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The unique presentation of the relationship between red blood cell folate and appendicular skeletal muscle mass: a cross-sectional study

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The overconsumption of folic acid has been associated with deleterious health effects; however, the extant body of research on this matter remains controversial. The principal objective of our investigation was to scrutinize the correlation between red blood cell (RBC) folate levels and appendicular skeletal muscle mass (ASM) among adult individuals. A total of 4117 adults aged over 20 years were included. The weighted prevalence of low muscle mass status (LMMS) was 14.50%. The correlation between RBC folate and ASM showed an inverted U-shaped curve. When the RBC folate concentration is below 500 nmol/L, ASM increases with increasing RBC folate concentration. However, when the RBC folate level exceeds 500 nmol/L, ASM decreases with increased RBC folate level. After correcting multiple confounding factors, a positive correlation was found between RBC folate and LMMS ($p < 0.001$). Compared with the RBC folate first quartile, the multivariable-adjusted ORs and 95% CIs of the second quartile, third quartile, and highest quartile were 1.08(0.81–1.44), 1.06(0.79–1.43), and 1.96(1.47–2.61), respectively. Our research suggested that excessive levels of RBC folate may be associated with an increased risk of LMMS in adults. Thus, being more cautious when considering folic acid supplementation is recommended.

Keywords Red blood cell folate, Muscle mass, Low muscle mass status, NHANES

It is well established that insufficient folate supply can markedly influence the proper development of the fetal neural tube and placental growth, thereby potentially leading to severe complications[1,2]. Furthermore, animal experiments and epidemiological inquiries have demonstrated that folate deficiency is a predisposing factor for conditions such as megaloblastic anemia, nervous system disorders, and cardiovascular ailments[3–5]. Therefore, in 1998, the United States instituted a policy mandating the fortification of grains with folic acid[6]. After three years of intervention, the report from the Centers for Disease Control and Prevention (CDC) in the United States indicated that the incidence of neonatal neural tube defects in 2001 significantly decreased to 10% before policy implementation[7,8]. However, certain epidemiological studies indicated that the consumption of elevated doses of folic acid may be implicated in the hastening of cancer progression, the onset of insulin resistance, the masking of vitamin B12 deficiency, and the induction of aortic calcification[9–14], and research on the association between folic acid and adverse health outcomes is still limited. Consequently, it is imperative to underscore the necessity for additional research elucidating the potential adverse health ramifications associated with the excessive fortification of folic acid.

Both animal and cell experiments have proven that folic acid not only plays a crucial role in maintaining normal cell division and immune function but is also closely related to energy metabolism in the body, both of which may directly or indirectly affect muscle strength and mass[15–17]. Recently, studies on the relationship between folic acid and muscle strength have yielded different results in different populations such as the United Kingdom, Korea, Singapore, and the United States[18–21], and similarly, the results of studies on the relationship between folic acid and muscle mass have been inconsistent[22,23]. In a survey of 58 elderly outpatient patients in

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the United States[23], the results showed a positive correlation between folate intake and muscle mass. However, in elderly Japanese women, it was found that there was no statistical correlation between serum folate and muscle[22]. Previous studies on the relationship between folic acid and muscle mass and strength were predominantly based on serum folate and dietary folate[18–23]. Due to the low bioavailability of dietary folate and the short cycle representativeness of serum folate, so neither of these indicators can reflect the overall folate storage in the human body[24,25]. By contrast, RBC folate can represent the intake of folate throughout the entire life cycle of red blood cells[25]. Despite the significance of RBC folate, there is a clear lack of research on its relationship with muscle mass. Accordingly, in the present investigation, our focus will center on examining the correlation between RBC folate levels and muscle mass.

