Note

Hexacosanoate Contents in Japanese Common Foods

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Summary Recently, a diet enriched in oleate and moderately restricted in hexacosanoate (C26:0) was found effective to reduce the plasma very long chain fatty acid (VLCFA) levels in patients with adrenoleukodystrophy (ALD), an X-linked disorder characterized by demyelination of the adrenal cortex and cerebral white matter, and accumulation of saturated VLCFA, particularly C26:0, in tissues of the demyelination. The information about the C26:0 content in Japanese food was, however, almost nil except for one report about foods in the USA, but this did not include some Japanese common foods. With the hope of treating an ALD patient in our hospital, C26:0 contents in Japanese common foods (42 items) were measured. In our case, a one-hour direct transesterification method was used to obtain methylesters of total fatty acids in foods and they were applied directly to a selected ion monitoring gas chromatography-mass spectrometry for the quantitative C26:0 analysis. The C26:0 content in nuts and seeds as well as in fats and oils was found to be significantly higher than in other foods; the content was highest in peanuts. The content in almost all kinds of examined fishes, the common protein foods in Japan, was relatively low. From these data and that in the national nutrition survey in 1986, the daily intake of C26:0 from the average Japanese diet could be estimated to be 12–36 mg. It can be recommended, therefore, that nuts and seeds as well as fats and oils should be restricted as severely as possible from the diet of ALD patients in Japan in order to keep daily C26:0 intake below 10 mg as recommended in the USA.

Key Words hexacosanoate, very long chain fatty acid, adrenoleukodys-

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Adrenoleukodystrophy (ALD) is an X-linked disorder characterized by demyelination of cerebral white matter and adrenal cortex as well as accumulation of saturated very long chain fatty acids (VLCFAs) composed of around 26 carbons in tissues of the demyelination. The pathogenesis of VLCFA accumulation in ALD is, however, not sufficiently elucidated; some reports (1, 2) attributed it to endogenous factors, especially a decrease in enzyme activity of the VLCFA-oxidation system, while another (3) demonstrated an exogenous C26:0 incorporation into the patient's brain. Regarding the treatment for this disease, Van Duyn and his colleagues (4) tried at first restricting VLCFA intake in the patients but found that it did not normalize the plasma levels of C26:0. Next, Rizzo *et al.* (5) and Moser *et al.* (6) achieved significant reductions in plasma VLCFA levels of the patients with diets enriched in oleate (C18:1) along with a restriction in C26:0. Recently we received one ALD patient in our hospital and we wanted to restrict the C26:0 content in his diet; however, information about C26:0 content in Japanese common foods was almost nil unless we refer to the report of Van Duyn group (4) about foods in the USA but this did not include some Japanese common foods, especially fishes. We thus measured the C26:0 contents in Japanese common foods, with the hope of treating ALD patients in Japan including ours.

METHODS

Foods examined. Ten food groups comprising 42 items easily obtainable in our district (Table 1) were examined for C26:0 content. We selected, for the examination, meat and fishes which were as fresh as possible, checking the slaughter or capture date. Vegetables, fats and oils, and all other foods examined were also selected on the same basis.

Reagents. The authentic methyl pentacosanoate (Me-C25:0), and Me-C26:0 were the product of Sigma Chemical Co. All organic solvents used were of the quality for HPLC. All other chemicals were reagent grade.

Preparation of methylesters of total fatty acids from foods. Fruits and vegetables were cut with a knife into small pieces and lyophilized overnight before use. Other foods as well as the lyophilized foods as above were weighed and duplicate samples (each 0.1–0.2 g) were used for the preparations of methylesters of total fatty acids. These preparations were carried out according to the method of Lepage and Roy (7) except for using pentacosanoate internal standard solution (5 µg C25:0 dissolved in 2 ml of methanol–benzene, 4:1, v/v), instead of tridecanoate, for the one sample and using non-internal standard solution (just 2 ml of methanol–benzene, 4:1, v/v, was used) for the other sample.

Quantitative analysis of C26:0 in the methylesters of total fatty acids. The

Table 1. Foods examined.

