



Dietary Riboflavin Intake and Riboflavin Status in Young Adult Women Living in Metro Vancouver, Canada

Abeer M Aljaadi,¹ Alejandra M Wiedeman,² Susan I Barr,³ Angela M Devlin,² and Tim J Green⁴

¹Clinical Nutrition Department, Umm Al-Qura University, Makkah, Saudi Arabia; ²Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada; ³Food, Nutrition, and Health, University of British Columbia, Vancouver, British Columbia, Canada; and ⁴Women and Kids Theme, South Australian Health and Medical Research Institute, and Discipline of Paediatrics, University of Adelaide, Adelaide, South Australia, Australia

ABSTRACT

Background: Nutrition surveys suggest that <10% of Canadian adults have inadequate riboflavin intakes. However, biochemical riboflavin deficiency [erythrocyte glutathione reductase activity coefficient (EGRac) ≥ 1.40] has been reported in 41% of young adult women living in Metro Vancouver. Canadian Chinese ethnicity comprise >25% of Vancouver's population and are postulated to have poorer riboflavin status than those of European ethnicity because they could be less likely to consume dairy products and fortified wheat.

Objectives: The objectives of this study were to determine dietary riboflavin intake and food sources, and to assess the association between riboflavin intake and status in young women of European ($n = 107$) and Chinese ($n = 91$) ethnicities living in Metro Vancouver, Canada.

Methods: This was a cross-sectional study conducted in women (aged 19–45 y). Women were healthy, not pregnant or breastfeeding, of European or Chinese ethnicities, and not taking riboflavin-containing supplements for the past 4 mo. Dietary riboflavin intake was assessed using the past-year Diet History Questionnaire II, and riboflavin status (EGRac) was measured in fasting venous blood samples.

Results: Only 7% of participants had dietary riboflavin intakes below the Estimated Average Requirement (0.9 mg/d), but 40% of women had biochemical riboflavin deficiency (EGRac ≥ 1.40). Although more Canadian women of European ethnicity than Chinese ethnicity had biochemical riboflavin deficiency (46% and 34%; $P < 0.001$), median dietary riboflavin intake did not differ (1.73 and 1.82 mg/d; $P = 0.587$). Dairy products and vegetables contributed the most to riboflavin intake. Energy-adjusted dietary riboflavin intake was inversely associated with EGRac ($B = -0.04$, 95% CI: $-0.07, -0.01$). However, after further adjustment the relation was not significant.

Conclusions: Overall, women of reproductive age living in Metro Vancouver, Canada, had a low prevalence of inadequate dietary riboflavin intake despite the high prevalence of apparent biochemical riboflavin deficiency. *Curr Dev Nutr* 2021;5:nzab021.

Keywords: riboflavin intake, riboflavin status, EGRac, reproductive age, women, C-DHQ II, food sources, Vancouver, Canada

© The Author(s) 2021. Published by Oxford University Press on behalf of the American Society for Nutrition. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Manuscript received December 21, 2020. Initial review completed March 1, 2021. Revision accepted March 5, 2021. Published online March 13, 2021.

The Dairy Farmers of Canada supported this research. AMA is supported by a scholarship from Umm Al-Qura University administered by the Saudi Arabian Cultural Bureau in Canada, and supported by a Bertram Hoffmeister Postdoctoral Fellowship from BC Children's Hospital Research Institute and support from Becas-Chile. AMD is supported by an Investigator Grant from BC Children's Hospital Research Institute. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author disclosures: The authors report no conflicts of interest.

Supplemental Tables 1–3 and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/cdn/>.

Address correspondence to AMA (e-mail: amjaadi@uqu.edu.sa) or AMW (e-mail: awiedeman@bcchr.ca).

Abbreviations used: BC, British Columbia; CCHS, Canadian Community Health Survey; C-DHQ II, Canadian version of the National Cancer Institute Diet History Questionnaire II; DHQ, Diet History Questionnaire; EAR, Estimated Average Requirement; EGRac, erythrocyte glutathione reductase activity coefficient; FPED, Food Patterns Equivalents Database; IPAQ, International Physical Activity Questionnaire; MPED, MyPyramid Equivalent Database; NDNS, National Diet and Nutrition Survey; US-DHQ II, the US Diet History Questionnaire II.

Introduction

Riboflavin in its coenzyme forms, FMN and FAD, participates in numerous redox reactions involved in energy metabolism and in different metabolic pathways (1). Severe riboflavin deficiency is associated with dermatological and visual abnormalities (1, 2). Milder forms of riboflavin deficiency, based on riboflavin biomarkers, have been associated with anemia and hypertension in women as well as pre-eclampsia in pregnancy (3–9). Riboflavin status has generally been assumed to be sufficient in Canadian women. However, a small study in Vancouver re-

ported that 41% of female university students (mean age = 26.3 ± 4.6 y; $n = 49$) had deficient [assessed using the functional indicator, erythrocyte glutathione reductase activity coefficient (EGRac) ≥ 1.40] and an additional 29% had marginal (EGRac = 1.30–1.40) riboflavin status (10).

Based on the results of this small study in Vancouver women, we conducted a larger study in 2015–2016 and measured EGRac in young Canadian women of Chinese ($n = 96$) and European ($n = 110$) ethnicity in Vancouver (3). Differences in nutrient intakes by ethnicity have been previously reported in women of reproductive age (11, 12). We

included women of Chinese ethnicity because we thought they would be at greater risk of riboflavin insufficiency due to a lower dairy intake, a major source of riboflavin (13–16). Overall, 40% of women were classified as riboflavin deficient ($EGRac \geq 1.40$) (3). Unexpectedly, the rate of marginal and deficient biochemical riboflavin status combined was lower in Canadian women of Chinese ethnicity compared with those of European ethnicity (57% compared with 85%, $P < 0.001$) (3).

Despite a high rate of biochemical riboflavin deficiency in our study, population representative data from the Canadian Community Health Survey (CCHS) in 2004 suggest that riboflavin intakes are mostly adequate, with <10% of women aged 19–50 y consuming less than the Estimated Average Requirement (EAR, 0.9 mg/d) (17). This survey is now >15 y old. Two recent studies in Canada on the contribution of dairy products and grains to nutrient intake reported that milk and alternatives contributed 24% of total riboflavin intake in adults, and that grain products contributed 19% of total riboflavin intake in participants aged ≥ 2 y, using data collected from the 2015 CCHS (18, 19); the contribution of other food sources to riboflavin intake in Canada remains unknown.

