

INTAKE OF RIBOFLAVIN (VITAMIN B2) AND THE OCCURRENCE OF MIGRAINE: AN
ANALYSIS OF THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY
(NHANES) 2001 TO 2004 DATABASE.

by

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LIST OF ABBREVIATIONS

Cortical spreading depression	CSD
Trigeminovascular system	TGVS
Reactive oxygen species	ROS
Malondialdehyde.....	MDA
Calcitonin Gene-Related Peptide	CGRP
Riboflavin	Vitamin B ₂ /B ₂
Flavin adenine dinucleotide	FAD
Flavin mononucleotide.....	FMN
Gastrointestinal	GI
United States Department of Agriculture	USDA
Recommended Dietary Allowance	RDA
National Health and Nutrition Examination Survey	NHANES
Oxidative stress	OS
Reduced glutathione.....	GSH
Oxidized glutathione.....	GSSG
Glutathione reductase.....	GR
Mitochondrial DN ¹⁻⁷ A	mtDNA
Randomized controlled trial.....	RCT
Mobile examination centers.....	MEC
Miscellaneous pain questionnaire	MPQ
Body mass index	BMI
Standard error.....	SE
Quartiles.....	Q
Odds ration.....	OR
95% confidence interval	CI

ABSTRACT

INTAKE OF RIBOFLAVIN (VITAMIN B2) AND THE OCCURRENCE OF MIGRAINE: AN ANALYSIS OF THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES) 2001 TO 2004 DATABASE.

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George Mason University, 2019

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Background:

Migraine is a common neurological disorder that causes moderate to severe headache attacks accompanied by other disabling symptoms. Previous clinical studies have found a daily single dose of 400 mg riboflavin to be effective as migraine prophylaxis for adult migraineurs. However, the dose and the administering pattern of riboflavin supplementation in previous studies were not supported by solid justification. Additionally, the average riboflavin consumption of migraine patients in the United States is not revealed in any previous literature to our knowledge.

Objective:

This study aimed to evaluate the average daily dietary, supplement, and total (diet + supplement) riboflavin consumption of adult migraine sufferers. Moreover, this study investigated the relationship between dietary, supplement, and total (diet + supplement) riboflavin consumption and the occurrence of migraine.

Methods:

This observational cross-sectional study analyzed adult participants aged from 20-50 years old using NHANES 2001-2002 and 2003-2004 datasets. After excluding disqualified individuals who were pregnant, breastfeeding, menopausal, diabetic, diagnosed with thyroid disease, having missing data, taking tricyclic medication, having unrealistic dietary intake, and having unrealistic dietary intake, a total of 3,634 participants were included, containing 884 probable migraineurs and 2,750 controls. Migraine status was determined based on the self-reported NHANES miscellaneous pain questionnaire. Dietary and supplement riboflavin intake was determined based on the 24-hour recall NHANES dietary interview and supplement section questionnaire. Riboflavin intake was divided into quartiles. Odds ratios and 95% confidence intervals were calculated using logistic regression model, adjusted for sex, BMI, and alcohol consumption. P value was computed using Wald test, adjusted for sex, BMI, and alcohol consumption. All statistical test and summary statistics were survey-weighted. The significance level was set at 0.05.

Results:

The mean dietary riboflavin consumption of adult migraineurs was significantly lower than the control. (2.14 mg/day vs. 2.36 mg/day, $p=0.0005$). In contrast, supplement riboflavin consumption (9.37 mg/day vs. 7.19 mg/day, $p=0.625$) and total riboflavin consumption (4.25 mg/day vs. 4.28 mg/day, $p=0.958$) of the migraine group and the control group were not statistically different. Among adult migraine sufferers, dietary riboflavin intake was associated with the occurrence of migraine ($p_{\text{overall}}=0.0013$), with the riboflavin intake level ranging from 2.07-2.87 mg/day showing the greatest reduction

in migraine occurrence ($OR_{Q3}=0.732$ [0.558-0.962]) compared to the lowest riboflavin intake quartile (0-1.45 mg/day). The relationship remained true between the total riboflavin consumption, which further included supplement intake, and migraine odds ($OR_{Q3}=0.630$ [0.463-0.859], $p_{overall}=0.032$). When considering supplement riboflavin intake alone, no relationship was observed between supplement riboflavin intake and the odds of migraine ($p_{overall}=0.1988$).

Conclusion:

The average dietary riboflavin intake of adult migraine patients (2.14 mg/day) meets the Recommended Dietary Allowance but is lower than the control. The mean riboflavin intake from supplement source only is 9.37 mg/day. The mean total riboflavin consumption of adult migraineurs, including both dietary and supplemental intakes, is 4.25 mg/day. Dietary riboflavin intake is associated with a 27% lower occurrence of migraine when the consumed amount is between 2.07 mg/day to 2.87 mg/day as compared to 0 mg/day to 1.45 mg/day. Total riboflavin consumption amount between 2.45 mg/day to 3.60 mg/day corresponds to 37% reduction in migraine odds as compared to 0 mg/day to 1.58 mg/day.

CHAPTER ONE: LITERATURE REVIEW

Migraine

Migraine is a disabling headache disorder characterized by moderate to severe headache attacks accompanied by a combination of other symptoms, including sensitivity to light, sound, and movement.¹ Additionally, nausea with or without vomiting affects 87% of migraine patients.^{1,2} As one of the most common neurological disorders, migraine affects 11% of the population world-wide.³ The prevalence is the highest among females at reproductive age, as measured in the U.S.⁴ In addition to the physical discomfort, the burden of migraine costs migraine patients \$2,571 more than non-migraine individuals per year in the U.S. in health-care expenditures.⁵ The national burden is estimated as \$11.07 billion per year.⁵ Migraine is also one of the leading causes of workplace absenteeism which results in approximately 4.4 missed work days per person annually in migraineurs who reported absenteeism.⁶

Diagnosis

The International Classification of Headache Disorder-3rd edition (ICHD-3) standardized the diagnosis and criteria of different types of headache disorder. Two major types of episodic migraine were identified by ICHD-3: Migraine with aura and migraine without aura.² *Migraine without aura* is characterized by unilateral location of the headache, with pulsating quality, aggravation after physical activity, and accompanied with nausea and/or photophobia and phonophobia, the symptoms of which can last

between 4-72 hours.² *Migraine with aura* usually lasts for 5-60 minutes with visual, sensory, speech, motor, brainstem, retinal symptoms, or other transient focal neurological symptoms, which may or may not be followed by a painful headache phase.²

Episodic migraine can progress to chronic migraine, starting from low-frequency episodic migraine (0-9 headache days/month) to high-frequency episodic migraine (10-14 headache days/month), and eventually to chronic migraine.⁷

Chronic Migraine is diagnosed when the number of headache days is greater than 15 days per month. The diagnostic criteria of chronic migraine include: non-episodic headache attacks, at least 5 of which fulfill the characteristics of migraine with or without aura, occurring on 15 days or more per month, for more than 3 months.² In chronic migraine patients, individual attacks may not be identifiable due to the high frequency and continuity of attacks.² In other words, individual migraine attacks may overlap, such that a clear beginning and end of individual attacks may not be discernable.

Prevalence

Migraine is most prevalence among women at reproductive age. In the United States, the prevalence of migraine ranges from 13.2% to 15.3% of the overall adult population, 17.5% to 20.7% of adult females, and 8.6% to 9.7% of the adult male population.⁸⁻¹⁰ Multiple studies have confirmed gender is one risk factor of migraine, with females at a 2-4 times higher risk of having migraine than males.¹⁰⁻¹² Migraine prevalence is also age-specific. The incident of migraine among female population accelerates after the age of 20 and peaks between the age of 20 to 24.¹⁰ Between the age of 40 to 50, the premenopausal period, the migraine rate accelerates again, forming a

bimodal trend of migraine incident among the female population.¹⁰ After menopause, approximately at the age of 50, the rate of migraine declines remarkably.¹³

Pathophysiology

Currently, the exact cause of migraine remains uncertain, but brain oversensitivity to environmental stimuli has long been proposed as a portion of the pathology. The dysfunction of the monoaminergic sensory control system in the brainstem and hypothalamus may be the cause of the hypersensitivity.¹ It has also been proposed that the trigeminovascular system (TGVS) and trigeminal-autonomic reflex act as key components in propagating migraine acute attack.¹⁴ Several factors can lead to the activation of the trigeminovascular system, such as cortical spreading depression (CSD).

Cortical spreading depression, the rapid depolarization of brain cells with redistribution of ions between intracellular and extracellular compartments, is one of the hallmarks of migraine, which is currently believed to be a physiological correlate of the clinical phenomena of migraine aura.¹⁵ The activation of the TGVS is coupled with CSD in a coherent process, as follows.

First, a high degree of reactive oxygen species (ROS) production is induced during and following CSD.¹⁶ ROS, especially H₂O₂, is able to interact with polyunsaturated fatty acid in cell membrane and plasma lipoproteins, which results in lipid peroxidation.¹⁷ Lipid peroxidation is responsible for multiple deleterious effects, such as cancer, inflammatory disease, atherosclerosis, and aging.¹⁸ In the migraine propagation process, ROS production increases the electrical activity in meningeal and trigeminal nerves, indicating an activation of these nerves.¹⁹

After the activation of trigeminal ganglia nerve activation, Calcitonin Gene-Related Peptide (CGRP), a vasoactive neuropeptide, is released. As a main migraine mediator, CGRP induces neurogenic inflammation, which leads to several cascade responses including arteriolar vasodilation, plasma protein extravasation, and degranulation of mast cells.^{19,20} Thus, CGRP is able to regulate blood flow to the brain and pain-sensitive meninges, and transmit painful stimuli from intracranial vessels to the central nervous system,²¹ resulting in head pain. The pain pathway involves trigeminovascular input from the meningeal vessel, which passes through the trigeminal ganglion and synapses in the trigeminocervical complex (TCC). The increased release of CGRP after ROS generation further confirms the overall propagation process.²²

The initial cause(s) of this cascade reaction is still uncertain. The inherited nature of migraine indicates the involvement of genetic factor(s) contributing to migraine. The comorbidity of migraine and other diseases, including obesity and inflammatory bowel disease, suggests that other health conditions may impact the migraine propagation process as well.^{23,24} Furthermore, food triggers, including caffeine at a high dosage (>400mg), alcohol, monosodium glutamate, gluten and histamine-containing food, and fasting are proposed to induce the cascade reaction.^{25,26} Thus, dietary interventions, such as low fat diet, high omega-3/omega-6 diet, ketogenic diet, various elimination diets, as well as nutrients supplementation have been proposed to ameliorate migraine, including riboflavin supplementation.^{27,28}

Riboflavin

Riboflavin (Vitamin B₂) is one of the B complex vitamins that was initially discovered in 1879 as a yellow fluorescent pigment in milk.^{29(p325)} It is a water-soluble,

photosensitive, and heat-stable vitamin that plays an essential role in energy metabolism.³⁰ The chemical structure of riboflavin contains a flavin (isoalloxazine) planar ring and a ribitol side chain. The molecular mass of riboflavin, with a molecular formula $C_{17}H_{20}N_4O_6$, is 376.4 g/mol.³¹ Although some plants have the ability to synthesize riboflavin, it is considered an essential nutrient to humans and must be acquired from exogenous sources, such as diet and microflora of the large intestine.^{29(p327),32} The primary and active forms of riboflavin are flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), two redox-active coenzymes. The structures of riboflavin, FAD, and FMN are shown in **Figure 1**.

