AA women 0). Dietary fibre intake was lower ($t = 2.46$ $p < 0.05$) and zinc intake higher ($t = 3.69$ $p < 0.05$) in Aboriginal women than in non-Aboriginal Australian women (Figure 1b).

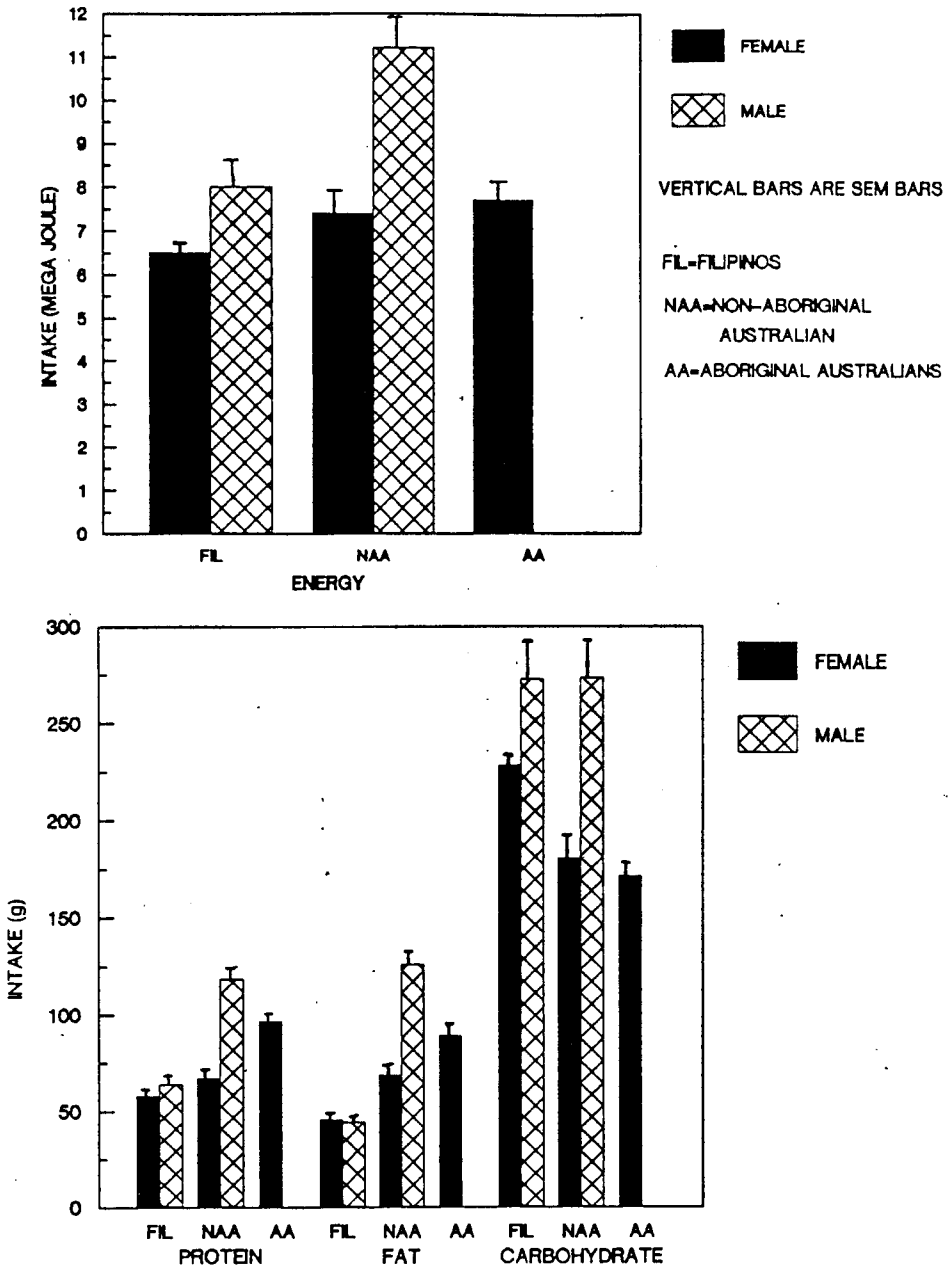

FIGURE 1a Energy and macronutrient intakes in 23 Filipinos (n = 12), non-Aboriginal (n = 6), Australian men and women and Aboriginal Australian women (n = 5). Studied at weekly intervals over 4 weeks. Means of all observations are depicted with SE bars. Fil=Filipino; non-AA = non-Aboriginal Australian and AA = Aboriginal Australian.

