

One-Carbon Metabolism Nutrients, Genetic Variation, and Diabetes Mellitus

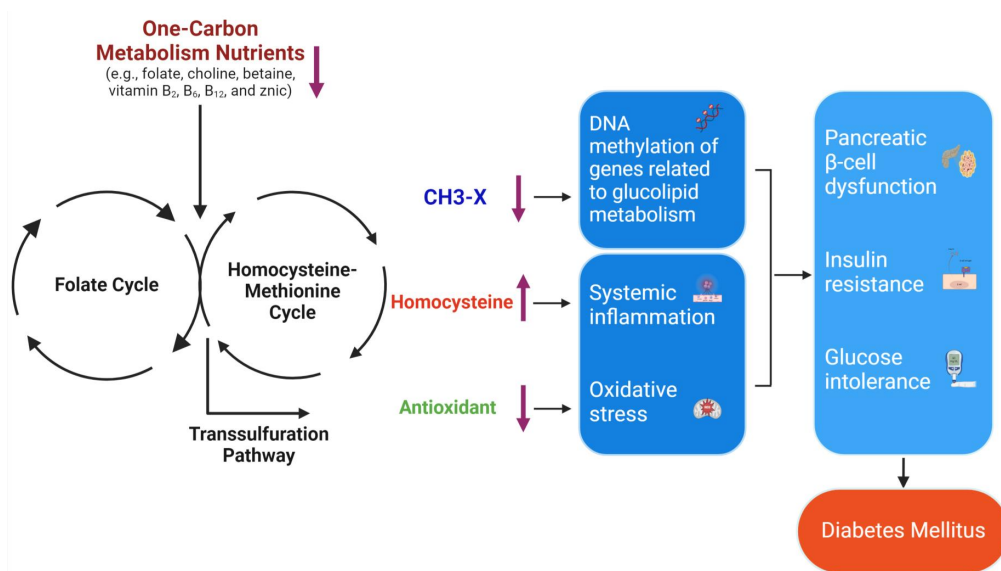
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Review

Metabolic Risk/Epidemiology

One-Carbon Metabolism Nutrients, Genetic Variation, and Diabetes Mellitus



Highlights

- OCM is driven by the folate cycle, the Hcy-methionine cycle, and the transsulfuration pathway.
- Low OCM nutrients result in fewer methyl groups, HHcy, and less antioxidation.
- HHcy and reduced antioxidation lead to oxidative stress and systemic inflammation.
- Low methyl group supply modifies DNA methylation of genes in glycolipid metabolism.
- These changes may cause β-cell dysfunction, IR, glucose intolerance, and DM development.



One-Carbon Metabolism Nutrients, Genetic Variation, and Diabetes Mellitus

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Diabetes mellitus (DM) affects about 9.3% of the population globally. Hyperhomocysteinemia (HHcy) has been implicated in the pathogenesis of DM, owing to its promotion of oxidative stress, β -cell dysfunction, and insulin resistance. HHcy can result from low status of one-carbon metabolism (OCM) nutrients (e.g., folate, choline, betaine, vitamin B6, B12), which work together to degrade homocysteine by methylation. The etiology of HHcy may also involve genetic variation encoding key enzymes in OCM. This review aimed to provide an overview of the existing literature assessing the link between OCM nutrients status, related genetic factors, and incident DM. We also discussed possible mechanisms underlying the role of OCM in DM development and provided recommendations for future research and practice. Even though the available evidence remains inconsistent, some studies support the potential beneficial effects of intakes or blood levels of OCM nutrients on DM development. Moreover, certain variants in OCM-related genes may influence metabolic handling of methyl-donors and presumably incidental DM. Future studies are warranted to establish the causal inference between OCM and DM and examine the interaction of OCM nutrients and genetic factors with DM development, which will inform the personalized recommendations for OCM nutrients intakes on DM prevention.


Keywords: Betaine; Choline; Diabetes mellitus; Folic acid; Genes; Homocysteine; Riboflavin; Vitamin B 6; Vitamin B 12; Zinc

INTRODUCTION

Diabetes mellitus (DM) affects approximately 9.3% (463 million people) of the population worldwide in 2019 with its prevalence projected to reach 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1]. DM is associated with increased risk of numerous chronic diseases, such as cardiovascular diseases (CVD) and diabetic retinopathy [2,3]. Thus, identifying potentially modifiable risk factors for DM may help develop more effective strategies for its prevention.

Hyperhomocysteinemia (HHcy) has emerged as a risk biomarker for type 2 diabetes mellitus (T2DM) [4]. HHcy was evi-

denced to promote oxidative stress, β -cell dysfunction, and insulin resistance (IR), which contributes to DM pathology [4-6]. Growing evidence suggests that HHcy can be due to the low status of one-carbon metabolism (OCM) nutrients [7-9]. OCM is a metabolic network that involves biochemical compounds to regulate nucleic acid synthesis and methylation reactions. Homocysteine (Hcy) in this OCM network can be either metabolized into cysteine or recycled into methionine with the aid of a group of OCM nutrients, which act as prerequisite substrate donors (folate, choline, betaine, and methionine) or essential coenzymes (vitamin B2, B6, B12, and zinc) [8,9]. Data directly linking the intake or circulating levels of these OCM

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nutrients to incident DM are sparse and results remain inconsistent [10-22]. For example, higher choline intake was associated with lower T2DM risk among men in eastern Finland [14], whereas dietary choline or betaine intake was not associated with risk of T2DM among the United States Black or White men [15]. Moreover, the etiology of HHcy may involve genetic variation encoding key enzymes in OCM, which may contribute to DM development [5,6]. A meta-analysis incorporating 68 studies indicated that methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism was correlated with T2DM in Asian populations, but not in White and Black populations [8]. Nevertheless, knowledge regarding the role of OCM in DM development remains in its infancy. Further investigations are warranted to better understand the role of OCM nutrients in the etiology of DM prevention and treatment.

The present review aims to provide an overview of the existing literature assessing the relationships between OCM nutri-

ents status as well as related genetic variation and risk of DM in the context of DM pathology. We will focus on understanding the hypothesized mechanism of OCM nutrients action on DM, limitations of the previous studies, and implications for dietetic practice and future research.

ONE-CARBON METABOLISM

OCM is a metabolic network driven by three interrelated metabolic pathways, which include the folate cycle, the Hcy-methionine cycle, and the transsulfuration pathway [2]. The complex set of biochemical reactions of OCM contributes to the generation or utilization of methyl groups (CH₃) [2,3].