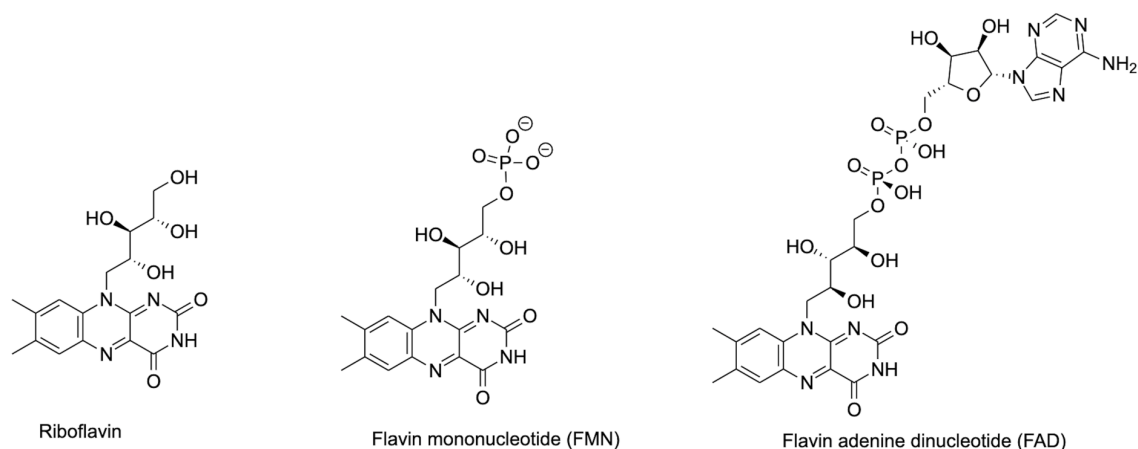


Figure 1 Structure of riboflavin, FMN, and FAD

Because of their chemical structures, flavins can accept a pair of hydrogen atoms and act as oxidizing agents. The isoalloxazine ring of the flavins is reduced to a semiquinone after receiving the first electron and forming a reduced isoalloxazine ring after accepting both hydrogen atoms (**Figure 2**). Due to their wide range of redox

potentials, flavoproteins function as coenzymes in several metabolic reactions, such as flavoprotein-catalyzed dehydrogenations, interaction with sulfur-containing compounds, hydroxylation, oxidative decarboxylation, deoxygenations, and reduction of oxygen to hydrogen peroxide.³³

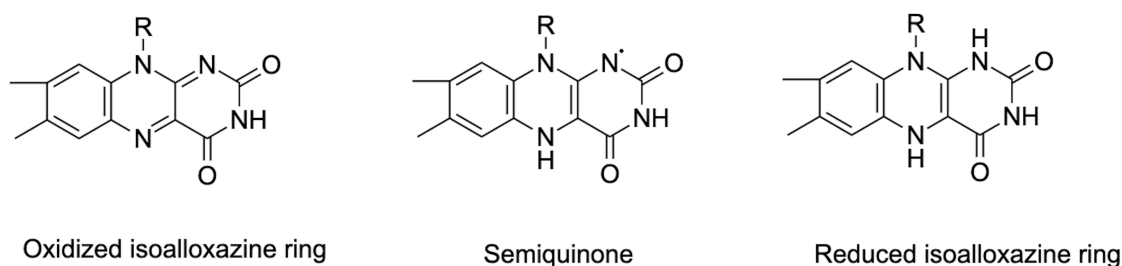


Figure 2 Structure of oxidized isoalloxazine ring, semiquinone, and reduced isoalloxazine ring

Absorption, transportation, and excretion

Riboflavin from dietary sources is primarily in the form of FAD and FMN, some of which is bound to phosphorus or amino acids.^{30(p325)} Through the digestive process, the protein-bound coenzymes are released by gastric acid, and later hydrolyzed to free riboflavin by nonspecific pyrophosphatase and phosphatase in the small intestine.³³ In the proximal small intestine, particularly the duodenum and jejunum, the main riboflavin absorption occurs through saturable, energy-dependent, sodium-independent carrier transportation.^{30(p326)} The absorption process occurs via active or facilitated transport at low intake levels, and through passive diffusion at higher intake levels.^{34(p276)} Riboflavin absorption rate increases when ingested along with food, as well as in the presence of bile

salts.^{30(p325)} Since its gastrointestinal (GI) uptake is a saturable process, the maximum absorption of riboflavin per dose or per meal is 27 mg.³³

To maintain the normal homeostasis of riboflavin in the human body, the kidneys reabsorbs the vitamin in the proximal tubule and controls the amount excreted in the urine.^{29(p327)} When absorbed in excess, free riboflavin and a wide variety of flavin-related products are filtered by the glomerulus and excreted in the urine, which is the primary excretion approach.³³ In healthy adult individuals, approximately 120 µg are excreted daily, with about 60 to 70 percent of the urinary flavins in the form of free riboflavin.^{29(p327),34} However, the amount of dietary intake, metabolic events, and age may influence the rate of urinary excretion.^{33,34} Diabetes mellitus, trauma, stress, and oral contraceptive use enhances the excretion of riboflavin.²⁹

The phosphorylation of riboflavin to FMN, an energy-consuming reaction, occurs upon the absorption of riboflavin into the intestinal cell, followed by the dephosphorylation of FMN to FAD at the serosal membrane of the intestinal cell.^{30(p327)} Once entered into the plasma, riboflavin mainly associates with carrier proteins, such as immunoglobulins and albumins for transport.³⁴ The availability of these carrier proteins increases during pregnancy, which results in a higher riboflavin uptake rate at the maternal surface of the placenta.³³

The cellular uptake in peripheral tissues is tightly regulated based on the riboflavin status of the individual. The riboflavin uptake into tissues depends on a carrier-mediated system, which is facilitated by riboflavin-binding protein.³⁰ A small quantity of riboflavin is found in various tissues, which is sufficient to maintain the needs of the human body for 2 to 6 weeks.³⁰

Deficiencies and DRIs

Animal products, especially lean meat, fish, organ meat and eggs, are good sources of dietary riboflavin. A cup of non-fat milk provides 0.45 mg riboflavin, and an egg provides 0.26 mg riboflavin.³⁰ Some fortified or enriched cereals and bread also provide a significant amount of riboflavin.²⁹ For the U.S adult population, milk and milk products contribute the greatest amount of dietary riboflavin intake.^{34(p277)} In addition to dietary source, dietary supplements containing vitamin B-complex vitamins are available, which include riboflavin in the form of free riboflavin or riboflavin 5'-phosphate.³⁵

Based on the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine, the Recommended Dietary Allowance (RDA) of riboflavin is 1.3 mg/day for adult men and 1.1 mg/day for adult women.^{34,36} The minimal intake to avoid clinical symptoms is 0.8 mg/day for both adult men and women.³³ According to the What We Eat In America 2013-2014 food survey data, the mean intake of riboflavin from food source of the U.S population aged 20 years or older is 2.54 mg/day for males and 1.83 mg/day for females.³⁷ The mean riboflavin intake from supplementation is 2.23 mg/day for males and 2.08 mg/day for females.³⁷ The mean total riboflavin intake from both dietary and supplement sources in the U.S population aged 20 years or older is 4.77 mg/day for males and 3.90 mg/day for females.³⁷ An analysis of 2003-2006 National Health and Nutrition Examination Survey (NHANES) indicated that less than 6% of the U.S residents aged greater than 2 years old had a total usual intake of riboflavin below the Estimated Average Requirement, which is 1.1 mg for males and 0.9 mg for females.³⁸

A deficiency of riboflavin, ariboflavinosis, is almost always caused by dietary deficiency. Although the deficiency is more common in developing countries, where

access to meat and dairy products is limited, acute deficiencies also appear in developed countries as the result of other health issues or an unbalanced diet. The high-risk population includes patients with long-standing infections, liver disease, alcoholism, malignancy, cardiac disease, diabetes mellitus, advanced age, and an impaired GI system.^{29,30(p329)} After 3 to 4 months of inadequate intake, the clinical symptoms of ariboflavinosis appear, including cheilosis (lesions on the outside lips), angular stomatitis (lesions on the corners of the mouth), glossitis (inflammation of the tongue), hyperemia (redness), seborrheic dermatitis (inflammatory skin condition), weakness, corneal vascularization, sore throat, edema, and anemia.^{29,30,33,39} The deficiency can be treated easily by orally administering 5-15 mg riboflavin per day or simply by dietary interventions depending on the severity.³⁰

Since kidneys maintain riboflavin homeostasis and excretes excessive riboflavin, high-levels of unabsorbed riboflavin have rarely been linked with any consequence. Thus, a Tolerable Upper Intake Level and a known toxicity are not established for riboflavin.^{30,39,40}

Riboflavin as a prophylaxis for migraine

For migraine patients, prophylactic therapy is commonly recommended because of the physical and economic benefits. On average, prophylaxis saves migraine patients \$559.71 per year.⁴¹ Riboflavin is categorized as one of the nonprescription pharmacologic prophylactics due to its neuroprotective potential to reduce oxidative stress, mitochondrial dysfunction, neuroinflammation, and glutamate excitotoxicity.⁴²

Riboflavin is recommended as an effective nutraceutical treatment for adult migraine patients by scholars and the American Migraine Foundation.^{43,44} The vitamin is

rated as level B (some supportive randomized clinical trials, but the evidence is not optimal), probably effective, for prophylaxis of migraine headache by American Academy of Neurology.⁴⁵ The following sections describe the research which has contributed to these recommendations.

Biochemical mechanisms

Oxidative stress

Though the human brain only represents 2% of the total body weight, its energy consumption is enormous, accounting for 20% of the total energy use.⁴⁶ Concerning the fact that the energy metabolism, mostly glucose metabolism, in the human brain is an aerobic process, which consumes approximately 20% of total body oxygen,⁴² neural tissues are highly susceptible to oxidative damage caused by free radicals.

