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B-vitamins and metabolic syndrome in Mesoamerican children and their adult parents

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Abstract

Objective: To examine the associations between vitamins of the methionine-homocysteine (Hcys) cycle (B_6, B_{12} and folate) and Hcys with metabolic syndrome (MetS) among Mesoamerican children and their adult parents.

Design: We conducted a cross-sectional study. Exposures were plasma vitamins B_6 and B_{12} concentrations, erythrocyte folate and plasma Hcys. In children, the outcome was a continuous metabolic risk score calculated through sex- and age standardisation of waist circumference, the homoeostatic model assessment for insulin resistance, mean arterial pressure (MAP), serum HDL-cholesterol and serum TAG. In parents, the outcome was the prevalence of MetS according to the Adult Treatment Panel III Criteria. We estimated mean differences in the metabolic risk score and prevalence ratios of MetS between quartiles of the exposures using multivariable-adjusted linear and Poisson regression models, respectively.

Setting: Capital cities of Belize, Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica and Chiapas State in Mexico.

Participants: In total, 237 school-aged children and 524 parents.

Results: Among children, vitamin B_{12} was inversely associated with the metabolic risk score (quartiles 4–1 adjusted difference = –0·13; 95 % CI: –0·21, –0·04; $P_{trend} = 0{\cdot}008$) through MAP, HDL-cholesterol and TAG. In contrast, folate was positively associated with the metabolic risk score (quartiles 4–1 adjusted difference = 0·11; 95 % CI: 0·01, 0·20; $P_{trend} = 0{\cdot}02$). In adults, vitamin B_6 was inversely associated with MetS prevalence, whereas vitamin B_{12} and folate were positively related to this outcome.

Conclusions: Vitamins of the methionine-Hcys cycle are associated with MetS in different directions. The associations differ between children and adults.

Keywords
Metabolic syndrome
Vitamin B_6
Vitamin B_{12}
Erythrocyte folate
Homocysteine

CVD is the leading cause of death worldwide[1]. The metabolic syndrome (MetS) is a cluster of independent risk factors for CVD, type 2 diabetes and mortality that includes abdominal obesity, impaired glucose tolerance, hypertension, elevated TAG and reduced HDL-cholesterol levels[2]. The prevalence of MetS is on the rise in many world regions; in Latin America, it ranges from 19 to 43 %[3,4] and is comparable with estimates from Europe[5] and the USA[6]. MetS components can become apparent from childhood[7] and are highly prevalent in children and adolescents from various Latin American settings[3,8–10] due, in part, to the growing childhood obesity epidemic in this region[11]. For example, at least one in every five children aged 6–12 years from the Chilean Araucania region had insulin resistance[8], and one-third of normal-weight Mexican children of the same age group had > 1 MetS component[10]. These early metabolic abnormalities track into adulthood,[12] increasing risk for CVD. Because MetS is mostly preventable, identifying potentially modifiable risk factors is an important research priority.

Vitamins B_6, B_9 (folate) and B_{12} are cofactors in the methionine–homocysteine (Hcys) cycle which is essential in the synthesis of methyl donors. Methyl donors play critical roles in multiple physiologic pathways including DNA methylation and lipid and protein metabolism. B-vitamin deficiencies could impair the synthesis of methyl groups and induce hyperhomocysteinaemia (H-Hcys), which increases the risk of atherosclerosis and CVD[13]. The

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potential role of these vitamins in MetS is less certain. In adults, vitamin B_6 serostatus[14] and intake[15] and vitamin B_{12}[16–18] and folate[16] biomarkers and supplementation[19] have been inversely related to MetS, whereas H-Hcys has been positively associated with this outcome[20,21]. In children, the evidence is scant. Vitamin B_{12} serostatus was inversely associated with MetS[22] and obesity[22,23] in cross-sectional studies of Turkish and Israeli school-aged children and adolescents. Maternal folate supplementation[24] and serostatus[25,26] were inversely associated with MetS[24], blood pressure[26] and BMI-for-age *Z* score[25] at ages 6–9 years in longitudinal studies from Nepal and the USA. A cross-sectional study of Czech adolescents found an inverse association of serum folate with hypertension[27], whereas H-Hcys was positively related to MetS prevalence in Nepalese school-aged children[28].