As shown in Fig. 1, folic acid (FA) from dietary intake can serve as a precursor to dihydrofolate (DHF) that is converted to tetrahydrofolate (THF) via dihydrofolate reductase (DHFR). DHF can also be converted to 5,10-methylenetetrahydrofolate

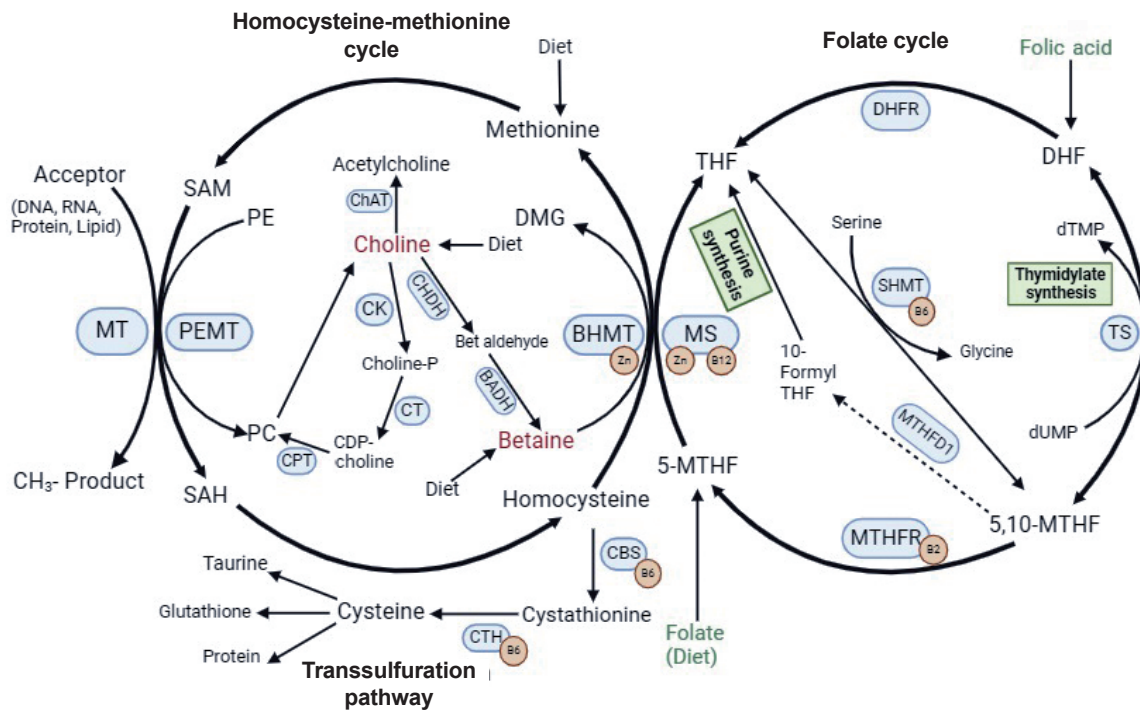


Fig. 1. One-carbon metabolism. SAM, S-adenosylmethionine; PE, phosphatidylethanolamine; ChAT, choline acetyltransferase; DMG, dimethylglycine; MT, methyltransferase; PEMT, phosphatidylethanolamine N-methyltransferase; CK, choline kinase; CHDH, choline dehydrogenase; BHMT, betaine-homocysteine S-methyltransferase; Zn, zinc; SAH, S-adenosylhomocysteine; PC, phosphatidylcholine; CPT, cholinephosphotransferase; CDP, cytidine diphosphate; Choline-P, phosphocholine; CT, cytidyltransferase; BADH, betaine aldehyde dehydrogenase; CBS, cystathionine β-synthase; CTH, cystathionine γ-lyase; THF, tetrahydrofolate; DHFR, dihydrofolate reductase; DHF, dihydrofolate; MS, methionine synthase; 10-formyl THF, 10-formyl-tetrahydrofolate; SHMT, serine hydroxymethyltransferase; dTMP, thymidine monophosphate; TS, thymidylate synthase; 5-MTHF, 5-methyl-tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; dUMP, deoxyuridine monophosphate; 5,10-MTHF, 5,10-methylenetetrahydrofolate.

(5,10-MTHF) via thymidylate synthase (TS), which is used for thymidylate synthesis. Conversion to 5,10-MTHF from THF requires serine via serine hydroxymethyltransferase (SHMT) with vitamin B6 as a cofactor. Methylenetetrahydrofolate dehydrogenase (MTHFD) catalyzes 5,10-MTHF to 10-formyltetrahydrofolate (10-formyl THF), which is used for purine synthesis. Conversion from 5,10-MTHF to 5-methyltetrahydrofolate (5-MTHF) requires MTHFR with vitamin B2 as a cofactor. Folate, the natural form from diet, can also contribute to 5-MTHF donating the methyl groups to Hcy to generate methionine and THF via methionine synthase (MS) with vitamin B12 and zinc as a cofactor [2-6].

In addition, choline and betaine act as other methyl-donors that can be supplied from diet and endogenously synthesized. Choline as an essential nutrient can be acetylated by choline acetyltransferase to produce acetylcholine, a pivotal neurotransmitter [4]. Also, choline can be phosphorylated by choline kinase to be converted to phosphocholine, and then to cytidine diphosphate-choline and phosphatidylcholine (PC) by cytidyltransferase (CT) and cholinephosphotransferase, sequentially. PC serves as an essential constituent of cell and mitochondrial membranes as well as a major component of lipoproteins [2-4]. In addition, choline can be endogenously synthesized through the conversion of phosphatidylethanolamine to PC, which is catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) [3]. Moreover, choline dehydrogenase catalyzes choline to synthesize betaine that can be oxidized by betaine-homocysteine S-methyltransferase with zinc as a cofactor to methylate Hcy to methionine. Then, methionine passes the methyl group to S-adenosylmethionine (SAM), which serves as a universal methyl-donor contributing to methylation modification of DNA, RNA, and protein [2-4]; After that, SAM is converted to S-adenosylhomocysteine and deadenylated to produce Hcy [20]. If there is abundant methionine, the transsulfuration pathway will become active, by which Hcy reacts with serine to form cystathionine by cystathionine β -synthase with vitamin B6 as a cofactor. Cystathionine is further processed by cystathionine γ -lyase with vitamin B6 as a cofactor to generate cysteine, which is used to produce taurine, glutathione (GSH), and other protein [2-4]. It has been evidenced that deficiency of the OCM nutrients (e.g., folate, choline, betaine, vitamin B6 and B12) and reduced activity of the key enzymes in OCM due to genetic variation (e.g., MS 2756 G>A, MTHFR 677C>T) contribute to HHcy, thus impairing the remethylation and/or transsulfuration pathways [5-9,20].

OCM NUTRIENTS AND RISK OF DM

Observational studies that directly related OCM nutrients to incident DM were limited and results remained contradictory (Table 1). One cross-sectional study reported an inverse relationship between serum folate level and DM prevalence among Chinese adults [20]. Consistently, a cross-sectional study demonstrated that serum choline or betaine was inversely correlated with fasting glucose levels and IR index in Canadian adults [18]. Another cross-sectional study conducted in Chinese adults showed that higher dietary consumptions of vitamin B6 and choline, but not folate, vitamin B12, methionine and betaine, were related to lower incident hyperglycemia [21]. However, a positive correlation between dietary methionine and the rate of DM was observed among Chinese adults [22].

