Micronutrients and Metabolic Syndrome in Children and Adults

by

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Dedicatoria

Esta tesis está dedicada a la memoria de mi papá. Gracias por enseñarme que “el esfuerzo que hagas hoy será el descanso de mañana”.

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Abstract

Background: Metabolic syndrome (MetS) is a cluster of independent risk factors for cardiovascular disease and its prevalence is increasing worldwide, including in the region of Latin America. Independent predictors of MetS include age, sex, socioeconomic characteristics, and diet. Micronutrients play vital roles in several biological pathways that could be associated with MetS. However, the associations between micronutrient status and MetS remains controversial, especially in children.

Objectives: To evaluate the association between the B-vitamins and homocysteine (Hcys) (aim 1), sodium and iodine (aim 2), and the trace minerals zinc, manganese, and copper (aim 3), with MetS in children and adults (aim 4) from Mesoamerica.

Methods: We conducted a cross-sectional study among 237 children and 524 parents from the capitals of Belize, Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Chiapas State in Mexico. Sociodemographic and health status information was collected through questionnaires. Anthropometric measures were done according to standardized protocols. Exposures for aim 1 were plasma concentrations of vitamin B6, B12, Hcys, and erythrocyte folate categorized as quartiles of the population distribution. For aim 2, urinary sodium and iodine were obtained from 24h urine samples and categorized as recommended sodium intake and excessive iodine intake according to the World Health Organization (WHO) definitions. For aim 3, the exposures were plasma concentrations of zinc, manganese, and cooper categorized as quartiles. For adults, the exposures were categorized as in children. The outcome in children was a continuous metabolic risk score calculated through sex-

and age-standardization of waist circumference, the homeostatic model assessment for insulin resistance (HOMA-IR), mean arterial pressure (MAP), serum high-density lipoprotein cholesterol (HDL-C), and serum triglycerides. In parents, the outcome was the prevalence of MetS according to the Adult Treatment Panel (ATP) III criteria[1]. We estimated mean differences in the metabolic risk score and prevalence ratios of MetS between the exposure categories using multivariable-adjusted linear and Poisson regression models, respectively.

Results: We found an inverse association between vitamin B6 and MetS, only in adults. Vitamin B12 and MetS were inversely associated in children, whereas they were positively associated in adults. Folate had a positive relation with MetS in both children and adults. Hcys was not associated with MetS in either children or adults. Exceeding the recommended sodium intake was positively associated with MetS and hypertension only in adults. Similarly, excessive iodine intake was positively associated with MetS in both children and adults. Plasma zinc was not associated with MetS in either children or adults. In contrast, we found an inverse association between plasma manganese with MetS only in children. Plasma copper had a positive association between the waist circumference score and abdominal obesity in children and adults, respectively.

Conclusions and significance: Micronutrient status biomarkers are associated with MetS and the associations differs between children and adults from the Mesoamerican region. Further evaluation of these associations is warranted along with closer monitoring of food fortification policies. Efforts to adhere to the recommended nutritional guidelines can help decrease the burden of cardiometabolic diseases that is affecting the region.

Chapter 1 . Introduction

Overview

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors composed of abdominal obesity, insulin resistance, hypertension, and atherogenic dyslipidemia (hypertriglyceridemia and low high-density lipoprotein-cholesterol (HDL-C))[1]. The presence of metabolic risk factors in childhood and adulthood is associated with an increased risk for cardiovascular diseases[2,3]. Moreover, cardiovascular disease is one of the leading causes of death worldwide[4], and MetS prevalence in increasing worldwide with estimates in Latin America ranging from 19 to 43%[5,6]. For these reasons, it is important to investigate the burden and causes of MetS in children and adults from under-studied populations like those living in Mesoamerica.

The causes of MetS are not completely clear but increased visceral adiposity and increased inflammation are well recognized.[7,8] Diet is perhaps the most salient modifiable factor associated with MetS. Total caloric intake[8], sodium,[9–13] vitamins B6,[14,15] B9 (folate)[16–18] and B-12[16–19], and trace elements, particularly zinc[20,21], could be related to the development of MetS and its components, but little research has been conducted in children and it is not clear if these dietary factors operate the same way as in adults. In addition, there is little research from low and middle-income countries where the nutrition transition is causing rapid changes in the prevalence of risk factors for non-communicable diseases. This dissertation focuses on addressing the gap of knowledge regarding the influence of micronutrients on MetS, with a special focus on middle childhood and the region of Mesoamerica.

Specific aims

This work aims to evaluate the associations between different micronutrient status and MetS in children and their adult parents using cross-sectional data from nine countries in Mesoamerica.

Aim 1. To determine the relation between plasma levels of the B-vitamins (vitamins B6, B12, and folate) and homocysteine (Hcys) with MetS and its components among school-aged children from Mesoamerica. As a secondary aim, we plan to evaluate the association of the B-vitamins and Hcys with MetS and its components using joint regression models to consider the impact of assesing children and adults together.

Aim 2. To determine the association between urinary sodium and iodine concentrations with MetS among school-aged children from Mesoamerica. Additionally, we will also examine each MetS component as independent outcomes.

Aim 3. To determine the association between plasma concentrations of trace minerals zinc, manganese, and copper with MetS and its components among school-aged children from Mesoamerica.

Aim 4. To determine the association between plasma levels of the B-vitamins and Hcys, urinary sodium and iodine, and plasma concentrations of zinc, manganese, and copper with MetS and its components among adults from Mesoamerica.

Metabolic Syndrome

Definition

In children, there is no unique definition of MetS and many classifications have been proposed; most definitions use the criteria for adult MetS based on percentile cutoffs[22,23]. The most widely used categorical definition was developed in 2007 by the International Diabetes Federation (IDF) which recommends no diagnosis of MetS in children younger than 10 years, the use of modified adult criteria for children between 10 to 16 years, and the use of adults criteria for children >16 years[24]. Because of the lack of a universal definition, Eisenmann[22] proposed the use of a metabolic risk score for diagnosis of MetS in children and adolescents. The score performs better compared to a dichotomous definition because of the relatively low prevalence of MetS in youth populations[22] and for children younger than 10 years. The metabolic risk score is constructed using Z scores of the sample distribution of the metabolic risk factors used to define MetS in adults and can be standardized for age, sex, and ethnicity. A higher score indicates a worse metabolic profile[22].

For adults, the most widely used definition for diagnosis of MetS is based on the 2005 criteria published by a joint statement of the American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) Adult Treatment Panel (ATP) III. Diagnosis is done with any 3 of 5 of the following: 1) elevated waist circumference ≥102 cm (≥40 inches) in men and ≥88 cm (≥35 inches) in women; 2) elevated triglycerides ≥150 mg/dL (1.7 mmol/L) or use of drug treatment for elevated triglycerides; 3) reduced HDL-C <40 mg/dL (1.03 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women, or use of drug treatment for reduced HDL-C; 4) elevated blood pressure ≥130 mm Hg systolic blood pressure or ≥85 mm Hg diastolic blood

pressure, or use of antihypertensive drug treatment in a patient with a history of hypertension; and 5) elevated fasting glucose ≥100 mg/dL, or use of drug treatment for elevated glucose[1].

Importance

The prevalence of MetS is increasing worldwide and in Latin America, it ranges from 19 to 43%[5,6] which is comparable to the prevalence in Europe[25] and the United States[26]. The importance of evaluating MetS in younger populations is controversial because of the challenges to define it, but evidence shows that MetS in children using several categorical definitions and a metabolic score was predictive of adult MetS and type 2 diabetes mellitus[2,3]. Moreover, evidence also support tracking of MetS components from childhood to adulthood[3]. In children, it is important to evaluate MetS because it constitutes a window of opportunity to intervene earlier in life and prevent the increase burden of cardiovascular diseases that is expected to affect most low and middle-income countries[27].

Previous research indicated that MetS in adults is associated with 27% higher risk for all-cause mortality, 65% higher risk for cardiovascular disease and 100% higher risk for type 2 diabetes mellitus[28]. This highlights the role of MetS as a tool to identify people at increased risk for developing more serious diseases later in life. Moreover, treatment focused on MetS components results in improvement of the metabolic profile and consequently, a reduction in cardiovascular disease[29].

The potential role of diet on MetS

Increased caloric intake is usually related to weight gain and obesity. In addition to an overall increase in energy intake, the composition of diet is more relevant when assessing its effects on metabolism[30], especially in children[31]. In adults, macronutrient content of diet has been associated with MetS, specifically carbohydrate content[32,33]. On the contrary, for children

there is no association of diet macronutrient composition with MetS rather, carbohydrate content of diet was associated with obesity[34,35]. This highlights the importance of diet composition when evaluating risk factors for MetS.

One of the main gaps in the research of MetS in adults and specially in children is the micronutrient content of diet. Therefore, I plan to evaluate the association between specific micronutrients and MetS during childhood and adulthood in the understudied population of Mesoamerica.

Micronutrient status and fortification policies in Latin America

The micronutrients are required in small quantities for various metabolic functions, and either deficiency or excess status can have deleterious health effects. In Mesoamerica mandatory food fortification policies exist for vitamin A, iron, B-vitamins including folate and B12, iodine, and zinc[36]. Therefore, it is important to evaluate the role of the micronutrients on MetS.

Fortification policies in Mesoamerica

The oldest fortification policy established in the region was salt iodization for the prevention of iodine-deficiency disorders (IDD) during the 60s after the WHO recommended salt iodization in iodine-deficient regions. However, it was until the 1990 World Summit for Children that the goal of virtual elimination of IDD was set[37]. In addition, the sociopolitical crisis of the 70s and 80s weakened the regional public health infrastructure and related policies. This led to an initial effort to decrease IDD in the region, with subsequent reestablishment of the policy during the 90s in most of the countries (**Table 1**). Also, during the 90s, fortification of wheat and corn flour with iron, folic acid, thiamin, and riboflavin was established in most countries of the region. Mexico, Guatemala, Costa Rica, Nicaragua, and Panama are the only countries in the region that have mandatory fortification of corn flour and rice with vitamin B12 and zinc[36,38]. Mexico is the only country with mandatory fortification of milk that is part of a large-scale incentive-based welfare program applied by the Mexican federal government since 1997[39]. Nicaragua and Panama are the only countries with mandatory rice fortification with vitamin B6.

Table 1.1. Fortification policies in the region of Mesoamerica[36,38,39]

		Mexico	Guatemala	El Salvador	Honduras	Nicaragua	Costa Rica	Panama	Dominican Republic	Belize
Folic acid	Year[1]	1996	1992	1967	2003	2003	1958	1999	1992	1992
	Vehicle	Wheat and corn flour, and milk	Wheat and corn flour	Wheat and corn flour, pasta	Wheat flour	Wheat flour and rice	Wheat and corn flour, rice, and milk	Wheat flour and rice[2]	Wheat flour	Wheat flour
	Amount[3]	2 mg/50 μg	1.6-1.8 mg	1-1.8 mg	1.8 mg	1-1.8mg	1.3 -1.8 mg/ 40 μg	1.1-1.8 mg	1.8 mg	1.8 mg
Vitamin B12	Vehicle	Milk	Corn flour			Rice	Rice	Rice		
	Amount	0.7 μg	5.1 μg			0.01 mg	10 μg	0.01 mg		
Vitamin B6	Vehicle					Rice		Rice		
	Amount					4 mg		5.4 mg		
Iodine	Year[4]	1963, 1993	1955, 1992	1961, 1993	1960, 1993	1977	1941	1970, 1997	1985	2007
	Vehicle	Salt	Salt	Salt	Salt	Salt	Salt	Salt	Salt	Salt
	Amount	20-40 mg	20-60 mg	20-60 mg	10-15 mg	33-60 mg	30-60 mg	10-40 mg	30-100 mg	20-60 mg
Zinc	Vehicle	Wheat and corn flour, and milk	Corn flour			Rice	Rice	Rice		
	Amount	10-20 mg	33 mg			25 mg	19 mg	36.6 mg		

[1]Year the fortification policy was introduced as national law. It applies for all micronutrients unless otherwise noted.
[2]Rice fortification was established in Panama in 2009.
[3]Dose of micronutrient per kg of food. The amount of micronutrients in milk is in the order of μg.
[4]In Central America, salt iodization was first established in the 1950s. It was reestablished during the 90s.

B-vitamins and Hcys

Vitamin B6 deficiency is considered rare and present in high risk groups like the elderly and alcoholics, but prevalence from the US ranged from 5% in males using supplements to 32% in women not using supplements[40]. There are no current estimates from Latin America. Prevalence of vitamin B12 deficiency is variable with ranges from 2.9% in Costa Rica to 22.5% in Guatemala[41]. In Latin America, food fortification with vitamin B12 is not ubiquitous as folate fortification, but voluntary fortification of prepared drinks and breakfast cereal is also available[36]. Moreover, estimates from Latin America described a decrease in vitamin B12 deficiency but some countries like Argentina still have a high prevalence of 49%[42]. Folate deficiency prevalence has decrease significantly after implementation of food fortification programs. In Mesoamerica, the impact of fortification of wheat and corn flour reduced prevalence ranges from 2 to 80% in the pre-fortification era to current estimates of 0.6% to 3.8%[42]. Fortification in the US had a similar effect on folate deficiency with post-fortification prevalence ≤ 1%[43]. In general, and based on estimated from Latin America, in the Mesoamerican region, vitamin B6 deficiency prevalence estimates are scarce, vitamin B12 deficiency is still present, and folate deficiency is practically non-existent. Moreover, concerns about negative health effects of excessive folic acid exposure through fortified foods on cancer[44] and immune function[45] have been described.

Sodium and iodine

In 2010, global average sodium consumption was 3.95 g/day, nearly twice the recommended intake level of 2 g/day established by the World Health Organization (WHO)[46]. In Latin America, the average was around 3.3 g/day, which was lower than the global estimate but comparable to the intake of the US and Canada. available[46]. This indicates that worldwide,

consumption of sodium is too high, and that further information about the situation in Latin America is warranted. Iodine deficiency is associated with multiple disorders related to inadequate thyroid hormone production including cretinism, a form of developmental delay and mental retardation[47]. In 1950, the WHO recommended salt iodization in iodine-deficient regions, but it was until after revision of this policy during the 1990s that a substantial decline of iodine deficiency prevalence occurred. By 2013, excessive iodine intake started to appear in several countries including Brazil, Colombia, and Honduras, while mild deficiency was still prevalent in other countries like Guatemala[48]. Moreover, data from the micronutrient survey in the Dominican Republic found an 8% prevalence of iodine deficiency with several population subgroups with excessive iodine intake[49]. Salt iodization is considered a successful public health intervention for the reduction of iodine deficiency disorders, but current sodium consumption levels could be responsible for the appearance of excessive iodine intake in some regions of the world.

Trace minerals

Of the trace minerals evaluated, only zinc has established cutpoints for deficiency. Zinc deficiency is associated with several non-specific clinical manifestations like reduced growth, increased infection rates, and skin lesions[50]. In Latin America, zinc deficiency is considered a public health problem since all the reported prevalences are greater that 20%, with the highest estimates around 40% for Colombian children. Additionally, rural-indigenous populations are most affected by zinc deficiency[51].

Based on the information about the fortification policies in the Mesoamerican region, it is important to evaluate the associations between micronutrient status and MetS, since the potential for either deficiency or excess has been previously described.

Micronutrients and Metabolic Syndrome

B-vitamins and Homocysteine

Vitamins B6, B9 (folate), and B12 are part of the methionine-homocysteine cycle which is responsible for the creation of methyl donors used in one-carbon reactions and leads to the production of Hcys. In this cycle, folate and vitamin B12 are responsible for Hcys remethylation to methionine, whereas vitamin B6 acts as a cofactor for the conversion of homocysteine to cystathionine[52]. Overall, there is an inverse relation between the B-vitamins and Hcys. Additionally, hyperhomocysteinemia is considered and independent risk factor for the development of atherosclerotic cardiovascular disease[53], and it has been considered a component of MetS[53]. Due to their role in one carbon metabolism and their effect on Hcys concentrations, the B-vitamins could have an inverse association with MetS, whereas Hcys is expected to be positively associated.

Previous research in children supports an inverse association between folate and vitamin B12 with MetS. An antenatal supplementation study and a observational studies found inverse associations between folate with MetS[54] and systolic blood pressure[55]; and between maternal folate with childhood overweight or obesity[56], and childhood systolic blood pressure[57]. Vitamin B12 also presented similar inverse associations with MetS and BMI-for-age Z scores[58]. Notably, obese adolescents presented lower levels of vitamin B12 when compared to normal weight adolescents[59,60]. These evidence supports a beneficial role of the B-vitamins on MetS, but there are no studies conducted in Mesoamerican children. Moreover, evidence about the role of vitamin B6 and Hcys with MetS is still scant.

In adults, although previous evidence suggest an inverse association between levels of vitamins B6, B12, and folate with Hcys serum concentrations[16,18,61], several clinical trials have

failed to demonstrate a beneficial role of supplementation with these vitamins to reduce risk of adverse cardiovascular outcomes[62,63]. Despite this, evidence from a randomized trial in Italy supported a beneficial effect of folate and vitamin B12 supplementation on levels of Hcys, insulin and the homeostatic model assessment (HOMA) when compared to placebo[18]. Similarly, evidence from observational studies indicated an inverse association between vitamin B6[14], folate, and vitamin B12[17] with MetS. Moreover, inverse associations between folate and vitamin B12, and positive associations between Hcys with some of the MetS components were described[17,19]

As shown above, evaluating the relation between B-vitamins and Hcys with MetS in adults could highlight potential avenues for prevention and reduction of cardiovascular disease burden, but evidence from low-middle income countries like the Mesoamerican region is virtually unknown.

Sodium and iodine

The effects of sodium consumption on blood pressure and cardiovascular outcomes has been thoroughly described[64]. Sodium metabolism has a direct effect on the control of blood pressure, causing an increment in intravascular volume that leads to increased blood pressure[64]. Independent of its effects on blood pressure, prolonged sodium exposure can also cause perivascular fibrosis and increased aldosterone levels, which leads to oxidative damage and changes in renal and cardiac function[64]. Moreover, hypertension is a component of MetS, and therefore, it is important to evaluate the association between sodium and MetS.

Iodine is a necessary element for the creation of thyroid hormones which are implicated in maintaining basal metabolic rate and have effects on the cardiovascular system and body weight. A complex regulation of iodine exists in normal metabolism; iodine deficiency can result

in decreased thyroid hormone levels, whereas iodine excess can result in either decreased or increased thyroid hormone levels[65]. Decreased thyroid function leads to decreased basal metabolic rate, weight gain, increased triglycerides and decreased HDL-C levels, abnormalities in carbohydrate metabolism, and hypertension[65,66]. Therefore, iodine could be associated with MetS and considering previous reports of excessive iodine intake in the Mesoamerica region, is it important to evaluate this association in this specific population.

In children, previous evidence found positive associations between sodium with MetS, BMI and blood pressure[67,68]. In contrast, there is no previous evidence about the association between iodine and MetS. With respect to the components, thyroid function was increased in obese children with MetS[69]. The importance of evaluating sodium and iodine in children is reflected by the profound lack of evidence and their potential metabolic effects later in life. Examining these associations in a Mesoamerican population can provide new insight due to their unique sociodemographic characteristics.

In adults, several prior studies have shown a direct association of sodium and MetS. In populations from Venezuela[11] and Korea[9,10,70] there was an increased sodium excretion in patients with MetS compared to those without. There are also descriptions of positive associations between sodium excretion and some MetS components[9,10,70–72]. Few studies have evaluated the relation between iodine and MetS, and different directions of the association with some of MetS components was described[73,74]. Additionally, observational studies have described associations between thyroid hormone levels and some of MetS components[66,75]. The bulk of evidence in adults comes from develop countries, and populations from low- and middle-income countries like Mesoamerica can present with different confounding structures and are important to investigate.

Trace minerals

Zinc is implicated in many biological functions like an enzyme catalyst and structural component of proteins[50]. Also, functions of zinc that could explain a relation with MetS include its insulin-like effect, blood pressure regulation[76] and anti-inflammatory and antioxidant properties[77]. Moreover, copper and zinc act as cofactors in the cytosolic enzyme superoxide dismutase which is responsible for elimination of reactive oxygen species (ROS) in the cytosol[78]. Manganese is a cofactor for the mitochondrial superoxide dismutase enzyme, responsible for ROS elimination in the mitochondria[78]. Hence, both copper and manganese play key roles in the antioxidant system. In MetS, a state low grade inflammation has been described[29] and this could explain the potential preventive role of the trace minerals in MetS development.

Prior evidence from an antenatal zinc supplementation trial found no association with cardiometabolic risk later in childhood[79]. However, one randomized crossover trial among obese school-aged children found a significant improvement of MetS components after zinc supplementation compared to placebo[80]. Evidence from observational studies is also mixed and varies by sex. One study found no association between zinc and MetS[81], others found inverse associations with MetS and some of the components[82–84], whereas another study found a positive association between zinc and insulin resistance[85]. Previous studies about manganese have only evaluated the association with some of the MetS components and positive and inverse relations were described[84,86]. Similarly, previous evidence in children about the association between copper and MetS is mixed. Cross-sectional studies found no significant associations between copper and MetS[83,87] or MetS components[81], but another study found a positive association with obesity[84].

The evidence in adults is also mixed, and a meta-analysis of observational studies described positive and null associations between zinc and MetS[88]. For manganese, inverse[89] and null[90,91] associations with MetS were found. Likewise, the evidence for copper described null[89,91–94], inverse[95,96], and possitive[97] associations with MetS. Previous evidence about the association between trace minerals is not consistent and most of the observational studies did not account for important confounders like age and sex. Additionally, there is no solid evidence from the Mesoamerican region and is critical to provide insight from a region that is readily exposed to fortified food but suffers from a high burden of malnutrition.

Overall, there is a need to evaluate the micronutrient status of the Mesoamerican population, that is suffering from the dual burden of disease and is expected to have an increase in cardiovascular disease. Moreover, fortification policies in the region are not a guaranty of elimination of deficiency status, neither an absence of excessive exposure, and therefore, a closer evaluation is warranted. Finally, childhood is a window of opportunity for potential early intervention that could help decrease the burden of cardiometabolic diseases in future generations.

