チアミンとビスフェノールAとの接点は不明である．一方，PIC排泄量の減少は，ビタミンB_6が関与する反応，例えば，アミノ酸の異化代謝をビスフェノールAが亢進させていることを示唆している．

アスコルビン酸はラットでは，ビタミンではない．ラットは生体異物を摂取するとアスコルビン酸の産生量を増大させることが知られている[27]．この増大は解毒に関係する薬物代謝系に関連する応答である．本実験においても，Fig. 6Dに示したように，ビスフェノールAの摂取により増大したことから，ラットはビスフェノールAを生体異物として認識し，水酸基を付加させて，脂溶性から水溶性物質に変化させて，積極的にビスフェノールAを尿中に排泄されているものと思われる．

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JSBA

The Necessity of Niacin in Rats Fed on a High Protein Diet

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It is known that niacin itself is not necessary in rats when tryptophan is given in adequate amounts, because rats can biosynthesize niacin from tryptophan. In our experiment, young rats were fed on a 20%, 40%, 60%, or 70% casein diet with or without niacin. The rats fed on the 20%, 40%, and 60% casein diets did not require niacin for growth, but the rats fed on the 70% casein diet needed it. This phenomenon was attributed to the supposition that liver aminocarboxymuconate-semialdehyde decarboxylase activities increased according with the dietary casein levels. The conversion ratio of tryptophan–niacin in rats fed on the 70% casein diet became extremely low, and then the rats needed niacin.

Key words: tryptophan; necessity of niacin; high protein diet; conversion ratio of tryptophan–niacin

Because more than 500 enzymes need niacin coenzymes, it is important to determine the control mechanisms of the coenzyme supply *in vivo*. Mammals including humans can biosynthesize niacin from an indispensable amino acid Trp. Therefore, many nutritionists including our group claim that niacin itself is not necessary when Trp is taken suitably. In fact, it lacks any influence on the growth of young rats even if they are given niacin-free diets containing a suitable amount of protein, such as 20% casein diets.[1] However, we found that rats needed niacin for maximum growth when they are fed a 70% casein diet. Our paper explains our methods and results.[1]

Materials and Methods

Chemicals. Vitamin-free milk casein, sucrose, L-methionine, Nam, and L-Trp were purchased from Wako Pure Chemical Industries (Osaka, Japan). Kynurenine sulfate, KA, and MNA chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick[2] and Shibata *et al.*[3] respectively. Corn oil was purchased from Ajinomoto (Tokyo, Japan). The mineral (AIN-93M-MX) and vitamin (AIN-93-VX) mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan), all the other chemicals used being of the highest purity available from commercial sources.

Animals. The care and treatment of the experimental animals conformed to The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Experiment 1 (70% casein diets with or without NiA in the presence of vitamin B_6). Male rats of the Wistar strain (4 weeks old with a body weight of around 60 g) were obtained from CLEA Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; CLEA Japan). To acclimatize the rats to these conditions, they were initially fed *ad libitum* for 7 d with a complete 20% casein diet[1] and water. They were then divided into the two groups and fed *ad libitum* for 19 d, with a 70% casein diet with or without NiA in the presence of vitamin B_6 (Table 1).

The room temperature was kept at 22 ± 2 °C at about 60% humidity, and a 12-h light/12-h dark cycle was maintained. Body weight and food intake were measured periodically, usually every other day at 9:00–10:00 a.m. Urine samples (24-h; 9:00 a.m.–9:00 a.m.) were collected for the last day of the experiment in amber bottles containing 1 ml of 1 M HCl, and were stored at −25 °C until needed. The rats were killed by decapitation after the collection of urine samples. The liver of each animal was removed, and a portion of it (approximately 1 g) was treated as described in the literature[4,5] to measure the enzyme activities involved in the metabolism of Trp to niacin.

Experiment 2 (70% casein diets with or without NiA in the absence of vitamin B_6). The same procedure was performed as with Experiment 1 except for the diet, from which was removed only vitamin B_6, as shown in Table 1.

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Abbreviations: Trp, L-tryptophan; XA, xanthurenic acid; KA, kynurenic acid; 3-HK, 3-hydroxyanthranilic acid; Nam, nicotinamide; MNA, N^1-methylnicotinamide; 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide; ACMSDase, aminocarboxymuconate-semialdehyde decarboxylase

Table 1. Composition of the 70% Casein Diets

	Experiment 1		Experiment 2	
	+NiA & +B_6 %	−NiA & +B_6 %	+NiA & −B_6 %	−NiA & −B_6 %
Vitamin-free milk casein	70	70	70	70
L-Methionine	0.5	0.5	0.5	0.5
Sucrose	18.5	18.5	18.5	18.5
Corn oil	5	5	5	5
Mineral mixture*	5	5	5	5
Vitamin mixture*	1	0	0	0
NiA-free vitamin mixture*	0	1	0	0
B_6-free vitamin mixture*	0	0	1	0
NiA and B_6-free vitamin mixture*	0	0	0	1

*AIN 93 was used (Reeves, P.G., Components of the AIN-93 diets as improvements in AIN-76A diet. *J. Nutr.*, 127, 838S–841S (1997)). The diet (+NiA & +B_6) contained 6 mg NiA and 0.8 mg of pyridoxine-HCl per 100 g of diet.

Table 2. Composition of the 40% and 60% Casein Diets

	40% Casein diet		60% Casein diet	
	+NiA %	−NiA %	+NiA %	−NiA %
Vitamin-free milk casein	40	40	60	60
L-Methionine	0.4	0.4	0.6	0.6
Sucrose	48.6	48.6	28.4	28.4
Corn oil	5	5	5	5
Mineral mixture*	5	5	5	5
Vitamin mixture*	1	0	1	0
NiA-free vitamin mixture	0	1	0	1

*AIN 93 was used (Reeves, P.G., Components of the AIN-93 diets as improvements in AIN-76A diet. *J. Nutr.*, 127, 838S–841S (1997)). The diet (+NiA & +B_6) contained 6 mg NiA and 0.8 mg of pyridoxine-HCl per 100 g of diet.

Experiment 3 (40% and 60% casein diets with or without NiA in the presence of vitamin B_6). The same procedure was performed as with Experiment 1 except for the diets was done. The composition of the diets used in Experiment 3 is shown in Table 2.

Analyses. To measure the conversion ratio of Trp to niacin, the urinary contents of Nam and its metabolites MNA, 2-Py, and 4-Py were measured. This method does not take account of the increased body store of Nam during growth, and the value does not, therefore, represent the net conversion ratio. However, this value is useful for the assessment of the apparent conversion ratio. The conversion ratio was calculated as the sum of the urinary excretions of {Nam + MNA + 2-Py + 4-Py(μmol/day)} × 100/Trp intake during urine collection (μmol/day). The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata *et al.*,[3] while the content of MNA in the urine was measured by the HPLC method of Shibata.[6]

The contents of KA[7] and XA[8] in the urine were measured by HPLC.

Trp oxygenase (EC 1.13.11.11),[9] kynureninase (EC 3.7.1.3: the reaction was done in the absence of added pyridoxal 5′-phosphate),[7] kynurenine aminotransferase (EC 2.6.1.7: the reaction was done in the absence of added pyridoxal 5′-phosphate),[10] 3-HA oxygenase (EC 1.13.1.1),[9] kynurenine 3-hydroxylase (EC 1.14.13.9: the reaction was done in the presence of added NADPH),[11] ACMSDase (EC 4.1.1.45),[12] NMN adenylyltransferase (EC 6.3.5.1),[13] Nam methyltransferase (EC 2.1.1.1),[14] 2-Py-forming MNA oxidase (EC 1.2.3.1),[14] and 4-Py-forming MNA oxidase (EC number not identified)[12] were measured as described in the literature.

Results

Experiment 1 (70% casein diets with or without NiA in the presence of vitamin B_6)

Table 3 shows the effects of feeding the 70% casein diet with or without NiA on the body weight gain, food intake, and food efficiency ratio. The food intake was almost the same between the two groups, but the body weight gain was significantly lower in the group fed on the NiA-free diet, as shown in Fig. 1. As a result, the food efficiency ratio was significantly lower in the −NiA group than in the +NiA group. That is, the necessity of niacin itself was observed in the 70% casein diet, even when a sufficient amount of Trp was taken.

The urinary excretion of KA and XA in terms of nmol/g of diet is shown in Table 4. The urinary

Table 3. Effects of Feeding the 70% Casein Diet with or without NiA on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 1)

	+NiA & +B_6	−NiA & +B_6
Initial body weight (g)	102 ± 2	105 ± 1
Final body weight (g)	205 ± 5	178 ± 4*
Body weight gain (g/19 days)	103 ± 5	73 ± 3*
Food intake (g/19 days)	231 ± 6	223 ± 3
FER[1]	0.45 ± 0.02	0.33 ± 0.01*

[1]FER, Food Efficiency Ratio.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

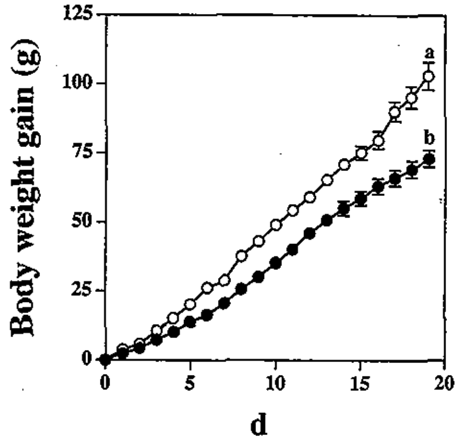

Fig. 1. Effects of Feeding the 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 1).