In the current study, we assessed dietary riboflavin intake using a semiquantitative FFQ. Our aims here were to determine riboflavin intake, the prevalence of dietary inadequacy, and food sources of riboflavin, as well as to examine the association between $EGRac$ and riboflavin intake in this group. Finally, we explored whether a difference in riboflavin intake could explain the lower rate of biochemical deficiency in women of Chinese ethnicity.

Methods

Study design and participants

This was a cross-sectional study of 206 women aged 19–45 y from Metro Vancouver, Canada, conducted between 2015 and 2016 (3). Women were recruited by convenience sampling through posters, e-mails, and advertisements on social media. To be included, women had to be non-pregnant or nonlactating, and not have taken a riboflavin-containing supplement in the 4 mo preceding the study. Women were excluded if they self-reported having β -thalassemia, untreated hypothyroidism, or glucose 6-phosphatase dehydrogenase deficiency (20–22). A sample size calculation indicated that a minimum of 84 participants in each ethnic group was required to provide 80% power to detect an association with medium effect size ($r = 0.30$) between dietary riboflavin intake and riboflavin status ($EGRac$) with $\alpha = 0.05$ (2-tailed) (23). Weight and height were measured in duplicate with use of standardized techniques (24). Demographic characteristics including self-reported ethnicity, marital status, education level, lactose intolerance, and household size and income were assessed by a questionnaire. Venous blood samples were collected after an overnight fast. Washed erythrocytes were collected and analyzed for $EGRac$ as previously described (3, 25). Physical activity data were obtained using the long-form of the International Physical Activity Questionnaire (IPAQ) and the IPAQ scoring protocol (26). Ethics approval was obtained from the University of British Columbia Clinical Research Ethics Board in Canada (H15-00,521). All participants were offered a gift card for participation and received their individual results. Written informed consent was obtained from all women.

Dietary riboflavin intake measurement

The Canadian version of the National Cancer Institute Diet History Questionnaire II (C-DHQ II) was used to assess dietary riboflavin intake over the previous year (27). The C-DHQ II consists of 165 questions (153 are related to food and 12 are nutritional supplement questions). The US version of the DHQ I has undergone extensive validation for energy and other nutrients in women, with correlations ranging from 0.51 for vitamin E to 0.78 for magnesium, and riboflavin at 0.66 (28). Dietary data were analyzed using Diet*Calc (version 1.5.0) and the estimated dietary riboflavin intakes were expressed as milligrams per day. Dietary riboflavin intakes were compared with the EAR of 0.9 mg/d set by the National Academy of Medicine (29).

Food source determination

To determine food sources of riboflavin in the Canadian diet, individual food items from the C-DHQ II were allocated into food groups. Because the C-DHQ II does not provide food groups, individual food items were manually grouped using Stata statistical software version SE/14.2 for Mac (StataCorp LLC). Main groups were based on the 2007 Canada's Food Guide (30) with the exception that Vegetables and Fruits were separated into 2 groups; accordingly, the groups were: 1) Grain Products; 2) Milk and Alternatives; 3) Meat and Alternatives; 4) Vegetables; and 5) Fruits. The rest of the food items were grouped into: 6) Fats and Oils; 7) Beverages; 8) Meal-replacement bars and drinks; and 9) Sweets and Desserts. Food items were also allocated into 29 subgroups (31, 32) as shown in **Supplemental Table 1**.

Mixed main dishes in the C-DHQ II were disaggregated into individual food items prior to grouping using the US Diet History Questionnaire II (US-DHQ II) nutrient database (33) or recipes. The US-DHQ II nutrient database provides food groups based on the Food Patterns Equivalents Database (FPED) and MyPyramid Equivalent Database (MPED). Mixed main dishes ($n = 19$) were defined as dishes that contain a mix of meats, dairy, grains, or vegetables, such as pizza, pasta dishes, and sandwiches. The disaggregation process is shown in **Supplemental Figure 1**. Briefly, each mixed main dish was broken down into servings of main groups (measured in US cups or ounces per 100 g). The MPED/FPED equivalent was then adjusted to MPED/FPED value per gram weight of C-DHQ II portion weight (grams). To determine the ingredients of mixed foods (e.g., type of meat, vegetable, grain), descriptors in the US-DHQ II nutrient database (33) were also used. The riboflavin content for each ingredient was then obtained from the C-DHQ II nutrients database. A threshold of $\pm 10\%$ difference between the calculated riboflavin of the disaggregated food and the actual/total riboflavin content obtained from the C-DHQ II nutrient database was considered acceptable.

When disaggregation was finalized, the amounts of riboflavin (milligrams) from ingredients of each disaggregated food were converted into proportions of total calculated riboflavin. For example, riboflavin from “pizza with meat” comes from grains (67%), cheese (26%), red meat (5%), and vegetables (3%). These proportions were applied to the corresponding mixed main dish in the Stata file for all participants. To create food group equivalents representing the 2007 Canada's Food Guide servings, the US-DHQ II nutrient database

TABLE 1 General characteristics of Canadian women (aged 19–45 y) of European and Chinese ethnicity living in Metro Vancouver¹

	All (n = 198)	European (n = 107)	Chinese (n = 91)	P
Age, y	27.60 (26.70, 28.49)	28.79 (27.63, 29.95)	26.20 (24.84, 27.56)	0.004
BMI, kg/m ²	22.41 (21.86, 22.96)	23.14 (22.35, 23.92)	21.56 (20.81, 22.30)	0.005
Overweight or obese (BMI ≥25), n (%)	38 (19)	25 (23)	13 (14)	0.106
Total physical activity scores, METs-min/wk	3193 [1514, 5580]	3999 [2337, 6458]	1835 [1016, 4128]	<0.001
Current smokers, n (%)	2 (1)	2 (2)	0	0.501
Education, n (%)				<0.001
Bachelor's or higher	131 (66)	85 (79)	46 (51)	
Marital status, n (%)				0.001
Single	146 (74)	71 (67)	75 (83)	
Married/common-law	47 (24)	35 (33)	12 (13)	
Separated/divorced	3 (2)	0	3 (3)	
Number in household	2 (2, 2)	2 (2, 2)	3 (2, 3)	0.003
Has children, n (%)	14 (7)	8 (7)	6 (7)	0.809
Generation in Canada, n (%)				<0.001
First generation	91 (46)	31 (29)	60 (66)	
Second generation	35 (18)	6 (6)	29 (32)	
Mixed generations	16 (8)	15 (14)	1 (1)	
Third generation	24 (12)	23 (22)	1 (1)	
Fourth generation or higher	31 (16)	31 (29)	0	
Annual household income, n (%)				0.621
<\$20,000	45 (23)	23 (22)	22 (24)	
\$20,000 to <\$40,000	40 (21)	17 (16)	23 (26)	
\$40,000 to <\$60,000	26 (13)	16 (15)	10 (11)	
\$60,000 to <\$80,000	31 (16)	18 (17)	13 (14)	
\$80,000 to <\$100,000	19 (10)	11 (11)	8 (9)	
≥\$100,000	34 (17)	20 (19)	14 (16)	
Lactose intolerant, ² n (%)	15 (8)	6 (6)	9 (10)	0.256
Excludes foods (vegetarian), ³ n (%)	30 (15)	17 (16)	13 (14)	0.754
Excludes meat	26 (13)	14 (13)	12 (13)	0.865
Excludes poultry	19 (10)	11 (10)	8 (9)	0.723
Excludes fish and shellfish	11 (6)	5 (5)	6 (7)	0.557
Excludes eggs	5 (3)	2 (2)	3 (3)	0.663
Excludes dairy products	7 (4)	4 (4)	3 (3)	1.000