Two case-control studies investigated the relationship between OCM nutrients and DM development. Al-Maskari et al. [10] observed that both dietary intake and serum level of folate and vitamin B12 were lower in Omani adult patients with T2DM, compared to the healthy controls. However, Nie et al. [19] reported a positive correlation between plasma zinc and the odds of DM in Chinese adults.

The inverse associations were reported between dietary folate intake and the rate of DM among Korean women aged ≥ 40 years with an average 4-year follow-up [11], and in Japanese women aged 40 to 79 years within a 5-year study period [12]. Similarly, our previous study found that higher intake of folate, but not vitamin B6 or vitamin B12, in young adulthood was associated with lower diabetes incidence in midlife among White and Black Americans over 30 years of follow-up [13]. Regarding other B vitamins in OCM, dietary vitamin B2 intake was inversely associated with risk of T2DM in Japanese women aged 40 to 79 years [12]. In addition, higher choline intake was inversely associated with lower T2DM risk among men aged 42 to 60 years in eastern Finland [14], while dietary choline or betaine intake was not significantly associated with risk of T2DM among the United States Black or White male participants aged 45 to 64 years [15]. In contrast, a study reported a positive trend for the association between choline consumption and DM risk among postmenopausal United States women aged 50 to 79 years [15]. As for the biomarkers of OCM nutrients, higher serum betaine was associated with lower T2DM risk in Chinese adults aged 40 to 75 years [17]. Presumably, the inconsistent findings from the aforementioned observational studies are mainly due to heterogeneities in exposure measures, time win-

Table 1. Observational studies examining association of one-carbon metabolism nutrients status with risk of DM

Study	Year	Location	Study population	Design	Exposure	Follow-up	Outcomes/Major results
Al-Maskari et al. [10]	2012	Oman	100 Omani adults aged 42-92-59.33 years (n = 50 diabetic cases, n = 50 healthy controls)	Case-control	Dietary intakes and serum levels of folate and vitamin B12	NA	Both dietary intakes and serum levels of folate and vitamin B12 were lower in patients with T2DM than those in the healthy controls (all P<0.05).
Hong et al. [11]	2017	South Korea	7,333 Adults aged ≥40 years	Prospective	Dietary folate intake (not including folate intake from supplements)	4.06 years	Dietary folate intake was inversely associated with risk of T2DM for women (RR in the highest vs. the lowest quartile was 0.64 [95% CI, 0.43-0.95; P _{trend} =0.0244]).
Eshak et al. [12]	2019	Japan	19,168 Healthy adults aged 40-79 years	Prospective	Intake of water-soluble vitamins	5 years	Higher dietary intakes of folate and vitamin B2 were associated with lower risk of T2DM in Japanese women (OR in the highest vs. the lowest quartile of intakes were 0.70 [95% CI, 0.46-0.98; P _{trend} =0.03] for folate and 0.56 [95% CI, 0.34-0.93; P _{trend} =0.03] for vitamin B2).
Zhu et al. [13]	2020	USA	4,704 White and Black adults aged 18-30 years	Prospective	Total intake of folate, vitamin B6, and vitamin B12	30 years	Intake of folate, but not vitamin B6, or vitamin B12, was inversely associated with DM incidence (HR in the highest vs. the lowest quartile of folate intake was 0.70 [95% CI, 0.51-0.97; P _{trend} =0.02]).
Virtanen et al. [14]	2020	Finland	2,332 Men aged 42-60 years	Prospective	Choline intake	19.3 years	Higher choline intake was associated with lower risk of T2DM among men in eastern Finland (HR in the highest vs. the lowest quartile of choline intake was 0.75 [95% CI, 0.57-0.98; P _{trend} =0.02]).
Dibaba et al. [15]	2020	USA	13,440 Men and women aged 45-64 years	Prospective	Intake of choline and betaine	9 years	Overall and among male participants, neither dietary choline nor betaine intake was associated with risk of T2DM. Among women, there was a trend for a modestly higher T2DM risk (HR in the highest vs. the lowest quartile of choline intake was 1.54 [95% CI, 1.06-2.25; P _{interaction} for sex=0.07]).
Greenberg et al. [16]	2021	USA	46,263 Postmenopausal women aged 50-79 years	Prospective	Intake of choline and betaine	13.3 years	Higher choline intake was associated with increase in DM risk (HR in the highest vs. the lowest quartile of choline intake was 1.30 [95% CI, 1.15-1.47; P _{trend} <0.0001]). There was a significant linear trend but no significant association between betaine intake and risk of DM (HR in the highest vs. the lowest quartile of betaine intake was 0.90 [95% CI, 0.81-1.002; P _{trend} =0.04]).
Lu et al. [17]	2022	China	1,565 Chinese adults aged 40-75 years	Prospective	Serum betaine	8.9 years	Higher serum betaine was associated with lower risk of T2DM (HR in the highest vs. the lowest quartile was 0.46 [95% CI, 0.31-0.69; P _{trend} <0.001]).
Gao et al. [18]	2019	Canada	1,081 Canadian adults aged 28-56 years	Cross-sectional	Serum choline and betaine levels	NA	Serum choline level was negatively correlated with serum fasting glucose levels in males (R=-0.121, P=0.006). Serum betaine level was negatively associated with insulin levels (R=-0.081, P=0.043), and HOMA-IR index (R=-0.086, P=0.021) in males. In females, serum betaine level was negatively associated with insulin levels (R=-0.104, P=0.016), and with HOMA-IR index (R=-0.092, P=0.034).
Nie et al. [19]	2022	China	188 Chinese adults aged 57-72 years (n = 94 diabetic cases, n = 94 healthy controls)	Case-control	Plasma metal levels	NA	Plasma Zn level was positively correlated with elevated DM risk (OR in the highest vs. the lowest quartile of plasma Zn levels was 2.37 [95% CI, 1.47-3.81; P<0.001]).

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Table 1. Continued

Study	Year	Location	Study population	Design	Exposure	Follow-up	Outcomes/Major results
Cheng et al. [20]	2022	China	772 Adults aged 43–82 years (n = 370 T2DM cases, n = 402 controls)	Cross-sectional	Serum folate	NA	Higher serum folate level was associated with lower risk of T2DM (OR in the highest vs. the lowest quartile of serum folate levels was 0.909 [95% CI, 0.840–0.983; $P_{\text{trend}} = 0.0177$]).
Ding et al. [21]	2022	China	901 Participants (n = 417 males, n = 484 females) aged 18–75 years	Cross-sectional	Dietary intake of betaine, total choline, methionine, folate, vitamins B6 and B12	NA	Higher dietary intakes of total choline and vitamin B6 were associated with a lower incidence of hyperglycemia (OR in the highest vs. the lowest quartile were 0.601 [95% CI, 0.365–0.988; $P_{\text{trend}} = 0.365–0.988$] for total choline; 0.575 [95% CI, 0.346–0.956; $P_{\text{trend}} = 0.038$] for vitamin B6). There were null associations of dietary intakes of folate, vitamin B12, methionine, and betaine with risk of hyperglycemia.
Sun et al. [22]	2023	China	12,489 Chinese adults aged ≥ 20 years	Cross-sectional	Methionine intake	NA	Higher intake of dietary methionine was associated with higher risk of DM (OR in the highest vs. the lowest quartile was 1.49 [95% CI, 1.12–1.98; $P_{\text{trend}} = 0.009$]).