Free radicals are common byproducts of aerobic metabolism. The molecules are highly reactive due to their unpaired electrons, which can impair nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins.¹⁷ The most damaging free radicals in biological systems are reactive oxygen species (ROS), particularly superoxide, hydroxyl, and perhydroxyl radicals.¹⁷ Similar to the excitatory neurotransmitters and amino acids, ROS activity is particularly high in the brain and neural tissues.⁴⁷ Oxidative stress (OS), a term used to describe elevated intracellular levels of ROS, contributes to free radical attack on glial cells and neurons, which results in protein and DNA injury, inflammation, tissue damage and subsequent cellular apoptosis.^{47,48}

As mentioned in the previous section, CSD, the hallmark of migraine aura, induces the production of ROS,¹⁶ indicating the potential role of ROS in propagating migraine attacks. Moreover, an elevated level of malondialdehyde (MDA), a biomarker

of lipid peroxidation, is observed among migraine patients when compared with the controls, suggesting a higher level of lipid peroxidation among migraineurs.⁴⁹ Lipid peroxidation is used to estimate the total body radical burdens, which further demonstrates the abnormal oxidative stress status among migraine individuals.¹⁷

Since ROS are normal byproducts of aerobic metabolism, the human body has a defense system in removing the ROS and preventing damage. Antioxidants are substances that can inhibit oxidation. Some widely recognized protective antioxidant nutrients are selenium, vitamins C and E, β -carotene and other carotenoids, and a variety of polyphenolic compounds.¹⁷ The antioxidant defense system in the human body is responsible for removing oxygen, scavenging of ROS or their precursors, binding catalysts of ROS generation, and inhibiting the formation of ROS.⁵⁰ With a balanced antioxidant defense system, damage from ROS can be effectively reduced.

Riboflavin has demonstrated its ability in activating antioxidant enzymes including endogenous superoxide dismutase (SOD), which neutralizes one of the main ROS in the cell.⁵¹ Clinical studies have shown that administering riboflavin balances ROS and decreases inflammation in some diseases.^{51–53} Another antioxidant that requires riboflavin for its activity is glutathione. Glutathione is a tripeptide that consists of three amino acids: cysteine, glycine, and glutamic acid. In the human body, glutathione exists in two states, reduced glutathione (GSH) and oxidized glutathione (GSSG). The active form of glutathione is GSH, which acts as an endogenous antioxidant.⁵⁴ GSH interacts with free radicals directly by acting as an electron donor. The regeneration of GSH is catalyzed by glutathione reductase (GR), an enzyme that requires riboflavin in the form

of FAD.⁵⁵ The glutathione system is extremely important for cellular defense against ROS.⁵⁶

The deficiency of riboflavin may therefore disrupt the endogenous antioxidant pathway, leading to elevated ROS. Consistent with this notion, lipid peroxidation, a result of ROS damage, has been demonstrated to be significantly enhanced in induced riboflavin deficiency in animal studies.^{57,58} Furthermore, riboflavin indicated the effectiveness in alleviating lipid peroxide formation on diabetic rats when administered orally.⁵⁹ Thus, the role of riboflavin in reducing ROS is essential, particularly in organs where oxygen utility is high, such as the brain.

Mitochondrial dysfunction

Mitochondrial dysfunction is one of the genetic disorders that has been linked to migraine for over a decade.⁶⁰ Mitochondria are membrane-bound organelles found in mammalian cells. The most crucial role of mitochondria in cells is producing energy, in the form of adenosine triphosphate (ATP), through aerobic respiration to supply the needs for cellular activities.⁶¹ Other secondary mitochondria functions include ROS production, apoptosis regulation, and homeostasis regulation. The mitochondrial gene, which codes for different mitochondria functions, is referred as Mitochondrial DNA (mtDNA). Migraine prevalence is significantly higher among individuals with point mutation on mtDNA (m.3243A >G) when compared to the general population.⁶² In addition, the primary symptoms of MELAS, a condition caused by a point mutation on mtDNA (m.3243A > G), is episodic headache.^{12,63,64} Other findings also suggest mtDNA polymorphisms (16519C→T and 3010G→A) are associated with migraine without aura.⁶⁵ These studies indicate the role of certain mtDNA changes in migraine pathology.

Although mtDNA is passed along the maternal lineage to all the offspring, some mtDNA disorder, such as Leber hereditary optic neuropathy, has demonstrated sex-biased transmission due to the influence of sex steroids, which implies the potential genetic and hormonal-associated impact on migraine prevalence since female are at a higher risk of migraine.¹²

Riboflavin is known to be an essential biochemical agent of efficient energy metabolism. The production of ATP in the mitochondria involves two main processes, the Tricarboxylic Acid Cycle (AKA the citric acid cycle, Krebs cycle, or TCA cycle) and the Electron Transport Chain (ETC).⁶⁶ In the TCA cycle, riboflavin, in the form of FAD, acts as an electron acceptor and receives a pair of hydrogen atoms to form FADH₂.^{30(p88)} In the ETC, both FMN and FAD are involved in electron transfer, which is important in generating the proton gradient.^{30(p93)} Thus, riboflavin is one of the critical biochemical agents that facilitate efficient mitochondrial energy metabolism and ATP production in mammalian cells, including the neural cells. In the case of mitochondrial dysfunction, excessive ROS are generated from the dysfunctional oxidative phosphorylation process of the ETC.⁶⁴ Superoxide anion can form from the impaired ETC by transferring one electron to oxygen.⁶⁷ The impact of ROS and the role of riboflavin in preventing their damage have been addressed in the previous discussion.

Although the complete mechanism is yet to be investigated, mutations in mtDNA are proposed to lead to impaired energy metabolism and ion homeostasis in neurons.⁶⁸ Also, plasma intermediate metabolites levels, lactic and pyruvic acid, are significantly higher among the migraineurs than controls, indicating an impaired energy metabolic

pathway.⁶⁹ One theory proposes that mitochondrial dysfunction leads to a reduction in ATP production, which decreases the threshold for CSD.⁶⁸

Though riboflavin does not affect the cortical information processing directly, it may inhibit the propagation of migraine by reducing ROS and improving energy metabolism.⁷⁰ Hence, the effectiveness of riboflavin in ameliorating migraine symptoms is theoretically feasible.

Clinical studies

The pilot clinical study on riboflavin as a migraine prophylaxis was conducted by Dr. Schoenen in 1994.⁷¹ In the study, a daily single oral dose of 400 mg riboflavin was administered for at least 3 months to the forty-nine migraine patients, whose gender and age were not specified. Forty-five patients had migraine without aura (10 with additional episodic tension-type headache distinguishable from migraine), and four had migraine with aura. The treatment decreased the score of migraine severity when comparing the month before riboflavin treatment and the last month of the treatment. The result of this pilot study requires careful interpretation, because the characteristics of the participants, as well as potential confounders or covariates are not examined and reported. More controlled randomized controlled trial (RCT) studies have been conducted to examine the efficacy of riboflavin as the single prophylaxis on adult migraine patients. A summary of available riboflavin studies in adults with migraines is provided in **Table 1** below.

A double-blind RCT, also conducted by Schoenen et al., investigated the efficacy of riboflavin on the migraine patients, aged 18-65 years.⁷² Individuals who had migraine of at least 1 year, had 2-8 attacks per month, had no more than 5 days of interval headaches per month, and fulfill the International Headache Society (IHS) diagnostic

criteria of migraine with and without aura, were recruited. During the 3-month treatment period, patients in the treatment group received a single oral dose of 400 mg riboflavin daily. Two out of the 28 participants in the treatment group experienced mild side effects, including diarrhea and polyuria, and one participant dropped out because of the side-effects. The results indicated that the migraine frequency, number of attack days, and the number of days with nausea were significantly decreased in the riboflavin group as compared to the placebo group. Breen et al. conducted another double-blind RCT using the same recruitment criteria and treatment method, which also found a significant reduction in attack frequency and attack days in the riboflavin group compared with placebo.⁷³ Compared to placebo, riboflavin group had 2 less migraine attacks/month and 3 less migraine days/month. The migraine index score was also lower than the placebo group. Only one participant experienced diarrhea in the riboflavin group.

An open-label study was conducted by Dr. Boehnke at a tertiary care center.⁷⁴ Adult migraine patients, aged 20-65 and had 2-8 attacks/month in the last 6 months, were recruited based on the IHS standard. Participants enrolled in a 4-week baseline period followed by a 3-month treatment period. During the treatment period, a single oral dose of 400mg riboflavin was administered daily. The result showed a reduction in attack frequency as well as attack duration, from 50 h/attack to 22-28 h/attack after the 3-month treatment. The research article noted the occurrence of side-effects, including diarrhea, upper abdominal pain, and facial erythema. However, the specific number of people that experienced side effects and the number of withdrawals were not reported.

Some studies compared the efficacy of riboflavin with other treatments using the RCT design. Sandor and his colleagues compared the effectiveness of β -Blockers and

riboflavin on the intensity dependence of auditory evoked cortical potentials, a potential that reflects sensory information processing in the cerebral cortex, among adult migraineurs.⁷⁰ Twenty women and five men aged 30.9 ± 14.4 years were recruited based on the IHS criteria. The study indicated the efficacy of high dose riboflavin (400 mg) in decreasing headache frequency, but not cortical information processing.

Another study conducted by Dr. Nambiar compared lower doses of riboflavin (100 mg/d) with 80 mg/d propranolol on adult population.⁷⁵ Participants were randomized to riboflavin (100 mg/d) treatment or 80 mg/d propranolol treatment for 3 months. Both groups showed a reduction in frequency, duration, severity, and disability. For the riboflavin group, migraine frequency decreased from 4 attacks/month to 2.3 attacks/month. Attack duration decreased from 2.8 hrs/attack to 2.1 hrs/attack. Visual analogue scale score, which is used to measure migraine severity, decreased from 4.9 to 3.6. Migraine disability questionnaire score decreased from 4.8 to 3.8. Though the effectiveness of propranolol and riboflavin were not significantly different, the side effects were significantly less in the riboflavin group than the propranolol group. The study suggested 100 mg riboflavin was an effective and well-tolerated alternative in migraine prophylaxis.

A more recent single-blinded study compared riboflavin with sodium valproate.⁷⁶ The study recruited 90 participants age 15-55 from a neurology clinic. Participants were divided into a riboflavin group (400 mg/day) and a sodium valproate group (500 mg/day). The result showed both groups had a significant reduction in the frequency, severity, and duration of migraine, but the difference between the two groups was not significant. For riboflavin group, the headache duration decreased from 15.1 hrs/month to 4.2 hrs/month.

The attack frequency decreased from 9.2 attacks/month to 2.4 attacks/month. The severity of migraine reduced in 71.8% of the participants. Additionally, the side effects observed in the riboflavin group were significantly less than the sodium valproate group (2.3% vs 17.7%). No drop out was reported. The article concluded that riboflavin could be administered to relieve migraine pain with less complications than sodium valproate.