The significantly higher intake of carotene in the non-Aboriginal Australians was primarily due to the consumption of carrots and broccoli (Figure 1a). In contrast, the Filipinos had a significantly higher retinol intake due largely to their intake of liver (Figure 1c). The Aborigines had a significantly lower intake of total vitamin A than the other two groups whose intakes did not differ significantly (Figure 1d).

The Aborigines' higher meat consumption explains their significantly higher

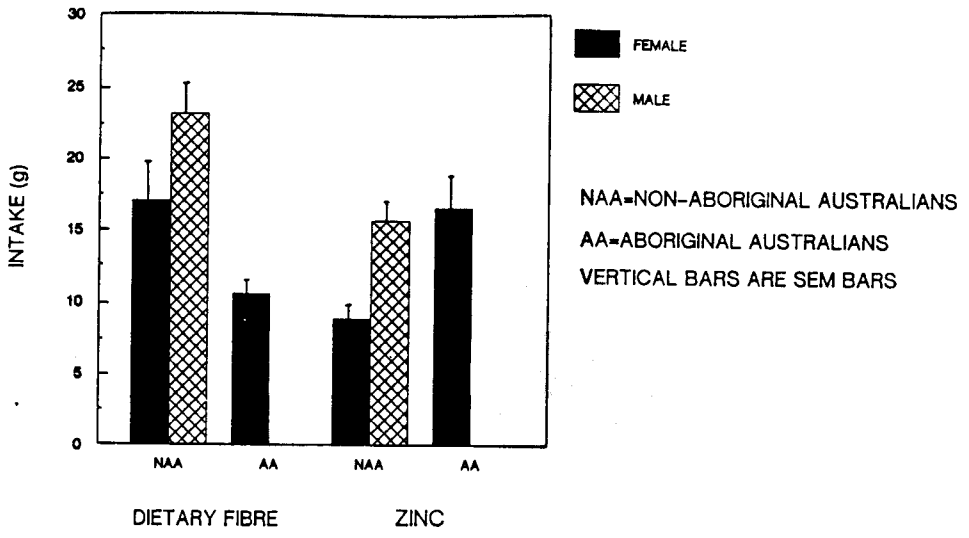

FIGURE 1b Dietary fibre and zinc intake in 11 non-Aboriginal Australian men and women (n = 6) and Aboriginal women (n = 5) studied at weekly intervals over 4 weeks. Notation as in Figure 1a.