○, +NiA & +B_6; ●, −NiA & +B_6. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p < 0.05$, as calculated by the Student–Newman–Keuls Multiple Comparisons test.

Table 4. Effects of Feeding the 70% Casein Diets with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 1)

	+NiA & +B_6	−NiA & +B_6
Food intake (g/day)	14.3 ± 0.3	15.0 ± 0.7
KA	198 ± 17	163 ± 15
XA	79 ± 5	62 ± 5
Nam	16 ± 1	3 ± 1*
MNA	87 ± 3	15 ± 3*
2-Py	80 ± 3	11 ± 2*
4-Py	331 ± 9	96 ± 5*
Sum[1]	514 ± 13	125 ± 10*
(2-Py + 4-Py)/MNA	4.7 ± 0.3	7.1 ± 0.8
NiA intake	487 ± 0	0
Trp intake	38783 ± 0	38783 ± 0
Conversion ratio of Trp to Niacin (%)	Not calculated	0.32 ± 0.03

[1]Sum, Nam + MNA + 2-Py + 4-Py.

Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and the conversion ratio of Trp to niacin and means ± SEM for five rats.

*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

excretion of KA and XA was almost the same between the two groups. The urinary excretion of Nam, MNA, 2-Py, 4-Py, and the sum of Nam + MNA + 2-Py + 4-Py in terms of g of diet respectively is also shown in Table 4. The higher values of each of these in the +NiA group than in the −NiA group was attributed to the intake of dietary NiA. The conversion ratio could not be calculated for the group fed on the +NiA diet, but it could be done on the group fed on −NiA diet by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 0.32 ± 0.03% (mean ± SEM for 5 rats), as shown in Table 4.

The next step was done to investigate the effects of the 70% casein diet with or without NiA on the enzyme activity of Trp to niacin. As Table 5 shows, none of the enzyme activities showed a difference between the two groups.

Table 5. Effects of Feeding the 70% Casein Diets with or without Niacin on the Enzyme Activities Involved in the Metabolism of Trp to Niacin (Experiment 1)

	+NiA & +B_6	−NiA & +B_6
Trp oxygenase	2.03 ± 0.22	1.77 ± 0.22
Kynureninase	1.57 ± 0.05	1.55 ± 0.04
Kynurenine aminotransferase	1.14 ± 0.10	1.03 ± 0.02
Kynurenine 3-hydroxylase	1.54 ± 0.15	1.96 ± 0.40
3-HA oxygenase	551 ± 35	550 ± 22
ACMSDase	11.3 ± 1.4	12.1 ± 1.1
NMN adenylyltransferase	8.97 ± 0.71	8.31 ± 0.46
NAD^+ synthetase	0.59 ± 0.12	0.61 ± 0.04
Nam methyltransferase	1.85 ± 0.03	1.89 ± 0.04
2-Py-forming MNA oxidase	0.68 ± 0.07	0.71 ± 0.06
4-Py-forming MNA oxidase	1.70 ± 0.08	1.59 ± 0.03

Values are expressed as μmol/h/g of liver and means ± SEM for five rats.

Experiment 2 (70% casein diets with or without NiA in the absence of vitamin B_6)

Table 6 shows the effects of feeding the vitamin B_6-free, 70% casein diet with or without NiA on the body weight gain, food intake, and food efficiency ratio. The food intake was significantly lower in the −NiA group than in the +NiA group and the body weight gain was greatly lower in the group fed on the NiA-free diet, as shown in Fig. 2. As a result, the food efficiency ratio was significantly lower in the −NiA group than in the +NiA group. That is, the necessity of niacin itself was also ascertained in the vitamin B_6-free and 70% casein diet.

Table 6. Effects of Feeding the Vitamin B_6-Free, and 70% Casein Diets with or without Niacin on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 2)

	+NiA & −B_6	−NiA & −B_6
Initial body weight (g)	106 ± 1	102 ± 2
Final body weight (g)	139 ± 5	115 ± 4*
Body weight gain (g/19 days)	33 ± 4	13 ± 4*
Food intake (g/19 days)	162 ± 2	141 ± 5*
FER[1]	0.20 ± 0.02	0.09 ± 0.01*

[1]FER, Food Efficiency Ratio.

Values are means ± SEM for five rats, expressed as μmol/h/g of liver and means ± SEM for five rats.

*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

The urinary excretion of KA and XA in terms of nmol/g of diet is shown in Table 7. The urinary excretion of KA and XA was almost the same between the two groups. But that of XA was much higher in Experiment 2 than in Experiment 1 (Tables 4 and 7).

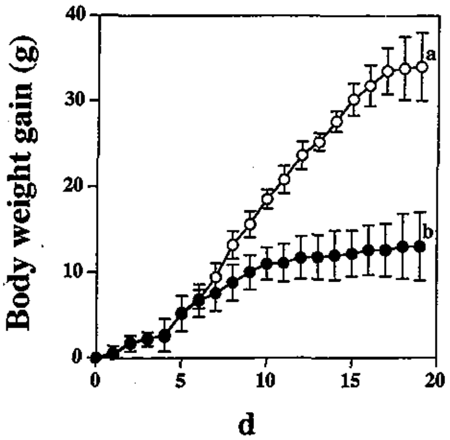

Fig. 2. Effects of Feeding the Vitamin B_6-Free and 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 2).

O, +NiA & $-B_6$; ●, −NiA & $-B_6$. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p < 0.05$, as calculated by the Student–Newman–Keuls Multiple Comparisons test.

Table 7. Effects of Feeding the Vitamin B_6-Free, and 70% Casein Diet with or without NiA on the Urinary Excretion of KA and XA, *Nam and Its Metabolites*, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 2)

	+NiA & $-B_6$	−NiA & $-B_6$
Food intake (g/day)	7.4 ± 1.3	5.8 ± 1.1
KA	145 ± 5	144 ± 34
XA	1775 ± 173	2100 ± 234
Nam	16 ± 2	11 ± 4
MNA	295 ± 30	65 ± 11*
2-Py	23 ± 3	7 ± 1*
4-Py	137 ± 18	70 ± 6*
Sum[1]	471 ± 48	153 ± 14*
(2-Py + 4-Py)/MNA	0.54 ± 0.04	1.2 ± 0.06*
NiA intake	487 ± 0	0
Trp intake	38783 ± 0	38783 ± 0
Conversion ratio of Trp to Niacin (%)	Not calculated	0.39 ± 0.05

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's *t* test.

The abnormal increase in XA means that the rats were in a vitamin B_6-deficient state. The urinary excretion of Nam, MNA, 2-Py, 4-Py, and the sum of Nam + MNA + 2-Py + 4-Py in terms of g of diet respectively is also shown in Table 7. The higher values of each of these in the +NiA group than in the −NiA group was attributed the intake of dietary NiA. The conversion ratio could not be calculated on the group fed on +NiA diet, but it could be done on the group fed on −NiA group by

Table 8. Effects of Feeding Vitamin B_6-Free, and 70% Casein Diet with or without NiA on the Enzyme Activities Involved in the Metabolism of Trp to Niacin (Experiment 2)

	+NiA & $-B_6$	−NiA & $-B_6$
Trp oxygenase	1.82 ± 0.09	1.81 ± 0.14
Kynureninase	0.39 ± 0.09	0.32 ± 0.03
Kynurenine aminotransferase	0.38 ± 0.04	0.35 ± 0.04
Kynurenine 3-hydroxylase	3.07 ± 0.17	2.79 ± 0.18
3-HA oxygenase	511 ± 39	565 ± 43
ACMSDase	11.1 ± 1.6	12.6 ± 1.5
NMN adenylyltransferase	8.16 ± 0.27	8.75 ± 0.57
NAD^+ synthetase	0.56 ± 0.07	0.56 ± 0.06
Nam methyltransferase	1.79 ± 0.02	1.82 ± 0.03
2-Py-forming MNA oxidase	0.05 ± 0.02	0.10 ± 0.04
4-Py-forming MNA oxidase	0.18 ± 0.09	0.74 ± 0.10*

Values are expressed as μmol/h/g of liver and means ± SEM for five rats.
* Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's *t* test.

comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 0.39 ± 0.05%, as shown in Table 7.

The next step was done to investigate the effects of the 70% casein diet with or without NiA on the enzyme activity of Trp to niacin. As Table 8 shows, none of the enzyme activities except for 2-Py- and 4-Py-forming MNA oxidases showed a difference between the two groups.

Experiment 3 (40% and 60% casein diets with or without NiA in the presence of vitamin B_6)

The body weight gain, food intake, and food efficiency ratio in the rats fed the 20%,[1] 40%, and 60% casein diets with or without NiA are shown in Table 9. These values are almost the same among all of the groups irrespective of dietary protein levels and NiA intake.

