The Association Between Serum Riboflavin and Flavin Mononucleotide With Pancreatic Cancer

Findings From a Prospective Cohort Study

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Objectives: Vitamin B2 (riboflavin) has a prime role in metabolic reactions imperative to cell cycle and proliferation. We investigated the associations between serum concentrations of riboflavin flavin mononucleotide with the risk of pancreatic cancer in a nested case-control study involving 58 cases and 104 matched controls.

Methods: The Singapore Chinese Health Study, an ongoing prospective cohort study of 63,257 Chinese Singaporeans. Conditional logistic regression method was used to evaluate these associations with adjustment for potential confounders including the level of education, body mass index, smoking status, alcohol consumption, history of diabetes, serum cotinine and pyridoxal 5′-phosphate, estimated glomerular filtration rate, and total methyl donors (ie, the sum of serum choline, betaine, and methionine).

Results: The risk of pancreatic cancer increased with increasing level of serum riboflavin in a dose-dependent manner, especially in men (P_{trend} = 0.003). The odds ratio (95% confidence intervals) of pancreatic cancer for the second and third tertiles of serum riboflavin, compared with the lowest tertile, were 9.92 (1.65–59.77) and 25.59 (3.09–212.00), respectively. This positive association was stronger in individuals with a longer follow-up period (≥7 years).

Conclusions: The findings suggest a potential role of riboflavin in the development of pancreatic cancer, especially in men.

Key Words: pancreatic cancer, risk factors, biomarkers, riboflavin, flavin mononucleotide

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Pancreatic cancer is the 12th most common cancer in men and 11th in women with an estimated 460,000 new cases worldwide in 2018.[1] In the United States, pancreatic cancer is the third leading cause of cancer death with 47,050 deaths in 2020.[2] It has a poor prognosis with a 5-year survival of only 8%.[3] The incidence and mortality rates of pancreatic cancer have been increasing in past 40 years.[4] With the decrease in mortality due to other major cancer types, it is projected that the share of pancreatic cancer among total cancer-related deaths will increase dramatically in the next ten years and would become the second leading cancer death behind only lung cancer in 2030.[5] A similar upward trend in pancreatic cancer incidence and mortality is present in Singapore. For example, the mortality rate of pancreatic cancer increased by 3-fold in the past 40 years to 5.5 per 100,000 men and 4.1 per 100,000 women in 2013 to 2017.[6] Established risk factors for pancreatic cancer are obesity, tobacco smoking, alcohol abuse, chronic pancreatitis, type 2 diabetes, and certain germline mutations, such as cyclin-dependent kinase inhibitor 2A (*CDKN2A*), tumor protein 53 (*TP53*), MutL homolog 1 (*MLH1*), breast cancer genes (*BRCA1*, *BRCA2*), and ataxia-telangiectasia mutate genes (*ATM*).[7] Collectively, less than half of the pancreatic cancer burden is attributable to these established risk factors.[8] The US Preventive Services Task Force recently reaffirmed the recommendation against screening for pancreatic cancer in asymptomatic adults.[9] Therefore, it is an urgent and unmet need to identify novel etiological factors for pancreatic cancer that would shed light on the potential biological mechanisms for the development of pancreatic cancer.

Riboflavin (or vitamin B2) is a water-soluble vitamin with a chemical structure defined as 7,8-dimethyl-10 isoalloxazine. Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are active riboflavin-derived coenzymes. Riboflavin is initially converted to FMN via the phosphorylation process, and subsequently, FAD is formed in an ATP-dependent reaction catalyzed by FAD synthase.[10] Flavin adenine dinucleotide serves as cofactors for methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase reductase (*MTRR*) in the one-carbon cycle.[11–13]

The folic acid pathway is known to play a supportive role in DNA synthesis and regulation of repair mechanisms.[14] Disturbed folate cycle affects nucleic acid metabolism via various potential mechanisms such as altered DNA methylation, disruption of DNA integrity, and interference with DNA repair.[15] There is evidence supporting an association between the polymorphisms in the *MTHFR* and *MTRR* genes and the risk of pancreatic cancer.[14,16,17] The *MTHFR* alleles with low enzymatic activity lead to hypomethylation of DNA, resulting in aberrant expression of proto-oncogenes and overexpression of proteins involved in tumor progression.[18]

Animal studies have shown that riboflavin species prevent the tumorigenesis process in pancreatic tissue.[19,20] One suggested mechanism is the degradation of carcinogens via flavin-driven enzymes.[19] Furthermore, the riboflavin-deficient diet increases the induction of DNA repair enzymes such as poly(ADP-ribose) polymerase, DNA polymerase β, and DNA ligase[19] and increased the

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binding of carcinogens to DNA.[20] However, these results are yet to be translated into human tumorigenesis pathophysiology.