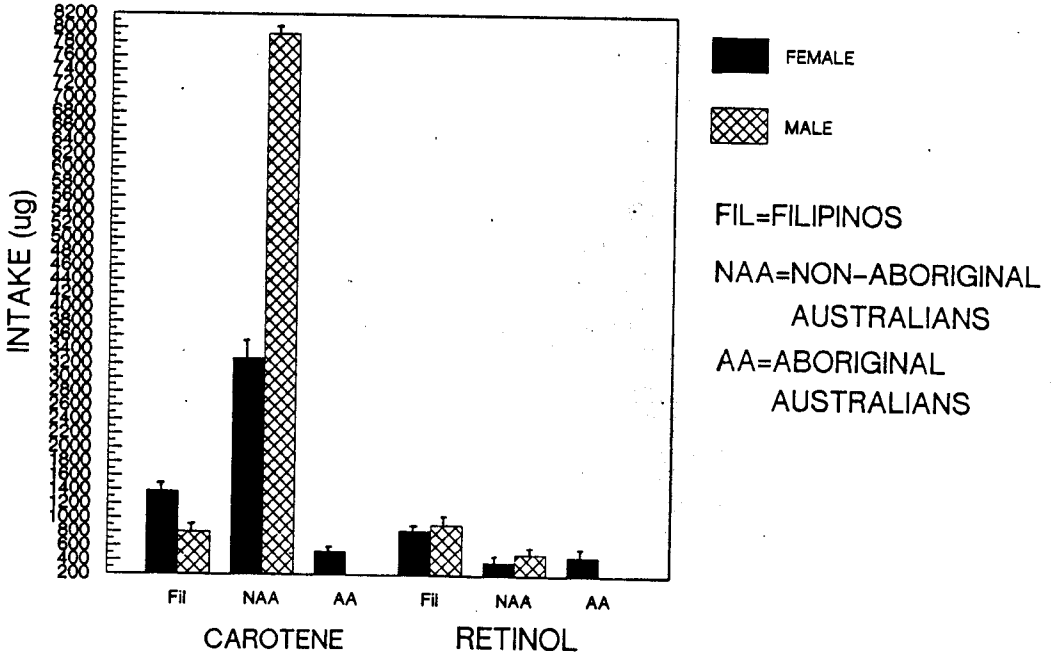

FIGURE 1c Retinol and β-carotene intakes (μg per day) for the same subjects as in Fig. 1a, with the same rotation.

protein and zinc intakes. The significantly lower fat intake of the Filipinos compared with the other two groups was due to minimal consumption of butter, margarine and oil (the oil used is principally coconut). Dietary fibre intake was higher in non-Aboriginal Australians than in the Aborigines since the former consumed more breakfast cereals, wholemeal bread and more vegetables in their diet.

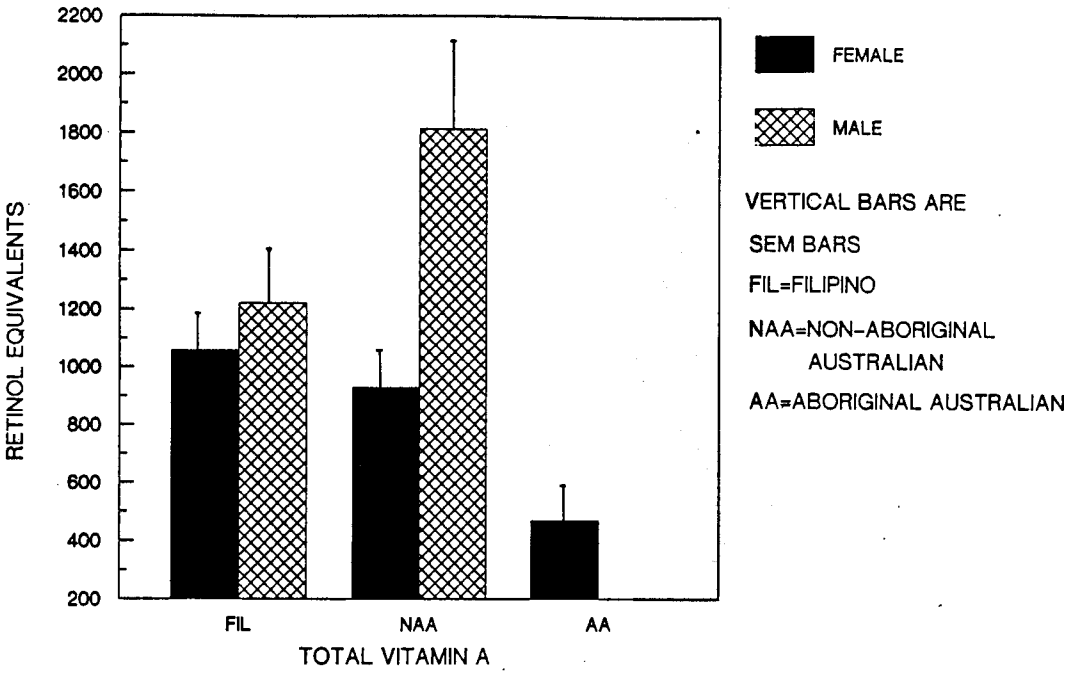

FIGURE 1d Vitamin A intakes, expressed as Retinol Equivalents per day, for the same subjects as in Figure 1a, with the same notation.

