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

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Abbreviations: AGP, α-1 acid glycoprotein; BMI, Body Mass Index; CRP, C-reactive protein; C-Chinese, Canadian Chinese; CHMS, Canadian Health Measures Survey; EAR, estimated average requirement; EGRac, erythrocyte glutathione reductase activity coefficient; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; G6PD,

glucose 6-phosphatase dehydrogenase; M-Chinese, Malaysian Chinese; MCV, mean corpuscular volume; RBP, retinol binding protein; sTfR, soluble serum transferrin receptor; UK, United Kingdom.

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ABSTRACT

Background: Riboflavin is required for several redox reactions. Clinical riboflavin deficiency occurs mainly in low-income countries, where it is associated with anemia. The functional significance of suboptimal riboflavin status in different populations and its role in anemia is not well understood.

Objectives: We assessed biomarker status of riboflavin and its association with hemoglobin concentration and anemia in women living in Vancouver, Canada and Kuala Lumpur, Malaysia.

Methods: Healthy non-pregnant, non-breastfeeding women (19-45 years) were recruited from Canada (n=206) and Malaysia (n=210) via convenience sampling. Fasting blood was collected to assess riboflavin status (erythrocyte glutathione reductase activity coefficient, EGRac), hematological indicators, soluble transferrin receptor (sTfR), ferritin, vitamin A, folate, and vitamin B-12 concentrations. Linear and logistic regression models were used to assess the association of riboflavin status with hemoglobin concentration and anemia.

Results: EGRac (mean±SD) values were higher, indicating poorer riboflavin status, in Malaysian versus Canadian women, 1.49±0.17 vs. 1.38±0.11. Likewise, riboflavin biomarker deficiency (EGRac ≥1.40) was significantly more prevalent among Malaysians than Canadians (71% vs. 40%). More Malaysian than Canadian women were anemic (hemoglobin <120 g/L; 18% vs. 7%). Using linear regression (pooled sample; n=416), EGRac values were negatively associated with hemoglobin concentration (r= -0.18; P <0.001). This relationship remained significant (P =0.029) after adjusting for age, parity, ethnicity, vitamin B-12, folate, sTfR, ferritin, and vitamin A. Women with riboflavin deficiency (EGRac ≥1.40) were twice as likely to present with anemia (adjusted OR= 2.38, 95% CI: 1.08, 5.27) compared to women with EGRac <1.40.

Conclusions: Biochemical riboflavin deficiency was observed in Canadian and Malaysian women, with higher rates of deficiency among Malaysian women. Deficient biomarker status of riboflavin was a weak but significant predictor of hemoglobin and anemia suggesting that the correction of riboflavin deficiency may potentially play a small protective role in anemia, but this requires further investigation.

Keywords: riboflavin; anemia; women; reproductive age

INTRODUCTION

Riboflavin, as flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), is an important enzymatic cofactor in several redox reactions. It is primarily obtained from dairy products, beef, chicken, fish, and in some countries, fortified wheat flour (1). Severe riboflavin deficiency (intakes of 0.5-0.6 mg/day in adults), which is uncommon in high-income countries, leads to clinical signs of deficiency such as cheilosis, angular stomatitis, and colored-swollen tongue (1). Milder forms of riboflavin deficiency, defined as the presence of biomarker deficiency without overt clinical deficiency symptoms (1), have been associated with anemia (2,3), which is more common in women (4). It is hypothesized that riboflavin deficiency impairs red blood cell synthesis by altering flavin-dependent release of iron from stores, decreasing iron absorption, and increasing the rate of iron loss from the gastrointestinal tract (2,5–7). In addition, riboflavin deficiency could affect hemoglobin production through impaired flavin-dependent synthesis of 5'pyridoxal phosphate that is required for the first step in heme biosynthesis (1,8). Most population studies of riboflavin deficiency and anemia have been conducted in regions with endemic riboflavin deficiency and inadequate intake, such as in rural Gambia, Thailand, and China (3,9–11). Less is known about populations with milder forms of riboflavin deficiency. One exception is the study of Powers et al.(2) in the United Kingdom (UK), which reported that riboflavin supplementation (2 or 4 mg/day) was associated with a median increase of 4.5g/L in hemoglobin concentrations among non-pregnant, non-lactating women in the lowest tertile of riboflavin status at baseline (erythrocyte glutathione reductase activity coefficient, EGRac >1.65) compared to supplemented women in the first and second