Summary of chapters

This dissertation seeks to elucidate the associations between important micronutrient levels and MetS in both children and adults from the understudied population of Mesoamerica. Overall, there is lack of evidence about these associations from this region and during childhood, and well conducted studies can help elucidate potentially modifiable risk factors that are amenable to interventions.

For this purpose, chapter 2 describes the associations between the plasma concentrations of the B-vitamins as biomarkers of intake and plasma Hcys concentrations with MetS and its components in children and their adult parents from nine countries in Mesoamerica. Following, chapter 3 describes the associations between urinary sodium and iodine as biomarkers of intake with MetS and its components. Next, chapter 4 evaluates the relations between plasma trace mineral zinc, manganese, and copper with MetS in the Mesoamerican families. To conclude, chapter 5 contains a summary of the dissertations' main findings and the public health implications they convey.

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Chapter 2 . B-vitamins and metabolic syndrome in children and their adult parents

Abstract

Background and aims: Vitamins of the methionine-homocysteine cycle (B6, B12, and folate) and homocysteine (Hcys) have been related to cardiovascular outcomes, but their role on the development of metabolic syndrome (MetS) is uncertain. We examined the associations of these factors with MetS among Mesoamerican children and their adult parents.

Methods: We conducted a cross-sectional investigation among 237 children and 524 parents from the capitals of Belize, Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Chiapas State in Mexico. Exposures were plasma vitamin B6 and B12 concentrations, erythrocyte folate, and plasma Hcys. In children, the outcome was a continuous metabolic risk score calculated through sex- and age-standardization of waist circumference, the homeostatic model assessment for insulin resistance, mean arterial pressure (MAP), serum high-density lipoprotein cholesterol, and serum triglycerides. In parents, the outcome was the prevalence of MetS according to the Adult Treatment Panel (ATP) III criteria. We estimated mean differences in the metabolic risk score and prevalence ratios (PR) of MetS between quartiles of the exposures using multivariable-adjusted linear and Poisson regression models, respectively.

Results: Among children, vitamin B12 was inversely associated with the metabolic risk score (adjusted difference of quartile 4 vs. 1=0.13, 95% CI: -0.21, -0.04; P, trend=0.008). By contrast, folate was positively associated with the metabolic risk score. In adults, vitamin B6 was

inversely associated with MetS prevalence whereas vitamin B12 and folate were positively related to this outcome.

Conclusions: Vitamins of the methionine-homocysteine cycle are associated with metabolic syndrome in different directions. The associations differ between children and adults. Mandatory fortification programs should be monitored and their potential role in these associations evaluated.

Keywords: Metabolic syndrome, metabolic risk score, vitamin B6, vitamin B12, erythrocyte folate, homocysteine.

Introduction

Cardiovascular disease is the leading cause of death worlwide[1]. The metabolic syndrome (MetS) is a cluster of independent risk factors for cardiovascular disease, type 2 diabetes, and mortality that includes abdominal obesity, insulin resistance, hypertension, elevated triglycerides, and reduced high-density lipoprotein (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 to estimates from Europe[5] and the United States[6]. MetS components can become apparent from childhood[7] and track into adulthood[8] increasing risk for cardiovascular disease[8]. Because MetS is mostly preventable, identifying potentially modifiable risk factors is a research priority.

Vitamins B6 (pyridoxal 5' phosphate), B9 (folate), and B12 are cofactors for the one-carbon metabolism which is essential for the synthesis of methyl donors. Methyl donors play critical roles in multiple pathways including DNA methylation and lipid and protein metabolism. B-vitamin deficiencies could impair the synthesis of methyl groups and induce hyperhomocysteinemia (H-Hcys), both of which could increase the risk of atherosclerosis and cardiovascular disease[9]. Previous evidence in adults found that vitamin B6 serostatus[10] and intake[11], and vitamin B12[12–14] and folate[12] biomarkers and supplementation[15] have been inversely related to MetS overall, whereas H-Hcys has been positively associated with this outcome[16,17]. In children, the evidence is scant. Vitamin B12 serostatus was inversely associated with MetS[18] and obesity[18,19] in cross-sectional studies of Turkish and Israeli school-aged children and adolescents. Similarly, maternal folate supplementation[20] and serostatus[21,22] were inversely associated with MetS[20], blood pressure[22], and body mass index (BMI)-for-age Z score[21] at ages 6 to 9 years in longitudinal studies from Nepal and the United States. Moreover, a cross-sectional

study of Czech adolescents found an inverse association of serum folate with hypertension[23], whereas H-Hcys was positively related to MetS prevalence in Nepalese school-aged children[24]. Therefore, the B-vitamins and Hcys could be potentially modifiable risk factors associated with MetS.

Additionally, because the prevalence of B-vitamin deficiencies is high in Latin-American children[25] and interventions during childhood may modulate cardiovascular risk in the long term[26] 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 vitamin B6, B9, and B12 biomarkers and Hcys with MetS and its components among school-aged children and their parents from Mesoamerica.

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 study have been described elsewhere[3]. In brief, we recruited 267 family groups each consisting of a school-aged child and their two parents from public primary schools in periurban areas of the capital cities, using convenience sampling. Eligibility criteria included a 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. The study protocol and procedures were approved by the Institutional Review Boards (IRB) of collaborating institutions in each country and by the University of Michigan Health and Behavioral Sciences IRB. 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 fast of at least 6 hours. At the visit, participants responded to a questionnaire on sociodemographic 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)[27], a 16 yes/no item survey about food security experiences during the past three months. Research assistants administered a 97-item semiquantitative food frequency questionnaire (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[28]. The FFQ characteristics have been detailed elsewhere[29]. Researchers measured height, weight, and waist circumference with the use of standardized 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[30]. Blood pressure was measured while seated, using Omron HEM-712C digital blood pressure monitors (Omron Healthcare, Inc., Lake Forest, IL, USA). Three measurements were taken, separated by at least one minute, 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. Samples were placed in refrigerated containers and transported on the day of collection to each country's collaborating laboratories where the serum, plasma, and red blood cells were separated, aliquoted, and cryostored at −20°C. Frozen stored samples from all countries were transported to the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City.

Laboratory methods

Quantification of insulin, glucose, lipids, vitamin B12, erythrocyte folate, and Hcys was conducted at INCAP. Plasma insulin, vitamin B12, and Hcys were measured using a chemiluminescent immunoassay on an Immulite 1000 system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Plasma glucose and serum lipids (total and HDL-cholesterol and triglycerides) were quantified on an automated chemistry analyzer (Cobas c111 system; Roche Diagnostics, Mannheim, Germany). Erythrocyte folate was measured in red blood cell lysates using competitive immunoassays on an Immulite 2000 system (Siemens Healthcare

Diagnostics Products GmbH, Marburg, Germany). Vitamin B6 biologically active metabolite, pyridoxal 5' phosphate (PLP), was measured in plasma using Liquid Chromatography-Mass Spectrometry on a Thermo Fisher/Cohesive Technologies Aria TLX Series HTLC System at the Mayo Medical Laboratories (Minnesota, USA).

Definition of outcomes

Children. 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 (HOMA-IR)[31], mean arterial pressure (MAP,) serum HDL-cholesterol, and serum triglycerides. MAP was calculated as [(2 x diastolic blood pressure) + systolic blood pressure]/3. HOMA-IR was used instead of high fasting blood glucose because the prevalence of this finding in children is low[32]. We used MAP as the blood pressure criteria to decrease the number of variables used to construct the score[32]. The metabolic risk score was created by regressing each log-transformed component on sex and log-transformed age using linear regression models to obtain the standardized regression residuals. The average of the residuals for the five components was used to create the score, with the residuals for HDL-cholesterol multiplied by -1 beforehand. Higher scores indicate worse metabolic profile.

Interpretability of the metabolic risk score. To assess the interpretability of the score, we compared it against a dichotomous MetS definition with the same components as in adults using percentile cutpoints for waist circumference[33], blood pressure[34], HDL-cholesterol, and triglycerides[35], and high fasting glucose as defined in adults. We also explored the relation between the metabolic score and the number of MetS factors based on the dichotomous definition. Because there were few children with 4 factors (n=4) and no children with all 5 factors, we collapsed the final three categories.

Adults. The presence of MetS was defined according to the Adult Treatment Panel (ATP) III criteria[2] as having any 3 of the following 5: 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 triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides.

Definition of exposures

Prevalences of B-vitamin deficiencies according to conventional cutpoints were low; thus, biomarker concentrations were categorized into quartiles.

Covariates

Children. Height-for-age and BMI-for-age Z scores were calculated using the World Health Organization (WHO) reference[36]. We categorized parental height 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 categorized by the number of affirmative responses in the ELCSA survey (no insecurity, 0; mild insecurity, 1-5; moderate insecurity, 6-10; severe insecurity, ≥11). The number of household assets was the sum of a car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color television, sound set, computer, or Internet, with a possible maximum number of assets of 12. Maternal parity was categorized as 1, 2, 3, or ≥ 4 births. We also considered as covariates food sources that may be predictors of the outcome. 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, which were

categorized as presented in Supplemental Table 2. 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 categorized as presented in Supplemental Table 3. Parental smoking was categorized as never, past, or current. Household income was categorized into country-specific quartiles. Dietary covariates were defined as described in children and categorized as presented in Supplemental Table 4.

Data analysis

The final analytic sample comprised of 237 children and 524 adults 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 of vitamin food sources, and total energy intake. Because the metabolic score is standardized 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 prevalences 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 generalized estimation equation (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. Robust variances were specified in all models to account for within-household correlations.

Joint family analysis. In supplement analyses, we fitted joint regression models including both children and adults. These models allow the estimation of associations with an outcome measured in different scales in populations subgroups simultaneously, with higher statistical power than separate models for each subgroup[37]. We included an interaction term to indicate the type of outcome distribution in children and adults to obtain the mean differences in the metabolic score and prevalence ratios of MetS, respectively. Multivariable-adjusted models included age, sex, education level, household assets, food security, and country of origin. Estimates for the vitamins were adjusted for each other but not for Hcys, whereas estimates for Hcys were adjusted for all vitamins. All models included an unstructured covariance matrix using the Cholesky parametrization to account for clustering by family membership.

All analyses were conducted using Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC, USA).

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), median, minimum, and maximum values of the metabolic risk score were 0.00 ± 0.22, -0.03, -0.44, and 0.82, respectively. Deficiency prevalence of vitamin B6 (<20 nmol/)[38], vitamin B12 (<148 pmol/L)[39], and folate (<305 nmol/L)[38] was 0.9%, 4.7%, and 2.1%, respectively; whereas 4.3% had hyperhomocysteinemia (Hcys >95th percentile for age)[40].

Interpretability of the metabolic risk score. The mean score (±SD) was 0.41 ± 0.18 and -0.03 ± 0.19 for children with and without MetS, respectively. The mean scores (±SD) for 0, 1, 2, ≥3 MetS factors were -0.14 ± 0.15, -0.01 ± 0.14, 0.18 ± 0.16, and 0.41 ± 0.18, respectively (**Supplemental Figure 1**). The average difference in metabolic risk score per one MetS component was 0.16 units (95% CI: 0.13, 0.18, P=<0.0001).

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 (**Supplemental Table 1**). Plasma vitamin B12 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, and maternal height, BMI, and parity; and inversely with parental education. It differed significantly by country. Plasma PLP was positively related to dairy intake (**Supplemental Table 2**); plasma

vitamin B12 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 or its components (**Table 1**). Plasma vitamin B12 was inversely related to the metabolic score (**Table 2**). Compared with children at the lowest vitamin B12 quartile, those at highest quartile had an adjusted 0.13 units lower metabolic score (P, trend=0.008). The metabolic score components associated with plasma vitamin B12 included MAP, serum HDL-cholesterol, and triglycerides (**Table 2**). Erythrocyte folate was positively associated with the metabolic risk score (**Table 3**). 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 (**Table 3**). Plasma Hcys was not associated with the metabolic score overall but was positively related to the waist circumference and arterial pressure components (**Table 4**).

Adults

Mean age (± SD) of participants was 38.7 ± 7.5 years, and 50.4% were women; 34.2% had obesity. The prevalence of MetS was 36.6%. Deficiency prevalence of vitamin B6 (<20 nmol/)[38], vitamin B12 (<148 pmol/L)[39], and folate (<305 nmol/L)[38] was 4.7%, 10.4%, and 8.4%, respectively; whereas 16.4% had hyperhomocysteinemia (Hcys >14 µmol/L)[41].

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 (**Supplemental Table 3**). Vitamin B12 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 B12 was positively associated with red meat, fish, and fortified foods intake (**Supplemental Table 4**) whereas erythrocyte folate was positively associated with intake of green leafy vegetables and fortified foods.

B-vitamin biomarkers and metabolic syndrome. Plasma PLP was inversely associated with MetS (**Table 5**); 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 (**Table 5**). Vitamin B12 was positively associated with MetS in a non-linear fashion (**Table 6**). Compared with parents at the lowest quartile of plasma vitamin B12, those at the highest three quartiles combined had a 49% higher prevalence of MetS (PR= 1.49; 95% CI: 1.07, 2.08; P = 0.02). Vitamin B12 was positively associated with high fasting blood glucose and high blood pressure (**Table 6**). Erythrocyte folate was positively associated with MetS prevalence (**Table 7**). 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. (**Table 7)**. Hcys was not significantly associated with MetS or its components (**Table 8**).

Joint family analysis. Results from the joint modeling of children and adults followed those from the stratified analyses. In children, erythrocyte folate was positively associated with the metabolic risk score (**Supplemental Table 5**). In adults, PLP was inversely related to MetS prevalence, whereas vitamin B12 was positively related to this outcome in a non-linear manner; participants in the lowest quartile had a 77% higher prevalence of MetS than those with higher

concentrations (PR=1.77; 95% CI: 1.25, 2.51; P=0.002). Erythrocyte folate was also positively related to MetS prevalence in adults.

Discussion

In this cross-sectional study, a metabolic risk score in children was inversely associated with plasma vitamin B12 and positively related to erythrocyte folate. In adults, plasma PLP was inversely associated with MetS prevalence, whereas plasma vitamin B12 and erythrocyte folate were positively related to this outcome.

Vitamin B6 was not associated with the metabolic risk score in children; however, we found an inverse association between plasma PLP with MetS and abdominal obesity prevalence in adults. The results in adults are consistent with previous cross-sectional studies in Nigeria[10] and Japan[11]. In a cross-sectional study, plasma PLP was lower in adults with morbid obesity than in healthy-weight controls[42]. Low plasma PLP has been associated with increased levels of inflammatory and oxidative stress biomarkers[43] which might contribute to explain an inverse association with MetS since inflammation and oxidative stress are considered underlying etiologic factors[2].

Vitamin B12 was inversely associated with the metabolic risk score in children; possibly through blood pressure and serum lipids. Previous studies of European adolescents[44] and preschool Japanese children[45] found similar inverse associations between vitamin B12 serostatus and blood pressure. Also, cross-sectional investigations of Turkish[18] and Israeli[46] children found inverse associations between vitamin B12 serostatus and body weight. Our findings in adults are in contrast to those in children, since, vitamin B12 was positively related to MetS prevalence, possibly through high fasting blood glucose and high blood pressure. These results were also inconsistent with prior cross-sectional studies that showed inverse associations between vitamin B12 and MetS[12–14]. Similarly, a randomized trial[15], a Mendelian randomization study[47], and a case control[12] study found inverse associations between vitamin B12 and measures of insulin

resistance, whereas a cross-sectional study found an inverse association of serum vitamin B12 with hypertension[48]. There are some possible explanations for these discrepancies. Although we were able to adjust for possible 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 B12 with MetS. Additionally, children had higher dairy consumption compared to adults and evidence has found an inverse association between dairy consumption and MetS[49]. Moreover, the discrepancy between our findings in children compared to adults could be explained by the different measures used to assess MetS, and direct comparisons may not be accurate. The strength of the mean adjusted difference in the metabolic score for vitamin B12 was moderate since the estimate was 80% of 0.16, which was the mean score difference per MetS component.

Erythrocyte folate was positively associated with the metabolic risk score in children, probably through abnormal waist circumference, blood pressure, and 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 and previous evidence has found inverse associations between maternal folate with MetS and some of its components. In a prenatal supplementation study conducted in rural Nepal, offspring from mothers who had received antenatal folic acid supplements had a lower risk of MetS at ages 6 to 8 years[20]. Likewise, results from the Boston Birth Cohort suggested an L-shaped association between maternal plasma folate levels at the end of pregnancy with childhood overweight or obesity at ages 3 to 9 years, with the highest risk among children from obese mothers with low folate compared to mothers with healthy weight and higher folate levels[21]. Data from the same cohort also found an inverse association of maternal folate levels with systolic blood pressure only

among children whose mothers presented any cardiometabolic risk factors[22]. Cross-sectional investigations have found different results, with two suggesting an inverse association between folate and blood pressure[23,45], but another suggesting a positive association between erythrocyte folate and systolic blood pressure among adolescent girls[44]. The discrepancy of the first two studies with our findings could be attributed to the evaluation of only adolescents with essential hypertension in the first study[23] and the measurement of dietary folate using recall methods in the second[45]. Our findings on both children and adults seem to contradict most of the existing evidence on folate and its relationship with MetS and its components. However, some previous evidence suggests a potential adverse effect of folate on MetS components; notably, cross-sectional evaluations of obese adults found a positive association between erythrocyte folate and HOMA-IR, with the highest levels of HOMA-IR among participants at the lowest tertile of vitamin B12 and highest tertile of erythrocyte folate[50]. Our results in both children and adults suggest folate might have a deleterious effect on metabolic functions. One proposed mechanism is the methyl-pool depletion caused by folic acid, the standard form of folate used for supplementation[51], which is mandatory for wheat and corn flour in the region of Mesoamerica[52]. Moreover, in our population, the consumption of green leafy vegetables and fortified food was significantly associated with erythrocyte folate levels, but consumption of fortified foods was higher compared to green leafy vegetables for both groups. The increased exposure to folic acid from fortified foods causes an imbalance in the methylation status of the body leading to increased oxidative stress and lipid abnormalities. Ultimately these changes manifest as obesity, dyslipidemia, insulin resistance, and MetS development[53]. These mechanisms support the notion that folic acid from fortified foods could have a deleterious role in metabolic health and require further study. Notably, the strength of the adjusted mean differences in the metabolic risk score

for folate was 67% of 0.16, the mean difference in the score associated with one additional MetS component.

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 H-Hcys was positively associated with having MetS[24], high blood pressure[24], and waist circumference >90th percentile[54]. Although most evidence suggests Hcys is positively associated with MetS, one previous cohort study found no association between serum Hcys with MetS components and insulin sensitivity among healthy men from London[55]. Recent evidence supports the notion that B-vitamins play a more significant role on MetS that is independent of Hcys. This theory is supported by supplementation trials, where folate and vitamin B12 supplementation was sufficient to lower Hcys levels, but no benefit was identified in the reduction of cardiovascular events[56].

Our study has several strengths. Dietary exposures were measured using biomarkers of intake, which removes recall bias when using FFQ or recall methods to assess intake and allows estimation of associations between specific cut points and MetS. Data collected on both parents and children allowed adjustment for parental characteristics that could be considered potential confounders for the associations in children. The specific dietary exposures evaluated are a unique investigation in children and generated new information regarding its association with MetS in a Mesoamerican population.

The main limitation of our study is its cross-sectional nature which limits causal inference. Additionally, the dietary confounders were measured using FFQ data that could be subject to non-differential misclassification, which could, in turn, result in reduced ability to control for confounding, and because we used categorial dietary confounders, the direction of

residual confounding bias is difficult to predict. Moreover, reverse causation could explain the associations we found, since the vitamin food sources, like fortified foods and red and processed meats are strong confounders. Therefore, our findings could instead reflect the association between specific food groups and MetS.

Because we used a metabolic risk score based on the specific distribution of the different components in this population, comparisons with other populations are limited. Since our study sample was not representative of the entire Mesoamerican population and deficiency prevalences varied by country, generalizability might be affected. Additionally, due to selection bias secondary to convenience sampling, this population comes from urban non-indigenous areas which could explain the lower B-vitamin deficiency prevalence compared to rural areas and estimates from nationally-representative surveys[25], resulting in a potentially lower strength of the associations of interest. Finally, due to small sample size, country-specific analyses were not possible.

In conclusion, we found that vitamin B6 was inversely associated with MetS in adults, but not in children. Vitamin B12 was inversely associated with a metabolic risk score in 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. Whether mandatory folate fortification could be driving this association deserves careful consideration in future studies.