TABLE II

Analysis of variance results for nutrient intakes of Filipino, non-Aboriginal and Aboriginal Australian women; and t-values for comparisons between Filipino and non-Aboriginal Australian men

	Women		Men	
Nutrient	*F-ratio*	*P-value*	*t-ratio*	*P-value*
Energy (non-alcohol)	2.47	NS	3.56	<0.005
Protein	12.60	<0.0001	4.68	<0.01
Fat	23.61	<0.0001	6.71	<0.01
Carbohydrates	12.54	<0.0001	0.04	NS
Retinol	3.07	NS	2.23	NS
Carotene	14.38	<0.0001	3.90	<0.05
Total vitamin A (retinol equivalents)	4.59	<0.05	1.60	NS

Comparison with the Recommended Daily Intakes (RDI)

RDIs for energy are Filipino men 10.8, women 8.1 MJ, for all Australians 11.6 for men and 8.4 MJ for women; for protein are Filipino men 63, women 54 g, for all Australians 70 for men and 58 g for women; for retinol are Filipino men 650, women 550 μg; for retinol equivalents are for all Australians 750 for men and 750 μg for women.

Figure 2 shows energy and nutrient intakes for the three groups in relation to the relevant RDI for energy, protein and vitamin A (FNRI 1976, NHMRC 1984). All three groups had energy intakes less than the average recommended level. The