112 tertiles (EGRac <1.51 and 1.51-1.65, respectively). EGRac is considered to be the gold-
113 standard measurement of biomarker status of riboflavin. It is a functional indicator of
114 riboflavin status, whereby the activity of erythrocyte glutathione reductase, an FAD-
115 dependent enzyme, is measured in red blood cells before and after the addition of FAD.
116 EGRac is a ratio of enzyme activity after FAD addition to enzymatic activity before FAD
117 addition. A higher EGRac indicates less endogenous FAD available for the enzyme, and
118 poorer riboflavin status. Although EGRac >1.40 generally equates with deficiency,
119 EGRac values between >1.20 and >1.70 have been previously used to define low status
120 (2,9,12–14).

121 Unlike other B vitamins (e.g. folate and vitamin B-12), biomarker status of
122 riboflavin is rarely measured in population studies. However, a report from the National
123 Diet and Nutrition Surveys (NDNS) from the UK reported that 53% of women (aged 19-
124 49 years) had biomarker evidence of riboflavin deficiency (EGRac >1.40) (15) although
125 only 9.3% of the women consumed less than the UK Lower Reference Nutritional Intake
126 for riboflavin of 0.8 mg/day (16). In Canada, where white wheat flour is fortified with
127 riboflavin (17), the prevalence of inadequate riboflavin intake is very low, with less than
128 5% of women of reproductive age consuming less than the estimated average requirement
129 (EAR) of 0.9 mg/day (18,19). However, in our recent small study in Vancouver, we
130 found that 41% of women of reproductive age (n=49) had riboflavin biomarker
131 deficiency (based on EGRac ≥1.4) (20).

132 The objectives of this study were to assess riboflavin biomarker status using
133 EGRac and to determine the relationship between EGRac and hemoglobin concentration
134 and anemia prevalence in a sample of women of reproductive age from Metro

Vancouver, Canada and Kuala Lumpur, Malaysia. Canada is a high-income country with mandatory riboflavin fortification and adequate riboflavin intake, but there is lack of evidence on the riboflavin status of women of reproductive age in this population; Malaysia is a middle-income country with no mandatory riboflavin fortification (21) and reported inadequate riboflavin intake, as well as low dairy consumption (22–24). To examine ethnic-specific differences in biomarker status, we assessed women of European and Chinese ethnicity in Canada, and women of Malay and Chinese ethnicity in Malaysia.

METHODS

Participants

Convenience samples of women (19-45 years) from Metro Vancouver, Canada, and from Kuala Lumpur, Malaysia, were recruited through posters, e-mails, and advertisement in the social media. Women were eligible if they were healthy, not pregnant or breastfeeding, not taking riboflavin-containing supplements for the past four months, and were of European or Chinese ethnicity (Canada) or Malay or Chinese ethnicity (Malaysia). Women who self-reported having β-thalassemia, untreated hypothyroidism, or glucose 6-phosphate dehydrogenase (G6PD) deficiency were excluded (25–27). To account for the possibility of a significant interaction between ethnicity and EGRac on hemoglobin concentrations, we aimed to recruit n=100 women from each ethnic group from each country based on consultation with a biostatistician using G*Power 3.1 for multiple linear regression (6 independent variables) and an

158 expected multiple regression coefficient of $R^2 = 0.13$ with 80% power and 95%CI. Ethics
159 approval was obtained from the University of British Columbia Clinical Research Ethics
160 Board in Canada [H15-00521] and from the Medical Research Ethics Committee of the
161 Faculty of Medicine and Health Sciences, Universiti Putra Malaysia [FPSK(FR15)P020].
162 Written informed consent was obtained from all women.
163
164 ***Data and blood collection***
165 Women attended a morning clinic following a 10-hour fast. Health and
166 demographic information was collected using a questionnaire. Body weight, height, and
167 waist circumference were measured in duplicate using standardized techniques (28).
168 Venous blood samples were collected into three evacuated tubes (Becton Dickinson), two
169 with EDTA and one without anticoagulant. A complete blood count was performed on
170 fresh EDTA blood using hematology analyzers (Beckman COULTER® Ac·T diff™
171 Analyzer in Malaysia and Sysmex XT2000i in Canada) and appropriate quality controls
172 were run daily. Blood samples were spun in a refrigerated (4°C) centrifuge; plasma or
173 serum were removed and divided into aliquots. Erythrocytes from a tube containing
174 EDTA were washed three times with PBS (SIGMA), for the EGRac assay. All samples
175 were shipped on dry ice and stored at -80°C until analyzed.
176
177 ***Biochemical analyses***
178 EGRac was measured at Ulster University, Coleraine, Northern Ireland, UK,
179 using established methods on Randox Daytona+ clinical chemistry analyzer (Randox
180 Laboratories) (9). EGRac was calculated as the ratio of FAD-stimulated to -unstimulated