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Table 2.1. Means (± SD), unadjusted and adjusted mean differences and 95% CI in metabolic syndrome score[1] and its components[2] by vitamin B6 (PLP[3]) quartiles among children from Mesoamerica

Median plasma PLP[3] (nmol/L)	Q1 36 (n=50)	Q2 49 (n=52)	Q3 65 (n=67)	Q4 107 (n=60)	P, trend[4]
Metabolic risk score					
Mean Z score ± SD	0.02 ± 0.21	-0.04 ± 0.20	0.03 ± 0.24	0.01 ± 0.24	
Unadjusted mean difference	Reference	-0.05 (-0.13, 0.02)	0.01 (-0.07, 0.09)	0.00 (-0.09, 0.08)	0.69
Adjusted difference - model 1[5]	Reference	-0.05 (-0.12, 0.02)	0.02 (-0.05, 0.09)	-0.01 (-0.09, 0.06)	0.88
Adjusted difference - model 2[6]	Reference	-0.07 (-0.14, 0.00)	0.00 (-0.07, 0.07)	-0.02 (-0.10, 0.06)	0.87
Waist circumference score					
Mean ± SD	0.02 ± 0.16	-0.02 ± 0.14	0.01 ± 0.17	0.02 ± 0.14	
Unadjusted mean difference	Reference	-0.04 (-0.10, 0.02)	-0.01 (-0.07, 0.04)	0.00 (-0.06, 0.05)	0.62
Adjusted difference - model 1	Reference	-0.03 (-0.08, 0.01)	-0.02 (-0.06, 0.03)	0.00 (-0.05, 0.04)	0.63
Adjusted difference - model 2	Reference	-0.03 (-0.08, 0.02)	-0.02 (-0.07, 0.02)	0.01 (-0.05, 0.06)	0.40
HOMA-IR score					
Mean ± SD	0.04 ± 0.53	-0.16 ± 0.57	0.11 ± 0.66	0.03 ± 0.58	
Unadjusted mean difference	Reference	-0.20 (-0.41, 0.01)	0.07 (-0.14, 0.28)	-0.02 (-0.22, 0.19)	0.55
Adjusted difference - model 1	Reference	-0.07 (-0.24, 0.11)	0.18 (0.00, 0.36)	-0.02 (-0.21, 0.16)	0.93
Adjusted difference - model 2	Reference	-0.10 (-0.29, 0.08)	0.15 (-0.03, 0.32)	-0.11 (-0.30, 0.08)	0.38
Mean arterial pressure (MAP) score					
Mean ± SD	0.03 ± 0.15	0.00 ± 0.15	-0.02 ± 0.12	-0.01 ± 0.16	
Unadjusted mean difference	Reference	-0.02 (-0.08, 0.04)	-0.04 (-0.10, 0.01)	-0.03 (-0.09, 0.03)	0.39
Adjusted difference - model 1	Reference	0.00 (-0.05, 0.05)	-0.03 (-0.08, 0.01)	-0.02 (-0.07, 0.03)	0.27
Adjusted difference - model 2	Reference	0.00 (-0.05, 0.05)	-0.02 (-0.07, 0.02)	-0.01 (-0.06, 0.04)	0.63
Serum HDL-cholesterol score					
Mean ± SD	-0.03 ± 0.27	0.00 ± 0.28	0.00 ± 0.28	0.02 ± 0.27	
Unadjusted mean difference	Reference	0.02 (-0.08, 0.13)	0.03 (-0.07, 0.13)	0.05 (-0.05, 0.15)	0.40
Adjusted difference - model 1	Reference	0.01 (-0.08, 0.11)	0.04 (-0.05, 0.13)	0.04 (-0.05, 0.13)	0.43
Adjusted difference - model 2	Reference	0.01 (-0.09, 0.12)	0.03 (-0.06, 0.12)	0.02 (-0.07, 0.11)	0.66
Serum triglycerides score					
Mean ± SD	-0.03 ± 0.45	-0.02 ± 0.37	0.05 ± 0.43	0.04 ± 0.47	
Unadjusted mean difference	Reference	0.01 (-0.15, 0.17)	0.08 (-0.08, 0.24)	0.07 (-0.10, 0.24)	0.37
Adjusted difference - model 1	Reference	0.02 (-0.14, 0.17)	0.07 (-0.08, 0.21)	0.02 (-0.14, 0.19)	0.83
Adjusted difference - model 2	Reference	-0.05 (-0.20, 0.10)	0.02 (-0.12, 0.16)	-0.01 (-0.18, 0.15)	0.94

Footnotes to Table 2.1

[1]The overall score was calculated as the average of the five component scores, after the HDL-cholesterol score was multiplied by -1

[2]Component scores (waist circumference, HOMA-IR, MAP, serum HDL-cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]PLP stands for pyridoxal 5' phosphate which is the biologically active metabolite of vitamin B6.

[4]Test for linear trend from linear regression models with the metabolic score or each component as the outcome and a variable representing medians of ordinal categories of vitamin B6 introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[5]From linear regression models. Model 1 for the metabolic score and waist circumference component was adjusted for vitamin B12, erythrocyte folate, height-for-age Z score, maternal height, highest parental education level, household food security, number of household assets, and country of origin. Model 1 for the rest of components was additionally adjusted for BMI-for age Z score.

[6]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of dairy, meat, fish, and green leafy vegetables.

Table 2.2. Means (± SD), unadjusted and adjusted mean differences and 95% CI in metabolic syndrome score[1] and its components[2] by vitamin B12 quartiles among children from Mesoamerica

Median plasma vitamin B12 (pmol/L)	Q1 191 (n=58)	Q2 297 (n=58)	Q3 404 (n=60)	Q4 719 (n=59)	P, trend[3]
Metabolic risk score					
Mean Z score ± SD	0.10 ± 0.24	-0.02 ± 0.23	-0.03 ± 0.21	-0.03 ± 0.20	
Unadjusted mean difference	Reference	-0.12 (-0.20, -0.03)	-0.13 (-0.21, -0.05)	-0.12 (-0.20, -0.04)	0.02
Adjusted difference - model 1[4]	Reference	-0.10 (-0.18, -0.02)	-0.14 (-0.22, -0.06)	-0.14 (-0.22, -0.05)	0.01
Adjusted difference - model 2[5]	Reference	-0.07 (-0.15, 0.01)	-0.12 (-0.20, -0.05)	-0.13 (-0.21, -0.04)	0.008
Waist circumference score					
Mean ± SD	0.03 ± 0.15	0.00 ± 0.14	-0.01 ± 0.15	-0.01 ± 0.17	
Unadjusted mean difference	Reference	-0.03 (-0.08, 0.02)	-0.04 (-0.10, 0.01)	-0.04 (-0.09, 0.02)	0.32
Adjusted difference - model 1	Reference	-0.01 (-0.06, 0.04)	-0.03 (-0.08, 0.01)	-0.02 (-0.08, 0.04)	0.51
Adjusted difference - model 2	Reference	0.01 (-0.04, 0.06)	-0.02 (-0.06, 0.03)	0.00 (-0.06, 0.05)	0.79
HOMA-IR score					
Mean ± SD	0.18 ± 0.65	-0.04 ± 0.62	-0.04 ± 0.54	-0.07 ± 0.54	
Unadjusted mean difference	Reference	-0.21 (-0.44, 0.02)	-0.22 (-0.43, 0.00)	-0.25 (-0.46, -0.03)	0.06
Adjusted difference - model 1	Reference	-0.19 (-0.38, 0.00)	-0.16 (-0.34, 0.01)	-0.21 (-0.41, -0.02)	0.11
Adjusted difference - model 2	Reference	-0.08 (-0.26, 0.10)	-0.09 (-0.28, 0.09)	-0.15 (-0.34, 0.04)	0.16
Mean arterial pressure (MAP) score					
Mean ± SD	-0.01 ± 0.14	0.02 ± 0.13	0.00 ± 0.17	-0.01 ± 0.15	
Unadjusted mean difference	Reference	0.03 (-0.02, 0.07)	0.01 (-0.04, 0.07)	0.00 (-0.05, 0.05)	0.69
Adjusted difference - model 1	Reference	0.00 (-0.05, 0.04)	-0.02 (-0.06, 0.03)	-0.06 (-0.11, 0.00)	0.02
Adjusted difference - model 2	Reference	-0.01 (-0.05, 0.04)	-0.03 (-0.07, 0.02)	-0.05 (-0.11, 0.00)	0.03
Serum HDL-cholesterol score					
Mean ± SD	-0.13 ± 0.28	0.02 ± 0.27	0.05 ± 0.32	0.03 ± 0.24	
Unadjusted mean difference	Reference	0.15 (0.05, 0.24)	0.17 (0.06, 0.28)	0.16 (0.06, 0.25)	0.009
Adjusted difference - model 1	Reference	0.09 (0.01, 0.18)	0.14 (0.05, 0.22)	0.11 (0.02, 0.21)	0.07
Adjusted difference - model 2	Reference	0.09 (0.01, 0.18)	0.15 (0.06, 0.24)	0.14 (0.05, 0.24)	0.01
Serum triglycerides score					
Mean ± SD	0.17 ± 0.48	-0.06 ± 0.44	-0.07 ± 0.37	-0.01 ± 0.39	
Unadjusted mean difference	Reference	-0.23 (-0.39, -0.06)	-0.24 (-0.39, -0.09)	-0.18 (-0.33, -0.02)	0.15
Adjusted difference - model 1	Reference	-0.18 (-0.33, -0.02)	-0.20 (-0.36, -0.05)	-0.20 (-0.36, -0.05)	0.04
Adjusted difference - model 2	Reference	-0.15 (-0.31, 0.00)	-0.21 (-0.37, -0.06)	-0.23 (-0.39, -0.07)	0.02

Footnotes to Table 2.2

[1]The overall score was calculated as the average of the five component scores, after the HDL-cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, MAP, serum HDL-cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Test for linear trend from linear regression models with the metabolic score or each component as the outcome and a variable representing medians of ordinal categories of vitamin B12 introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[4]From linear regression models. Model 1 for the metabolic score and waist circumference component was adjusted for vitamin B6, erythrocyte folate, height-for-age Z score, maternal height, highest parental education level, household food security, number of household assets, and country of origin. Model 1 for the rest of components was additionally adjusted for BMI-for age Z score.

[5]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of dairy, meat, fish, and green leafy vegetables.

Table 2.3. Means (± SD), unadjusted and adjusted mean differences and 95% CI in metabolic syndrome score[1] and its components[2] by folate quartiles among children from Mesoamerica

Median erythrocyte folate (nmol/L)	Q1 531 (n=58)	Q2 735 (n=59)	Q3 858 (n=59)	Q4 1169 (n=59)	P, trend[3]
Metabolic risk score					
Mean Z score ± SD	0.02 ± 0.23	-0.01 ± 0.22	-0.02 ± 0.19	0.03 ± 0.26	
Unadjusted mean difference	Reference	-0.03 (-0.11, 0.06)	-0.03 (-0.11, 0.04)	0.01 (-0.08, 0.10)	0.75
Adjusted difference - model 1[4]	Reference	-0.03 (-0.11, 0.04)	0.00 (-0.08, 0.07)	0.04 (-0.05, 0.14)	0.21
Adjusted difference - model 2[5]	Reference	0.01 (-0.07, 0.08)	0.04 (-0.02, 0.11)	0.11 (0.01, 0.20)	0.02
Waist circumference score					
Mean ± SD	0.02 ± 0.16	0.01 ± 0.15	-0.04 ± 0.14	0.01 ± 0.16	
Unadjusted mean difference	Reference	-0.01 (-0.06, 0.05)	-0.06 (-0.11, 0.00)	-0.01 (-0.07, 0.05)	0.60
Adjusted difference - model 1	Reference	0.00 (-0.05, 0.05)	-0.01 (-0.05, 0.04)	0.07 (0.01, 0.13)	0.01
Adjusted difference - model 2	Reference	0.03 (-0.02, 0.08)	0.02 (-0.02, 0.07)	0.10 (0.05, 0.16)	0.0003
HOMA-IR score					
Mean ± SD	0.03 ± 0.69	-0.04 ± 0.51	0.01 ± 0.53	0.03 ± 0.62	
Unadjusted mean difference	Reference	-0.07 (-0.29, 0.15)	-0.02 (-0.24, 0.20)	-0.01 (-0.24, 0.23)	0.92
Adjusted difference - model 1	Reference	-0.24 (-0.43, -0.05)	-0.08 (-0.28, 0.12)	-0.21 (-0.45, 0.04)	0.26
Adjusted difference - model 2	Reference	-0.21 (-0.40, -0.03)	-0.04 (-0.24, 0.15)	-0.16 (-0.41, 0.09)	0.46
Mean arterial pressure (MAP) score					
Mean ± SD	0.00 ± 0.14	-0.01 ± 0.14	0.01 ± 0.15	0.00 ± 0.16	
Unadjusted mean difference	Reference	-0.01 (-0.06, 0.04)	0.01 (-0.04, 0.07)	0.01 (-0.05, 0.06)	0.73
Adjusted difference - model 1	Reference	-0.02 (-0.06, 0.03)	0.05 (0.00, 0.10)	0.02 (-0.04, 0.09)	0.24
Adjusted difference - model 2	Reference	0.01 (-0.04, 0.06)	0.09 (0.03, 0.14)	0.06 (-0.01, 0.13)	0.04
Serum HDL-cholesterol score					
Mean ± SD	-0.11 ± 0.34	0.05 ± 0.27	0.04 ± 0.25	-0.01 ± 0.26	
Unadjusted mean difference	Reference	0.16 (0.05, 0.27)	0.15 (0.04, 0.25)	0.10 (-0.01, 0.21)	0.15
Adjusted difference - model 1	Reference	0.02 (-0.08, 0.12)	-0.01 (-0.09, 0.08)	-0.06 (-0.17, 0.04)	0.14
Adjusted difference - model 2	Reference	-0.02 (-0.11, 0.08)	-0.04 (-0.13, 0.04)	-0.11 (-0.22, 0.00)	0.04
Serum triglycerides score					
Mean ± SD	-0.07 ± 0.44	0.05 ± 0.46	-0.03 ± 0.38	0.09 ± 0.42	
Unadjusted mean difference	Reference	0.12 (-0.04, 0.28)	0.04 (-0.11, 0.19)	0.17 (0.01, 0.32)	0.07
Adjusted difference - model 1	Reference	0.06 (-0.09, 0.21)	0.03 (-0.12, 0.18)	0.08 (-0.09, 0.25)	0.45
Adjusted difference - model 2	Reference	0.11 (-0.05, 0.27)	0.07 (-0.08, 0.22)	0.14 (-0.04, 0.33)	0.19

Footnotes to Table 2.3

[1]The overall score was calculated as the average of the five component scores, after the HDL-cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, MAP, serum HDL-cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Test for linear trend from linear regression models with the metabolic score or each component as the outcome and a variable representing medians of ordinal categories of erythrocyte folate introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[4]From linear regression models. Model 1 for the metabolic score and waist circumference component was adjusted for vitamin B6, vitamin B12, height-for-age Z score, maternal height, highest parental education level, household food security, number of household assets, and country of origin. Model 1 for the rest of components was additionally adjusted for BMI-for age Z score.

[5]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of dairy, meat, fish, and green leafy vegetables.

Table 2.4. Means (± SD), unadjusted and adjusted mean differences and 95% CI in metabolic syndrome score[1] and its components[2] by homocysteine (Hcys) quartiles among children from Mesoamerica

Median plasma Hcys (μmol/L)	Q1 3.8 (n=57)	Q2 5.5 (n=60)	Q3 7.5 (n=59)	Q4 10.5 (n=59)	P, trend[3]
Metabolic risk score					
Mean Z score ± SD	0.00 ± 0.26	0.02 ± 0.23	-0.02 ± 0.19	0.02 ± 0.23	
Unadjusted mean difference	Reference	0.02 (-0.07, 0.11)	-0.03 (-0.11, 0.05)	0.01 (-0.08, 0.10)	0.99
Adjusted difference - model 1[4]	Reference	0.05 (-0.03, 0.13)	0.05 (-0.03, 0.14)	0.07 (-0.02, 0.17)	0.18
Adjusted difference - model 2[5]	Reference	0.02 (-0.06, 0.10)	0.03 (-0.05, 0.12)	0.06 (-0.04, 0.16)	0.22
Waist circumference score					
Mean ± SD	0.01 ± 0.16	0.00 ± 0.14	-0.02 ± 0.17	0.03 ± 0.15	
Unadjusted mean difference	Reference	-0.01 (-0.06, 0.05)	-0.03 (-0.09, 0.03)	0.02 (-0.03, 0.08)	0.44
Adjusted difference - model 1	Reference	0.01 (-0.03, 0.06)	0.05 (-0.01, 0.10)	0.07 (0.01, 0.12)	0.01
Adjusted difference - model 2	Reference	0.02 (-0.03, 0.06)	0.05 (-0.01, 0.11)	0.07 (0.01, 0.13)	0.02
HOMA-IR score					
Mean ± SD	0.08 ± 0.64	0.06 ± 0.62	-0.09 ± 0.55	-0.02 ± 0.56	
Unadjusted mean difference	Reference	-0.01 (-0.24, 0.21)	-0.16 (-0.38, 0.05)	-0.09 (-0.31, 0.12)	0.27
Adjusted difference - model 1	Reference	0.08 (-0.11, 0.26)	-0.01 (-0.21, 0.18)	-0.06 (-0.26, 0.15)	0.34
Adjusted difference - model 2	Reference	0.05 (-0.15, 0.25)	-0.03 (-0.22, 0.16)	-0.09 (-0.30, 0.13)	0.26
Mean arterial pressure (MAP) score					
Mean ± SD	-0.05 ± 0.13	0.00 ± 0.14	0.00 ± 0.16	0.04 ± 0.14	
Unadjusted mean difference	Reference	0.04 (-0.01, 0.09)	0.05 (0.00, 0.10)	0.09 (0.04, 0.14)	0.0005
Adjusted difference - model 1	Reference	0.04 (-0.01, 0.09)	0.06 (0.01, 0.12)	0.06 (0.01, 0.12)	0.05
Adjusted difference - model 2	Reference	0.03 (-0.02, 0.08)	0.06 (0.01, 0.12)	0.07 (0.01, 0.12)	0.02
Serum HDL-cholesterol score					
Mean ± SD	0.01 ± 0.28	0.01 ± 0.27	0.00 ± 0.28	-0.04 ± 0.33	
Unadjusted mean difference	Reference	0.00 (-0.09, 0.10)	-0.01 (-0.11, 0.09)	-0.04 (-0.15, 0.07)	0.40
Adjusted difference - model 1	Reference	-0.03 (-0.12, 0.06)	0.00 (-0.10, 0.10)	-0.04 (-0.14, 0.07)	0.61
Adjusted difference - model 2	Reference	0.00 (-0.09, 0.09)	0.01 (-0.09, 0.12)	-0.04 (-0.15, 0.07)	0.42
Serum triglycerides score					
Mean ± SD	-0.01 ± 0.48	0.07 ± 0.46	-0.01 ± 0.38	-0.01 ± 0.40	
Unadjusted mean difference	Reference	0.07 (-0.09, 0.24)	-0.01 (-0.16, 0.15)	0.00 (-0.16, 0.16)	0.69
Adjusted difference - model 1	Reference	0.12 (-0.04, 0.28)	0.05 (-0.13, 0.22)	0.03 (-0.15, 0.21)	0.87
Adjusted difference - model 2	Reference	0.08 (-0.08, 0.24)	0.02 (-0.15, 0.20)	0.05 (-0.14, 0.23)	0.83

Footnotes to Table 2.4

[1]The overall score was calculated as the average of the five component scores, after the HDL-cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, MAP, serum HDL-cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Test for linear trend from linear regression models with the metabolic score or each component as the outcome and a variable representing medians of ordinal categories of plasma Hcys introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[4]From linear regression models. Model 1 for the metabolic score and waits circumference component was adjusted for vitamin B6, vitamin B12, erythrocyte folate, height-for-age Z score, maternal height, highest parental education level, household food security, number of household assets, and country of origin. Model 1 for the rest of components was additionally adjusted for BMI-for age Z score.

[5]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of dairy, meat, fish, and green leafy vegetables.

Table 2.5. Prevalence, unadjusted and adjusted prevalence ratios (PR) and 95% CI of metabolic syndrome[1] and its components by vitamin B6 (PLP[2]) quartiles among adults from Mesoamerica

Median plasma PLP[2] (nmol/L)	Q1 24 (n=110)		Q2 36 (n=119)		Q3 53 (n=142)		Q4 89 (n=141)	P, trend[3]
Metabolic Syndrome								
Prevalence (%)	44.6		44.5		28.9		30.5	
Unadjusted PR	Reference	1.00	(0.74, 1.36)	0.65	(0.46, 0.91)	0.68	(0.49, 0.95)	0.009
Adjusted PR - model 1[4]	Reference	0.97	(0.71, 1.34)	0.61	(0.43, 0.86)	0.66	(0.46, 0.93)	0.01
Adjusted PR - model 2[5]	Reference	0.95	(0.69, 1.31)	0.59	(0.41, 0.83)	0.67	(0.47, 0.95)	0.02
Abdominal obesity[6]								
Prevalence (%)	63.6		52.1		40.1		36.2	
Unadjusted PR	Reference	0.82	(0.66, 1.02)	0.63	(0.50, 0.80)	0.57	(0.44, 0.74)	<0.0001
Adjusted PR - model 1	Reference	0.81	(0.66, 1.01)	0.73	(0.57, 0.92)	0.71	(0.54, 0.94)	0.03
Adjusted PR - model 2	Reference	0.81	(0.64, 1.02)	0.71	(0.55, 0.91)	0.71	(0.54, 0.95)	0.04
High fasting blood glucose[7]								
Prevalence (%)	10.0		11.8		8.5		6.4	
Unadjusted PR	Reference	1.18	(0.54, 2.56)	0.85	(0.36, 1.96)	0.64	(0.26, 1.56)	0.18
Adjusted PR - model 1	Reference	1.56	(0.67, 3.66)	1.10	(0.45, 2.68)	0.62	(0.22, 1.74)	0.13
Adjusted PR - model 2	Reference	1.44	(0.59, 3.52)	0.87	(0.34, 2.23)	0.60	(0.22, 1.63)	0.12
High blood pressure[8]								
Prevalence (%)	25.5		22.7		21.3		17.7	
Unadjusted PR	Reference	0.89	(0.56, 1.43)	0.84	(0.54, 1.30)	0.70	(0.43, 1.13)	0.14
Adjusted PR - model 1	Reference	0.80	(0.45, 1.42)	0.74	(0.46, 1.21)	0.60	(0.37, 0.98)	0.05
Adjusted PR - model 2	Reference	0.85	(0.49, 1.48)	0.80	(0.50, 1.29)	0.65	(0.41, 1.04)	0.08
Low serum HDL-cholesterol[9]								
Prevalence (%)	84.6		84.9		81.0		75.2	
Unadjusted PR	Reference	1.00	(0.90, 1.12)	0.96	(0.86, 1.06)	0.89	(0.79, 1.00)	0.03
Adjusted PR - model 1	Reference	0.99	(0.89, 1.11)	0.96	(0.87, 1.07)	0.93	(0.82, 1.05)	0.19
Adjusted PR - model 2	Reference	0.97	(0.87, 1.09)	0.97	(0.87, 1.08)	0.92	(0.81, 1.04)	0.21
High serum triglycerides[10]								
Prevalence (%)	50.9		53.8		52.1		50.4	
Unadjusted PR	Reference	1.06	(0.82, 1.36)	1.02	(0.81, 1.30)	0.99	(0.77, 1.26)	0.75
Adjusted PR - model 1	Reference	1.12	(0.87, 1.45)	1.03	(0.81, 1.29)	0.99	(0.77, 1.27)	0.60
Adjusted PR - model 2	Reference	1.08	(0.83, 1.40)	1.04	(0.82, 1.32)	0.96	(0.74, 1.25)	0.54

Footnotes to Table 2.5

[1]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 triglycerides.