DM, diabetes mellitus; NA, not applicable; T2DM, type 2 diabetes mellitus; RR, risk ratio; CI, confidence interval; OR, odds ratio; HOMA-IR, homeostasis model assessment of insulin resistance; Zn, zinc.

dows of exposure, and diverse study populations.

Randomized clinical trials (RCT) investigating the effects of OCM nutrients supplementation on incident DM were scanty (Table 2). A double-blind RCT reported that Hcy-lowering intervention by daily B vitamins supplementation (FA [2.5 mg], vitamin B6 [50 mg], and vitamin B12 [1 mg]) for 4.5 years failed to decrease incident T2DM among 5,442 United States female health professionals at high risk for CVD [7]. Likewise, another double-blind RCT reported that daily supplementation of FA (0.8 mg) with enalapril (10 mg) for 7.3 years exerted no significant impact on risk of new-onset diabetes among 20,702 Chinese hypertensive adults [8]. Notably, a double-blind RCT involving 200 Sri Lankan participants with prediabetes had noted that 12-month zinc supplementation (20 mg zinc daily) decreased T2DM incidence [9]. These discrepant results are probably explained by different supplementation formulas and duration, and various health statuses across different study populations. Moreover, the participants in these studies were those who were >40 years old and at high risk for metabolic disorders. Thus, the generalizability of these findings is limited.

EFFECT OF OCM NUTRIENTS SUPPLEMENTATION ON GLUCOSE METABOLISM INDICES

Although it is insufficient to draw a firm conclusion as to whether OCM nutrients can prevent incident DM, a growing body of interventional trials have demonstrated beneficial effects of OCM nutrients consumption on glucose metabolism indices (Table 2).

Fasting blood glucose (FBG) is a common glucose metabolism index and the easiest way to monitor blood glucose levels for DM diagnosis. The present review identified 26 previous studies that explored the effects of OCM nutrients supplementation on FBG and the results remain elusive (Table 2). Single administration of FA (400 $\mu\text{g/day}$ to 15 mg/day), betaine (100 mg/kg/day), and zinc (30 mg/day) failed to result in significant changes in FBG values [23–36]. In addition, no remarkable alterations in FBG values were reported following the joint supplementation of FA with Fe^{2+} [37], FA with other B vitamins [38–40], FA with metformin [41], B vitamins complex with metformin [42]. However, FA (5 mg/day) administration alone or joint supplementation of FA (0.4 or 0.8 mg/day) and enalapril (10 mg/day) decreased fasting plasma glucose (FPG) among Iranian patients with metabolic syndrome (MetS) [43] and Ira-

Table 2. Intervention studies examining the effect of one-carbon metabolism nutrients on DM development and glucose metabolism index

Study	Year	Location	Study population	Design	Intervention	Duration	Outcomes/Major results
DM risk							
Song et al. [7]	2009	USA	5,442 Female health professionals aged 54–72 years with history of CVD or ≥3 CVD risk factors	Double-blind RCT	Treatment group: daily intake of a combination pill containing 2.5 mg FA, 50 mg vitamin B6, and 1 mg vitamin B12 (n=2,132) Placebo group: a matching placebo pill daily (n=2,120)	7.3 years	No significant effect on risk of DM ↓ Blood Hcy
Qin et al. [8]	2016	China	20,702 Hypertensive patients aged 52.3–57.5 years	Double-blind RCT	Daily treatment with tablets containing: (1) 10 mg enalapril and 0.8 mg FA (n=10,348); or (2) 10 mg enalapril alone (n=10,354)	4.5 years	No significant effect on FPG and risk of DM
Ramasinghe et al. [9]	2018	Sri Lanka	200 Participants with prediabetes aged 44–59.4 years	Double-blind RCT	Treatment group: capsules containing 20 mg zinc daily (n=100) Placebo group: capsules containing inactive ingredients (n=100)	12 months	↓ FPG, ↓ 2-hour glucose after the OGTT, ↓ HOMA-IR, ↓ T2DM development ↑ HOMA-β
Glucose metabolism index							
Aarsand et al. [37]	1998	Norway	28 Diabetic patients with metformin treatment for more than 1 year, aged 53.9–64.1 years	Double-blind RCT	Treatment group: tablets containing 0.25 mg folic acid+60 mg Fe ²⁺ daily (n=14) Placebo group: tablets containing 60 mg Fe ²⁺ daily (n=14)	12 weeks	No significant effect on FSG and HbA1c ↓ Serum Hcy ↑ Serum vitamin B12 and folate
Doshi et al. [23]	2001	UK	50 Patients with coronary artery disease, aged 46–65 years	A randomized, double-blind, placebo-controlled crossover trial	Treatment group: 5 mg FA supplement per day Placebo group: matched placebo	Two 6-week treatments separated by a washout period of 4 months	No significant effect on FPG ↑ Plasma folate ↓ Plasma Hcy
Doshi et al. [24]	2002	UK	33 Patients with coronary artery disease, aged 46–65 years	Double-blind RCT	Treatment group: 5 mg FA supplement per day (n=16) Placebo group: matched placebo (n=17)	6 weeks	No significant effect on FPG ↑ Plasma folate ↓ Plasma Hcy
Setola et al. [38]	2004	Italy	50 Patients with metabolic syndrome+hyperinsulinemia, aged 66.1–68.5 years	Double-blind, parallel, identical placebo-drug RCT	Group 1: treated with diet+placebo for 2 months Group 2: treated with diet+placebo (1 month) then diet+FA (5 mg/day)+vitamin B12 (500 µg/day) for 2nd month	2 months	↓ Insulin levels, ↓ HOMA-IR, ↓ Hcy levels No significant effect on fasting glucose
Kilicdag et al. [48]	2005	Turkey	49 Female patients with PCOS (group 1, 17.22–31.06 years; group 2, 18.27–25.73 years; group 3, 18.27–31.61 years)	RCT	Group 1 received metformin (850 mg twice daily) (n=20) Group 2 received metformin (850 mg twice daily)+B-group vitamins (vitamin B1 250 mg, vitamin B6 250 mg, vitamin B12 1,000 µg twice daily) (n=20) Group 3 received metformin (850 mg twice daily)+FA (174 µg), vitamin D (1,200 µg) and calcium (666.670 mg) twice daily (n=20)	12 weeks	No significant effect on HOMA-IR ↓ Serum Hcy (groups 1 & 2) ↑ Plasma FA (group 3), ↑ plasma vitamin B12 (group 2)
Mangoni et al. [25]	2005	Australia	26 T2DM patients aged 46–65 years	Double-blind, parallel-group RCT	Treatment group: 5 mg FA supplement per day (n=13) Placebo group: matched placebo (n=13)	4 weeks	No significant effect on plasma glucose, HbA1c, and serum vitamin B12 ↓ Plasma Hcy, ↑ serum folate