enzyme activity, which indicates the degree of sample saturation with riboflavin (9,29). Quality control was provided by repeated analysis of stored aliquots of pooled and characterized erythrocytes, with known EGRac values corresponding to adequate and deficient status. To adjust for confounding effects of micronutrient deficiencies and inflammation on hematologic status, the following parameters were measured: indicators of iron status, folate, vitamin B-12, vitamin A [retinol binding protein (RBP)], C-reactive protein (CRP), and α-1 acid glycoprotein (AGP). Serum ferritin, soluble transferrin receptor (sTfR), RBP, CRP, and AGP were assessed by sandwich ELISA at the VitMin Laboratory (30). Plasma folate concentrations were quantified by a microbiological assay using 96-microtitre plates and the chloramphenicol-resistant strain of *Lactobacillus rhamnosus* (ATCC 27773) at the Trace Elements Lab in the University of Otago, New Zealand (31,32). Plasma vitamin B-12 was analyzed by competitive immunoassay using direct chemiluminescent technology at Vancouver General Hospital Chemistry lab (Siemens ADVIA Centaur, Erlangen, Germany).

For EGRac analysis, the inter-assay CVs were 2.3% for the high control sample (high riboflavin; mean±SD EGRac: 1.17±0.03) and 2.7% for the low control sample (low riboflavin; mean±SD EGRac: 1.42±0.04). Inter-assay CVs for the folate assay were calculated for the concentration of high, medium, and low control samples and were <15% (12.87%, 6.58%, 9.37%, respectively). For ferritin, sTfR, RBP, CRP, and AGP, inter-assay CVs were 2.25%, 3.59%, 3.61%, 5.84%, and 8.09%, respectively.

Statistical analyses

Results are presented as frequencies (%) for categorical variables, mean± SD for normally distributed continuous variables, and median (IQR) for non-normally distributed continuous variables. Country- and ethnic-specific differences were determined by independent sample T-tests (for parametric) and Wilcoxon-rank sum tests (for non-parametric) to compare concentrations of biomarkers (continuous variables) and Fischer's exact tests were used to compare prevalence rates across groups. Ferritin and RBP concentrations were corrected for inflammation using the inflammation biomarkers, CRP and AGP (33,34).

Riboflavin status was classified as deficient (EGRac ≥1.4), suboptimal (1.3≤ EGRac <1.4), and adequate (EGRac <1.3) (13,29,35). Anemia was defined as hemoglobin <120 g/L (36). Depleted iron stores were defined as ferritin <15 µg/L, and tissue iron deficiency was defined as sTfR >8.3 mg/L (36). Acute inflammation was defined as CRP >5 mg/L and chronic inflammation as AGP >1 g/L (33). Plasma folate status was categorized as deficient (<6.8 nmol/L), possible deficiency (6.8-13.4 nmol/L), normal (13.5-45.3 nmol/L), and high (>45.3 nmol/L) (37). Vitamin B-12 status was classified into adequate (>220 pmol/L), marginal (148-220 pmol/L), and deficient (<148 pmol/L) categories (38,39).

Linear regression was used to assess the association between hemoglobin concentration and multiple independent variables. We included interaction terms for EGRac by country and by ethnicity in the models in order to explore whether country or ethnicity modified the relationship between EGRac and hemoglobin concentrations. There were no significant interactions (P >0.05) between EGRac and country or EGRac and ethnicity on hemoglobin concentrations. We decided *a priori,* regardless of an