¹Data are expressed as means (95% CI) or n (%), except for physical activity (median [25th, 75th percentiles] and n = 186). Independent samples t test or Wilcoxon rank-sum test (continuous) and χ^2 test (proportions) were used to compare groups. MET, metabolic equivalent.

²Self-identified as lactose intolerant.

³Excluding meat included beef, pork, lamb, etc.; poultry included chicken, turkey, duck, etc.; and dairy products included milk, cheese, etc.

file was used with modifications; details are shown in **Supplemental Figure 2**.

Statistical analyses

Descriptive statistics were used to report means (95% CI) and medians (25th and 75th percentiles). For comparisons between Canadian women of European and Chinese ethnicity, independent samples *t* test or Wilcoxon rank-sum test were used for continuous variables, and χ^2 test was used for comparison of proportions; Fisher exact test was used when expected cell frequencies in the 2 × 2 table were ≤5. A linear regression model was used to examine the association between dietary riboflavin intake (continuous) and riboflavin status (EGRac; continuous outcome). Total energy intake was included in the models as a continuous variable to control for a potential confounding effect (34). Other variables were included in the model based on a change-in-estimate approach, in which a variable is included in the model if eliminating it led to ≥10% change in the coefficient of dietary riboflavin intake and riboflavin status (35). Models included energy intake, ethnicity, physical activity, marital status, and household income as confounders. One-

factor ANOVA with post hoc Bonferroni adjustments was used to estimate differences in EGRac by tertiles of dietary riboflavin intake or by dietary riboflavin intake per 1000 kcal.

Results

Dietary data were available for 205 women. After excluding 7 women with extreme energy intakes (<600 and >3500 kcal/d) (28), data from 198 women were used in the analyses. Participant characteristics are given in **Table 1**. Overall, >60% of the women had a bachelor's degree or higher level of education, >70% were single, and >40% were first-generation immigrants to Canada. Only 3.5% of the women reported excluding dairy products from their diet.

Ethnic differences between European and Chinese women were observed in age, BMI, total physical activity scores, education, marital status, household size, and generation of immigration to Canada. The Canadian women of Chinese ethnicity were more likely to be younger and single, and almost all were first- or second-generation immigrants,

TABLE 2 Riboflavin status and dietary riboflavin and energy intakes in Canadian women (aged 19–45 y) of European and Chinese ethnicity living in Metro Vancouver¹

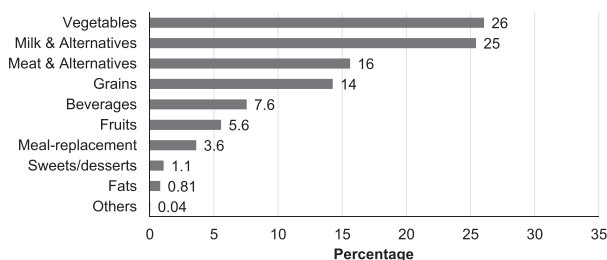
	All (n = 198)	European (n = 107)	Chinese (n = 91)	P
EGRac, ratio	1.38 (1.36, 1.40)	1.40 (1.38, 1.42)	1.36 (1.33, 1.38)	0.016
Riboflavin status, n (%)				<0.001
Adequate, EGRac < 1.30	56 (28)	16 (15)	40 (44)	
Marginal, 1.30 ≤ EGRac < 1.40	62 (31)	42 (39)	20 (22)	
Deficient, EGRac ≥ 1.40	80 (40)	49 (46)	31 (34)	
Dietary riboflavin intake, mg/d				
Median [25th, 75th percentiles]	1.76 [1.30, 2.33]	1.73 [1.32, 2.22]	1.82 [1.30, 2.64]	0.587
Mean (95% CI)	1.90 (1.78, 2.01)	1.84 (1.70, 1.98)	1.96 (1.77, 2.15)	0.320
Total energy intake, kcal/d				
Median [25th, 75th percentiles]	1654 [1233, 2077]	1714 [1278, 2096]	1588 [1142, 2070]	0.252
Mean (95% CI)	1708 (1623, 1793)	1759 (1640, 1878)	1647 (1524, 1770)	0.197
Dietary riboflavin intake, mg/1000 kcal				
Median [25th, 75th percentiles]	1.05 [0.94, 1.21]	1.03 [0.93, 1.14]	1.13 [0.94, 1.37]	0.010
Mean (95% CI)	1.11 (1.07, 1.14)	1.05 (1.01, 1.08)	1.18 (1.11, 1.24)	<0.001
Inadequate dietary riboflavin intake (<0.9 mg/d), n (%)				0.244
95% CI	13 (7) (4, 11)	5 (5) (2, 11)	8 (9) (4, 17)	

¹Data were analyzed by independent samples t test or Wilcoxon rank-sum test (continuous) and χ^2 test (proportions). Prevalence of inadequate intake was estimated as percentage less than the Estimated Average Requirement of 0.9 mg/d. EGRac, erythrocyte glutathione reductase activity coefficient.

whereas the women of European ethnicity were more likely to have higher BMI, education level, and physical activity scores.

