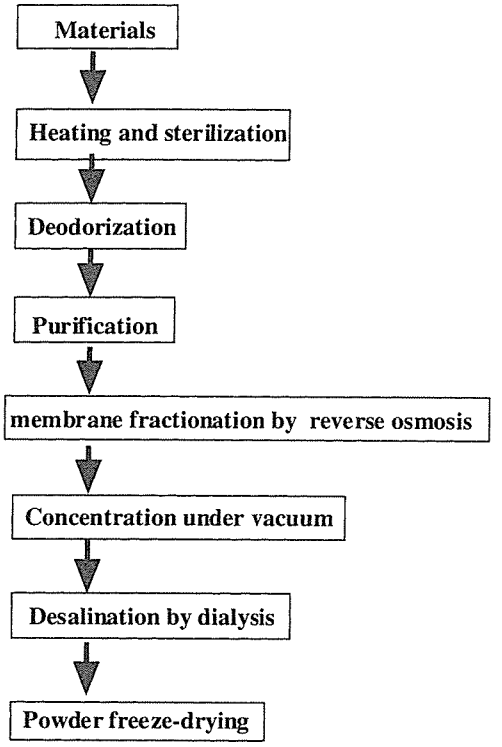

Fig. 1. Flow chart for making the bonito powder high in nicotinamide content

Table 1. Major components of the bonito power high in nicotinamide content

	%
Water	0.79
Total nitrogen	14.37
Lipid	0.12
Ash	14.18
NaCl	0.08

含まれるナイアシンの生物有効性について検討した．

さらに，ニコチンアミドには糖尿病を予防する作用があること（ニコチン酸にはこの作用はない）が実験用小動物で証明されている[10]．そこで，本実験では，カツオパウダーをラットに摂取させ，STZ 誘発性糖尿病を予防できるか否かについても検討した．

Table 2. Amino acid composition of the bonito powder high in nicotinamide content

Amino acid	mg/100 g
Phosphoserine	74
Taurine	531
Aspartic acid	318
Hydroxyproline	140
Threonine	409
Serine	498
Glutamic acid	942
Sarcosine (N-methylglycine)	348
α-Aminoadipic acid	0
Proline	735
Glycine	2,425
Alanine	1,604
α-Aminobutyric acid	15
Valine	561
Cystine	19
Methionine	369
Isoleucine	374
Leucine	831
Thyrosine	385
Phenylalanine	469
β-Alanine	1,177
γ-Aminobutyric acid	15
Histidine	19,593
1-Methylhistidine	2,480
Hydroxylysine	30
Ornithine	92
Lysine	1,353
Arginine	5,234
Total	41,021

2. 実験方法

(1) ナイアシン高含有カツオパウダー製造方法の概略

カツオ節製造時に副産する煮汁を出発物質として，Fig. 1 に示した製造フローに従って製造した．Table 1 に主な成分含量を，Table 2 にアミノ酸含量を示した．

(2) 第一実験（ヒトを用いた生物有効性を求めるための実験）

1) 被 験 者

21～23 歳の健康な女性 8 名（身長，159.5±1.2 cm；体重，53.0±0.8 kg）を対象とした．厚生労働省が示

Table 3. Composition of the control diet

	(g/day)	Remarks
Vitamin-free milk casein	22.6	The casein contained 87.5% protein, so the net protein amount was 19.8 g. The tryptophan content was 1.3%, so 257 mg of tryptophan was supplied.
Gluten	43.2	The gluten contained 81.6% protein, so the net protein amount was 35.3 g. The tryptophan content was 1.1%, so 388 mg of tryptophan was supplied.
		Total protein=55 g
		Total tryptophan=645 mg
Cornstarch	250	
Sucrose	50	**Total carbohydrate=300 g**
Fat		**Total fat=46 g**
Soybean oil	10.6	
Rapeseed oil	16.0	
Coconut oil	6.1	
Lard	13.3	
Dietary fiber		Soluble dietary fiber, Fibersol, was obtained from Matsutani Chemical
Soluble	3.6	Industry Co. Ltd. (Osaka, Japan), and insoluble dietary fiber, Ramie, was ob-
Insoluble	14.4	tained from Tosco Co., Ltd. (Tokyo, Japan).
Mineral mixture	13.8	The composition is shown below.
Total amount (g)	425.6	
Total energy (kcal)	1,834	

For breakfast and supper, 128 g of the diet was added to 90 ml of water, mixed well, and baked for 9 min at 250℃. The weight of the baked meal was *ca.* 175 g (170–180 g). The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 170 g of the diet was added to 120 ml of water, mixed well, and was baked for 10 min at 250℃. The weight of the baked meal was *ca.* 233 g (225–240 g). The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects. Composition of the mineral mixture: 1,100 mg of $CaHPO_4 \cdot 2H_2O$, 860 mg of $CaCO_3$, 2,200 mg of KH_2PO_4, 3,500 mg of $KHCO_3$, 2,100 mg of $MgCl_2 \cdot 2H_2O$, 60 mg of $FeSO_4 \cdot 5H_2O$, 13 mg of $MnSO_4 \cdot 5H_2O$, 19 mg of $ZnCl_2$, 6.3 mg of $CuSO_4 \cdot 5H_2O$, 0.2 mg of KI, and 4,000 mg of NaCl. Composition of the vitamin mixture: 3.6 mg (1,800 IU) of retinal acetate reagent (500,000 IU/g), 2.5 μg of cholecalciferol, 5.1 mg of *dl*-α-tocopherol (4.6 mg was supplied from oils), 16 μg of phylloquinone (39 μg was supplied from oils), 0.9 mg of thiamin-HCl, 1.0 mg of riboflavin, 1.5 mg of pyridoxine-HCl, 2.4 μg of cyanocobalamin, 5.5 mg of calcium panthotenate, 200 μg of pteroylmonoglutamic acid, 30 μg of D(+)-biotin, and 100 mg of ascorbic acid, made up to 1 g with sucrose.

す食事摂取基準[11]（生活活動強度Ⅱの18～29歳女性，約1,800 kcal/day，タンパク質55 g/day，炭水化物300 g/day，脂質46 g/day）を満たす食事（Table 3）を4日間与えた．食事中にはビタミン体のナイアシンは0であるが，トリプトファンが645 mg含まれ，この量はナイアシン当量として，約11 mgである．被験者は，一定の生活スケジュール（起床，6時；朝食，7時30分；昼食，12時30分；夕食，18時30分；就寝，11時）に従って行動させた．飲料については，市販の水を自由摂取させた．実験開始日をday 1とした．day 3に5 gのカツオパウダー（ナイアシン17 mgを含む）を朝食後，昼食後，夕食後の計3回，食事終了後，別途摂取させた．カツオパウダー由来のナイアシン摂取量は51 mg，食事由来のナイアシン当量摂取量は11 mgであった．起床後2回目から翌日起床直後までの尿を24時間尿とし，実験期間中4日分の24時間尿を採集した．

2) 尿中のニコチンアミド代謝産物の測定

N^1-メチルニコチンアミド（MNA）については，尿中のMNAをアセトフェノンと縮合させることにより蛍光物質に変換し，HPLCを用いて定量した[12]．

N^1-メチル-2-ピリドン-5-カルボキサミド（2-Py）およびN^1-メチル-4-ピリドン-3-カルボキサミド（4-Py）については，尿に炭酸カリウムを飽和量加えた後，ジエチルエーテルで抽出し，乾固させた抽出物を水に溶解させた．HPLCを用いてこの溶解物を定量し

Table 4. Composition of the diets

	Control diet (NiA-free 22% casein) (%)	Test diet (control diet+bonito powder) (%)
Vitamin-free casein	22	20
L-Methionine	0.2	0.2
Gelatinized cornstarch	44.5	42.5
Sucrose	22.3	21.3
Corn oil	5	5
Mineral mixture	5	5
Vitamin mixture*	1	1
Bonito powder	0	5

*The vitamin mixture was niacin-free.

た[13].

3) カツオパウダー中のニコチンアミド量の測定

カツオパウダー1gに水1lを加え，室温で10分間撹拌した溶液をニコチンアミド測定用試料とした．試料に炭酸カリウムを飽和量加えた後，ジエチルエーテルで抽出し，乾固させた抽出物を水に溶解させた．HPLCを用いて定量を行った[13]．その結果，本実験に使用したカツオパウダー中のニコチンアミド量は3.4 mg/gであった．この含量（3.4 mg/g）は五訂に本食品標準成分表に記載されているカツオ節中のナイアシン含量の約7.5倍であった．

(3) 第二実験（STZ-誘発糖尿病ラットに対する影響）

本実験は滋賀県立大学動物実験委員会の承認を受けたものである．

1) 動物の飼育方法

5週齢のWistar系雄ラット20匹を日本クレア㈱より購入し，体重が均等になるよう10匹ずつ2群に分けた．試験食群として，20%カゼイン食に5%となるようカツオパウダー（ナイアシン含量340 mg/100 gカツオパウダー）を添加したカツオ食群（ナイアシン含量；17 mg/100 g飼料）を設定した（Table 4）．対照群として，カツオ食中のアミノ酸含量と等しくなるよう22%カゼイン食群を設定した（Table 4）．ラットは1匹ずつ金網ケージにて29日間飼育し，飼料と水は自由摂取とした．動物室の温度は20℃前後，湿度は50%前後，明暗サイクルは6時から18時を明，18時から6時までを暗とした．飼育開始日をday 0とした．

Day 8の午前9時に対照群，試験食群をそれぞれ5匹ずつ，対照食-生理食塩水群及び対照食-STZ群，試験食-生理食塩水群及び試験食-STZ群の4群に分けた．対照食-STZ群及び試験食-STZ群には，生理食塩水0.5 mlに70 mg/kg body weightとなるように溶解させたストレプトゾトシン（STZ）を腹腔内注射した．また，対照食-生理食塩水群及び試験食-生理食塩水群には生理食塩水0.5 mlを腹腔内注射した．

Day 28の9時より6時間絶食し，15時に尾静脈から採血した．小型血糖測定機グルテストエース（㈱三和化学研究所）を用いて空腹時血糖を測定した．

Day 29にラットを断頭屠殺し，直ちに頸動脈血を採取し，血中NAD含量及び血中NADP含量を測定した．また，肝臓を摘出し，肝NAD含量，肝NADP含量及び肝α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase（ACMSD）活性を測定した[14].