226 interaction, to examine each country separately. Variables were included in the model if
227 they had a bivariate correlation of $P \geq 0.2$ with hemoglobin or if they are known to be
228 associated with anemia. Age, ferritin, RBP, sTfR, vitamin B-12, and folate were analyzed
229 as continuous variables and parity (≥ 1 child born, yes/no) and ethnicity as categorical
230 variables in the regression models. Logistic regression models were used to determine the
231 association between anemia (binary outcome) and EGRac (continuous variable) or
232 riboflavin deficiency (categorical variable). A maximum of 5 independent variables were
233 included in the logistic regression models as anemia cases in the total sample were n=53
234 (40). Significance was indicated by two-sided *P* values of <0.05. Data were analyzed
235 using Stata software version SE/14.2 for Mac (Stata Corp, College Station, Texas).
236
237 **RESULTS**
238 We recruited n=110 women of European ethnicity and n=96 women of Chinese
239 ethnicity living in Metro Vancouver, Canada, and n=105 women of Malay ethnicity and
240 n=105 of Chinese ethnicity living in Kuala Lumpur, Malaysia. Demographic and
241 anthropometric characteristics are presented by country and ethnicity in **Table 1**. Women
242 from both countries were comparable in age, smoking status, education level, and
243 prevalence of acute (CRP >5mg/L) and chronic (AGP >1g/L) inflammation. More
244 Malaysian women had children and the prevalence of overweight/obesity (BMI ≥25
245 kg/m^2) was higher among Malaysian than Canadian women. In Canada, women of
246 Chinese ethnicity were younger and had a lower prevalence of overweight/obesity than
247 women of European ethnicity. In Malaysia, women of Chinese ethnicity were younger,

were less likely to have children, and had lower prevalences of overweight/obesity and acute inflammation than women of Malay ethnicity.

Mean hemoglobin concentrations were not different between Canadian and Malaysian women (**Table 2**). In Malaysia, women of Chinese ethnicity had higher hemoglobin concentrations than Malay women. The prevalence of anemia (hemoglobin <120g/L) was higher in Malaysian women compared to Canadian women. Compared to Canadian women, EGRac values were higher and riboflavin biomarker deficiency (EGRac ≥1.4) was more prevalent in Malaysian women.

Although serum ferritin and the prevalence of depleted iron stores (ferritin <15 µg/L) did not differ between Canadian and Malaysian women, more Malaysian women had tissue iron deficiency (sTfR >8.3 mg/L) than Canadian women. There was no biochemical evidence of vitamin A deficiency (RBP <0.7µmol/L), plasma folate deficiency (<6.8 nmol/L), or macrocytic anemia (hemoglobin <120 g/L and mean corpuscular volume (MCV) >98 fL). Less than 1% of women had vitamin B-12 deficiency (<148 pmol/L) in both countries. However, more Canadian women had marginal vitamin B-12 status and high plasma folate concentration (>45.3 nmol/L) than Malaysian women.

Few ethnic-specific differences in micronutrient status in women from each country were observed (**Table 2**). In Canada, European women had higher EGRac values and were less likely to be classified as adequate; they also had lower median plasma vitamin B-12 concentrations and higher serum RBP compared to Chinese women. In Malaysia, Malay women had higher mean EGRac values, lower median plasma folate

concentrations, and higher median plasma vitamin B-12 concentrations compared to Chinese women (**Table 2**).

An inverse relationship between hemoglobin concentrations and EGRac was observed in the entire population of women (r= -0.18; P <0.001). Further, multivariable linear regression analyses found that a 1-SD increase in EGRac was associated with a 0.10-SD decrease in hemoglobin concentrations (**Table 3**). EGRac contributed 1% of the variance in hemoglobin concentrations in the multivariable linear regression model. RBP, ferritin, and sTfR were all predictors of hemoglobin. There was no significant interaction between country and EGRac (P = 0.75) on hemoglobin concentrations. Models for each country are shown separately (**Supplemental Tables 1 and 2**). When analyses were conducted by country, EGRac was negatively associated with hemoglobin concentrations in Canada, but was not a significant predictor of hemoglobin concentrations in Malaysia. Ethnicity, sTfR, and RBP were significant predictors of hemoglobin concentrations in Malaysia, whereas EGRac and sTfR remined significant predictors of hemoglobin concentrations in Canada. We further analyzed the relationship between riboflavin status and the prevalence of anemia by logistic regression. EGRac was not associated with anemia in the adjusted model (**Table 4**). However, deficient riboflavin status (EGRac ≥1.4) was positively associated with a greater risk of anemia as shown in **Table 4**.