Table 10 shows the urinary excretions of Trp–niacin metabolites in the groups of 20%,[1] 40%, and 60% casein diets with and without NiA. The urinary excretions of KA and XA in terms of nmol/g of diet increased with dietary protein intake irrespective of the presence or absence of vitamin B_6. The higher values of Nam, MNA, 2-Py, and 4-Py in the +NiA group than in the −NiA group between each protein level were attributed the intake of dietary NiA. The conversion ratio could not be calculated on the group fed on +NiA diet, but it could be done on the group fed on −NiA diet by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 1.90 ± 0.25% for the 20% casein diet,[1] 1.13 ± 0.07 for the 40% casein diet, and 0.60 ± 0.08% for the 60% casein diet.

Table 11 shows the activities of ACMSDase in the liver. The activities were not different irrespective of presence or absence of vitamin B_6 between the same protein levels, while the activities increased with dietary protein levels.

Table 9. Effects of NiA Addition to the 20%, 40%, and 60% Casein Diets on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
Initial body weight (g)	103 ± 2	106 ± 2	102 ± 2	102 ± 2	102 ± 1	102 ± 1
Final body weight (g)	216 ± 4	216 ± 4	217 ± 5	223 ± 5	213 ± 4	212 ± 4
Body weight gain (g/19 d)	113 ± 4	110 ± 3	115 ± 4	121 ± 4	111 ± 3	110 ± 4
Food intake (g/19 d)	273 ± 5	287 ± 5	284 ± 5	294 ± 6	262 ± 6	260 ± 3
Food efficiency ratio*	0.41 ± 0.01	0.38 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.42 ± 0.02

*FER, body weight gain (g/19 d)/food intake (g/19 d).
Each value is the mean ± SEM for five rats.
[1]Data were drawn from reference 1.

Table 10. Effects of Feeding the 20%, 40%, and 60% Casein Diets with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
Food intake (g/day)	16.7 ± 0.6	18.0 ± 0.8	17.9 ± 0.6	18.5 ± 0.7	17.9 ± 0.6	16.5 ± 0.4
KA	35 ± 2.1	40 ± 5.6	105 ± 7.5	126 ± 20.5	156 ± 26.7	191 ± 8.7
XA	28 ± 2.5	36 ± 4.7	55 ± 6.4	51 ± 4.2	67 ± 7.8	81 ± 3.1
Nam	16 ± 0.8	13 ± 1.3	17 ± 1.4	9 ± 0.4	9 ± 1.4	7 ± 0.3
MNA	55 ± 1.1	18 ± 1.8*	57 ± 4.9	22 ± 2.2*	84 ± 8.1	24 ± 1.1*
2-Py	61 ± 2.4	16 ± 2.7*	55 ± 5.3	20 ± 1.9	66 ± 4.1	21 ± 0.9*
4-Py	484 ± 19	161 ± 21*	449 ± 53	199 ± 14*	257 ± 30	149 ± 19*
Sum[1]	616 ± 18	208 ± 27*	578 ± 57	250 ± 17*	416 ± 35	201 ± 20*
(2-Py + 4-Py)/MNA	9.9 ± 0.4	9.8 ± 0.5	8.8 ± 1.5	10.0 ± 1.1	3.8 ± 0.6	7.1 ± 0.7
NiA intake	487 ± 0	0	487 ± 0	0	487 ± 0	0
Trp intake	11081 ± 0	11081 ± 0	22162 ± 0	22162 ± 0	33242 ± 0	33242 ± 0
Conversion ratio of Trp to niacin (%)	Not calculated	1.90 ± 0.25	Not calculated	1.13 ± 0.07	Not calculated	0.60 ± 0.08

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the respective the +NiA group, as evaluated by Student's t test.
[1]Data were drawn from reference 1.

Table 11. Effects of Feeding the 20%, 40%, and 60% Casein Diets with or without NiA on the ACMSDase Activity in the Liver (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
ACMSDase (μmol/h/g of liver)	2.4 ± 0.6	2.3 ± 0.6	3.81 ± 0.37	3.69 ± 0.57	8.85 ± 0.36	7.90 ± 1.16

[1]Data were drawn from reference 1.

Discussion

Sanada[15] and our group[5,16] have reported that the ACMSDase activity increases with dietary protein levels. We also found that the conversion ratio of Trp to niacin decreases with dietary protein levels.[5,16] Hence we thought that the ACMSDase controls niacin formation to shunt the excessive niacin supply. However, from the present experimental findings, we learned that the view of the Trp–niacin relationship that the ACMSDase controls the niacin formation is not right.

In a previous report,[1] we found that the body weight gains between the rats fed on a diet containing NiA and those fed on the diet minus only NiA were exactly the same. That is, when rats were fed on the 20% casein diet, they do not need niacin. Vitamin B_6 is important in the Trp–niacin metabolism, especially in the metabolism of kynurenine.[17] The body weight gain in young rats was significantly lower in the group fed on the 20% casein diet without vitamin B_6 than in the group fed on the 20% casein diet with vitamin B_6.[1] The lower body weight gain in the vitamin B_6-free group was due to a deficiency of vitamin B_6.[1] In fact, the urinary excretion of XA, which is an indicator of vitamin B_6 deficiency,[18] was significantly higher in the vitamin B_6-free group than in the vitamin B_6-containing group.[1] However, no necessity of niacin on the 20% casein diet was observed in the diet without vitamin B_6.[1]

Feeding the NiA-free 70% casein diets rats caused a decrease in body weight gain as compared with the 70% casein diets containing NiA (Fig. 1).

In the groups fed on the NiA-free 20%, 40%, and 60% casein diets, the urinary excretion of the sum were 200–250 nmol/g of diet (Table 10), while it was around 130 nmol/g of diet in the groups on the NiA-free 70% casein diet (Tables 4 and 7). These results indicate that the rats fed on the 70% casein diet without NiA were niacin deficient. So the supplementation of NiA to the rats fed on the 70% casein diet caused the growth promoting action. These findings are very curious, since niacin is believed to be supplied from dietary Trp.[19] Under the 20% casein diet conditions, about 2% of Trp is converted to niacin.[1] On the contrary, when rats were fed on 70% protein diets, the conversion ratio was very low, about 0.3% (Table 4), and the rats, therefore, needed niacin for normal growth. The intake of Trp in the group fed on the 70% protein diet increased by 7/2 in comparison with the 20% casein diet, while the conversion decreased by 2/0.3. Therefore, the absolute formation of niacin was about half ($7/2 \times 0.3/2 = 0.525$) that of the rats fed on the 20% casein diet.

The conversion ratio was not affected by the presence or absence of vitamin B_6 on the 70% casein diets (Tables 4 and 7), although it was severely affected by the presence or absence of vitamin B_6 on the 20% casein diets.[1] In the experiment with the 70% casein diets, the urinary excretion of XA was much more increased by feeding the vitamin B_6-free diets (Table 7) than by feeding the B_6-containing diets (Table 4). That is, in Experiment 1, the rats were not vitamin B_6 deficient even when they were fed the 70% casein diet. Therefore, the necessity of niacin in the 70% casein diet was not associated with the nutritional state of vitamin B_6.[20]

The excretion of KA was almost the same among the four groups (Tables 4 and 7). The formations of KA and XA are catalyzed by the same enzyme, kynurenine aminotransferase, which is a PLP-dependent enzyme. This enzyme activity was much lower in the groups fed on the vitamin B_6-free diets (Table 8) than in those fed on the vitamin B_6-containing diets (Table 5). Nevertheless, the flux of Trp to XA increased greatly (Tables 4 and 7). The mechanism in the case of increased XA only can be explained as follows: Kynurenine was more efficiently converted to 3-hydroxykynurenine in the vitamin B_6-deficient rats than in the normal rats because the activity of kynurenine 3-hydroxylase was increased on the vitamin B_6-free diets (Tables 5 and 8), and 3-hydroxykynurenine, therefore, accumulates because the activity of kynureninase, which catalyzes the reaction of 3-hydroxykynurenine to 3-hydroxyanthranilic acid, decreased on the vitamin B_6-free diets (Tables 5 and 8). The accumulated 3-hydroxykynurenine in the group fed on two vitamin B_6-free diets in Experiment 2 was converted to XA by kynurenine aminotransferase. The reason the urinary excretion of KA did not increase might be increased kynurenine 3-hydroxylase.

The side flux of Trp increased in the groups fed on the two vitamin B_6-free diets in experiment 2, but the conversion ratio of Trp to niacin did not change between the groups fed on the diets with or without vitamin B_6 (Tables 4 and 7). This phenomenon has not been explained. The decreased conversion ratio in the high protein diets was due to the increased activity of ACMSDase as compared with that of the 20% protein diets (Tables 5 and 8 and Reference 1). It is a question why the activity of ACMSDase so increased on the high protein diets. High protein diets mean low carbohydrate diets, so that under the conditions, amino acids can be catabolized into energy formation pathways but not into protein synthesis and other biofactors. But in the present experiments it was clearly shown that the conversion ratio of Trp to niacin is subjected to the reaction α-amino-β-carboxymuconate-ε-semialdehyde $\rightarrow$ α-amino muconate-ε-semialdehyde, which is catalyzed by ACMSDase, and α-aminomuconate-ε-semialdehyde is then catabolized into acetyl-CoA, but not into niacin. On the contrary, the reaction α-amino-β-carboxymuconate-ε-semialdehyde $\rightarrow$ quinolinic acid is non-enzymatic, and quinolinic acid is then metabolized into niacin. Accordingly, quinolinic acid formation from Trp is subjected to the activity of ACMSDase. The administration of an inhibitor of ACMSDase causes the greatly increased conversion ratio of Trp to niacin.[21]

In conclusion, we found that rats need dietary niacin when they are fed a 70% casein diet for maximum growth, while they do not need it when they are fed 20%, 40%, or 60% diets. This phenomenon is attributed to changes in the Trp–niacin conversion ratio due to the amount of protein intake. Therefore, when evaluating niacin requirements or status, protein intake must be considered.