Results

The demographic characteristics of the study are delineated in Table 1. A total of 4117 individuals participated in this analysis, comprising 2049 males and 2068 females, with a weighted mean age of 44.0 ± 14.5 years. Among these participants, 597 adults were identified as presenting with LMMS, representing a weighted prevalence rate of 14.9%. Notably, age, serum folate, vitamin B12, waist circumference, and BMI demonstrated a positive correlation with ascending quartiles of RBC folate concentration. Conversely, homocysteine peaked in the second quartile of erythrocyte folate concentrations and then declined in the third quartile. In terms of categorical variables, individuals categorized as having high RBC folate levels exhibited a higher likelihood of being female, married, white, non-smoking, and drinking when compared to their counterparts with low RBC folate levels.

Characteristics	Quartile levels of red blood cell folate (nmol/L)				
	Q1 (< 457.5)	Q2 (457.5-579.8)	Q3 (579.8-747.5)	Q4 (> 747.5)	P value
N	1016	1053	1014	1034	
Age (years)	44.2 ± 0.5	45.1 ± 0.5	45.9 ± 0.5	48.8 ± 0.5	< 0.001
Sex (%)					< 0.001
Male	529 (52.1)	570 (54.1)	482 (47.5)	468 (45.3)	
Female	487 (47.9)	483 (45.9)	532 (52.5)	566 (54.7)	
Ethnicity (%)					< 0.001
Hispanic	233 (22.9)	291 (27.6)	238 (23.5)	225 (21.8)	
White	379 (37.3)	485 (46.1)	548 (54.0)	590 (57.1)	
Black	354 (34.8)	244 (23.2)	181 (17.9)	171 (16.5)	
Others	50 (4.9)	33 (3.1)	47 (4.6)	48 (4.6)	
Education (%)					< 0.001
Less than high school	295 (29.0)	288 (27.4)	219 (21.6)	233 (22.5)	
High school	262 (25.8)	235 (22.3)	242 (23.9)	250 (24.2)	
More than high school	459 (45.2)	530 (50.3)	553 (54.5)	551 (53.3)	
Family poverty levels (%)					0.001
≤ 1.30	299 (29.4)	283 (26.9)	227 (22.4)	250 (24.2)	
1.31 ~ 1.85	130 (12.8)	132 (12.5)	106 (10.5)	121 (11.7)	
> 1.85	587 (57.8)	638 (60.6)	681 (67.2)	663 (64.1)	
Marry status					< 0.001
married	691 (68.0)	766 (72.7)	791 (78.0)	844 (81.6)	
unmarried	325 (32.0)	287 (27.3)	223 (22.0)	190 (18.4)	
Alcohol status (%)					0.036
Yes	675 (66.4)	761 (72.3)	711 (70.1)	723 (69.9)	
No	341 (33.6)	292 (27.7)	303 (29.9)	311 (30.1)	
Smoking status (%)					0.010
Yes	545 (53.6)	509 (48.3)	474 (46.7)	497 (48.1)	
No	471 (46.4)	544 (51.7)	540 (53.3)	537 (51.9)	
LMMS (%)					0.003
Yes	145(14.3)	140 (13.3)	128 (12.6)	186 (18.0)	
No	871 (85.7)	913 (86.7)	886 (87.4)	848 (82.0)	
Vitamin B12(pmol/L)	363.7 ± 17.6	382.4 ± 8.7	404.0 ± 9.9	545.4 ± 54.6	< 0.001
Homocysteine(umol/L)	9.9 ± 0.2	8.5 ± 0.2	8.2 + 0.1	8.5 ± 0.1	< 0.001
Serum folate (nmol/L)	18.8 ± 0.3	24.6 ± 0.3	31.2 ± 0.4	42.9 ± 0.9	< 0.001
Waist (cm)	96.5 ± 0.5	97.6 ± 0.5	98.2 ± 0.5	100.2 ± 0.5	< 0.001
BMI	28.3 ± 6.7	28.6 ± 6.1	28.7 ± 6.0	29.4 ± 6.5	< 0.001

Table 1. Characteristics of the study population. LMMS: low muscle mass status.