DISCUSSION

In this sample of healthy women of reproductive age, we found that 40% of Canadian women and 70% of Malaysian women had EGRac values ≥1.40, indicating riboflavin biomarker deficiency. We also report that EGRac was inversely correlated with

293 hemoglobin concentrations and that the odds of anemia were 2-fold greater in women
294 with riboflavin biomarker deficiency (EGRac ≥1.40) than women with EGRac <1.40.
295 The high rate of riboflavin biomarker deficiency in Canadian women was
296 unexpected given that white wheat flour is fortified with riboflavin and the prevalence of
297 dietary inadequacy is very low (18). Riboflavin is naturally found in grain products in
298 small quantities, but the milling and processing of cereal grains cause the loss of many
299 nutrients, including riboflavin. Canada has required the addition of 0.40 mg of riboflavin
300 to each 100 g (equivalent to 4 ppm) of white flour and all foods made from white flour
301 since 2009 (17). Our findings are consistent with Whitfield et al. (20), who reported 41%
302 of female university students living in Vancouver (n=49, mean age= 26.3 ± 4.6 years)
303 had deficient (EGRac ≥1.40) and 29% had suboptimal (1.30≤ EGRac <1.40) riboflavin
304 status (20). In contrast, the finding of higher rates of riboflavin biomarker deficiency in
305 Kuala Lumpur compared to Metro Vancouver were expected because of the assumed
306 lower intake of riboflavin due to low dairy consumption and lack of mandatory food
307 enrichment or fortification (21–23). Given that less than 5% of women of reproductive
308 age in Canada had riboflavin intakes less than the EAR for riboflavin (18), the high
309 prevalence of EGRac ≥1.4 raises the question as to whether it is the current cutoff or the
310 EAR is set too low.
311 We chose EGRac cutoffs that are widely used to defining riboflavin deficiency
312 and suboptimal status (13,20,35,41,42), but there remains controversy around which
313 EGRac cutoff is optimal. An EGRac of 1.20 suggests 20% stimulation of the enzyme and
314 EGRac >1.20 has been considered an indication for inadequate riboflavin intake by many
315 researchers (43–46). However, Tillotson and Baker suggested that EGRac values up to

316 1.30 are considered normal based on their riboflavin depletion-repletion trial conducted
317 on n=6 adult men (47). Sadowski set an upper limit for adequate riboflavin status of 1.34
318 based on the mean EGRac +2 SD of a group of healthy older adult men and women (aged
319 ≥ 60 years; n=927) from the Boston Nutritional Status Survey (48). Accordingly, many
320 have considered EGRac >1.40 (2,14,49) and ≥1.40 (13,20,29,35,42) as a cutoff point for
321 deficiency, but even higher cutoffs have been also used (12,50).
322 The prevalence of anemia (hemoglobin <120 g/L) in women (20-49 y) was lower
323 in the Canadian Health Measures Survey 2009-2011 (CHMS) than our study, 3.7%
324 compared with 7.3%, respectively (51). Likewise, the prevalence of iron deficiency,
325 based on a low serum ferritin (<15 µg/L), was lower in the CHMS (9.1%) than our study
326 (14.6%). Based on national data, 22.8% of Malaysian non-pregnant women (15-49 y)
327 were anemic (hemoglobin <120 g/L) in 2015 (52), which is similar to our sample at
328 18.1%. Mirroring our findings, rates of anemia are higher among ethnic Malays
329 compared to Chinese Malay (53). There are no national data on iron deficiency in
330 Malaysian non-pregnant women, but a study in Kuala Lumpur reported that 24.1% of
331 women (18-40 y; n=135 Malays and n=130 Chinese) had depleted iron stores (serum
332 ferritin <15 µg/L) which is comparable to our 20.8% (54).
333 In the current study, EGRac was inversely and independently associated with
334 hemoglobin concentrations. The relationship was significant but weak, explaining about
335 1% of the variance, and was the third most important modifiable predictor of hemoglobin
336 after iron indicators (ferritin and sTfR) and vitamin A (RBP concentrations). There is
337 little published data available for comparison. A prospective survey carried out in China
338 (2002-2007) reported a positive association between inadequate riboflavin intake (<