Biochemical riboflavin status (EGRac) and dietary riboflavin and energy intakes are shown in **Table 2**. Overall, mean EGRac was 1.38 (95% CI: 1.36, 1.40) for all women, and 40% were classified as biochemically riboflavin deficient (EGRac ≥ 1.40). Riboflavin status was poorer in Canadian women of European compared with Chinese ethnicity: their mean EGRac was significantly higher, and only 15% of Canadian women of European ethnicity had “adequate” riboflavin status (EGRac < 1.30) compared with 44% of Canadian women of Chinese ethnicity. For all women, median (25th, 75th percentiles) dietary riboflavin intake was 1.76 (1.30, 2.33) mg/d, and only 7% of all women had dietary riboflavin intakes less than the EAR (0.9 mg/d). Dietary riboflavin intake was similar in Canadian women of European and Chinese ethnicity. However, when expressed on a per 1000 kcal basis, Canadian women of European ethnicity had lower median dietary riboflavin intake than Canadian women of Chinese ethnicity.

Food sources of riboflavin are presented for all women in **Figure 1** and by ethnicity in **Figure 2**. The predominant sources of dietary riboflavin were vegetables (26%) and milk and alternatives (25%).

**FIGURE 1** Food sources of riboflavin intake in young adult women (aged 19–45 y) living in Metro Vancouver (n = 198).

Riboflavin from grain products, and meat and alternatives accounted for 14% and 16%, respectively. Other groups that provided ≥5% of total riboflavin intake were fruits (7%) and beverages (6%). Although Canadian women of Chinese ethnicity appeared to consume less riboflavin from milk and milk alternatives and more from vegetables, they were not statistically different from Canadian women of European ethnicity. Dark-green vegetables and milk contributed 18% and 14%, respectively, to total riboflavin intake from food sources (**Supplemental Table 2**). Eggs and nonalcoholic beverages (e.g., caffeinated beverages, energy and sport drinks, and soft drinks) each contributed ~7% to dietary riboflavin intake. Breakfast cereals contributed <5% to dietary riboflavin intake. No significant ethnic-specific differences were found.

The mean number of daily servings from each of the 2007 Canada's Food Guide food groups is shown in **Supplemental Table 3**. Women reported consuming, on average, 1.5 servings of milk and alternatives per day, but the median was 1.3 servings. Median consumption of fluid milk, including plant-based beverages, was 0.6 cups/d. Further, median

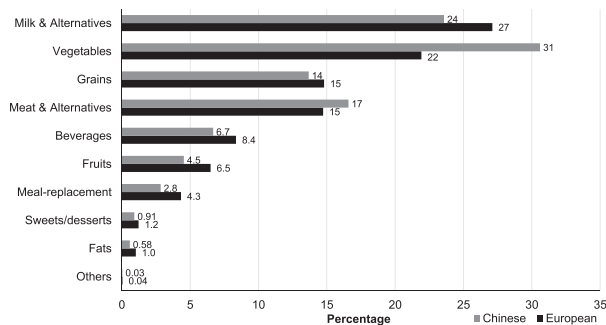
**FIGURE 2** Food sources of riboflavin intake in young adult Canadian women of European (n = 107) and Chinese (n = 91) ethnicity living in Metro Vancouver.

TABLE 3 Relation between dietary riboflavin intake and riboflavin status (EGRac) in women (aged 19–45 y) living in Metro Vancouver ($n = 198$)¹

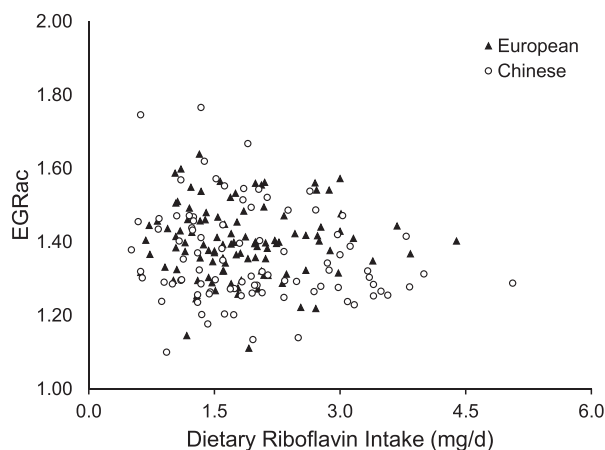
	B (95% CI) for dietary riboflavin	P
Model 1 (unadjusted)	−0.02 (−0.04, 0.00)	0.081
Model 2	−0.04 (−0.07, −0.01)	0.021
Model 3	−0.03 (−0.07, 0.00)	0.082
Model 4	−0.03 (−0.07, 0.01)	0.100

¹Linear regression was used with EGRac as a continuous outcome and dietary riboflavin intake as the independent predictor ($n = 183$ – 198). Model 1 $R^2 = 0.02$. Model 2 was adjusted for total energy intake ($n = 198$; model $R^2 = 0.03$; partial R^2 for dietary riboflavin was 0.03). Model 3 was adjusted for total energy intake and ethnicity ($n = 198$; model $R^2 = 0.05$; partial R^2 for dietary riboflavin was 0.02). Model 4 was adjusted for energy intake, ethnicity, physical activity, marital status, and income ($n = 183$; model $R^2 = 0.09$; partial R^2 for dietary riboflavin was 0.02). EGRac, erythrocyte glutathione reductase activity coefficient.

servings of total fruits and vegetables consumed was 4.6 servings/d, with a median of 1 serving of dark-green vegetables per day. Women reported a median of 4.6 servings/d of grain products and 2.1 servings/d of meat and alternatives.

There was no association between dietary riboflavin intake and EGRac (B: −0.02; 95% CI: −0.04, 0.00; $P = 0.08$) as shown in **Table 3** and **Figure 3**. After adjustment for energy intake, dietary riboflavin intake was inversely associated with EGRac (B: −0.04; 95% CI: −0.07, −0.01; $P = 0.02$), and dietary riboflavin explained 3% of the variance in EGRac. Dietary riboflavin was no longer associated with EGRac after further adjustments (**Table 3**).

Using ANOVA, EGRac was compared between tertiles of dietary riboflavin intake or dietary riboflavin intake per 1000 kcal (**Figure 4**). There was no difference in EGRac across tertiles of total riboflavin intake (milligrams per day), but EGRac did vary across tertiles of riboflavin intake when expressed as milligrams per 1000 kcal ($P = 0.04$). Post hoc analysis showed that only women in the highest tertile of dietary riboflavin intake per 1000 kcal (>1.14 mg/1000 kcal) had significantly lower EGRac (better riboflavin status) compared with

**FIGURE 3** Scatterplot of the association between dietary riboflavin intake and EGRac in Canadian women of European and Chinese ethnicities. $n = 198$. EGRac, erythrocyte glutathione reductase activity coefficient.

women in the lowest tertile of dietary riboflavin intake per 1000 kcal (<0.98 mg/1000 kcal).