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Table 2. Continued

Study	Year	Location	Study population	Design	Intervention	Duration	Outcomes/Major results
Sheu et al. [26]	2005	Taiwan	84 Obese women who were 20% over their ideal weight	Double-blind RCT	Treatment group: 5 mg FA supplement per day (n = 36) Placebo group: matched placebo (n = 38) This program also included caloric restriction and light exercises to promote weight loss.	12 weeks	↓ HOMA-IR, ↓ fasting plasma insulin, ↓ serum Hcy No significant effect on FPG and fasting serum vitamin B12 and folate
Villa et al. [27]	2005	Italy	20 Healthy postmenopausal women aged 48–61 years	RCT	Treatment group: 7.5 mg FA supplement per day (n = 10) Placebo group: commercial calcium capsule (n = 10)	8 weeks	No significant effect on fasting glucose and insulin, and vitamin B12, methionine ↓ Hcy levels
Moat et al. [28]	2006	USA	128 Patients with angiographically proven CAD aged 53–68 years	Double-blind, parallel, RCT	84 Patients were randomly divided into 3 groups for FA study (placebo, n = 29; low-dose FA 400 µg/day, n = 30; and high-dose FA 5 mg/day, n = 25). Parallely 44 patients were randomly divided into 2 groups for betaine study (Placebo, n = 23 or betaine, 100 mg/kg/day, n = 21).	6 weeks	↓ Total plasma Hcy levels, ↑ plasma folate, and no significant difference in blood glucose and serum vitamin B12 for FA study ↓ Plasma folate but no significant difference in blood glucose, serum vitamin B12, or plasma Hcy for betaine study
Solimi et al. [29]	2006	Italy	60 Healthy overweight adults aged 29–61 years	Unmasked randomized, placebo-controlled trial	All patients were put on a hypocaloric diet and were randomly assigned to either a placebo or FA (2.5 mg/day) group.	12 weeks	↓ Fasting plasma insulin, ↓ HOMA-IR, ↑ serum folate No significant difference in serum vitamin B12, Hcy, or FPG
Title et al. [30]	2006	Canada	19 T2DM patients aged 35–65 years	Randomized, double-blind, placebo-controlled, crossover trial	Patients were randomly assigned to receive either oral FA (10 mg/day) or a matching placebo for 2 weeks. This was followed by 8 weeks of washout period and then patients were crossed over to receive alternate treatment for another 2 weeks.	2 weeks+8 weeks wash-out+2 weeks	No significant effect on plasma Hcy and plasma glucose ↑ Serum folate
Moens et al. [31]	2007	Belgium	40 Patients with acute myocardial infarction, aged 42–70 years	Randomized, double-blind, placebo-controlled crossover trial	Patients were randomly divided into 2 groups: Group A (n = 20) received FA (10 mg/day) for initial 6 weeks then a placebo for another 6 weeks. For group B (n = 20), the order was reversed. There was a washout of 2 weeks between the treatments.	6 weeks+2 weeks wash-out+6 weeks	No significant difference in plasma vitamin B12, and FPG ↑ Plasma and RBC folate, ↓ plasma Hcy
Mao et al. [50]	2008	China	443 Patients with mild to moderate hypertension, aged 27–75 years	Double-blind RCT	Participants were randomly assigned to 3 groups: control (10 mg of enalapril, n = 149); low-FA group (10 mg enalapril+0.4 mg of FA, n = 146); high-FA group (10 mg enalapril+0.8 mg of FA, n = 148).	8 weeks	↓ FPG in low-FA and high-FA groups ↑ Serum folate
Mashavi et al. [42]	2008	Israel	57 T2DM patients aged 54.1–66.1 years	Double-blind RCT	Group 1: 1,500 mg metformin+folate (1,000 µg), vitamin B12 (400 µg), and vitamin B6 (10 mg) daily (n = 28) Group 2: 1,500 mg metformin+placebo daily (n = 29)	4 months	No significant difference in HOMA-IR and FPG ↑ Serum FA, ↑ serum vitamin B12 ↓ Serum Hcy
Potter et al. [39]	2008	Australia	162 Patients with history of stroke, aged 52–80 years	Double-blind RCT	Treatment group: a single daily tablet containing FA (2 mg), vitamin B6 (25 mg), and vitamin B12 (500 µg) (n = 83) Placebo group: matched placebo (n = 79)	104 weeks	No significant difference in FGB and HbA1c ↓ Blood Hcy ↑ Serum B6, ↑ serum vitamin B12, ↑ RBC folate

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Table 2. Continued

Study	Year	Location	Study population	Design	Intervention	Duration	Outcomes/Major results
Cagnacci et al. [32]	2009	Italy	30 Healthy White postmenopausal women aged 48–58 years	Double-blind RCT	Treatment group: 15 mg/day 5-methyltetrahydrofolate (n=15) Placebo group: matched placebo (n=15)	3 weeks	↓ HOMA-IR, ↓ blood insulin, ↓ blood Hcy No significant effect on FBG
Palomba et al. [41]	2010	Italy	47 Females with PCOS, aged 23.6–30 years	Non-randomized placebo-controlled double-blind trial	Experimental group: 1,700 mg metformin+400 µg FA daily (n=25) Control group: 1,700 mg metformin+a placebo daily (n=25)	25 weeks	↓ Fasting serum insulin, ↓ HOMA-IR ↑ Serum Hcy No significant difference in serum folate, vitamin B12, or FBG
Kurt et al. [40]	2010	Turkey	44 Adults aged >65 years with vitamin B12 deficiency	Double-blind RCT	Treatment group: FA (5 mg)+vitamin B12 (500 µg) daily (n=24) Placebo group: matched placebo (n=20)	8 weeks	↓ HOMA-IR, ↓ serum Hcy ↑ Serum folate, ↑ serum vitamin B12 No significant difference in FBG
Gargari et al. [33]	2011	Iran	48 Overweight and obese men with T2DM and under metformin treatment, aged 46.9–67.7 years	Double-blind RCT	Treatment group: FA (5 mg) supplementation daily (n=24) Placebo group: matched placebo (n=24)	8 weeks	↓ HOMA-IR, ↓ serum HbA1c, ↓ serum insulin ↑ Serum folate, ↑ serum vitamin B12 No significant difference in FBG
Grigoletti et al. [34]	2013	Brazil	30 HIV-infected individuals aged 43–47 years	Double-blind RCT	Treatment group: FA (5 mg) supplementation daily (n=15) Placebo group: matched placebo (n=15)	4 weeks	No significant difference in FSG ↑ Serum vitamin B12, ↑ serum folate ↓ Plasma Hcy
Asemi et al. [35]	2014	Iran	81 Overweight or obese women with PCOS aged 18–40 years	Double-blind RCT	Group 1: 1 mg FA daily (n=27) Group 2: 5 mg FA daily (n=27) Group 3: matched placebo (n=27)	8 weeks	↓ Serum insulin, ↓ HOMA-IR, ↓ plasma Hcy No significant difference in FBG
Karamali et al. [45]	2015	Iran	58 Females with GDM aged 18–40 years	Double-blind RCT	Treatment group: 233 mg zinc gluconate daily (n=29) Placebo group: matched placebo (n=29)	6 weeks	↓ FPG, ↓ serum insulin, ↓ HOMA-IR, ↓ HOMA-β ↑ QUICKI, ↑ serum zinc
Asemi et al. [49]	2016	Iran	58 Females with cervical intraepithelial neoplasia grade 1, aged 18–55 years	Double-blinded RCT	Treatment group: 5 mg FA daily (n=29) Placebo group: matched placebo (n=29)	6 weeks	↓ Serum insulin levels, ↓ HOMA-β, ↓ plasma Hcy
Hashemi et al. [36]	2016	Iran	79 Pregnant women with preeclampsia, aged 21–41 years	Randomized, triple-blind, clinical trial	Treatment group: 5 mg FA daily Placebo group: matched placebo	8 weeks	No significant difference in FBG
Talari et al. [43]	2016	Iran	60 Patients with metabolic syndrome, aged 40–85 years	Double-blind RCT	Treatment group: tablets containing 5 mg FA daily (n=30) Placebo group: daily placebo tablets (n=30)	12 weeks	↓ FPG, ↓ serum insulin, ↓ HOMA-IR, ↓ plasma Hcy No significant difference in HOMA-β
Bahmani et al. [44]	2018	Iran	60 Women with endometrial hyperplasia	Double-blind RCT	Treatment group: 5 mg FA daily (n=30) Placebo group: matched placebo (n=30)	12 weeks	↓ FPG, ↓ serum insulin, ↓ HOMA-IR, ↑ QUICKI
Attia et al. [46]	2022	Australia	98 Prediabetic participants aged 40–70 years	Double-blind RCT	Treatment group: a daily capsule containing 30 mg elemental zinc gluconate (n=48) Placebo group: a daily capsule containing cellulose (n=50)	12 months	No significant difference in FBG, HbA1c, and HOMA-β