[2]PLP stands for pyridoxal 5' phosphate which is the biologically active metabolite of vitamin B6.

[3]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing medians of ordinal categories of vitamin B6 introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[4]From Poisson regression models. Model 1 for MetS and abdominal obesity was adjusted for vitamin B12, erythrocyte folate, age, sex, smoking and education level, number of household assets, household food security, and country of origin. Model 1 for the rest of the components was additionally adjusted for BMI category.

[5]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of meat, fish, fortified foods, and green leafy vegetables.

[6]Waist circumference >102 cm in men and >88 cm in women.

[7]Fasting blood glucose ≥100 mg/dL

[8]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[9]HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL-cholesterol.

[10]Triglycerides ≥150 mg/dL or receiving treatment for hyperlipidemia.

Table 2.6. Prevalence, unadjusted and adjusted prevalence ratios (PR) and 95% CI of metabolic syndrome[1] and its components by vitamin B12 quartiles among adults from Mesoamerica

Median plasma vitamin B12 (pmol/L)	Q1 155 (n=130)		Q2 232 (n=130)		Q3 345 (n=130)		Q4 619 (n=130)	P, trend[2]
Metabolic Syndrome								
Prevalence (%)	25.4		43.9		37.7		39.2	
Unadjusted PR	Reference	1.73	(1.20, 2.49)	1.48	(1.01, 2.18)	1.55	(1.06, 2.24)	0.15
Adjusted PR - model 1[3]	Reference	1.61	(1.12, 2.31)	1.40	(0.96, 2.06)	1.42	(0.97, 2.10)	0.39
Adjusted PR - model 2[4]	Reference	1.67	(1.14, 2.43)	1.47	(0.99, 2.17)	1.50	(1.00, 2.26)	0.28
Abdominal obesity[5]								
Prevalence (%)	41.5		50.0		51.5		44.6	
Unadjusted PR	Reference	1.20	(0.92, 1.58)	1.24	(0.95, 1.61)	1.07	(0.82, 1.41)	0.98
Adjusted PR - model 1	Reference	1.12	(0.87, 1.43)	1.14	(0.87, 1.50)	0.98	(0.74, 1.28)	0.56
Adjusted PR - model 2	Reference	1.10	(0.85, 1.43)	1.15	(0.87, 1.52)	0.97	(0.74, 1.29)	0.56
High fasting blood glucose[6]								
Prevalence (%)	3.1		10.8		9.2		13.1	
Unadjusted PR	Reference	3.50	(1.21, 10.13)	3.00	(0.98, 9.18)	4.25	(1.51, 11.98)	0.01
Adjusted PR - model 1	Reference	4.06	(1.45, 11.37)	2.88	(0.98, 8.42)	5.04	(1.88, 13.48)	0.02
Adjusted PR - model 2	Reference	4.29	(1.40, 13.12)	2.52	(0.75, 8.44)	5.08	(1.89, 13.67)	0.03
High blood pressure[7]								
Prevalence (%)	20.0		21.7		21.5		26.2	
Unadjusted PR	Reference	1.09	(0.66, 1.78)	1.08	(0.67, 1.74)	1.31	(0.83, 2.07)	0.21
Adjusted PR - model 1	Reference	1.03	(0.63, 1.68)	0.95	(0.58, 1.54)	1.37	(0.86, 2.20)	0.13
Adjusted PR - model 2	Reference	1.14	(0.68, 1.90)	1.11	(0.64, 1.92)	1.74	(0.99, 3.05)	0.03
Low serum HDL-cholesterol[8]								
Prevalence (%)	83.9		77.7		81.5		83.1	
Unadjusted PR	Reference	0.93	(0.82, 1.05)	0.97	(0.86, 1.09)	0.99	(0.88, 1.11)	0.73
Adjusted PR - model 1	Reference	0.90	(0.79, 1.03)	0.95	(0.83, 1.08)	0.94	(0.83, 1.06)	0.64
Adjusted PR - model 2	Reference	0.88	(0.77, 1.01)	0.94	(0.82, 1.06)	0.94	(0.83, 1.07)	0.82
High serum triglycerides[9]								
Prevalence (%)	48.5		53.9		48.5		55.4	
Unadjusted PR	Reference	1.11	(0.87, 1.41)	1.00	(0.77, 1.29)	1.14	(0.91, 1.44)	0.36
Adjusted PR - model 1	Reference	1.17	(0.92, 1.51)	1.00	(0.77, 1.30)	1.21	(0.94, 1.56)	0.25
Adjusted PR - model 2	Reference	1.16	(0.90, 1.49)	1.01	(0.77, 1.32)	1.22	(0.93, 1.60)	0.21

Footnotes to Table 2.6

[1]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 triglycerides.

[2]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing medians of ordinal categories of vitamin B12 introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[3]From Poisson regression models. Model 1 for MetS and abdominal obesity was adjusted for vitamin B6, erythrocyte folate, age, sex, smoking and education level, number of household assets, household food security, and country of origin. Model 1 for the rest of the components was additionally adjusted for BMI category.

[4]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of meat, fish, fortified foods, and green leafy vegetables.

[5]Waist circumference >102 cm in men and >88 cm in women.

[6]Fasting blood glucose ≥100 mg/dL

[7]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[8]HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL-cholesterol.

[9]Triglycerides ≥150 mg/dL or receiving treatment for hyperlipidemia.

Table 2.7. Prevalence, unadjusted and adjusted prevalence ratios (PR) and 95% CI of metabolic syndrome[1] and its components by folate quartiles among adults from Mesoamerica

Median erythrocyte folate (nmol/L)	Q1 413 (n=130)		Q2 699 (n=130)		Q3 891 (n=130)		Q4 1218 (n=132)	P, trend[2]
Metabolic Syndrome								
Prevalence (%)	32.3		30.0		38.5		45.5	
Unadjusted PR	Reference	0.93	(0.65, 1.34)	1.19	(0.84, 1.68)	1.41	(1.02, 1.94)	0.02
Adjusted PR - model 1[3]	Reference	1.19	(0.77, 1.84)	1.51	(0.96, 2.37)	1.83	(1.18, 2.84)	0.003
Adjusted PR - model 2[4]	Reference	1.29	(0.83, 2.02)	1.62	(1.02, 2.57)	1.97	(1.22, 3.17)	0.003
Abdominal obesity[5]								
Prevalence (%)	44.6		43.9		50.0		50.0	
Unadjusted PR	Reference	0.98	(0.76, 1.27)	1.12	(0.88, 1.43)	1.12	(0.87, 1.44)	0.27
Adjusted PR - model 1	Reference	1.06	(0.80, 1.41)	1.21	(0.89, 1.64)	1.21	(0.86, 1.70)	0.25
Adjusted PR - model 2	Reference	1.09	(0.81, 1.46)	1.24	(0.89, 1.73)	1.25	(0.88, 1.80)	0.20
High fasting blood glucose[6]								
Prevalence (%)	6.2		6.2		11.5		12.1	
Unadjusted PR	Reference	1.00	(0.39, 2.56)	1.88	(0.83, 4.26)	1.97	(0.87, 4.48)	0.05
Adjusted PR - model 1	Reference	1.00	(0.33, 3.04)	1.75	(0.54, 5.69)	1.76	(0.55, 5.65)	0.27
Adjusted PR - model 2	Reference	1.30	(0.38, 4.42)	2.48	(0.64, 9.57)	2.83	(0.75, 10.68)	0.08
High blood pressure[7]								
Prevalence (%)	23.9		24.8		20.0		20.5	
Unadjusted PR	Reference	1.04	(0.68, 1.59)	0.84	(0.52, 1.35)	0.86	(0.54, 1.37)	0.41
Adjusted PR - model 1	Reference	0.97	(0.56, 1.70)	0.84	(0.46, 1.56)	1.10	(0.56, 2.14)	0.70
Adjusted PR - model 2	Reference	0.89	(0.49, 1.60)	0.74	(0.38, 1.44)	1.09	(0.53, 2.25)	0.63
Low serum HDL-cholesterol[8]								
Prevalence (%)	76.9		79.2		80.0		88.6	
Unadjusted PR	Reference	1.03	(0.91, 1.17)	1.04	(0.92, 1.17)	1.15	(1.03, 1.29)	0.01
Adjusted PR - model 1	Reference	1.09	(0.95, 1.26)	1.06	(0.91, 1.23)	1.20	(1.02, 1.40)	0.03
Adjusted PR - model 2	Reference	1.13	(0.97, 1.31)	1.07	(0.93, 1.24)	1.19	(1.01, 1.39)	0.06
High serum triglycerides[9]								
Prevalence (%)	41.5		43.9		60.8		59.9	
Unadjusted PR	Reference	1.06	(0.81, 1.37)	1.46	(1.16, 1.85)	1.44	(1.14, 1.82)	0.0002
Adjusted PR - model 1	Reference	0.93	(0.70, 1.24)	1.31	(0.97, 1.77)	1.33	(0.97, 1.81)	0.02
Adjusted PR - model 2	Reference	0.91	(0.68, 1.22)	1.27	(0.93, 1.73)	1.25	(0.90, 1.74)	0.06

Footnotes to Table 2.7

[1]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 triglycerides.

[2]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing medians of ordinal categories of erythrocyte folate introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[3]From Poisson regression models. Model 1 for MetS and abdominal obesity was adjusted for vitamin B6, vitamin B12, age, sex, smoking and education level, number of household assets, household food security, and country of origin. Model 1 for the rest of the components was additionally adjusted for BMI category.

[4]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of meat, fish, fortified foods, and green leafy vegetables.

[5]Waist circumference >102 cm in men and >88 cm in women.

[6]Fasting blood glucose ≥100 mg/dL

[7]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[8]HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL-cholesterol.

[9]Triglycerides ≥150 mg/dL or receiving treatment for hyperlipidemia.

Table 2.8. Prevalence, unadjusted and adjusted prevalence ratios (PR) and 95% CI of metabolic syndrome[1] and its components by homocysteine (Hcys) quartiles among adults from Mesoamerica

Median plasma Hcys (μmol/L)	Q1 5.2 (n=129)	Q2 7.8 (n=131)		Q3 10.2 (n=130)		Q4 15.2 (n=130)		P, trend[2]
Metabolic Syndrome								
Prevalence (%)	42.6	32.8		36.59		33.9		
Unadjusted PR	Reference	0.77	(0.56, 1.05)	0.87	(0.65, 1.16)	0.79	(0.57, 1.10)	0.30
Adjusted PR - model 1[3]	Reference	0.71	(0.51, 0.98)	0.82	(0.58, 1.14)	0.78	(0.54, 1.12)	0.39
Adjusted PR - model 2[4]	Reference	0.71	(0.50, 1.00)	0.75	(0.52, 1.07)	0.72	(0.48, 1.09)	0.24
Abdominal obesity[5]								
Prevalence (%)	59.7	52.7		38.5		36.9		
Unadjusted PR	Reference	0.88	(0.72, 1.09)	0.64	(0.49, 0.84)	0.62	(0.48, 0.80)	0.0002
Adjusted PR - model 1	Reference	1.09	(0.88, 1.36)	1.06	(0.81, 1.38)	1.30	(0.98, 1.72)	0.10
Adjusted PR - model 2	Reference	1.11	(0.88, 1.40)	1.01	(0.77, 1.34)	1.27	(0.95, 1.69)	0.18
High fasting blood glucose[6]								
Prevalence (%)	13.2	6.1		8.5		8.5		
Unadjusted PR	Reference	0.46	(0.21, 1.04)	0.64	(0.31, 1.35)	0.64	(0.30, 1.36)	0.43
Adjusted PR - model 1	Reference	0.40	(0.16, 0.98)	0.72	(0.28, 1.91)	0.88	(0.37, 2.09)	0.87
Adjusted PR - model 2	Reference	0.33	(0.12, 0.90)	0.57	(0.19, 1.71)	0.72	(0.28, 1.85)	0.85
High blood pressure[7]								
Prevalence (%)	17.1	20.0		26.9		25.4		
Unadjusted PR	Reference	1.17	(0.72, 1.92)	1.58	(1.01, 2.47)	1.49	(0.90, 2.45)	0.08
Adjusted PR - model 1	Reference	0.65	(0.36, 1.17)	0.83	(0.48, 1.43)	0.57	(0.30, 1.11)	0.15
Adjusted PR - model 2	Reference	0.70	(0.39, 1.27)	0.85	(0.47, 1.52)	0.56	(0.28, 1.14)	0.12
Low serum HDL-cholesterol[8]								
Prevalence (%)	84.5	77.1		80.8		83.9		
Unadjusted PR	Reference	0.91	(0.81, 1.03)	0.96	(0.85, 1.07)	0.99	(0.89, 1.11)	0.76
Adjusted PR - model 1	Reference	0.95	(0.83, 1.09)	1.01	(0.89, 1.14)	1.04	(0.91, 1.18)	0.36
Adjusted PR - model 2	Reference	0.95	(0.83, 1.08)	1.01	(0.88, 1.14)	1.06	(0.92, 1.20)	0.24
High serum triglycerides[9]								
Prevalence (%)	49.6	44.3		54.6		57.7		
Unadjusted PR	Reference	0.89	(0.68, 1.17)	1.10	(0.87, 1.40)	1.16	(0.92, 1.47)	0.08
Adjusted PR - model 1	Reference	0.78	(0.59, 1.02)	0.87	(0.68, 1.12)	0.86	(0.66, 1.12)	0.62
Adjusted PR - model 2	Reference	0.77	(0.58, 1.02)	0.86	(0.66, 1.11)	0.83	(0.63, 1.10)	0.48

Footnotes to Table 2.8

[1]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 triglycerides.

[2]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing medians of ordinal categories of Hcys introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[3]From Poisson regression models. Model 1 for MetS and abdominal obesity was adjusted for vitamin B6, vitamin B12, erythrocyte folate, age, sex, smoking and education level, number of household assets, household food security, and country of origin. Model 1 for the rest of the components was additionally adjusted for BMI category.

[4]Model 2 includes covariates from model 1 plus log-transformed total energy intake and consumption of meat, fish, fortified foods, and green leafy vegetables.

[5]Waist circumference >102 cm in men and >88 cm in women.

[6]Fasting blood glucose ≥100 mg/dL

[7]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[8]HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL-cholesterol.

[9]Triglycerides ≥150 mg/dL or receiving treatment for hyperlipidemia.

Supplemental Table 2.1. Mean (± SD) plasma concentrations of vitamins B6 and B12, erythrocyte folate and homocysteine (Hcys) according to sociodemographic characteristics among children from Mesoamerica

Characteristics	N[1]	Plasma PLP[2] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)	Plasma Hcys (µmol/L)
Overall	237	68 ± 40	406 ± 234	828 ± 270	7.1 ± 3.3
Sex					
Female	124	66 ± 34	428 ± 230	839 ± 290	6.6 ± 2.9
Male	113	70 ± 46	381 ± 237	815 ± 247	7.6 ± 3.7
P[3]		0.98	0.02	0.49	0.05
Age (years)					
<9	79	72 ± 47	423 ± 251	839 ± 293	6.5 ± 3.2
9 - <11	82	68 ± 39	412 ± 186	841 ± 270	6.4 ± 2.5
≥11	76	64 ± 34	383 ± 262	801 ± 246	8.4 ± 3.9
P, trend[4]		0.31	0.16	0.39	0.0006
Height-for-age Z score					
<-2	13	58 ± 26	317 ± 172	867 ± 211	7.7 ± 1.6
-2 - <-1	45	66 ± 26	442 ± 315	863 ± 215	7.2 ± 2.7
-1 - <0	89	78 ± 54	389 ± 187	852 ± 308	7.3 ± 4.0
0 - <1	55	59 ± 30	427 ± 249	771 ± 277	6.7 ± 3.2
≥1	35	66 ± 30	402 ± 218	796 ± 229	7.0 ± 2.9
P, trend		0.42	0.53	0.04	0.15
BMI-for-age Z score					
<-1	34	65 ± 44	494 ± 332	890 ± 265	6.7 ± 3.0
-1 - <0	49	57 ± 20	417 ± 197	803 ± 286	6.5 ± 2.0
0 - <1	71	75 ± 48	351 ± 173	800 ± 221	7.4 ± 3.9
≥1	83	69 ± 39	412 ± 244	840 ± 299	7.3 ± 3.5
P, trend		0.24	0.15	0.64	0.32
Maternal age (years)					
<30	36	60 ± 32	366 ± 199	823 ± 277	7.6 ± 3.1
30 - <35	51	61 ± 25	369 ± 181	815 ± 273	7.0 ± 2.4
35 - <40	78	73 ± 42	423 ± 229	857 ± 282	7.2 ± 3.6
40 - <45	45	74 ± 51	440 ± 261	794 ± 246	6.6 ± 3.2
≥45	27	71 ± 45	426 ± 322	827 ± 269	7.2 ± 4.6
P, trend		0.13	0.24	0.91	0.11
Maternal height (cm)					
Q1 (148.9)	62	63 ± 37	337 ± 168	832 ± 255	6.4 ± 2.6
Q2 (153.2)	58	79 ± 46	416 ± 292	881 ± 286	6.4 ± 2.9
Q3 (157.1)	60	65 ± 42	474 ± 240	828 ± 294	7.8 ± 4.2
Q4 (162.0)	57	65 ± 35	399 ± 206	769 ± 237	7.8 ± 3.2
P, trend		0.67	0.02	0.11	0.003
Maternal body mass index (kg/m^2)					
<25	52	67 ± 48	401 ± 260	802 ± 272	6.2 ± 2.3
25 - <30	92	73 ± 44	412 ± 211	846 ± 265	6.9 ± 3.4
≥30	93	64 ± 31	403 ± 242	825 ± 276	7.8 ± 3.6
P, trend		0.82	0.87	0.74	0.006
Maternal parity					
1	16	81 ± 58	421 ± 214	750 ± 370	6.8 ± 2.6
2	77	69 ± 38	414 ± 251	868 ± 275	6.7 ± 2.8
3	81	70 ± 40	434 ± 216	815 ± 278	6.7 ± 3.1
≥4	62	62 ± 38	348 ± 229	807 ± 211	8.2 ± 4.1
P, trend		0.08	0.04	0.62	0.04

Characteristics	N[1]	Plasma PLP[2] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)	Plasma Hcys (μmol/L)
Paternal height (cm)					
Q1 (159.0)	60	73 ± 35	387 ± 272	854 ± 261	7.7 ± 4.0
Q2 (165.0)	61	67 ± 41	361 ± 180	857 ± 254	7.1 ± 3.1
Q3 (169.6)	56	78 ± 54	425 ± 252	782 ± 269	6.6 ± 3.3
Q4 (176.4)	56	57 ± 23	462 ± 222	814 ± 305	6.9 ± 2.8
P, trend		0.03	0.009	0.23	0.21
Paternal body mass index (kg/m^2)					
<25	62	67 ± 39	407 ± 236	821 ± 256	6.5 ± 2.6
25 - <30	102	69 ± 35	422 ± 273	843 ± 261	7.7 ± 4.0
≥30	69	70 ± 49	387 ± 167	811 ± 302	6.7 ± 2.8
P, trend		0.85	0.80	0.83	0.74
Parental smoking history					
Neither parent ever smoked	87	74 ± 47	414 ± 219	804 ± 287	7.1 ± 3.1
One parent ever smoked	116	66 ± 38	394 ± 238	857 ± 269	7.1 ± 3.5
Both parents ever smoked	28	62 ± 26	397 ± 177	800 ± 220	7.3 ± 3.4
P		0.18	0.75	0.59	0.97
Parental metabolic syndrome					
No parent	91	68 ± 34	431 ± 234	839 ± 231	6.8 ± 3.1
Mother only	56	67 ± 36	398 ± 292	837 ± 286	7.6 ± 4.2
Father only	49	73 ± 51	415 ± 197	801 ± 311	6.7 ± 2.5
Both parents	34	66 ± 47	363 ± 186	822 ± 305	7.6 ± 3.4
P, trend		0.48	0.17	0.54	0.31
Highest parental education level					
Incomplete elementary	21	54 ± 22	267 ± 112	747 ± 272	9.2 ± 4.5
Complete elementary	27	53 ± 20	312 ± 125	821 ± 187	7.3 ± 2.6
Incomplete secondary	64	72 ± 46	410 ± 235	870 ± 280	7.2 ± 3.3
Complete secondary	44	74 ± 38	505 ± 332	832 ± 312	6.0 ± 2.9
Post secondary	81	71 ± 44	417 ± 193	814 ± 261	7.0 ± 3.3
P, trend		0.02	<0.0001	0.79	0.003
Number of household assets[5]					
0-4	45	49 ± 18	296 ± 155	813 ± 233	7.8 ± 4.2
5-7	91	73 ± 46	413 ± 282	832 ± 279	6.7 ± 2.9
8-9	45	59 ± 25	390 ± 167	771 ± 303	7.0 ± 3.2
10-12	56	82 ± 46	492 ± 213	878 ± 253	7.2 ± 3.4
P, trend		0.0002	<0.0001	0.36	0.61
Household income					
Lower <25%	45	57 ± 27	369 ± 170	806 ± 273	7.7 ± 3.7
Medium 25-75%	108	69 ± 40	406 ± 270	842 ± 282	6.8 ± 3.0
Higher >75%	79	73 ± 47	433 ± 217	822 ± 260	7.1 ± 3.6
P, trend		0.04	0.07	0.85	0.18
Food insecurity					
No insecurity	77	75 ± 45	445 ± 205	908 ± 292	7.1 ± 3.0
Mild insecurity	65	69 ± 38	428 ± 278	826 ± 280	6.8 ± 3.1
Moderate insecurity	54	67 ± 43	404 ± 243	777 ± 224	7.2 ± 4.0
Severe insecurity	40	56 ± 28	305 ± 166	738 ± 226	7.5 ± 3.4

Characteristics	N[1]	Plasma PLP[2] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)	Plasma Hcys (μmol/L)
P, trend		0.005	<0.0001	0.0002	0.50
Country of origin					
Guatemala	29	66 ± 48	306 ± 164	891 ± 224	6.2 ± 1.7
El Salvador	29	83 ± 60	285 ± 115	760 ± 190	4.5 ± 2.0
Dominican Republic	28	63 ± 33	354 ± 189	556 ± 205	7.5 ± 3.4
Honduras	28	77 ± 50	517 ± 209	1057 ± 216	6.5 ± 2.8
Nicaragua	31	52 ± 22	349 ± 165	768 ± 236	8.5 ± 3.6
Panama	18	58 ± 32	475 ± 183	678 ± 216	9.0 ± 2.2
Costa Rica	20	77 ± 33	513 ± 183	949 ± 223	9.2 ± 2.8
Mexico	27	76 ± 35	527 ± 356	1025 ± 281	5.4 ± 2.3
Belize	27	60 ± 26	399 ± 302	757 ± 206	8.3 ± 4.7
P		0.99	0.0002	0.07	0.002

Footnotes to Supplemental Table 2.1

[1]Total may be less than 237 due to missing values.

