Association between mixed dietary B vitamin intake and insulin resistance in US middle-aged and older adults without diabetes: The Bayesian kernel machine regression approach

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ABSTRACT

Background and Objectives: In daily life, the intake of dietary nutrients is mixed. However, evidence for the association between mixed dietary B vitamin intake and insulin resistance is limited. In this study, we estimated the joint effect of intake of various dietary B vitamins on insulin resistance. **Methods and Study Design:** This cross-sectional study used data from the National Health and Nutrition Examination Survey 2011-2018. We included 1,628 middle-aged and 1,058 older adults without diabetes. Multivariable logistic regression and Bayesian kernel machine regression models were constructed. **Results:** In the multivariable logistic regression, when all B vitamins were included in the model, the ORs (95% CIs) of insulin resistance were 3.06 (1.00-9.37) and 0.42 (0.19-0.93) for the highest quartile of vitamin B-1 and B-12 intake in the middle-aged group when the lowest quartile was the reference. In the older group, no significant association was observed. In the Bayesian kernel machine regression analysis, a negative trend was noted between mixed B vitamin intake and insulin resistance in both examined groups. The univariate exposure-response function indicated that vitamin B-12 intake was negatively associated with insulin resistance in the middle-aged group, and that vitamin B-6 and dietary folate equivalent intakes were negatively associated with insulin resistance in older group. The bivariate exposure-response function indicated a potential interaction effect between dietary intake of vitamin B-12 and those of vitamin B-1, B-2, niacin, and dietary folate equivalent on insulin resistance in older people. **Conclusions:** Our results suggest that mixed dietary B vitamin intake tends to decrease the OR of insulin resistance both in middle-aged and older people.

Key Words: dietary B vitamin intake, insulin resistance, joint effect, cross-sectional study, Bayesian Kernel Machine Regression (BKMR)

INTRODUCTION

Diabetes mellitus, a chronic metabolic disease characterized by hyperglycemia, is a worldwide public health concern.[1] Since 1980, the global prevalence of diabetes has increased from 4.7% to 8.5% in the adult population.2 In the United States, 34.2 million adults had diabetes in 2018,[2] with type 2 diabetes mellitus (T2DM) accounting for 90% to 95% of cases.[3] Insulin resistance, a major metabolic abnormality, plays a pivotal role in the pathogenesis of T2DM.[3] Patients with T2DM typically develop insulin resistance before hyperglycemia occurs.[4] Individuals with insulin resistance have higher risk of T2DM, obesity, hypertension, cardiovascular disease, nonalcoholic fatty liver disease, and metabolic

syndrome than those without insulin resistance.[5-7] In addition, insulin resistance affects 85% of people aged ≥45 years.[2] Therefore, exploring the risk factors for insulin resistance in the population aged over 45 years may contribute to the prevention of a wider spectrum of metabolic diseases. Research has increasingly focused on the role of dietary factors in insulin resistance. In particular, higher dietary fiber intake,[8-11] higher dietary vitamin D intake,[12] lower caffeine intake[13] and higher dietary protein quality[14,15] have been associated with reduced risk of insulin resistance.

B vitamins, which are water-soluble vitamins, usually act as coenzymes to maintain cell function and energy metabolism.[15] Numerous studies have explored the protective roles of B vitamins in several diseases, such as metabolic syndrome,[16] depression,[17] and cardiovascular disease.[18] Although an association between B vitamin intake and insulin resistance has been reported in diverse populations,[19-21] some knowledge gaps remain. First, evidence regarding the association between B vitamin intake and insulin resistance is inconsistent.[20,22] A cross-sectional study indicated that serum vitamin B-6 and folate levels were negatively associated with insulin resistance in US adults without diabetes, but the association between serum vitamin B-12 level and insulin resistance was non significant.[22] By contrast, Cigerli et al employed the homeostatic model assessment of insulin resistance (HOMA-IR) and discovered that insulin resistance was negatively correlated with serum vitamin B-12 level, however, no association was found between folic acid level and insulin resistance.[20] Second, one study revealed that insulin secretion appears to decrease with age, even after adjusting for differences in obesity, fat distribution, and physical activity, moreover, insulin resistance in the older people may not be related to behavior, but to age.[23] The modifying effect of age on the association between B vitamin intake and insulin resistance remains unclear. Third, in daily life, dietary nutrient intake is mixed, and nutrients have distinct interactions with each other. Related studies have only focused on the association between dietary intake of a single vitamin B and insulin resistance; they have failed to consider the overall effect of mixed vitamin B intake on insulin resistance and the interaction between different B vitamins.[19,20,22,24]

To address the aforementioned gaps in the literature, we conducted a cross-sectional study using a large data set derived from the National Health and Nutrition Examination Survey (NHANES) 2011-2018. We evaluated (1) the association between dietary intake of a single B vitamin and insulin resistance in US nondiabetic middle-aged and older populations; (2) the effect of mixed dietary B vitamin intake on insulin resistance; and (3) the interaction between different B vitamins.

MATERIALS AND METHODS

Data source and study population

The NHANES is an ongoing cross-sectional study designed to assess the health and nutritional status of adults and children in the United States. The survey is conducted by the National Center for Health Statistics (NCHS) and a complex multistage probability design is employed to obtain a representative sample of the United States civilian noninstitutionalized population.[24] Data are collected through standardized home interviews followed by physical examination and biological specimen collection at a mobile examination center. Data collection for NHANES was approved by the NCHS Research Ethics Review Board, and written informed consent was obtained from all participants.

Data from four survey cycles were used in this study, covering 8 consecutive years (2011-2018). Although 39,156 individuals participated in the NHANES from 2011 to 2018, our analyses were limited to 13,173 individuals aged ≥45 years. Among them, participants with incomplete or unreliable 24-hour recall dietary data (n=1,771), and unavailable serum insulin or glucose data (n=7,125) were excluded. We also excluded participants who had diabetes (n=1,327). Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL, 2-h plasma glucose level ≥200 mg/dL following the 75-g oral glucose tolerance test, or glycated hemoglobin level ≥6.5%.[19,22,25] In addition, those who responded in the affirmative to any of the following questions were also regarded as having diabetes: “Did a doctor tell you that you have diabetes?”, “Are you taking insulin?”, and “Do you take pills to lower your blood sugar?”.[19,22,25] Twelve participants with extreme daily energy intake (men with daily energy intake of <500 kcal or >8,000 kcal and women with daily energy intake of <500 kcal or >5,000 kcal)[16] and 252 participants with unavailable covariant data were excluded. Finally, 2,686 participants aged ≥45 years were included in this study (1,628 and 1,058 individuals aged 45-64 and ≥65 years, respectively).