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Recommended Dietary Allowance for Vitamin C in the United States is Also Applicable to a Population of Young Japanese Women

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The recommended dietary allowance (RDA) for ascorbic acid (AA) in Canada and the United States has been set for several years at 75 mg/day for women 19–30 years old. Recently this level was questioned, and an increase to 90 mg/day was suggested. For Japanese women in the same age group, we found that the RDA for AA is currently 100 mg/day. Our goal was to determine which RDA is sufficient for maintaining a serum concentration of AA in young Japanese women above the lower reference limit of 7.0 mg/L. We measured serum AA concentrations by an ascorbate oxidase method in 176 healthy Japanese women (19–26 years old). We also performed an ROC analysis to estimate the optimal cutoff value for oral dosage to distinguish individuals with hypovitaminosis-C (<7.0 mg/L) from those with a normal serum AA. We evaluated the Japanese RDA using the 75 or 90 mg/day U.S. RDA and the weight ratio between Japanese and U.S. women, and discovered that the RDA value ranged between 66 and 79 mg/day. From the ROC analysis, we found that the optimal daily dosage of AA is approximately 75 mg/day. This value gave the highest efficiency, sensitivity, negative predictive value, and positive likelihood ratio, and the lowest negative likelihood ratio. Therefore, an RDA of 100 mg/day may be unnecessarily high for young Japanese women. J. Clin. Lab. Anal. 18:305–308, 2004.

Key words: vitamin C (ascorbic acid); recommended dietary allowance (RDA); ROC analysis; serum ascorbic acid; dietary intake

INTRODUCTION

Recently the recommended dietary allowance (RDA) for several vitamins was set in Canada, the United States, and Japan. The RDA is based on the intake required to maintain serum or blood concentrations above a lower reference limit (1,2). For vitamin C (ascorbic acid (AA)), the Japanese RDA is 100 mg/day for both men and women >18 years old. The Japanese RDA is set to maintain a serum AA concentration of >7.0 mg/L based on evidence by Levine et al. (3) that AA in leukocytes is saturated at 100 mg/day intake without urinary excretion. In Levine et al.'s (3) study, seven healthy men (20–26 years old) were given AA doses of 30, 60, 100, 200, 400, 1,000, and 2,500 mg/day. They reported that neutrophils, monocytes, and lymphocytes saturated at 100 mg/day, and that six of the seven volunteers did not excrete AA in urine until the 100-mg dosage level was reached. Their report indicated that plasma AA levels in six of seven volunteers were 2.6–4.2 mg/L at the 60-mg dosage. At the 100-mg dosage, plasma AA levels in all volunteers increased to 8.9–10.9 mg/L. Therefore, we suggest that the serum AA level of 7.0 mg/L used for the Japanese RDA does not require a 100-mg dosage. In addition, the U.S. RDA is lower: 90 mg/day for men, and 75 mg/day for women, even though the reference body weight in the U.S. is 11 kg and 10 kg greater for men and women, respectively, than that of Japanese (1,2). Therefore, in this study, we aimed to determine the appropriateness of the U.S. and Japanese RDAs for producing serum AA levels above 7.0 mg/L in young Japanese women.

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MATERIALS AND METHODS

Subjects

We collected blood specimens by venipuncture from fasting individuals. The subjects were 176 female Japanese university students (median age = 19.0 years; range = 19–26 years), all in good health, with body weights of 51 ± 6 kg (mean ± SD). They provided a 3-day dietary history prior to blood collection. None had a history of smoking, regular use of medications, use of dietary supplements, or alcohol consumption. Written informed consent was obtained from all enrolled volunteers, and our study was conducted in compliance with the rules for human experimentation at our institution.

For this study, we selected a life-stage group of 19–26 years. Since it is known that higher nutrient intakes during early adulthood are important for achieving optimal genetic potential and energy metabolism, the U.S. and Japanese RDAs were set for life-stage groups of 19–30 years and 18–29 years, respectively.

Analyses

We measured serum AA concentrations in all subjects by an ascorbate oxidase method (4) that involves protein precipitation with metaphosphoric acid. Ascorbate oxidase converts all AA in a deproteinized supernatant to dehydro-AA (the latter is measured with o-phenylenediamine used as the chromogen at 340 nm). The reference value for serum AA by the ascorbate oxidase method is quoted elsewhere as 7.0–13.0 mg/L (lower confidence interval = 6.6–7.4 mg/L) (5,6). In the automated version of this assay, a Cobas Mira S instrument (Roche Diagnostic System, Montclair, NJ) was employed. The assay was validated with a linear concentration range of 0.4–80 mg/L, and the within-day and between-day assays gave a mean ± SD of 11.7 ± 0.1 mg/L (10 consecutive assays) and 11.7 ± 0.4 mg/L (10 consecutive days), respectively. No effects of potential interferences were observed. Hemoglobin at 5 g/L, and conjugated and unconjugated bilirubin at 400 mg/L had no significant effects on the values of AA.

Statistical Analysis

We prepared ROC curves (7) to obtain the optimal cutoff value (i.e., daily intake) for the sensitivity [(true positive (TP))/(TP + false negative (FN))] and specificity [(true negative (TN))/(TN + false positive (FP))]. The value was calculated from the highest sum of sensitivity and specificity from the ROC curve. The efficiency of the dietary intake in maintaining serum AA concentrations above the lower reference limit of 7.0 mg/L was calculated as: efficiency = (TP + TN)/(TP + FP + TN + FN). Predictive values and likelihood ratios for the test were calculated according to Sasse (7) and Simel et al. (8), respectively. The 95% confidence intervals for sensitivity, specificity, efficiency, predictive values, and likelihood ratios were also calculated according to Simel et al. (8). Here the TP is the number of individuals who had serum AA concentrations >7.0 mg/L and were classified correctly as having adequate AA in their diet by dietary assessment. The FP is the number of individuals who had serum AA concentrations <7.0 mg/L but were misclassified as having adequate AA in their diet by dietary assessment. The FN is the number of individuals who had serum AA concentrations >7.0 mg/L but were misclassified as having inadequate AA in their diet by dietary assessment. The TN is the number of individuals who had serum AA concentrations <7.0 mg/L and were correctly classified as having inadequate AA in their diet by dietary assessment.

RESULTS

The 3-day mean of the volunteers' intake of AA ranged from 3 to 368 mg/day (median = 99 mg/day); only 88 volunteers (50%) ingested more AA than the Japanese RDA (100 mg/day). For the entire group of 176 volunteers, the concentration of AA in serum ranged from 4.9 to 17.2 mg/L (median = 10.5 mg/L and mean ± SD, 10.8 ± 2.4 mg/L) (Fig. 1). The serum AA concentrations were significantly ($P < 0.05$) correlated to the mean intake of AA; however, the correlation coefficient was low (r = 0.173). Despite AA intakes

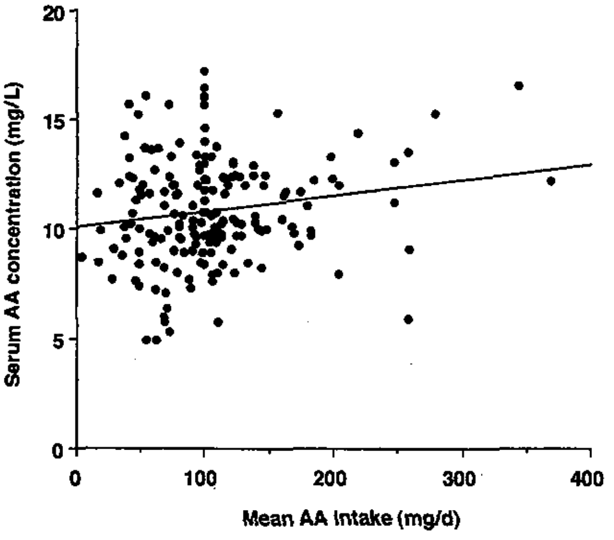

Fig. 1. Relationship of AA intake (mean intake over 3 days) to serum AA concentration in 176 healthy volunteers. The regression equation was: $y = 0.007x + 10.0$ mg/L ($Sy/x = 2.4$ mg/L; $r = 0.173$, $P < 0.05$).

<100 mg/day, 82 volunteers represented normal serum AA concentrations >7.0 mg/L. Volunteers who occasionally reported a lower AA intake during their 3-day dietary history were daughters from middle-class homes, and blood analysis revealed that their nutritional levels of carbohydrates, proteins, and lipids were sufficient. Several volunteers (who were not vegetarians) consumed two or three times more AA than the RDA, because they consumed fruits, vegetables, juice, and soft drinks containing a great deal of AA.