Food group	Food	Producing districts or Manufactories	The date of produced	Food group	Food	Producing districts or Manufactories	The date of produced
Meats	Beef (chuck, separable fat)	Okayama	1987, 8, 17	Cereals	White bread	Okayama	1987, 12, 9
	Beef (chuck, separable lean)	Okayama	1987, 9, 16		Somen	Okayama	—
	Chicken (broiler, breast, skin)	Kagoshima	1987, 9, 17	Potatoes	White rice	Okayama	1987 annual production
	Chicken (broiler, breast, separable lean)	Kagoshima	1987, 9, 17		Potato chips (fried)	Tokyo	1986, 9, 5
	Pork (inside ham, separable fat)	Okayama	1987, 7, 17		Mashed potato (dehydrated)	Hokkaido	1987, 12, 9
	Pork (inside ham, separable lean)	Okayama	1987, 7, 17	Milk and Dairy Products	Milk	Okayama	1987, 12, 19
	Japanese pichard	Wakayama	1987, 10, 21		Yogurt	Hiroshima	1987, 12, 5
	Yellow tail (back)	Kagoshima	1987, 10, 19	Nuts and Seeds	Icecream	Tokyo	—
	Skipjack (back)	Kagoshima	1987, 10, 26		Processed cheese	Hyogo	1987, 12, 9
	Cod (back)	Hokkaido	1987, 11, 19		Sesame seed	Mie	—
Fishes and Selffishes	Spanish mackerel (back)	Ooita	1987, 12, 1		Sweet almond (dried)	U.S.A.	1987, 11, 17
	Flat fish (back)	Okayama	1987, 12, 3	Vegetables and Fruits	Peanut (dried)	Japan	1987, 12, 19
	Mackerel (back)	Aomori	1987, 12, 8		Squash (raw)	Miyazaki	1988, 1, 12
	Bluefin tuna (back)	Shizuoka	1987, 12, 8		Cabbage (raw)	Okayama	1988, 1, 18
	Shrimp	Okayama	1987, 11, 19		Tomato (raw)	Kumamoto	1988, 1, 19
	Tofu (momen)	Okayama	1988, 1, 18	Fat and Oils	Banana (raw)	Philippines	1988, 1, 25
	Abura-age	Okayama	1988, 1, 22		Butter	Hokkaido	1986, 8, 21
					Corn oil	Tokyo	1986, 10, 3
					Sunflower oil	Tokyo	1986, 5, 24
					Safflower oil	Tokyo	1986, 5, 21
Processed Soybean Foods					Salad oil (Soybean and Rapeseed)	Tokyo	1986, 6, 9
					Lard	Hokkaido	1986, 9, 18
					Peanut butter	Tokyo	—
				Confectioneries	Mayonnaise	Tokyo	—
					Milk chocolate	Tokyo	—

Table 2. Calculation of real peak-area ratio (R).

$$R = \frac{ab}{b-a}$$

a : apparent peak-area ratio with internal standard.

b : apparent peak-area ratio without internal standard.

Fig. 1. Calibration curve.

Fig. 2. One example (yogurt) of selected ion monitoring gas chromatato-mass-spectra (IS, internal standard).

Table 3. C 26:0 contents in foods (mg/100 g).

Food group	Food	Observed value	The value given in the literature ⁴⁾
Fat and Oils	Peanut butter	63.46	—
	Butter	34.87	—
	Corn oil	21.63	—
	Salad oil	11.61	—
	Sunflower oil	8.14	8.81
	Mayonnaise	6.29	5.25
	Safflower oil	2.78	5.57
	Lard	1.17	3.40
	Peanut	119.329	—
	Sesame seed	18.504	—
Nuts and Seeds	Sweet almond (dried)	2.516	—
	Processed cheese	16.420	—
	Ice cream	5.987	—
	Yogurt	1.610	1.760
Milk and Dairy Products	Ordinary liquid milk	0.381	—
	White bread	4.064	1.390
	Somen (dry form)	2.385	—
	White rice	1.705	1.020
Cereals	Chicken (broiler, breast, skin)	2.458	—
	Beef (chuck, separable fat)	1.060	1.230
	Pork (inside ham, separable fat)	0.638	—
	Chicken (broiler, breast) (separable lean)	0.076	0.020
	Pork (inside ham, separable lean)	0.047	0.130
	Beef (chuck, separable lean)	undetectable	—
Fishes and Selffishes	Skipjack (back)	2.429	—
	Yellow tail (abdomen)	1.676	—
	Spanish mackerel (back)	0.950	—
	Bluefin tuna (back)	0.227	—
	Japanese pilchard (back)	0.201	—
	Mackerel (back)	0.182	(baked) 0.480
	Cod (back)	0.147	—
	Flat fish (back)	0.134	(baked) 0.068
	Shrimp	0.077	(baked) 0.760
	Banana (raw)	1.790	0.780
Vegetables and Fruits	Cabbage (raw)	0.775	2.190
	Squash (raw)	0.177	0.960
	Tomato (raw)	0.098	0.290
	Abura-age (momen)	1.21	—
Processed Soybean Foods	Tofu	0.362	0.850
	Potato chips (fried)	9.64	7.46
Potatoes	Mashed potato (dehydrated)	0.262	0.580
Confectioneries	Milk chocolate	6.19	3.33