Insulin resistance

HOMA-IR scores, based on fasting glucose and fasting insulin concentrations, were used to assess insulin resistance.[13,26] Participants’ glucose and insulin levels after 9 hours of fasting were determined using a hexokinase enzymatic method and two-site immunoenzymometric assay, respectively.[28,29] We calculated HOMA-IR scores by multiplying the fasting glucose level (mmol/L) by the fasting insulin level (uU/mL) and dividing the result by 22.5. Quartiles of the HOMA-IR scores were calculated for the full sample. Insulin resistance was defined as a HOMA-IR score in the upper quartile (HOMA-IR ≥3.7).[22,27]

Daily dietary B vitamin intakes

Dietary B vitamin intake was assessed using two 24-hour dietary recall interviews. The first interview was conducted in a mobile examination center, whereas the second was conducted by telephone 3 to 10 days later. Detailed instructions for dietary interviews and data processing procedures are provided in the NHANES dietary interviewer procedures manual.[28,31] The daily intake of dietary B vitamins was calculated by summing the mean of the two 24-hour dietary intakes and the mean of two 24-hour dietary supplement intakes if participants completed two interviews. Otherwise, only dietary recall data from one interview were used.[16,28]

Study covariates

Factors highlighted in the literature as been associated with insulin resistance were included in regression models as potential confounders. The following demographic characteristics were considered: age (years, continuous), sex (male or female), race (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black, or other), educational level (below high school, high school, or above high school), and annual household income (<$20000 or ≥$20000). Other covariates were smoking status (current, former, or never), drinking status (yes or no), physical activity (vigorous or moderate), BMI (normal: <25 kg/m^2, overweight: 25-30 kg/m^2, or obese: ≥30 kg/m^2), waist circumference (cm, continuous variable), hypertension status (yes or no), total cholesterol level (mg/mL, continuous variable), LDL-cholesterol level (mg/mL, continuous variable), triglyceride level (mg/mL, continuous variable), and daily energy intake (kcal/day, continuous variable). Smoking status was determined using the question "Do you now smoke cigarettes?". Individuals who responded in the affirmative to the question "Do you have at least 12 alcoholic drinks per year?" were defined as alcohol drinkers. Respondents were categorized as having hypertension if they had a mean systolic blood pressure of ≥130 mmHg, or mean diastolic blood pressure of ≥80 mmHg, or if they self-reported a hypertension diagnosis.[29]

Statistical analysis

All analyses were stratified for the middle-aged (aged 45-64 years) and older (aged ≥65 years) groups. Means and standard deviations (SD) are used to describe the characteristics of normally distributed continuous variables, otherwise, the median and interquartile range (IQR) are used. Number (percentages) is used to describe the characteristics of categorical variables. To analyze the differences between variables, Student's t-test (normally distributed

continuous variables), Mann-Whitney U test (nonnormally distributed continuous variables), or Chi-Square tests (categorical variables) was employed.

Multivariable logistic regression

B vitamin dietary intake was divided into quartiles (quartile 1: <25th percentile, quartile 2: 25th to 50th percentile, quartile 3: 50th to 75th percentile, quartile 4: ≥75th percentile), with quartile 1 serving as the reference. Multivariable logistic regression models were used to investigate the association between dietary intake of individual B vitamins and the odds of insulin resistance. Moreover, to adjust for the confounding effects of dietary intake of other B vitamins, we fitted the multivariable logistic regression by including all the six B vitamins. ORs and their 95% CIs are presented. Models were adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, BMI, waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake.

Bayesian kernel machine regression

Bayesian kernel machine regression (BKMR) is an advanced statistical analysis method that can be used to consider complex interactions and nonlinear relations through Gaussian kernel iterative regression using a high-dimensional exposure-response function.30 BKMR is performed using the following equation:

$$Y_i = h(z_i) + x^T_i\beta + \epsilon_i$$

where Y_i is the health endpoint, i refers to the individual (i=1, 2, 3…n), the function h(.) is a high-dimensional exposure-response function allowing nonlinear and interaction terms to be included, z_i is the log-transformed B vitamin intake, x_i is a set of potential confounders, β represents the coefficients of the confounders, and ϵ_i is the residual.

The model was fit with 50,000 iterations by using the Markov chain Monte Carlo method. We used noninformative priors for all model parameters. Because the distribution of dietary B vitamin intake was skewed, we log-transformed all B vitamin intake values into approximately a normal distribution. The joint effect of B vitamin consumption was estimated by comparing individual B vitamin intake at the 60th percentile or higher with overall B vitamin intake at the 50th percentile level. We also estimated the exposure-response relationship and the interaction between each B vitamin pair by fixing the intake of other B vitamins to their median levels. In addition, considering the impact of excessive nutritional supplementation on dietary B vitamin intake, sensitivity analysis was conducted to restrict the study population to those not taking any nutritional supplements.

RESULTS

Participant characteristics

The characteristics of participants with and without insulin resistance in the two age groups are summarized in Table 1. We discovered significant differences between individuals with versus without insulin resistance in terms of BMI, triglycerides level, and daily energy intake. In the middle-aged group, those with insulin resistance tended to have higher BMI, a higher triglycerides level, and lower daily energy intake than those without insulin resistance (p<0.05). In the older group, those with insulin resistance were more likely to have higher BMI, and a higher triglycerides level (p<0.05) than those without insulin resistance.

The distribution of dietary B vitamin intakes is presented in Table 2. No significant difference was found in the intake of dietary vitamin B-1, niacin, B-6, or dietary folate equivalents (DFE) between the middle-aged and older groups. However, the intakes of dietary vitamin B-2 and B-12 in the middle-aged group were significantly lower than those in the older group (p<0.05). The median (IQR) intakes of vitamin B-2 and B-12 were 2.10 (1.85) mg/day and 5.99 (12.54) mcg/day in the middle-aged adults, respectively. For the older adults, the median (IQR) intakes of vitamin B-2 and B-12 were 2.21 (2.04) mg/day and 7.77 (25.44) mcg/day, respectively.