Because the prevalence of B-vitamin deficiencies is high in Latin American children[29] and interventions during childhood may modulate cardiovascular risk in the long term[30], there is a need to clarify the role of the B-vitamins and Hcys on MetS in this understudied population. This study aimed to examine the associations of vitamins B_6, B_9 and B_{12} biomarkers and Hcys with MetS and its components among school-aged Mesoamerican children and their parents. The family design offered an opportunity to account for measured parental confounders in the estimation of associations among children.

Methods

Study population

The Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMeS) Study was a cross-sectional investigation on nutrition and cardiovascular health conducted in the capital cities of Belize, Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica and Chiapas State in Mexico. Details of the NiMeCoMeS study have been described elsewhere[3]. In brief, we recruited a convenience sample of 267 family groups, a number determined by the availability of funding, each consisting of a school-aged child and their two parents from public primary schools in periurban areas of the cities, using convenience sampling. Eligibility criteria included child's age between 7 and 12 years, living with both biological parents, not being pregnant or having a pregnant mother and not having a sibling already recruited into the study. We chose only few eligibility criteria to enhance generalisability of the findings. The study protocol and procedures were approved by the Institutional Review Boards of collaborating institutions in each country and by the University of Michigan Health and Behavioral Sciences Institutional Review Boards. Written parental informed consent and children's assent to participate were obtained before enrollment.

Data collection

Data collection took place at a single home or clinic visit after a minimum 6-h fasting. At this visit, participants responded to a questionnaire on socio-demographic characteristics including age, education level, household assets and income, home ownership and past and current health status. The mothers answered the Latin American and Caribbean Food Security Scale (ELCSA)[31], a 15 yes/no item survey about food security experiences during the past 3 months; seven questions only concern households with children. Each affirmative response corresponded to one point and a household's food insecurity status was classified as: none (sum = 0), mild (sum = 1–3 or 1–5 in households without or with children, respectively), moderate (sum = 4–6 or 6–10) or severe (sum = 7–8 or 11–15). Research assistants administered a 97-item semiquantitative FFQ separately to mothers, fathers and children to estimate average intake during the past 12 months. The FFQ was based on a previously validated instrument developed for Costa Rican adults[32]. The FFQ characteristics have been detailed elsewhere[33]. In brief, items included major sources of energy and micronutrients in food groups such as fruits, vegetables, animal protein (dairy, eggs and meat), carbohydrates (breads, flours and cereals), beverages (dairy, sugar-sweetened beverages, coffee and alcohol), fast foods, desserts, sauces and dressings, cooking fats and oils and dietary supplements. For each item, research assistants described reference portion sizes in natural units or standard measures for commonly consumed servings among this population. There were nine frequency of intake options: ≥ 6 times/d, 4–5 times/d, 2–3 times/d, 1 time/d, 5–6 times/week, 2–4 times/week, 1 time/week, 1–3 times/month or < 1 time/month. Researchers measured height, weight and waist circumference with the use of standardised methods and calibrated instruments. Waist circumference was measured at the end of an unforced exhalation to the nearest millimeter, at the midpoint between the lower edge of the ribcage and the iliac crest in adults and above the uppermost lateral border of the right ilium in children. All anthropometric measures were obtained in triplicate, and the median of the three values was used[34]. Blood pressure was measured while seated, using Omron HEM-712C digital blood pressure monitors (Omron Healthcare, Inc.). Three measurements were taken, separated by at least 1 min, and the average of the second and third measures was the value of blood pressure used. At the end of the visit, phlebotomists obtained 7·5 ml of blood through antecubital venipuncture. The median (interquartile range) estimated fasting time at the time of sample collection was 12·9 (12·0–14·0) h. Samples were placed in refrigerated containers and transported on the day of collection to each country's collaborating laboratories where the serum, plasma and RBC were separated, aliquoted and cryostored at −20 °C. Stored samples from all countries were transported to the

Institute of Nutrition of Central America and Panama in Guatemala City.