DM, diabetes mellitus; CVD, cardiovascular diseases; RCT, a randomized placebo-controlled trial; FA, folic acid; Hcy, homocysteine; FBG, fasting blood glucose; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; T2DM, type 2 diabetes mellitus; HOMA-β, homeostatic model assessment of β-cell function; FSG, fasting serum glucose; HbA1c, glycosylated hemoglobin; PCOS, polycystic ovarian syndrome; CAD, coronary artery disease; RBC, red blood cell; FGB, fibrinogen beta chain; FBG, folate-binding protein; HIV, human immunodeficiency virus; GDM, gestational diabetes mellitus; QUICKI, quantitative insulin sensitivity check index.

nian women with endometrial hyperplasia [44]. Likewise, administration of zinc (20 or 233 mg/day) reduced FPG among Sri Lankan participants with prediabetes [9] and Iranian women with gestational diabetes [45].

Glycosylated hemoglobin (HbA1c) is commonly assayed to indicate average blood glucose level over the past 3 months, which is also used for DM diagnosis [25,37,39,46]. Among five studies identified in the present review, only one reported that FA (5 mg/day) supplementation led to serum HbA1c reduction in overweight and obese Iranian men with T2DM [33]. No significant changes in HbA1c values were observed following administration of FA in T2DM patients [25], supplementation of zinc in prediabetic patients [46], the joint supplementation of FA with Fe²⁺ [37] in diabetic patients, or FA administration with other B vitamins in patients with history of stroke [39].

The homeostasis model assessment of insulin resistance (HOMA-IR) value is calculated by an equation derived from FBG and insulin levels. Higher HOMA-IR was independently associated with an increased DM risk [47]. Single FA administrations at high doses (ranging from 2.5 to 15 mg/day) were inversely associated with HOMA-IR among obese women in Taiwan [26] and overweight adults [29] as well as postmenopausal women in Italy [32]. Similar inverse associations were reported in overweight and obese men with T2DM [33], overweight or obese women with polycystic ovarian syndrome (PCOS) [35], men and women with MetS [43], and women with endometrial hyperplasia in Iran [44]. Likewise, a single administration of zinc (20 mg/day or 233 mg zinc gluconate/day) reduced HOMA-IR in prediabetic patients [9] and women with gestational diabetes mellitus (GDM) [45]. In addition, concomitant administration of FA (5 mg/day) with vitamin B12 (500 µg/day) or FA (400 µg/day) with metformin (1,700 mg/day) decreased HOMA-IR in patients with MetS and hyperinsulinemia [38], and elderly adults with vitamin B12 deficiency [40]. However, two other studies reported no effect of joint supplementation of B vitamins with metformin on HOMA-IR among Turkish women with PCOS [48] and Israeli patients with T2DM [42].

The quantitative insulin sensitivity check index (QUICKI) is another surrogate biomarker of IR, the higher of which reflects a lower degree of IR [44,45]. In the study by Karamali et al. [45], 6-week zinc supplementation (233 mg/day zinc gluconate) increased QUICKI in women with GDM. Likewise, Bahmani et al. [44] demonstrated that 6-week supplemental FA at 5 mg/day augmented QUICKI in women with endometrial hyperplasia.

Homeostatic model assessment of β -cell function (HOMA- β), derived from fasting plasma insulin and glucose levels, is applied as an index of the insulin secretory function of pancreatic β -cells [47]. Lower HOMA- β was independently associated with an increased DM risk [47]. Increase of HOMA- β were observed under administration of zinc (20 mg/day) in prediabetic patients [9], whereas zinc (233 mg zinc gluconate/day) or FA (5 mg/day) supplementation decreased HOMA- β among women with GDM [45] or women with cervical intraepithelial neoplasia [49]. However, no significant effect on HOMA- β was found under single supplementation of FA (5 mg/day) in patients with MetS or zinc (30 mg zinc gluconate/day) in prediabetic patients [46].

The heterogeneous effects of either the single or the combined supplementation of OCM nutrients on the above glucose metabolism indices are due to the various dosages and combinations of the OCM nutrients with various durations [23-46,48-50]. In addition, the medication (e.g., enalapril, metformin) that was co-ingested with the OCM nutrients may also counterbalance the beneficial impact of the OCM nutrients on the glucose metabolism indices [41,42,50]. Moreover, most of the studies were conducted among patients with various health problems which contributed to the disputed results [23-46,48-50]. Whether the OCM nutrients intake prevents the public at an early age from developing prediabetic status or incident DM later in life merits further investigation. Furthermore, interventions aimed at optimizing balanced OCM nutrients status and preventing HHcy may help mitigate IR and improve insulin signaling and glucose homeostasis. While further research is warranted, incorporating OCM nutrients into holistic lifestyle strategies may provide a valuable avenue for DM management and prevention.