Multivariable logistic regression analyses of the association between dietary B vitamin intake and insulin resistance

The results of the multivariable logistic regression are presented in Table 3. After we adjusted for all covariates, in the middle-aged group, the ORs (95% CIs) of insulin resistance for vitamin B-2, niacin, vitamin B-6, DFE, and vitamin B-12 were 0.66 (0.49-0.89), 0.55 (0.34-0.88), 0.67 (0.50-0.90), 0.64 (0.48-0.86) and 0.83 (0.72-0.94), respectively. In the older group, compared with the lowest quartile, we found that the second (OR: 2.00; 95% CI: 1.07-3.74) and fourth quartiles (OR: 1.86; 95% CI: 1.07-3.25) of vitamin B-12 intake were positively associated with the odds of insulin resistance; however, no significant associations were discovered between vitamin B-1, B-2, niacin, B-6, and DFE intakes and the odds of insulin resistance.

To adjust for the confounding effects of dietary intake of other B vitamins, multivariable logistic regression including all the six B vitamins was conducted; the results are displayed in Table 4. For the middle-aged adults, when the lowest quartile was the reference group, the ORs (95% CIs) of insulin resistance for the second and fourth quartiles of vitamin B-1 intake were 3.21 (1.51-6.86) and 3.06 (1.00-9.37), respectively. Furthermore, the results indicated

that the second (OR: 0.42; 95% CI: 0.23-0.78), third (OR: 0.47; 95% CI: 0.24-0.94) and fourth quartiles (OR: 0.42; 95% CI: 0.19-0.93) of vitamin B-12 intake were negatively associated with insulin resistance. In the older adults, no significant associations were found.

BKMR model to evaluate the combined association between dietary B vitamin intakes and insulin resistance

We included the intakes of the six B vitamins in the BKMR model simultaneously to visually evaluate the combined effect of such intake on insulin resistance. The overall association between dietary B vitamin intake and insulin resistance is illustrated in Figure 1. When all the dietary B vitamin intakes were at their 60th percentile or higher relative to their median intake levels, we identified negative trends between mixed B vitamin intakes and insulin resistance in both the middle-aged and older groups, but no significant differences were found.

The trends in the exposure-response functions of the six B vitamins are illustrated in Figure 2. In the middle-aged group, when all the other dietary B vitamins intakes were at their median levels, dietary vitamin B-12 intake exhibited a negative association with insulin resistance, whereas dietary vitamin B-1 intake exhibited a positive association (Figure 2A). In the older group, dietary vitamin B-6 and DFE intakes demonstrated a negative association with insulin resistance when the other dietary B vitamins intakes were at their median levels (Figure 2B).

We also investigated the association between insulin resistance and single dietary B vitamin intakes, when fixing the dietary intakes of other B vitamins at their 25th, 50th, and 75th quantiles (with the remaining predictors fixed at their median level); the results are presented in Figure 3. We found no evidence of an interaction between dietary B vitamin intakes in middle-aged people (Figure 3A). For older people, when dietary vitamin B-12 intake was fixed at the 25th, 50th and 75th quantiles, the slopes between dietary vitamin B-1, B-2, niacin, and DFE intakes and insulin resistance were different; this result suggests that interactions may exist between dietary intake of vitamin B-12 and those of vitamin B-1, B-2, niacin, and DFE (Figure 3B).

In the sensitivity analysis, we excluded participants with vitamin B supplementation (532 and 478 individuals in the middle-aged and older groups, respectively). The results of the joint effect and univariate exposure-response functions were consistent with that in the main analysis (Supplementary Figures 1 and 2). The bivariate exposure-response function revealed no evidence of an interaction effect of dietary B vitamin intakes on insulin resistance in both the middle-aged and older groups.

DISCUSSION

To our knowledge, this is the first epidemiological study using the BKMR model to examine the associations between mixed daily dietary B vitamin intakes and the risk of insulin resistance. This study yielded three main findings. First, we discovered a negative trend between overall dietary intake of B vitamins and insulin resistance in both middle-aged and older adults. Second, in the middle-aged group, the results indicated that dietary vitamin B-12 and B-1 intakes were negatively and positively associated with insulin resistance, respectively. For the older adults, increases in dietary vitamin B-6 and DFE intakes helped reduce the odds of insulin resistance. Third, our results indicate potential interaction effects between dietary intake of vitamin B-12 and those of vitamin B-1, B-2, niacin, and DFE on insulin resistance in older adults.

Multivariable logistic regression, which is the most common approach for assessing health effects, is advantageous because of its fast modeling, straightforward results, and easy interpretations.[31-33] In this study, we found that the protective effect of dietary intake of a single B vitamin on insulin resistance was non significant in older people, even though their intakes of dietary vitamins B-2 and B-12 were significantly higher than those of middle-aged adults. Notably, studies using multivariable logistic regression analysis have generally included only single B vitamins, without considering overall B vitamin intake and the complex nonlinear interactions between individual B vitamins, which could lead to false positives or false negatives.[34] According to the bivariate dose-response relationships in the BKMR model, vitamin B-12 may have an interactive effect with vitamin B-1, B-2, niacin, and DFE on insulin resistance in older people. This may explain why we found no significant association between single dietary B vitamin intake and insulin resistance, whereas mixed intake tended to reduce insulin resistance in the older population.

However, the aforementioned problem was effectively addressed in the BKMR model, which is an advanced method for analyzing the health effects of mixed exposures.[31] The BKMR model can be used to consider the interaction between the mixed intake of dietary B vitamins, enabling researchers to evaluate the joint effect of various B vitamins in various action directions and the dose–response relationship between B vitamin intake and insulin resistance.[35] In our analysis, we found that overall dietary B vitamin intake was significantly associated with insulin resistance in middle-aged and older people. Vitamin B-12 intake had a negative association with insulin resistance, whereas vitamin B-1 intake was positively associated with insulin resistance in the middle-aged population; this was consistent with our findings in the multivariable logistic regression model for which all the B vitamins were

included. However, the BKMR model also has disadvantages. One limitation of this model relates to its kernel algorithm, which fits the exposure-response function by fixing other B vitamin intakes at specific levels (in this study, median levels) while ignoring estimates of the effects of coexposure patterns at other levels.[31] Therefore, the advantages and disadvantages of the two statistical analysis methods should be carefully considered to ensure effective analysis of the association between dietary B vitamin intake and insulin resistance.