The association between RBC folate and ASM is illustrated in Figs. 1, which displays an inverted U-shape. At RBC folate concentrations below 500 nmol/L, ASM demonstrates an upward trajectory with increasing RBC folate concentration. Conversely, upon surpassing this threshold, ASM exhibits a declining trend. Analogously, the same outcomes were observed for RBC folate and ASMI. (Supplementary Fig. 1). Furthermore, considering the gender and age differences in ASM, we conducted a stratified analysis. In stratified analysis, the results indicated that this inverted U-shaped relationship between RBC folate and ASM does not change due to gender or age (Supplementary Fig. 2), which is similar to the stratification results of ASMI (Supplementary Fig. 3).

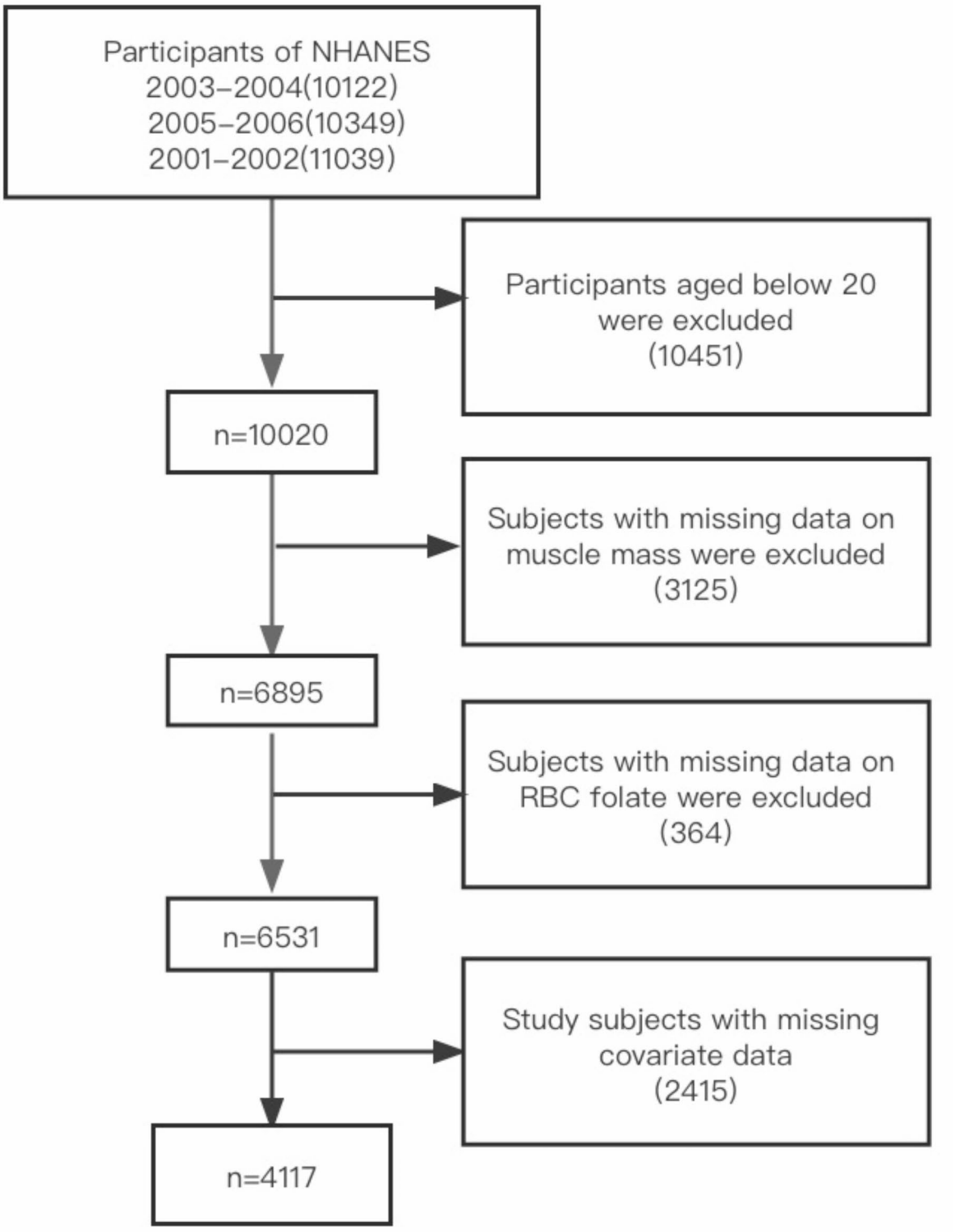

Fig. 1. Flow chart of the screening process for the selection of eligible participants from NHANES.