Laboratory methods

Quantification of insulin, glucose, lipids, vitamin B_{12}, erythrocyte folate and Hcys was conducted at Institute of Nutrition of Central America and Panama. Plasma insulin, vitamin B_{12} and Hcys were measured using a chemiluminescent immunoassay on an Immulite 1000 system (Siemens Healthcare Diagnostics Products GmbH). Plasma glucose and serum lipids (total and HDL-cholesterol and TAG) were quantified on an automated chemistry analyser (Cobas c111 system; Roche Diagnostics). Erythrocyte folate was measured in RBC lysates using competitive immunoassays on an Immulite 2000 system (Siemens Healthcare Diagnostics Products GmbH). Vitamin B_6 biologically active metabolite, pyridoxal 5' phosphate, was measured in plasma using Liquid Chromatography–MS on a Thermo Fisher/Cohesive Technologies Aria TLX Series HTLC System at the Mayo Medical Laboratories.

Definition of outcomes

Children

Because the MetS is undefined in children < 10 years of age, we calculated a metabolic risk score using MetS components that correspond to the definition in adults: waist circumference, the homeostatic model assessment for insulin resistance[35], mean arterial pressure (MAP), serum HDL-cholesterol and serum TAG. MAP was calculated as ((2 × diastolic blood pressure) + systolic blood pressure)/3. The score was created by regressing each log-transformed component on sex and log-transformed age using linear regression models to obtain standardised regression residuals. We then averaged the residuals of the components with the negative of HDL to create the score. Higher scores indicate worse metabolic profile.

Adults

The presence of MetS was defined according to the Adult Treatment Panel III criteria[2] as having any three of the following: (1) waist circumference ≥ 102 cm in men and ≥ 88 cm in women; (2) fasting blood glucose ≥ 100 mg/dl; (3) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or treatment with an antihypertensive drug; (4) serum HDL-cholesterol < 40 mg/dl in men and < 50 mg/dl in women or drug treatment for low HDL-cholesterol and (5) serum TAG ≥ 150 mg/dl or drug treatment for elevated TAG.

Definition of exposures

Exposures were plasma concentrations of PLP and vitamin B_{12}, erythrocyte folate and plasma Hcys. Prevalence of B-vitamin deficiencies according to conventional cutpoints was low; thus, biomarker concentrations were categorised into quartiles.

Covariates

Children

Height-for-age and BMI-for-age *Z* scores were calculated using the WHO reference[36]. Parental height was categorised into quartiles and parental BMI according to the WHO classification. Household education was the maximum number of years of schooling achieved by either parent. Household food insecurity was categorised by the number of affirmative responses in the ELCSA survey. The number of household assets was the sum of car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, colour television, sound set, computer or Internet. Maternal parity was categorised as 1, 2, 3 or ≥ 4 births. We also considered as covariates food sources that may be predictors of the outcome. Using information collected with the FFQ, we created five food intake frequency groups (dairy, meat, fish, green leafy vegetables and fortified foods) by adding intake frequency weights of individual foods corresponding to each group. Total energy intake was estimated by multiplying the intake frequency of each food by the energy contents of the specific food portion using values from the USDA's Standard Reference food composition database.

Adults

Height, BMI, education level, home ownership, number of household assets and household food security were categorised as shown in Supplemental Table 3. Parental smoking was categorised as never, past or current. Household income was categorised into country-specific quartiles. Dietary covariates were defined as described in children.

Data analysis

The final analytic sample comprised 237 children and 524 adult parents who had information on both the exposures and MetS.

Children

In bivariate analysis, we compared the distribution of MetS and MetS components by quartiles of exposures using means and SD. We estimated tests for trend by introducing a variable representing the median value of each quartile as a continuous predictor in a linear regression model with MetS or each MetS component as the outcome. In multivariable analyses, we estimated adjusted differences with 95 % CI in mean metabolic scores between quartiles of exposures using linear regression models with robust variances. Adjustment covariates were independent predictors of the outcome including height-for-age *Z* score, maternal height, parental education, household food security, number of household assets, country of origin, habitual intake

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of vitamin food sources and total energy intake. Because the MetS is standardised for the age and sex distributions of the study population, the inclusion of these variables in multivariable models is unnecessary. Estimates for each vitamin were adjusted for the others but not for Hcys as this could be an intermediate variable. Estimates for Hcys were further adjusted for all vitamins. Models for each component, except waist circumference, were further adjusted for the child's BMI-for-age *Z* score.