2) NAD・NADPの定量方法[15]~[18]

i) 測定機器

測定に使用したマイクロプレート用吸光測定装置はLabsystemsのMultiskan Ascent（Thermo Bioanalysis Company, FIN-00811 Helsinki, Finland. 輸入元：サーモバイオアナリシスジャパン株式会社．販売元：大日本製薬株式会社　ラボラトリープロダクツ部）を使用し，570 nmのフィルターを使用した．

マイクロプレートタイターは住友ベークライト㈱のELISA用プレートSを使用した．

ii) 抽出方法

血液及び肝臓からのNAD・NADP測定用試料の作製方法はShibataら[15]が開発した0.1 Mニコチンアミドを含む50 mMリン酸カリウム緩衝液，pH 6.0を用

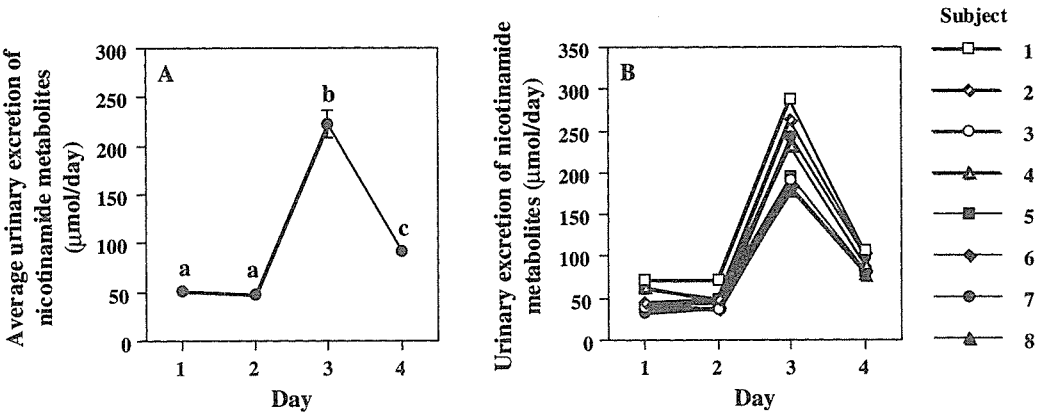

Fig. 2. Average urinary excretion (A) and individual excretion (B) of nicotinamide metabolites (Experiment 1)

Each value is the mean±SEM ($n=8$) (A). A different superscript letter means significant difference at $p<0.05$ as determined by paired ANOVA with post hoc testing with Tukey's multiple-comparison test.

いる熱抽出法で行った.

iii) 測定方法

NAD・NADP の測定方法は，柴田ら[15)~18)]が報告した酵素サイクリング法に従って行った.

3. 結　果

(1) 第一実験

ヒトにおいてナイアシンは MNA，2-Py，4-Py のいずれかの化合物として尿中に排泄されることから，MNA，2-Py，4-Py の合計を総ニコチンアミド代謝産物量とし，カツオパウダーに含まれるナイアシンがどの程度利用されるのか調べた．規定の食事を与えると尿中の総ニコチンアミド代謝産物量は day 1 では 53±5 μmol/day，day 2 では 47±4 μmol/day であったが，カツオパウダーを服用した day 3 では 222±14 μmol/day に増加した（Fig. 2A）．翌日の day 4 でも 93±4 μmol/day と，投与前の値よりも高い値を示した．この現象は被験者 8 名全員に共通していた（Fig. 2B）．Day 1 および day 2 の平均尿中総ニコチンアミド代謝産物量は 49±4 μmol/day（day 1 および day 2 のナイアシン当量摂取量は約 11 mg，すなわち約 90 μmol である．したがって，摂取量に対するニコチンアミド異化代謝産物の排泄量比は 54%である）であったことから，カツオパウダーの摂取により増大した量は 217 μmol/day（222+93−49−49=）と推定した．この値はカツオパウダー 15 g 中に含まれるナイアシン 51 mg（418 μmol）の 52%であった.

(2) 第二実験

1) 飼料摂取量と体重増加量

飼料摂取量と体重の変化量を各々 Fig. 3A と Fig. 3B に示した．Day 8 までの予備飼育期間において，対照食群と試験食群との間に飼料摂取量及び体重増加量の違いは認められなかった．対照食-STZ 群及び試験食-STZ 群の飼料摂取量は STZ 投与直後に減少したが，day 11 より増加しはじめ，積算飼料摂取量は対照食-生理食塩水群及び試験食-生理食塩水群の約 1.5 倍となった．しかし，対照食-STZ 群及び試験食-STZ 群の体重増加量は，対照食-生理食塩水群及び試験食-生理食塩水群より低く，30～40%であった．飼料摂取量及び体重増加量において，対照食-生理食塩水群と試験食-生理食塩水群間に，また対照食-STZ 群及び試験食-STZ 群間に有意な差異は認められなかった.

2) 血 糖 値

対照食-STZ 群及び試験食-STZ 群の血糖値は対照食-生理食塩水群及び試験食-生理食塩水群間に比べ，著しく高い値となった（Table 5）．また，対照食-STZ 群と試験食-STZ 群間の血糖値に差は認められなかった.

3) 血中 NAD・NADP 含量

ナイアシン摂取量が必要量を満たさない場合には血中 NAD 含量は低くなり，必要量に達すると飽和値を示すことから[19)]，血中 NAD 含量はナイアシン栄養状態を反映する．対照食-生理食塩水群，試験食-生理食

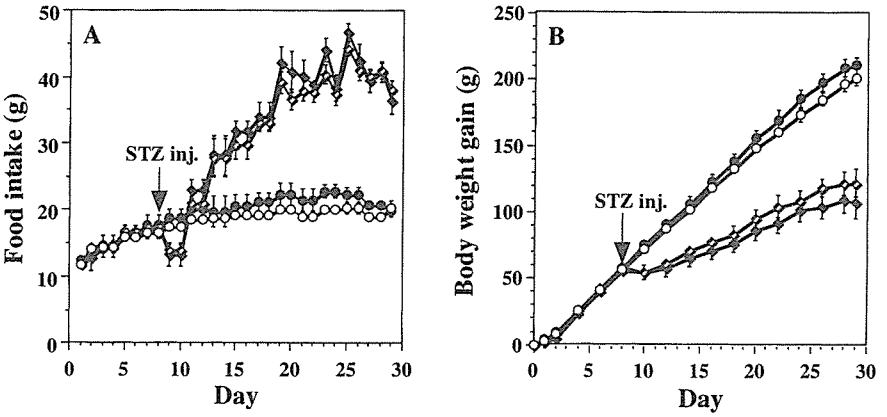

Fig. 3. Food Intake (A) and Body Weight Gain (B) (Experiment 2)

○, Control diet with saline injection; ◇, Control diet with STZ injection; ●, Test diet with saline injection; ◆, Test diet with STZ injection. Each value is the mean±SEM (n=5).

Table 5. Effects of the bonito powder intake and STZ injection on the body weight, food intake, liver weight, blood glucose level, and niacin metabolism

	Control diet saline injection	Control diet STZ injection	Test diet saline injection	Test diet STZ injection
Initial body weight (g)	111.9±1.3	111.9±1.3	112.0±1.4	112.3±1.6
Body weight at day 8 (g)	168.5±1.5	170.6±2.2	170.1±3.7	169.1±5.7
Final body weight (g)	313.6±5.6^{a}	234.4±10.2^{b}	323.0±7.0^{a}	219.4±12.8^{b}
Food intake* (g/21 days)	403.6±4.0^{a}	686.1±19.9^{b}	440.5±14.4^{a}	711.4±28.9^{b}
Body weight gain* (g/21 days)	145.1±4.8^{a}	63.8±10.9^{b}	153.0±4.9^{a}	50.3±9.3^{b}
Food efficiency ratio**	0.359±0.008^{a}	0.094±0.014^{b}	0.348±0.006^{a}	0.070±0.012^{b}
Liver weight (g)	15.1±0.4	12.7±0.8	14.4±0.6	12.2±0.6
ACMSD activity (μmol/h/g of liver)	2.1±0.4^{a}	23.6±3.0^{b}	2.2±0.5^{a}	29.5±2.2^{b}
Blood glucose level (mg/dl)	94±3^{a}	433±11^{b}	99±3^{a}	482±40^{b}
Blood NAD level (nmol/ml)	80.9±2.1^{a}	66.0±2.3^{b}	84.7±2.9^{a}	85.5±5.7^{a}
Blood NADP level (nmol/ml)	14.3±0.6^{ab}	15.9±0.9^{bc}	15.5±0.7^{bc}	18.3±0.4^{c}

* Data were calculated as total from day 8 to day 29. ** FER=body weight gain (g/21 days)/food intake (g/21 days). Each values is the mean±SEM (n=4-5). Values with different superscript letters are statistically different at $p<0.05$ by Tukey's multiple-comparison test.