duplicate samples of methylesters of total fatty acids as prepared above were applied directly to a selected ion monitoring gas chromatography-mass spectrometry (SIM-GC-MS). The M-80B-type GC-MS instrument, Hitachi Co., was used in electron-impact mode, monitoring selected ions at m/z 410 (molecular ion of C26:0), m/z 367 (molecular ion of C26:0-43), and m/z 396 (molecular ion of C25:0). The OV-1 bonded capillary column (0.25 mm $\times$ 25 m) of Gaskurokōgyo Co. and a split-less cooled-on-column injector of the Gaskurokōgyo Co. were used. The raising rates of column temperature were 30°C/min between 85 and 250°C and 1°C/min between 250 and 270°C. The MS was operated at an ionization voltage of 70 eV, an accelerating voltage of 3 V, and an ion source temperature of 210°C. Calibration curve for quantitative analysis of C26:0 was made by plotting the peak-area ratio (m/z 410/396) against the weight ratio (MeC26:0/MeC25:0), as shown in Fig. 1. The real peak-area ratio (m/z 410/396) was calculated by the formula shown in Table 2 where a and b represent apparent area ratios obtained by SIM-GC-MS with methylesters of total fatty acids in the presence and absence of internal standard (C25:0), respectively. One example of SIM-GC-MS is shown in Fig. 2.

RESULTS

The C26:0 contents (mg/100 g food) in foods examined by procedures as described above are shown in Table 3. The data of Van Duyn group (4) on the food items which were the same as ours are also presented in that table. Among the examined foods, peanuts have the highest content (much higher than any others), and many kinds of fats and oils, cheese, potato chips, milk chocolate, ice cream, and bread have relatively high contents. Fishes have generally low contents. Comparing our data with that of Van Duyn group (4), C26:0 contents in about half of the food items (sunflower oil, mayonnaise, yogurt, *etc.*) are similar whereas in the other items (safflower oil, lard, cabbage, tofu, *etc.*) they differ.

DISCUSSION

Before our present work, Van Duyn group (4) reported the C26:0 contents in 135 American food items. However, their study did not include some Japanese common foods, especially several kinds of fishes. Accordingly, C26:0 contents in Japanese common foods were measured. The method used for preparing methylesters of total fatty acids from food is a very convenient one from the viewpoints of both precision and time-saving. The SIM-GC-MS for quantitative analysis of C26:0 in the methylesters of total fatty acids is also a well-known procedure as the most rapid, accurate, and sensitive method. As shown in Table 3, some of our data do not coincide well with those of Van Duyn group (4). The reason for the discrepancy is not clear at present, because many experimental conditions, for example, measuring method, food-producing place, food-processing, *etc.*, differed. It is conceivable that contents of some elements in foods might vary depending on

the place produced, especially if the distance were considerable.

From data presented here and the national nutrition survey in 1986(8), the daily intake of C26:0 from the average Japanese diet could be estimated to be 12–36 mg, which is close to that from American diet (15–40 mg intake). Moser's group(6) achieved significant reduction in plasma VLCFA levels of 25 out of 36 ALD patients with a daily diet enriched in C18:1 (45–90 ml of glyceryl trioleate oil containing about 90% of C18:1) along with a restriction in C26:0 below 10 mg for about a hundred days. Presumably C18:1 competed with saturated fatty acids for their microsomal chain elongation system. Now, we can recommend that, beside the oleate-enrichment as described above, nuts and seeds as well as fats and oils should be restricted as severely as possible from the diet of ALD patients in Japan also, in order to keep daily C26:0 intake below 10 mg as recommended by Moser's group(6). We hope our present data will prove beneficial in the treatment of ALD as well as other VLCFA-accumulating disorders in Japan and other countries which consume food items similar to the ones in our study.

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