GENETIC VARIATION ENCODING KEY ENZYMES OF OCM IN RELATION TO RISK OF DM

Emerging evidence indicates that the etiology of HHcy may also involve genetic variation encoding key enzymes of OCM, which may contribute to diabetes development [6,13]. The transformation of the methyl group from these OCM nutrients to Hcy is catalyzed by an array of key enzymes including MTHFR, methionine synthase reductase (MTRR), and MS [51]. Genetic single nucleotide polymorphisms (SNPs) may alter these key enzymes activities, thus changing the enzymes catalytic efficiencies of metabolizing OCM nutrients after dietary intake. SNPs encoding these enzymes such as MS 2756A>G, MTRR 66A>G,

MTHFR 677C>T, and 1298A>C, have been evidenced to alter blood Hcy level [51-53]. Therefore, these genetic variants may also be potential genetic markers for incident diabetes development. A meta-analysis incorporating 68 studies indicated that MTHFR 677C>T polymorphism was correlated with T2DM in Asian populations, but not in White and Black populations [54]. Additionally, MTRR 66A>G polymorphism was related to T2DM risk in overweight and obese Chinese individuals [55]. Another study showed that only those Chinese adults who carry MTHFR 1793 GA+AA genotype or MTHFR 1298 AC+CC genotype appeared to have lower T2DM risk [17]. In addition, in the middle-age Han Chinese, those with the genotype CC of MTHFR 1470 A>C had a significantly higher likelihood of T2DM, whereas those with the genotype AA of MTHFR 1958G>A or carrying CT+TT of PEMT (rs4646356) had a significantly lower likelihood of T2DM [53]. Moreover, MTHFR CTCCGA haplotype (rs12121543-rs13306553-rs9651118-rs1801133-rs2274976-rs1801131) was found to be related to decreased risk of T2DM in a Chinese population, compared with CTTTGA haplotype [56]. Nevertheless, the frequencies of these SNPs appear different among people with diverse ethnicities, which may partially explain the inconsistent results from previous studies that a direct relationship between these SNPs and diabetes remains controversial among different study populations. However, previous studies investigating the interplay between OCM nutrients status and genetic variation encoding key enzymes of OCM on DM risk were limited. Lu et al. [17] reported the joint effects of higher serum betaine levels (>47.82 $\mu\text{mol/L}$) and heterozygous or homozygous variants of MTHFR (G1793A, A1298C) could be found influencing risk of T2DM among Chinese adults aged 40 to 75 years. The biological relevance of these OCM nutrients and genetic variation to the efficiency of the OCM pathway and Hcy homeostasis makes it necessary to consider interrelationships of the OCM nutrients intakes/circulation levels and SNPs with DM risk.

HYPOTHESIZED MECHANISM OF ACTION

Emerging evidence from *in vivo* and *in vitro* studies suggests that OCM nutrients are essential for facilitating energy and glucose metabolism through multifactorial mechanisms. Low status of the OCM nutrients (e.g., folate, choline, or vitamin B12) has been evidenced to induce HHcy [4,10,57], which has been implicated in the pathogenesis of DM [5,13]. HHcy increases reactive oxygen species (ROS) and C-reactive protein (CRP)

levels to promote oxidative stress and systemic inflammation, which have been reported to activate various stress-sensitive signaling pathways and eventually lead to pancreatic β -cells dysfunction [5,10], glucose intolerance [58], and IR [21,59,60]. OCM nutrients can directly scavenge ROS, decrease CRP, and promote production of GSH, the major intracellular antioxidant [10,21,61-64], which can counteract the disturbance in glucose metabolism by HHcy. Moreover, OCM nutrients (e.g., folate, choline, betaine) provide the methyl group to the universal methyl-donor, SAM, the change of which can modify methylation status of genetic loci signals involved in insulin signaling and glucose homeostasis [2,6,10,13,65,66]. These alterations in DNA methylation patterns may generate different gene expression profiles that facilitate the development or progression of DM [2,65,66]. However, the molecular mechanisms by which OCM nutrients contribute to DM pathology are only partially understood. The plausible mechanisms remain to be elucidated in human studies. Thus, more future studies exploring the underlying mechanisms are warranted.

IMPLICATION FOR PRACTICE

Based on the existing literature, it is premature to infer the causal relationship between OCM nutrients intake and incident DM. However, the beneficial effect of OCM nutrients on the major glucose metabolism indices indicates that consumption of natural food rich in OCM nutrients should be stimulated for maintaining optimal glucose homeostasis and preventing DM development. Co-ingestion of OCM nutrients-enriched foods, such as green leafy vegetables, legumes, fruit, nuts, whole-grain products, eggs, less-processed dairy products, and deep-sea fish, may exert a synergistic beneficial effect on better glucose control and insulin sensitivity. Common foods rich in OCM nutrients are listed in Supplementary Tables 1-7 [67-69]. In addition, there is no sufficient evidence to establish personalized OCM nutrient recommendations for DM prevention, based on the genetic variation information. Lower blood concentrations of these OCM nutrients may be indicators of a higher risk of DM. Therefore, it is worth monitoring these OCM nutrients biomarkers (e.g., plasma/serum levels of OCM nutrients) regularly to adjust their intake for optimal health.

CONCLUSIONS

The present review summarizes the existing evidence of wheth-

er OCM nutrients status influences the occurrence of DM. Currently, our knowledge of how OCM nutrients play a role in protesting against DM development in humans is in its early stages. Although limited RCTs using treatment with single/multiple OCM nutrient(s) reported different onsets on DM, the majority of observational studies manifested that intakes or blood biomarkers of OCM nutrients, particularly folate and betaine, were inversely associated with abnormal glucose metabolism indices and affect the progression of DM. In addition, association studies suggest that several SNPs in OCM-related genes may influence the metabolic handling of methyl-donors and presumably the risk of DM. While OCM nutrients interventions displayed promise, current human studies were mainly conducted among participants with different underlying medical conditions or middle-aged and elderly populations who may have already had disease onset. Future well-designed RCTs are warranted to examine whether balanced OCM nutrients intakes at an early age prevent DM later in life among the general population. Moreover, it is essential to examine whether the OCM nutrients intakes/circulating levels interplay with genetic risk factors to influence DM development in multi-ethnic populations, which will inform the personalized recommendations for OCM nutrients intakes in terms of DM prevention and management.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at <https://doi.org/10.4093/dmj.2023.0272>.

CONFLICTS OF INTEREST

Ka Kahe is an international editorial board member of the *Diabetes & Metabolism Journal*. He was not involved in the review process of this review. Otherwise, there is no conflict of interest.