塩水群及び試験食-STZ 群間の血中 NAD 含量に差異は認められなかった（Table 5）．対照食-STZ 群の血中 NAD 含量は他の 3 群に比べ，有意に低い値を示した．

一方，血中 NADP 含量はナイアシン摂取量の多少に関わらず容易に変動しないことが報告されている[20]．しかしながら，今回の実験においては，試験食-STZ 群の血中 NADP 含量は対照食-生理食塩水群よりも高い値を示した．なお，他の群とは有意な差異は認められなかったことから，やはり NADP 含量は変動しにくいものと思われた．

4） 肝 ACMSD 活性

STZ 誘発糖尿ラットでは肝 ACMSD 活性が著しく高くなることが報告されている[14]．今回の実験においても，対照食-STZ 群及び試験食-STZ 群の肝 ACMSD 活性は対照食-生理食塩水群及び試験食-生理食塩水群間の約 12 倍の値を示した（Table 5）．

4. 考　察

体内のナイアシンプールが飽和に達すると，余剰のナイアシンはニコチンアミド代謝産物として尿に排泄される[19]．したがって，尿中の総ニコチンアミド代謝産物量を指標として，食品中のナイアシンの生物有効性を調べることが可能となる．本実験において，女子学生にナイアシンを高濃度で含むカツオパウダーを投与したところ，投与量の52%がニコチンアミド代謝産物として尿中に排泄された．一方，女子学生にニコチンアミド標品50 mgをそれぞれ朝食後，昼食後，夕食後の計3回，総量で150 mg服用させると，ニコチンアミド服用量の64%が投与日の24時間尿中に排泄されることが報告されている[20]．また，ニコチンアミド75 mgを含む総合ビタミン剤を男子学生に服用させると，ニコチンアミド服用量の55%が投与日の24時間尿中に排泄されることが報告されている[21]．これらの既報の報告は[20][21]，本研究のニコチンアミド51 mgの負荷に比べ多い摂取量であるが，カツオパウダー中のニコチンアミド代謝産物排泄率はこれらの報告と近い値であった．したがって，カツオパウダー中のナイアシンはサプリメントやビタミン剤として使用されているニコチンアミド標品と同等の生物有効性を持つと考えられる．

自己免疫的機序により膵β細胞が傷害を受けると，インスリン依存型糖尿病を発症する．そのモデル動物を作製するために，膵β細胞に対して特異的に毒性を示すストレプトゾトシン（STZ）が用いられている[10]．一方，STZ投与時に大量のニコチンアミドを同時投与することにより，STZ誘発性糖尿病を予防することが報告されている[22]．そこで，カツオパウダー投与がどの程度STZ-誘発糖尿ラットの予防に有効であるか否かを調べてみた．その結果，STZ誘発糖尿ラットの血糖値及び体重増加量に対し，カツオパウダー摂取による予防，改善は見られなかった．STZ誘発性糖尿病の予防効果を示したニコチンアミドの投与量は500 mg/kg body weightと大量であり[22]，β細胞内のNAD濃度は一過性に高濃度になることが推察される．本実験においてSTZ投与までのニコチンアミド摂取量は約20 mg/kg body weightであり，また経口摂取したことと併せ，ニコチンアミド腹腔内投与時に比べβ細胞内NAD濃度が低いためにカツオパウダー摂取による糖尿病予防・改善効果が見られなかったことが推察される．なお，今回用いたカツオパウダー中にはニコチン酸は含まれておらず，ニコチンアミドのみが含まれている．

トリプトファン-NAD生合成経路において，α-amino-β-carboxymuconate-ε-semialdehydeはACMSDによって代謝されるとアセチルCoAにまで代謝され，さもなくば自己環状化してキノリン酸になることによってNADにまで代謝される．STZ誘発糖尿ラットではACMSD活性が著しく高くなるため，トリプトファンからのNAD生合成量が減少し，ナイアシン栄養状態が悪くなる．対照食-STZ群の血中NAD含量は対照食-生理食塩水群に比べ有意に低く，ナイアシン栄養状態の悪化を示している．しかし，試験食-STZ群はカツオパウダーを摂取したため，血中NAD含量は対照食-生理食塩水群及び試験食-生理食塩水群と同じレベルを維持しており，ナイアシン栄養状態の悪化を防いでいる．この結果から，カツオパウダーはナイアシン栄養状態の悪化を予防・改善するためのナイアシン供給源となりうる可能性がある．

結論として，我々が前に報告した小麦ふすま[23]とコーヒー抽出液乾固物（インスタントコーヒーパウダー）[24]と同様に，今回作製したカツオ由来のパウダーは，ナイアシンを生物有効性の高い遊離型のニコチンアミドとして含有していることが示唆された．また，カツオパウダーはカツオ節と同様に味質的にも優れ，かつ保存性にも優れていることから，通常食品として，さらにはトウモロコシ多食地域のペラグラ予防食品としても有用であると考えられる．

5. 要　約

(1) ヒトを用いて，カツオ由来ナイアシン高濃度含有パウダー（カツオパウダー）中のナイアシンの生物有効性について検討した．21～23歳の健康な女子学生8名を対象として，食事摂取基準を満たす食事を2日間与えた後，ナイアシン51 mgを含むカツオパウダー15 gを摂取させた．カツオパウダーを摂取した日に，摂取カツオパウダー中のナイアシンの52%がニコチンアミド代謝産物として尿中に排泄された．カツオパウダー中のナイアシンはニコチンアミド標品に近い，高い生物有効性を持つことが示唆された．

(2) カツオパウダーがストレプトゾトシン（STZ）誘発性糖尿病の予防・改善効果を有するか検討した．5週齢のWistar系雄ラットにカツオパウダー添加食を8日間与えた後，STZ 70 mg/kg body weightを腹腔内注射し，さらにカツオパウダー添加食を21日間与えた．カツオパウダー摂取によるSTZ誘発性の糖

尿病の予防・改善は見られなかった．しかし，STZ誘発性糖尿病によるナイアシン栄養状態の悪化を防止した．

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ノ ー ト

トリプトファン-ナイアシン代謝に関与する酵素活性から推定したラット乳仔のトリプトファン-ナイアシン転換率

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Estimated Conversion Ratio of Tryptophan-Niacin in Nursing Rats Using the Enzyme Activities Involved in the Tryptophan-Niacin Metabolism

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Although the conversion ratio of tryptophan-niacin has been reported to be 1/60 by the weight basis in human adults, there is no data on nursing infants of humans. It is estimated at 0 in 0-5 month-old nursing infants of humans and at 1/120 in 6-11month-old of humans from extrapolating data obtained in the weaning rats and the adult rats. Then, in order to estimate the conversion ratio of tryptophan-niacin in nursing infants of humans, the rats from newborn to before weaning were used; the enzyme activities involved in the tryptophan-niacin metabolism were measured and compared with those of the adult rats. Consequently, tryptophan-2,3-dioxygenase (TDO) activity which is the first enzyme in the tryptophan-niacin conversion pathway was very weak in the 1st day immediately after birth, and also in the 7th day. These results mean that niacin is hardly biosynthesized from tryptophan in the early stages of nursing infants in rats. Moreover, since the TDO activity on the 14th and 21st increased with age, when applying these data to humans, it was thought in the latter period of nursing infants of humans i.e., 6-11 month-old, that this conversion pathway begins to operate and it is about 1/120 which is the half of the human adults.

Keywords: *nursing infant, weaning rat, tryptophan, enzyme activity, metabolism*

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緒 言

乳児(0～11カ月)の水溶性ビタミン必要量の算定精度が低いことが指摘されている[1]．乳児の水溶性ビタミン必要量を求めるための根本的な考え方は，「乳児(0～5カ月)は，母乳を適当量摂取している限り，健常に発育する．」[1]というものである．また，6カ月から11カ月の乳児は離乳食を摂るようになるので，必要量を求めるのは複雑であるため，精度の高いデータを得ることが困難であり，我々が調べた限り報告は認められなかった．現在の必要量の求め方の考え方は，「0～5カ月児の必要量からの外挿値と成人の必要量からの外挿値を，平均化したものが適当であろう」[1]である．具体的に述べれば，乳児(0～5

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カ月)の必要量に体表面積比，(6～11カ月の基準体重/0～5カ月の基準体重)$^{0.75}$をかけた数値と成人(18～29歳)の必要量に，{(6～11カ月)の基準体重/成人(18～29歳)の基準体重}$^{0.75}$×(1+成長因子の0.3)}をかけた数値の，二つの値の平均値である[1]．これらの6～11カ月の乳児の必要量の外挿値は，若年成人から外挿した値よりも乳児から外挿した値の方がかなり低くなる．単位重量当たりの代謝回転は乳児の方が若年成人よりも高いと思われるのに，なぜであろうかという疑問を解決するために，このシリーズの研究を開始した．