Although the high-dose riboflavin demonstrated encouraging effectiveness in alleviating migraine symptoms on adult migraineurs, these clinical studies did not take baseline riboflavin status and riboflavin intake of the migraine population into consideration. If the study participants are not consuming sufficient riboflavin or having low riboflavin status at baseline, the high-dose riboflavin could be a treatment for nutrient deficiency instead of or in addition to migraine. On the other hand, if the participants were already taking a substantial amount of riboflavin from dietary or supplement sources before or during the study, the validity of the treatment could be affected. Furthermore, other covariates, such as alcohol use, medication use, and pre-existing health conditions, were also not taken into account in most of the clinical studies. Alcoholism, tricyclic antidepressant, thyroid disease, and diabetes are known to influence riboflavin metabolism in human body, which also impact the validity of the riboflavin treatment in the clinical studies.^{29(p325)} Only one study excluded individual who were taking dietary vitamin supplement and/or experiencing alcohol abuse.⁷⁵

Table 1 Summary of clinical trials of high-doses riboflavin on adult migraine population

Study	Participants	Method	Result	Side-effect	Drop-out due to B ₂ treatment	Limitations

Schoenen, J ⁷¹ 1994	<ul style="list-style-type: none"> • n=49 • age not specified 	<ul style="list-style-type: none"> • Paired-test • 400 mg B₂/day • 3 month-5month 	<ul style="list-style-type: none"> • 68.2% mean global improvement • 8% patients had >50% reduction in migraine days/month • 4 MA^b symptom disappeared 	<ul style="list-style-type: none"> • B₂ n=1 • B₂ + aspirin group: GI discomfort 	n=1	<ul style="list-style-type: none"> • Participant's characteristics not specified • No baseline B₂ information
Schoenen, J ⁷² 1998	<ul style="list-style-type: none"> • n=55 • Age 18-65 	<ul style="list-style-type: none"> • Double-blind RCT^a • 3 months • 400 mg B₂/day 	<ul style="list-style-type: none"> • Less attack days/month compared to placebo • Less days with nausea compared to placebo • 59% patients improved symptoms for >50% 	<ul style="list-style-type: none"> • B₂ n=2 • B₂ group: diarrhea, polyuria 	n=1	<ul style="list-style-type: none"> • No baseline B₂ information • Not considering confounding factors
Sandor ⁷⁰ 2000	<ul style="list-style-type: none"> • n=26 • Age 18-26 	<ul style="list-style-type: none"> • RCT • 4 months • 400 mg B₂/day 	<ul style="list-style-type: none"> • No change in cortical information processing • 53% patients had reduction in attack frequency for >50% 	<ul style="list-style-type: none"> • Unknown 	Unknown	<ul style="list-style-type: none"> • No baseline B₂ information • Not considering confounding factors
Breen ⁷³ 2003	<ul style="list-style-type: none"> • n=55 • Age 18-65 	<ul style="list-style-type: none"> • Double-blind RCT • 3 months • 400 mg B₂/day 	<ul style="list-style-type: none"> • Reduction in attack frequency (2 less migraine/month) compared to placebo • 3 less migraine days/month compared to placebo • Decreased migraine index score compared to placebo 	<ul style="list-style-type: none"> • B₂ n=1 • B₂ group: diarrhea 	Unknown	<ul style="list-style-type: none"> • No baseline B₂ information • Not considering confounding factors

Boehnke ⁷⁴ 2004	<ul style="list-style-type: none"> • n=23 • Age 20-65 	<ul style="list-style-type: none"> • Paired-test • 3 months • 400 mg B₂/day 	<ul style="list-style-type: none"> • Reduction in attack frequency from 4 to 2 migraine/month • Attack duration decreased from 50 to 22-28 hrs/attack 	<ul style="list-style-type: none"> • B₂ n=3 • B₂ group: diarrhea, upper abdominal pain, facial erythema 	Unknown	<ul style="list-style-type: none"> • No baseline B₂ information • Not considering confounding factors
Nambair ⁷⁵ 2011	<ul style="list-style-type: none"> • n=100 • Age 18-65 	<ul style="list-style-type: none"> • RCT • 3 months • 100 mg B₂/day 	<ul style="list-style-type: none"> • Attack frequency decreased from 4 to 2.3 migraine/month • Attack duration decreased from 2.8 to 2.1 hrs/attack. • Reduction in headache intensity: visual analogue scale score decreased from 4.9 to 3.6, migraine disability questionnaire score decreased from 4.8 to 3.8 	<ul style="list-style-type: none"> • B₂ n=12 • B₂ group: Vomiting (1), orange discoloration (10), diarrhea (1). 	Unknown	<ul style="list-style-type: none"> • No baseline B₂ information
Rahimdel ⁷⁶ 2015	<ul style="list-style-type: none"> • n=90 • Age 15-55 	<ul style="list-style-type: none"> • Single-blind • 3 months • 400 mg B₂/day 	<ul style="list-style-type: none"> • Reduction in attack frequency (from 9.2 to 2.4 attack/month) • Decreased median attack duration 	<ul style="list-style-type: none"> • B₂ n= 1 • Not differentiated between 2 groups: weight gain, dizziness, and gastrointestinal problem. 	n=5	<ul style="list-style-type: none"> • No baseline B₂ information • Not considering confounding factors

			from 15.1 to 4.2 hrs/month • 71.8% had reduction in headache severity			
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^aRCT: Randomized control trial

^bMA: Migraine with aura

As described above, high-dose riboflavin showed some promising results in alleviating migraine symptoms among the adult population with mild or no side-effects. Yet, studies on adolescents and children had indicated inconclusive results. A double-blind RCT, conducted by Athaillah, investigated 98 adolescents age 12-19, who met the IHS criteria for migraine.⁷⁷ The treatment group was administrated 400 mg riboflavin per day for 3 months. Assessments of migraine frequency, duration, and disability were based on Pediatric Migraine Disability Assessment (PedMIDAS) questionnaire. At the end of the study, migraine frequency, duration as well as the disability were significantly lower in the riboflavin group than the placebo group. Thus, the study concluded riboflavin might be used as migraine preventive therapy for adolescents. In contrast, a double-blind RCT conducted by Dr. MacLennan indicated a negative result.⁷⁸ Forty-eight children ages 5-15 were recruited from schools in the Western Sydney area and referred by pediatricians based on IHS criteria. Children were randomized to a riboflavin group (200 mg/d) or the placebo group for 3 months. Stratification was also performed based on the attack frequency during a 4-weeks baseline measure. No significant difference was detected between two groups in the number of patients achieving 50% or greater

frequency reduction, and no change in migraine severity was detected. The article thus concluded that riboflavin was not an effective agent for prophylaxis in children. Overall, riboflavin showed a less promising effect on children and adolescent migraine patients than the adult population.

CHAPTER TWO: RESEARCH METHOD

Rationale

Though some clinical studies discussed in the previous section indicated some effects of riboflavin, the baseline riboflavin status of the participants and the dietary and supplement riboflavin intake were not assessed. It is possible that individuals with migraine had higher riboflavin needs. Additionally, several confounding variables were not considered in previous clinical studies, which might impact the effectiveness of the riboflavin treatment and the validity of these results. Therefore, this observational cross-sectional study analyzed data collected from a large-scale population from NHANES to examine whether a relationship exists between total riboflavin intake from both dietary and supplement source and the occurrence of migraine.

Moreover, the necessity of administering 400 mg riboflavin, an amount used in several clinical studies, is uncertain. If the maximum absorption per dose or meal is 27 mg, and about 95% of riboflavin intake from food is absorbed, a single dose of 400 mg riboflavin exceeds the bioavailable dose, in theory.³³ The pilot riboflavin prophylaxis study did not justify the reason for the megadose.⁷¹ Based on the discussion of the pilot study, it is possible that the 400 mg riboflavin is used to obtain rapid estimation of the therapeutic potential of riboflavin. Some subsequent clinical studies adopted the megadose method, daily single dose of 400 mg, from the pilot studies because it seemed to be effective. However, one clinical study demonstrated that 100 mg riboflavin

treatment was effective in reducing migraine frequency, severity, and duration, suggesting that the effective dose may be lower than the 400 mg dose used in the original exploratory trial and most subsequent clinical trials exploring the potential relationship between riboflavin and migraine occurrence.⁷⁵ Therefore, recommendations made based on these clinical trials need to reconsider and reevaluate the suggestions. A lower dose or multiple doses with a smaller amount might be a better option in generating effect and lower the cost, implying that higher consumption via food throughout the day may also be effective.

In a larger picture, previous studies that investigated the dietary pattern of migraineurs did not report the average riboflavin intake of migraineurs.^{79,80} It is possible that migraine sufferers are consuming insufficient amount of riboflavin as suggested by the RDA. Hence, this study aimed to fill a gap in the literature by providing a general understanding of the average riboflavin consumption of migraine patients. For the purpose of this study, the NHANES dataset was used because it contained both dietary and migraine-related data and was assessible to the public. The dietary questionnaires incorporated questions developed by the USDA and were delivered by trained professionals. Moreover, the NHANES collected a large sample, approximately 5000 people per year, and provided nationally-representative information, making the analysis more generalizable.⁸¹

Objective

The overall aim of this study was to examine the relationship between riboflavin intake and the risk of migraine as stated in the following objectives:

Objective 1: To assess the dietary, supplement, and total riboflavin intake of adults age 20-50 years, who reported having migraine or severe headache in the past 3 months, compared to those who reported not having migraine or severe headache in the past 3 months.

Strategy:

The average dietary, supplement, and total (diet + supplement) riboflavin intake of self-reported migraineurs was estimated, which was not examined in previous literature.

Objective 2: To examine the relationship between the dietary, supplement, and total riboflavin intake and the occurrence of migraine or severe headache in the past 3 months among adult participants age 20-50 years.

Strategy:

The relationship between dietary, supplement, and total (diet + supplement) riboflavin intake and odds of migraine were investigated, with the intent of assisting future evaluations of the effectiveness of riboflavin as a migraine prophylaxis.

Methods

National Health and Nutrition Examination Survey (NHANES)

All data used in this study was downloaded from National Health and Nutrition Examination Survey (NHANES) database. The NHANES is a nation-wide continuous program of cross-sectional surveys designed to assess the health and nutritional status of adults and children in the United States.⁸¹ The program was first established in 1960 and started continuously collecting data in 1999. As a major program of the National Center for Health Statistics (NCHS), part of the Center of Disease Control and Prevention, NHANES produces crucial health statistics and information, which are used to determine

the prevalence of major diseases and risk factors for diseases. Findings from the program expand the understanding of health for the nation and assist the development of public health policies.⁸¹

The population-based survey has two parts, home interviews and health examinations. The home interviews include demographic socioeconomic, dietary, and health-related questions. The health examinations contain medical, dental, physiological measurements, and laboratory tests. The physical examinations were performed in Mobile Examination Centers (MEC).