Because the dietary assessment of AA nutrition did not agree well with the serum concentrations (r=0.173, Fig. 1), we wished to determine the intake of AA required to produce a normal AA in serum of >7.0 mg/L. Toward that end, we used the ROC curve analysis (Fig. 2). The optimal cutoff (highest sum of sensitivity and specificity from the ROC curve) for discriminating individuals with normal serum AA was obtained at 75 mg/day of AA intake, with an efficiency of 0.72. We compared operating characteristics at three cutoff levels (e.g., 75, 90, and 100 mg/day). Although the confidence intervals overlapped each other, the ROC analysis also gave the highest sensitivity, negative predictive value, and positive likelihood ratio, and the lowest negative likelihood ratio at 75 mg/day of AA intake (Table 1). The specificity and positive likelihood ratio were the same at any cutoff level.

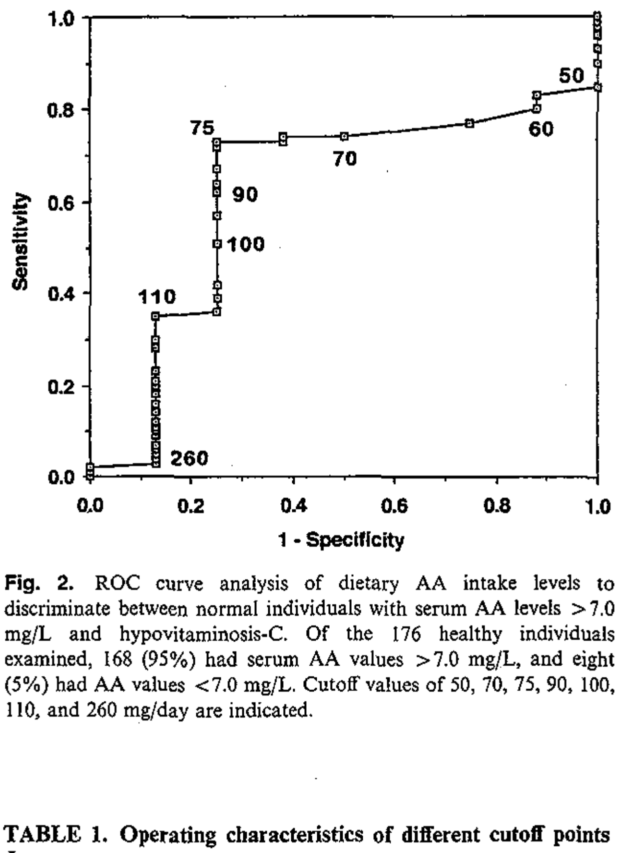

Fig. 2. ROC curve analysis of dietary AA intake levels to discriminate between normal individuals with serum AA levels >7.0 mg/L and hypovitaminosis-C. Of the 176 healthy individuals examined, 168 (95%) had serum AA values >7.0 mg/L, and eight (5%) had AA values <7.0 mg/L. Cutoff values of 50, 70, 75, 90, 100, 110, and 260 mg/day are indicated.

DISCUSSION

The aim of this study was to determine which RDA (75 mg/day, 90 mg/day, or 100 mg/day) is sufficient to maintain the serum concentration of AA in young Japanese women above the lower reference limit of 7.0 mg/L.

The U.S. RDA for AA is set to provide the antioxidant protection of this vitamin as derived from the correlation of such protection with neutrophil AA concentration according to the report by Anderson and Lukey (9). In addition, the U.S. RDA referenced Levine et al.'s (3) report that a near-maximum neutrophil AA concentration (1.25 mmol/L) was observed at a 100-mg dosage of AA. However, since neutrophil AA>1.0 mmol/L is lost in urine, the U.S. RDA uses a neutrophil AA concentration of 1.0 mmol/L. From the regression curve illustrated in Levine et al.'s report, a 75-mg dosage of AA (for an average of seven male volunteers) was needed to maintain the neutrophil AA level at 1.0 mmol/L. Thus, the National Research Council cites 75 mg/day AA as the estimated average intake (EAR) for men,

TABLE 1. Operating characteristics of different cutoff points for producing serum AA concentration above 7.0 mg/L in young Japanese women

	Cutoff point (daily intake of AA, mg/day)		
Characteristics	75	90	100
Sensitivity	0.72 (0.65–0.79)	0.62 (0.55–0.69)	0.51 (0.44–0.59)
Specificity	0.75 (0.45–1.05)	0.75 (0.45–1.05)	0.75 (0.45–1.05)
Efficiency	0.72 (0.66–0.79)	0.63 (0.55–0.70)	0.52 (0.45–0.60)
Positive predictive value	0.98 (0.96–1.01)	0.98 (0.96–1.01)	0.98 (0.95–1.01)
Negative predictive value	0.11 (0.03–0.20)	0.09 (0.02–0.15)	0.07 (0.02–0.12)
Positive likelihood ratio	2.88 (0.86–9.60)	2.48 (0.74–8.27)	2.05 (0.61–6.86)
Negative likelihood ratio	0.37 (0.23–0.60)	0.51 (0.33–0.79)	0.65 (0.42–1.00)

Values in parentheses are 95% confidence interval.

because 1.0 mmol/L is the average value. Since the daily intake at the EAR level is set to meet the nutrient requirement of 50% of healthy individuals, and 97–98% at the RDA level (1.2 × EAR for AA) (1), the U.S. RDA for men is 90 mg/day. Correcting by 0.75 power based on body weight ratio between women and men (90 mg/day × $(61\,\text{kg}/76\,\text{kg})^{0.75}$), the RDA for women is then computed at 75 mg/day. Recently, however, Levine et al. (10) performed an experiment similar to that performed on men on 13 healthy women (19–27 years old), and suggested 60 mg EAR and 90 mg RDA (here, 1.5 × EAR) for young women.

In Levine et al.'s report (10), 12 of 13 women showed plasma AA concentrations >7.0 mg/L at a dosage of 90 mg/day. If one calculates the Japanese RDA using either 75 or 90 mg U.S. RDA, based on the weight ratio between Japanese and U.S. women (51 kg/61 kg), amounts of 66 mg and 79 mg are obtained, respectively. We can not conclude at this time which U.S. RDA is correct for U.S. and Canadian women, but we believe that an AA intake of 66–79 mg/day is sufficient to provide antioxidant protection for young Japanese women. This conclusion is supported by the finding from the ROC curve analysis that the 75 mg/day intake (corresponding to the present U.S. RDA) was most effective as a cutoff point for young Japanese women to produce a serum AA concentration above the lower reference limit (7.0 mg/L). The 100 mg/day dosage may be unnecessarily high as an RDA for young Japanese women. For the U.S. RDA, it is assumed that women have a lower AA requirement than men due to their smaller lean-body mass, total body water, and body size, and thus women maintain higher plasma AA concentrations than men at an equal AA intake (1). Plasma AA concentrations in Japanese given 100 mg/day of AA for 7 days were reported for young men (20.4±1.26 years old; 61.4±7.53 kg) and women (20.5±1.11 years old; 57.3±3.45 kg) to be 10.9±1.8 mg/L and 11.8±2.5 mg/L, respectively (11). Since the Japanese RDA of AA for men and women is set to a similar value (2), this requires further investigation.

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資　料

離乳前乳児の哺乳量に関する研究

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Milk Intake by Breast-fed Infants before Weaning

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A questionnaire survey was conducted on the milk intake by 77 breast-fed infants born during the two years from January 2002 to December 2003.

Individual information on each infant and mother (weight, health condition, etc.), the amount of milk breast-fed each day, and the amount of liquids other than breast milk taken each day were recorded.

The intake data were recorded from the age of one month old to 5 months old. The intake of milk in a day varied from case to case. Although a relationship emerged between the intake of breast milk and the increase in body weight during the early months, this relationship was not maintained as the infants grew.

The mean intake of breast milk per day was 777.8m*l*, this being higher than the figure of 750m*l* recommended before weaning in the *6-th Revised Edition of the Japanese* Nutrition Intake.