Discussion

In this convenience sample of young adult women from Metro Vancouver, the majority of women had adequate dietary riboflavin intake despite the high rates of apparent biochemical riboflavin deficiency (as assessed by $EGRac \geq 1.40$). Although Canadian women of European ethnicity had poorer riboflavin status compared with Canadian women of Chinese ethnicity, there were no significant ethnic differences in the prevalence of inadequate dietary riboflavin intake or dietary riboflavin intake. Moreover, dietary sources for riboflavin intake did not differ by ethnicity. Lastly, dietary riboflavin intake was not associated with riboflavin status (EGRac).

In this study, the prevalence of inadequate dietary riboflavin intake was similar to results previously reported in the CCHS (2004) and British Columbia (BC) Nutrition Survey (1999). In the 2004 CCHS, $<3\%$ of women aged 31–50 y in BC had dietary riboflavin intakes below the EAR, but data were not reported for women aged 19–30 y due to extreme sampling variability (17). Median (25th, 75th percentiles) dietary riboflavin intake in the BC Nutrition Survey was 1.6 (1.4, 2.0) and 1.5 (1.3, 1.8) mg/d in women aged 19–30 y ($n = 266$) and 31–50 y ($n = 282$), respectively; only 2% and 8% of females aged 19–30 y and 31–50 y, respectively, reported dietary riboflavin intakes below the EAR (36). In our study, median riboflavin intake was 1.8 (1.3, 2.3) mg/d, which is slightly higher than that reported in the BC Nutrition Survey, but the intake distribution and prevalence of inadequate dietary intake are comparable. Similar findings are reported in US populations (11), where riboflavin fortification is mandatory and similar to Canada (37).

In this study, the major sources of dietary riboflavin were vegetables (26%) and milk and alternatives (25%), followed by meat and alternatives (16%) and grains (14%). The contribution of grains was slightly lower than that reported by a study of the contribution of nutrients from grain-based foods from the 2015 CCHS, in which grain-based foods contributed 19% of riboflavin intake in Canadians; however, the results included both males and females, and children (≥ 2 y; $n = 19,797$) (19). It is not surprising that 25% of dietary riboflavin intake was from milk and alternative sources. A study of the contribution of nutrients from milk and alternatives from the 2015 CCHS reported that milk and alternatives contributed 24% of riboflavin intake in adults (aged ≥ 19 y; $n = 13,616$), with milk being the top milk and alternative source, contributing $\sim 15\%$ to total dietary riboflavin intake (18).

In the current study, it was unexpected that vegetables contributed 26% of dietary riboflavin intake. Although some vegetables, such as dark-green vegetables, are good sources of riboflavin, the maximum contribution of vegetables and vegetable products in European surveys was only 13% (range: 3–13%) of total dietary riboflavin intake (38). Details of food items categorized as vegetables are provided in Supplemental Table 1. Median daily servings of total fruits and vegetables (4.6 servings) in the current study is similar to the mean (\pm SE) of 4.7 ± 0.1 servings of total fruits and vegetables reported by adults (18–54 y) in the 2015 CCHS (39). We reported that median daily servings of dark-green vegetables was 1.03, which is higher than the mean daily intake (0.7 servings) of dark-green and orange vegetables in adults in the 2015

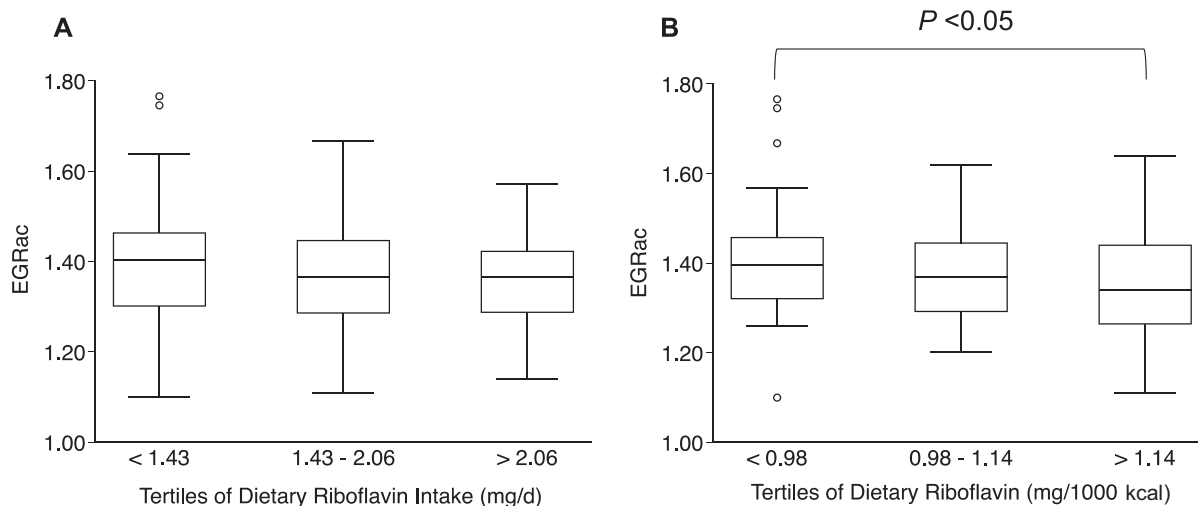


FIGURE 4 Median (interquartile range) of EGRac according to tertiles of (A) daily dietary riboflavin intake and (B) dietary riboflavin intake per 1000 kcal with riboflavin status (EGRac) for Canadian women of European and Chinese ethnicities. Data were analyzed by ANOVA with Bonferroni correction ($n = 198$). EGRac, erythrocyte glutathione reductase activity coefficient.

CCHS (39). Overall, our findings showed that daily servings of total fruits and vegetables are similar to national estimates, but intakes of dark-green vegetables, which are a good source of riboflavin, appeared to be higher.