Several studies that used conventional multiple linear regression or logistic regression models have reported association between metabolites or other indicators of B vitamins and insulin resistance in various populations.[36,37] Consistent with our findings, some related studies have reported a negative association of serum vitamin B-6, folate, and vitamin B-12 levels with insulin resistance.[19-22,24] However, several studies have failed to find a significant association between niacin, folate, and vitamin B-12 levels and the risk of insulin resistance.[19,20,22,37] A meta-analyses of randomized controlled trials revealed that folate supplementation may be beneficial for mitigating insulin resistance.[38] Disparities in the findings of previous studies may be attributed to the different ethnic backgrounds of participants or different evaluation methods. Moreover, these studies used traditional linear statistical analyses and failed to account for false negative or false positive errors caused by possible interactions between dietary B vitamin intakes.[34] To our knowledge, an association between mixed dietary B vitamin intake and insulin resistance has yet to be reported in the literature. The present study provides new evidence for the joint effect of total dietary B vitamin intake on insulin resistance.

The mechanisms underlying the association between B vitamin intake and insulin resistance have yet to be fully clarified. One possible mechanism is the anti-inflammatory and antioxidant effects of B vitamins.[39,40] Vitamin B-2 insufficiency induces a pathological proinflammatory response of functional macrophages, leading to intensification of their proinflammatory, pro-insulin-resistance, and prodiabetes effects.[41-43] Moreover, vitamin B-2 can help counter act oxidative stress, especially lipid peroxidation, protein carbonylation, and oxidative DNA damage,[15,44] all of which have been demonstrated to play crucial roles in the pathogenesis of insulin resistance.[5,45,46] In addition, vitamin B-6 and folate intakes have been reported to be negatively associated with inflammation, oxidative stress, and chronic inflammatory conditions.[47-50] Animal experiments have revealed that N-methylnicotinamide (a product of niacin) can increase insulin sensitivity through activation of SIRT1 and inhibition of FOXO1 acetylation.[48,50] Vitamin B-6, folic acid, and B-12 are involved in homocysteine degradation, thus a deficiency in these vitamins may lead to

hyperhomocysteinemia.[51-55] Numerous studies have reported that hyperhomocysteinemia aids the development of insulin resistance by inducing endoplasmic reticulum stress, promoting proinflammatory cytokine production, and facilitating macrophage infiltration.[51-55] In other words, deficiencies in vitamin B-6, folate, and B-12 may lead to hyperhomocysteinemia, which can promote insulin resistance.[56-58] Moreover, folate is a key source of the one-carbon group used to methylate DNA.[59] Because of the role of folate in DNA methylation, those deficient in this vitamin may be at increased risk of insulin resistance.60

The present study has several shortcomings. First, because NHANES yields cross-sectional data, making causal inferences regarding dietary B vitamin intake and insulin resistance is challenging. Thus, further prospective longitudinal studies and trials should be conducted to confirm those associations. Second, we assessed insulin resistance only through HOMA-IR. Nevertheless, HOMA-IR scores have been demonstrated to correlate well with the results of established methods for determining insulin resistance, such as hyperglycemia and euglycemic clamps, and the approach has been widely used in epidemiological studies of large populations.[13,26] Third, dietary intake variables were represented by the average of two 24-hour dietary recalls; this approach may not fully reflect long-term intake at the individual level. In addition, the 24-hour dietary recall data in NHANES were collected using the US Department of Agriculture automated multichannel approach (AMPM).[61] Although the AMPM has been proven to be valid for measuring sodium and total energy intakes;[62,63] its validity and accuracy for measuring dietary B vitamin intakes have yet to be reported. Fourth, although we adjusted for various confounders, residual confounding remains possible, especially from other nutrients, nonnutrients, phytonutrients, food structures, pollutants, dietary patterns, and food culture.

Despite the aforementioned limitations, the present results have two key public health implications. First, this may be the first study to examine the association between mixed dietary B vitamin intakes and insulin resistance by using the BKMR model. Because dietary nutrient intake in daily life is complex and varied, complex nonlinear and nonadditive relationships are likely to exist between nutrients and health outcomes. This study provides new insights into the relationship between nutrient intakes and health outcomes. Second, we found potential interactions of dietary vitamin B-12 with vitamin B-1, B-2, niacin, and DFE in the older population. Although no significant association was observed between single dietary B vitamin intakes and insulin resistance, mixed B vitamin intakes tended to result in a lower OR of insulin resistance. These findings suggest that an intake of foods rich in a

mixture of dietary B vitamins may help mitigate insulin resistance in middle-aged and older people.

Conclusion

This cross-sectional study revealed a negative trend between the mixed dietary intake of B vitamins and insulin resistance in middle-aged and older people. These findings provide evidence of a relationship between dietary B vitamin intakes and insulin resistance. Furthermore, our findings demonstrate the importance of linear and nonlinear models when evaluating the health effects of mixed dietary nutrient intakes. Further prospective longitudinal and intervention studies should be conducted to confirm this association.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare that there is no conflict of interest.