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Supplementary Table 1. Common food sources of folate and folic acid [67,68]

Food	DFE per serving, μg^{a}	DV, %
Black-eyed peas (cowpeas), boiled, ½ cup	105	26
Breakfast cereals, fortified with 25% of the DV ^b	100	25
Rice, white, medium-grain, cooked, ½ cup ^b	90	22
Asparagus, boiled, 4 spears	89	22
Brussels sprouts, frozen, boiled, ½ cup	78	20
Spaghetti, cooked, enriched, ½ cup ^b	74	19
Lettuce, romaine, shredded, 1 cup	64	16
Avocado, raw, sliced, ½ cup	59	15
Spinach, raw, 1 cup	58	15
Broccoli, chopped, frozen, cooked, ½ cup	52	13
Mustard greens, chopped, frozen, boiled, ½ cup	52	13
Bread, white, 1 slice ^b	50	13
Green peas, frozen, boiled, ½ cup	47	12
Kidney beans, canned, ½ cup	46	12
Wheat germ, 2 tablespoons	40	10

DFE, dietary folate equivalent; DV, daily value.

^aDFE (μg) = naturally occurring folate + (1.7 × folic acid), ^bFortified with folic acid as part of the folate fortification program.

Supplementary Table 2. Common food sources of choline [67]

Food	Per serving, mg	DV, %
Beef liver, pan fried, 3 ounces	356	65
Egg, hard boiled, 1 large egg	147	27
Beef top round, separable lean only, braised, 3 ounces	117	21
Soybeans, roasted, ½ cup	107	19
Chicken breast, roasted, 3 ounces	72	13
Beef, ground, 93% lean meat, broiled, 3 ounces	72	13
Fish, cod, Atlantic, cooked, dry heat, 3 ounces	71	13
Potatoes, red, baked, flesh and skin, 1 large potato	57	10
Wheat germ, toasted, 1 ounce	51	9
Beans, kidney, canned, ½ cup	45	8
Quinoa, cooked, 1 cup	43	8
Milk, 1% fat, 1 cup	43	8
Yogurt, vanilla, nonfat, 1 cup	38	7
Brussels sprouts, boiled, ½ cup	32	6
Broccoli, chopped, boiled, drained, ½ cup	31	6

DV, daily value.

Supplementary Table 3. Common food sources of betaine [69]

Food	Food, mg/100 g
Wheat bran	1,339.35
Breakfast cereals wheat germ, toasted	1,240.48
Spinach, cooked	645.06
Spinach, raw	599.81
Beets (canned)	296.73
Pretzel, hard, plain, salted	236.45
Finfish and shellfish shrimp, canned	218.74
Baked products wheat bread	201.41
Wheat cracker	198.71
Graham cracker, plain	172.59
Beet, raw	114.42
English muffins	95.42
White bread	93.20
Pasta/rice	89.86
Plain muffins	82.12

Supplementary Table 4. Common food sources of vitamin B2 [67]

Food	Per serving, mg	DV, %
Beef liver, pan fried, 3 ounces	2.9	223
Breakfast cereals, fortified with 100% of the DV for riboflavin, 1 serving	1.3	100
Oats, instant, fortified, cooked with water, 1 cup	1.1	85
Yogurt, plain, fat free, 1 cup	0.6	46
Milk, 2% fat, 1 cup	0.5	38
Beef, tenderloin steak, boneless, trimmed of fat, grilled, 3 ounces	0.4	31
Clams, mixed species, cooked, moist heat, 3 ounces	0.4	31
Almonds, dry roasted, 1 ounce	0.3	23
Cheese, Swiss, 3 ounces	0.3	23
Mushrooms, portabella, sliced, grilled, ½ cup	0.2	15
Rotisserie chicken, breast meat only, 3 ounces	0.2	15
Egg, whole, scrambled, 1 large	0.2	15
Quinoa, cooked, 1 cup	0.2	15
Bagel, plain, enriched, 1 medium (3½"-4" diameter)	0.2	15
Salmon, pink, canned, 3 ounces	0.2	15

DV, daily value.

Supplementary Table 5. Common food sources of vitamin B6 [67]

Food	Per serving, µg	DV, %
Chickpeas, canned, 1 cup	1.1	65
Beef liver, pan fried, 3 ounces	0.9	53
Tuna, yellowfin, fresh, cooked, 3 ounces	0.9	53
Salmon, sockeye, cooked, 3 ounces	0.6	35
Chicken breast, roasted, 3 ounces	0.5	29
Breakfast cereals, fortified with 25% of the DV for vitamin B6	0.4	25
Potatoes, boiled, 1 cup	0.4	25
Turkey, meat only, roasted, 3 ounces	0.4	25
Banana, 1 medium	0.4	25
Marinara (spaghetti) sauce, ready to serve, 1 cup	0.4	25
Ground beef, patty, 85% lean, broiled, 3 ounces	0.3	18
Waffles, plain, ready to heat, toasted, 1 waffle	0.3	18
Bulgur, cooked, 1 cup	0.2	12
Cottage cheese, 1% low-fat, 1 cup	0.2	12
Squash, winter, baked, ½ cup	0.2	12

DV, daily value.

Supplementary Table 6. Common food sources of vitamin B12 [67]

Food	Per serving, μg	DV, %
Beef liver, cooked, pan fried, 3 ounces	70.7	2,944
Clams (without shells), cooked, 3 ounces	17	708
Nutritional yeast, fortified, from several brands (check label), about $\frac{1}{4}$ cup	8.3 to 24	346–1,000
Salmon, Atlantic, cooked, 3 ounces	2.6	108
Tuna, light, canned in water, 3 ounces	2.5	104
Beef, ground, 85% lean meat/15% fat, pan-browned, 3 ounces	2.4	100
Milk, 2% milkfat, 1 cup	1.3	54
Yogurt, plain, fat free, 6-ounce container	1	43
Breakfast cereals, fortified with 25% of the DV for vitamin B12, 1 serving	0.6	25
Cheese, cheddar, $1\frac{1}{2}$ ounces	0.5	19
Egg, whole, cooked, 1 large	0.5	19
Turkey, breast meat, roasted, 3 ounces	0.3	14
Tempeh, $\frac{1}{2}$ cup	0.1	3

DV, daily value.

Supplementary Table 7. Common food sources of zinc [67]

Food	Per serving, mg	DV, %
Oysters, Eastern, farmed, raw, 3 ounces	32	291
Oysters, Pacific, cooked, 3 ounces	28.2	256
Beef, bottom sirloin, roasted, 3 ounces	3.8	35
Blue crab, cooked, 3 ounces	3.2	29
Breakfast cereals, fortified with 25% of the DV for zinc, 1 serving	2.8	25
Cereals, oats, regular and quick, unenriched, cooked with water, 1 cup	2.3	21
Pumpkin seeds, roasted, 1 ounce	2.2	20
Pork, center loin (chops), bone-in, broiled, 3 ounces	1.9	17
Turkey breast, meat only, roasted, 3 ounces	1.5	14
Cheese, cheddar, 1.5 ounces	1.5	14
Shrimp, cooked, 3 ounces	1.4	13
Lentils, boiled, ½ cup	1.3	12
Sardines, canned in oil, drained solids with bone, 3 ounces	1.1	10
Greek yogurt, plain, 6 ounces	1	9
Milk, 1% milkfat, 1 cup	1	9

DV, daily value.