Participants in the NHANES were selected using random sampling method. Certain populations, including individuals over the age of 60 years, African American, and Hispanics, were over-sampled to ensure minority groups are represented. A numerical weight is assigned to each participant to assure the collected information can be used to create nationally representative estimates. Annually, the survey collects samples of approximately 5,000 persons. The public data is released on a biannual basis. Therefore, dataset of each NHANES cycle contains information from a length of 2 years (e.g. 2001-2002 is considered as one cycle). The sample weight is created for each 2-year cycle, making the information in each NHANES cycle nationally representative.⁸¹

For the purpose of this study, the public dataset from two continuous cycles (2001-2002 and 2003-2004) were used for the following reasons. The migraine questionnaire that was used to assess migraine status was only included in three NHANES cycles: 1999-2000, 2001-2002, and 2003-2004. Beginning in 2002, the major component of the NHANES dietary assessment method, a 24-hr dietary recall interview, was modified to include an automated dietary interview system.⁸² The new method is

demonstrated to reduce bias in reporting intake compared to older methods.⁸³ Moreover, additional quality assurance and quality control were applied starting from 2001-2002 cycle, which matched reported supplement to know supplement, included additional supplement label when possible, removed inappropriate products, and assigned matching codes.⁸⁴ Thus, only 2001-2002 and 2003-2004 datasets were included to maintain the consistency and validity of dietary data during the years that migraine data was available.

Study population

Based on the prevalence and characteristics of migraine patients, the sample of this study was limited to adults, aged from 20-50 years, and non-menopausal if the gender was female.⁴ Since estrogen is associated with migraine incidence, females who were pregnant or breastfeeding were excluded due to their hormonal change.⁸⁵ A total of 5,528 participants aged 20-50 years old participated in NHANES 2001-2002, and 2003-2004 cycles. Individuals who were pregnant (n=505), breastfeeding (n=83), and menopausal (n=308) were excluded.

Chlorpromazine, a tricyclic phenothiazine drug found in many antidepressants, inhibits the intestinal riboflavin uptake by competitively binding to the riboflavin carriers.^{29(p326)} Hence, individuals that indicated tricyclic medication use (n=160) were excluded using the Prescription Medications Questionnaire. Thyroid hormone appears to affect the conversion of riboflavin to its coenzyme forms, FMN and FAD.^{29(p325)} Thus, individuals who had thyroid disease (n=71) were excluded as determined based on the Medical Condition Questionnaire. Participants who answered “Yes” to question MCQ170M “Do you still have a thyroid problem?” were excluded. Alcoholism and diabetes may lead to suboptimal riboflavin level or riboflavin deficiency.^{29(p325)} Based on

the National Institute on Alcohol Abuse and Alcoholism, heavy alcohol use is binge drinking having 5 or more drinks in one occasion for male and 4 or more drinks for female.⁸⁶ Therefore, individuals who reported consuming 150 or more drinks per month (n=40) were excluded because of the heavy alcohol use, with the understanding that this criterion is not an indication of alcoholism. Diabetic participants (n=155) were excluded according to responses from the Diabetes Questionnaire.

Individuals with missing data (n=576) for Body Mass Index (BMI), gender, caffeine intake, migraine status, and riboflavin intake were also excluded based on the Demographic Variables & Sample Weights Questionnaire, Body Measures Questionnaire, Miscellaneous Pain Questionnaire, and Dietary Interview - Total Nutrient Intakes Questionnaire. Unrealistic dietary intake was determined based on the daily calorie intake. Though energy need is different for each individual, the recommended average daily calorie intake is 1600-2400 calories for adult women and 2000-3000 calories for adult men.⁸⁷ Thus, participants who consumed less than 500 calories per day or over 5000 calories per day were excluded (n=137).

After excluding disqualified individuals (n=1,894), a total of 3,634 individuals were included in the analysis of this study (**Figure 3**).

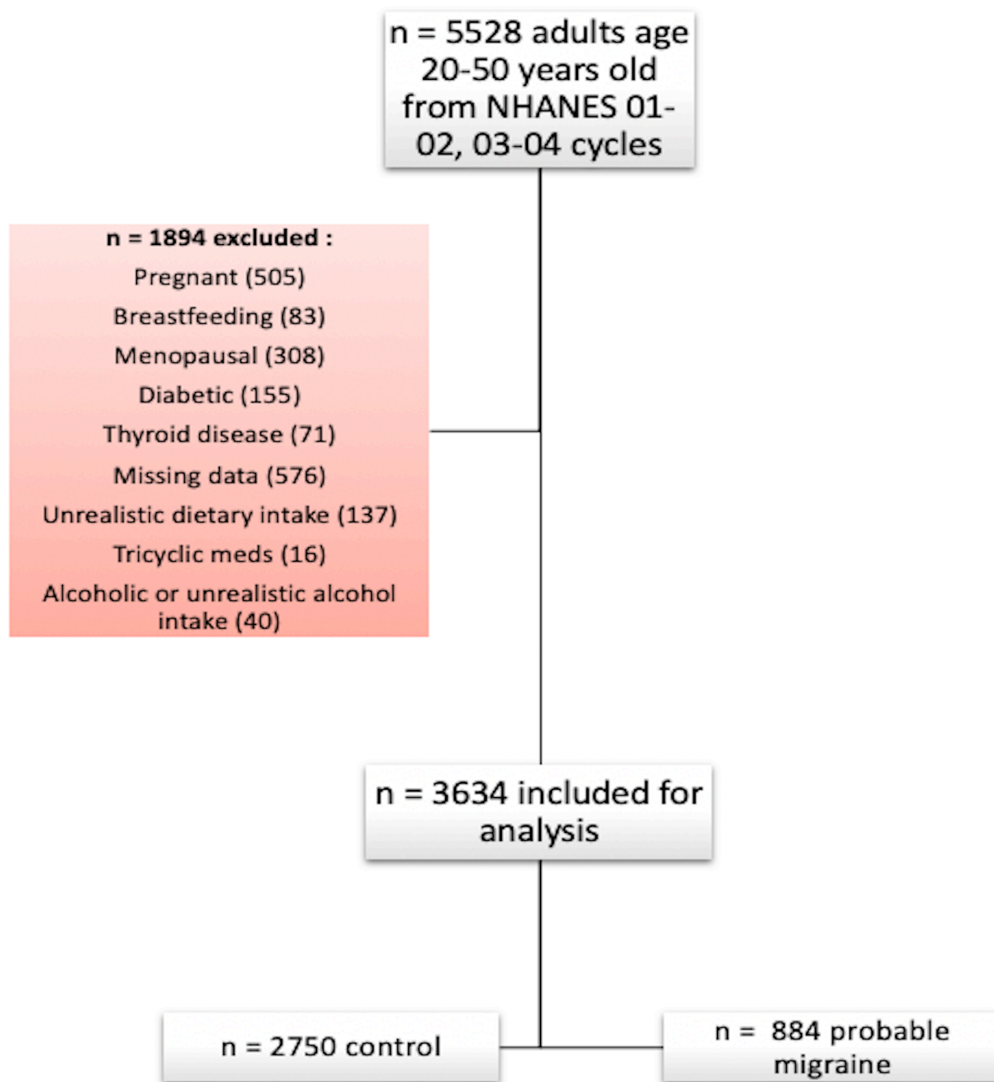


Figure 3 Flow diagram of selecting participants

Migraine assessment

Migraine status was determined based on the self-reported answer to the NHANES Miscellaneous Pain Questionnaire (MPQ).^{88,89} Survey participants at the age of 20 or older completed the MPQ. The questionnaire was only administered in 3 continuous cycles, 1999-2000, 2001-2002, and 2003-2004. The question MPQ090 asked

“During the past 3 months, did you have severe headaches or migraines?” Participants who answered “Yes” were categorized as having probable migraine and classified in the migraine group.

Riboflavin intake assessment

Dietary intake of riboflavin was determined from an in-person 24-hour recall that was administered during the MEC exam. Starting in 2002, 24-hour diet recalls were collected using Automated Multiple Pass Method developed by the USDA.⁹⁰ Since only one 24-hour recall data is available from the 2001-2002 cycle, only the first day 24-hour recall data of the 2003-2004 cycle was used in this study to remain the dataset consistency of the two cycles. The numerical value of the daily riboflavin intake was used in this study, which was computed from the 24-hour recall and reported in milligram in the NHANES Dietary Interview- Total Nutrient Intakes data file.

Supplemental riboflavin intake was acquired from the dietary supplements section (DSQ), which contained data on the use of vitamins, mineral, herbals and other dietary supplements. Supplements that contain riboflavin in their ingredients were included. Since most clinical studies mentioned in previous chapter administered riboflavin for 3 months or more, only individuals who reported taking the supplement for 90 days or more (n=789) were analyzed for supplemental riboflavin intake in this study. The daily supplemental riboflavin intake was calculated based on the supplemental riboflavin intake on the past 30 days. The numerical value of average total daily amount of supplemental riboflavin intake within the past 30 days was calculated using the following information: servings taken per day \times number of pills per serving \times dosage per pill in mg

× percentage of riboflavin content per pill in mg (as needed) × total days using supplement / past 30 days.

The total daily riboflavin intake was calculated by combining the supplemental intake and dietary intake. Total riboflavin intakes of individuals who were not meeting the 90-day threshold or had missing supplemental intake information only included their dietary intakes, and were thus equivalent to their dietary riboflavin intakes.