ナイアシンは必須アミノ酸のトリプトファンから体内で合成される．その量は，成人では，重量比で1/60である[2]．ところが，この比率は年齢によって変動し，「第六次改定日本人の栄養所要量—食事摂取基準—」では，離乳直後のラットと成熟期のラットにおける本転換経路に関与する酵素活性の比較から，0～5カ月の乳児では0，6～11カ月の乳児では1/120としている[3]．離乳前のデータに関しては，GreengardとDewey[4]が，ラットの乳仔についてトリプトファン2,3-ジオキシゲナーゼ(TDO)活性について調べた成績が報告されている．出生12日まではTDOが全く検出されなかったが，離乳期の21日ではすでに成熟ラットの値と同じ程度の活性が検出されたことを報告している．しかしながら，他のトリプトファン-ナイアシン転換経路の酵素活性に関する報告はない．そこで，今回は，乳児期のトリプトファン-ナイアシン転換率の数値の精度を高めるために，ラット新生仔におけるトリプトファン-ナイアシン転換率に関与する酵素活性の測定を行ったので報告する．

実　験　方　法

1．ラットの飼育方法

妊娠20日目のWistar系，雌ラットを日本クレア(株)より購入後，ただちに飼育ケージに入れ，固形飼料と水を自由に与えた．新生仔が出生した日を1日目とし，7日目，14日目，21日目の肝臓をそれぞれ摘出した．ラットの乳児期は離乳する前の0～3週齢未満とした．また，日本クレア(株)よりWistar系，雄ラットも購入し，同様に固形飼料と水を自由に与えて成熟(15週齢)させ，対照として用いた．動物室は温度20°C前後，湿度60％前後を維持し，明暗サイクルは6時～18時を明，18時～6時を暗とした．なお，本実験は滋賀県立大学動物実験委員会の承認を得たものである．

2．トリプトファン-ナイアシン代謝に関わる酵素活性の測定方法

断頭によりラットをと殺後，肝臓を摘出した．直ちに肝臓を細切し，5倍量の冷却した50 mMのリン酸カリウム緩衝液(pH7.0)を加え，テフロンホモゲナイザーで均一化した．このホモジネートを酵素源とし，TDO[5]，キヌレニナーゼ(Ky-ase)[5]，3-ヒドロキシアンスラニル酸オキシゲナーゼ(3-HAO)[5]，キノリン酸ホスホリボシルトランスフェラーゼ(QPRT)[6]の活性測定を行った．

実　験　結　果

トリプトファン-ナイアシン転換率

ナイアシンは必須アミノ酸のトリプトファンから体内で合成されている．このトリプトファン-ナイアシン転換率は成人では，重量比で1/60というデータがあるが[4]，ヒト乳児のデータはなく，離乳直後(21日齢)のラットにおける実験値と成熟ラットにおける実験値の外挿から，ヒト乳児ではこの転換経路は作動しておらず，転換率は0とされている[3][7]．この推測の精度を高めるために，出産当日の1日目，7日目，14日目，21日目のラットの肝臓を取り出し，トリプトファン-ナイアシン転換経路の主要な酵素活性を測定した．その結果を表1にまとめた．

TDOはトリプトファン-ナイアシン転換経路の初発酵素である．この酵素活性が，1日目および7日目では非常に弱かった．14日，21日目と日齢とともに活性は増大し，21日目では成熟ラットの約1/2であった．測定したキヌレニナーゼ，3-ヒドロキシアンスラニル酸オキシゲナーゼ，およびキノリン酸ホスホリボシルトランスフェラーゼ活性は，出産直後の1日目でも，すでに成熟ラットの酵素活性に匹敵あるいはそれ以上の活性が検出された．

考　　察

トリプトファン-ナイアシン転換率は，年齢によって変

表1．出生後のトリプトファン-ナイアシン転換経路に関わる主要な酵素の活性変動(ラット)．

	1日目	7日目	14日目	21日目	成熟ラット
TDO	0.08 ± 0.01	0.06 ± 0.01	0.28 ± 0.04	0.48 ± 0.02	1.18 ± 0.17
Ky-ase	0.50 ± 0.01	0.41 ± 0.02	0.50 ± 0.06	1.02 ± 0.03	1.39 ± 0.18
3-HAO	528 ± 38	684 ± 25	638 ± 30	570 ± 20	627 ± 54
QPRT	1.34 ± 0.03	1.73 ± 0.03	1.06 ± 0.05	0.67 ± 0.02	0.62 ± 0.03

値は，3匹のラットの平均値±標準偏差である．単位はμmol/hr/g liverで示した．TDO = Tryptophan dioxygenase, Ky-ase = kynureninase, 3-HAO = 3-Hydroxyanthranilic acid oxygenase, QPRT = Quinolinate phosphoribosyltransferase.

動し，「第六次改定日本人の栄養所要量-食事摂取基準-」では，離乳直後のラットと成熟期のラットにおける本転換経路に関与する酵素活性の比較から，0～5カ月の乳児では0，6～11カ月の乳児では1/120としている[3]．このデータの精度を高めるために，出産当日の1日目，7日目，14日目，21日目のラットの肝臓を取り出し，トリプトファン-ナイアシン転換経路の主要な酵素活性を測定した．その結果，表1に示したように，TDOを除く他の酵素活性は，すでに出生直後においても，成熟ラットのそれらの活性とほぼ同程度の活性を有していることがはじめて明らかとなった．一方，本転換経路の初発酵素であるTDO活性が，1日目，7日目ではわずかに検出されたにすぎなかった．この事実は，ヒト乳児の初期においては，ほとんどトリプトファンからナイアシンは生合成されていないことを裏付けるものである．また，14日，21日目と日齢とともに活性は増大し，21日目では成熟ラットの約1/2であった．

この結果は，GreengardとDewey[7]が1971年に報告した結果と類似していた．今回の結果(表1)と彼らの結果[4]との間で異なる点は，今回の結果では，離乳時期に相当する出生21日後でも成熟ラットの活性の約半分であったのに対し，GreengardとDewey[4]の報告では，出生21日後で，すでに成熟ラットと同じ活性を有していたという点である．この違いは，定性的な考え方をする生化学的観点においては，重要な相違点とはなりにくいが，定量的な考え方をする栄養学的観点からは，非常に重要なことである．つまり，離乳期ですでに成熟ラットと同程度のトリプトファン-ナイアシン転換能力を有しているのか，あるいは成熟ラットの1/2程度の能力を有しているのか，という相違である．

出生1日目から約21日目までがラットの乳児期にあたることから，ラットでの転換率が，出生14日後で成熟ラットの約1/4，21日後で約1/2であったこと，および他のトリプトファン-ナイアシン転換経路に関わる酵素活性が，すでに成熟ラットとほぼ同程度まで発現していることを考えると，乳児期(0～11カ月)の後期，すなわち6～11カ月では，本転換経路が作動しはじめており，成人のトリプトファン-ナイアシン転換率の1/60の半分である1/120程度としてもさしつかえないと思われた．

結　論

ヒトの離乳期(6カ月～11カ月)のトリプトファン-ナイアシン転換率の精度を高めるために，ラットを用いて実験を行った．出産後，経日的にトリプトファン-ナイアシン転換経路の主要な酵素活性を測定した．その結果，本転換経路の初発酵素であるトリプトファン-2,3-ジオキシゲナーゼ活性が1日目，7日目ではわずかに検出されたにすぎなかった．14日目，21日目と日齢が進むとともに本酵素活性は増大し，成熟ラットの約1/2に達した．したがって，このデータをヒトにあてはめれば，乳児の離乳期すなわち6～11カ月では本転換経路は作動しはじめており，成人の半分であると算定してもさしつかえないと思われた．

謝　辞

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(平成16. 12. 22受付)

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Values of Water-Soluble Vitamins in Blood and Urine of Japanese Young Men and Women Consuming a Semi-Purified Diet Based on the Japanese Dietary Reference Intakes

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Summary We investigated the levels of water-soluble vitamins except for vitamin B_6 in the blood and urine of Japanese college male (n=10) and female (n=10) students. They consumed for 7 d a semi-purified diet based on Japanese Dietary Reference Intakes to assess the Recommended Dietary Allowances (RDA) for water-soluble vitamins and to present some new normal values for blood and urine levels of water-soluble vitamins in Japanese. The blood and the 24-h urine samples were collected on the last day of the experiment and measured. The values of total vitamin B_1 in whole blood, total vitamin B_2 in whole blood, total cyanocobalamin in serum, total nicotinamide in whole blood, total pantothenic acid in whole blood, total folates in serum, total biotin in serum, and ascorbic acid in plasma were 104±17 pmol/mL (mean±SD), 216±25 pmol/mL, 0.34±0.05 pmol/mL, 59.1±5.0 nmol/mL, 2.45±0.37 nmol/mL, 15.6±4.6 pmol/mL, 8.3±0.5 pmol/mL, and 62±10 nmol/mL, respectively, in males, and 90±23, 234±18, 0.67±0.20, 61.9±6.0, 2.48±0.30, 30.2±8.6, 8.4±0.3, and 67±14, respectively, in females. There was a significant difference in the values of cyanocobalamin and total folates between men and women. The urinary excretion of vitamin B_1, vitamin B_2, cyanocobalamin, sum of the catabolic metabolites of nicotinamide, pantothenic acid, folates, biotin, and ascorbic acid were 665±114 nmol/d, 562±325 nmol/d, 93±31 pmol/d, 84±26 μmol/d, 9.3±2.3 μmol/d, 19.4±2.8 nmol/d, 83±18 pmol/d, and 148±51 μmol/d, respectively, in males, and 495±212, 580±146, 145±49, 83±19, 16.9±1.3, 22.7±2.7, 83±23, and 140±51, respectively, in females. There was a significant difference in the urinary excretion of cyanocobalamin, pantothenic acid and total folates between men and women. These values will be useful for the nutritional assessment of water-soluble vitamins for Japanese, although the examination period was short. In future, an experiment with various age groups and re-evaluation by repeated experiments will provide more accurate values.