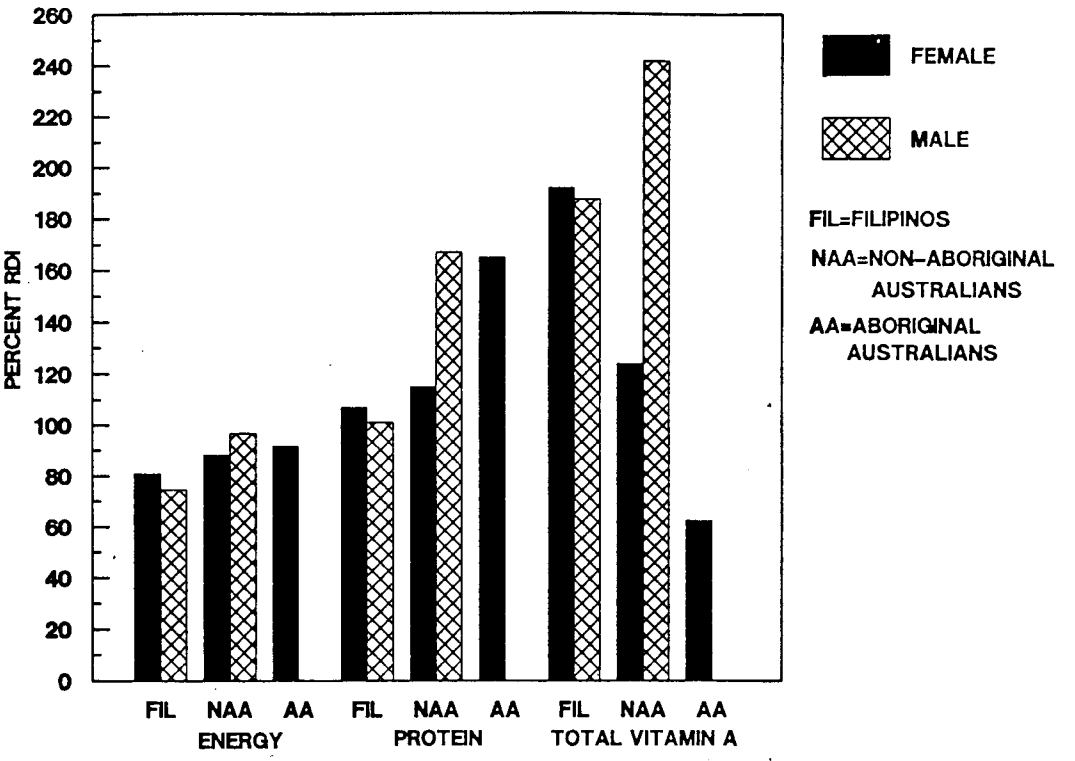

FIGURE 2 Energy and nutrient intakes expressed as a percentage of the Recommended Dietary Intakes (RDIs) as issued either by the Philippines or Australia, as appropriate, at the time of the investigation.

lower energy intake of the Filipinos, at least in part, reflects a difference in recommendations for energy intake (see Figure 1a).

The overall mean protein intake in the Filipinos in agreement with previous reports (Corpus and Ramos 1979, FNRI 1984) was similar to the RDI. The protein intakes of both the Australian groups exceeded the RDI. This is not surprising since both groups consumed considerably more meat than the Filipinos.

Suggested guidelines for the percentage contribution of the macronutrients are 10–14% for protein, 50–60% for carbohydrates and not more than 35% for fat (Bruce 1980). Table III shows that the contribution of fat to the total energy intake was least among the Filipinos (25%) while that from carbohydrate was highest (60%). Fat contributed more (38%) and carbohydrate less (44%) in the non-Aboriginal group, while, in the Aborigines, fat provided the highest contribution (42%). The contribution of protein was also highest (21%) in this group, because of the high meat consumption.

The total vitamin A intake in Filipinos exceeded the RDI due to the consumption of liver by most of the subjects during the study period. The non-Aboriginal Australians also exceeded the RDI for vitamin A, but mainly due to carotene intake. The vitamin A intake of the Aborigines studied met only 60% of the RDI.

If vitamin A intake is expressed in terms of μg retinol equivalents/kg body weight (BW) and compared with the recent FAO/WHO report (FAO, 1988) which recommends a level of 9.3 μg/kg BW, the conclusions reached are similar to those obtained when country specific RDI values of 750 μg for Australians and 650 μg and 550 μg for male and female Filipinos are used. The mean (± SEM) of

TABLE III
Percentage contribution (mean and range) of protein, fat and carbohydrate to total non-alcohol energy intake in twelve Filipinos, six non-Aboriginal and five Aboriginal Australians

	Filipinos	Non-Aboriginal Australians	Aboriginal Australians
Protein	15 (9–20)	18 (14–26)	21 (11–34)
Fat	25 (10–40)	38 (24–48)	42 (31–59)
Carbohydrate	60 (49–76)	44 (33–54)	37 (17–47)

vitamin A intake expressed per kg body weight was as follows: Fil women 22 ± 3; men 20 ± 3; NAA women 16 ± 2; men 26 ± 4 and AA women 8 ± 3. Thus both men and women Fil and NAA mean intakes exceeded the recommended amount while the AA had a lower mean intake.

Contrary to expectations, the percentage contribution of carotene to total vitamin A intake (expressed as Retinol Equivalents) was highest in the non-Aboriginal group 68% (28–95%), compared with 30% (0–98%) in Filipinos and 22% (4–87%) in the Aborigines. The objective of obtaining a wide range of intake was nevertheless achieved.

Plasma Retinol and β-carotene

The plasma retinol and β-carotene concentrations in the three groups studied are shown in Figure 3. The average plasma retinol in all the groups was above 30 μg/100 ml although levels between 20–29 μg/100 ml were obtained in one Filipino woman and one Aboriginal Australian woman. All subjects had levels of plasma retinol above 20 μg/100 ml (WHO 1982). Despite a higher intake of retinol, the Filipinos had lower plasma retinol levels (women F-ratio = 25.25 $p < 0.0005$; men $t = 2.80$ $p < 0.005$). Filipino women had lower plasma retinol concentrations than Filipino men ($p < 0.01$), but this was not so for the non-Aboriginal Australians.