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Table 1. Demographic characteristics of insulin resistance and non-insulin resistance in middle-aged and elderly people, 2011-2018 NHANES

Variables	Middle-aged (45-64 years old), N=1628			Elderly group (≥ 65 years old), N=1058		
	Without IR (N=1208)	With IR (N=420)	*p*	Without IR (N=806)	With IR (N=252)	*p*
Age (years), mean ± SD	54.79±5.83	55.01±5.86	0.502 †	72.95±5.29	72.84±5.31	0.780 †
Sex, n (%)			0.827 ‡			0.746 ‡
Men	573 (47.43)	203 (48.33)		395 (49.01)	137(54.37)	
Women	635 (52.57)	217 (51.67)		411 (50.99)	115(45.63)	
Race, n (%)			0.907 ‡			0.472 ‡
Mexican American	180 (14.9)	56 (13.33)		85 (10.55)	24 (9.52)	
Other Hispanic	127 (10.51)	47 (11.19)		75 (9.31)	24(9.52)	
Non-Hispanic White	413 (34.19)	142 (33.81)		416 (51.61)	129(51.19)	
Non-Hispanic Black	304 (25.17)	114 (27.14)		161 (19.98)	45(17.86)	
Other Race	184 (15.23)	61 (14.52)		69 (8.56)	30(11.9)	
Education level, n (%)			0.077‡			0.019 ‡
<High school	254 (21.03)	86 (20.48)		220 (27.3)	66(26.19)	
High school	283 (23.43)	116 (27.62)		183 (22.7)	68(26.98)	
>High school	671 (55.55)	218 (51.9)		403 (50)	118(46.83)	
Annual family income, n (%)			0.668 ‡			0.645 ‡
Under $20000	262 (22.96)	105 (26.45)		206 (26.96)	59(25.11)	
$20000 and over	879 (77.04)	292 (73.55)		558 (73.04)	176(74.89)	
Smoking status, n (%)			0.619 ‡			0.129 ‡
Current	183 (17.96)	55 (15.15)		125 (18.14)	42(20.69)	
Former	226 (22.18)	67 (18.46)		147 (21.34)	56(27.59)	
Never	610 (59.86)	241 (66.39)		417 (60.52)	105(51.72)	
Physical activity, n (%)			0.825 ‡			0.806 ‡
Vigorous	419 (34.69)	153 (36.43)		277 (34.37)	90(35.71)	
Moderate	264 (21.85)	85 (20.24)		194 (24.07)	53(21.03)	
Others	525 (43.46)	182 (43.33)		335 (41.56)	109(43.25)	
Hypertension status, n (%)			0.512 ‡			0.360 ‡
Yes	787 (65.15)	270 (64.29)		597 (74.07)	176(69.84)	
No	421 (34.85)	150 (35.71)		209 (25.93)	76(30.16)	
BMI (kg/m^2), n (%)			0.016 ‡			0.033 ‡
<25	253 (20.94)	113 (26.90)		220 (27.30)	65(25.79)	
25-30	449 (37.17)	124 (29.52)		266 (33.00)	95(37.70)	
≥30	506 (41.89)	183 (43.57)		320 (39.70)	92(36.51)	
Waist circumference (cm), mean±SD	102.27±16.10	101.94±16.17	0.664 †	102.87±14.16	102.24±14.18	0.074 †
Serum triglycerides (mg/dL), mean±SD	90.28±53.54	128.48±67.52	0.000 †	89.18±51.60	117.99±66.65	0.009 †
Serum cholesterol (mg/dL), mean±SD	199.02±41.34	200.75±42.54	0.216 †	185.72±40.48	187.73±43.53	0.927 †
Serum LDL-cholesterol (mg/dL), mean±SD	106.88±34.42	108.41±32.00	0.701†	104.82±33.47	107.35±34.37	0.896 †
Daily energy intake (kcal/day), mean±SD	2064.72±22.33	1969.50±39.56	0.014 †	1789.30±24.16	1762.75±39.08	0.337 †

NHANES: National Health and Nutrition Examination Survey; IR: Insulin Resistance.
†*p* value are generated by Student's t-test or Mann-Whitney U test. ‡*p* value are generated by Chi-Square tests

Table 2. Distribution percentiles of dietary B vitamin intakes for middle-aged and elderly people, 2011-2018 NHANES

Total intake	Middle-aged (45-64 years old), N=1628					Elderly group (≥65 years old),N=1058					*p*
	*p*5	*p*25	*p*50	*p*75	*p*95	*p*5	*p*25	*p*50	*p*75	*p*95	
Daily dietary vitamin B-1 intake (mg/day)	0.66	1.17	1.69	2.69	12.52	0.64	1.16	1.77	2.82	26.43	0.247
Daily dietary vitamin B-2 intake (mg/day)	0.86	1.44	2.10	3.29	11.43	0.82	1.48	2.21	3.52	15.43	0.048
Daily dietary niacin intake (mg/day)	10.68	18.79	26.84	39.18	69.64	9.64	17.37	26.52	40.90	77.25	0.274
Daily dietary vitamin B-6 intake (mg/day)	0.77	1.42	2.17	3.74	10.73	0.73	1.34	2.24	4.31	17.77	0.404
Daily dietary DFE intake (mcg/day)	179.00	353.25	557.75	989.25	1733.00	176.00	337.00	590.50	1045.00	1692.00	0.419
Daily dietary vitamin B-12 intake (mcg/day)	1.12	3.00	5.99	15.54	354.89	1.13	3.10	7.77	28.54	1009.87	0.000

NHANES: National Health and Nutrition Examination Survey; DFE: Dietary Folate Equivalent.

Table 3. Association between single dietary B vitamin intakes and insulin resistance in middle-aged and elderly people, 2011-2018 NHANES