Variable	Model1		Model2		Model3	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Q1	1(Referent)	-	1 (Referent)	-	1 (Referent)	-
Q2	0.92 (0.72–1.18)	0.522	1.03 (0.78–1.36)	0.856	1.08 (0.81–1.44)	0.816
Q3	0.87 (0.68–1.13)	0.300	1.04 (0.78–1.39)	0.774	1.06 (0.79–1.43)	0.986
Q4	1.32 (1.04–1.67)	0.025	1.97 (1.49–2.61)	< 0.001	1.96 (1.47–2.61)	< 0.001
Per-SD	1.02 (1.01–1.03)	< 0.001	1.03 (1.02–1.04)	< 0.001	1.03 (1.02–1.04)	< 0.001

Table 2. Odds ratios and 95% confidence intervals of RBC folate levels associated with LMMS. Model 1 adjusted for age and sex. Model 2 additionally adjusted for ethnicity, education, family poverty levels, marital status, activity, alcohol drinking, smoking, vitaminB12 and waist. Model 3 further adjusted for hypertension, diabetes, high cholesterol, homocysteine.

Variable	Quartile levels of red blood cell folate				*p* for interaction
	Q1	Q2	Q3	Q4	
Age					0.106
< 60	1(Ref)	0.88(0.65–1.18)	0.99(0.74–1.33)	1.32(0.99–1.76)	
≥ 60	1(Ref)	1.03(0.66–1.65)	0.57(0.34–0.95)	1.20(0.78–1.85)	
Gender					0.717
Men	1(Ref)	0.92(0.65–1.29)	0.82(0.57–1.18)	1.44(1.03–2.10)	
Women	1(Ref)	0.92(0.64–1.33)	1.22(0.87–1.71)	1.22(0.87–1.71)	

Table 3. Subgroup analyses of odds ratios and 95% confidence intervals for LMMS with RBC folate.

The relationship between RBC folate and LMMS is shown in Table 2. After adjusting for age and gender, compared with the lowest quartile of RBC folate, only the highest quartile of RBC folate was associated with LMMS (OR: 1.32, 95%CI: 1.04–1.67, $p = 0.025$), and this association persisted after adjusting for factors such as smoking, alcohol consumption, and other factors on the basis of age and gender, and this association persisted after additionally adjusting for ethnicity, education, family poverty levels, marital status, activity, alcohol drinking, smoking, vitaminB12 and waist on the basis of age and sex (OR: 1.97, 95%CI: 1.49–2.59, $p < 0.001$). Association of RBC folate with LMMS remained significant (OR: 1.96, 95%CI: 1.47–2.61, $p < 0.001$) after additional adjustment for hypertension, diabetes, high cholesterol, homocysteine in model 2. In addition, we also included the concentration of RBC folate as a continuous variable in the regression analysis and found that regardless of which model, the risk of LMMS increased for every 1 SD increase in RBC folate concentration (Model1: OR, 1.02, 95%CI, (1.01–1.03), $p < 0.001$; Model2: OR, 1.03, 95%CI, (1.02–1.04), $p < 0.001$; Model3: OR, 1.03, 95%CI, (1.02–1.04), $p < 0.001$.).

To explore whether the relationship between RBC folate and LMMS is affected by age and sex, we analyzed the interaction between age, sex, and RBC folate. The results showed that there was no interaction between RBC folate and the two factors (p for year interaction = 0.106, p for gender interaction = 0.717) (Table 3).