Adults

In bivariate analysis, we compared unadjusted MetS prevalence by quartiles of the exposures. We conducted tests for linear trend by introducing a variable representing the median value of each exposure quartile as a continuous predictor into a generalised estimating equations (GEE) model with the Poisson distribution. In multivariable analysis, we estimated prevalence ratios with 95 % CI between quartiles of the exposures from GEE models using an analogous approach to adjustment as described for children. Adjustment variables were independent predictors of the outcome that could be related to the exposures but not be their consequence. Robust variances were specified in all models to account for within-household correlations.

All analyses were conducted using Statistical Analysis Software version 9·4 (SAS Institute).

Results

Children

Mean age (± SD) of children was 9·9 (1·6) years; 52·3 % were girls. Thirty-five percent of children were overweight (BMI *Z* > 1 SD) and 19·4 % were obese (BMI *Z* > 2 SD). The mean (± SD) metabolic risk score was 0·00 ± 0·22. Deficiency prevalence of vitamin B_6 (< 20 nmol/l), vitamin B_{12} (< 148 pmol/l) and folate (< 305 nmol/l)[37] was 0·9 %, 4·7 % and 2·1 %, respectively, whereas 4·6 % had H-Hcys (Hcys > 95th percentile for age)[38].

Correlates of B-vitamin status biomarkers

Plasma PLP was inversely associated with paternal height and food insecurity and positively related to parental education, number of household assets and household income (see online supplementary material, Supplemental Table 1). Plasma vitamin B_{12} was positively related to female sex, parental height and education and number of household assets, and inversely to maternal parity and household food insecurity. It varied significantly between countries. Erythrocyte folate was inversely related to the child's height-for-age *Z* score and household food insecurity. Hcys was positively associated with male sex, child's age, maternal height, BMI and parity, and inversely with parental education. It differed significantly by country. Plasma PLP was positively related to dairy intake (see online supplementary material, Supplemental Table 2); plasma vitamin B_{12} was associated with dairy, red meat and fish intake, and erythrocyte folate was positively related to dairy, green leafy vegetables and fortified foods intake.

B-vitamin biomarkers and metabolic score

PLP was not significantly associated with the metabolic score (Table 1) or its components (see online supplementary material, Supplemental Table 3). Plasma vitamin B_{12} was inversely related to the metabolic score (Table 1), compared with children at the lowest vitamin B_{12} quartile, those at the highest quartile had an adjusted 0·13 units lower metabolic score ($P_{trend} = 0·008$). The metabolic score components associated with plasma vitamin B_{12} included MAP, serum HDL-cholesterol and TAG (see online supplementary material, Supplemental Table 3). Erythrocyte folate was positively associated with the metabolic risk score (Table 1); the mean adjusted difference between quartiles 4 and 1 was 0·11 units ($P_{trend} = 0·02$). Folate was positively related to waist circumference and MAP and inversely to HDL-cholesterol (see online supplementary material, Supplemental Table 3). Plasma Hcys was not associated with the metabolic score overall but was positively related to the waist circumference and arterial pressure components.

Adults

Mean age (± SD) of participants was 38·7 ± 7·5 years and 50·4 % were women; 34·2 % had obesity (BMI > 30 kg/m^2). The prevalence of MetS was 36·6 %. Deficiency prevalence of vitamin B_6 (< 20 nmol/), vitamin B_{12} (< 148 pmol/l) and folate (< 305 nmol/l)[37] was 4·7 %, 10·4 % and 8·4 %, respectively, whereas 16·4 % had H-Hcys (Hcys > 14 μmol/l)[39].