Statistical analysis

The continuous variables in NHANES dataset included age, alcohol intake, BMI, caffeine intake, riboflavin intake. The categorical variable was sex, including male and female. For the purpose of this study, alcohol intake, caffeine intake, riboflavin intake data were categorized into alcohol intake groups, dietary caffeine intake groups, and riboflavin intake quartiles to minimize the influence of outliers. Alcohol consumption was categorized as <1 drink per month, 1-19 drinks per month, and ≥ 20 drinks per month. Dietary caffeine consumption was categorized to as <5 mg per day (effectively no consumption), 5-99 mg per day, 100-399 mg per day, and ≥ 400 mg per day, where over 400 mg is considered the safe and healthy limit of consumption.⁹¹ Body Mass Index (BMI) was also categorized based on the scores that defines underweight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²), overweight (25.0-29.9 kg/m²), and obese (≥ 30.0 kg/m²).⁹²

Self-reported occurrence of migraine was a binary outcome in the study. Summary statistics were used for presenting the data. For demographic characteristics, the number of observations (n) and the proportions (%) of the corresponding category were included. For daily riboflavin intake quartile comparison between the migraine

group and control group, the number of observations and the proportions of the corresponding quartile were reported. For gender-specific daily riboflavin intake quartile analysis, the range, mean, and median of daily riboflavin intake were shown. Demographic comparisons between migraine group and controls were performed using a t-test of the mean for continuous variables and a Chi-square (χ^2) test for categorical variables. Dietary, supplemental, and total riboflavin intakes were divided into quartiles. Adjusted odds ratio (OR) and the corresponding 95% confidence intervals (CI) were computed using a logistic regression model, controlling for covariates that were found to be significantly different between the migraine and control group. Dietary, supplement, and total riboflavin quartiles were further stratified by gender in the subgroup analysis. An adjusted Wald test was used to compute the p-overall value, adjusting for covariates that were found to be significantly different between the migraine and control group. For the total riboflavin intake quartile analysis, an additional adjustment of riboflavin supplement use was performed to examine the potential influence of the source of riboflavin intake. For all statistical tests used in this study, a p-value less than 0.05 was defined as statistically significant. STATA/IC 15.1 (StataCorp, College Station, TX, USA) was used for statistical analysis.⁹³

Sample weighting

To ensure the samples are nationally representative, a numerical sample weight was assigned to each individual sampled in NHANES, which measures the number of people in the population represented by that specific participant. The assignment of sample weight adjusts unequal selection probabilities, certain types of non-response, and independent estimates of population size for specific age, sex, and race categories. Since

NHANES suggests that weights for the detailed sample must be used if variables from the detailed examination are used, the full sample 2 year MEC exam weight (WTMEC2YR) was applied to all the summary statistics as well as all the statistical tests in this study to represent the U.S. population.⁹⁴

CHAPTER THREE: RESULTS AND DISCUSSION

Results

Demographics

The data of 3,634 NHANES participants from the 2001- 2002 and 2003-2004 cycles were analyzed in this study. Of these 3,634 participants, 884 were classified in the probable migraine group, and 2,750 were in the control group. The demographic characteristics of the study participants were summarized in **Table 2**. The majority of the study sample was male (54.60%). Most of the participants had a normal BMI (36.70%), followed by the overweight group (32.56%). The most common level of alcohol consumption was 1 to 19 drinks per month, with 39.76% of the study sample consuming this level, which was similar to the proportion of individuals that consumed less than 1 drink per month (38.67%). Individuals that ingested 100 to 399 mg caffeine per day contributed to the largest proportion (44.37%) of the caffeine groups. The average age \pm Standard Error (SE) of the overall sample was 34.65 ± 0.24 years old. The overall mean riboflavin intake was 2.30 ± 0.04 mg/day from dietary source, and 7.66 ± 0.89 mg/day from supplement source. The total riboflavin intake was 4.27 ± 0.20 mg/day for the overall study sample.

When comparing the migraine and control group, there were more female (62.94%) than male (37.06%) in the probable migraine group, while more male (60.26%)

than female (39.74%) in the control group. The difference in sex between groups was highly statistically significant ($p < 0.0001$) between the two groups. BMI was significantly different between the migraine group and the control group ($p=0.0224$). The migraine group had a larger proportion of obese participants ($\text{BMI} \geq 30 \text{ kg/m}^2$) than the control group (31.62% vs. 27.33%), and a higher percentage of underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$) individuals than the control group (3.56% vs. 2.05%). For alcohol consumption, a higher percentage ($p = 0.0005$) of participants consumed less than 1 drink per month in the migraine group (44.97%) when compared to the control group (36.64%). The caffeine consumption of the migraine and control group was similar ($p=0.184$).

The mean age of the migraine (34.26 ± 0.40 years) and control group (34.76 ± 0.32 years) was not significantly different ($p= 0.37$). The mean daily dietary riboflavin intake of migraine group (2.14 ± 0.06 mg) was very highly significantly lower ($p<0.0001$) than the mean intake of the control group (2.36 ± 0.04 mg). The mean daily supplement intake was not statistically different between the migraine group and the control group ($p=0.652$). The migraine group had a numerically higher mean daily supplement riboflavin intake (9.37 ± 1.63 mg) than the control group (7.19 ± 0.97 mg). The mean daily total riboflavin intake of the migraine group was 4.25 ± 0.38 mg, which was not significantly different ($p=0.958$) from the mean total intake of the control group (4.28 ± 0.23 mg).

Table 2 Demographic characteristics of study participants across migraine and control groups

	Total		Migraine^a		Control		
	(n=3634)		(n=884)		(n=2750)		
Variable	n	%^b	n	%	n	%	P^b
Sex							<0.0001
Male	1984	54.6	328	37.06	1657	60.26	
Female	1650	45.4	556	62.94	1093	39.74	
BMI (kg/m²)							0.0224
<18.5	88	2.42	31	3.56	56	2.05	
18.5 – <24.9	1334	36.7	306	34.61	1026	37.31	
25 - <30	1183	32.56	267	30.22	916	33.31	
≥30	1031	28.38	280	31.62	752	27.33	
Alcohol Consumption							<0.0001
<1 drinks/mo	1405	38.67	398	44.97	1008	36.64	
1-19 drinks/mo	1445	39.76	360	40.69	1085	39.45	
≥20 drinks/mo	784	21.57	127	14.34	658	23.91	
Dietary Caffeine							0.1844
< 5 mg/day	637	17.54	150	16.94	488	17.73	
5-99 mg/day	954	26.24	219	24.76	735	26.72	
100-399 mg/day	1612	44.37	389	44.04	1223	44.47	
≥400 mg/day	431	11.85	126	14.26	305	11.08	
	Mean	SE^c	Mean	SE	Mean	SE	P
Age (year)	34.65	±0.24	34.26	±0.40	34.77	±0.32	0.370
Mean daily dietary B₂^d intake (mg/day)	2.30	±0.04	2.14	±0.05	2.36	±0.04	=0.0005
Mean daily supplement B₂ intake (mg/day)	7.66	±0.89	9.37	±1.63	7.19	±0.97	0.652

Mean Daily total B₂ intake (mg/day)	4.27	±0.20	4.25	±0.38	4.28	±0.23	0.958
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^aMigraine group: who answered yes to question “During the past 3 months, did you have severe headaches or migraines?”

^bp: Chi-square test for categorical variables, and t-test for continuous variables.

^cSE: Standard Error

^dB₂: Riboflavin

Demographics were further stratified by the total daily riboflavin intake quartiles and presented using summary statistics as shown in **Table 3**. The ranges of the total daily riboflavin intake quartile 1 (Q1), quartile 2 (Q2), quartile 3 (Q3), and quartile 4 (Q4) were 0-1.58 mg, 1.58-2.45 mg, 2.45-3.60 mg, and 3.60 -205.16 mg. There were more females than males in Q1 (60.35% vs. 39.65%), while more males than females in Q3 (62.83% vs 37.17) and Q4 (65.39% vs. 34.61%). Normal weight individuals (BMI 18.5-24.9) contributed to the largest proportion to all quartiles (33.62% Q1, 35.83% Q2, 37.52% Q3, 39.64% Q4). Alcohol consumption in moderate consumption (1-19 drinks/month) represented the greatest percentage of the population in Q3 (41.57%) and Q4 (42.09%), while individuals who consumed less than 1 drink per month contributed to the largest proportion in Q1 (45.83%) and Q2 (39.12%). Individuals consuming moderate caffeine intake (100-399 mg per day) represented the largest population in all four quartiles (38.20% Q1, 47.88% Q2, 48.61% Q3, 42.78% Q4).

Table 3 Demographics stratified by total daily riboflavin intake quartiles

Daily total riboflavin intake quartiles								
	Q1		Q2		Q3		Q4	

	(0- 1.581 mg)		(1.58- 2.45 mg)		(2.45- 3.60 mg)		(3.60- 205.16 mg)	
	n ^a	% ^a	n	%	n	%	n	%
Sex								
Males	360	39.65	460	50.57	570	62.83	594	65.39
Females (pre- menopause)	549	60.35	449	49.43	338	37.17	314	34.61
BMI								
<18.5 kg/m ²	21	2.3	28	3.12	19	2.06	20	2.18
18.5 – 24.9 kg/m ²	306	33.62	326	35.83	341	37.52	360	39.64
25 - <30 kg/m ²	278	30.57	279	30.69	310	34.13	316	34.83
≥30 kg/m ²	305	33.51	276	30.36	239	26.29	212	23.35
Alcohol intake								
<1 drinks/mo	417	45.83	356	39.12	321	35.38	312	34.35
1-19 drinks/mo	338	37.16	347	38.2	377	41.57	382	42.09

≥ 20 drinks/mo	155	17	206	22.69	209	23.05	214	23.56
Caffeine intake								
< 5 mg/day	243	26.68	137	15.12	120	13.24	137	15.1
5-99 mg/day	284	31.19	231	25.44	239	26.35	200	21.98
100-399 mg/day	347	38.2	435	47.88	441	48.61	388	42.78
≥400 mg/day	36	3.93	105	11.55	107	11.8	183	20.14

Riboflavin intake

Comparisons of the daily dietary, supplemental, and total riboflavin intake were made between the probable migraine group and the control group using weighted summary statistics and Wald test controlling for sex, BMI, and alcohol intake (**Table 4**). The range of each dietary riboflavin intake quartile was: 0-1.45 mg in Q1, 1.45 - 2.07 mg in Q2, 2.07- 2.87 mg in Q3, and 2.87-18.59 mg in Q4. The first quartile was the reference when computing the ORs and 95% CIs of other quartiles. An association was found in daily dietary riboflavin consumption and the odds of migraine ($p_{\text{overall}} = 0.0013$), with Q3 showing a significantly lower OR when compared to Q1 ($\text{OR}_{\text{Q3}} = 0.732$, $p = 0.026$). Approximately 27% reduction in the odds of migraine was observed in Q3 compared to Q1. Both the ORs of Q2 ($\text{OR}_{\text{Q2}} = 1.053$, $p = 0.607$) and Q4 ($\text{OR}_{\text{Q4}} = 0.977$, $p = 0.847$) were

not significantly different from Q1. Upon a casual comparison of the daily dietary riboflavin intake, the migraine group showed a higher percentage of the population than the control group in Q1 (29.64% vs. 23.51%) and Q2 (28.68% vs. 23.89%), while the control group had a larger percentage of the population than the migraine group in Q3 (26.64% vs. 19.82%) and Q4 (25.96% vs 21.87%).

For supplemental riboflavin intake, a total of 789 individuals were included, consisting approximately 21.7% of the overall study sample. The range of Q1 for riboflavin intake from supplement sources was 0.17-1.17 mg, Q2 was 1.19-1.70 mg, Q3 was 1.98-5.00 mg, and Q4 was 5.10-202.00 mg. Although, the ORs increased numerically as the consumption increased, $OR_{Q2}=0.881$ [0.523-1.483], $OR_{Q3}=1.228$ [0.520-2.904], and $OR_{Q4}=1.565$ [0.905-2.707], no statistically significant association between the supplement riboflavin intake and the odds of migraine was detected ($p_{overall}=0.1988$). A greater percentage of the population was present in Q1 (25.30%) and Q2 (41.75%) in the control group. Migraine group showed a higher percentage of the population in Q3 (10.62%) and Q4 (30.89%) when compared to the control group (9.69% and 23.27%).