Discussion

Folate stands as a pivotal nutritional factor recognized for its potential to facilitate muscle development[15,17]. Nonetheless, preceding findings on the relationship between folate and indicators of muscular strength or mass have demonstrated inconsistency[18–23]. In our investigation, a notable observation emerged revealing an inverted U-shaped dose-response relationship between RBC folate levels and muscle mass. Specifically, when the RBC folate concentration fell below 500 nmol/L, an increment in ASM was noted with an escalating RBC folate concentration. However, entering into a high folate state, marked by a concentration of 745.5 nmol/L, was found to significantly elevate the risk of LMMS.

Previous research has predominantly focused on the relationship between folate and muscular strength, with multiple studies from the United Kingdom, Singapore, and South Korea underscores the significance of folate in maintaining skeletal muscle strength. Such investigations typically emphasize the role of folate in neuromuscular transmission, protein synthesis, and energy metabolism[18,19,21]. However, the study conducted by Zhang et al. indicates a decline in corrected grip strength when serum folate levels exceed 40 nmol/L among 9,079 adults aged ≥ 20 in the United States[20]. While these findings provide crucial insights into our understanding of the physiology of folate and muscles, research directly addressing the correlation between muscle mass and folate remains relatively limited[22,23]. Yeung et al. showed that serum folate levels are positively correlated with muscle mass in the elderly population of the United States[23]. Misora Ao et al. reported that there is no correlation between serum folate and muscle mass[22]. Our research results indicated an inverted U-shaped relationship between RBC folate and ASM, which is different from the findings of Yeung et al. and Misora Ao et al., possibly because the RBC folate used in this study was different from the serum folate they used, or because our study population was younger (4117 adults, age 44.0 ± 14.5 years old)[22,23]. In addition, we explored the interaction