There are few epidemiological studies with inconclusive findings on the associations between circulating riboflavin and flavin mononucleotide levels and the risk of pancreatic cancer.[21–23] To our knowledge, the only prospective epidemiologic study was conducted in Europeans[21,23] and no such study had been conducted in an Asian population. The objective of the current analysis was, therefore, to examine these associations in the Singapore Chinese Health Study, an ongoing prospective cohort of more than 63,000 participants in Singapore. The findings may have potential public health implications in identifying individuals with high exposure to riboflavin and developing a risk reduction strategy. A better understanding of riboflavin in relation to the risk of pancreatic cancer also provides supportive evidence for mechanistic experimental studies.

MATERIALS AND METHODS

Study Population

The present study was based on the Singapore Chinese Health Study that was described in detail previously.[24] Briefly, 63,257 Chinese men and women were enrolled in the Singapore Chinese Health Study between April 1993 and December 1998. The subjects were residents of the Singapore government-built housing estates, 45 to 74 years of age, and belonged to either the Hokkiens or Cantonese groups, the 2 main dialects in Singapore. The Hokkiens originated from the Fujian province and the Cantonese from Guangdong province, both in southern China. All study participants provided written informed consent. The Singapore Chinese Health Study is approved by the institutional review boards of the National University of Singapore and the University of Pittsburgh.

At baseline, each study participant was interviewed in person by a trained interviewer using a predesigned structured questionnaire. This questionnaire asked for information on the subject's demographics, lifetime use of tobacco, the current consumption of alcoholic beverages, menstrual and reproductive histories (women only), current physical activity occupational exposure, medical history, family history of cancer, and dietary habits during the past 12 months using a validated[25] semi-quantitative food frequency questionnaire. Non-fasting blood (20 mL) and single-void urine specimens were requested from a random 3% sample of cohort participants during April 1994 to December 1999. From January 2000, the request for biospecimens was extended to all surviving cohort participants. Overall, biospecimens were obtained from 32,535 participants, representing approximately 60% of eligible subjects by April 2005. Blood components (ie, plasma, serum, buffy coat, and red blood cells) were separated and stored at −80°C until further analysis.

Identification of Pancreatic Cancer Cases

The incident cancer cases and deaths among the cohort participants were identified via linkage analyses with the databases of the nationwide Singapore Cancer Registry and the Birth and Death Registry. Pancreatic cancer was defined by the International Classification of Diseases-Oncology, 9th edition (ICD-9) code 157 or ICD 10th edition (ICD-10) 2nd revision code C25 of the cancer registry files. Any subject with recorded ICD code corresponding with pancreatic cancer within the study timeframe was entered in the cases subgroup. The follow-up for cancer incidence and death is virtually completed. Overall, 56 (<0.1%) of cohort participants were lost to annual linkage analyses due to their migration out of Singapore.

Nested Case-Control Studies

For the present case-control study, we identified 58 incident pancreatic cancer cases among participants who provided a prediagnostic blood sample as of December 2013. We also randomly selected up to 2 control subjects for each cancer case among all eligible participants who provided a baseline blood sample and were alive and free of cancer during the time from blood collection to cancer diagnosis of the index case. The controls were individually matched to the index case by age at enrollment (±3 years), date of baseline interview (±2 year), sex, dialect group (Hokkien, or Cantonese), and date of biospecimen collection (±6 months).

We excluded 12 control subjects without serum samples, leaving 104 control subjects for the current analysis. Consequently, the current study included a total of 58 incident pancreatic cancer cases and 104 individually matched controls.