339 Chinese EAR) (55); assessed by 3-day weighed food record) and anemia (hemoglobin
340 <120 g/L) in women at baseline (11). Those with anemia but with riboflavin intake in the
341 highest quartile at baseline (1.3 mg/d) were less likely to have anemia at the 5-year
342 follow up [RR=0.52 (95%CI: 0.28, 0.98)]. However, no biochemical indicators of
343 riboflavin status were measured in this survey. A study conducted in the UK in n=123
344 women (aged 19-25 years), all of whom had baseline EGRac >1.40, reported a negative
345 correlation between hemoglobin and EGRac (n=117; r= -0.22, P= 0.016) (2). The study
346 also showed a significant improvement in riboflavin status (decreased EGRac values)
347 after 8 weeks of riboflavin supplementation (2 or 4 mg/day) with a dose-dependent
348 response. This improvement in riboflavin status was associated with an increase in
349 hemoglobin concentrations, but this was observed only among women with the poorest
350 riboflavin status at baseline (EGRac >1.65) (2). We found 1.5% and 14.8% of women
351 above this cutoff in Canada and Malaysia, respectively.
352 Our study had a number of strengths, including the use of a robust functional
353 marker of riboflavin status, EGRac, and including women of different ethnicities from
354 different countries. Moreover, we were able to adjust for a number of important
355 nutritional and non-nutritional confounders. However, we did not measure biomarkers of
356 vitamin B-6, vitamin C, and zinc, which have been shown to be associated with anemia
357 (56–58). Our use of convenience samples makes it difficult to generalize findings of this
358 study to non-pregnant women living in Metro Vancouver and Kuala Lumpur. For
359 example, the women were of higher education than the general population. Over 60% of
360 our sample had obtained a bachelor's degree or higher, compared with 39% of women
361 aged 25-64 y in Metro Vancouver (59) and 18% of women aged 20-44 y in Selangor

362 state, Malaysia (60). Therefore, studies on representative samples of Canadian and
363 Malaysian women are needed. There were very few cases of anemia (n=53) to draw
364 definitive conclusions. Exploring the relationship between riboflavin status and anemia in
365 populations with higher rates of anemia is warranted.

366 In conclusion, we found high rates of suboptimal and deficient riboflavin
367 biomarker status (EGRac ≥1.30) in women from both Canada and Malaysia with higher
368 rates observed in Malaysian women. The findings in Canada were surprising given
369 riboflavin fortification and the low rate of dietary riboflavin inadequacy. The unexpected
370 higher rates of riboflavin biomarker deficiency in European than Chinese women in
371 Canada require further research. Riboflavin status was found to be a predictor for
372 hemoglobin concentrations, albeit to a lesser extent than iron or vitamin A. Although
373 EGRac is widely recognized as the gold standard biomarker for assessing riboflavin
374 status, standardized protocols and cutoffs are required to allow for valid comparisons
375 between different populations.

376

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384 TJG and AMD reviewed and edited the final protocol; AMA conducted the research,

385 analyzed the data, and drafted the research manuscript. SPL and GLK provided essential
386 logistic support for the study execution in Malaysia; REH and SEH helped with data
387 collection in Malaysia. LM, MW, and HM developed the method and measured EGRac;
388 CDK and SIB contributed to data analyses. AMA, AMD, SIB and TJG contributed to the
389 data interpretation and to the review and editing of the manuscript to its final stage.
390 AMA, AMD and TJG had primary responsibility for final content. All authors read and
391 approved the final manuscript.

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TABLE 1 Characteristics of women aged 19-45 years in Vancouver, Canada, and Kuala Lumpur, Malaysia[1]

	Vancouver, Canada				**Kuala Lumpur, Malaysia**				***P***
	All (n=206)	European (n=110)	Chinese (n=96)	*P*	All (n=210)	Malay (n=105)	Chinese (n=105)	*P*	*Canada vs. Malaysia*
Age, years	27.6 ± 6.4	28.7 ± 6.0	26.2 ± 6.6	0.004	27.3 ± 5.3	28.3 ± 5.4	26.3 ± 5.1	0.006	0.72
Parity (≥1 child born)	14 (6.8)	8 (7.3)	6 (6.3)	1.00	31 (14.8)	24 (23.1)	7 (6.7)	0.001	0.011
Smokers	2 (<1)	2 (1.8)	0	0.50	2 (0.96)	0	2 (1.9)	0.24	1.00
Education				<0.001				0.34	0.43
Secondary or less	28 (13.6)	5 (4.6)	23 (24.0)		29 (13.9)	18 (17.5)	11 (10.5)		
Some post-Secondary	45 (21.8)	19 (17.3)	26 (27.1)		35 (16.8)	17 (16.5)	18 (17.1)		
Bachelor's or higher	133 (64.6)	86 (78.2)	47 (49.0)		144 (69.2)	68 (66.0)	76 (72.4)		
BMI, kg/m^2	22.5 ± 3.9	23.1 ± 4.1	21.7 ± 3.6	0.008	23.6 ± 5.0	25.1 ± 6.0	22.1 ± 3.3	<0.001	0.021
Overweight/obesity ≥25	41 (20.0)	26 (23.6)	15 (15.6)	0.17	58 (27.8)	43 (41.4)	15 (14.3)	<0.001	0.039
Waist circumference, cm	73.0 ± 9.8	74.4 ± 10.5	71.4 ± 8.6	0.026	74.7 ± 11.2	77.0 ± 13.3	72.5 ± 8.0	0.004	0.10
Waist circumference ≥80 cm	34 (16.6)	19 (17.3)	15 (16.8)	0.85	50 (23.9)	35 (33.7)	15 (14.3)	0.001	0.07
Inflammation									
Acute, CRP >5 mg/L	11 (5.3)	8 (7.3)	3 (3.1)	0.23	17 (8.1)	15 (14.3)	2 (1.9)	0.002	0.33
Chronic, AGP >1 g/L	4 (1.9)	4 (3.6)	0	0.13	5 (2.4)	4 (3.8)	1 (<1.0)	0.37	1.00