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Key words : breast milk, Japanese Nutrition Intake, infant milk intake

緒　言

母乳は，乳児にとって最適な栄養成分を，最適な割合で含むものである。母乳育児は乳児に対して栄養的機能面だけではなく，母子間の心理面においても有益であることが明らかにされている[1]。また，先進国と途上国を問わず国際的な母乳育児推進の動きも盛んである[2]。乳児期における母乳の摂取量は，乳児の栄養状態を判定する上で重要な指標であり，「日本人の栄養所要量」においてもその値は乳児期のエネルギーやビタミン摂取量等を算出する際に利用されている。

「日本人の栄養所要量」は，1975年以来5年毎に改定が行われていることは既によく知られているが，平成12年からは第6次改定による内容が使用されている。この第6次改定では，授乳婦の1日当たりの授乳量，つまり乳児の母乳哺乳量に大幅な変更が施された。すなわち，第5次改定までは1日当たり850m*l*とされていたが，第6次改定で750m*l*とされ，母乳哺乳量が約12%減となった。そして，乳児の栄養所要量もこの母乳哺乳量で算出された数値が表示された。

近年，日本人を対象に月齢を追った乳児母乳哺乳量についての報告は，調査対象数が十数例と少なく，100例近いものはほとんどない状態である。本論文では乳児母乳哺乳量についての最新情報を得るために，100例程度の対象を予定して検討を行ったので報告する。

方　法

1．調査対象

平成14年2月4日に昭和女子大学倫理委員会の審査を完了した上で，協力病院を通して被験者を募集した。応募者113名には本研究の主旨と方法を文書で説明した。インフォームド・コンセントが得られた応募者の中で，母乳哺育を選択した母親とその児89名のうち，各満月齢日から1週間前後間に測定ができた77名（男児43名，女児34名）を調査対象とした。対象者はすべて単胎児である。在胎期間は全児37週以上，妊婦の妊娠経過は概ね正常で，軽度の妊娠中毒症，貧血が数名見られた以外に顕著な異常はなかった。対象者の在住地域は静岡県45名，大阪府9名，東京都8名，愛知県6名，長野県4名，和歌山県2名，神奈川県2名，兵庫県1名であった。

キーワード：母乳，日本人の栄養所要量，哺乳量
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2. 調査方法

調査は満月齢1～5ヶ月の期間とし，1）母子の個人情報（対象母子の健康状態等），2）乳児の1日の母乳哺乳量，3）乳児が母乳以外に摂取した水分量，について，郵送での紙面調査を行った。

母乳哺乳量調査は，月に1回とし合計5回で，生後1～5ヶ月齢までの各満月齢日から1週間前後の間に重量計量法で測定した。満月齢日の母乳哺乳量を当該月の母乳哺乳量として記録した。なお，今回の調査では，出生後1～5ヶ月齢までの期間に連続した調査を原則として行ったが，被験者ならびにその母親の都合により一部の測定値に欠損を生じた場合があった。その結果，各月齢群間に標本数のばらつきを生じた。

母乳哺乳量の測定日は，当日午前0時から24時間すべての授乳について授乳前後に乳児の体重を計量し，その体重の差の合算により算出した値を母乳哺乳量とした。体重計測には，全調査対象にタニタデジタルベビースケール「すこやか」（0～5kgは5g，5～10kgは10g単位の精度）を無償貸与し，測定精度の安定化に配慮した。

乳児が母乳以外に摂取した水分量は，全調査対象に対して当方が用意した同一社製のベビースプーンにより計量することを依頼した。

3. 統計処理

統計処理はMicrosoft Excelを用いて行った。統計学的検討は，乳児の月齢数・母乳哺乳量・体重を要因として，t検定および相関係数の検討にて行った。

結　　果

1. 乳児の身体状況

調査対象児の月齢別身長・体重の平均値を表1に示した。また，厚生労働省による平成12年乳幼児身体発育調査の値を使い，各満月齢における身長・体重のパーセンタイル値のグラフを作成し，このグラフから各児の満月齢におけるパーセンタイル値を読み取った。この際，米山[3]が行った方法と同様に各満月齢時の値になるように補正を行った。このグラフの各月齢における50パーセンタイル値を全国平均として表1に併記した。

身長は，男児女児とも本調査平均と全国平均はほぼ近似値であった。体重は，女児は出生時及び満4ヶ月齢以外は全国平均よりも軽く，男児は満5ヶ月齢以外は全国平均よりも重かった。

2. 母乳哺乳量について

（1） 乳児の1日平均母乳哺乳量

各月齢別の乳児の1日平均母乳哺乳量を図1に示した。この結果から満1～5ヶ月齢までの平均母乳哺乳量は791gであった。各月齢群間のt検定による有意差は見られなかった。

表1　調査対象児の月齢別身体状況

月齢		身長（cm）		体重（g）	
		男	女	男	女
出生時	平均	48.8	48.2	3,052	2,982
	標準偏差	2	2	345	311
	全国平均	49.0	48.5	3,000	2,950
1ヶ月齢	平均	54.2	53.2	4,482	3,848
	標準偏差	2	2	771	223
	全国平均	54.0	52.6	4,240	4,010
2ヶ月齢	平均	58.4	55.9	5,574	4,912
	標準偏差	2	3	680	630
	全国平均	58.1	56.6	5,480	5,160
3ヶ月齢	平均	61.4	60.0	6,541	5,817
	標準偏差	2	3	881	637
	全国平均	61.4	60	6,370	5,910
4ヶ月齢	平均	63.9	62.2	7,147	6,762
	標準偏差	2	3	810	886
	全国平均	64.2	62.6	7,070	6,510
5ヶ月齢	平均	66.1	64.1	7,293	6,969
	標準偏差	2	3	556	913
	全国平均	66.2	64.6	7,580	6,970

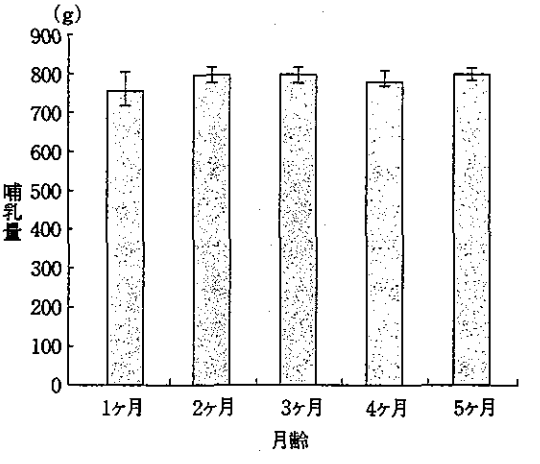

図1　乳児の1日平均母乳哺乳量

（2） 各乳児の月齢別母乳哺乳量の推移

図2に各乳児の月齢別母乳哺乳量の推移をパターンごとに示した。パターン1では，月齢1～5ヶ月の間に母乳哺乳量が増え続けた乳児，パターン2では，同期間中に最多の母乳哺乳量を記録し，その後母乳哺乳量が減った乳児の母乳哺乳量の推移を示した。パターン3では，母乳哺乳量が同期間中に減少した後に再び増えた乳児の母乳哺乳量の推移を示した。

3. 母乳哺乳量と体重の関係

各月齢時における母乳哺乳量と前1ヶ月間体重増加量との関係を図3-1～3に示した。満1・2ヶ月齢では強

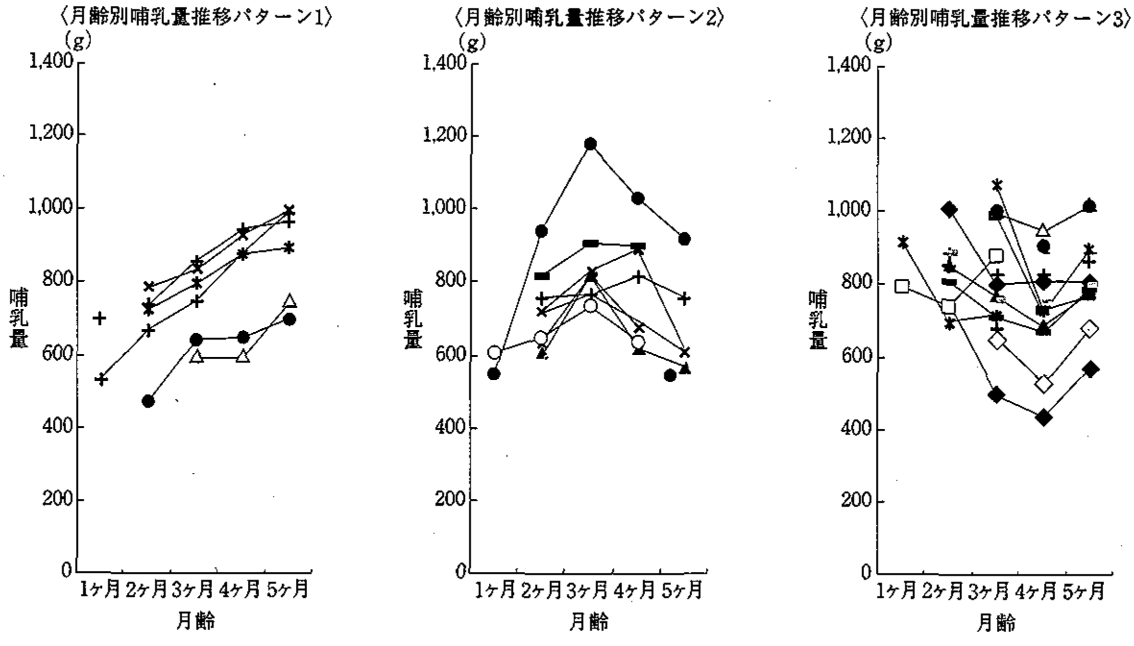

図2　月齢別母乳哺乳量の推移

い相関が見られ（$r=0.720$・0.667），満3ヶ月齢ではやや相関が見られ（$r=0.487$），満4・5ヶ月齢では相関は見られなかった。

考　　察

対象となった乳児の体位と平成12年厚生労働省幼児身体発育調査結果（以下，全国平均と略す）との比較は表1に示すようにほぼ同値となった。しかし，体重において各児のパーセンタイル値を見ると，月齢を経るにつれ，男児女児ともパーセンタイル値が小さくなる傾向が見られた。母乳栄養児の体位は，人工栄養児と母乳栄養児のデータで作成されている全国平均よりも低い値になることは知られており[4]，本調査におけるこの結果も，母乳栄養児の体位の特徴のためと考えられた。また，1〜5ヶ月齢の体重のパーセンタイル値の変動と出生時体重のパーセンタイル値との関連について検討したが，この2群間において明確な差異は認められなかった。