Assessment of Serum Biomarkers

Serum specimens of cases and their matched controls were processed, aliquoted, shipped in the dry-ice box, and assayed together at Bevital A/S, Bergen, Norway (www.bevital.no). The serum samples of each matched case-control set (1 case and up to 2 controls) were placed next to each other in random order and tested in the same batch. The case/control status of all test samples was blind to laboratory personnel. Riboflavin and flavin mononucleotide were detected using electrospray ionization tandem mass spectrometry (ESI-MS/MS).[26] We also used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure methyl donors (ie, serum methionine, betaine, choline), creatinine,[27] pyridoxal 5′-phosphate (PLP), and cotinine.[26]

Serum creatinine was used for the calculation of estimated glomerular filtration rate (eGFR), a renal function measurement. Serum cotinine is a metabolite of nicotine, an indicator of recent exposure to tobacco smoking and the use of other nicotine-containing products.[28] For quality control purposes, 14 duplicated samples derived from a pool of serum samples collected from cohort participants at the same period as the study sample collection were dispersed in 7 batches of the test samples (2 per batch). The within-batch coefficients of variation for riboflavin and flavin mononucleotide were 3.5% and 11.8% respectively. The between-batch coefficients of variation for riboflavin and flavin mononucleotide was 11.7% and 13.4%, respectively (Supplemental Table 1, http://links.lww.com/MPA/B10).

Statistical Analysis

We used natural logarithmic transformation of original values for statistical analysis to reduce their skewness and improve normal distribution. The analysis of covariance was used to examine the difference in concentrations among controls according to demographic characteristics and lifestyles, as well as between cases and controls. χ^2 Test was used to compare the difference in distributions of categorical variables and *t* test statistic for continuous variables.

Conditional logistic regression method was used to examine the associations for serum concentrations of riboflavin, flavin mononucleotide, and the sum of riboflavin and flavin mononucleotide with pancreatic cancer risk. Study subjects were divided into tertiles according to the distribution of individual biomarkers among control subjects. Odds ratios (ORs) and respective 95% confidence intervals (CIs) were used to evaluate the magnitude for the association between levels of serum biomarkers and pancreatic cancer risk. Ordinal values of tertiles for riboflavin and flavin mononucleotide were used for linear trend test in the biomarker-pancreatic cancer risk association.

Potential confounders included in the multivariable regression models were level of education (ie, no formal schooling, primary school, secondary school, or above), body mass index (BMI) (ie, <18.5, 18.5 to <23, or ≥23 $kg \cdot m^2$), smoking status (ie, never,

former, or current smokers), serum cotinine (nmol·L^{-1}), alcohol consumption (ie, number of drinks per week), history of diabetes (yes versus no), serum pyridoxal 5-phosphate-PLP (nmol·L^{-1}),[29] eGFR,[29] and total methyl donors (ie, the sum of serum choline, serum betaine, and serum methionine).

Stratified analysis was conducted based on the duration of follow-up (<7 years vs ≥7 years). Statistical analyses were carried out using SAS software version 9.4 (SAS Institute, Cary, NC). All *P* values reported are 2-sided. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

The mean (standard deviation [SD]) age at blood draw for pancreatic cancer cases and controls was 64.9 (SD, 7.6) years and 64.0 (SD, 7.1) respectively. Among cases, the average (range) time interval from blood draw to cancer diagnosis was 6.8 years (5 months to 13.0 years).

The characteristics of pancreatic cancer cases and matched controls are presented in Table 1. There were no statistically significant differences between cases and controls in age, BMI, eGFR, cotinine, PLP, level of education, alcohol intake, and history of diabetes.

The geometric means of riboflavin and flavin mononucleotide and their sum in cases of pancreatic cancer were comparable with those in the controls (all $P > 0.05$) (Table 2). In the analysis of the categorical variables, elevated levels of serum riboflavin were associated with a significantly increased risk of pancreatic cancer (Table 3). Compared with the lowest tertile, ORs for the second and third tertiles of riboflavin in the total cohort (both men and women) were 3.97 (95% CI, 1.33–11.81) and 5.91 (95% CI, 1.84–19.05), respectively ($P_{trend} = 0.004$). This positive riboflavin-pancreatic cancer risk association was only present in male participants. Accordingly, the ORs for the second and third tertiles were 9.92 (95% CI, 1.65–59.77) and 25.59 (95% CI, 3.09–212.00), respectively, compared with the lowest tertile ($P_{trend} = 0.003$). There was no association between serum flavin mononucleotide and pancreatic cancer risk (Table 3).