[2]PLP stands for pyridoxal phosphate which is the biologically active metabolite of vitamin B6.

[3]χ^2 score statistic from linear regression models with each vitamin and homocysteine as the outcome and indicator variables of the characteristic as predictors.

[4]Test for linear trend from linear regression models with each vitamin and homocysteine as the outcome and a variable representing ordinal categories of each characteristic as a continuous predictor. An independent covariance structure was specified in all models to obtain empirical variances.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

Supplemental Table 2.2. Mean (± SD) concentrations of plasma vitamins B6 and B12, folate, and homocysteine (Hcys) according to dietary intake of food groups among children from Mesoamerica

	N[1]	Plasma PLP[2] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)
Dairy[3]				
≤ 4 servings per day	52	61 ± 30	317 ± 154	766 ± 243
5-6 servings per day	79	65 ± 41	411 ± 207	794 ± 267
7 servings per day	43	71 ± 34	485 ± 295	888 ± 277
≥ 8 servings per day	52	79 ± 53	445 ± 266	886 ± 294
P-trend[4]		0.02	0.005	0.007
Meat[5]				
≤ 4 servings per week	61	64 ± 34	364 ± 240	793 ± 184
5-6 servings per week	59	70 ± 49	409 ± 203	835 ± 287
1.5 servings per day	28	70 ± 36	439 ± 256	867 ± 305
> 1.5 serving per day	78	71 ± 53	439 ± 227	762 ± 306
P-trend		0.44	0.005	0.36
Fish[6]				
≤ 3 servings per month	46	62 ± 28	337 ± 179	799 ± 227
One serving per week	59	72 ± 54	380 ± 204	802 ± 276
2-4 servings per week	72	68 ± 35	453 ± 265	910 ± 296
≥ 5 servings per week	49	71 ± 42	456 ± 258	759 ± 252
P-trend		0.34	0.0006	0.63
Green leafy vegetables[7]				
One serving per day	62	69 ± 47	402 ± 216	779 ± 272
2-3 servings per day	72	69 ± 42	392 ± 249	807 ± 255
4-5 serving per day	43	63 ± 28	453 ± 284	834 ± 300
≥ 6 servings per day	49	71 ± 40	413 ± 194	910 ± 265
P-trend		0.54	0.29	0.01
Fortified foods[8]				
≤ 4 servings per day	41	60 ± 22	384 ± 252	723 ± 258
5-6 servings per day	59	64 ± 32	477 ± 290	832 ± 308
7-8 servings per day	51	72 ± 48	431 ± 210	799 ± 242
> 8 servings per day	75	75 ± 48	359 ± 180	899 ± 256
P-trend		0.06	0.22	0.002

Footnotes to Supplemental Table 2.2

[1]N may be less than 237 due to missing values on specific items of the FFQ.

[2]PLP stand for pyridoxal phosphate which is the biologically active metabolite of vitamin B6.

[3]Dairy includes the following portion sizes: milk (one glass), American (one slice), fresh (one piece) and cream cheese (one tablespoon), and cream (one tablespoon).

[4]Test for linear trend from linear regression models with each vitamin concentration as the outcome and a variable representing ordinal categories of each food group as a continuous predictor.

[5]Meat includes beef or pork as main and side dishes (one portion), ham (one slice), hot dog (one portion), and hamburger (one portion).

[6]Fish includes canned tuna or sardines (one portion) and fish (one portion).

[7]Green leafy vegetable includes herbs and green leaves (1/2 cup), broccoli (1/2 cup), and spinach (1/2 cup).

[8]Fortified food includes white and sweet bread (one portion), flour and corn tortillas (one unit) and breakfast cereal (one cup).

Supplemental Table 2.3. Mean (± SD) plasma concentrations of vitamins B6 and B12, erythrocyte folate and homocysteine (Hcys) by sociodemographic characteristics among adults from Mesoamerica

Characteristics	N	Plasma PLP[1] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)	Plasma Hcys (µmol/L)
Overall	524	64 ± 72	390 ± 345	800 ± 349	10.2 ± 6.0
Sex					
Female	264	55 ± 60	423 ± 395	853 ± 356	8.4 ± 5.0
Male	260	74 ± 81	356 ± 283	745 ± 333	12.0 ± 6.4
P[2]		<0.0001	0.07	<0.0001	<0.0001
Age (years)					
<30	56	62 ± 60	376 ± 359	808 ± 380	9.8 ± 6.3
30 - <35	110	65 ± 82	359 ± 296	759 ± 298	10.2 ± 5.8
35 - <40	163	62 ± 66	403 ± 364	816 ± 349	9.2 ± 4.0
40 - <45	106	60 ± 64	348 ± 260	759 ± 390	11.2 ± 7.8
45 - <5	69	69 ± 87	484 ± 447	832 ± 327	10.6 ± 6.2
55+	19	93 ± 72	398 ± 391	985 ± 326	11.3 ± 8.0
P, trend[3]		0.28	0.14	0.22	0.15
Height quartile (mothers/fathers medians, cm)					
Q1 (148.9/159.0)	130	56 ± 37	374 ± 345	859 ± 348	11.1 ± 8.1
Q2 (153.1/165.0)	132	72 ± 96	423 ± 426	810 ± 358	9.8 ± 4.9
Q3 (157.0/169.7)	133	63 ± 66	366 ± 273	773 ± 348	9.7 ± 4.8
Q4 (162.7/176.4)	129	67 ± 75	396 ± 319	757 ± 337	10.1 ± 5.6
P, trend		0.72	0.22	0.01	0.65
Body mass index (kg/m^2)					
<25	129	63 ± 61	373 ± 314	759 ± 361	10.3 ± 6.1
25-<30	216	73 ± 89	365 ± 313	809 ± 349	10.3 ± 5.6
≥30	179	55 ± 53	431 ± 399	817 ± 340	9.9 ± 6.5
P, trend		0.02	0.14	0.20	0.40
Education level					
Incomplete elementary	73	66 ± 96	415 ± 366	781 ± 307	10.6 ± 7.3
Complete elementary	74	55 ± 32	375 ± 378	840 ± 293	11.3 ± 7.8
Incomplete secondary	154	69 ± 84	407 ± 362	807 ± 374	10.1 ± 5.4
Complete secondary	80	70 ± 85	418 ± 408	813 ± 422	8.9 ± 4.2
Post secondary	131	63 ± 48	357 ± 256	760 ± 323	9.8 ± 5.2
P, trend		0.20	0.84	0.42	0.15
Smoking status					
Never	331	61 ± 62	393 ± 334	801 ± 373	9.3 ± 4.7
Past	144	69 ± 68	368 ± 345	793 ± 307	11.3 ± 7.4
Current	47	74 ± 128	437 ± 425	817 ± 298	12.8 ± 8.3
P		0.49	0.67	0.92	<0.0001
Home ownership					
Yes	367	69 ± 82	399 ± 360	827 ± 333	9.9 ± 5.6
No	157	53 ± 38	369 ± 308	735 ± 376	10.7 ± 6.8
P		0.05	0.47	0.03	0.20
Number of household assets[4]					

Characteristics	N	Plasma PLP[1] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)	Plasma Hcys (µmol/L)
0-4	96	47 ± 36	355 ± 317	773 ± 294	11.2 ± 8.6
5-7	196	67 ± 68	368 ± 328	820 ± 379	10.1 ± 5.9
8-9	102	63 ± 85	415 ± 418	694 ± 336	10.0 ± 5.5
10-12	130	74 ± 83	429 ± 326	872 ± 331	9.6 ± 3.9
P, trend		0.03	0.003	0.27	0.43
Household income					
Lower <25%	100	52 ± 38	357 ± 322	769 ± 378	10.8 ± 5.5
Medium 25-75%	233	58 ± 54	389 ± 348	805 ± 354	10.0 ± 6.5
Higher >75%	179	81 ± 100	417 ± 361	807 ± 336	9.9 ± 5.7
P, trend		0.002	0.03	0.54	0.15
Food security					
No insecurity	173	69 ± 77	432 ± 379	883 ± 333	9.8 ± 4.2
Mild insecurity	138	59 ± 41	352 ± 298	789 ± 369	10.1 ± 5.4
Moderate insecurity	124	71 ± 96	372 ± 334	754 ± 323	9.9 ± 5.0
Severe insecurity	87	55 ± 59	398 ± 359	706 ± 350	11.3 ± 10.0
P, trend		0.22	0.23	0.0005	0.64
Country of origin					
Guatemala	60	51 ± 38	324 ± 255	883 ± 285	10.9 ± 6.7
El Salvador	58	85 ± 85	395 ± 457	753 ± 285	8.6 ± 4.5
Dominican Republic	60	65 ± 59	318 ± 162	344 ± 198	12.0 ± 8.5
Honduras	59	68 ± 67	315 ± 310	953 ± 262	10.9 ± 4.5
Nicaragua	62	71 ± 114	369 ± 274	754 ± 241	10.6 ± 8.5
Panama	52	51 ± 32	387 ± 248	509 ± 249	10.3 ± 3.9
Costa Rica	54	50 ± 28	403 ± 277	949 ± 178	11.4 ± 3.4
Mexico	62	58 ± 43	627 ± 536	1167 ± 250	7.8 ± 4.3
Belize	57	78 ± 106	366 ± 322	856 ± 356	8.9 ± 5.5
P		0.76	<0.0001	<0.0001	0.03

Footnotes to Supplemental Table 2.3

[1]N may be less than 524 due to missing values.

[2]PLP stands for pyridoxal phosphate which is the biologically active metabolite of vitamin B6.

[3]χ^2 score statistic from linear regression models with each vitamin and homocysteine as the outcome and indicator variables for each level of the characteristic as predictors.

[4]Test for linear trend from linear regression models with each vitamin and homocysteine as the outcome and a variable representing ordinal categories of each characteristic introduced as a continuous predictor. An independent covariance structure was specified in all models to account for clustering by family membership.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

Supplemental Table 2.4. Mean (± SD) concentrations of plasma vitamins B6 and B12, and folate according to dietary intake of food groups among adults from Mesoamerica

	N[1]	Plasma PLP[2] (nmol/L)	Plasma vitamin B12 (pmol/L)	Erythrocyte folate (nmol/L)
Dairy[3]				
≤ 2 servings per day	56	73 ± 98	373 ± 244	779 ± 361
3-4 servings per day	155	60 ± 48	406 ± 354	846 ± 345
5 servings per day	153	64 ± 75	377 ± 308	828 ± 341
> 5 servings per day	149	63 ± 70	400 ± 411	742 ± 345
P-trend[4]		0.52	0.31	0.14
Meat[5]				
≤ 1 serving per week	51	60 ± 48	290 ± 238	769 ± 276
2-4 servings per week	133	54 ± 41	375 ± 394	858 ± 325
5-6 servings per day	156	79 ± 107	459 ± 385	831 ± 359
> 6 serving per day	173	57 ± 43	376 ± 291	745 ± 366
P-trend		0.94	0.003	0.10
Fish[6]				
≤ 3 servings per month	84	66 ± 90	361 ± 349	846 ± 349
One serving per week	151	60 ± 56	361 ± 313	788 ± 346
2-4 servings per week	173	67 ± 75	411 ± 370	859 ± 341
> 4 servings per week	105	62 ± 58	433 ± 358	697 ± 338
P-trend		0.75	0.009	0.07
Green leafy vegetables[7]				
≤ 2 serving per day	149	62 ± 54	311 ± 161	703 ± 367
3 servings per day	86	55 ± 41	406 ± 345	886 ± 344
4 serving per day	121	63 ± 61	431 ± 384	844 ± 331
> 4 servings per day	157	70 ± 96	432 ± 431	819 ± 323
P-trend		0.98	0.06	0.02
Fortified foods[8]				
≤ 3 servings per day	125	58 ± 75	366 ± 271	715 ± 312
4-5 servings per day	122	73 ± 93	429 ± 354	851 ± 355
6-7 servings per day	124	59 ± 53	436 ± 406	791 ± 397
> 7 servings per day	142	64 ± 52	346 ± 346	850 ± 309
P-trend		0.36	0.03	0.01

Footnotes to Supplemental Table 2.4

[1]N may be less than 524 due to missing values on specific items of the FFQ.

[2]PLP stand for pyridoxal phosphate which is the biologically active metabolite of vitamin B6.

[3]Dairy includes the following portion sizes: American cheese (one slice), cream cheese (one tablespoon), and cream (one tablespoon).

[4]Test for linear trend from linear regression models with each vitamin concentration as the outcome and a variable representing ordinal categories of each food group as a continuous predictor.

[5]Meat includes beef or pork as main and side dishes (one portion), ham (one slice), and hamburger (one portion).

[6]Fish includes canned tuna or sardines (one portion) and fish (one portion).

[7]Green leafy vegetables include herbs and green leaves (1/2 cup), broccoli (1/2 cup), and spinach (1/2 cup).

[8]Fortified foods include white and sweet bread (one portion), flour and corn tortillas (one unit), and breakfast cereal (one cup).

Supplemental Table 2.5. Unadjusted and adjusted joint estimates of metabolic syndrome[1] by vitamins B6 (PLP[2]) and B12, erythrocyte folate and homocysteine (Hcys) among children and adults from Mesoamerica

		Children		Adults	
	N[3]	Unadjusted mean difference (95% CI)[4]	Adjusted mean difference (95% CI)[5]	Unadjusted prevalence ratio (95% CI)[4]	Adjusted prevalence ratio (95% CI)[5]
Plasma PLP[2] (nmol/L)					
Q1 (28)	183	Reference	Reference	Reference	Reference
Q2 (41)	191	-0.04 (-0.13, 0.06)	-0.03 (-0.11, 0.06)	0.99 (0.74, 1.31)	0.98 (0.73, 1.31)
Q3 (57)	172	-0.01 (-0.11, 0.08)	-0.01 (-0.09, 0.07)	0.75 (0.54, 1.06)	0.71 (0.51, 1.00)
Q4 (97)	195	-0.01 (-0.10, 0.09)	-0.01 (-0.09, 0.08)	0.78 (0.57, 1.05)	0.71 (0.51, 1.00)
P, trend[6]		0.70	0.84	0.04	0.01
Plasma vitamin B12 (pmol/L)					
Q1 (160)	187	Reference	Reference	Reference	Reference
Q2 (251)	189	-0.06 (-0.16, 0.03)	-0.07 (-0.17, 0.04)	1.83 (1.32, 2.55)	1.83 (1.29, 2.59)
Q3 (364)	190	-0.16 (-0.25, -0.08)	-0.18 (-0.27, -0.09)	1.43 (0.99, 2.08)	1.35 (0.93, 1.96)
Q4 (643)	189	-0.11 (-0.20, -0.02)	-0.14 (-0.24, 0.04)	1.50 (1.06, 2.13)	1.40 (0.97, 2.01)
P, trend		0.005	0.001	0.08	0.26
Erythrocyte folate (nmol/L)					
Q1 (456)	189	Reference	Reference	Reference	Reference
Q2 (714)	189	-0.01 (-0.06, 0.09)	0.02 (-0.06, 0.09)	1.13 (0.78, 1.64)	1.17 (0.79, 1.73)
Q3 (884)	190	0.00 (-0.08, 0.08)	0.03 (-0.05, 0.11)	1.38 (0.99, 1.93)	1.32 (0.92, 1.89)
Q4 (1208)	189	0.06 (-0.03, 0.15)	0.10 (-0.01 0.20)	1.48 (1.07, 2.05)	1.57 (1.11, 2.20)
P, trend		0.22	0.05	0.009	0.007
Hcys (µmol/L)					
Q1 (4.6)	188	Reference	Reference	Reference	Reference
Q2 (7.1)	189	-0.04 (-0.11, 0.03)	-0.02 (-0.08, 0.04)	0.84 (0.60, 1.18)	0.81 (0.56, 1.17)
Q3 (9.3)	186	-0.03 (-0.11, 0.04)	-0.03 (-0.11, 0.05)	0.95 (0.68, 1.33)	0.89 (0.60, 1.31)
Q4 (13.9)	192	-0.03 (-0.13, 0.07)	0.03 (-0.07, 0.14)	0.84 (0.59, 1.18)	0.94 (0.64, 1.38)
P, trend		0.40	0.83	0.48	0.99

Footnotes to Supplemental Table 2.5

[1]Metabolic syndrome was defined in children using a metabolic risk score. The score was computed as the average of the standardized residuals of the 5 component scores (waist circumference, HOMA-IR, MAP, serum HDL-cholesterol, and serum triglycerides) that were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models. In adults, we defined metabolic syndrome 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 triglycerides.

[2]PLP stands for pyridoxal phosphate which is the biologically active metabolite of vitamin B6.

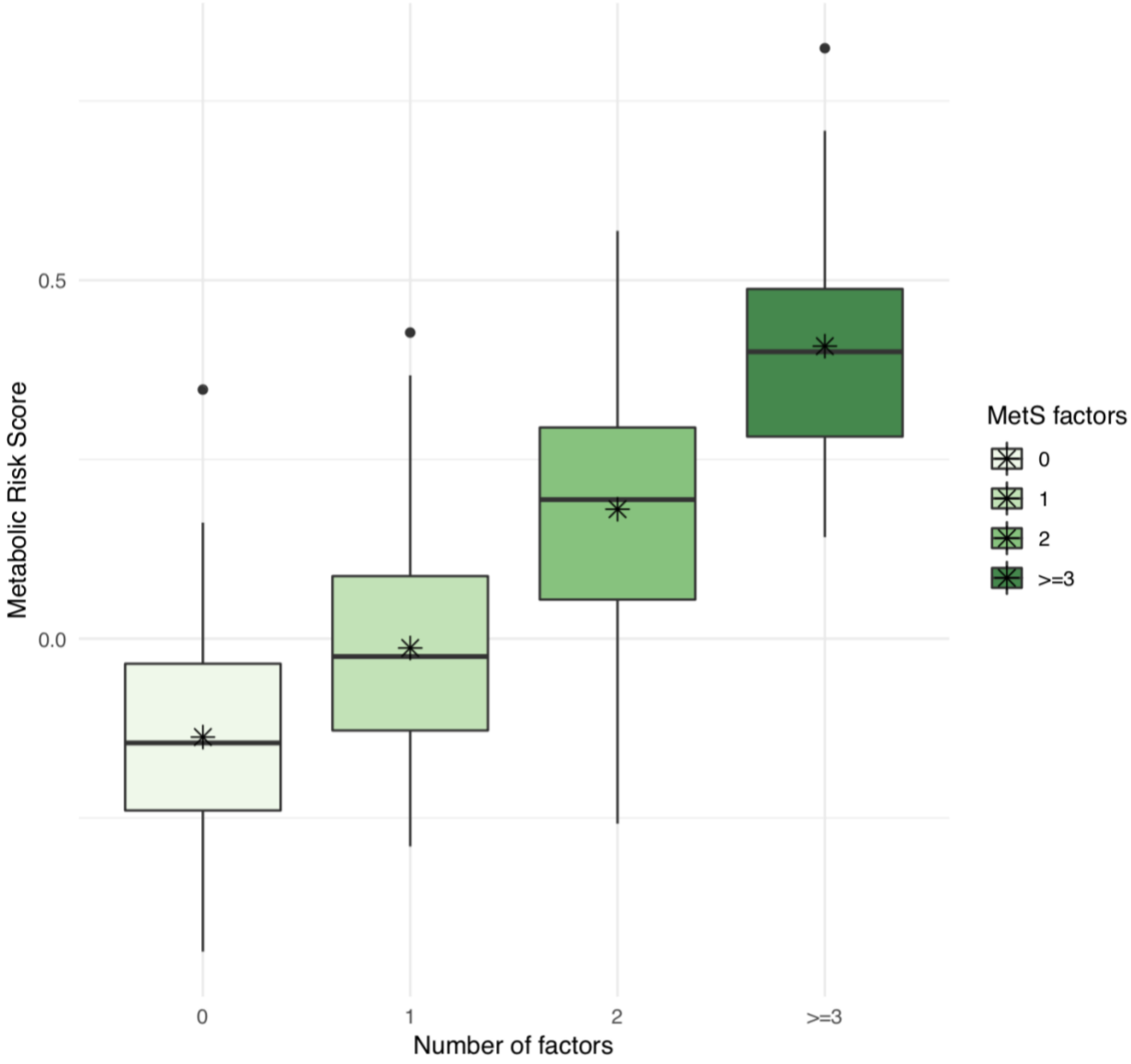
[3]N may be less than 761 due to missing values. It includes 260 fathers, 264 mothers, and 237 children.