Correlates of B-vitamin biomarkers

Plasma PLP was inversely associated with female sex and BMI and positively with home ownership, number of assets and income (see online supplementary material, Supplemental Table 4). Vitamin B_{12} was positively related to the number of household assets and income; it was highest in Mexico and lowest in Honduras and the Dominican Republic. Erythrocyte folate was inversely associated with male sex and height and positively to home ownership and food security. It was highest in Mexico and lowest in the Dominican Republic. Hcys was positively associated with male sex and smoking status and was highest in the Dominican Republic and lowest in Mexico. Vitamin B_{12} was positively associated with red meat, fish and fortified foods intake (see online supplementary material, Supplemental Table 5), whereas erythrocyte folate was positively associated with intake of green leafy vegetables and fortified foods.

Table 1 Metabolic syndrome score* according to plasma vitamins B_6 and B_{12}, erythrocyte folate and plasma homocysteine concentrations among school-aged children from Mesoamerica

Biomarker quartile (median)	*n*†	Mean	SD	Unadjusted difference 95 % CI		Adjusted difference 95 % CI Model 1‡		Adjusted difference 95 % CI Model 2§	
Plasma PLP (nmol/l)									
Q1 (36)	50	0·02	0·21	Reference		Reference		Reference	
Q2 (49)	52	–0·04	0·20	–0·05	–0·13, 0·02	–0·05	–0·12, 0·02	–0·07	–0·14, 0·00
Q3 (65)	67	0·03	0·24	0·01	–0·07, 0·09	0·02	–0·05, 0·09	0·00	–0·07, 0·07
Q4 (107)	60	0·01	0·24	0·00	–0·09, 0·08	–0·01	–0·09, 0·06	–0·02	–0·10, 0·06
P_{trend}‖				0·69		0·88		0·87	
Plasma vitamin B_{12} (pmol/l)									
Q1 (191)	58	0·10	0·24	Reference		Reference		Reference	
Q2 (297)	58	–0·02	0·23	–0·12	–0·20, –0·03	–0·10	–0·18, –0·02	–0·07	–0·15, 0·01
Q3 (404)	60	–0·03	0·21	–0·13	–0·21, –0·05	–0·14	–0·22, –0·06	–0·12	–0·20, –0·05
Q4 (719)	59	–0·03	0·20	–0·12	–0·20, –0·04	–0·14	–0·22, –0·05	–0·13	–0·21, –0·04
P_{trend}				0·02		0·01		0·008	
Erythrocyte folate (nmol/l)									
Q1 (531)	58	0·02	0·23	Reference		Reference		Reference	
Q2 (735)	59	–0·01	0·22	–0·03	–0·11, 0·06	–0·03	–0·11, 0·04	0·01	–0·07, 0·08
Q3 (858)	59	–0·02	0·19	–0·03	–0·11, 0·04	0·00	–0·08, 0·07	0·04	–0·02, 0·11
Q4 (1169)	59	0·03	0·26	0·01	–0·08, 0·10	0·04	–0·05, 0·14	0·11	0·01, 0·20
P_{trend}				0·75		0·21		0·02	
Plasma Hcys (μmol/l)									
Q1 (3·8)	57	0·00	0·26	Reference		Reference		Reference	
Q2 (5·5)	60	0·02	0·23	0·02	–0·07, 0·11	0·05	–0·03, 0·13	0·02	–0·06, 0·10
Q3 (7·5)	59	–0·02	0·19	–0·03	–0·11, 0·05	0·05	–0·03, 0·14	0·03	–0·05, 0·12
Q4 (10·5)	59	0·02	0·23	0·01	–0·08, 0·10	0·07	–0·02, 0·17	0·06	–0·04, 0·16
P_{trend}				0·99		0·18		0·22	

PLP, pyridoxal phosphate; Hcys, homocysteine.
*Component scores (waist circumference, homoeostatic model assessment for insulin resistance, mean arterial pressure, serum HDL-cholesterol and serum TAG) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardised residuals. After the HDL-cholesterol score was multiplied by −1, the overall score was calculated as the average of the five component scores.
†*n* may be less than 237 due to missing values.
‡From linear regression models adjusted for height-for-age Z score, maternal height, highest parental education level, household food security, number of household assets and country of origin. Estimates for the vitamins were adjusted for each other but not for Hcys; estimates for Hcys were adjusted for all vitamins.
§Adjusted for covariates in model 1 plus log-transformed total energy intake and intake of dairy, meat, fish and green leafy vegetables.
‖From linear regression models with the metabolic score as the outcome and a variable representing medians of ordinal categories of the predictor introduced as a continuous covariate. Robust estimates of variance were specified in all models.