The high prevalence of biochemical riboflavin deficiency observed in women with adequate dietary intakes has been previously reported in other populations. The UK's National Diet and Nutrition Survey (NDNS) 2014–2016 reported a median (2.5th, 97.5th percentile) intake of 1.39 (0.48, 2.79) mg/d, and that 14% of women aged 19–64 y had riboflavin intakes below the Lower Reference Nutrient Intake of 0.8 mg/d (40). However, 61% of women had EGRac >1.30 (data on prevalence of EGRac >1.40 or consumption less than the EAR were not reported in recent NDNSs) (40). A survey of German adults ($n = 2006$) reported a median EGRac of 1.37 (41) and median dietary riboflavin intake was 1.3 mg/d (2.5th, 97.5th percentiles: 0.5, 2.9 mg/d) in women (42). Another study conducted in older adults (aged ≥ 65 y; $n = 62$; 82% women) from Northern Ireland reported that dietary riboflavin intake was not correlated with EGRac ($r = 0.12$; $P = 0.35$) (43). The study also reported that 12% had an EGRac >1.40 and 33% had marginal status (EGRac = 1.20–1.40) (43). A subsequent 12-wk riboflavin intervention trial ($n = 41$) in the same population reported that only supplementation with 25 mg/d of riboflavin resulted in a significant reduction in EGRac; no significant change from baseline was observed in subjects who received 1.6 mg/d riboflavin or placebo (43).

The lack of association between dietary riboflavin intake and riboflavin status that we report is similar to previous studies (43–45). A study of Canadian adults (≥ 65 y; $n = 60$, 50% women) from Edmonton reported that the majority of participants had adequate riboflavin intake (assessed by nonconsecutive 3-d food record) and found no relation between EGRac and dietary riboflavin intake (45). Similarly, a study conducted in Spanish adults (25–60 y, $n = 372$, 51% women) reported no significant correlation between intake (assessed by 48-h recall) and EGRac (44).

A lack of relation between nutrient intakes and biomarkers of status can occur when intakes exceed requirements, and variability in the biomarker status can be due to factors other than the nutrient. In addition, the ability of dietary assessment tools to accurately capture riboflavin intake could be a confounder in our study. However, the riboflavin intakes in our study are comparable to those reported by the 2004 CCHS, 1999 BC Nutrition Survey, and 2003–2006 US NHANES. Moreover, the EGRac values used to define marginal and deficient riboflavin status could be too low. Studies that established EGRac ≥ 1.30 or ≥ 1.40 as the threshold of normality (46–48) employed older methods of the EGRac assay, which used higher FAD concentrations ($>5 \mu\text{M}$) than current methods, which use $\leq 2 \mu\text{M}$ FAD. As such, high FAD concentrations can lead to an underestimation of EGRac due to the potential inhibitory effect of high FAD concentrations on glutathione reductase (49). Many studies employing the older methods report EGRac values < 1 , which has been criticized (49). Some gut microbiota produce riboflavin, but their contribution to riboflavin status is not well established in humans and riboflavin is predominantly absorbed in the proximal small intestine (50).

Our study had a number of strengths. We provided data on both dietary riboflavin intake and riboflavin status in a relatively large sample, including 2 major ethnic groups in Metro Vancouver that allowed for the exploration of ethnic-specific differences. We used a standardized FFQ (C-DHQ II), which could facilitate comparisons of these findings with future studies. Some limitations include that the C-DHQ II has not been validated; however, it is expected to perform similar to the validated US-DHQ I (28, 51) because only minor modifications were incorporated (52). In addition, the use of self-reported methods of dietary assessment such as C-DHQ II is associated with systematic and random reporting errors although values reported in this study were comparable to values reported by major national surveys (17, 36). Errors in intake estimation can occur due to serving sizes and ethnic foods limitations. We are only reporting on dietary riboflavin intake, not total riboflavin intake, because supplement users were excluded at recruitment; median

riboflavin intakes and status are commonly higher with supplement use (12, 53, 54). Lastly, the use of a nonrepresentative convenience sample limits the ability to control for selection bias and to generalize these findings to the entire population of young adult women living in Metro Vancouver. The majority of the women in this study were of high education and income levels, limiting the generalizability of the findings to all women in Vancouver, including those with low socioeconomic status. For example, 27% of the women reported an annual household income \geq \$80,000, compared with 10% in the 2016 census reported by women (aged \geq 15 y) living in Vancouver (55). Over 60% of our sample had obtained a bachelor's degree or higher, compared with 39% of women aged 25–64 y in Metro Vancouver reported in the 2016 census (55). Others have reported associations of low education levels (9) and low socioeconomic status (11, 56) with lower riboflavin intakes.

Overall, this study suggested that a low prevalence of inadequate riboflavin intake can occur despite a high prevalence of apparent biochemical riboflavin deficiency. The discrepancy between riboflavin intake and status reported in our study, along with previous studies, raises the question of whether the deficiency cutoffs for EGRac are too low and highlights the need to consider a review of the current dietary recommendations for riboflavin that were established in 1998, using current biochemical assessment methods. Future research is also needed to investigate potential determinants of EGRac and study more closely the association between dietary riboflavin intake and different biomarkers of riboflavin status in women.

Acknowledgments

We thank members of the Department of Cancer Epidemiology and Prevention Research at Alberta Health Services, Calgary, Canada, for their assistance in the processing of dietary information of the Canadian Diet History Questionnaire II. We also thank Liadhan McAnena, Mary Ward, and Helene McNulty in the Nutrition Innovation Centre for Food and Health, Ulster University, Coleraine, Northern Ireland, for conducting the EGRac analysis.

The authors' responsibilities were as follows—AMA, SIB, TJG: designed the research and overall research plan; AMA: drafted the research protocol; TJG, AMD: reviewed and edited the final protocol; AMA: conducted the research, analyzed the data, and drafted the research manuscript; AMA, AMW: developed the mixed dishes disaggregation protocol; AMA, SIB, AMW, AMD, TJG: contributed to data interpretation and manuscript revision; and all authors: read and approved the final manuscript.