Daily dietary intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Total
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Middle-aged (45-64 years old)					
Vitamin B-1 (mg/day)	1 (Ref.)	1.67 (0.89-3.13)	0.88 (0.44-1.77)	0.64 (0.33-1.26)	0.81 (0.63-1.05)
Vitamin B-2 (mg/day)	1 (Ref.)	0.93 (0.52-1.67)	0.90 (0.44-1.81)	0.41 (0.21-0.79)**	0.66 (0.49-0.89)**
Niacin (mg/day)	1 (Ref.)	0.87 (0.47-1.64)	0.74 (0.40-1.38)	0.35 (0.17-0.73)**	0.55 (0.34-0.88)*
Vitamin B-6 (mg/day)	1 (Ref.)	0.89 (0.48-1.66)	0.73 (0.39-1.37)	0.45 (0.25-0.81)**	0.67 (0.50-0.90)**
DFE (mcg/day)	1 (Ref.)	0.54 (0.30-0.97)*	0.50 (0.28-0.90)*	0.42 (0.24-0.75)**	0.64 (0.48-0.86)**
Vitamin B-12 (mcg/day)	1 (Ref.)	0.46 (0.28-0.78)**	0.48 (0.26-0.88)*	0.34 (0.19-0.61)**	0.83 (0.72-0.94)**
Elderly group (≥65 years old)					
Vitamin B-1 (mg/day)	1 (Ref.)	0.62 (0.24-1.58)	0.87 (0.35-2.18)	0.83 (0.35-1.99)	1.01 (0.79-1.27)
Vitamin B-2 (mg/day)	1 (Ref.)	0.84 (0.36-1.97)	0.78 (0.29-2.13)	0.88 (0.35-2.22)	0.96 (0.70-1.31)
Niacin (mg/day)	1 (Ref.)	1.35 (0.63-2.91)	0.79 (0.32-1.93)	1.06 (0.48-2.32)	1.14 (0.76-1.70)
Vitamin B-6 (mg/day)	1 (Ref.)	1.08 (0.40-2.88)	0.81 (0.32-2.06)	0.91 (0.38-2.21)	0.92 (0.70-1.21)
DFE (mcg/day)	1 (Ref.)	1.46 (0.71-3.01)	1.12 (0.56-2.21)	1.32 (0.61-2.86)	1.01 (0.69-1.49)
Vitamin B-12 (mcg/day)	1 (Ref.)	2.00 (1.07-3.74)*	0.83 (0.40-1.74)	1.86 (1.07-3.25)*	1.11 (0.98-1.26)

NHANES: National Health and Nutrition Examination Survey; OR: Odds Ratios; CI: Confidence Interval; DFE: Dietary Folate Equivalent.
Model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake. Quartiles were based on all the participants in our study.
*p<0.05, **p<0.01.

Table 4. Association between dietary B vitamin intakes and insulin resistance with all the B vitamin included, 2011-2018 NHANES

Daily dietary intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Total
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Middle-aged (45-64 years old)					
Vitamin B-1 (mg/day)	1 (Ref.)	3.21 (1.51-6.86)**	1.70 (0.72-3.99)	3.06 (1.00-9.37)*	1.36 (0.93-1.99)
Vitamin B-2 (mg/day)	1 (Ref.)	1.15 (0.56- 2.38)	1.50 (0.58-3.86)	0.55 (0.15- 1.98)	0.68 (0.34-1.37)
Niacin (mg/day)	1 (Ref.)	0.81 (0.43-1.54)	0.75 (0.37-1.54)	0.37 (0.11-1.19)	0.94 (0.37-2.41)
Vitamin B-6 (mg/day)	1 (Ref.)	0.98 (0.55-1.74)	1.16 (0.47-2.89)	1.18 (0.43-3.28)	0.85 (0.46-1.59)
DFE (mcg/day)	1 (Ref.)	0.39 (0.20-0.77)**	0.54 (0.23-1.23)	0.91 (0.36-2.36)	0.85 (0.57-1.27)
Vitamin B-12 (mcg/day)	1 (Ref.)	0.42 (0.23-0.78)**	0.47 (0.24-0.94)*	0.42 (0.19-0.93)*	0.93 (0.80-1.07)
Elderly group (≥65 years old)					
Vitamin B-1 (mg/day)	1 (Ref.)	0.53 (0.19-1.48)	0.74 (0.22-2.53)	0.75 (0.17- 3.35)	1.18 (0.72-1.93)
Vitamin B-2 (mg/day)	1 (Ref.)	0.72 (0.28-1.86)	0.84 (0.28-2.52)	0.95 (0.30-3.09)	0.78 (0.40-1.52)
Niacin (mg/day)	1 (Ref.)	1.56 (0.59-4.16)	0.80 (0.28-2.30)	1.00 (0.27-3.74)	1.34 (0.82-2.19)
Vitamin B-6 (mg/day)	1 (Ref.)	1.30 (0.56-3.06)	1.20 (0.46-3.15)	0.70 (0.21-2.30)	0.79 (0.55-1.15)
DFE (mcg/day)	1 (Ref.)	1.62 (0.66-3.97)	1.90 (0.62-5.81)	2.23 (0.62-8.03)	0.93 (0.56-1.55)
Vitamin B-12 (mcg/day)	1 (Ref.)	1.72 (0.88-3.38)	0.72 (0.31-1.65)	1.55 (0.72-3.35)	1.15 (1.00-1.33)

NHANES: National Health and Nutrition Examination Survey; OR: Odds Ratios; CI: Confidence Interval; DFE: Dietary Folate Equivalent
Model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake. Quartiles were based on all the participants in our study.
*p<0.05, **p<0.01.