We also examined the joint effect of riboflavin and flavin mononucleotide on the risk of pancreatic cancer. We compared various groups based on riboflavin and flavin mononucleotide with the subset with lower level (below median) of riboflavin and higher levels of flavin mononucleotide as the reference group. None of the 3 subgroups showed a significant difference in the risk of pancreatic cancer (Table 4).

TABLE 1. Baseline Demographic Characteristics and Lifestyle Factors of Pancreatic Cancer Cases and Control Subjects, the Singapore Chinese Health Study

Baseline Characteristics	Controls	Cases	*P*
N	104	58	
Age at blood draw, mean (SD), y	64.0 (7.1)	64.9 (7.6)	0.47*
BMI, mean (SD), kg·m^2	23.1 (3.2)	23.2 (3.6)	0.79*
Glomerular filtration rate, mean (SD), mL·min^{-1}·1.73 m^2	77.4 (16.6)	78.4 (17.3)	0.81*
Cotinine, geometric mean (95% CI), nmol·L^{-1}	12.2 (6.7–22.5)	15.1 (6.9–33.0)	0.68†
Pyridoxal 5′ phosphate, geometric mean (95% CI), nmol·L^{-1}	69.2 (58.9–81.2)	60.3 (48.7–74.7)	0.32†
Total methyl donors, mean (SD), μmol·L^{-1}	98.4 (19.6)	93.2 (16.8)	0.09†
Sex, n (%)			
Male	63 (61.6)	35 (61.3)	0.98‡
Female	41 (39.4)	23 (39.7)	
Education level, n (%)			0.42‡
No formal schooling	21 (20.2)	7 (12.1)	
Primary school	45 (43.3)	28 (48.3)	
Secondary school or above	38 (36.5)	23 (39.7)	
Smoking status, n (%)			0.87‡
Never	63 (60.6)	34 (58.6)	
Former	23 (22.1)	12 (20.7)	
Current	18 (17.3)	12 (20.7)	
Alcohol intake, drinks/week, n (%)			0.61‡
0	86 (82.7)	51 (87.9)	
<7	11 (10.6)	5 (8.6)	
≥7	7 (6.7)	2 (3.5)	
Diabetes, n (%)			0.88‡
No	94 (90.4)	52 (89.7)	
Yes	10 (9.6)	6 (10.3)	
Weekly multivitamin use, n (%)			0.69‡
No	93 (89.4)	53 (91.4)	
Yes	11 (10.6)	5 (8.62)	

*2-sided *P* values were based on the Student *t* test.

†2-sided *P* values were based on the Mann-Whitney test.

‡2-sided *P* values were based the Chi-square test.

TABLE 2. Geometric Means of Serum Concentrations of Riboflavin and Flavin Mononucleotide in Pancreatic Cancer Cases and Control Subjects, the Singapore Chinese Health Study

	Controls (n = 104)	Cases (n = 58)	P*
Riboflavin, nmol·L^{-1}	30.18	33.60	0.39
Flavin mononucleotide, nmol·L^{-1}	8.50	7.96	0.60
Sum of riboflavin and flavin mononucleotide, nmol·L^{-1}	40.28	43.10	0.57

*P values were based on analysis of covariance comparing geometric means between cases and controls with adjustment for age at blood draw, sex, level of education (no formal schooling, primary school, and secondary school or above), BMI (<18.5, 18.5 to < 23.0, ≥23.0 kg·m^2), smoking status (never, former, and current smokers), number of alcoholic drinkers per week (continuous), history of diabetes (no, yes), serum cotinine concentration (nmol·L^{-1}), serum pyridoxal 5′-phosphate concentration (nmol·L^{-1}), eGFR (mL·min^{-1}·1.73 m^2) and total methyl donors.

In the sensitivity analysis on subjects separated by the median time period of follow-up, elevated levels of both riboflavin and flavin mononucleotide were associated with a statistically significant increase in the risk of pancreatic cancer among all participants with a longer duration of follow-up (≥7 years). The respective ORs for the third tertile, compared with the first tertile, for riboflavin and flavin mononucleotide were 7.13 (95% CI, 1.18–43.03) and 5.78 (95% CI, 1.28–28.89) respectively (both P_{trends} = 0.03). There was no significant association observed among participants with a shorter duration of follow-up (<7 years) (Table 5).