B-vitamin biomarkers and metabolic syndrome

Plasma PLP was inversely associated with MetS (Table 2); adults in the highest quartile of plasma PLP had a 33 % lower adjusted prevalence of MetS compared with adults in the lowest quartile ($P_{trend} = 0·02$). Of the MetS components, only abdominal obesity was related to PLP (see online supplementary material, Supplemental Table 6). Vitamin B_{12} was positively associated with MetS in a non-linear fashion (Table 2). Compared with parents at the lowest quartile of plasma vitamin B_{12}, those at the highest three quartiles combined had a 49 % higher prevalence of MetS (95 % CI: 7 %, 108 %; $P = 0·02$). Vitamin B_{12} was positively associated with high fasting blood glucose and high blood pressure (see online supplementary material, Supplemental Table 6). Erythrocyte folate was positively associated with MetS prevalence (Table 2); compared with adults at the lowest quartile, those in the highest had twice the prevalence of MetS ($P_{trend} = 0·003$). Erythrocyte folate was not related to any of the components (see online supplementary material, Supplemental Table 6). Hcys was not significantly associated with MetS (Table 2) or its components (see online supplementary material, Supplemental Table 6).

Discussion

In this cross-sectional study, a metabolic risk score in children was inversely associated with plasma vitamin B_{12} and positively related to erythrocyte folate. In adults, plasma PLP was inversely associated with MetS prevalence, whereas plasma vitamin B_{12} and erythrocyte folate were positively related to this outcome.

Vitamin B_6 was not associated with the metabolic risk score in children; however, we found an inverse association of plasma PLP with MetS prevalence and abdominal obesity in adults. These results are consistent with previous cross-sectional studies in Nigeria[14] and Japan[15]. In a cross-sectional study in Norway, plasma PLP was lower in adults with morbid obesity than in healthy weight controls[40]. Low plasma PLP has been associated with increased levels of inflammatory and oxidative stress biomarkers[41] which might contribute to explain an inverse association with MetS since inflammation and oxidative stress are considered underlying aetiologic factors[2].

Vitamin B_{12} was inversely associated with the metabolic risk score in children, possibly through blood pressure and

Table 2 Metabolic syndrome* according to plasma vitamins B_6 and B_{12}, erythrocyte folate and plasma homocysteine concentrations among adults from Mesoamerica

					Adjusted PR 95% CI			
Biomarker quartile (median)	*n*†	Prevalence %	Unadjusted PR	95 % CI	Model 1‡		Model 2§	
Plasma PLP (nmol/l)								
Q1 (24)	110	44·6	Reference		Reference		Reference	
Q2 (36)	119	44·5	1·00	0·74, 1·36	0·97	0·71, 1·34	0·95	0·69, 1·31
Q3 (53)	142	28·9	0·65	0·46, 0·91	0·61	0·43, 0·86	0·59	0·41, 0·83
Q4 (89)	141	30·5	0·68	0·49, 0·95	0·66	0·46, 0·93	0·67	0·47, 0·95
P_{trend}‖			0·009		0·01		0·02	
Plasma vitamin B_{12} (pmol/l)								
Q1 (155)	130	25·4	Reference		Reference		Reference	
Q2 (232)	130	43·9	1·73	1·20, 2·49	1·61	1·12, 2·31	1·67	1·14, 2·43
Q3 (345)	130	37·7	1·48	1·01, 2·18	1·40	0·96, 2·06	1·47	0·99, 2·17
Q4 (619)	130	39·2	1·55	1·06, 2·24	1·42	0·97, 2·10	1·50	1·00, 2·26
P_{trend}			0·15		0·39		0·28	
Erythrocyte folate (nmol/l)								
Q1 (413)	130	32·3	Reference		Reference		Reference	
Q2 (699)	130	30·0	0·93	0·65, 1·34	1·19	0·77, 1·84	1·29	0·83, 2·02
Q3 (891)	130	38·5	1·19	0·84, 1·68	1·51	0·96, 2·37	1·62	1·02, 2·57
Q4 (1218)	132	45·5	1·41	1·02, 1·94	1·83	1·18, 2·84	1·97	1·22, 3·17
P_{trend}			0·02		0·003		0·003	
Plasma Hcys (μmol/l)								
Q1 (5·2)	129	42·6	Reference		Reference		Reference	
Q2 (7·8)	131	32·8	0·77	0·56, 1·05	0·71	0·51, 0·98	0·71	0·50, 1·00
Q3 (10·2)	130	36·9	0·87	0·65, 1·16	0·82	0·58, 1·14	0·75	0·52, 1·07
Q4 (15·2)	130	33·9	0·79	0·57, 1·10	0·78	0·54, 1·12	0·72	0·48, 1·09
P_{trend}			0·30		0·39		0·24	