References

- Gallagher ML. The water-soluble vitamins. In: Mahan LK, Escott-Stump S, Raymond JL, editors. *Krause's food and the nutrition care process*. 13th ed. St Louis (MO): Elsevier; 2012. p. 76–8.
- Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR. *Vitamins*. In: Said HM, Ross AC, editors. *Modern nutrition in health and disease*. 11th ed. Baltimore (MD): Wolters Kluwer Lippincott Williams & Wilkins; 2012. p. 325.
- Aljaadi AM, How RE, Loh SP, Hunt SE, Karakochuk CD, Barr SI, McAnena L, Ward M, McNulty H, Khor GL, et al. Suboptimal biochemical riboflavin status is associated with lower hemoglobin and higher rates of anemia in a sample of Canadian and Malaysian women of reproductive age. *J Nutr* 2019;149:1952–9.
- Powers HJ, Hill MH, Mushtaq S, Dainty JR, Majsak-Newman G, Williams EA. Correcting a marginal riboflavin deficiency improves hematologic status in young women in the United Kingdom (RIBOFEM). *Am J Clin Nutr* 2011;93:1274–84.
- Wacker J, Fruhauf J, Schulz M, Chiwora FM, Volz J, Becker K. Riboflavin deficiency and preeclampsia. *Obstet Gynecol* 2000;96:38–44.
- Wilson CP, Ward M, McNulty H, Strain JJ, Trouton TG, Horigan G, Purvis J, Scott JM. Riboflavin offers a targeted strategy for managing hypertension in patients with the MTHFR 677TT genotype: a 4-y follow-up. *Am J Clin Nutr* 2012;95:766–72.
- Wilson CP, McNulty H, Ward M, Strain JJ, Trouton TG, Hoefft BA, Weber P, Roos FF, Horigan G, McAnena L, et al. Blood pressure in treated hypertensive individuals with the MTHFR 677TT genotype is responsive to intervention with riboflavin: findings of a targeted randomized trial. *Hypertension* 2013;61:1302–8.
- Horigan G, McNulty H, Ward M, Strain JJ, Purvis J, Scott JM. Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→T polymorphism in MTHFR. *J Hypertens* 2010;28:478–86.
- Liu M, Zhou C, Zhang Z, Li Q, He P, Zhang Y, Li H, Liu C, Qin X. Inverse association between riboflavin intake and new-onset hypertension: a nationwide cohort study in China. *Hypertension* 2020;76:1709–16.
- Whitfield KC, Karakochuk CD, Liu Y, McCann A, Talukder A, Kroeun H, Ward M, McNulty H, Lynd LD, Kitts DD, et al. Poor thiamin and riboflavin status is common among women of childbearing age in rural and urban Cambodia. *J Nutr* 2015;145:628–33.
- Rai D, Bird JK, McBurney MI, Chapman-Novakofski KM. Nutritional status as assessed by nutrient intakes and biomarkers among women of childbearing age – is the burden of nutrient inadequacies growing in America? *Public Health Nutr* 2014;18:1658–69.
- Malek AM, Newman JC, Hunt KJ, Marriott BP. Race/ethnicity, enrichment/fortification, and dietary supplementation in the U.S. population, NHANES 2009–2012. *Nutrients* 2019;11:1005.
- Shi Z, Zhen S, Wittert GA, Yuan B, Zuo H, Taylor AW. Inadequate riboflavin intake and anemia risk in a Chinese population: five-year follow up of the Jiangsu Nutrition Study. *PLoS One* 2014;9:e88862.
- Shi Z, Yuan B, Taylor AW, Zhen S, Zuo H, Dai Y, Wittert GA. Riboflavin intake and 5-year blood pressure change in Chinese adults: interaction with hypertensive medication. *Food Nutr Bull* 2014;35:33–42.
- Brun TA, Chen J, Campbell TC, Boreham J, Feng Z, Parpia B, Shen TF, Li M. Urinary riboflavin excretion after a load test in rural China as a measure of possible riboflavin deficiency. *Eur J Clin Nutr* 1990;44:195–206.
- Shaw NS, Prentice A, Watkinson M, Morrell P, Foord F, Watkinson A, Cole T, Whitehead R. Riboflavin status in late pregnancy, postpartum and cord blood. *Nutr Res* 1993;13:147–55.
- Health Canada. Canadian Community Health Survey, cycle 2.2, nutrition – nutrient intakes from food, provincial, regional and national summary data tables. Vol. 2. Ottawa: Statistics Canada; 2004.
- Auclair O, Han Y, Burgos SA. Consumption of milk and alternatives and their contribution to nutrient intakes among Canadian adults: evidence from the 2015 Canadian Community Health Survey—Nutrition. *Nutrients* 2019;11:1948.
- Hosseini SH, Papanikolaou Y, Islam N, Rashmi P, Shamloo A, Vatanparast H. Consumption patterns of grain-based foods among adults in Canada: evidence from Canadian Community Health Survey–Nutrition 2015. *Nutrients* 2019;11:784.
- Prentice AM, Bates CJ, Prentice A, Welch SG, Williams K, McGregor IA. The influence of G-6-PD activity on the response of erythrocyte glutathione reductase to riboflavin deficiency. *Int J Vitam Nutr Res* 1981;51:211–15.
- Anderson BB, Clements JE, Perry GM, Studds C, Vullo C, Salsini G. Glutathione reductase activity and its relationship to pyridoxine phosphate activity in G6PD deficiency. *Eur J Haematol* 1987;38:12–20.
- Becker K, Krebs B, Schirmer RH. Protein-chemical standardization of the erythrocyte glutathione reductase activation test (EGRAC test). Application to hypothyroidism. *Int J Vitam Nutr Res* 1991;61:180–7.