Serum concentrations of riboflavin and flavin mononucleotide were reasonably correlated with each other (r = 0.44, P < 0.0001) among control subjects given their inter-relationship in the metabolic pathway. We also investigated the correlation for selected demographic and lifestyle factors with serum levels of riboflavin and flavin mononucleotide. Overall, there was a modestly inverse correlation for serum eGFR and smoking status with riboflavin (r = −0.20 and −0.21 respectively) (both P < 0.05). On the other hand, PLP was positively correlated with riboflavin (r = 0.32, P < 0.05) (Supplemental Table 1, http://links.lww.com/MPA/B10).

DISCUSSION

In this nested case-control analysis, we evaluated the associations between serum levels of riboflavin and flavin mononucleotide and the risk of pancreatic cancer in a well-phenotyped Asian cohort. We found that study participants with higher riboflavin levels at baseline had a significantly higher risk of developing pancreatic cancer during the subsequent years of follow-up up to 7 years. The association between riboflavin and pancreatic cancer risk was only present in male subjects.

A number of previous studies have investigated the impact of riboflavin, among other nutrients, on the risk of pancreatic cancer.[21–23] The findings of these studies were inconsistent.[17,22]

TABLE 3. Associations Between Serum Concentrations of Riboflavin and Flavin Mononucleotide and Pancreatic Cancer Risk, the Singapore Chinese Health Study

	All		Men		Women		
	Controls/ Cases, n	OR (95% CI)*	Controls/ Cases, n	OR (95% CI)*	Controls/ Cases, n	OR (95% CI)*	$P_{sex\ diff}$
Riboflavin							
Tertile 1	38/10	1.00	21/3	1.00	17/7	1.00	
Tertile 2	32/22	**3.97 (1.33–11.81)**	21/14	**9.92 (1.65–59.77)**	11/8	3.27 (0.38–27.88)	0.43
Tertile 3	34/26	**5.91 (1.84–19.05)**	21/18	**25.59 (3.09–212.00)**	13/8	3.63 (0.41–32.51)	0.12
P_{trend}		**0.004**		**0.003**		0.27	
Continuous (log_2)	104/58	1.22 (0.83–1.81)	63/35	1.34 (0.81–2.18)	41/23	1.24 (0.55–2.81)	
Flavin mononucleotide							
Tertile 1	35/21	1.00	23/10	1.00	12/11	1.00	
Tertile 2	35/21	0.92 (0.40–2.10)	19/14	1.40 (0.39–5.01)	16/7	0.30 (0.07–1.38)	0.12
Tertile 3	34/16	0.80 (0.31–2.06)	21/11	0.97 (0.25–3.71)	13/5	0.45 (0.06–3.27)	0.53
P_{trend}		0.65		0.87		0.26	
Continuous (log_2)		0.90 (0.578–1.39)	63/35	0.81 (0.45–1.47)	41/23	1.01 (0.47–2.06)	
Riboflavin + flavin mononucleotide							
Tertile 1	35/13	1.00	20/3	1.00	15/10	1.00	
Tertile 2	35/24	2.55 (0.98–6.60)	20/19	**10.65 (2.01–56.43)**	15/5	0.33 (0.05–2.19)	**0.0007**
Tertile 3	34/21	2.65 (0.91–7.73)	23/13	**11.94 (1.56–91.51)**	11/8	1.37 (0.27–6.80)	**0.04**
P_{trend}	104/58	0.09		**0.02**		0.80	
Continuous (log_2)		1.15 (0.78–1.68)	63/35	1.19 (0.74–1.91)	41/23	1.23 (0.55–2.72)	

Presented in bold are ORs and the respective CIs with 2-sided P < 0.05.

*ORs were derived from conditional logistic regression models adjusting for level of education (no formal schooling, primary school, and secondary school or above), BMI (<18.5, 18.5 to < 23.0, ≥23.0 kg·m^2), smoking status (never, former, and current smokers), number of alcoholic drinkers per week (continuous), history of diabetes (no, yes), serum cotinine concentration (nmol·L^{-1}), serum pyridoxal 5′-phosphate concentration (nmol·L^{-1}), eGFR (mL·min^{-1}·1.73 m^2) and total methyl donors.