Key Words water-soluble vitamins, blood vitamin concentrations, DRIs (Dietary Reference Intakes), humans, Japanese

In Japan, revision of the "Recommended Dietary Allowance (RDA)" has been done every 5 y since 1970 by the Ministry of Health, Labor, and Welfare. During these 3 decades, the life span of Japanese has extended and retained the first position in the world. This distinction may be attributed to the fact that the nutritional guidance and health promotion programs are excellent in Japan. In particular, in June 1999, "The 6th revision of the Japanese RDA—Dietary Reference Intakes (DRIs)—" (*1*) was laid down by the Ministry of Health, Labor, and Welfare. In this revision, the dietary reference intakes of all of the water-soluble vitamins (vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, pantothenic acid, folic acid, biotin, and vitamin C) were first established. However, many RDAs were determined based on reports from foreign countries. So, we mea-

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sured the values for the blood and urine of Japanese adult male and female subjects, who ingested a diet prescribed by "The 6th revision of Japanese RDA—DRIs—" (*1*) to assess the validity for the DRIs of water-soluble vitamins.

In clinical examination, blood values are used for assessment of vitamins, but in a nutritional examination, those values would not be suitable sometimes. As a general rule, the concentrations of nutrients in the body are well controlled in healthy subjects. We think that the concentrations of water-soluble vitamins in the blood are kept almost constant in healthy adult subjects but that the urinary amounts are relatively varied, because the excess water-soluble vitamins are excreted into the urine. In other words, the blood concentrations of water-soluble vitamins would be kept constant when the subjects have ingested a more than adequate amount of nutrients, whereas the urinary excretion of water-soluble vitamins would vary, because the requirements of nutrients are different among subjects. Therefore, the measurement of urinary excretion of water-soluble vitamins should be the most reliable index for nutritional status of water-soluble vitamins. So, we investigated the water-soluble vitamin contents in the urine when subjects were given a diet based on the recommended Japanese DRIs. As far as we know, this study is the first of its kind. The values listed in the present paper will be useful as indices for evaluating the nutritional status of water-soluble vitamins.

MATERIALS AND METHODS

Subjects. Healthy Japanese college students, consisting of 10 males and 10 females, participated in the present experiment. Their ages, body weights and heights are shown in Table 1. Prior to the experiment, they had physical checkups, and their hematological and blood biochemical analyses showed normal values. This study was reviewed and approved by The Ethical Committee of The Incorporated Administrative Agency of Health and Nutrition.

Diet and experimental design. All subjects were housed in the same facility for 9 d. The experimental design is shown in Fig. 1. The 24-h urine samples were collected from the second urinary excretion on day 7 to the first one on day 8. After the volumes of the urine samples had been measured, the collected urine samples were immediately treated as described under "Analyses" to avoid destruction of water-soluble vitamins, and then stored at −20°C until needed. The blood was taken from a cubital vein at 08:30 on day 8 before breakfast, and treated immediately to avoid destruction of water-soluble vitamins, and stored at −20°C until needed.

The daily schedule was partly restricted: The lights

Table 1. Characteristics of the subjects.

Subjects	Age (y)	Height (cm)	Body weight (kg)	BMI
Male 1	19	160.2	58.50	22.79
Male 2	19	173.0	56.05	18.73
Male 3	21	176.1	70.40	22.70
Male 4	21	170.1	61.00	21.08
Male 5	21	170.5	60.95	20.97
Male 6	19	180.5	55.55	17.05
Male 7	20	166.0	57.10	20.72
Male 8	23	170.0	56.80	19.65
Male 9	20	182.0	58.70	17.72
Male 10	21	183.6	79.00	23.43
Mean	20.4	173.2	61.40	20.48
SD	1.3	7.4	7.52	2.18
SEM	0.4	2.4	2.38	0.69
Female 1	21	161.8	52.55	20.27
Female 2	22	164.8	60.90	22.42
Female 3	20	159.9	57.40	22.45
Female 4	21	162.5	51.10	19.35
Female 5	21	166.4	52.70	19.03
Female 6	21	167.7	56.50	20.09
Female 7	21	163.4	51.90	19.44
Female 8	20	158.3	51.00	20.35
Female 9	20	170.9	56.05	19.19
Female 10	20	163.6	51.35	19.19
Mean	20.7	163.9	54.15	20.08
SD	0.7	3.7	3.37	1.28
SEM	0.2	1.2	1.07	0.41

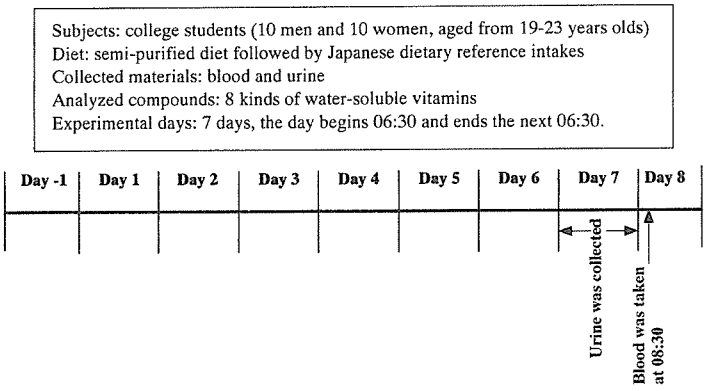

Fig. 1. Scheme of the experimental design.

Table 2. Composition of the male diet.

	(g/d)	Remarks
Wheat flour (Soft flour), first grade	315	Protein: 315 g×0.08=25.2 g. Tryptophan content was 1.1%, so 277 mg of tryptophan were supplied. Carbohydrate: 315 g×0.759=239 g Lipid: 315 g×0.017=5.4 g
Gluten	56	The gluten contained 81.6% protein, so the net protein amount was 45.7 g. Tryptophan content was 1.1%, so 503 mg of tryptophan were supplied. Total protein=70.9 g Total tryptophan=780 mg
Cornstarch	118	
Sucrose	30	Total carbohydrate=387.1 g
Fats		Total fat=50.3 g
Soybean oil	7.0	
Rapeseed oil	21.0	
Coconut oil	8.5	
Lard	8.4	
Dietary fiber		Soluble dietary fiber used, "Fibersol" was obtained from Matsutani Chemical Industry Co., Ltd. (Osaka, Japan), and insoluble dietary fiber used, Ramie powder, was obtained from Tosco Co., Ltd. (Tokyo, Japan).
Soluble	0.8	
Insoluble	14.3	
Mineral mixtures	14.6	The composition is shown below.
Total amount	592.5	Total energy=ca. 2,300 kcal=9,614 kJ

For breakfast and supper, 177.75 g of the above powder mixture was added to 110 mL of water, and mixed well, and was baked for 9 min at 250°C. The weight of the baked meal was ca. 214 g. The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 237.0 g of the above mixture was added to 150 mL of water, and mixed well, and was baked for 10 min at 250°C. The weight of the baked meal was ca. 285 g. The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects.

Composition of the mineral mixture: 1,200 mg of $CaHPO_4$-$2H_2O$, 1,049 mg of $CaCO_3$, 2,124 mg of KH_2PO_4, 3,558 mg of $KHCO_3$, 2,594 mg of $MgCl_2$-$2H_2O$, 49.8 mg of $FeSO_4$-$5H_2O$, 17.6 mg of $MnSO_4$-$5H_2O$, 22.9 mg of $ZnCl_2$, 7.1 mg of $CuSO_4$-$5H_2O$, 0.2 mg of KI, and 4,000 mg of NaCl.

Composition of the vitamin mixture: 4.0 mg (2,000 IU) of retinal acetate reagent (500,000 IU/g), 2.5 μg of cholecalciferol, 4.47 mg of *dl*-α-tocopherol (7 mg was supplied from oils), 24 μg of phylloquinone (41 μg was supplied from oils), 1.2 mg of thiamin-HCl, 1.2 mg of riboflavin, 2.0 mg of pyridoxine-HCl, 2.4 μg of cyanocobalamin, 4.2 mg of nicotinamide (13.0 mg of nicotinamide was supplied from 780 mg of tryptophan in protein), 5.5 mg of calcium panthotenate, 200 μg of pteroylmonoglutamic acid, 30 μg of D(+)-biotin, 100 mg of ascorbic acid, made up to 1 g with sucrose.

were turned off at 22:00 in order to promote sleep and the subjects got up at 06:00. The breakfast time was 08:00–09:00, lunch 12:30–13:10, and dinner 18:30–19:00. They consumed a semi-purified diet based on Japanese DRIs during experiment. The composition and the amount of the semi-purified diet are shown in Table 2 for males and in Table 3 for females. The experiment was carried out from March, 1st through March, 8th, 2002 for females, and from August 27th through September 3rd, 2002 for males.

Chemicals. Wheat flour (soft flour, first grade) was obtained from Nisshin Flour Milling Inc. (Tokyo). Wheat gluten, raw cornstarch, soybean oil, 13 kinds of vitamins (see Tables 2 and 3), and minerals (see Tables 2 and 3) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rapeseed oil was purchased from Ajinomoto Co. Ltd. (Tokyo, Japan). Coconut oil and lard were both obtained from Clea Japan (Tokyo, Japan). "Fibersol," used as a soluble dietary fiber, was obtained from Matsutani Chemical Industries (Osaka, Japan) and "Ramie powder," used as an insoluble dietary fiber, was from Tosco (Tokyo, Japan).

Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS$-HCl=337.27), riboflavin ($C_{17}H_{20}N_4O_6$=376.37), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$=1355.40), nicotinamide (Nam; $C_6H_6N_2O$=122.13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca=476.54), folic acid ($C_{19}H_{19}N_7O_6$=441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$=244.31), and L(+)-ascorbic acid ($C_6H_8O_6$=176.13) were purchased from Wako Pure Chemical Industries. N^1-Methylnicotinamide (MNA) chloride ($C_7H_9N_2O$-HCl=159.61) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py, $C_7H_8N_2O_2$=152.15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py, $C_7H_8N_2O_2$=152.15) were synthesized by the methods of Pullman and Colowick (*2*) and Shibata et al. (*3*), respectively.

All other chemicals used were of the highest purity available from commercial sources.

Analyses

Blood and urine

Vitamin B_1 (thiamin): The concentrations of total vitamin B_1 in whole blood and urine were measured by the HPLC-post labeled fluorescence method of Kimura et al. (*4*).

Vitamin B_2 (riboflavin): The concentration of total vitamin B_2 in whole blood was determined by the

Table 3. Composition of the female diet.

	(g/d)	Remarks
Wheat flour (Soft flour), first grade	315	Protein: 315 g×0.08=25.2 g. Tryptophan content was 1.1%, so 277 mg of tryptophan were supplied. Carbohydrate: 315 g×0.759=239 g Lipid: 315 g×0.017=5.4 g
Gluten	37.3	The gluten contained 81.6% protein, so the net protein amount was 29.8 g. Tryptophan content was 1.1%, so 328 mg of tryptophan was supplied. Total protein=55 g Total tryptophan=605 mg
Cornstarch	33	
Sucrose	20	Total carbohydrate=292 g
Fats		Total fat=40 g
Soybean oil	4.8	
Rapeseed oil	16.8	
Coconut oil	7.1	
Lard	5.9	
Dietary fiber		Insoluble dietary fiber used, Ramie powder, was obtained from Tosco Co., Ltd. (Tokyo, Japan).
Soluble	0	
Insoluble	10.1	
Mineral mixtures	12.0	The composition is shown below.
Total amount	462.0	Total energy=ca. 1,800 kcal=7,524 kJ

For breakfast and supper, 139 g of the above powder mixture was added to 91 mL of water, and mixed well, and was baked for 9 min at 250°C. The weight of the baked meal was ca. 175 g. The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 185 g of the above mixture was added to 122 mL of water, and mixed well, and was baked for 10 min at 250°C. The weight of the baked meal was ca. 233 g. The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects.

Composition of the mineral mixture: 1,100 mg of $CaHPO_4$-$2H_2O$, 900 mg of $CaCO_3$, 2,200 mg of KH_2PO_4, 3,500 mg of $KHCO_3$, 2,083 mg of $MgCl_2$-$2H_2O$, 60 mg of $FeSO_4$-$5H_2O$, 12.2 mg of $MnSO_4$-$5H_2O$, 19 mg of $ZnCl_2$, 6.3 mg of $CuSO_4$-$5H_2O$, 0.2 mg of KI, and 2,120 mg of NaCl.

Composition of the vitamin mixture: 3.6 mg (1,800 IU) of retinal acetate reagent (500,000 IU/g), 2.5 μg of cholecalciferol, 4.47 mg of *dl*-α-tocopherol (5 mg were supplied from oils), 24 μg of phylloquinone (31 μg was supplied from oils), 0.9 mg of thiamin-HCl, 1.0 mg of riboflavin, 1.5 mg of pyridoxine-HCl, 2.8 mg of nicotinamide (10.0 mg of nicotinamide was supplied from 605 mg of tryptophan in protein), 2.4 μg of cyanocobalamin, 5.5 mg of calcium panthotenate, 200 μg of pteroylmonoglutamic acid, 30 μg of D(+)-biotin, 100 mg of ascorbic acid, made up to 1 g with sucrose.

HPLC-lumiflavin method of Ohkawa et al. (5) modified slightly. Urinary concentration of riboflavin was analyzed according to the method of Ohkawa et al. (6).

Vitamin B_{12} (cyanocobalamin): Serum vitamin B_{12} concentration was determined by using a fully automated chemiluminescence analyzer (ACS 180; Beckman Coulter, Inc.) according to the manufacturer's instructions. The serum, prepared in the usual way, was directly applied to the analyzer.

Vitamin B_{12} concentration in urine was assayed by the microbiological method with *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830 (7).

Niacin: The total nicotinamide content in whole blood was measured by the method of Shibata et al. (8).

The quantities of Nam, 2-Py and 4-Py in urine were measured simultaneously by the HPLC method of Shibata et al. (3). The content of MNA was measured by the method of Shibata (9).

Pantothenic acid: The heat-extracted supernatant of whole blood was treated with a mixture of 2 enzymes (phosphatase and amidase) for decomposing bound pantothenic acid such as CoA, phosphopantetheine, and pantetheine to form free pantothenic acid (10). The enzyme-treated materials were directly used for measuring total pantothenic acid by using *Lactobacillus plantarum* ATCC 8014 (11). Total pantothenic acid means the sum of free pantothenic acid, and that derived from pantetheine, phosphopantethine, CoA, and acyl CoA including acetyl CoA.

The content of free pantothenic acid in urine was directly measured by using *Lactobacillus plantarum* ATCC 8014 (11).

Folates: Serum concentrations of folates were determined by an automated method based on the competitive protein-binding assay using the ACS at the Clinical Laboratory of Mitsubishi BCL in Kobe. Herbert (12) suggested that there was no significant loss of serum folate activity after storage at −20°C for up to 106 d. We also found that the solution of folic acid was stable for at least 3 wk in the freezer at −20°C. Therefore, ascorbic acid was not added prior to storage of serum and urine samples in this study.

The concentrations of urinary folates were determined by an automated method based on the competitive protein-binding assay using the ACS as mentioned above.

Biotin: Biotin levels in the serum were microbiologically quantified with *Lactobacillus plantarum* ATCC 8014 according to the agar plate assay developed by Fukui et al. (*13*).

The content of free biotin in urine was directly measured by using *Lactobacillus plantarum* ATCC 8014.

Vitamin C: Total ascorbic acid (vitamin C) was determined by an HPLC-UV method according to Fujiwara et al. (*14*).

Vitamin C in urine was determined by the 2,4-dinitrophenylhydrazine (DNPH) method according to Shigeoka et al. (*15*).

Statistical analysis. For the statistical evaluation, the significance of the differences in the mean concentrations of water-soluble vitamins in blood and urine between two sexes was tested by using Student's paired, two-tailed *t*-test and non-parametric Mann-Whitney *U* test. Differences of $p<0.05$ were considered to be statistically significant. Instat software (version 2.00; obtained from GraphPad Software, Inc., San Diego, CA, USA) was used for all analyses.

RESULTS AND DISCUSSION

Vitamin B_1

The content of total vitamin B_1 (free thiamin+thiamin monophosphate: TMP+thiamin diphosphate: TDP+thiamin triphosphate: TTP) in whole blood is shown in Table 4. Vitamin B_1 concentrations in males were 104±17 pmol/mL (range 81–122) and in females 90±23 pmol/mL (range 38–122) on the final day of the study. No significant difference between males and females was observed. However, a wide distribution range in blood vitamin B_1 concentrations of females was found; in particular, very low vitamin B_1 concentrations (38 pmol/mL) could be found. On the first day of the study, blood vitamin B_1 concentrations in males were 82±10 pmol/mL (range 74–106) and in females 88±19 pmol/mL (range 48–112).

The reference value of erythrocyte vitamin B_1 (70–90 pmol/mL) was proposed by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (USA and Canada) (*16*). However, the reference value in whole blood has not yet been proposed. In Japan, the content of vitamin B_1 in whole blood is frequently used. Kimura et al. (*17*) reported that the total vitamin B_1 concentration of farm village inhabitants was 109±10 pmol/mL and that of fishing village inhabitants was 119±19 pmol/mL in a survey of rural areas in 1982. And after these subjects were supplied with vitamin B_1 (1.5 mg/d) in daily foods for 1 wk, the total vitamin B_1 concentration of farm village inhabitants was 137±7 pmol/mL and that of fishing village inhabitants was 157±16 pmol/mL. The total vitamin B_1 concentration in whole blood of young men (29.4±4.5 y old) was 140±36 pmol/mL ($n=10$) and that of young women (23.5±3.1 y old), 117±21 pmol/mL ($n=10$), which was reported by Itokawa et al. (*18*). Takeda et al. (*19*) reported that the total vitamin B_1 concentration in whole blood of Japanese adult males and females was 119±33 pmol/mL ($n=524$) and 104±27 pmol/mL ($n=345$), respectively. In the survey study of other countries by Kimura et al. (*20*), the total vitamin B_1 concentration in the whole blood of Chinese adult males and females living in urban areas was 164±34 pmol/mL ($n=100$) and 159±37 pmol/mL ($n=100$). In the survey for vitamin B_1 status of inhabitants in northeast rural Thailand by Kimura et al. (*21*), the total vitamin B_1 concentration in whole blood of

Table 4. Blood values of water-soluble vitamins for Japanese college students who consumed on a semi-purified diet based on the Japanese dietary reference intakes.