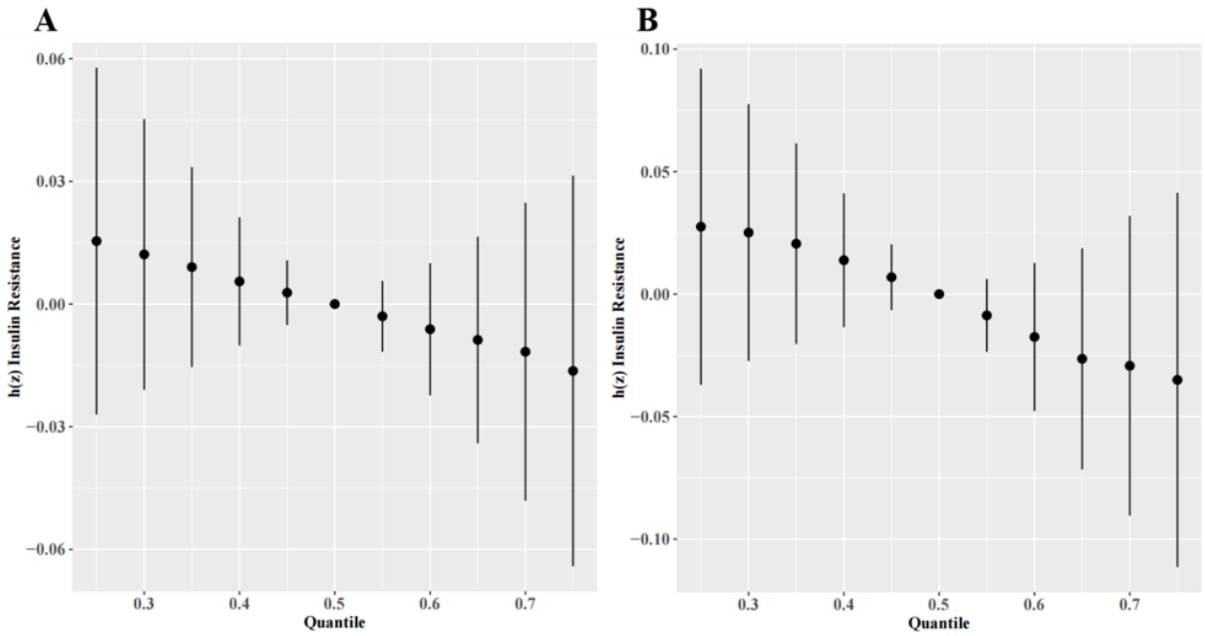


Figure 1. Joint effects of dietary B vitamin intakes on insulin resistance for the (A) middle-aged, and (B) older groups (estimated value and 95% CIs). All dietary B vitamin intakes at particular percentiles (from 0.25 to 0.75, increments of 0.05) were compared with overall intake at the 50th percentile. The association between dietary B vitamin intakes and insulin resistance is represented by h(z). The model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake.

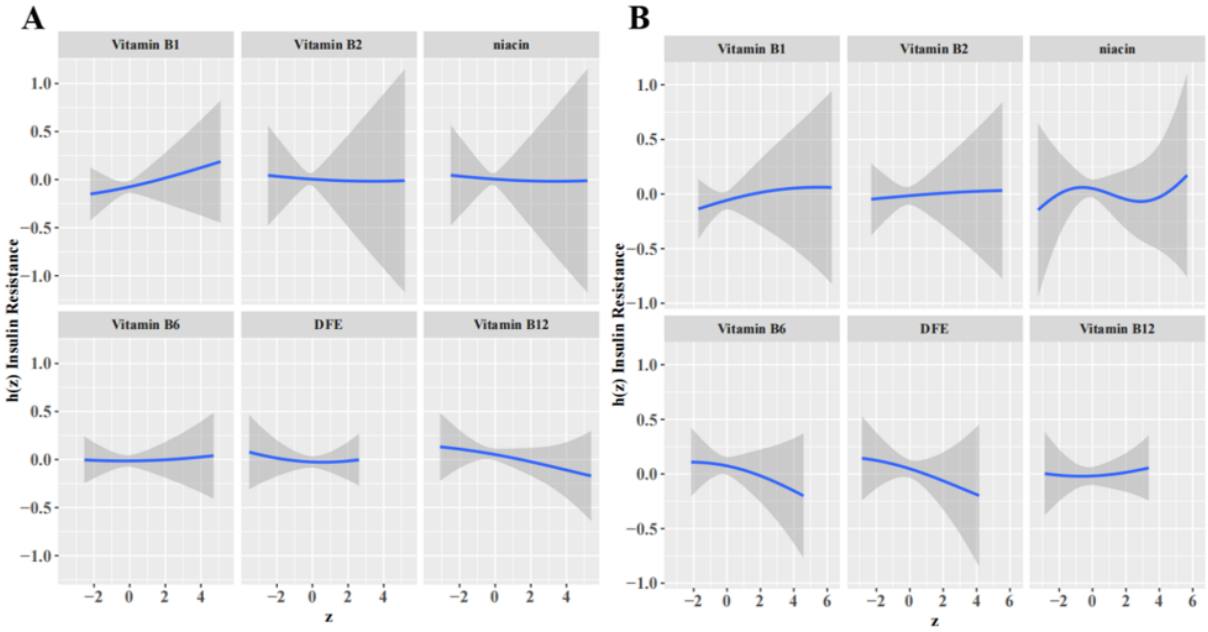


Figure 2. Estimated univariate exposure-response functions (with 95% CIs) for the (A) middle-aged, and (B) older groups. Associations between each dietary B vitamin intake and insulin resistance were assessed using BKMR models with the other dietary B vitamin intakes set at their median value. The association between dietary B vitamin intakes and insulin resistance is represented by h(z). The model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake.