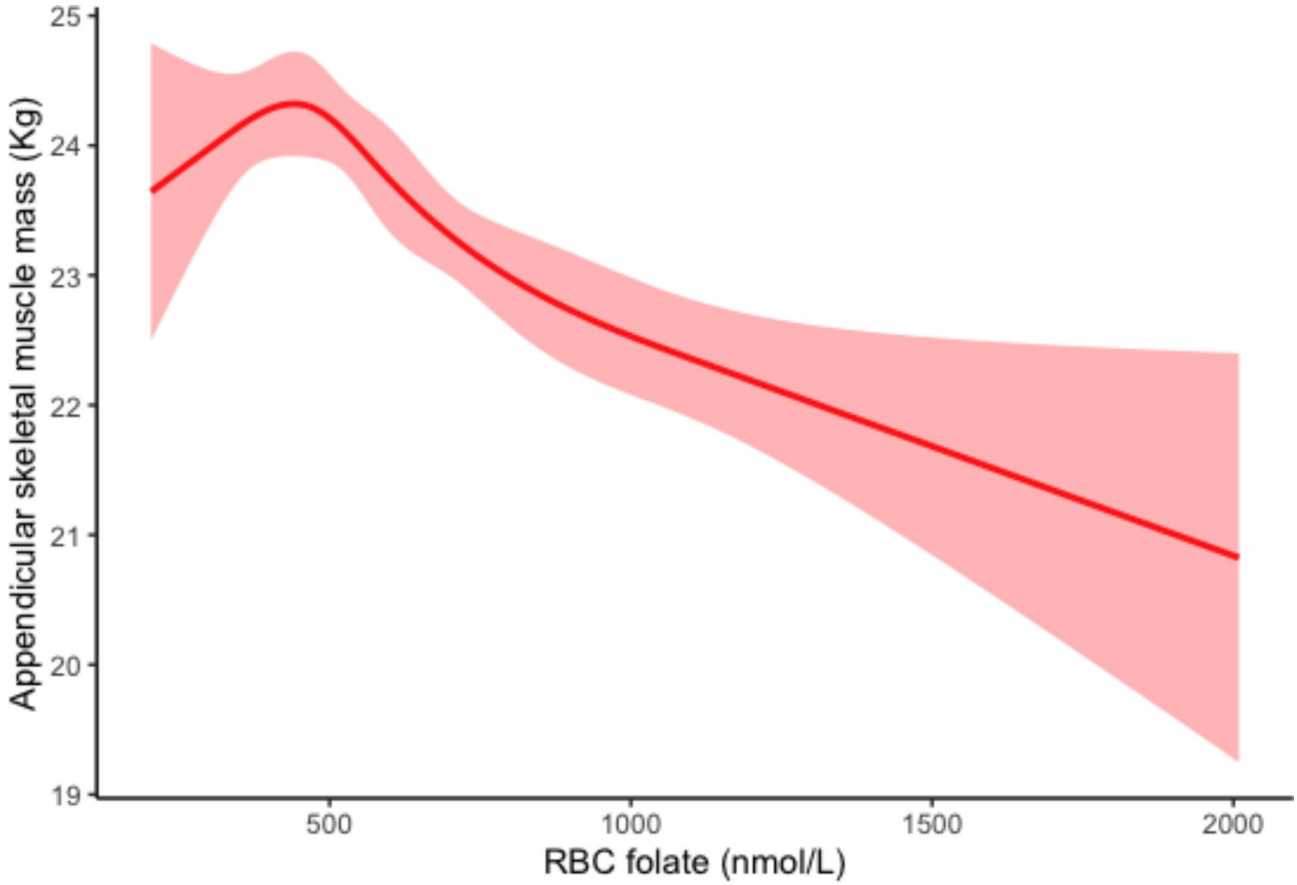

Fig. 2. Relationship between RBC folate and appendicular skeletal muscle mass (p-nonlinear < 0.001).

between RBC folate and age and gender. However, the stratified results showed that the trends of RBC folate and ASM were the same in both gender and age groups.

In the current discussion, we need to recognize that supplementing folic acid may involve a range of potential health risks. Regarding this, Marta Ebbing and colleagues observed that[26] following a seven-year follow-up period, ischemic heart disease patients who consumed 0.8 mg/day of folic acid experienced a substantial increase in the incidence of lung cancer, cancer-related mortality, and overall mortality risk. Furthermore, a decade-long randomized controlled trial indicated a significant elevation in the risk of prostate cancer among males supplementing with 1 mg/day[27]. The folic acid intake in these studies notably approached or exceeded the upper tolerable intake level of 1000 ug/day[28]. However, we do not yet know how long or how large a dose of folic acid intake is needed to reach concentrations of RBC folate that may be potentially harmful. Therefore, before considering folic acid supplementation, various potential risks should be taken into account. In our study, we discovered a significant increase in the risk of LMMS when the concentration of red blood cell folate exceeded 745.5 nmol/L. Similarly, Zhou et al.'s study presented that when the concentration of RBC folate exceeded 1809nmol/L, the risk of abdominal aortic calcification increased[14]. The dosage of RBC folate reported by our research is different from that in previous studies, which may due to be adverse health reactions that can affect the threshold of RBC folate[29]. Additionally, compared with the microbiological assay used in previous studies, the Bio-Rad assay used for measuring RBC folate in our study underestimated the concentration of RBC folate by 45%[30]. Accordingly, more accurate measurement methods are needed to explore the RBC folate threshold for different health effects. Our findings are along the lines of previous evidence suggesting that excessive RBC folate levels may be associated with decreased muscle mass. However, more direct evidence is needed in the future to confirm this speculation.

Although the mechanism between folic acid and muscle mass is not yet clear, the following possible mechanisms can help understand the relationship between folic acid and muscle mass. First, studies have shown that folate deficiency can affect the inhibition of S-adenosylmethionine in muscle cells, resulting in DNA hypomethylation, which can affect the proliferation and differentiation of muscle cells, and accelerate the aging and apoptosis of muscle cells[15,24,31]. Second, van Dijk M et al. have shown that the reduction of folic acid intake in the diet will reduce antioxidant capacity and increase oxidative stress, which is one of the risk factors for decreased muscle mass[32,33]. Moreover, folic acid intake can reduce high levels of homocysteine associated with poor physical performance, decreased muscle strength, and decreased muscle density[34,35]. However, previous studies have reported a U-shaped relationship between folate levels and homocysteine[36,37], which is supported by the results of our study (Supplementary Fig. 3). Studies have shown that folate plays an important role in the

