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.

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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 $\pm$ 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 $\pm$ 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 $\pm$ 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. 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.

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.

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

Characteristics	Cutoff point (daily intake of AA, mg/day)		
	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 ($1.2 \times \text{EAR}$ 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 ($90 \text{ mg/day} \times (61 \text{ kg}/76 \text{ kg})^{0.75}$), 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, $1.5 \times \text{EAR}$) for young women.

In Levine et al.'s report (10), 12 of 13 women showed plasma AA concentrations $> 7.0 \text{ mg/L}$ 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; $61.4 \pm 7.53 \text{ kg}$) and women (20.5 ± 1.11 years old; $57.3 \pm 3.45 \text{ kg}$) to be $10.9 \pm 1.8 \text{ mg/L}$ and $11.8 \pm 2.5 \text{ mg/L}$, 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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