[4]From joint regression models, using an interaction term with each of the predictors to indicate a normal distribution for the metabolic syndrome score and a Poisson distribution for metabolic syndrome presence.

[5]From joint regression models. Models for the vitamins were adjusted for each other but not for Hcys, and the model for Hcys was adjusted for all vitamins. Additionally, models were adjusted for age, sex, education level, food security, household assets, and country of origin.

[6]Test for linear trend from joint regression models with a variable representing medians of ordinal categories of the predictor introduced as continuous. An unstructured covariance matrix using the Cholesky parametrization was used in all models to account for clustering by family membership.

Supplemental Figure 2.1. Boxplot of the metabolic risk score by the number of metabolic syndrome (MetS) in children from Mesoamerica



Chapter 3 . Urinary sodium and iodine and metabolic syndrome in children and their adult parents

Abstract

Background and aims: High sodium consumption is a known risk factor for hypertension and cardiovascular disease, but its role on Metabolic Syndrome (MetS) remains controversial. Additionally, there is scant evidence about the role of iodine on MetS. We evaluated the associations between these factors with MetS among Mesoamerican children and their adult parents.

Methods: We conducted a cross-sectional study among 217 children and 478 parents from the capitals of Belize, Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Chiapas State in Mexico. Exposures were urinary sodium and iodine obtained from 24h urine samples. In children, the outcome was a continuous metabolic risk score calculated through sex- and age-standardization of waist circumference, the homeostatic model assessment for insulin resistance (HOMA-IR), mean arterial pressure (MAP), serum high-density lipoprotein cholesterol (HDL-C), and serum triglycerides. In parents, the outcome was the prevalence of MetS according to the Adult Treatment Panel (ATP) III criteria[1]. We estimated mean differences in the metabolic risk score and prevalence ratios of MetS between recommended sodium and excessive iodine intakes based on the World Health Organization (WHO) definitions using multivariable-adjusted linear and Poisson regression models, respectively.

Results: Among children, excessive iodine intake was positively associated with the metabolic risk score. Children with excessive intake had an adjusted 0.12 units higher metabolic score compared to those without excessive intake (95% CI: 0.05, 0.19; P, trend = 0.0008). Similarly, excessive iodine intake was positively associated with insulin resistance and triglyceride component scores. In adults, exceeding the recommended sodium intake was positively associated with MetS and high blood pressure prevalence. Similarly, excessive iodine intake was positively associated with MetS prevalence.

Conclusions: In adults, exceeding the recommended sodium intake was associated with MetS and high blood pressure, therefore, achieving the WHO recommendations should be encouraged. An excessive iodine intake showed adverse metabolic effects in both children and adults. Hence salt iodization fortification programs should be closely monitored.

Keywords: Metabolic syndrome, urinary sodium, urinary iodine, recommended sodium intake, iodine excess.

Introduction

The metabolic syndrome (MetS) is a cluster of independent risk factors for cardiovascular disease, type 2 diabetes, and mortality that includes abdominal obesity, insulin resistance, hypertension, elevated triglycerides, and reduced high-density lipoprotein (HDL)-cholesterol levels[1]. The prevalence of MetS is rising in many world regions, and in Latin America, it ranges from 19 to 43%[2,3]. These estimates are comparable to those from Europe[4] and the United States[5]. Additionally, MetS components can start to appear in childhood[6] and track into adulthood[7], increasing the risk for cardiovascular disease[7]. Because MetS is mostly preventable, a research priority is identifying potentially modifiable risk factors.

The effects of sodium consumption on blood pressure and cardiovascular outcomes have been thoroughly described[8,9]. Sodium metabolism has a direct effect on blood pressure levels as higher renal tubular absorption of sodium in response to high consumption increases intravascular volume, which leads to increased blood pressure[10]. Because hypertension is one of the MetS components, interest in evaluating the association between sodium consumption and MetS has arisen. Several cross-sectional studies have found a positive association between sodium intake (estimated from urinary sodium excretion) and MetS[11–16]. Similarly, a recent meta-analysis by Soltani et al[17], found a positive association between sodium intake (from urinary excretion or dietary intake) and MetS, with a bias-corrected standardized mean difference (Hedges' g) of 0.12, indicative of a small positive effect of sodium intake on MetS. Previous evidence in children found a positive association between sodium and MetS prevalence in Korean boys aged 10 to 18[18]. The adverse effects of sodium have become evident in the past years since worldwide consumption has increased substantially[19], with average consumption estimates that double the recommended intake established by the WHO[20].

Iodine is an essential element for the production of thyroid hormones, which are implicated in maintaining basal metabolic rate and affect the cardiovascular system and body weight[21]. The association between iodine and thyroid function is complex. Iodine deficiency can cause hypothyroidism and iodine excess can cause both hypothyroidism and hyperthyroidism[22]. These disorders could explain why iodine could be associated with MetS. However, no previous studies have evaluated the association between iodine and MetS. Some previous evidence in adults described associations with some of the MetS components. One cross-sectional study in postmenopausal Turkish women found an inverse association between iodine with triglyceride levels and a positive association with fasting blood glucose[23]. Similarly, another cross-sectional study conducted in US adults showed an inverse association between iodine and elevated low-density lipoprotein (LDL)-cholesterol and lower HDL-cholesterol/LDL-cholesterol ratio[24]. Additionally, salt iodization policies were implemented in the region during the 90s to decrease iodine deficiency disorders (IDD)[25]. Currently, iodine deficiency has substantially decreased, but excessive iodine levels have been recently reported[26,27], which could result in metabolic abnormalities. Therefore, further investigation of the role of iodine on MetS in Mesoamerica is warranted.

The bulk of the current evidence about the association between sodium and MetS comes from developed countries[17], and thus there is a need to assess sodium status in populations facing the epidemiological transition like Mesoamerica, where various forms of malnutrition are likely to occur[28]. Furthermore, there is scant evidence about the association between iodine and MetS. We aimed to study the association between sodium and iodine with MetS in children and adults from Mesoamerica.

Methods

Study population

The Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMes) Study was a cross-sectional study 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 study have been described elsewhere[2]. Briefly, using convenience sampling, we recruited 267 family groups each consisting of a school-aged child and their two parents from public primary schools in periurban areas of the capital cities. Eligibility criteria included a 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. The study protocol and procedures were approved by the Institutional Review Boards (IRB) of collaborating institutions in each country and by the University of Michigan Health and Behavioral Sciences IRB. We obtained written parental informed consent and children's assent to participate before enrollment.

Data collection

Data collection took place at a single home or clinic visit after fasting for at least 6 hours. At the visit, participants responded to a questionnaire on sociodemographic 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)[29], a 16 yes/no item survey about food security experiences during the past three months. Research assistants administered a 97-item semiquantitative food frequency questionnaire (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[30]. The FFQ characteristics have been detailed elsewhere[31]. Researchers measured height, weight, and waist circumference using standardized 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 taken in triplicate, and the median of the three values was used[32]. Blood pressure was measured while seated, using Omron HEM-712C digital blood pressure monitors (Omron Healthcare, Inc., Lake Forest, IL, USA). Three measurements were obtained, separated by at least one minute, and the value of blood pressure used was the average of the second and third measures. At the end of the visit, phlebotomists obtained 7.5 ml of blood through antecubital venipuncture, and all participants received detailed instructions on the 24h urine collection procedure along with sterile flasks. Blood samples were placed in refrigerated containers and transported on the day of collection to each country's collaborating laboratories where the serum, plasma, and red blood cells were separated, aliquoted, and cryostored at −20°C. Urine samples were collected the next day. The total volume of each flask was measured, and two aliquots of 5 ml each were transferred into separate tubes to be transported to the country's laboratory and cryostored at −20°C. Frozen stored samples from all countries were transported to the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City.

Laboratory methods

Plasma insulin was measured using a chemiluminescent immunoassay on an Immulite 1000 system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Plasma glucose and serum lipids (total and HDL-cholesterol and triglycerides) were quantified on an automated chemistry analyzer (Cobas c111 system; Roche Diagnostics, Mannheim, Germany).

Urinary sodium was measured using Ion Selective Electrode (ISE) technology with a Medica EasyLyte Na/K/Cl analyzer (Medica Corporation, Bedford, MA). Urinary iodine was measured with ammonium persulfate using method A as previously described[33]. Quantifications for all metabolites were conducted at INCAP.

Definition of outcomes

Children. We calculated a metabolic risk score using MetS components that are consistent with the definition in adults: waist circumference, the homeostatic model assessment for insulin resistance (HOMA-IR)[34], mean arterial pressure (MAP,) serum HDL-cholesterol, and serum triglycerides. MAP was calculated as [(2 x diastolic blood pressure) + systolic blood pressure]/3. The metabolic risk score was created by regressing each log-transformed component on sex and log-transformed age using linear regression models to obtain the standardized regression residuals. The average of the residuals for the five components was used to create the score, with the residuals for HDL-cholesterol multiplied by -1 beforehand. Higher scores indicate a worse metabolic profile.

Adults. The presence of MetS was defined according to the Adult Treatment Panel (ATP) III criteria[1] as having any 3 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 triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides.

Definition of exposures

Sodium excretion (mg/day) from 24h urine samples is the biomarker of choice for assessing dietary sodium intake as around 90% of all sodium consumed is excreted in urine[35].

Because the variability in sodium excretion was low, we evaluated the recommended sodium intake using the World Health Organization (WHO) as urinary sodium excretion ≤2000 mg/day. This recommendation is suggested for blood pressure control in children and the reduction of blood pressure and risk of cardiovascular disease, stroke, and coronary artery disease in adults[20].

Similarly, urinary iodine excretion (μg/day) measured with 24h urine samples is a widely used biomarker of dietary iodine intake as 90% of iodine consumed from all sources is excreted in urine[36]. We assessed excessive iodine intake as urinary iodine concentrations ≥300μg/L, based on the WHO definition[33]. The definition uses concentration (μg/L) instead of excretion (μg/day) because the original derivation of the cut points came from urine spot samples. This cut point indicates an increased risk of adverse health consequences, like iodine-induced hyperthyroidism and autoimmune hyperthyroidism[33].

Covariates

Children. Height-for-age and BMI-for-age Z scores were calculated using the WHO reference[37]. Parental height was categorized into quartiles and parental BMI according to the WHO classification. Household education was the maximum number of schooling years achieved by either parent. Household food insecurity was categorized by the number of affirmative responses in the ELCSA survey (no insecurity, 0; mild insecurity, 1-5; moderate insecurity, 6-10; severe insecurity, ≥11). The number of household assets was the sum of a car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color television, sound set, computer, or Internet, with a potential maximum number of assets of 12. Maternal parity was categorized as 1, 2, 3, or ≥ 4 births. For the evaluation of potential dietary confounders, we used a stepwise regression algorithm approach to obtain a set of foods that best explained the variability of each exposure and adjusted for those in the main models for

the metabolic score and each component. We used the log-transformed intake frequency weights of individual foods as predictors and the log-transformed urinary concentrations of each exposure as the outcome. For sodium, the predictors included common food sources[38,39]. For iodine, the predictors were sodium intake (as log-transformed urinary excretion) and typical food sources[40]. The results of this procedure are presented in Supplemental Tables 5 and 6. 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 categorized as presented in Supplemental Tables 2 and 4. Parental smoking was categorized as never, past, or current. Household income was categorized into country-specific quartiles. Dietary covariates were assessed as described for children and are presented in Supplemental Tables 5 and 6.

Data analysis

The final analytic sample was comprised of 217 children with information on both the exposures and MetS. Fifty children were excluded because 20 had no outcome information with adequate urine samples (average volume of 1L), 23 did not provide any sample, and 7 had inadequate/insufficient urine sample. The average urine volume for these 7 samples was 323 ml (range 100-500 ml). Four hundred and seventy-eight adults had information on both the exposures and MetS. Fifty-six adults were excluded because 2 had no outcome information with adequate urine samples (volumes of 900 and 1260 ml), 53 did not provide a urine sample and 1 sample was inadequate in a female with her menstrual period.

Children. In bivariate analysis, we compared the distribution of MetS and MetS components by recommended sodium and excessive iodine intakes using means and SD. We estimated χ^2 tests by introducing an indicator variable for recommended sodium or excessive iodine intake introduced as predictors in a linear regression model with MetS or each component as the outcome.

In multivariable analyses, we estimated adjusted differences with 95% CI in mean metabolic scores between exposure levels using linear regression models with robust variances. Adjustment covariates were independent predictors of the outcome including height-for-age Z score, parental smoking, country of origin, total energy intake, and specific food items. Because the metabolic score is standardized for the age and sex distributions of the study population, the inclusion of these variables in multivariable models is unnecessary.

Adults. In bivariate analysis, we compared unadjusted MetS prevalences by levels of the exposures. We conducted χ^2 tests by introducing an indicator variable representing levels of the exposures into a generalized estimation equation (GEE) model using the Poisson distribution. In multivariable analysis, we estimated prevalence ratios with 95% CI between exposure levels from GEE models using an analogous approach to adjustment as described for children. The adjustment covariates included were age, sex, smoking history, and country of origin. Robust variances were specified in all models to account for within-household correlations.

Results

Children. The mean age (± SD) was 9.9 ± 1.6 years, range 7 to 12 years, with 51.2% females. The mean of the metabolic risk score (± SD) was 0.01 ± 0.23. The prevalence of overweight (BMI Z >1) and obese (BMI Z >2) children was 34.6% and 20.3%, respectively. The mean (± SD) urinary sodium excretion was 2316 ± 1309 mg/day. The mean iodine excretion and concentration were 172 ± 110 µg/day and 242 ± 171 µg/L, respectively. Forty-eight percent of children met the recommended sodium intake (≤2000 mg/day), 15.2% had iodine deficiency (<100 µg/L), and 23.7% had excessive iodine intake (≥300 µg/L).

Adults. The mean age (±SD) was 38.5 ± 7.4; 50.8% were females. The overall prevalence of MetS was 36.6%. Forty-one percent of adults were overweight, and 33.7% were obese. The mean (± SD) urinary sodium excretion was 3627 ± 2179 mg/day. The mean urinary iodine excretion and concentration were 260 ± 197 µg/day and 232 ± 169 µg/L, respectively. The prevalence of the recommended sodium intake (≤2000 mg/day), iodine deficiency (<100 µg/L), and excessive iodine intake (≥300 µg/L) was 21.6%, 18.8%, and 23.0%, respectively.

Correlates of sodium status biomarkers. In multivariable analysis, age and BMI-for-age Z score were positively associated with urinary sodium concentrations in children. Similarly, there was a positive association between age and exceeding the recommended sodium intake (**Supplemental Table 1**). For adults, male sex and BMI were positively associated with urinary sodium concentrations (**Supplemental Table 2**). Similarly, there was a positive association between BMI with exceeding the recommended sodium intake. The food items that best explained the variability of sodium intake in children were breakfast cereal, tomato sauce, coated sandwich cookies, corn products, American cheese, added butter, and chorizo (**Supplemental**

Table 5). In adults, these foods included: rice, fried and boiled chicken, corn products, atole, hard cheese, and salami.

Correlates of iodine status biomarkers. In children, age and BMI-for-age Z score were positively associated with urinary iodine concentrations (**Supplemental Table 3**). Country of origin was a significant predictor of urinary iodine concentrations; the Dominican Republic had the highest mean concentration whereas Panama had the lowest. In adults, male sex, BMI, and the number of household assets were positively associated with the urinary iodine concentrations. As in children, urinary iodine concentrations were significantly different among countries (**Supplemental Table 4**). The Dominican Republic had the highest, whereas Panama had the lowest mean urinary iodine concentration. Similarly, male sex was positively associated with excessive iodine intake. Compared to Guatemala, the country with the highest prevalence ratio for excessive iodine intake was the Dominican Republic, and the country with the lowest was Costa Rica. The food items that best explained the variability of iodine intake in children were sodium intake and cream (**Supplemental Table 6**). For adults, the list of food included: sodium intake, salami, fried chicken, cream cheese, sausage, and beef or pork as side dish. Notably, sodium intake was the most important dietary predictor for both children and adults.

Urinary sodium and metabolic syndrome. For children, surpassing the daily recommended sodium intake was not associated with the metabolic score or any of the components including blood pressure (**Table 1**). In adults, exceeding the recommended sodium intake was positively associated with MetS (**Table 2**). Compared to adults with the recommended sodium intake, those with higher intake had a 53% higher adjusted prevalence of

MetS (95% CI: 1.05, 2.24; P = 0.02). The blood pressure component was positively associated with exceeding the recommended sodium intake (**Table 2**).

Urinary iodine and metabolic syndrome. In children, an excessive iodine intake was positively associated with the metabolic score (**Table 3**). Compared to children without excessive iodine intake, those with excessive intake had an adjusted 0.12 units higher metabolic score (95% CI: 0.05, 0.19; P = 0.0008). Similarly, there was a positive association between excessive iodine intake with the HOMA-IR and the serum triglycerides scores (**Table 3**). In adults, having an excessive iodine intake was positively associated with MetS (**Table 4**). Adults with excessive iodine intake had a 41% higher adjusted prevalence of MetS compared to those without excessive intake (95% CI: 1.04, 1.92; P = 0.03). None of the components were associated with an excessive iodine intake (**Table 4**).

Discussion

In this cross-sectional study, we found that excessive iodine intake in children was positively associated with the metabolic risk score, insulin resistance, and triglyceride component scores. In adults, we found that sodium intake above the current recommendation was positively associated with MetS prevalence and high blood pressure. Additionally, adults with excessive iodine intake had a higher MetS prevalence compared to those with lower iodine intakes.

In children, our findings are not directly comparable to previous work, as there are few evaluations of the association between sodium intake and Mets. Of note, a cross-sectional study in Korean boys aged 10 to 18 years found that sodium intake assessed with the urinary sodium excretion to urinary specific gravity ratio was positively associated with MetS prevalence[18]. In contrast with those results, we found no association in children between the recommended sodium intake and the metabolic risk score. The reasons may be that the variability in sodium consumption in our population was low since around 50% of children meet the recommendation for sodium intake, which limits power to detect associations. Additionally, the previous study was done in a population with different consumption patterns, restricted to older boys, using a different biomarker for sodium intake, and defined MetS prevalence according to Cook et al[41], making direct comparisons difficult.

In adults, as expected, we found a positive association between sodium intake and MetS prevalence, possibly through increased blood pressure. High sodium intake is a known risk factor for hypertension[10], stroke, and cardiovascular disease[9]. Sodium intake has a direct effect on blood pressure, where higher intakes cause an increase in the intravascular volume that leads to higher blood pressure levels and hypertension[10]. Similarly, several studies have described a

positive association between sodium intake and MetS. Cross-sectional studies of Venezuelan[11], Chilean[12], Chinese[13], and Korean[14–16] adults found a positive association between sodium excretion and MetS. Thus, our findings are consistent with previous evidence, supporting the negative health impacts of high sodium consumption.

In children, we found that excess iodine consumption was positively associated with the metabolic risk score, possibly through the insulin resistance and triglycerides components, and with MetS prevalence in adults. Very few studies have evaluated these associations, especially in pediatric populations. Similar to our findings in children, a cross-sectional study of postmenopausal Turkish women found that urinary spot iodine was positively associated with fasting blood glucose[23]. In contrast, another study in US adults found that compared to participants above the 10th percentile, those with urinary iodine concentrations below the 10th percentile had higher odds of elevated LDL-cholesterol and lower HDL-cholesterol/LDL-cholesterol ratio[24]. Iodine is an essential component of thyroid hormones and several cross-sectional studies conducted in euthyroid adults from Mexico[42], Iran[43], and the Netherlands[44] have described a positive association of thyroid stimulating hormone (TSH) with triglycerides[42–44] and HOMA-IR[43] levels, and an inverse association between free thyroxine (fT4) and MetS[43], triglycerides[43] and HOMA-IR[42–44] levels. Moreover, another study in euthyroid Korean children and adolescents found a U-shaped association between urinary iodine and serum TSH, and an inverted U-shaped association between urinary iodine and fT4[45]. From these findings, we can infer that either low or high iodine levels could be associated with high levels of TSH and low levels of fT4, which in turn, could explain the positive association between urinary iodine with MetS, lipid profiles and insulin resistance in previous studies, including ours.

Furthermore, the adverse effects of excess iodine are more likely to occur in areas that suffered from iodine deficiency in the past[46]. The mechanisms for iodine-induced hypothyroidism are not entirely clear, but autoimmunity and previous damage to the thyroid gland are described as potential explanations, and increased rates of these conditions have been reported in previously iodine-deficient regions where later salt iodization programs occured[46]. This scenario characterizes the changes that have occurred in the Mesoamerican region, where iodine deficiency was highly prevalent before the 1990s and has substantially decreased since then due to the implementation of salt iodization, leading to an increase in iodine excess[26,27]. Likewise, similar populations with salt iodization programs have found excessive iodine associated with thyroid disorders[45,47]. Additionally, our results found that sodium intake was the most significant dietary determinant of iodine concentration. This situation highlights the importance of strict monitoring of salt iodization programs as the policy implications of our findings and the need for further research to elucidate the role of iodine in the development of MetS.

Our study has several strengths. Dietary exposures were measured using biomarkers of intake, which removes recall bias when using FFQ or recall methods to assess intake and allows estimation of associations between specific cut points and MetS. Data collected on both parents and children allowed adjustment for parental characteristics that could be considered potential confounders for the associations in children. Our study adds to the current body of literature on the negative impact of sodium consumption on hypertension and MetS and emphasizes the importance of adhering to the recommended intake proposed by the WHO. Additionally, the evaluation of the role of iodine in MetS is unique and provides with new information for the understudied region of Mesoamerica.

Due to its cross-sectional design, causal inference is limited in our study. It is important to highlight that since sodium consumption is highly correlated with iodine intake, and that high sodium consumption is associated with increased caloric intake, our findings could be explained by the positive association between caloric intake and MetS. Comparisons of the metabolic risk with other populations are limited because the score is based on the specific distribution of the different components. Since our study sample was not representative of the entire Mesoamerican population, generalizability might be affected. Finally, due to the small sample size, country-specific analyses were not possible. This is especially important to evaluate iodine status since the geographical location is a significant independent predictor of iodine availability[26].