conversion of homocysteine to S-adenosylmethionine - as a coenzyme in the methyl transfer reaction[38]. However, excessive folate levels may reduce methyl donor utilisation or impair the activity of methylenetetrahydrofolate reductase, which is responsible for homocysteine methylation[36,37]. Finally, it has been proven that the intake of folic acid can improve skeletal muscle blood flow, which is a key component in maintaining muscle mass[39]. Nevertheless, some studies have shown that excessive folate in red blood cells is associated with arteriosclerosis, which can hinder muscle blood supply and reduce muscle mass[14,40].

This is the first study to investigate the relationship between RBC folate and muscle mass. The present study has several deficiencies as follows. First, the cross-sectional nature of our research made it difficult to determine a causal relationship between RBC folate and decreased muscle mass. Second, the data for this study were derived from the NHANES database for the years 2002–2006. This relatively early time frame may not fully reflect erythrocyte folate and muscle mass status in the current population. In addition, due to the time constraints of the database, we could not include other indicators that may reflect high levels of folate status (e.g., unmetabolized folate) in our analyses, which somewhat limits our ability to provide a comprehensive assessment of folate status. Eventually, although we adjusted for several potential factors, we cannot completely rule out the influence of residual confounding of unknown or unmeasured variables.

In conclusion, our study demonstrates that among American adults, the relationship between RBC folate and muscle mass exhibits an inverted U-curve. Specifically, when RBC folate concentrations exceed 745.5 nmol/L, there is a significant association with an increased risk of LMMS. This finding highlights the need for further investigation into the potential adverse health effects of elevated folate levels. Future research should delve into the broader health implications of high RBC folate concentrations, enhancing our understanding of the complex and potentially dual-edged nature of this essential nutrient.

Methods

Study population

The present study utilized data derived from the National Health and Nutrition Examination Survey (NHANES) spanning the years 2003–2006, information about this cross-sectional study can be described in detail elsewhere[25]. Briefly, NHANES is a nationally representative cross-sectional study on the nutrition and health of respondents living in the United States. In total, 20,471 participants completed the interviews in the NHANES 2003–2006, in which the muscle mass of adult individuals over the age of 20 was evaluated. Individuals who met the following criteria in the analysis were excluded: (1) age < 20 years ($n = 10451$); (2) subjects with missing muscle mass data ($n = 3125$); (3) subjects who failed to obtain RBC folate data ($n = 364$); and (4) subjects who missed covariate data ($n = 2415$). Finally, a total of 4117 adults were retained for analysis. The specific exclusion process of the research object is shown in Fig. 2. All participants provided written informed consent and the protocol was approved by the Ethics Review Board of National Center for Health Statistics(Protocol #98–12, Protocol #2005–06).

Data collection

The demographic information of NHANES was collected by professionally trained staff using computer-assisted personal interview programs. The information collected included demographics, health status, lifestyle, and serum indicators such as physical activity, homocysteine, and vitamin B12. Other variables included ethnicity (to facilitate statistical analysis classifying Mexican and Hispanic as Hispanic, white, black, and other races[41], educational attainment (below high school graduates, high school graduates, and above high school education level), and family poverty level (based on the poverty criteria, poverty to income ratio was divided into three categories: ≤1.30, 1.31–1.85, and > 1.85)[14]. By collecting the questionnaire data about the lifestyle of the researchers, at least 100 times of smoking in the previous year were defined as smokers, and at least 100 times of drinking in the previous year were classified as drinkers.