The range of total daily riboflavin intake quartiles was 0-1.58 mg in Q1, 1.58-2.45 mg in Q2, 2.45-3.60 mg in Q3, and 3.60 -205.16 mg in Q4. Only the OR of Q3 (0.630 [0.463-0.859]) was highly significantly lower when compared to Q1 ($p=0.005$), representing a 37% decrease in the odds of migraine for riboflavin consumers in Q3 as compared to Q1. Similar to dietary intake levels, both the ORs of Q2 (0.780 [0.579-1.052]) and Q4 (0.809 [0.604-1.086]) of total riboflavin intake were not significantly different from Q1. There was an association of the total daily riboflavin intake and the

odds of migraine ($p_{\text{overall}} = 0.032$). After further adjusting for whether riboflavin supplement was used, the association remained consistent ($p_{\text{overall}} = 0.034$). When comparing the total daily riboflavin intake, migraine group indicated a higher proportion of people falling in Q1 (32.29%) and Q2 (25.14%) than the control group (22.66% and 24.97%).

Table 4 Daily riboflavin intake quartiles and the odds of migraine

Riboflavin Intake Quartiles						
	Migraine group^a (n=884)		Control group (n=2750)		OR^b[CI]^c	
	n	%	n	%		
Dietary daily B₂^d intake						p_{overall}^e =0.0013
Q1 (0 - 1.45 mg)	262	29.64	647	23.51	1.000 (reference)	
Q2 (1.45-2.07 mg)	254	28.68	657	23.89	1.053 [0.824-1.345]	p =0.67
Q3 (2.07-2.87 mg)	175	19.82	733	26.64	0.732 [0.558-0.962]	p =0.026
Q4 (2.87-18.59 mg)	193	21.87	714	25.96	0.977 [0.769-1.243]	p =0.847
Supplement daily B₂ intake						p_{overall} =0.1988
Q1 (0.17-1.17 mg)	42	24.03	156	25.3	1.000 (reference)	

Q2 (1.19-1.70 mg)	60	34.46	257	41.75	0.881 [0.523-1.483]	p =0.623
Q3 (1.98-5.00 mg)	18	10.62	60	9.69	1.228 [0.520-2.904]	p =0.629
Q4 (5.10-202.00 mg)	54	30.89	143	23.27	1.565 [0.905-2.707]	p =0.105
Total daily B₂ intake						p_{overall} =0.032
Q1 (0-1.58 mg)	285	32.29	623	22.66	1.000 (reference)	
Q2 (1.58-2.45 mg)	222	25.14	687	24.97	0.780 [0.579-1.052]	p =0.101
Q3 (2.45-3.60 mg)	172	19.5	736	26.77	0.630 [0.463-0.859]	p =0.005
Q4 (3.60 - 205.16 mg)	204	23.07	704	25.61	0.809 [0.604-1.086]	p =0.152

^aMigraine group: who answered yes to question “During the past 3 months, did you have severe headaches or migraines?”

^bOR: Odds Ratio

^cCI: 95% Confidence Interval

^dB₂: Riboflavin

^ep_{overall}: Wald test. Adjusted for sex, BMI, and alcohol consumption

Subgroup analysis further stratified dietary, supplement, and total daily riboflavin intake by gender (**Table 5**). A total of 1,617 females and 2,017 males were included in

the stratified analysis. The sample size of supplement users was $n=384$ for female and $n=405$ for male.

Male population in this study illustrated higher mean and median dietary riboflavin intakes than female population in all four dietary intake quartiles. There was no association between daily dietary riboflavin intake and the odds of migraine after stratification by sex ($p_{\text{overall:female}} = 0.594$, $p_{\text{overall:male}} = 0.543$). Although no statistical significance was found, the ORs of the male population followed the same pattern as the ORs of the overall sample, with Q3 showing the lowest OR (0.775 [0.523-1.148]) compared to Q1.

For the daily supplement intake quartiles, females showed higher mean supplemental riboflavin intakes in Q1 and Q2, while lower mean intakes in Q3 and Q4 when compared to males. In the female population, a positive association was found between the supplement riboflavin intake and the odds of reporting migraine ($p = 0.0039$). The OR of the female population decreased in Q1 (0.627 [0.342-1.150]) but increased in Q3 (1.340 [0.440-4.076]) and Q4 (1.953 [0.969-3.938]). In contrast, no significant association was found between the supplement riboflavin intake and the odds of reporting migraine in the male population ($p=0.653$).

For the total daily riboflavin intake quartiles, the male population reported greater mean and median total riboflavin intakes than the female population in all four quartiles. The ORs of the female population in Q3 indicating the lowest OR (0.653 [0.437-0.976]), which was significantly lower than the reference Q1 ($p=0.038$). Yet, there was no statistically significant association between the total riboflavin intake and the odds of migraine in both male and female population ($p_{\text{female}} = 0.127$, $p_{\text{male}} = 0.511$). The result

remained consistent after further adjusting for whether riboflavin supplement was used

($p_{\text{female}} = 0.103$, $p_{\text{male}} = 0.677$).

Table 5 Summary statistics of riboflavin intake quartiles stratified by sex

	Female					Male				
	(n=1617)					(n=2017)				
	range(mg)	mean(mg)	median(mg)	OR^a [CI]^b	p	range(mg)	mean(mg)	median(mg)	OR[CI]	p
Dietary daily B₂^c intake										
Q 1	0-1.26	0.9	0.94	1.000 (reference)	p_{overall}^d = 0.594	0.16-1.68	1.22	1.3	1.000 (reference)	p_{overall} = 0.543
Q 2	1.26-1.78	1.51	1.51	1.137 [0.807-1.602]	p=0.449	1.68-2.40	2.04	2.05	0.967 [0.611-1.530]	p=0.882
Q 3	1.78-2.39	2.06	2.04	0.931 [0.684-1.268]	p=0.641	2.41-3.24	2.79	2.78	0.775 [0.523-1.148]	p=0.196
Q 4	2.39-9.85	3.21	2.9	0.936 [0.650-1.347]	p=0.713	3.24-18.59	4.45	4.05	0.933 [0.670-1.300]	p=0.674
Supplement daily B₂ intake										
Q 1	0.02-1.13	0.71	0.74	1.000 (reference)	p_{overall} = 0.0039	0.06-1.28	0.69	0.85	1.000 (reference)	p_{overall} = 0.653
Q 2	1.20-1.70	1.68	1.7	0.627 [0.342-1.150]	p=0.127	1.30-1.70	1.67	1.7	1.531 [0.642-3.649]	p=0.325

Q 3	1.98-3.89	3	3.33	1.340 [0.44 0-4.076]	p=0.596	2.04-5.10	3.77	3.4	0.779 [0.25 5-2.376]	p=0.651
Q 4	4.17-202	24.88	12	1.953 [0.96 9-3.938]	p=0.061	5.33-150	31.5	17.5	1.263 [0.47 3-3.368]	p=0.630
Total daily B₂ intake										
Q 1	0-1.37	0.97	1.02	1.000 (reference)	p _{overall} =0.127	0.16-1.83	1.3	1.35	1.000 (reference)	p _{overall} =0.511
Q 2	1.37-2.05	1.71	1.71	0.959 [0.69 4-1.327]	p=0.796	1.83-2.76	2.3	2.3	0.839 [0.57 4-1.227]	p=0.354
Q 3	2.05-3.21	2.57	2.55	0.653 [0.43 7-0.976]	p=0.038	2.76-3.96	3.29	3.27	0.873 [0.57 0-1.339]	p=0.532
Q 4	3.21-205.16	10.47	4.3	0.808 [0.54 6-1.195]	p=0.274	3.97-155.86	11.36	5.42	0.745 [0.50 7-1.095]	p=0.129

^aOR: Odds Ratio of migraine occurrence

^bCI: 95% Confidence Interval

^cp_{overall}: Wald test. Adjusted for sex, BMI, and alcohol consumption

^dB₂: Riboflavin

Discussion

In this observational cross-sectional study, daily dietary riboflavin intake was associated with the odds of migraine. A statistically significant 27% reduction was observed in the odds of migraine for individual consuming dietary riboflavin intake in the

third quartile (2.07 to 2.87 mg/day) compared to the first quartile (0 to 1.45 mg/day), while no significant change in migraine odds of individuals in the fourth quartile, whose intake exceeded 2.87 mg. A wide range of riboflavin intake was observed in the fourth quartile when compared with other quartiles, from 2.87 to 18.59 mg/day, demonstrating a high between-person variation in riboflavin intake. The eating pattern of an individual who consumed 18.59 mg riboflavin might differ substantially from the eating pattern of the individual who consumed only 2.87 mg. Furthermore, migraine sufferers with more severe symptoms might adopt dietary interventions that incorporate higher riboflavin content in an attempt to relieve migraine symptoms. Thus, the potential dietary intervention adoption and varying dietary pattern of individuals in the fourth quartile might impact the observed odds ratio in this group.

Consistent with findings of the dietary riboflavin intake, a relationship was also observed between daily total riboflavin intake, which considered riboflavin intake from both diet and supplement, and the occurrence of migraine. A 37% reduction in the migraine occurrence was observed in the third quartile, daily total riboflavin consumption between 2.45 mg/day to 3.60 mg/day, as compared to the reference quartile consumption (below 1.58 mg/day). Nonetheless, the association was not observed in the highest-consuming quartile, since there was no significant difference observed between quartile 4, whose total riboflavin intake surpassed 3.60 mg/day, and the reference group. After further adjusting supplement intake, the association remained significant, indicating that the source of riboflavin did not impact the association between riboflavin intake and migraine occurrence. A decrease in migraine odds was observed in the third quartile but not in the fourth, which might imply that migraine sufferers were more likely to adopt

dietary interventions or use supplements in an attempt to alleviate their symptoms, and therefore showed a higher riboflavin intake while experiencing migraine.

When stratified by sex, no association was detected in both the dietary and total riboflavin intake analysis. While not rising to statistical significance, it can be noted that the migraine odds in the male population followed a similar pattern to the overall sample, with odds of migraine decreasing when the dietary riboflavin intake increased through the third quartile. Moreover, the trend of migraine occurrence in the female population was similar to the trend in the overall sample when the total riboflavin intake increased, with the migraine odds in the third quartile being significantly lower than the first quartile. Thus, the smaller sample size after gender stratification might decrease the power of detecting any difference.

