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Original Research

Plasma riboflavin is a useful marker for studying riboflavin requirement in Chinese male adults

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ABSTRACT

Urinary riboflavin excretion and erythrocyte glutathione reductase activation coefficient are frequently applied in determining riboflavin requirement. Previously, we found that plasma riboflavin is a sensitive marker in the assessment of riboflavin status in rat models. Here, we hypothesize that plasma riboflavin is a useful maker in studying riboflavin requirement. This study examines the changes of fasting plasma riboflavin and urinary riboflavin excretion in response to different riboflavin intake levels in Chinese male adults. The estimated average requirement (EAR) of riboflavin was extrapolated. Seventy-eight participants were randomly divided into the control and 5 riboflavin-supplemented groups. A 6-week riboflavin supplementation was performed at the doses of 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mg daily. The energy expenditure was 15.4 ± 1.9 MJ/d, as estimated by the 24-hour physical activity recording method. Dietary riboflavin intake was 1.0 ± 0.2 mg/d, based on chemical analysis. The fasting plasma riboflavin was increased significantly in a dose-dependent manner when the supplemented riboflavin exceeded 0.4 mg/d and the EAR of riboflavin was suggested to be between 1.3 and 1.5 mg/d. In addition, we found a significant increase in fasting urinary riboflavin excretion when the supplemented riboflavin exceeded 0.6 mg/d. The critical point was calculated as 1.4 mg/d, based on the intersecting point of the 2 regression lines at lower and higher riboflavin intakes. These findings demonstrate that plasma riboflavin is a sensitive marker for riboflavin status, and the EAR of riboflavin for Chinese male adults is 1.4 mg.

1. Introduction

Riboflavin acts as coenzymes in the forms of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) for numerous oxidases, reductases, and dehydrogenases in metabolic processes. Riboflavin deficiency results in low FMN and FAD contents in the cell and reduced activity of a series of flavin-containing enzymes [1,2]. Mitochondrial β-oxidation was impaired remarkably by riboflavin deficiency because several flavin-containing enzymes, such as acyl-CoA dehydrogenase, are involved in mitochondrial β-oxidation [3]. Riboflavin also plays a role in glutathione synthesis, in which FAD is a cofactor for glutathione reductase. The erythrocyte glutathione reductase activation coefficient (EGRAC) is frequently used as a marker in the assessment of riboflavin status [4]. Moreover, riboflavin is required for methylenetetrahydrofolate reductase and plays an

Abbreviations: CNS, Chinese Nutrition Society; EAR, estimated average requirement; EGRAC, erythrocyte glutathione reductase activation coefficient; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide.

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Table 1 – Baseline characteristics of the study participants

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Participants (n)	13	13	13	13	13	13
Age (y)	21 ± 2	20 ± 1	20 ± 2	21 ± 2	21 ± 2	20 ± 1
Height (cm)	175.2 ± 3.9	174.8 ± 7.0	174.9 ± 5.9	170.0 ± 5.2	176.8 ± 6.1	172.5 ± 5.8
Body weight (kg)	62.9 ± 6.5	68.8 ± 13.8	67.5 ± 6.4	66.1 ± 6.0	67.9 ± 8.7	65.4 ± 5.8
Body mass index (kg/m^2)	20.5 ± 1.6	22.5 ± 3.1	22.1 ± 1.9	22.9 ± 1.4	22.0 ± 2.8	22.0 ± 2.4

Values are expressed as means ± SD.

important role in homocysteine metabolism, especially in individuals with a methylenetetrahydrofolate reductase 677 TT genotype [5].

The present estimated average requirement (EAR) of riboflavin in the United States is based mainly on studies conducted more than 50 years ago, in which 24-hour urinary riboflavin excretion was used as an indicator [6]. The critical point of urinary riboflavin excretion, a point of intersection of the regression lines fitted to the excretion at higher and lower riboflavin intakes, is considered the level of intake at which organ or tissue riboflavin saturation occurs [7]. Later, the EGRAC method was developed and applied in determining riboflavin requirement in children, women, and the elderly [8–10]. However, the acceptable range of activity coefficient values is too small in magnitude (from 1.0 to 1.2), as recommended by Sauberlich et al [11]. Boisvert et al [10] found that the changes of the EGRAC value were without a clear delineation between inadequate and adequate riboflavin status, although a decreasing trend was recorded with increasing riboflavin intake in aged participants. In 2005, Xu et al [12] reported that fasting plasma riboflavin was decreased rapidly in response to a riboflavin-deficient diet and recovered soon after a riboflavin-containing diet was provided in rats. In riboflavin malnourished humans, it was confirmed that fasting plasma riboflavin was increased significantly after 1 week of riboflavin supplementation [13]. A consistent result was also reported by Hustad et al [14] in a human study, in which plasma riboflavin was demonstrated to be useful in the assessment of riboflavin status. Therefore, it appears that plasma riboflavin is a sensitive index for riboflavin status.

The purpose of this study is to validate the effectiveness of plasma riboflavin in assessing riboflavin status and further to determine the EAR of riboflavin for Chinese male adults. We hypothesize that plasma riboflavin is a useful maker in studying riboflavin requirement. To test this hypothesis, the changes of fasting plasma riboflavin in response to different riboflavin intake levels were determined in Chinese male adults after 6 weeks of intervention. In addition, fasting urinary riboflavin excretion was simultaneously measured.

2. Methods and materials

2.1. Participants and study design

Seventy-eight male adults aged 18 to 22 years from an army unit in Tianjin, PR China, were recruited. They were nonsmokers and physically healthy and volunteered to participate in this study. No classic signs of riboflavin deficiency, such as cheilosis, angular stomatitis, and glossitis, were found in a routine physical examination. A written informed consent was signed by all participants. Approval for this study was granted by the ethical committee of the Institute of Health and Environmental Medicine. The participants were randomly assigned to the control and 5 riboflavin-supplemented groups by selection of a random number. The general characteristics of the study participants are presented in Table 1. During the 6-week experimental period, all participants led a similar lifestyle and were engaged in routine physical training. They were asked to abstain from any riboflavin-containing supplements and were group fed in a dining room on a menu designed by a dietitian. Riboflavin supplementation was performed via the drinks containing different amounts of riboflavin for the different groups. The drinks were prepared freshly by dissolving riboflavin (≥98% in purity, provided by Tianjin Feiying Pharmaceutical Co, Ltd, Tianjin, PR China) in 200 mL of water and consumed by the participants every morning under the supervision of the field staff. The doses of riboflavin for different riboflavin-supplemented groups were 0.2, 0.4, 0.6, 0.8, and 1.0 mg daily, respectively. At these low concentrations, riboflavin was completely soluble in the water. During the experimental period, 5 participants withdrew from groups 1 (control), 2 (0.2 mg), 3 (0.4 mg), 5 (0.8 mg), and 6 (1.0 mg), respectively, and their data were excluded from the final statistical analyses (Fig. 1).

2.2. Assessment of dietary intakes

A 4-day dietary survey was performed in the last week, using a food-weighting method. Dietary intakes of energy and nutrients (including riboflavin) were calculated based on food intakes and Chinese Food Composition, which was compiled by the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention [15]. From the second week to the last week, daily duplicate food samples consumed by the participants were selected randomly from each group, collected, and stored at –80°C before being analyzed fluorophotometrically for riboflavin content by a standardized procedure approved by the Ministry of Public Health, PR China [16].

2.3. Estimation of daily energy expenditure

From the third week to the fifth week, 3 to 4 participants were selected randomly from each group for estimation of daily energy expenditure by the 24-hour physical activity recording method [17]. The field staff followed the participants from the