TABLE 4. Joint Effect of Riboflavin and Flavin Mononucleotide on the Risk of Pancreatic Cancer in the Singapore Chinese Health Cohort Study

Riboflavin	Flavin Mononucleotide	Controls	Cases	OR (95% CI)*
Low (<26.05)†	High (≥7.51)†	23	8	1.00
Low (<26.05)	Low (<7.51)	34	16	1.35 (0.50–3.68)
High (≥26.05)	High (≥7.51)	16	15	1.76 (0.66–4.73)
High (≥26.05)	Low (<7.51)	31	19	2.69 (0.92–7.85)

*ORs were derived from conditional logistic regression models adjusting for level of education (no formal schooling, primary school, and secondary school or above), BMI (<18.5, 18.5 to < 23.0, ≥23.0 kg·m^2), smoking status (never, former, and current smokers), number of alcoholic drinkers per week (continuous), history of diabetes (no, yes), serum cotinine concentration (nmol·L^{-1}), serum pyridoxal 5′-phosphate concentration (nmol·L^{-1}), eGFR (mL·min^{-1}·1.73 m^2) and total methyl donors.

†Low or high was defined as below or above median values of the corresponding analytes among all control subjects, respectively.

In a retrospective case-control study of 212 pancreatic cancer patients and 220 controls among the White male population in Minnesota, Olsen et al[22] reported a positive association between circulating riboflavin concentration and risk of pancreatic cancer. On the contrary, 2 case-control studies in Europe, one with 463 incident cases of pancreatic cancer and 463 individually matched controls within the European Prospective Investigation into Cancer and Nutrition[21] and the other hospital-based study of 181 pancreatic cancer cases and 181 controls in Greece,[23] did not find a significant association between riboflavin or flavin mononucleotide and the risk of pancreatic cancer.[21–23] The present study shows a positive association that was consistent with the finding by Olsen et al.[22] It is noteworthy that all previous studies were conducted among Caucasian populations. The different findings among various studies might be attributable to the difference in dietary intake and the different ethnicities of the studied subjects.

Similar to pancreatic cancer, studies in other solid tumors such as lung cancer, esophageal cancer, and gastric cancer have reported conflicting findings. A randomized prevention trial in esophageal and gastric cancer reported no change in cancer incidence among the study participants treated with riboflavin and niacin.[30] On the other hand, an experiment using non–small cell lung cancer cell lines revealed high dose of riboflavin increased cancer cell proliferation, invasion, and migration.[31] This aggravating impact on the lung cancer cell lines was attributed to the overexpression of mediators such as intercellular adhesion molecule-1, interleukin 6 (IL-6), matrix metalloproteinase (MMP) 2 and 9, fibronectin, and tumor necrosis factor-alpha (TNF-α).

TABLE 5. Associations Between Serum Riboflavin, and Flavin Mononucleotide With Pancreatic Cancer Risk in the Singapore Chinese Health Study Cohort, Stratified by the Median Time Period From Blood Draw to Cancer Diagnosis

	<7 Years of Follow-Up		7+ Years of Follow-Up	
	Controls/Cases, n	OR (95% CI)*	Controls/Cases, n	OR (95% CI)*
Riboflavin				
Tertile 1	20/5	1.00	18/5	1.00
Tertile 2	16/12	6.79 (1.31–35.04)	16/10	3.80 (0.71–20.23)
Tertile 3	18/12	5.61 (0.93–33.8)	16/14	**7.13 (1.18–43.03)**
P_{trend}		0.09		**0.03**
OR for continuous $\log_2$		1.30 (0.73–2.33)		1.25 (0.68–2.27)
Flavin mononucleotide				
Tertile 1	18/12	1.00	17/9	1.00
Tertile 2	19/9	2.65 (0.89–14.45)	16/12	3.28 (1.87–19.89)
Tertile 3	17/8	3.1 (0.97–21.85)	17/8	**5.78 (1.28–28.89)**
P_{trend}		0.13		**0.03**
OR for continuous $\log_2$		1.195 (0.69–2.069)		0.63 (0.25–1.56)
Riboflavin + flavin mononucleotide				
Tertile 1	18/8	1.00	17/5	1.00
Tertile 2	18/10	1.72 (0.38–7.67)	17/14	3.58 (0.78–16.33)
Tertile 3	18/11	1.76 (0.41–7.60)	16/10	4.30 (0.71–26.20)
P_{trend}		0.48		0.14
OR for continuous $\log_2$		1.282 (0.72–2.27)		1.12 (0.62–10.43)

Presented in bold are ORs and the respective CIs with 2-sided $P < 0.05$.