Statistically significant association was not detected between the supplement riboflavin intake and the odds of migraine, but the numerical value of the odds ratio indicated a trend of increasing migraine occurrence as the supplement riboflavin intake increased. The small sample size of the supplement intake population (n=789) might affect the power of detecting any difference. The percentage of riboflavin supplement user in this study is 21.7% using NHANES 2001-2004 data, which is close to 20% riboflavin supplement user (aged 2 over) as reported in What We Eat in America (WWEA) food survey using NHANES 2013-2014 data.³⁷

When stratified by gender, a statistically positive association was observed between the supplement riboflavin intake and the odds of migraine in the female population, but not in the male population. However, the sample size of supplement users after stratification was further decreased in both male and female groups. Additionally,

the 95% confidence interval of the odds ratios of each supplement riboflavin intake quartiles varied substantially in both male and female population, indicating a low precision of the odds ratios. Therefore, the trend of increasing odds of reporting migraine in the female population as the daily supplement intake increased, and the absent association between supplement riboflavin intake and migraine occurrence in the male population may not accurately reflect the true relationship between riboflavin intake from supplemental source and the odds of migraine. Assuming the findings in this study reflects the actual relationship, the increasing migraine odds when supplement riboflavin consumption increases may indicate that individuals with migraine are intentionally consuming supplement with more riboflavin to alleviate the symptoms.

Findings in this study suggest the prudence of reevaluating the effective dose of riboflavin supplements as migraine prophylaxis since this study found a significant migraine occurrence reduction when dietary and total riboflavin intake reached 2.07-2.87 mg/day and 2.45-3.60 mg/day, which are approximately 100 times less than the amount administered in previous clinical studies (400 mg). Additionally, the riboflavin ingestion pattern (daily single dose or multiple doses at different times) may be reconsidered, because dietary riboflavin intake was distributed in each meal and indicated an association with migraine odds, as observed in this study.

When examining the average riboflavin intake, the mean dietary riboflavin intake of probable migraine group was found to be highly significantly lower than the control group. Yet, the numerical difference between two groups, 0.22 mg, is approximately equivalent to the riboflavin content in $\frac{1}{2}$ cup non-fat milk, an egg, or $\frac{1}{2}$ cup spinach, which can be achieved with minor changes in diet. No difference was found in the mean

daily total riboflavin intake and the mean daily supplement riboflavin intake across groups.

The average dietary riboflavin consumption in both the total population (2.30 mg) and the control group (2.36 mg) are slightly higher than the mean riboflavin intake from food source reported in 2013-2014 WWEA survey (2.14 mg),³⁷ whereas the migraine group in this study has the same mean dietary riboflavin intake (2.14 mg) as reported in WWEA survey. In the WWEA survey, the study sample included individuals aged 2 or over, which is a much wider age range than study sample in this study. Additionally, the WWEA survey uses more recent 2013-2014 NHANES dataset, while this study uses 2001-2004 NHANES dataset. Furthermore, the demographic characteristics of the samples included in this study are different from the WWEA survey, since multiple exclusion criteria are applied in this study. Thus, the deviations of the average intakes are expected.

The overall mean supplement riboflavin intake reported in WWEA survey is 8.64 mg, which is higher than the mean intake of our total sample (7.66 mg) and control group (7.19 mg), while lower than the mean intake of our migraine group (9.37 mg). The reported average total riboflavin consumption from both food and supplement source is 3.85 mg in WWEA survey, which is lower than the intake of the overall samples (4.27 mg), migraine group (4.25 mg), and control group (4.28 mg) in this study.

The mean daily total riboflavin consumption of both the migraine group and the control group achieved the RDA recommendation, 1.3 mg/day for adult men and 1.1 mg/day for adult women.³⁶ When determining the riboflavin RDA levels, the Food and Nutrition Board of the Institute of Medicine considered multiple indicators, including

erythrocyte glutathione reductase (coenzyme activity), erythrocyte flavin (cellular riboflavin concentration), and urinary flavin (absorption saturation).³⁴ The RDA provides recommended riboflavin intake levels to maintain regular metabolism and prevent riboflavin deficiency-related clinical symptoms. Yet, it is possible that a higher amount riboflavin consumption (2.45-3.60 mg/day) than the RDA recommendation promotes beneficial effect by decreasing the oxidative stress and assisting energy production, or through some unknown biochemical pathways. Furthermore, the RDA guidelines are intended for healthy individuals, but not individuals with a medical condition such as migraine. In this study, starting from the second quartile (total riboflavin intake 1.58-2.45 mg/day) none of the individuals consume riboflavin lower than the RDA, but only the third quartile (total riboflavin intake 2.45-3.60 mg/day) has a significantly lower odds of migraine. Thus, migraine patients may require a different, presumably higher riboflavin intake, than the healthy population.

Study population characteristics

In this study, there were more males than females in the overall study sample. Some exclusion criteria of this study, including pregnancy, breastfeeding, and menopause, specifically targeted females, which might result in the gender disproportion observed in the overall sample. The migraine group characteristics of sample in this study were consistent with the migraine population characteristics identified in previous literatures.

In this study, the probable migraine group contained a higher percentage of females than males, which is consistent with the previous finding that migraine is more prevalent in female than male in the U.S. population.⁸ When stratified by total riboflavin

intake quartiles, more females were in the first quartile, while more males were in the third and fourth quartiles, indicating more females are consuming lower amount of riboflavin than males. Such distribution was further confirmed in the later gender-specific analysis, which showed that male population had higher means and medians in both the dietary riboflavin intake quartiles and the total riboflavin intake quartiles. In general, males have a higher dietary intake and higher RDA than females in order to fulfill their physiological need, which may lead to the distribution found in this study.³⁴

A higher percentage of underweight and obese individuals were found in the migraine group when compared to the control ($p=0.02$). Since studies have consistently suggested that the risk of migraine increases among obese and underweight individuals, the higher frequency of obese and underweight individuals in the probable migraine group in this study is in agreement with previous literature.^{95,96} Normal weight individuals contributed to the largest proportion of all four dietary riboflavin intake quartiles. However, the percentage of obese individuals tends to decrease from the first quartile to the fourth quartile. Previous study found that the Healthy Eating Index score of obese population is significantly lower than normal weigh population, indicating the association between poor diet quality and obesity.⁹⁷ A poor quality diet usually provides excessive empty calories and lower essential nutrients, such as riboflavin, than a high quality diet, which is consistent with the distribution found in this study.

A higher proportion of the migraine group illustrated low or no alcohol consumption ($p < 0.0001$). Because alcohol is a commonly reported migraine trigger,^{28,80,98} migraine patients may avoid alcohol consumption to prevent migraine

symptoms, which may lead to different alcohol consumption between migraine group and the control in this study.

No difference in caffeine consumption was detected across groups ($p=0.1844$). The greatest proportion of both the migraine and control group consumed 100-399 mg caffeine per day. Individuals who consumed 100-399 mg caffeine per day also consisted of the largest proportion in all four riboflavin intake quartiles. Approximately 85% of the U.S. population consumes at least one caffeinated beverage per day.⁹⁹ In the U.S., the mean caffeine intake of adults ages 20 years and over is 171.5 mg per day.¹⁰⁰ The social norm of caffeine consumption may explain the similarities in the caffeine intake pattern between the migraine and control groups in this study.

Strengths and Limitations

The observational cross-sectional design of this study is one limitation, which cannot provide any causal effect between the variable and outcome. In this study, the classification of migraine group is limited, since only one question in NHANES study asked whether the participant had severe headaches or migraines in the past 3 months. With no additional information, severe headache and migraine are not distinguishable, and the specific subtypes of migraine are not identifiable. Individuals who were classified in the migraine group may not fulfill the clinical diagnosis of migraine. Indeed, the percentage of migraine population in this study was 24.4%, which is slightly higher than the migraine prevalence in the U.S, 20.7% of females and 9.7% of males.⁸ Other studies using NHANES database also reported a higher proportion of migraine patients.^{4,79} Since some non-migraineurs were classified as migraineurs, a higher odds of migraine might be observed in this study sample than the actual population using the same predictor.

However, this approximately 5% difference would have a minor impact on the analysis and results in this study, because the odds ratios were compared within this sample.

With limited information about the migraine variable, we were not able to identify any individual migraine symptom or the severity of the symptoms. Since the outcome in this study is binary, we could not evaluate the relationship between riboflavin intake and the occurrence or the severity of the specific migraine symptoms. Several clinical studies discussed in background section found that 400 mg daily single dose of riboflavin can effectively reduce migraine symptoms but may not eliminate migraine. Therefore, it is possible that individuals with higher frequency and/or severity of migraine may be aware of this prior research, making them more likely to consume or take a higher amount of riboflavin supplement to relieve the symptoms. Even with some resulting decrease in the severity, the migraines would not be expected to be completely prevented, which would lead the migraineur to still answer “yes” to the NHANES migraine assessment question. Such a limitation may result in higher odds of migraine when supplement riboflavin consumption increases. Since this data cannot provide solid interpretations regarding supplement riboflavin intake and migraine occurrence, this study cannot support nor deny the effectiveness of the mega dose riboflavin supplementation in reducing migraine symptoms as observed in previous clinical studies.

Another limitation of this study is the distinct riboflavin needs of different individuals. The RDA of riboflavin varies by sex, age and pregnancy status.³⁴ This study excluded pregnant and breastfeeding individuals, limited the age to only include adults, and performed sex-specific analysis. However, the riboflavin needs are still subjected to between person variation.

The strength of this study includes the large sample size, strict inclusion and exclusion criteria, and novel findings regarding dietary riboflavin intake. Using the large sample from NHANES allowed for the generalization of findings to the actual population. The sample was carefully selected by excluding most of the confounding health conditions and behaviors reported in previous literature. The overall characteristics of the migraine sample agree with the previous literature, indicating the representativeness of the sample. A validated dietary information collecting method is used by NHANES, which further increases the generalizability of the study findings. To our knowledge, this is the first study that reveals the average daily riboflavin intake among migraine patients.

Conclusion

In summary, this study reveals the average daily dietary riboflavin consumption of adult migraine patients (2.14 mg/day), which meet the RDA for both male and female, but was lower than the control participants. The total riboflavin consumption of adult migraine patients, considering both dietary and supplemental sources, was 4.25 mg/day. The study observed a relationship between the daily dietary riboflavin consumption and the occurrence of migraine. The relationship was also found between the total daily riboflavin consumption, which considered supplement riboflavin intake, and the odds of migraine. Dietary riboflavin intake in the range of 2.07-2.87 mg/day corresponded to a 27% reduction in migraine occurrence as compared to the lowest intake quartile (<1.45mg/day). In addition, total riboflavin consumption in the range of 2.45-3.60 mg/day contributed to a 37% reduction in migraine odds compared to the lowest quartile (<1.58 mg/day).

With limited migraine diagnostic information, findings in this study cannot support nor deny the effectiveness of daily mega-dose (400 mg) of riboflavin supplement used in previous clinical migraine prophylaxis studies. However, future studies aiming to make recommendations of riboflavin intake as migraine prophylaxis or examine the effectiveness of riboflavin in alleviating migraine should consider reevaluating the effective dose and intake pattern of riboflavin and the influence of baseline dietary riboflavin intake on the symptoms of migraine.

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