[1]Values are presented as means±SDs or n (%). Data are analyzed by Fisher's exact test (proportion) or two-sample t-test (continuous). AGP, α-1 acid glycoprotein; BMI, body mass index; CRP, C-reactive protein

TABLE 2 Anemia and micronutrients status of women aged 19–45 years in Malaysia and Canada[1]

	Vancouver, Canada				Kuala Lumpur, Malaysia				***P***
	All (n=206)	European (n=110)	Chinese (n=96)	*P*	All (n=210)	Malay (n=105)	Chinese (n=105)	*P*	*Canada vs. Malaysia*
Hemoglobin, g/L[2]	130.9 ± 8.4	131.4 ± 9.2	130.2 ± 7.5	0.29	129.4 ± 12.2	127.2 ± 12.3	131.5 ± 11.8	0.011	0.15
Anemia (hemoglobin <120 g/L)[3]	15 (7.3)	7 (6.4)	8 (8.3)	0.60	38 (18.1)	24 (22.9)	14 (13.3)	0.11	0.001
Microcytic anemia (hemoglobin <120 g/L and MCV <80 fL)[3]	7 (3.4)	2 (1.8)	5 (5.2)	0.26	26 (12.5)	17 (16.5)	9 (8.6)	0.096	0.001
EGRac, ratio[2]	1.38 ± 0.11	1.40 ± 0.10	1.36 ± 0.13	0.022	1.49 ± 0.17	1.52 ± 0.17	1.46 ± 0.15	0.005	<0.001
Adequate, EGRac <1.3[3]	58 (28.2)	17 (15.5)	41 (42.7)	<0.001	20 (9.5)	8 (7.6)	12 (11.4)	0.021	<0.001
Suboptimal, 1.3≤ EGRac <1.4[3]	66 (32.0)	44 (40.0)	22 (22.9)		40 (19.0)	13 (12.4)	27 (25.8)		
Deficient, EGRac ≥1.4[3]	82 (39.8)	49 (44.5)	33 (34.4)		150 (71.4)	84 (80.0)	66 (62.9)		
Serum ferritin, µg/L[4]	36.9 (38.9)	34.1 (32.1)	38.9 (50.9)	0.31	38.4 (49.4)	40.4 (52.2)	37.1 (44.1)	0.22	0.73
Ferritin <15 µg/L[3]	30 (14.6)	14 (12.7)	16 (16.7)	0.44	43 (20.5)	18 (17.1)	25 (23.8)	0.31	0.12
Serum sTfR, mg/L[4]	4.43 (1.42)	4.35 (1.29)	4.58 (1.55)	0.19	5.08 (1.99)	5.09 (1.98)	4.89 (1.97)	0.34	<0.001
sTfR >8.3 mg/L[3]	8 (3.9)	2 (1.8)	6 (6.3)	0.15	19 (9.1)	9 (8.6)	10 (9.5)	1.00	0.045
Plasma vitamin B-12, pmol/L[4]	307.5 (147.0)	254.0 (137)	352.0 (135.5)	<0.001	360.0 (152.0)	372.0 (167.0)	336.0 (128.0)	0.017	<0.001
Deficiency, <148 pmol/L[3]	2 (<1)	2 (1.8)	0 (0)	0.50	1 (<1)	0 (0)	1 (<1.0)	1.00	0.620
Marginal, 148-220 pmol/L[3]	41 (19.9)	36 (32.7)	5 (5.2)	<0.001	7 (3.3)	1 (1.0)	6 (5.7)	0.07	<0.001
Plasma folate, nmol/L[4]	31.5 (15.5)	31.5 (17.6)	31.6 (14.8)	0.73	13.7 (8.4)	11.8 (6.5)	16.6 (9.8)	<0.001	<0.001
Folate >45.3 nmo/L[3]	38 (18.5)	20 (18.2)	18 (18.8)	1.00	2 (1.0)	1 (1.0)	1 (1.0)	1.00	<0.001
Serum RBP, µmol/L[2]	1.8 ± 0.5	1.9 ± 0.5	1.6 ± 0.4	<0.001	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	0.30	<0.001
RBP <7 µmol/L[3]	0.00	0.00	0.00	1.00	1 (<1)	1 (<1)	0.00	1.00	1.00