In conclusion, we found that exceeding the recommended sodium intake is associated with MetS and high blood pressure in adults. Similarly, excessive iodine intake is associated with MetS in both children and adults. Efforts should be made to emphasize the need for a decrease in sodium consumption and stricter monitoring of salt iodization programs to prevent the possible deleterious effect of iodine excess.

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Table 3.1. Mean and adjusted mean differences in metabolic syndrome score[1] and its components[2] by recommended sodium intake among children from Mesoamerica

	Sodium excretion, mg/d		
	≤2000[3]	>2000	
	N = 104	N = 113	*P*[4]
Overall metabolic score			
Mean ± SD	0.01 ± 0.22	0.01 ± 0.23	
Unadjusted difference (95% CI)	Reference	0.00 (-0.06, 0.06)	0.92
Adjusted difference (95% CI) - model 1[5]	Reference	-0.01 (-0.07, 0.04)	0.65
Adjusted difference (95% CI) - model 2[6]	Reference	-0.01 (-0.07, 0.05)	0.79
Waist circumference score			
Mean ± SD	-0.02 ± 0.16	0.02 ± 0.16	
Unadjusted difference (95% CI)	Reference	0.04 (0.00, 0.08)	0.06
Adjusted difference (95% CI) - model 1	Reference	0.02 (-0.02, 0.05)	0.34
Adjusted difference (95% CI) - model 2	Reference	0.02 (-0.02, 0.05)	0.32
HOMA-IR score			
Mean ± SD	0.04 ± 0.59	0.00 ± 0.59	
Unadjusted difference (95% CI)	Reference	-0.04 (-0.20, 0.11)	0.60
Adjusted difference (95% CI) - model 1	Reference	-0.07 (-0.23, 0.08)	0.34
Adjusted difference (95% CI) - model 2	Reference	-0.07 (-0.22, 0.08)	0.37
Mean arterial pressure (MAP) score			
Mean ± SD	0.01 ± 0.16	-0.01 ± 0.14	
Unadjusted difference (95% CI)	Reference	-0.01 (-0.05, 0.02)	0.45
Adjusted difference (95% CI) - model 1	Reference	-0.02 (-0.06, 0.02)	0.28
Adjusted difference (95% CI) - model 2	Reference	-0.02 (-0.06, 0.02)	0.35
Serum HDL-cholesterol score			
Mean ± SD	0.02 ± 0.27	-0.03 ± 0.30	
Unadjusted difference (95% CI)	Reference	-0.05 (-0.12, 0.03)	0.21
Adjusted difference (95% CI) - model 1	Reference	-0.04 (-0.10, 0.03)	0.28
Adjusted difference (95% CI) - model 2	Reference	-0.03 (-0.09, 0.04)	0.45
Serum triglycerides score			
Mean ± SD	0.04 ± 0.43	0.00 ± 0.44	
Unadjusted difference (95% CI)	Reference	-0.05 (-0.16, 0.07)	0.43
Adjusted difference (95% CI) - model 1	Reference	-0.03 (-0.14, 0.09)	0.67
Adjusted difference (95% CI) - model 2	Reference	-0.01 (-0.12, 0.13)	0.93

Footnotes to Table 3.1

[1]The overall score was calculated as the average of the five component scores after the HDL cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Urinary sodium excretion ≤2000 mg/d corresponds to the daily recommended sodium intake[20].

[4]χ^2 score statistic from linear regression models with the metabolic score as the outcome and an indicator variable for recommended sodium intake introduced as the predictor. An independent covariance structure was indicated in all models to obtain empirical variances.

[5]From linear regression models. Model 1 was adjusted for height-for-age Z score, parental smoking, and country of origin.

[6]Model 2 includes covariates from model 1 plus log-transformed total energy intake and log-transformed frequency intake weights of cereal, tomato sauce, coated sandwich cookies, corn products, American cheese, added butter, and chorizo introduced as continuous predictors.

Table 3.2. Prevalence and prevalence ratios (PR) of metabolic syndrome[1] and its components by recommended sodium intake among adults from Mesoamerica

	Sodium excretion, mg/d			
	≤2000[2] N = 103	>2000 N = 375		*P*[3]
Metabolic Syndrome				
Prevalence (%)	27.2	39.2		
Unadjusted PR (95% CI)	Reference	1.44	(1.02, 2.04)	0.04
Adjusted PR (95% CI) - model 1[4]	Reference	1.43	(1.00, 2.04)	0.05
Adjusted PR (95% CI) - model 2[5]	Reference	1.53	(1.05, 2.24)	0.03
Abdominal obesity[6]				
Prevalence (%)	41.8	48.0		
Unadjusted PR (95% CI)	Reference	1.15	(0.90, 1.47)	0.26
Adjusted PR (95% CI) - model 1	Reference	1.19	(0.96, 1.47)	0.11
Adjusted PR (95% CI) - model 2	Reference	1.16	(0.94, 1.44)	0.17
High fasting blood glucose[7]				
Prevalence (%)	5.8	9.3		
Unadjusted PR (95% CI)	Reference	1.60	(0.68, 3.75)	0.28
Adjusted PR (95% CI) - model 1	Reference	1.52	(0.62, 3.72)	0.36
Adjusted PR (95% CI) - model 2	Reference	1.71	(0.70, 4.19)	0.24
High blood pressure[8]				
Prevalence (%)	10.7	24.9		
Unadjusted PR (95% CI)	Reference	2.33	(1.29, 4.21)	0.005
Adjusted PR (95% CI) - model 1	Reference	2.50	(1.37, 4.57)	0.003
Adjusted PR (95% CI) - model 2	Reference	2.85	(1.46, 5.54)	0.002
Low serum HDL-cholesterol[9]				
Prevalence (%)	74.8	82.9		
Unadjusted PR (95% CI)	Reference	1.11	(0.98, 1.25)	0.09
Adjusted PR (95% CI) - model 1	Reference	1.09	(0.97, 1.23)	0.14
Adjusted PR (95% CI) - model 2	Reference	1.11	(0.99, 1.26)	0.08
High serum triglycerides[10]				
Prevalence (%)	50.5	52.0		
Unadjusted PR (95% CI)	Reference	1.03	(0.83, 1.28)	0.79
Adjusted PR (95% CI) - model 1	Reference	1.05	(0.84, 1.32)	0.68
Adjusted PR (95% CI) - model 2	Reference	1.11	(0.88, 1.40)	0.37

Footnotes to Table 3.2

[1]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 triglycerides.

[2]Urinary sodium excretion ≤2000 mg/d corresponds to the daily recommended sodium intake[20].

[3]χ^2 score statistic from Poisson regression models with metabolic syndrome as the outcome and indicator variable for recommended sodium intake as the predictor. An independent covariance structure was specified in all models to account for clustering by family membership.

[4]From Poisson regression models. Model 1 is adjusted for age, sex, smoking history, and country of origin.

[5]Model 2 is adjusted for covariates in model 1 plus log-transformed total energy intake and log-transformed frequency intake weights of rice, fried and roasted chicken, corn products, atole, hard cheese, and salami introduced as continuous predictors.

[6]Waist circumference >102 cm in men and >88 cm in women.

[7]Fasting blood glucose ≥100 mg/dL

[8]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[9]HDL-C <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL cholesterol.

[10]TG ≥150 mg/dL or receiving treatment for hyperlipidemia.

Table 3.3. Mean and adjusted mean differences in metabolic syndrome score[1] and its components[2] by excessive iodine intake among children from Mesoamerica

	Iodine concentration, µg/L			*P*[4]
	<300	≥300[3]		
	N = 165	N = 52		
Overall metabolic score				
Mean ± SD	-0.01 ± 0.22	0.06 ± 0.23		
Unadjusted difference (95% CI)	Reference	0.07	(0.00, 0.14)	0.05
Adjusted difference (95% CI) - model 1[5]	Reference	0.09	(0.02, 0.16)	0.009
Adjusted difference (95% CI) - model 2[6]	Reference	0.12	(0.05, 0.19)	0.0008
Waist circumference score				
Mean ± SD	0.00 ± 0.16	0.00 ± 0.16		
Unadjusted difference (95% CI)	Reference	0.00	(-0.05, 0.05)	0.99
Adjusted difference (95% CI) - model 1	Reference	0.02	(-0.02, 0.07)	0.29
Adjusted difference (95% CI) - model 2	Reference	0.03	(-0.02, 0.07)	0.25
HOMA-IR score				
Mean ± SD	0.00 ± 0.58	0.08 ± 0.61		
Unadjusted difference (95% CI)	Reference	0.08	(-0.11, 0.26)	0.43
Adjusted difference (95% CI) - model 1	Reference	0.21	(0.03, 0.38)	0.02
Adjusted difference (95% CI) - model 2	Reference	0.28	(0.09, 0.46)	0.003
Mean arterial pressure (MAP) score				
Mean ± SD	0.00 ± 0.15	0.00 ± 0.14		
Unadjusted difference (95% CI)	Reference	0.00	(-0.05, 0.04)	0.86
Adjusted difference (95% CI) - model 1	Reference	0.03	(-0.02, 0.07)	0.26
Adjusted difference (95% CI) - model 2	Reference	0.04	(-0.01, 0.08)	0.11
Serum HDL-cholesterol score				
Mean ± SD	0.04 ± 0.25	-0.16 ± 0.33		
Unadjusted difference (95% CI)	Reference	-0.20	(-0.29, -0.10)	<0.0001
Adjusted difference (95% CI) - model 1	Reference	-0.04	(-0.14, 0.05)	0.35
Adjusted difference (95% CI) - model 2	Reference	-0.05	(-0.14, 0.05)	0.35
Serum triglycerides score				
Mean ± SD	-0.01 ± 0.42	0.09 ± 0.48		
Unadjusted difference (95% CI)	Reference	0.10	(-0.05, 0.24)	0.19
Adjusted difference (95% CI) - model 1	Reference	0.15	(0.00, 0.30)	0.05
Adjusted difference (95% CI) - model 2	Reference	0.20	(0.05, 0.35)	0.008

Footnotes to Table 3.3

[1]The overall score was calculated as the average of the five component scores after the HDL cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Urinary iodine concentrations ≥300 μg/L correspond to excessive iodine intake[33].

[4]χ^2 score statistic from linear regression models with the metabolic score as the outcome and an indicator variable for excessive iodine intake introduced as predictor. An independent covariance structure was indicated in all models to obtain empirical variances.

[5]From linear regression models. Model 1 is adjusted for height-for-age Z score, parental smoking, and country of origin.

[6]Model 2 includes covariates from model 1 plus log-transformed total energy intake, log-transformed frequency intake weights of cream, and log-transformed urinary sodium introduced as continuous predictors.

Table 3.4. Prevalence and prevalence ratios (PR) of metabolic syndrome[1] and its components by excessive iodine intake among adults from Mesoamerica

	Iodine concentration, µg/L			
	<300	≥300[2]		
	N = 368	N = 110		*P*[3]
Metabolic Syndrome				
Prevalence (%)	34.5	43.6		
Unadjusted PR (95% CI)	Reference	1.26	(0.98, 1.63)	0.07
Adjusted PR (95% CI) - model 1[4]	Reference	1.36	(1.00, 1.84)	0.05
Adjusted PR (95% CI) - model 2[5]	Reference	1.41	(1.04, 1.92)	0.03
Abdominal obesity[6]				
Prevalence (%)	46.7	46.4		
Unadjusted PR (95% CI)	Reference	0.99	(0.80, 1.24)	0.94
Adjusted PR (95% CI) - model 1	Reference	1.12	(0.88, 1.44)	0.35
Adjusted PR (95% CI) - model 2	Reference	1.14	(0.88, 1.47)	0.32
High fasting blood glucose[7]				
Prevalence (%)	9.0	7.3		
Unadjusted PR (95% CI)	Reference	0.81	(0.40, 1.65)	0.56
Adjusted PR (95% CI) - model 1	Reference	1.09	(0.47, 2.53)	0.84
Adjusted PR (95% CI) - model 2	Reference	1.19	(0.49, 2.89)	0.69
High blood pressure[8]				
Prevalence (%)	20.4	26.6		
Unadjusted PR (95% CI)	Reference	1.31	(0.91, 1.88)	0.15
Adjusted PR (95% CI) - model 1	Reference	1.34	(0.89, 2.02)	0.16
Adjusted PR (95% CI) - model 2	Reference	1.27	(0.85, 1.90)	0.24
Low serum HDL-cholesterol[9]				
Prevalence (%)	79.6	86.4		
Unadjusted PR (95% CI)	Reference	1.08	(0.99, 1.19)	0.08
Adjusted PR (95% CI) - model 1	Reference	1.06	(0.95, 1.18)	0.30
Adjusted PR (95% CI) - model 2	Reference	1.07	(0.95, 1.19)	0.26
High serum triglycerides[10]				
Prevalence (%)	50.8	54.6		
Unadjusted PR (95% CI)	Reference	1.07	(0.88, 1.31)	0.49
Adjusted PR (95% CI) - model 1	Reference	1.13	(0.91, 1.41)	0.26
Adjusted PR (95% CI) - model 2	Reference	1.17	(0.93, 1.46)	0.17

Footnotes to Table 3.4

[1]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 triglycerides.

[2]Urinary iodine concentrations ≥300 μg/L correspond to excessive iodine intake[33].

[3]χ^2 score statistic from Poisson regression models with metabolic syndrome as the outcome and indicator variable for excessive iodine intake as predictor. An independent covariance structure was specified in all models to account for clustering by family membership.

[4]From Poisson regression models. Model 1 is adjusted for age, sex, smoking history, and country of origin.

[5]Model 2 is adjusted for covariates in model 1 plus log-transformed total energy intake, log-transformed frequency intake weights of salami, fried chicken, cream cheese, sausage, and beef or pork as side dish, and log-transformed urinary sodium introduced as continuous predictors.

[6]Waist circumference >102 cm in men and >88 cm in women.

[7]Fasting blood glucose ≥100 mg/dL

[8]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[9]HDL-C <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL cholesterol.

[10]TG ≥150 mg/dL or receiving treatment for hyperlipidemia.

Supplemental Table 3.1. Mean (± SD) and adjusted mean differences, and prevalence and prevalence ratios (PR) of urinary sodium concentrations according to sociodemographic characteristics among children from Mesoamerica

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean difference (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 113	Adjusted PR (95% CI)[4]
Sex					
Female	113	2321 ± 1133	54 (-288, 397)	55.8	0.83 (0.57, 1.21)
Male	104	2311 ± 1482	Reference	48.1	Reference
P[5]		0.96	0.76	0.43	0.34
Age (years)					
<9	73	2052 ± 1505	Reference	37.0	Reference
9 - <11	74	2358 ± 1148	304 (-105, 712)	56.8	1.55 (0.95, 2.51)
≥11	70	2548 ± 1214	562 (143, 980)	62.9	1.79 (1.11, 2.91)
P, trend[6]		0.02	0.009	0.03	0.02
Height-for-age Z score					
<-2	13	1947 ± 1167	Reference	38.5	Reference
-2 - <-1	40	1990 ± 1018	52 (-743, 846)	42.5	1.14 (0.42, 3.11)
-1 - <0	84	2426 ± 1464	549 (-201, 1298)	54.8	1.58 (0.62, 4.02)
0 - <1	48	2432 ± 1299	368 (-435, 1170)	54.2	1.41 (0.53, 3.76)
≥1	32	2413 ± 1246	318 (-542, 1179)	59.4	1.63 (0.58, 4.57)
P, trend		0.10	0.32	0.26	0.30
BMI-for-age Z score					
<-1	32	2000 ± 778	Reference	43.8	Reference
-1 - <0	43	2143 ± 1168	299 (-290, 889)	48.8	1.23 (0.62, 2.44)
0 - <1	66	2317 ± 1303	454 (-101, 1009)	54.6	1.34 (0.71, 2.55)
≥1	76	2547 ± 1528	616 (44, 1188)	55.3	1.26 (0.65, 2.43)
P, trend		0.02	0.03	0.42	0.54
Maternal age (years)					
<30	33	2089 ± 1057	Reference	42.4	Reference
30 - <35	48	2153 ± 1243	165 (-414, 744)	45.8	1.19 (0.59, 2.38)
35 - <40	72	2515 ± 1544	413 (-121, 947)	61.1	1.44 (0.77, 2.67)
40 - <45	39	2295 ± 1178	229 (-366, 825)	51.3	1.25 (0.62, 2.51)
≥45	25	2394 ± 1187	271 (-411, 954)	52.0	1.25 (0.57, 2.75)
P, trend		0.26	0.37	0.48	0.53

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean difference (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 113	Adjusted PR (95% CI)[4]
Maternal height (cm)					
Q1 (149.0)	56	2440 ± 1586	Reference	53.6	Reference
Q2 (153.1)	56	2193 ± 1125	-344 (-819, 131)	48.2	0.85 (0.50, 1.44)
Q3 (157.0)	56	2383 ± 1363	-200 (-691, 290)	51.8	0.88 (0.51, 1.51)
Q4 (162.3)	49	2241 ± 1096	-427 (-970, 116)	55.1	0.86 (0.48, 1.56)
P, trend		0.62	0.20	0.87	0.66
Maternal body mass index (kg/m^2)					
<25	49	2231 ± 1158	Reference	53.0	Reference
25 - <30	85	2216 ± 1516	-141 (-596, 315)	41.2	0.73 (0.44, 1.24)
≥30	83	2470 ± 1155	-17 (-495, 462)	62.7	1.08 (0.65, 1.79)
P, trend		0.25	0.98	0.31	0.58
Maternal parity					
1	16	1808 ± 885	Reference	37.5	Reference
2	69	2285 ± 1480	242 (-479, 964)	50.7	1.00 (0.40, 2.51)
3	77	2362 ± 1294	424 (-267, 1115)	53.3	1.15 (0.47, 2.79)
≥4	54	2395 ± 1158	471 (-269, 1210)	55.6	1.18 (0.47, 3.00)
P, trend		0.20	0.15	0.44	0.49
Paternal age					
<35	53	2062 ± 959	Reference	47.2	Reference
35 - <40	63	2552 ± 1639	517 (45, 989)	57.1	1.20 (0.71, 2.04)
40 - <45	46	2275 ± 1225	131 (-383, 645)	50.0	1.03 (0.57, 1.84)
45 - <55	38	2388 ± 1354	319 (-223, 862)	52.6	1.07 (0.58, 1.97)
≥55	13	2300 ± 968	205 (-571, 982)	61.5	1.25 (0.56, 2.83)
P, trend		0.51	0.66	0.68	0.82
Paternal height (cm)					
Q1 (159.0)	53	2167 ± 1253	Reference	45.3	Reference
Q2 (164.7)	57	2319 ± 1300	205 (-298, 709)	52.6	1.19 (0.67, 2.11)
Q3 (169.7)	53	2466 ± 1559	293 (-214, 799)	52.8	1.15 (0.65, 2.04)
Q4 (176.5)	51	2343 ± 1126	103 (-444, 651)	58.8	1.19 (0.65, 2.18)
P, trend		0.40	0.65	0.36	0.65
Paternal body mass index (kg/m^2)					

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean difference (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 113	Adjusted PR (95% CI)[4]
<25	57	2302 ± 1283	Reference	52.6	Reference
25 - <30	93	2273 ± 1084	-191 (-625, 242)	54.8	0.95 (0.60, 1.52)
≥30	64	2415 ± 1629	-120 (-607, 367)	48.4	0.83 (0.48, 1.42)
P, trend		0.62	0.66	0.74	0.48
Parental smoking history					
Neither parent ever smoked	80	2475 ± 1554	Reference	52.5	Reference
One parent ever smoked	106	2249 ± 1198	-217 (-590, 155)	50.9	0.97 (0.64, 1.46)
Both parents ever smoked	26	2174 ± 974	-353 (-923, 218)	57.7	1.08 (0.59,1.97)
P		0.21	0.16	0.85	0.90
Parental metabolic syndrome					
No parent	85	2373 ± 1516	Reference	49.4	Reference
Mother only	48	2552 ± 1286	134 (-314, 583)	64.6	1.30 (0.81, 2.07)
Father only	47	2022 ± 974	-400 (-859, 59)	46.8	0.94 (0.55, 1.61)
Both parents	32	2268 ± 1112	-284 (-815, 247)	50.0	0.95 (0.52, 1.74)
P, trend		0.29	0.11	0.89	0.80
Highest parental education level					
Incomplete elementary	18	2156 ± 1001	Reference	55.6	Reference
Complete elementary	24	2114 ± 1369	-36 (-822, 750)	37.5	0.67 (0.27, 1.68)
Incomplete secondary	61	2321 ± 1123	88 (-613, 788)	52.5	0.86 (0.40, 1.81)
Complete secondary	41	2260 ± 1209	25 (-731, 781)	53.7	0.89 (0.39, 2.00)
Post secondary	73	2450 ± 1549	212 (-492, 915)	54.8	0.90 (0.42, 1.91)
P, trend		0.26	0.42	0.61	0.85
Home ownership					
Yes	150	2335 ± 1377	30 (-343, 402)	54.0	1.14 (0.75, 1.73)
No	67	2274 ± 1152	Reference	47.8	Reference
P		0.75	0.88	0.56	0.55
Number of household assets[7]					
0-4	42	2198 ± 1172	Reference	57.1	Reference
5-7	84	2477 ± 1536	229 (-256, 714)	52.4	0.93 (0.55, 1.58)
8-9	41	1948 ± 994	-348 (-898, 201)	39.0	0.63 (0.33, 1.20)
10-12	50	2449 ± 1189	154 (-380, 687)	58.0	0.96 (0.54, 1.69)

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean difference (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 113	Adjusted PR (95% CI)[4]
P, trend		0.88	0.79	0.89	0.65
Household income					
Lower <25%	42	2224 ± 1357	Reference	47.6	Reference
Medium 25-75%	100	2281 ± 1160	65 (-417, 548)	53.0	1.15 (0.67, 1.97)
Higher >75%	71	2435 ± 1505	185 (-331, 702)	53.5	1.11 (0.63, 1.96)
P, trend		0.38	0.45	0.70	0.79
Food insecurity					
No insecurity	66	2449 ± 1583	Reference	54.6	Reference
Mild insecurity	61	2338 ± 1293	-13 (-463, 437)	52.5	1.03 (0.63, 1.68)
Moderate insecurity	51	2060 ± 1059	-291 (-776, 193)	41.2	0.76 (0.43, 1.33)
Severe insecurity	38	2418 ± 1103	37 (-479, 552)	63.2	1.23 (0.72, 2.10)
P, trend		0.49	0.73	0.92	0.78
Country of origin					
Guatemala	28	1861 ± 1185	Reference	32.1	Reference
El Salvador	29	3004 ± 1825	1035 (397, 1673)	72.4	2.17 (0.96, 4.92)
Dominican Republic	27	2260 ± 1073	220 (-451, 891)	55.6	1.65 (0.68, 3.99)
Honduras	28	2479 ± 1304	502 (-155, 1160)	53.6	1.60 (0.66, 3.86)
Nicaragua	30	2171 ± 922	205 (-446, 855)	56.7	1.78 (0.76, 4.19)
Panama	17	1397 ± 870	-715 (-1460, 30)	23.5	0.63 (0.19, 2.13)
Costa Rica	20	2483 ± 936	350 (-354, 1053)	60.0	1.55 (0.63, 3.80)
Mexico	26	2231 ± 1128	73 (-581, 726)	50.0	1.33 (0.55, 3.20)
Belize	12	3038 ± 1797	1236 (405, 2066)	58.3	2.02 (0.72, 5.64)
P		0.85	0.74	0.94	0.81

Footnotes to Supplemental Table 3.1

[1]Total may be less than 217 due to missing values.