本調査結果では1日の平均母乳哺乳量は791gとなったが，この数値を五訂食品成分表に記載されている人乳の比重1.017で補正換算すると，満1〜5ヶ月齢の平均母乳哺乳量は777.8mlとなった。この値は米山[3]の結果よりも高値となったが，小林[5]の値，金[6]の値，Ferrisら[7]の値とほぼ同じである。現行の第6次改定日本人の栄養所要量において乳児，授乳婦の食事摂取基準を算定する際に使われている1日平均母乳哺乳量750mlは，本調査の1日の平均母乳哺乳量を満たしていなかった。日本人の栄養所要量の，乳児，授乳婦の食事摂取基準値は，乳児の母乳哺乳量を基準にして所要量を決めている栄養素があり，この1日平均母乳哺乳量の値により左右される。したがって，1日平均母乳哺乳量750mlは，多少低い数値なのではないかと考えた。なお，乳児が経口摂取したものとして，離乳食や補水を目的とする水分がある。それらの量については，方法の項に示したように統一したベビースプーンを用いて計量を依頼した。

その結果は1〜20杯（0.5〜10ml）であり，各対象者間での内容に差異が見られた。この値は1日哺乳量に相対すると最大でも5%程度の変化量であり，1日哺乳量に大きな影響を与えないと判断して，母乳哺乳量への補水量等の補正は加えないこととした。

母乳栄養児における1日母乳哺乳量のうち満1〜2ヶ月齢の値が高くなるという報告[5,8〜10]が見られるので，1日平均母乳哺乳量の各月齢群間の有意差を，t検定を用いて検討したが，本調査ではこのような傾向は確認できなかった。また，母乳哺乳量の月齢に伴う推移について他の特徴を検討してみた。各乳児の月齢を追った母乳哺乳量の推移は，いくつかにパターン化すると，図2に示す内容となった。すなわち，各被験者の微細な差異が見られるものの，それらの一部を抽出し，代表的な内容として3群にとりまとめることとした。この結果から，月齢に伴う一定の変動傾向は見られないと結論づけた。米山[3]も母乳栄養児において月齢1〜7ヶ月では月齢に伴う一定の変動傾向は見られないとの報告をしている。

各月齢時における母乳哺乳量と前1ヶ月間体重増加量との関係（図3）は，月齢に伴い相関関係がなくなった。これは，月齢が経つにつれて母乳哺乳量と1ヶ月体重増加量との正の関係が薄れていることを意味している。この現象は，母乳哺乳によるエネルギーは月齢が経つにつれて，主に身体の成長のみならず基礎代謝のためのエネ

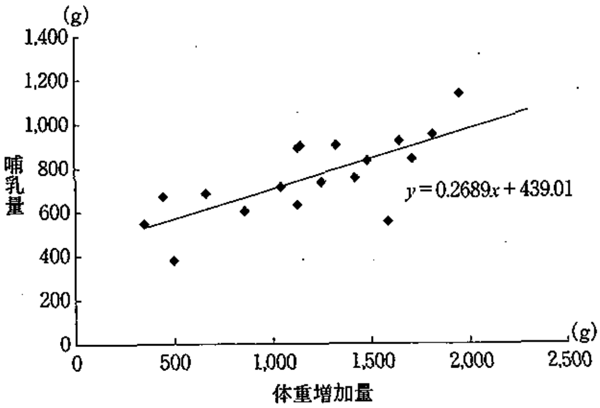

図3－1　月齢1ヶ月時における母乳哺乳量と前1ヶ月間体重増加量との関係

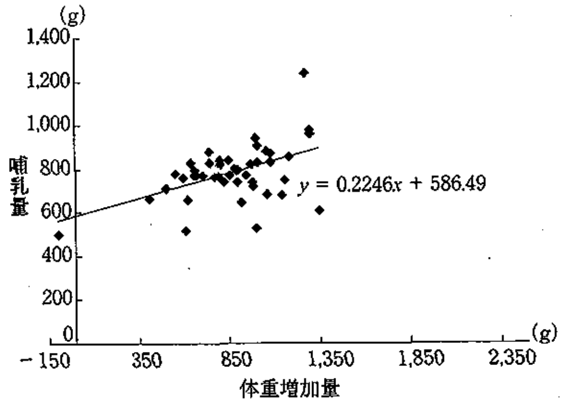

図3－2　月齢3ヶ月時における母乳哺乳量と前1ヶ月間体重増加量との関係

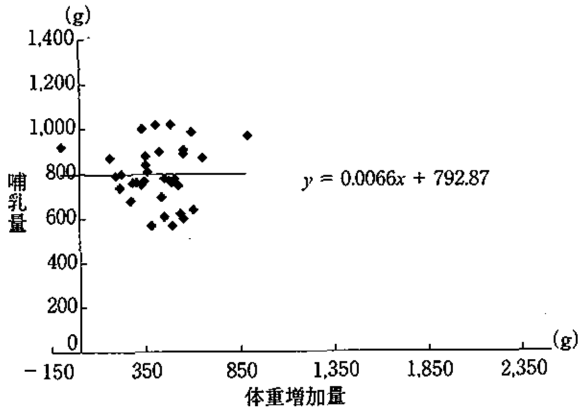

図3－3　月齢5ヶ月時における母乳哺乳量と前1ヶ月間体重増加量との関係

ルギーに注がれ[11]，また，月齢が進むと児の活動が活発になるため，身体活動のエネルギーにも多く使われるためと考えられた。

以上のことから，乳児の1日平均母乳哺乳量は約780mlが妥当と判断した。

ま　と　め

母乳哺育児77例につき，1日の母乳哺乳量および体重の変化について，離乳期前までの期間の各月齢（1～5ヶ月）に調査を行った。離乳期前の乳児における1日の平均母乳哺乳量は777.8mlとなり，現行の第6次改定日本人の栄養所要量において乳児，授乳婦の食事摂取基準を算定する際に使われている1日の平均母乳哺乳量750mlよりもやや多い結果となった。この結果から，離乳期前の乳児における1日の平均母乳哺乳量は780mlが妥当とする判断に至った。

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Underreporting of energy intake among Japanese women aged 18–20 years and its association with reported nutrient and food group intakes

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Abstract

Objectives: To evaluate the ratio of energy intake to basal metabolic rate (EI/BMR) among young female Japanese adults, and to compare the lifestyle and dietary characteristics between relatively low and high reporters.
Design: Dietary intakes were assessed over a 1-month period with a validated, self-administered, diet history questionnaire, and lifestyle variables were assessed by a second questionnaire designed for this survey. The ratio of EI/BMR was calculated from reported energy intake and estimated basal metabolic rate.
Subjects: In total, 1889 female Japanese university students aged 18–20 years who were enrolled in dietetics courses.
Results: Ninety-five per cent of the subjects were classified into a non-obese group (body mass index (BMI) $<25\,kg\,m^{-2}$; mean ± standard deviation (SD): $20.8 \pm 2.6\,kg\,m^{-2}$). EI/BMR was 1.43 ± 0.40 (mean ± SD). Sixty-eight per cent of the subjects showed an EI/BMR level below the possibly balanced value of 1.56, 37% showed EI/BMR below the minimum survival value of 1.27 and 2% of the subjects showed EI/BMR exceeding the maximum value for a sustainable lifestyle of 2.4. BMI, body weight and BMR decreased significantly with the increase in EI/BMR ($P < 0.001$). The percentage of energy from carbohydrate was significantly higher, whereas those from fat and protein were significantly lower, among the lower EI/BMR groups. As for food groups, a significantly declining trend from the lowest to the highest EI/BMR groups was observed for cereals.
Conclusion: Underreporting, rather than overreporting, of energy intake was predominant in this relatively lean Japanese female population. BMI was the most important factor affecting the reporting accuracy of energy intake.

Keywords
Dietary questionnaire
Underreporting
Energy intake
Japanese women
Epidemiology

An accurate assessment of habitual dietary intake is very important in determining the association between diet and disease. Several dietary assessment methods have been developed, validated and used in dietary surveys. However, any method used to assess self-reported dietary intake is not entirely able to avoid reporting errors[1]. Most dietary surveys may include not only random errors but also systematic errors, such as the misreporting of true intake by certain subject groups[2,3].

In the 1980s, the development of the doubly labelled water technique, which measured the total energy expenditure of subjects in free-living situations[4,5], made it possible to validate reported energy intake as an external biomarker[6–8]. However, the high cost of the technique has restricted its use to relatively small-scale studies. As an alternative approach to detect misreporting of energy intake, Goldberg *et al.*[9] introduced the ratio of reported energy intake to basal metabolic rate (EI/BMR). Many investigators who have used the Goldberg cut-off value to identify underreporters[10] have indicated that reporting errors have been associated with subject characteristics[3]. However, almost all studies on this issue were conducted in Western countries such as in Europe[11–14], the USA[15] and Australia[16]. No studies have been performed in Asian countries except one dealing with pregnant Indonesian women[17].

The purpose of the present study was to evaluate EI/BMR values in order to examine the prevalence of misreporting of energy intake in female Japanese students and the relationship between reported energy intake and body mass index (BMI) and nutrient intakes.