	Mean	SD	Minimum	Maximum	Median
Males ($n=10$)					
Total thiamin in whole blood (pmol/mL)	104	17	81	122	106
Total riboflavin in whole blood (pmol/mL)	216	25	175	259	211
Cyanocobalamin in serum (pmol/mL)	0.34	0.05	0.26	0.43	0.34
Total Nam in whole blood (nmol/mL)	59.1	5.0	52.8	69.9	60.0
Total pantothenic acid in whole blood (nmol/mL)	2.45	0.37	1.86	3.27	2.36
Folates in serum (pmol/mL)	15.6	4.6	9.9	23.1	15.0
Biotin in serum (pmol/mL)	8.3	0.5	7.8	9.0	8.2
Ascorbic acid in plasma (nmol/mL)	62	10	47	80	60
Females ($n=10$)					
Total thiamin in whole blood (pmol/mL)	90	23	38	122	91
Total riboflavin in whole blood (pmol/mL)	234	18	205	259	238
Cyanocobalamin in serum (pmol/mL)	0.67*	0.20	0.41	0.92	0.67
Total Nam in whole blood (nmol/mL)	61.9	6.0	55.4	75.4	61.0
Total pantothenic acid in whole blood (nmol/mL)	2.48	0.30	2.08	2.83	2.51
Folates in serum (pmol/mL)	30.2*	8.6	21.0	51.7	29.0
Biotin in serum (pmol/mL)	8.4	0.3	7.8	9.0	8.6
Ascorbic acid in plasma (nmol/mL)	67	14	47	100	61

* Significant difference from male value at $p<0.05$, calculated by Student's *t* test.

Table 5. Urinary values of water-soluble vitamins for Japanese college students who consumed a semi-purified diet based on the Japanese dietary reference intakes.

	Mean	SD	Minimum	Maximum	Median
Males (n=10)					
Thiamin (nmol/d)	665	114	467	821	669
Riboflavin (nmol/d)	562	325	155	1,208	492
Cyanocobalamin (pmol/d)	93	31	69	170	85
Sum (MNA+2-Py;4-Py) (μmol/d)	84	26	54	128	74
Pantothenic acid (μmol/d)	9.3	2.3	6.2	12.2	10.2
Folates (nmol/d)	19.4	2.8	14.5	23.9	19.6
Biotin (pmol/d)	83	18	59	112	78
Ascorbic acid (μmol/d)	148	51	87	231	134
Females (n=10)					
Thiamin (nmol/d)	495*	212	286	988	458
Riboflavin (nmol/d)	580	146	366	849	569
Cyanocobalamin (pmol/d)	145*	49	94	252	112
Sum (MNA+2-Py;4-Py) (μmol/d)	83	19	53	111	81
Pantothenic acid (μmol/d)	16.9*	1.3	14.8	18.6	17.3
Folates (nmol/d)	22.7*	2.7	20.4	29.0	22.3
Biotin (pmol/d)	83	23	54	120	78
Ascorbic acid (μmol/d)	140	51	89	257	131

* Significant difference from male value at $p<0.05$, calculated by Student's t test.

males and females working on farms was 74±37 pmol/mL (n=59) and 76±46 pmol/mL (n=47), and the total vitamin B_1 concentration in the whole blood of males and females working in factories was 96±34 pmol/mL (n=279) and 105±27 pmol/mL (n=21), respectively.

The urinary excretion of free vitamin B_1 in males and females is shown in Table 5. A significant difference between sexes was observed. The reference value of urinary excretion of vitamin B_1 (133–333 nmol/d) has been proposed by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (USA and Canada) (*16*). All of the present values were over this reference value. So, the RDA of vitamin B_1 in the 6th revision was evaluated as good in adult men and women.

Murata et al. (*22*) reported that vitamin B_1 levels of 24 h-urine samples collected from 13 women in summer and winter were 545±402 nmol/d and 522±445 nmol/d, respectively. Itokawa et al. (*23*) reported that the urinary excretion of vitamin B_1 in adult men (21–24 y old) was 472±339 nmol/d (n=21). Therefore, the present values were almost the same as the previously reported ones (*22*, *23*).

Based on the results of a thiamin deficiency study, Nishio et al. (*24*) concluded that the urinary excretion of vitamin B_1 would not be a useful index of thiamin depletion because the urinary excretion decreased so quickly before the clinical deficiency was revealed. But the use of the urinary values helps to assess the vitamin B_1 nutritional status, because the urinary excretion reflects the immediate vitamin B_1 intake.

Vitamin B_2

The total vitamin B_2 content expressed in whole blood is shown in Table 4. The values were almost the same between the 2 groups. Hiraoka (*25*) reported that the mean value and the reference interval were 225 pmol/mL and 154–311 pmol/mL, respectively, for the blood riboflavin levels of healthy female students aged 21–22 y whose intake of vitamin B_2 was above the RDA. The value obtained in the present study coincides with their data. There is a paper stating that over 180 pmol/mL of vitamin B_2 in whole blood indicates a well-nourished individual (*16*). In the present experiment, only one person was below the value. Therefore, the RDA of vitamin B_2 in the 6th revision would be evaluated as good.

The vitamin B_2 content in urine is shown in Table 5. The values were almost the same between the male and female students. Roughead and McCormick (*26*) reported that the amount of urinary riboflavin determined by the HPLC procedure exceeded 319 nmol/d under conditions of riboflavin intake of ca. 1.5 mg/d in healthy residents of rural Georgia, USA. Two of our subjects were below the value of 319 nmol/d. Urinary riboflavin separated from other flavin derivatives including catabolites by HPLC is considered to be useful for estimation of the vitamin B_2 requirement. The average in our study was ca. 550 nmol/d and the range was 155 to 1,208 nmol/d.

Vitamin B_{12}

The concentration of vitamin B_{12} in serum reflects both vitamin B_{12} intake and storage. The lower limit (cut-off value) of the serum vitamin B_{12} concentration is considered to be approximately 0.12–0.18 pmol/mL for adults (*16*). The serum concentrations of vitamin B_{12} found in the present study are shown in Table 4. These values are over the cut-off value, and so the RDA in the 6th revision was evaluated as good. But, the diet

used for the experiment contained a small amount of vitamin B_{12}. So, the accurate amount of vitamin B_{12} consumed was 4.2 μg/d. The mean values for males and females were 0.34 pmol/mL and 0.67 pmol/mL, respectively, and this difference was significant. Those values at day −1 were 0.34±0.07 pmol/mL and 0.48±0.14 pmol/mL, respectively. Therefore, daily intake of 2.4 μg of cyanocobalamin for 7 d maintained the serum vitamin B_{12} level in the male subjects, and increased it in the female subjects. Fernandes-Costa et al. (*27*) reported mean serum vitamin B_{12} values for young men (n=77) and women (n=82) to be 0.477 and 0.604 pmol/mL, respectively. Similar results have been also obtained by other workers (*28*, *29*). At the present time, we cannot explain why there is such a difference in the serum vitamin B_{12} concentration between men and women and do not know whether there is any gender-based difference in vitamin B_{12} requirements.

Amounts of urinary vitamin B_{12} are shown in Table 5. The mean value in men and women was 93 pmol/d and 145 pmol/d, respectively, and this difference was significant.

Mollin and Ross (*30*) reported that the daily excretion of vitamin B_{12} in urine ranged from 81.2 to 199.3 pmol/d in 6 normal subjects, but decreased significantly [to less than 36.9 pmol] in 7 patients with pernicious anemia.

Adams (*31*) demonstrated that the mean value of urinary vitamin B_{12} was 121 pmol/d in non-vitamin B_{12}-deficient subjects and found a linear correlation between the serum vitamin B_{12} level and daily loss of vitamin B_{12} in the urine. From the present data, the urinary excretion of vitamin B_{12} in Japanese would be ca. 100 pmol/d in men and 150 pmol/d in women.

The values for serum and urinary vitamin B_{12} between men and women were significantly different, as stated above. However, the RDA of vitamin B_{12} in the 6th revision is the same for men and women. This problem should be resolved in the future.

Niacin (total Nam)

The content of total Nam in whole blood is shown in Table 4. There was no difference in the value between sexes. The present mean value for men and women was ca. 60 μmol/mL of whole blood. This value is about the same as was reported previously (*32*), in a study in which the subjects were female college students (n=19), who consumed self-selected foods. In a report published in 1950, Nose (*33*) reported that the content of total niacin in whole blood was 62.7±5.3 nmol/mL (n=10). In 1953, Katsura and Sakakida (*34*) reported a mean value of 61±5 nmol/mL for 10 men (24–29 y old) and 56±6 nmol/mL for 10 women (19–50 y old). Therefore, the RDA of niacin equivalent intake in the 6th revision was evaluated as good.

In humans, nicotinamide itself is minimally excreted into urine, but its catabolic metabolites, MNA, 2-Py, and 4-Py are excreted. The sum of the urinary excretion of MNA+2-Py+4-Py for the male and female students is shown in Table 5. Shibata and Matsuo (*35*) reported a value of ca. 100 μmol/d, for female college students who consumed self-selected foods. Okamoto et al. (*36*) reported that when female college students consumed a diet containing 25 mg niacin equivalent/d, the urinary excretion of the sum was ca. 80 μmol/d. The present and previous data (*35*, *36*) indicate that the urinary excretion of the sum of MNA+2-Py+4-Py changes according to the niacin equivalent intake. In fact, Shibata and Matsuo (*35*) and Moyer et al. (*37*) revealed a high correlation coefficient between the sum and the niacin equivalent intake. Therefore, determination of the sum of MNA, 2-Py, and 4-Py would provide an excellent index for a niacin nutritional assessment. We would like to propose that adult humans (18–69 y olds) including men and women should ingest sufficient niacin equivalent to maintain this urinary sum at a level over 50 μmol/d.