*ORs were derived from conditional logistic regression models adjusting for level of education (no formal schooling, primary school, and secondary school or above), BMI (<18.5, 18.5 to < 23.0, ≥23.0 kg·m^2), smoking status (never, former, and current smokers), number of alcoholic drinkers per week (continuous), history of diabetes (no, yes), serum cotinine concentration (nmol·L^{-1}), serum pyridoxal 5′-phosphate concentration (nmol·L^{-1}), and eGFR (mL·min^{-1}·1.73 m^2) and total methyl donors.

These mediators are expressed in elevated levels in pancreatic cancer and are implicated in oncogenesis, cancer progression, and treatment resistance.[32–35]

Flavin mononucleotide is 1 of 2 active coenzymes derived from riboflavin with a pivotal role in a number of oxidation-reduction reactions.[36] In the folate cycle, FAD is a cofactor for the *MTHFR* enzyme and functions as an electron carrier in the conversion of folic acid to its active form.[37] The polymorphism in the *MTHFR* genotype is reported to be associated with the risk of pancreatic cancer in some specific populations.[14,16] Liu et al reported a strong association between *MTHFR C677T* variant genotype and pancreatic cancer risk in East Asians but not in Whites.[38] Recent randomized trial of riboflavin supplementation has be reported to perturb one-carbon metabolism in individuals with the homozygous variant alleles (ie, *MTHFR 677TT*) who were assigned to the riboflavin treatment arm.[39] These subjects demonstrated increased S-adenosylmethionine (SAM), cystathionine, and altered methylation in response to riboflavin supplementation. Aberrant DNA methylation has a detrimental effect on gene expression, and genomic stability.[40,41] Higher levels of SAM upregulate the DNA methyltransferase, which may enhance pancreatic cancer cell self-renewal, leading to tumorigenesis and metastasis.[42] This genotype-specific one-carbon metabolite response is a possible mechanism through which riboflavin or flavin mononucleotide might aggravate the risk of pancreatic cancer.

In addition to its impact on one-carbon metabolism, riboflavin may enhance the risk of pancreatic cancer formation and progression via increased production of inflammatory mediators, cell surface glycoproteins, and extracellular components. Previous studies have revealed a positive association between levels of inflammatory cytokines (IL-6, TNFα),[43] extracellular matrix components (MMP-9, MMP2, fibronectin),[34,44] and cell surface glycoproteins, such as ICAM-1, which facilitate the intercellular interactions of cancer cells and stimulate tumor growth.[45] Another potential impact of riboflavin on pancreatic cancer may be through its effect on the tumor microenvironment where the extracellular matrix is mainly composed of the collagen content.[46] Previous studies have shown the role of the extracellular matrix in the regulation of the invasive behavior of cancer cells.[47] Riboflavin has demonstrated its potency to upregulate specific types of collagen including collagen type 1A1 in human stromal cells.[48] The differential expression of elements of the extracellular matrix including collagen I has been observed in proliferation and migration of pancreatic cancer cells.[49] The crosslink between riboflavin and collagen type-1 and the increased matrix rigidity may be an underlying mechanism of riboflavin-induced risk of pancreatic cancer. Further studies are thus warranted to elucidate this underlying mechanism.

A notable physiologic aspect of riboflavin and its coenzymes is the difference in their absorption through gut epithelia. This process is mainly regulated via the number of transporters in the plasma membrane. Three main membrane riboflavin transporters are identified in pancreatic β-cells: hRFVT-1, hRFVT-2, and hRFVT-3.[50] Prior studies have shown that these transporters control the riboflavin uptake or efflux while they hardly contribute to FMN hemostasis.[51]

These notions of a direct effect of riboflavin on extracellular matrix and the specific riboflavin transporters may partly explain a stronger association for riboflavin than flavin mononucleotide with pancreatic cancer risk observed in our study. Yet, further studies are warranted to validate this postulation.