Subjects and methods

Subjects

The subjects were freshmen who were enrolled in dietetics courses at 22 colleges and technical schools in Japan in April 1997 ($n = 2069$). All the questionnaires were

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distributed between 7 and 21 April 1997. A total of 2063 students (2017 women and 46 men) returned the answered questionnaires within 1 week (response rate of 99.7%). Faculty members of each school checked the submitted questionnaires. When missing replies and/or errors were found, the subjects were requested to answer the questions again. All questionnaires were checked at least once by local staff and once by staff of the study centre. The entire survey was completed before the end of May.

Assessment of dietary habits

We used a self-administered diet history questionnaire (DHQ). The DHQ is a validated, 16-page questionnaire assessing dietary habits in the previous month. Intakes of 147 food items, 16 nutrients and total energy intake were calculated using an *ad hoc* computer algorithm developed to analyse the questionnaire. More detailed descriptions of the questionnaire, methods of calculating nutrients and the validity are given elsewhere[18,19]. The 147 foods from the DHQ were grouped into 17 food groups, mainly according to the food composition tables of Japanese foods, 4th revised edition[20]. In this study, sugar, nuts, and mushrooms and sea vegetables were categorised into confectioneries, pulses and vegetables, respectively, because the mean intakes of these items were much lower than those of other food groups.

Assessment of lifestyle variables

Lifestyle variables were obtained from the 4-page questionnaire designed for this survey. It included the frequency of sports club activity and smoking habits. The physical activity level was assessed by the monthly frequency of sports club activity only, without inquiring into the types of sport, their intensity or duration. The subjects who engaged in sports club activity at least once per week in the previous month were defined as 'physically active' and the others as 'sedentary'. Smoking habits were divided into three categories: never, former and current smokers. Data on birth date, and self-reported body weight and height – to the nearest kg and cm, respectively, were obtained from the DHQ. BMI was calculated as body weight (kg) divided by the square of body height (m^2). We classified BMI into three categories according to the Japan Society for the Study of Obesity[21]: $<18.5\,kg\,m^{-2}$, $18.5–25\,kg\,m^{-2}$ and $\geq 25\,kg\,m^{-2}$ as 'lean', 'normal' and 'obese', respectively.

Estimation of BMR

BMR was estimated for each subject using the formula for women aged 18–30 years based on body weight, given by the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU)[22] as follows:

$$\text{Estimated BMR (MJ day}^{-1}) = 0.0615 \times \text{body weight (kg)} + 2.08.$$

Statistical analysis

For the purpose of statistical analysis we selected only women who completed the questionnaires ($n = 2017$), and we included 1889 subjects (93.7%) who satisfied the following three criteria in the analysis:

1. Those aged 18–20 years on the surveyed day ($n = 1960$);
2. Those with information on sports club activity and smoking habits ($n = 1988$); and
3. Those with reported energy intake of more than or equal to half of the energy requirement of the lowest physical activity category and less than 1.5 times the energy requirement of the highest physical activity category[23], i.e. the subjects with reported energy intake of 3.0–14.4 MJ day^{-1} ($n = 1980$).

We calculated the EI/BMR ratio to evaluate the validity of energy intake. To compare the relative degree of under- and overreporting, we temporarily used the values defined by FAO/WHO/UNU[22]: the minimum survival level of 1.27, the sedentary level for women of 1.56, and the maximum sustainable lifestyle level of 2.0–2.4. We classified the subjects into quintiles of EI/BMR. Distribution of anthropometric and dietary variables across quintiles of EI/BMR was evaluated by calculating the means of these variables for each quintile.

Nutrient intakes were energy-adjusted using the energy density model, i.e. the percentage of energy intake for macronutrients and g/mg/μg per 10 MJ energy intake for micronutrients and food groups. The results are given only with the adjustment for sports club activity, because other variables such as smoking and alcohol drinking habits were not statistically different across quintiles of EI/BMR.

We tested the differences across quintiles of EI/BMR by using the PROC GLM procedure with the LSMEANS statement. The chi-square test was used to test for proportionate differences between categories. All statistical analyses were performed using version 8.2 of the SAS software package (SAS Institute, Inc., Cary, NC, USA). A P-value of <0.05 was considered significant.

Results

The characteristics of the subjects are shown in Table 1. BMI for all subjects was $20.8 \pm 2.6\,kg\,m^{-2}$ (mean ± standard deviation (SD)). Ninety-five per cent of the subjects were classified into a non-obese group (BMI $<25\,kg\,m^{-2}$). Energy intake was 7.5 ± 2.0 MJ day^{-1} (mean ± SD). The frequency of sports club activity was 1.7 ± 4.1 days per month (mean ± SD). Eighty-eight per cent of the subjects participated in sports club activity

Table 1 Characteristics of the subjects ($n = 1889$). Values are expressed as mean ± standard deviation, unless specified otherwise

Age (years)	18.1 ± 0.4
Body weight (kg)	51.8 ± 7.3
Body height (cm)	157.9 ± 5.2
Reported EI (MJ day^{-1})	7.5 ± 2.0
BMR (MJ day^{-1})*	5.3 ± 0.5
EI/BMR	1.43 ± 0.40
BMI (kg m^{-2})	20.8 ± 2.6
< 18.5 (%)	16
18.5–25.0 (%)	79
≥ 25.0 (%)	5
Sports club activity (days/month)	1.7 ± 4.1
Sedentary (%)	88
Active (%)†	12
Smoking habits (%)	
Current	3
Former	3
Never	94
Alcohol drinking habits (%)	
Non-drinker	80
Drinker	20

EI – energy intake; BMR – basal metabolic rate; BMI – body mass index.
*BMR was calculated by the Food and Agriculture Organization/World Health Organization/United Nations University formula (1985)[22].
†Subjects who participated in sports club activity at least once per week were defined as 'active'.

less than once per week during the previous month. Regarding smoking habits, most of the subjects (97%) were current non-smokers. Eighty per cent were non-drinkers. EI/BMR for all subjects was 1.43 ± 0.40 (mean ± SD). Figure 1 shows the distribution of EI/BMR values. The distribution is slightly skewed to the right. Some 68% and 37% of subjects showed lower EI/BMR when we compared EI/BMR with the possibly balanced value of 1.56 and the minimum survival level of 1.27[22], respectively. On the other hand, 2% of the subjects showed EI/BMR exceeding the maximum value for a sustainable lifestyle of 2.4.

Table 2 shows mean values of body weight and height, BMI, BMR and EI by quintile of EI/BMR. A significant declining trend from the lowest to the highest quintile of EI/BMR was observed for body weight, BMI and BMR. As for sports club activity, the proportion of the physically active group increased slightly with increasing EI/BMR. The percentage of current smokers and alcohol drinkers was not statistically different between quintiles of EI/BMR.

Table 3 presents mean energy and nutrient intakes by quintile of EI/BMR. Mean fat intake expressed as a percentage of total energy increased with increasing EI/BMR. A similar tendency was seen for saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. On the other hand, the energy intake derived from carbohydrate decreased with increasing EI/BMR. Vitamin C did not correlate significantly with EI/BMR.

Table 4 presents the mean intakes of food groups by quintile of EI/BMR. When intake was expressed per 10 MJ of energy intake, a significant declining trend from the lowest to the highest quintile of EI/BMR was seen for cereals. A significantly positive correlation was observed for confectioneries, fats and oil, fish, and meats. As for pulses and non-sugar containing soft drinks, neither correlated significantly with EI/BMR.

Discussion

This is the first study to report an inverse relationship between BMI and EI/BMR among young Japanese women. Some previous papers reported that obese subjects in Western countries tended to underreport their energy intake[2,3,24]. Despite the fact that the subjects of the present study were relatively lean, 37% of them showed an EI/BMR level below the minimum survival value of 1.27, whereas 2% of the subjects showed EI/BMR exceeding the maximum value for a sustainable lifestyle of 2.4. In the six previous studies dealing with adult populations with cut-off values for EI/BMR from < 1.20 to < 1.28, the mean ratio of underreporters was 40%[3], which was similar to the rate of possible underreporters in this female Japanese population. This indicates that they tended to underreport,

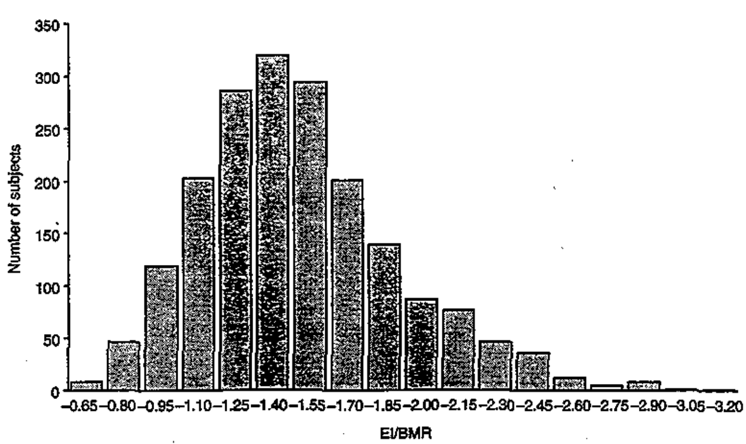

Fig. 1 Distribution of the ratio of energy intake to basal metabolic rate (EI/BMR). Values on horizontal axis show the upper limit of each range ($n = 1889$)