Pantothenic acid

The content of total pantothenic acid in whole blood is shown in Table 4. No difference in value was observed between sexes. The present mean value was ca. 2.5 nmol/mL of whole blood. Wittwer et al. (*38*) reported that a normal value for pantothenic acid in the whole blood ranged from 1.57 to 2.66 nmol/mL, and Song et al. (*39*) reported the concentration in adult women to be 2.40±0.05 (n=47) nmol/mL. They also reported that the correlation between total pantothenic acid in blood and the intake of pantothenic acid was weak. As to the paper by Fry et al. (*40*), the total pantothenic acid did not decrease even when the subjects were fed a diet without pantothenic acid for 9 wk. Therefore, the total pantothenic acid in blood does not reflect the intake of pantothenic acid and should not be used for assessing the nutritional status of pantothenic acid.

The urinary content of pantothenic acid in the males and the females is shown in Table 5. The value was about 2 times higher in the females than in the males, and this difference was significant. There is clear evidence that the urinary excretion of pantothenic acid is a reliable index for assessing the nutritional status of pantothenic acid. For example, Fox and Linkswiler (*41*) reported that the correlation coefficient between dietary pantothenic acid intake and the urinary excretion of pantothenic acid was 0.805. Previously reported values (*40*, *41*) for urinary pantothenic acid were ca. 10–20 μmol/d in adults. So, we conclude the RDA of pantothenic acid in the 6th revision is good, and propose that the urinary excretion of pantothenic acid is a suitable index for the nutritional assessment of pantothenic acid. A value of 10 μmol/d would be a suitable intake of pantothenic acid for maintaining health in males, whereas 15 μmol/d would be appropriate for females.

Folates

The serum content of folates is presented in Table 4. The serum concentrations of folates were 15.6±4.6 pmol/mL for males and 30.2±8.6 pmol/mL for females. The large sex difference was observed with the females showing a significantly higher level. Those values at

day −1 were 19.6±6.2 pmol/mL and 25.4±5.8 pmol/mL, respectively. Therefore, daily intake of 200 μg of pteroylmonoglutamic acid for 7 d maintained the serum level of folates in the male subjects, and increased it in the female subjects. No subjects with extremely low amounts of serum folates (6.8 pmol/mL) were found in either group.

Milne et al. (*42*) reported that the serum content of folates was 18.9±6.1 pmol/mL in 40 male volunteers, aged 19 to 54 y, without underlying disease in the USA. The mean plasma concentration of folates was 23.3±6.3 pmol/mL (n=39) and 23.3±7.5 pmol/mL (n=41) in 80 free-living men and women aged 50–87 y in the USA (*43*). In 6 healthy Caucasian males (aged 22–31 y) the serum value was 18 pmol/mL (*44*). It was 17.1±1.3 pmol/mL in 30 apparently healthy subjects (12 males and 18 females from 37 to 69 y of age) in Turkey (*45*). The Framingham Heart Study of 885 elderly people found the plasma folates concentrations to be greater for women (*46*): The level was 12.7 pmol/mL for men, which was significantly different from the 16.3 pmol/mL for women. Thus, a significant sexual difference was observed, which is in accordance with the present findings. On the other hand, Yukawa et al. (*47*) reported that in a folate-deficient group of the neurological patients, serum folates levels were significantly lower in females (5.0±2.0 pmol/mL, n=11) than in males (6.6±2.0 pmol/mL, n=25). A normal value for serum folates has not yet been proposed, but from these findings, the baseline values of folates in serum (or plasma) ranged from approximately 15 to 25 pmol/mL. There was also no difference of race among Japanese, Asians and Caucasians. From these findings, we conclude the RDA of folic acid in the 6th revision value of 200 μg/d is good.

The urinary content of folates is shown in Table 5. The urinary concentrations of folate were 19.4±2.8 nmol/d in the males and 22.7±2.7 nmol/d in the females. A significant difference in the values of the two groups was observed. The urinary excretion of folates ranged from 15 to 30 nmol/d in the present study, which is closely in accord with the previously reported value (11.3–90.7 nmol/d) (*48*). This would be a normal value for Japanese subjects.

Biotin

The amount of biotin supplemented in the semi-purified diet in this study was 30 μg/d, which followed the Japanese DRIs. As biotin is widely distributed in many foodstuffs, biotin is also contained in natural sources such as wheat gluten and raw cornstarch compounded as ingredients of the semi-purified diet. Therefore, the daily biotin intake was 142.7 μg for young men and 118.2 μg for young women.

Table 4 presents the serum biotin concentrations in both groups. The serum concentrations of biotin were 8.3±0.5 pmol/mL in men and 8.4±0.3 pmol/mL in women.

In a large Japanese population of 190 healthy adults (18 to 66 y, mean 34.4 y), Fukui et al. (*13*) found the mean total biotin level in their serum to be 10.9±2.2 pmol/mL. From these findings, normal values for total biotin in serum were from 7.8 to 12.2 pmol/mL. Watanabe et al. (*49*) also demonstrated that the serum biotin level in 685 elderly people was 10.2±7.2 pmol/mL, and that in 2,004 reference people, 9.4±1.4 pmol/mL, and the mean and SD of biotin levels did not differ between men and women. The normal value for serum biotin in reference people was determined to range from 6.5 to 12.1 pmol/mL. So, the present data are within this range. However, the ingested biotin was over 30 μg/d because the ingredients, raw cornstarch and gluten, contained a great deal of biotin. The evaluation of the present RDA (30 μg/d) of biotin could not be precisely made in the present experiment.

The biotin content in the urine is shown in Table 5. The urinary concentrations of biotin were 83.0±18.3 pmol/d in men and 83.2±22.5 pmol/d in women. No sex difference was observed. These results are in accord with the findings of previous studies in the USA and UK: Sweetman et al. (*50*) reported that 600 adults, aged 20–50 y, excreted biotin at the rate of 24.6–204.7 pmol/d. Similar findings were also obtained by Jung et al. (*51*) and Bitsch et al. (*52*), who demonstrated 187.1±44.6 pmol/d in 40 adults (45.7±10.9 y) and 40.9 pmol/d in 28 adults, respectively. As the ingestion of biotin was over 30 μg/d, the evaluation of the present RDA of biotin should be made carefully.

Vitamin C

The plasma level of vitamin C is shown in Table 4. No difference in the values of vitamin C was observed between sexes. The mean value of plasma vitamin C was ca. 64.5 nmol/mL. Levine et al. (*53*) reported a mean value of vitamin C in plasma of 62.0±10.2 nmol/mL for 15 women. Kobata et al. (*54*) reported that when 7 female subjects (average age of 25 y) were given a diet containing 111.0±33.0 mg/d of vitamin C, the plasma level of vitamin C remained constant through out the experimental period, at 56.8±10.8 nmol/mL. It was also reported that the mean value of vitamin C in plasma was 56.0±4.0 nmol/mL for 7 men (20–26 y old), who were administered 50 mg of the vitamin twice daily in the fasting state, at least 2-h before breakfast and dinner (*53*, *54*). Levine et al. (*55*) demonstrated that the plasma saturation of vitamin C occurred between 200 and 400 mg daily doses, whereas a 100 mg daily dose resulted in saturation of vitamin C in neurophils, monocytes, and lymphocytes. Furthermore, they indicated that a 100 mg daily dose would be a suitable intake of vitamin C for maintaining the steady-state plasma concentration at a value corresponding to the RDA of vitamin C (*56*). Recently Ihara et al. (*57*) performed an experiment with 176 young Japanese women (19–26 y old) and reported that a vitamin C intake of ca. 99-mg daily is sufficient to produce a serum vitamin C concentration above the lower reference limit of 40 nmol/mL (7 mg/L). The data reported here clearly agree with the RDA of vitamin C for the Japanese population i.e., 100 mg daily (*19*). Healthy subjects require 100 mg daily to keep the plasma con-

centration at more than 40 nmol/mL.

The sum of urinary excretion of vitamin C in men and women is shown in Table 5. No sex difference was observed, and the mean value was ca. 144 μmol/d. Levine et al. (*53*, 55) reported the effect of 30–1,250 mg doses daily on the urinary excretion of vitamin C. In their report (*53*, 55), no vitamin C was excreted in the urine of men or women until a 100-mg dose has been given orally or intravenously. With intravenous administration, the entire dose was excreted at the 500–1,250 mg doses. At an oral dose of 500 mg daily and higher, vitamin C excretion increased linearly. It was also reported that bioavailability or gastrointestinal absorption declined at intravenously delivered doses above 200 mg or at oral doses above 500 mg (*53*, 55). Our data presented here and the data reported previously indicate that a 100-mg daily dose would be a suitable intake of vitamin C to maintain the steady-state plasma concentration without affecting the urinary excretion.

CONCLUSION

We measured the values of water-soluble vitamins except for vitamin B_6 in the blood and urine of Japanese young men and women who consumed a diet based on the DRIs in the 6th revision of Recommended Dietary Allowance in the Japanese population (*1*). The RDAs of water-soluble vitamins except for vitamin B_{12} and biotin were evaluated. All of the values of water-soluble vitamins indicated these RDAs to be good. So, we propose that the present values except for vitamin B_{12} and biotin might be used as normal values for Japanese adult men and women. The requirement of vitamin B_{12} and biotin were so low that we could not precisely control them in the present diet.

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