PR, prevalence ratios; PLP, pyridoxal phosphate; Hcys, homocysteine.
*According to the National Cholesterol Education Program's Adult Treatment Panel III definition as having three or more of the following components: abdominal obesity, high fasting glucose, high blood pressure, low serum HDL-cholesterol and high serum TAG.
†*n* may be less than 524 due to missing values.
‡From Poisson regression models adjusted for age, sex, smoking and education level, number of household assets, household food security and country of origin. Estimates for the vitamins were adjusted for each other but not for Hcys; Hcys estimates were adjusted for all vitamins.
§Adjusted for covariates in model 1 plus log-transformed total energy intake and intake of meat, fish, fortified foods and green leafy vegetables.
‖From Poisson regression models with metabolic syndrome as the outcome and a variable representing medians of ordinal categories of the predictor introduced as a continuous covariate. Robust estimates of variance were specified in all models to account for clustering by family membership.

serum lipids. Previous studies of European adolescents[42] and Japanese preschool children[43] found inverse associations between vitamin B_{12} serostatus and blood pressure. Also, cross-sectional investigations of Turkish[22] and Israeli[44] children found inverse associations between vitamin B_{12} serostatus and body weight. Our findings in children are in contrast to those in adults since vitamin B_{12} was positively related to MetS prevalence, possibly through high fasting blood glucose and high blood pressure. This finding was also inconsistent with prior cross-sectional studies that showed inverse associations between vitamin B_{12} and MetS[16–18]. Similarly, a randomised trial[19], a Mendelian randomisation study[45] and a case–control investigation[16] found inverse associations between vitamin B_{12} and measures of insulin resistance in adults, whereas a cross-sectional study found an inverse association of serum vitamin B_{12} with hypertension[46]. There are some possible explanations for the discrepancies. Although we were able to adjust for foods like red and processed meats that could also be associated with increased risk of cardiometabolic disease, there is a possibility of residual confounding which could explain the positive association of vitamin B_{12} with MetS. The discrepancy between our findings in children compared with adults could be explained by the different measures used to assess MetS, and direct comparisons may not be accurate. Additionally, children had higher dairy consumption compared with adults, and there is evidence of an inverse association between dairy consumption and MetS in other populations[47].

Erythrocyte folate was positively associated with the metabolic risk score in children, probably trough increased waist circumference and blood pressure, and lowered HDL-cholesterol. Similarly, erythrocyte folate was positively associated with MetS prevalence in adults. Direct evaluations of the role of folate on MetS in children are scarce, although some studies had reported inverse associations between maternal folate and MetS or some of its components. In a prenatal supplementation study conducted in rural Nepal, offspring from mothers who had received antenatal folic acid with vitamin A supplements had a lower risk of MetS at ages 6–8 years compared with vitamin A alone[24]. Likewise, results from the Boston Birth Cohort suggested an L-shaped association of maternal plasma folate levels at the end of pregnancy with childhood overweight or obesity at ages 3–9 years; the highest risk