Laboratory tests and clinical definition

Whole blood and blood serum were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, GA for analysis. Both folate (whole blood folate and serum folate) and vitamin B12 were measured by using the Bio-Rad Laboratories "Quantaphase II Folate/vitamin B12" radio assay kit. Homocysteine in plasma was measured by the Abbott homocysteine assay, a fully automated fluorescence polarization immunoassay from Abbott Diagnostics. Long-term estimated coefficients of variation (CV) for homocysteine, vitamin B12, serum and RBC folate measured or calculated by the above methods fluctuated within 3–7%. The concentration of RBC folate was calculated using the following formula:

$$((whole\ blood\ folate \times 22) - serum\ folate\,(1 - hematocrit)) \Big/ \left(\mathrm{hematocrit} \Big/ 100\right)$$

Hypertension is defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg and/or currently used antihypertensive drugs[42]. Diabetes is defined as glycosylated hemoglobin ≥ 6.5% and/or currently treated with hypoglycemic drugs or insulin[43]. Hypercholesterolemia is defined as TC ≥ 6.2 mmol/l or current medication use[44].

Body test data and low muscle mass status evaluation

The participant's height and weight data were measured using a Toledo electronic weight scale and a Seca electronic stadiometer, respectively. The body mass index (BMI) was calculated by the following formula based on the measured height and weight data: weight(kg)/[height (m)]^2. The waist circumference data of the participants were measured using an inelastic tape measure with a minimum graduation of 1 mm, at the highest

point of the iliac crest, horizontally around the abdomen, and at the end of normal breathing, with an accuracy of 0.01 cm.

In this investigation, Dual-energy X-ray absorptiometry (DXA) was employed for comprehensive scanning of participants' anatomical regions including the head, arms, legs, and trunk, providing data on bone mineral content (kg), fat content (kg), and lean body weight (kg). Participants were required to remove metallic accessories such as earrings and assume a supine position on the Hologic QDR 4500 A fan beam X-ray bone densitometer (Hologic Inc., equipped with Hologic Discovery software, version 12.1), with bare feet and arms extended alongside their bodies. The scanning procedure typically lasted approximately 3 min. Within the scope of this research, ASM represented the combined muscle mass of both the upper and lower limbs. In addition, to exclude the effect of height, the ASM was divided by the square of height to obtain the appendicular skeletal muscle mass index (ASMI). Low muscle mass status (LMMS) was established according to the diagnostic criteria proposed by EWGSOP. LMMS was defined as ASMI of less than 7.9 kg/m^^{2} in men and 5.8 kg/m^^{2} in women.

Statistical analysis

Data were presented as weighted mean ± standard deviations (SDs) for continuous variables and as frequency (weighted percentages) for categorical variables according to NHANES analytic guidelines. Baseline characteristics of the population were compared using one-way analysis of variance and Chi-square tests. RBC folate levels were used as quartile variables (Q1: <475.5 nmol/L; Q2: 457.5-579.8 nmol/L, Q3: 579.8-747.5 nmol/L, Q4: >747.5 nmol/L) to explore the relationship with LMMS. Conditional logistic regression analysis was performed to estimate odds ratios (ORs) and 95% CIs. Covariates included age, sex, race, education levels, marital status, family poverty levels, alcohol consumption, smoking status, activity, waist circumference, vitamin B12, homocysteine, hypertension, diabetes, and hyperlipidemia. In addition, this study analyzed the interaction between RBC folate and age and gender. The restricted cubic spline model was used for the correlation analysis. The covariates included in the analysis of the relationship between RBC folate and ASM using the restricted cubic spline method are the same covariates used in Model 3. A two-tailed $p < 0.05$ was considered significant. All analyses were performed using R version 4.2.1.

Data availability

The datasets analysed during the current study are available in the NHANES repository [https://www.cdc.gov/nchs/nhanes/index.htm].

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Author contributions

L.Z. and Y.P. designed the current study; L.Z. performed data cleaning and analysis and wrote the manuscript; Y.P. contributed to data cleaning and interpretation; H.J. critically revised and edited the manuscript for important intellectual content; and all authors reviewed and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This data was retrieved from the public database of the National Health and Nutrition Examination Survey. The ethics approval was granted by the National Center for Health Statistics Ethics Review Committee. All procedures were carried out by relevant guidelines and regulations (Declaration of Helsinki). All individuals provided written informed consent before participating in the study.

Additional information

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