The Aboriginal women had a significantly lower plasma β-carotene than the other two groups (F ratio = 37.65 $p < 0.001$) whilst Filipino women and men had lower plasma β-carotene than non-Aboriginal Australians ($p < 0.005$ for men and women). The intake of carotene rich foods by Aborigines during the study period was minimal. As with retinol, there were again gender differences for Filipinos (the females had higher plasma β-carotene than the males, $p < 0.001$) but not for non-Aboriginal Australians.

DISCUSSION

Ethnic Differences in Vitamin A Intake

The objective in selecting individuals from three different ethnic backgrounds (Filipino, non-Aboriginal and Aboriginal Australians) was to obtain a wide range of nutrient intakes with respect to retinol and β-carotene. The observed differences

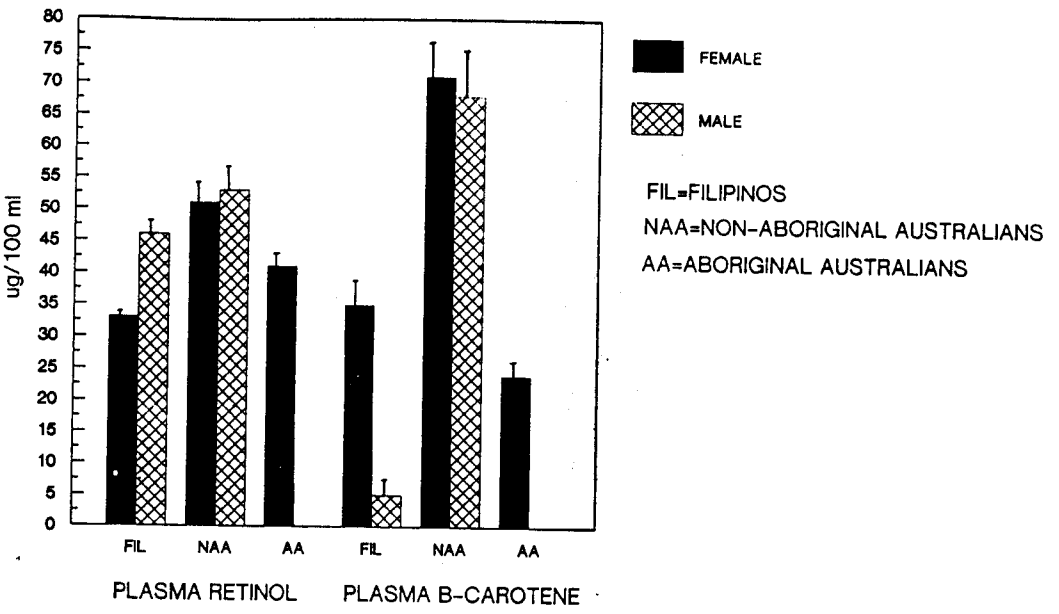

FIGURE 3 Plasma retinol and β-carotene concentrations in the same 23 Filipino (Fil) non-Aboriginal Australian (non-AA) and Aboriginal Australian (AA) as depicted in Figure 1, and with the same notation.

in dietary patterns and in nutrient intakes thus reflect ethnic differences in how vitamin A status is achieved. Caution must be exercised in extrapolating from the subjects studied to these communities at large. Nevertheless, distinct cultural differences in food intake provide a basis for consideration of how the overall diet affects plasma vitamin A level and what this means in terms of the vitamin A status of the individual.

A recent survey (FNRI 1987) indicates that the average intake of energy by Filipinos is unusually low despite the fact that the relative contribution of the macronutrients to total energy intake is of the same order as that reported in other population groups (Margetts *et al.* 1981, Baghurst and Record 1983). In the present study the highest contribution of carbohydrate to energy intake was in the Filipinos, principally from refined carbohydrate as rice, bread, bakery products or native cakes (Table I and Figures 1a and 1b). Use of butter, margarine and oil was limited among Filipinos. The macronutrient profiles, along with dietary fibre, reflect vitamin A sources and may themselves influence vitamin A absorption.