was among children from mothers with obesity and low folate compared with children from mothers with normal weight and folate[25]. In the same cohort, there was also an inverse association of maternal folate levels with systolic blood pressure among children from mothers with cardiometabolic risk factors[26]. Two cross-sectional investigations in children found an inverse association between folate and blood pressure[27,43], but another suggested a positive relation between erythrocyte folate and systolic blood pressure among adolescent girls[42]. Our findings on both children and adults seem to contradict existing evidence on folate's relation with MetS and its components. However, some previous evidence suggests a potential adverse effect of folate on MetS components; notably, cross-sectional evaluations of adults with obesity found a positive association between erythrocyte folate and homoeostatic model assessment for insulin resistance, especially among participants with low vitamin B_{12} status[48]. One proposed mechanism to explain a potential deleterious effect of folate on metabolism is the methyl-pool depletion caused by folic acid, the standard form of folate used for fortification[49], which is mandatory for wheat and maize flour in Mesoamerica[50]. In our population, fortified foods intake was significantly associated with erythrocyte folate and folate concentrations were generally high. Whether folic acid from fortified foods could have a deleterious role on metabolic health warrants further investigation.

Hcys was positively associated with waist circumference and blood pressure in children, but there was no association with MetS or any of its components in adults. Previous cross-sectional studies in school-aged children found that H-Hcys was positively associated with MetS, high blood pressure[28] and waist circumference > 90th percentile[51]. Nonetheless, one cohort study found no association between serum Hcys with MetS components or insulin sensitivity among healthy men from London[52]. A supplementation trial suggested that, while folate and vitamin B_{12} might reduce Hcys, this effect may not extend to cardiometabolic outcomes[53].

Our study has several strengths. We used valid biomarkers of intake, which precludes recall bias in the ascertainment of exposure compared with methods that rely on memory. The prevalence of B-vitamin deficiencies was within the range of other studies in comparable populations, which enhances external validity. For example, 4·7 % of children in this study were vitamin B_{12} deficient, compared with 2·6 % in Mexico in 2012[54] and 2·8 % in Colombia in 2010[55]; and only 2·1 % of children were folate deficient, consistent with reports from Mexico[54] and Guatemala[56], where prevalence was zero. Among adults, the prevalence of vitamin B_{12} deficiency, 10·4 %, was also within range of reports among women from Costa Rica (4·8 % in 2008)[57], Mexico (8·5 % in 2012)[58], Argentina (11·9 % in 2007)[59] and Colombia (18·5 % in 2010)[55]. Prevalence of folate deficiency in adults, 8·4 %, was slightly higher than that reported among women from Mexico (1·9 % in 2012)[58], Costa Rica (3·8 % in 2008)[57], Guatemala (3·1 % in 2008)[60] and Argentina (1·3 % in 2007)[57]. No previous studies in the region have reported on the prevalence of vitamin B_6 deficiency according to PLP concentrations; the figure among adults, 4·7 %, was lower than that reported in the USA for people ≥ 9 years old, 11 %[61]. The prevalence of MetS in adults, 37 %, was close to that in Mexico, 41 %[62], but lower than that in Guatemala, 64 %[63]. The family design involving children with their parents allowed adjustment of the associations in children for parental confounders. Evaluating the associations of B-vitamins with MetS in Mesoamerican children is a novel endeavour in an understudied population.

Some limitations are also noteworthy. The cross-sectional design limits the making of causal inference. Comparisons of the absolute value of the children's metabolic risk score with other populations are not possible because it was standardised for the population-specific sex and age distributions. Since the study sample was not representative of the entire Mesoamerican population, generalisability of the findings is limited. Finally, due to small sample size, country-specific analyses were not possible.

In conclusion, vitamin B_{12} was inversely associated with a metabolic risk score in Mesoamerican children but was positively associated with MetS prevalence in adults. Unexpectedly, erythrocyte folate was positively associated with MetS and some of its components in both children and adults. The potential role of mandatory folate fortification on these findings deserves careful consideration in future studies.

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Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980020003936

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