[1]Data are analyzed by two-sample t-test or Wilcoxon rank-sum test (continuous) and Fisher's exact test (proportions). MCV, mean corpuscular volume; EGRac, erythrocyte glutathione reductase activity coefficient; sTfR, soluble transferrin receptor, RBP, retinol binding protein.
[2]Values are means±SDs. [3]Values are n (%). [4]Values are medians (IQRs).
[5]Ferritin and RBP were adjusted for levels of subclinical inflammation by using AGP and CRP biomarkers (33,34)

TABLE 3 Association between hemoglobin concentrations and riboflavin status (EGRac) [1]

	B (95% CI)	**Standardized coefficient β**	***P***
EGRac	-7.02 (-13.30, -0.74)	-0.10	0.029
Age (y)	-0.12 (-0.30, 0.06)	-0.07	0.20
Parity (Yes versus No)	-0.32 (-3.82, 3.18)	-0.01	0.86
Ethnicity			
European	Reference		
C-Chinese	-0.57 (-3.31, 2.18)	-0.02	0.69
Malay	0.47 (-2.97, 3.91)	0.02	0.79
M-Chinese	4.47 (1.35, 7.58)	0.18	0.005
Folate (nmol/L)	0.03 (-0.05, 0.11)	-0.04	0.51
Vitamin B-12 (pmol/L)	0.01 (0.00, 0.01)	0.08	0.09
Ferritin (μg/L)[2]	0.03 (0.00, 0.06)	0.09	0.035
sTfR (mg/L)	-1.60 (-1.94, -1.27)	-0.42	<0.001
RBP (μmol/L)[2]	5.03 (2.73, 7.34)	0.22	<0.001

[1]Multiple linear regression was used with hemoglobin concentrations (g/L) as a dependent variable; n=415. Model R^2= 0.29 and adjusted R^2= 0.27; EGRac contributed to 1% of the variance in hemoglobin concentrations; the correlation matrix and variance inflation factors showed no signs of multicollinearity between variables included in the model. EGRac, erythrocyte glutathione reductase activity coefficient; C-Chinese, Canadian Chinese; M-Chinese, Malaysian Chinese; sTfR, soluble transferrin receptor; RBP, retinol binding protein.
[2]Ferritin and RBP were adjusted for levels of subclinical inflammation by using AGP and CRP biomarkers (33,34)

TABLE 4 Association between anemia and riboflavin status (EGRac) and deficiency[1]

Anemia	OR	SE	z	*P*	95% CI
Model 1: EGRac[2]	6.03	6.37	1.70	0.09	(0.76, 47.86)
Model 2: Riboflavin deficiency[3]	2.38	0.96	2.14	0.032	(1.08, 5.27)

[1]Logistic regression was used with anemia (yes/no) as an outcome variable and both models were adjusted for concentrations of folate, vitamin B-12, sTfR, and RBP; n=416. EGRac, erythrocyte glutathione reductase activity coefficient.
[2]EGRac was added to the model as a continuous variable; Model Pseudo R^2= 0.27; An OR of 6.03 indicates that the odds of being anemic are 6.03 times higher with one-unit increase in EGRac after holding vitamin B-12, folate, sTfR, and RBP concentrations at fixed values.
[3]Riboflavin deficiency (EGRac ≥1.4) was added to the model as a categorical variable and EGRac <1.4 was the reference category; Model Pseudo R^2= 0.27. An OR=2.38 indicates that the odds of anemia in women with riboflavin deficiency (EGRac ≥1.4) is 2.38 times that of women with EGRac <1.4 after holding vitamin B-12, folate, sTfR, and RBP concentrations at fixed values.