23. Paul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009;41:1149–60.
24. World Health Organization. STEPS manual: section 5: collecting step 2 data: physical measurements [Internet]. WHO; 2017 [cited April 19, 2018]. p. 2–4. Available from: https://www.who.int/ncds/surveillance/steps/Part3_Section5.pdf.
25. Powers HJ, Bates CJ, Prentice AM, Lamb WH, Jepson M, Bowman H. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. *Hum Nutr Clin Nutr* 1983;37:413–25.
26. IPAQ Group. Guidelines for the data processing and analysis of the International Physical Activity Questionnaire [Internet]. 2005 [cited August 1, 2017]. Available from: <https://sites.google.com/site/theipaq/scoring-protocol>.
27. National Cancer Institute. Diet history questionnaire II: Canadian version (C-DHQ II) – forms and diet calc files [Internet]. 2015 [cited April 15, 2015]. Available from: <https://epi.grants.cancer.gov/DHQ/forms/canadian/#files>.
28. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, Mcnutt S. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table study. *Am J Epidemiol* 2001;154:1089–99.
29. Institute of Medicine. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC): National Academies of Sciences; 1998.
30. Health Canada. Eating well with Canada's food guide [Internet]. Health Canada; 2007 [cited December 27, 2019]. Available from: <https://www.canada.ca/en/health-canada/services/canada-food-guide/about/history-food-guide/eating-well-with-canada-food-guide-2007.html>.
31. U.S. Department of Agriculture, Agricultural Research Service. What we eat in America food categories 2015–2016 [Internet]. USDA; 2018 [cited October 15, 2019]. Available from: www.ars.usda.gov/nea/bhnrc/fsrg.
32. Auestad N, Hurley J, Fulgoni V, Schweitzer C, Auestad N, Hurley JS, Fulgoni VL, Schweitzer CM. Contribution of food groups to energy and nutrient intakes in five developed countries. *Nutrients* 2015;7:4593–618.
33. National Cancer Institute. Diet History Questionnaire II (DHQ II) database file [Internet]. National Cancer Institute, Epidemiology and Genomics Research Program; 2018 [cited March 21, 2019]. Available from: <https://epi.grants.cancer.gov/dhq2/database/current.html>.
34. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;65:1220–8.
35. Greenland S, Pearce N. Statistical foundations for model-based adjustments. *Annu Rev Public Health* 2015;36:89–108.
36. Forster-Coull L, Milne RL, Barr SI. British Columbia Nutrition Survey: report on energy and nutrient intakes [Internet]. 2004 [cited February 24, 2015]. p. 94–5. Available from: <http://www.health.gov.bc.ca/library/publications/year/2004/nutrientsreport.pdf>.
37. US Food and Drug Administration. Nutritional quality guidelines for foods: subpart B—fortification policy, 21CFR104.20 [Internet]. FDA; 2019 [cited July 30, 2020]. Available from: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=104.20>.
38. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle HJEFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), et al. Scientific opinion on Dietary Reference Values for riboflavin. *EFSA J* 2017;15:17–19.
39. Tugault-Lafleur CN, Black JL. Differences in the quantity and types of foods and beverages consumed by Canadians between 2004 and 2015. *Nutrients* 2019;11:1–24.
40. Public Health England. Results of the National Diet and Nutrition Survey (NDNS) rolling programme for 2014 to 2015 and 2015 to 2016. [Internet]. London: Public Health England and the Food Standards Agency; 2018 [cited May 15, 2019]. Available from: <https://www.gov.uk/government/statistics/ndns-results-from-years-7-and-8-combined>.
41. Hesecker H, Schneider R, Moch K, Kohlmeier M, Kübler W. Vitaminversorgung Erwachsener in der Bundesrepublik Deutschland. VERA-Schriftenreihe Band IV. Niederkleen, Germany: Wissenschaftlicher Fachverlag Dr. Fleck; 1992.
42. Hesecker H, Adolf T, Eberhardt W, Hartmann S, Herwig A, Kübler W, Matiaske B, Moch K, Schneider R, Zipp A. Lebensmittel und Nährstoffaufnahme Erwachsener in der Bundesrepublik Deutschland. VERA Schriftenreihe Band III. Niederkleen, Germany: Wissenschaftlicher Fachverlag Dr. Fleck; 1994.
43. Madigan SM, Tracey F, McNulty H, Eaton-Evans J, Coulter J, McCartney H, Strain JJ. Riboflavin and vitamin B-6 intakes and status and biochemical response to riboflavin supplementation in free-living elderly people. *Am J Clin Nutr* 1998;68:389–95.
44. Mataix J, Aranda P, Sánchez C, Montellano MA, Planells E, Llopis J. Assessment of thiamin (vitamin B1) and riboflavin (vitamin B2) status in an adult Mediterranean population. *Br J Nutr* 2003;90:661–6.
45. Toh SY, Thompson GW, Basu TK. Riboflavin status of the elderly: dietary intake and FAD-stimulating effect on erythrocyte glutathione reductase coefficients. *Eur J Clin Nutr* 1994;48:654–9.
46. Tillotson JA, Baker EM. An enzymatic measurement of the riboflavin status in man. *Am J Clin Nutr* 1972;25:425–31.
47. Boisvert WA, Mendoza I, Castaneda C, De Portocarrero L, Solomons NW, Gershoff SN, Russell RM. Riboflavin requirement of healthy elderly humans and its relationship to macronutrient composition of the diet. *J Nutr* 1993;123:915–25.
48. Sadowski JA. Riboflavin. In: Hartz SC, Rosenberg IH, Russell RM, editors. *Nutrition in the elderly: the Boston Nutritional Status Survey*. London: Smith-Gordon; 1992. p. 119–25.
49. Hill MH, Bradley A, Mushtaq S, Williams EA, Powers HJ. Effects of methodological variation on assessment of riboflavin status using the erythrocyte glutathione reductase activation coefficient assay. *Br J Nutr* 2009;102:273–8.
50. Zempleni J, Galloway JR, McCormick DB. Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* 1996;63:54–66.
51. Thompson FE, Subar AF, Brown CC, Smith AF, Sharbaugh CO, Jobe JB, Mittl B, Gibson JT, Ziegler RG. Cognitive research enhances accuracy of food frequency questionnaire reports: results of an experimental validation study. *J Am Diet Assoc* 2002;102:212–25.
52. National Cancer Institute. Validation studies for the Diet History Questionnaire II [Internet]. 2018 [cited June 20, 2019]. Available from: <https://epi.grants.cancer.gov/dhq2/about/validation.html>
53. Choi JY, Kim YN, Cho YO. Evaluation of riboflavin intakes and status of 20–64-year-old adults in South Korea. *Nutrients* 2014;7:253–64.
54. Whitfield KC, da Silva L, Feldman F, Singh S, McCann A, McAnena L, Ward M, McNulty H, Barr SI, Green TJ. Adequate vitamin B12 and riboflavin status from menus alone in residential care facilities in the Lower Mainland, British Columbia. *Appl Physiol Nutr Metab* 2018;44:414–19 [Internet]. Available from: <http://www.nrcresearchpress.com/doi/10.1139/apnm-2018-0459>
55. Statistics Canada. Census profile, 2016 census. Vancouver [census metropolitan area], British Columbia and British Columbia [province] [Internet]. 2017 [cited May 14, 2019]. Available from: <https://www12.statcan.gc.ca/census-recensement/2016/dp-pd/prof/details/page.cfm?Lang=E&Geo1=CMACA&Code1=933&Geo2=PR&Code2=59&Data=Count&SearchText=vancouver&SearchType=Begins&SearchPR=01&B1=Education&TABID=1>.
56. Bates CJ, Prentice A, Cole TJ, van der Pols JC, Doyle W, Finch S, Smithers G, Clarke PC. Micronutrients: highlights and research challenges from the 1994–5 National Diet and Nutrition Survey of people aged 65 years and over. *Br J Nutr* 1999;82:7–15.