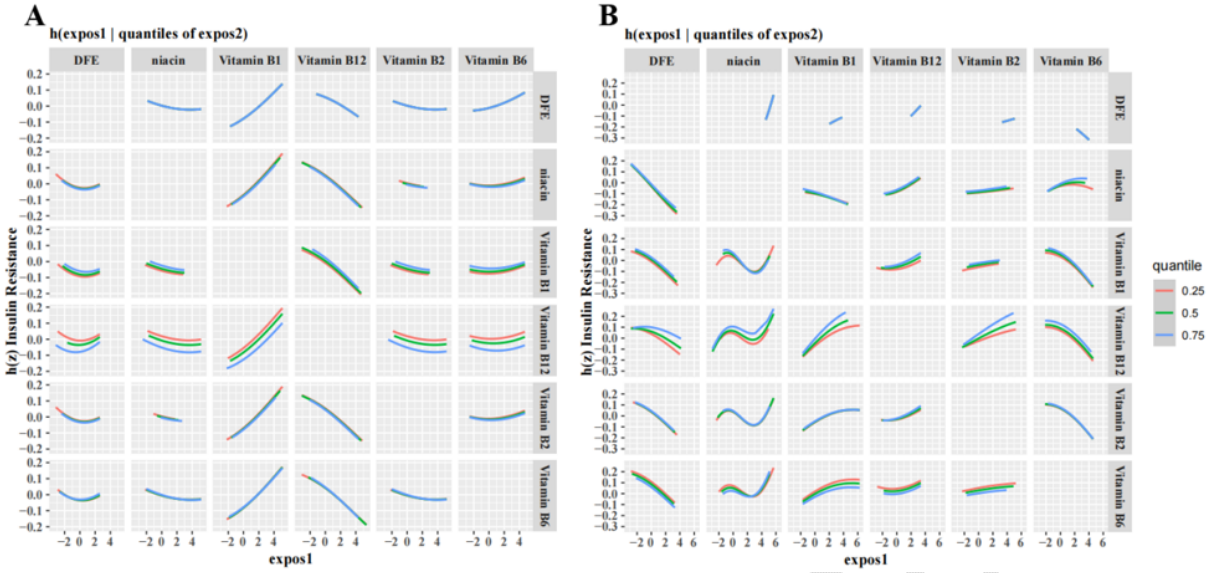
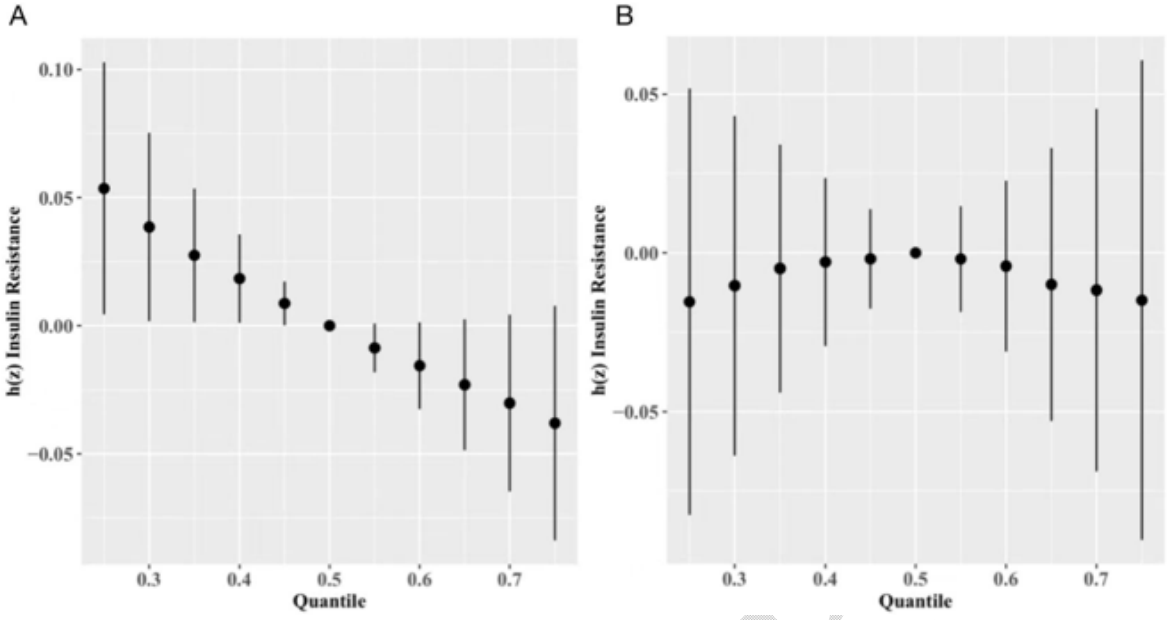
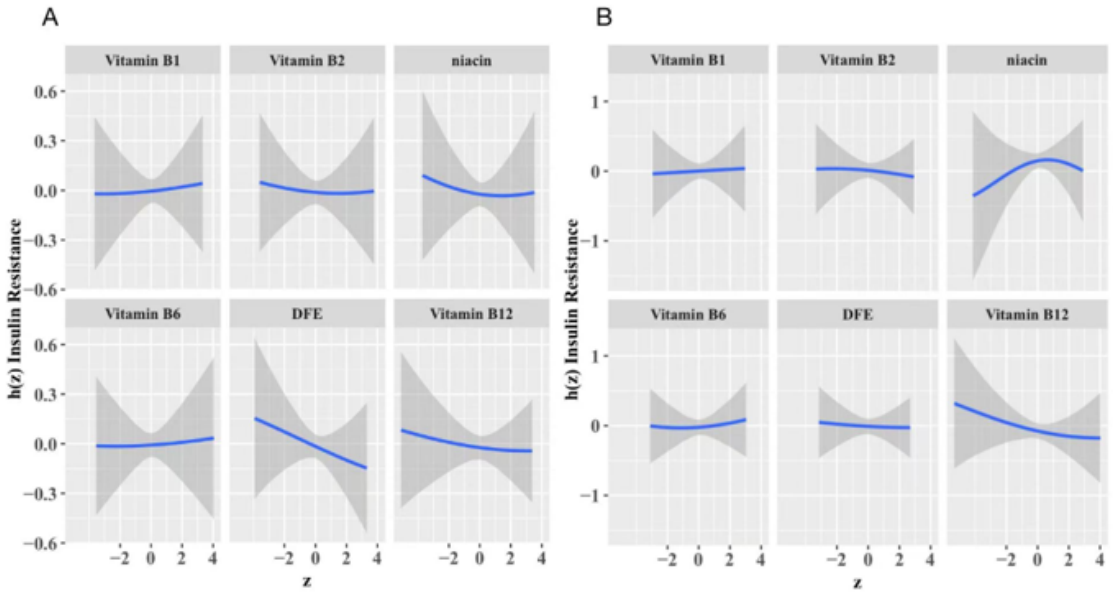


Figure 3. Bivariate exposure-response functions of the BKMR model for the (A) middle-aged, and (B) older groups. Association between exposure 1 and insulin resistance was assessed, with exposure 2 fixed at the 25th, 50th, and 75th quantiles (and fixing the remnant predictors to their median level). The association between dietary B vitamin intakes and insulin resistance is represented by h(z). The model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake.



Supplementary figure 1. Joint effects of dietary B vitamin intakes (without dietary supplement intakes) on insulin resistance for the (A) middle-aged, and (B) older groups. All dietary B vitamin intakes at particular percentiles (from 0.25 to 0.75, increments of 0.05) were compared with overall intake at the 50th percentile. The association between dietary B vitamin intakes and insulin resistance is represented by h(z). The model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake.



Supplementary figure 2. Estimated univariate exposure-response functions (with 95% CIs) for the (A) middle-aged, and (B) older groups (without dietary supplement intakes). Associations between each dietary B vitamin intake and insulin resistance were assessed using BKMR models with the other dietary B vitamin intakes set at their median value. The association between dietary B vitamin intakes and insulin resistance is represented by h(z). The model was adjusted for age, sex, race, educational level, annual household income, smoking status, drinking status, physical activity, body mass index (BMI), waist circumference, hypertension status, total cholesterol level, LDL-cholesterol level, triglyceride level, and daily energy intake.