In practice, over 60% of the vitamin A intake of non-Aboriginal Australians was from pro-vitamin A sources while the Filipinos derived their vitamin A mainly from preformed sources particularly liver. (This may have some implication for the bioavailability of retinol when fat intake is relatively low as in Filipinos.)

The results of the present study are in line with the general observation that dietary fibre intake is low in western diets including that of Australians. The average dietary fibre intake of 23.4 and 17.0 g for females and males respectively was similar to that reported by other authors (Rutishauser 1985).

Adequate dietary fibre compositional data for Filipino foods are not available, but the total intake from a diet based on polished rice and refined wheat flour noodles, together with limited leguminous vegetables is unlikely to exceed that of the Australian western style diet.

Ethnic Differences in Plasma Vitamin A

Vitamin A status, using plasma retinol concentration as the criterion, exceeded the WHO acceptable level of 20 μg/100 ml (WHO 1982) in all the individuals studied. The plasma retinol values obtained for the Aborigines indicate that this group despite a low vitamin A intake had stores of the vitamin sufficient to maintain plasma concentrations within the acceptable range. It is possible that they consume rich sources of vitamin A such as liver intermittently.

Reports on plasma β-carotene, as distinct from total carotenoid level, are still relatively few. In recent studies, it has been found that only 9–43% of total carotenoids in plasma are α- and β-carotenes. In the present study, the mean plasma β-carotene in the non-Aboriginal Australians was more than twice the concentration found in the two other cultures studied and in line with their dietary intake of β-carotene.

The observation that Filipino men showed only 5 μg/100 ml of β-carotene in the plasma was due to non-detectable levels in three of the subjects. It is unlikely that this is due to technical problems since this would have been detected by the use of quality control sera. It may be that the predominant carotenoids in the food of these men were carotenoids other than β-carotene such as α-carotene, lycopene and lutein.

Food Intake as a Determinant of Vitamin A Biochemistry

This study found that in a group whose retinol intakes were relatively low, Australians compared with Filipinos (who eat more liver), the plasma retinol concentrations were higher. Thus, factors other than retinol intake, which may be both nutritional and non-nutritional appear to affect the plasma retinol even when within the physiological range above 30 μg per 100 ml. One of these might be β-carotene or a dietary co-variate, since β-carotene intake was higher in the group whose plasma retinol was higher. Covariates of a higher β-carotene intake in this study include higher fat intake and a lower carbohydrate intake. In the group with the higher carbohydrate intake, the Filipinos, β-carotene was not obtained from β-carotene rich plant foods, but in the main from refined rice and wheat flour-based products.

Lower dietary fibre intakes in Aboriginal women than in their non-Aboriginal counterparts were also associated with a lower serum retinol. This is consistent with the observations of Kasper *et al.* (1979) who found that various types of dietary fibre increased absorption of pharmacological amounts of retinyl palmitate. The higher zinc intakes of Aboriginal Australian women compared with their non-Aboriginal counterparts, were, however, not associated with a higher serum retinol as might have been expected from studies which show that zinc increases transport of retinol.

Other dietary factors did not appear to influence the plasma vitamin A levels although subjects whose fat intakes were low (FIL) had lower plasma retinol values. Nutritional status with respect to vitamin A would appear, therefore, to require an assessment of more constituents of the diet than pro- and preformed vitamin A and concomitant assessment of vitamin A biochemistry.

Factors, other than dietary intake of vitamin A have been shown to influence plasma levels of retinol (Underwood 1983). Among these factors are fluctuating steroid hormone levels that occur as part of the normal female menstrual cycle, in which case two peaks in plasma vitamin A levels are observed, one that occurs during the ovulation period and the second but lower peak at the end of the cycle

(26–27 days). These increases were not observed among the subjects of the present study.

A broader nutritional and non-nutritional approach to the assessment of vitamin A status is more likely to be cross-culturally robust.

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