The apparent associations between riboflavin and the risk of pancreatic cancer among men but not among women remained to be clarified. One possible explanation for the sex difference may be due to the possible interference between riboflavin and estrogen via conjugation and methylation of this hormone. There is supportive evidence from in vivo and in vitro studies implicating the influence of estrogen on promoting pancreatic cancer.[52] Riboflavin can impede the harmful estrogen-induced carcinogenic pathway by detoxification mechanism through excretion of catechol estrogens.[53] Future studies are thus warranted to elucidate this phenomenon. It is also particularly interesting that the positive association between serum riboflavin and pancreatic cancer development in participants with a longer-time interval from blood draw to pancreatic cancer diagnosis, suggesting that potential effect of riboflavin on the development of pancreatic cancer is slow with long-term exposure.

Also, in a methodologic study[54] among 40 postmenopausal women, aged 51 to 68 years (median, 62 years), randomly selected from the Nurses' Health Study (NHS) who provided at least 2 fasting blood samples, we found that the geometric mean of riboflavin at first collection and second collection were 26.2 (95% CI, 21.7–31.5) and 28.5 (95% CI, 23.7–34.2) nmol·L^{-1}, respectively. The intraclass correlation coefficient was 0.87 (95% CI, 0.76–0.93), considered to be an excellent reproducibility. In the same study, we analyzed samples from 633 participants of the Western Norway B Vitamin Intervention Trial study with 4 timepoints of serum samples within 3.5 years and found that the geometric mean of riboflavin at first visit and last visit were 12.5 (95% CI, 11.8–13.2) and 11.5 (95% CI, 10.8–12.2) nmol·L^{-1}, respectively, and that the intraclass correlation coefficient was 0.70 (95% CI, 0.67–0.73). These results suggest that even though riboflavin has a short half-life (ie, 1.1 hours),[55] one-time point of assessment would provide a relatively long-term representative concentration of riboflavin for a given individual.

In the Singapore Chinese Health Study, dietary sources of riboflavin are diverse, from bread (10.72%), dairy products (9.87%), green vegetables (7.83%), fresh fish and shellfish (7.59%), noodles and pasta (7.46%), fresh pork (7.02%), dark green vegetables (5.44%), and cruciferous vegetables (4.50%).

Our study has several strengths. First, the measurement of biomarkers was conducted before the diagnosis of pancreatic cancer. The prospective design of the study and long-term follow-up of the cohort allowed us to explore the potential impact of riboflavin and its derivative coenzyme on the risk of developing pancreatic cancer, which would minimize the impact of the disease progression, status or diagnosis/treatment for pancreatic cancer on serum levels of riboflavin or its metabolite. Second, all the biochemical procedures were performed in a single laboratory with rigorous quality control procedure producing reproducible and precise data, which minimized potential measurement errors. Third, the statistical analyses were conducted with adjustment for carefully selected potential confounders including eGFR, alcohol consumption, smoking, and preexisting diabetes. Fourth, our study is the first prospective epidemiological study in an Asian population that examined the associations for serum riboflavin and flavin mononucleotide with pancreatic cancer risk. The main limitation of our study is the modest sample size, especially for women.

The findings related to riboflavin and flavin mononucleotide have several implications and warrants future mechanistic studies. Considering the potential impact of riboflavin vitamin B2 on risk of pancreatic cancer in humans, future experimental studies are warranted to unravel the biological mechanism for the tumor-promoting role of riboflavin which may provide an evidence-based strategy for risk reduction through modification of dietary intake of vitamin B2. The interconnection of riboflavin with other members in the one-carbon metabolism prompts a wide variety of targetable enzymes or mediators that can be investigated for the chemoprevention of pancreatic cancer.

In summary, we found a statistically positive association between serum levels of riboflavin and the risk of pancreatic cancer.

The association was found only in men and more apparent in subjects with a prolonged period of follow-up. These findings warrant future prospective epidemiological studies with a larger sample size and experimental studies to elucidate the underlying biological mechanism for the tumor-promoting role of riboflavin vitamin B2 on the development of pancreatic cancer.

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