[2]From linear regression models. All models were adjusted for age, sex, height-for-age Z score, and BMI-for-age Z score.

[3]Urinary sodium excretion ≤2000 mg/d corresponds to the daily recommended sodium intake[20].

[4]From Poisson regression models. All models were adjusted for age, sex, height-for-age Z score, and BMI-for-age Z score.

[5]χ^2 score statistic with urinary concentrations of sodium or recommended sodium intake as the outcome from linear or Poisson regression models, respectively, and indicator variables for the characteristics as predictors.

[6]Test for linear trend with urinary concentrations of sodium or recommended sodium intake as the outcome from linear or Poisson regression models, respectively, and a variable representing ordinal categories of each characteristic as a continuous predictor.

[7]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

Supplemental Table 3.2. Mean (± SD) and adjusted mean differences, and prevalence and prevalence ratios (PR)of urinary sodium concentrations, according to sociodemographic characteristics among adults from Mesoamerica

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean differences (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 103	Adjusted PR (95% CI)[4]
Sex					
Female	243	3465 ± 2067	Reference	78.6	Reference
Male	235	3794 ± 2282	413 (99, 726)	78.3	1.00 (0.91, 1.09)
P[5]		0.04	0.01	0.93	0.93
Age (years)					
<30	53	3695 ± 2652	Reference	77.4	Reference
30 - <40	252	3658 ± 2150	-210 (-937, 516)	79.0	1.01 (0.86, 1.18)
40 - <50	139	3535 ± 2042	-343 (-1130, 444)	75.5	0.97 (0.81, 1.16)
50+	33	3685 ± 2251	-195 (-1194, 803)	87.9	1.14 (0.93, 1.39)
P, trend[6]		0.75	0.50	0.68	0.65
Height quartile (mothers/fathers medians, cm)					
Q1 (148.9/159.1)	114	3444 ± 2274	Reference	75.4	Reference
Q2 (153.0/164.9)	127	3660 ± 2244	280 (-275, 835)	80.3	1.08 (0.95, 1.22)
Q3 (157.0/169.7)	122	3741 ± 2215	289 (-257, 835)	78.7	1.05 (0.91, 1.20)
Q4 (162.7/176.5)	115	3651 ± 1981	220 (-337, 778)	79.1	1.06 (0.92, 1.22)
P, trend		0.45	0.47	0.60	0.56
Body mass index (kg/m^2)					
<25	119	3058 ± 1738	Reference	71.4	Reference
25-<30	198	3651 ± 2246	591 (166, 1017)	78.8	1.10 (0.96, 1.26)
≥30	161	4017 ± 2308	1000 (546, 1455)	83.2	1.17 (1.03, 1.33)
P, trend		<0.0001	<0.0001	0.02	0.02
Smoking status					
Never	303	3672 ± 2162	Reference	80.5	Reference
Past	129	3570 ± 2125	-320 (-811, 172)	76.0	0.92 (0.82, 1.03)
Current	44	3541 ± 2506	-420 (-1204, 363)	72.7	0.89 (0.73, 1.09)
P		0.62	0.17	0.15	0.12

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean differences (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 103	Adjusted PR (95% CI)[4]
Education level					
Incomplete elementary	64	3801 ± 2387	Reference	75.0	Reference
Complete elementary	71	3390 ± 2050	-453 (-1232, 327)	81.7	1.12 (0.94, 1.33)
Incomplete secondary	146	3705 ± 2429	-176 (-895, 542)	81.5	1.11 (0.93, 1.32)
Complete secondary	74	3372 ± 1922	-389 (-1139, 360)	73.0	0.99 (0.79, 1.23)
Post secondary	114	3844 ± 1991	11 (-694, 715)	79.0	1.07 (0.89, 1.28)
P, trend		0.73	0.71	0.94	0.97
Home ownership					
Yes	327	3625 ± 2145	-62 (-550, 427)	79.2	1.02 (0.91, 1.14)
No	151	3630 ± 2260	Reference	76.8	Reference
P		0.98	0.80	0.62	0.79
Number of household assets[7]					
0-4	90	3471 ± 2457	Reference	74.4	Reference
5-7	183	3542 ± 2107	32 (-649, 713)	76.5	1.02 (0.86, 1.22)
8-9	92	3815 ± 2412	297 (-526, 1119)	81.5	1.08 (0.90, 1.30)
10-12	113	3735 ± 1849	225 (-434, 883)	82.3	1.10 (0.92, 1.30)
P, trend		0.31	0.34	0.15	0.17
Household income					
Lower <25%	94	3734 ± 2629	Reference	75.5	Reference
Medium 25-75%	215	3682 ± 2097	-45 (-718, 627)	82.3	1.09 (0.94, 1.27)
Higher >75%	159	3520 ± 2038	-329 (-1039, 381)	74.8	0.97 (0.83, 1.14)
P, trend		0.52	0.31	0.69	0.48
Food security					
No insecurity	149	3461 ± 1937	Reference	81.9	Reference
Mild insecurity	127	3931 ± 2234	536 (-5, 1078)	81.9	1.02 (0.90, 1.15)
Moderate insecurity	117	3316 ± 2052	-89 (-623, 445)	73.5	0.90 (0.78, 1.04)
Severe insecurity	83	3940 ± 2587	494 (-219, 1206)	74.7	0.91 (0.78, 1.07)
P, trend		0.52	0.46	0.12	0.11
Country of origin					

Characteristics	N[1]	Urinary sodium (mg/d)	Adjusted mean differences (95% CI)[2]	Sodium excretion[3] >2000 mg/d N = 103	Adjusted PR (95% CI)[4]
Guatemala	58	2529 ± 1343	Reference	69.0	Reference
El Salvador	58	4224 ± 2112	1513 (780, 2245)	86.2	1.20 (0.96, 1.49)
Dominican Republic	58	4098 ± 1772	1476 (844, 2108)	84.5	1.21 (0.97, 1.51)
Honduras	59	3926 ± 2126	1194 (463, 1924)	81.4	1.13 (0.89, 1.43)
Nicaragua	62	4788 ± 2966	1956 (1054, 2858)	85.5	1.17 (0.94, 1.46)
Panama	46	2287 ± 1315	-482 (-1069, 105)	54.4	0.76 (0.55, 1.05)
Costa Rica	52	4189 ± 1991	1505 (862, 2147)	90.4	1.27 (1.04, 1.56)
Mexico	59	2892 ± 2009	69 (-585, 723)	71.2	0.97 (0.74, 1.26)
Belize	26	3164 ± 1874	466 (-334, 1267)	80.8	1.10 (0.86, 1.43)
P		0.39	0.12	0.71	0.37

Footnotes to Supplemental Table 3.2

[1]N may be less than 478 due to missing values.

[2]From linear regression models. All models were adjusted for age, sex, and BMI.

[3]Urinary sodium excretion ≤2000 mg/d corresponds to the daily recommended sodium intake[20].

[4]From Poisson regression models. All models were adjusted for age, sex, and BMI.

[5]χ^2 score statistic with urinary sodium or recommended sodium intake from linear o Poisson regression models as the outcome, and indicator variables for each level of the characteristics as predictors.

[6]Test for linear trend with urinary sodium or recommended sodium intake as the outcome from linear or Poisson regression models, respectively, and a variable representing ordinal categories of each characteristic introduced as a continuous predictor. An independent covariance structure was specified in all models to account for clustering by family membership.

[7]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

Supplemental Table 3.3. Mean (± SD) and adjusted mean differences, and prevalence and prevalence ratios (PR) of urinary iodine concentrations according to sociodemographic characteristics among children from Mesoamerica

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentration[3] ≥300 µg/L N = 52	Adjusted PR (95% CI)[4]
Sex					
Female	113	166 ± 89	Reference	26.6	Reference
Male	104	178 ± 129	12 (-15, 39)	21.2	1.04 (0.59, 1.83)
P[5]		0.40	0.38	0.42	0.90
Age (years)					
<9	73	144 ± 105	Reference	21.9	Reference
9 - <11	74	168 ± 103	28 (-3, 60)	23.0	1.22 (0.60, 2.44)
≥11	70	205 ± 115	68 (36, 101)	27.1	1.28 (0.65, 2.50)
P, trend[6]		0.0006	<0.0001	0.53	0.48
Height-for-age Z score					
<-2	13	150 ± 104	Reference	38.5	Reference
-2 - <-1	40	151 ± 88	11 (-52, 73)	30.0	0.62 (0.21, 1.84)
-1 - <0	84	169 ± 114	27 (-32, 85)	20.2	0.45 (0.16, 1.29)
0 - <1	48	193 ± 120	47 (-18, 111)	27.1	0.44 (0.15, 1.36)
≥1	32	183 ± 110	40 (-28, 109)	15.6	0.30 (0.08, 1.11)
P, trend		0.08	0.10	0.20	0.08
BMI-for-age Z score					
<-1	32	141 ± 85	Reference	21.9	Reference
-1 - <0	43	158 ± 93	34 (-12, 79)	27.9	1.53 (0.59, 3.95)
0 - <1	66	167 ± 107	32 (-11, 76)	18.2	1.03 (0.40, 2.67)
≥1	76	197 ± 126	66 (21 ,110)	27.6	1.37 (0.57, 3.30)
P, trend		0.007	0.005	0.75	0.68
Maternal age (years)					
<30	33	164 ± 93	Reference	15.2	Reference
30 - <35	48	160 ± 93	10 (-35, 54)	22.9	1.78 (0.60, 5.30)
35 - <40	72	175 ± 133	16 (-26, 58)	29.2	2.33 (0.85, 6.39)
40 - <45	39	168 ± 89	22 (-26, 69)	15.4	1.47 (0.44, 4.91)
≥45	25	204 ± 117	22 (-32, 75)	36.0	3.28 (1.04, 10.39)
P, trend		0.18	0.32	0.30	0.09
Maternal height (cm)					

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentration[3] ≥300 µg/L N = 52	Adjusted PR (95% CI)[4]
Q1 (149.0)	56	182 ± 140	Reference	26.8	Reference
Q2 (153.1)	56	163 ± 100	-36 (-74, 1)	30.4	1.19 (0.58, 2.45)
Q3 (157.0)	56	167 ± 79	-19 (-57, 20)	17.9	0.65 (0.28, 1.51)
Q4 (162.3)	49	176 ± 114	-16 (-60, 28)	20.4	0.61 (0.25,1.48)
P, trend		0.83	0.60	0.29	0.16
Maternal body mass index (kg/m^2)					
<25	49	171 ± 99	Reference	20.4	Reference
25 - <30	85	166 ± 116	-2 (-38, 34)	29.4	1.74 (0.82, 3.68)
≥30	83	178 ± 110	-4 (-42, 34)	20.5	1.26 (0.56, 2.88)
P, trend		0.66	0.83	0.84	0.71
Maternal parity					
1	16	147 ± 75	Reference	25.0	Reference
2	69	170 ± 116	-15 (-71, 41)	20.3	0.80 (0.25, 2.57)
3	77	178 ± 125	1 (-53, 55)	23.4	0.71 (0.23, 2.20)
≥4	54	172 ± 89	-9 (-68, 50)	29.6	1.15 (0.36, 3.67)
P, trend		0.54	0.85	0.42	0.54
Paternal age					
<35	53	158 ± 86	Reference	22.6	Reference
35 - <40	63	172 ± 131	17 (-21, 54)	23.8	1.22 (0.55, 2.71)
40 - <45	46	176 ± 109	3 (-39, 45)	26.1	1.00 (0.43, 2.34)
45 - <55	38	190 ± 112	17 (-26, 60)	29.0	1.16 (0.49, 2.70)
≥55	13	180 ± 94	9 (-52, 69)	15.4	0.85 (0.18, 3.95)
P, trend		0.19	0.69	0.85	0.97
Paternal height (cm)					
Q1 (159.0)	53	163 ± 110	Reference	24.5	Reference
Q2 (164.7)	57	174 ± 93	21 (-18, 60)	22.8	0.86 (0.38, 1.97)
Q3 (169.7)	53	179 ± 126	12 (-28, 52)	28.3	1.00 (0.44, 2.27)
Q4 (176.5)	51	175 ± 114	24 (-19, 68)	21.6	0.81 (0.34, 1.92)
P, trend		0.53	0.41	0.92	0.74
Paternal body mass index (kg/m^2)					
<25	57	167 ± 97	Reference	33.3	Reference
25 - <30	93	163 ± 92	4 (-30, 38)	19.4	0.86 (0.44, 1.70)

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentration[3] ≥300 µg/L N = 52	Adjusted PR (95% CI)[4]
≥30	64	192 ± 141	25 (-13, 63)	23.4	0.87 (0.43, 1.76)
P, trend		0.20	0.18	0.29	0.69
Parental smoking history					
Neither parent ever smoked	80	191 ± 123	Reference	31.3	Reference
One parent ever smoked	106	159 ± 100	-29 (-60, 1)	20.8	1.04 (0.53, 2.04)
Both parents ever smoked	26	173 ± 108	-5 (-53, 42)	15.4	1.09 (0.33, 3.58)
P		0.16	0.37	0.09	0.88
Parental metabolic syndrome					
No parent	85	167 ± 118	Reference	21.2	Reference
Mother only	48	187 ± 116	6 (-30, 42)	29.2	1.38 (0.68, 2.81)
Father only	47	156 ± 81	-16 (-52, 20)	27.7	1.13 (0.54, 2.36)
Both parents	32	190 ± 118	-11 (-53, 30)	21.9	0.81 (0.33, 1.98)
P, trend		0.66	0.41	0.74	0.78
Highest parental education level					
Incomplete elementary	18	162 ± 105	Reference	33.3	Reference
Complete elementary	24	155 ± 131	3 (-59, 66)	12.5	0.56 0.13 2.44
Incomplete secondary	61	172 ± 110	26 (-31, 82)	26.2	0.91 0.34 2.46
Complete secondary	41	178 ± 101	22 (-40, 85)	26.8	0.84 0.29 2.43
Post secondary	73	176 ± 111	23 (-36, 82)	21.9	0.69 0.25 1.92
P, trend		0.44	0.49	0.79	0.55
Home ownership					
Yes	150	175 ± 117	15 (-15, 44)	23.3	0.99 (0.54, 1.79)
No	67	166 ± 92	Reference	25.4	Reference
P		0.58	0.33	0.78	0.96
Number of household assets[7]					
0-4	42	159 ± 118	Reference	23.8	Reference
5-7	84	187 ± 121	22 (-18, 61)	25.0	0.93 (0.42, 2.05)
8-9	41	161 ± 102	9 (-37, 54)	26.8	0.91 (0.37, 2.25)
10-12	50	166 ± 88	20 (-29, 70)	20.0	1.06 (0.40, 2.85)
P, trend		0.76	0.64	0.72	0.91
Household income					
Lower <25%	42	174 ± 121	Reference	26.2	Reference

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentration[3] ≥300 µg/L N = 52	Adjusted PR (95% CI)[4]
Medium 25-75%	100	180 ± 105	-4 (-41, 34)	26.0	0.77 (0.37, 1.60)
Higher >75%	71	159 ± 112	-15 (-54, 25)	19.7	0.71 (0.31, 1.59)
P, trend		0.38	0.42	0.44	0.42
Food insecurity					
No insecurity	66	170 ± 114	Reference	18.2	Reference
Mild insecurity	61	174 ± 92	-6 (-43, 31)	27.9	1.44 (0.67, 3.08)
Moderate insecurity	51	154 ± 93	-10 (-53, 33)	21.6	1.43 (0.56, 3.66)
Severe insecurity	38	197 ± 146	6 (-41, 53)	31.6	1.43 (0.58, 3.55)
P, trend		0.51	0.88	0.29	0.49
Country of origin					
Guatemala	28	192 ± 129	Reference	28.6	Reference
El Salvador	29	212 ± 159	6 (-46, 58)	13.8	0.49 (0.15, 1.62)
Dominican Republic	27	214 ± 97	3 (-51, 58)	74.1	2.65 (1.15, 6.12)
Honduras	28	183 ± 107	-17 (-70, 37)	17.9	0.64 (0.21, 1.96)
Nicaragua	30	146 ± 95	-56 (-109, -4)	10.0	0.36 (0.10, 1.37)
Panama	17	77 ± 54	-146 (-206, -85)	5.9	0.21 (0.03, 1.66)
Costa Rica	20	153 ± 66	-65 (-123, -8)	5.0	0.17 (0.02, 1.37)
Mexico	26	167 ± 73	-55 (-108, -2)	34.6	1.19 (0.46, 3.08)
Belize	12	148 ± 87	-52 (-119, 16)	8.3	0.30 (0.04, 2.45)
P		0.002	<0.0001	0.08	0.07

Footnotes to Supplemental Table 3.3

[1]Total may be less than 217 due to missing values.

[2]From linear regression models. All models were adjusted for age, sex, height-for-age Z score, BMI-for-age Z score, and country of origin.

[3]Urinary iodine concentrations ≥300 μg/L correspond to excessive iodine intake[33].

[4]From Poisson regression models. All models were adjusted for age, sex, and country of origin.

[5]χ^2 score statistic from linear or Poisson regression models with urinary concentrations or excessive intake of iodine, respectively as the outcome, and indicator variables of the characteristics as predictors.

[6]Test for linear trend with urinary concentrations of iodine or excessive iodine intake as the outcome and a variable representing ordinal categories of each characteristic as a continuous predictor.

[7]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

Supplemental Table 3.4. Mean (± SD) and adjusted means, and prevalence and prevalence ratios (PR) of urinary iodine concentrations according to sociodemographic characteristics among adults from Mesoamerica

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentrations[3] ≥300 µg/L N = 110	Adjusted PR (95% CI)[4]
Sex					
Female	243	227 ± 157	Reference	19.3	Reference
Male	235	294 ± 226	66 (38, 93)	26.8	1.48 (1.10, 1.99)
P[5]		<0.0001	<0.0001	0.02	0.009
Age (years)					
<30	53	244 ± 182	Reference	24.5	Reference
30 - <40	252	249 ± 172	-22 (-72, 28)	23.4	0.89 (0.56, 1.42)
40 - <50	139	277 ± 214	-11 (-68, 46)	23.7	0.72 (0.44, 1.18)
50+	33	301 ± 298	14 (-124, 152)	15.2	0.51 (0.17, 1.49)
P, trend[6]		0.18	0.74	0.48	0.08
Height quartile (mothers/fathers medians, cm)					
Q1 (148.9/159.1)	114	217 ± 128	Reference	18.4	Reference
Q2 (153.0/164.9)	127	270 ± 186	29 (-9, 68)	26.8	1.24 (0.78, 1.98)
Q3 (157.0/169.7)	122	274 ± 219	39 (-11, 89)	19.7	0.87 (0.53, 1.45)
Q4 (162.7/176.5)	115	276 ± 232	42 (-7, 92)	27.0	1.08 (0.68, 1.72)
P, trend		0.03	0.11	0.33	0.83
Body mass index (kg/m^2)					
<25	119	234 ± 169	Reference	21.0	Reference
25-<30	198	264 ± 191	43 (6, 80)	23.2	1.23 (0.84, 1.79)
≥30	161	274 ± 221	58 (16, 99)	24.2	1.29 (0.89, 1.89)
P, trend		0.11	0.01	0.54	0.19
Smoking status					
Never	303	258 ± 189	Reference	22.4	Reference
Past	129	282 ± 218	12 (-24, 48)	27.9	1.69 (1.20, 2.38)
Current	44	216 ± 185	-61 (-120, -1)	13.6	0.82 (0.37, 1.80)
P		0.64	0.20	0.71	0.31

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentrations[3] ≥300 µg/L N = 110	Adjusted PR (95% CI)[4]
Education level					
Incomplete elementary	64	251 ± 199	Reference	15.6	Reference
Complete elementary	71	228 ± 174	-8 (-67, 51)	19.7	1.65 (0.89, 3.06)
Incomplete secondary	146	268 ± 224	-10 (-74, 54)	25.3	1.29 (0.77, 2.18)
Complete secondary	74	257 ± 178	-14 (-82, 54)	27.0	1.40 (0.78, 2.51)
Post secondary	114	285 ± 187	-4 (-72, 64)	25.4	1.23 (0.71, 2.10)
P, trend		0.13	0.94	0.12	0.91
Home ownership					
Yes	327	253 ± 180	-14 (-63, 34)	21.1	0.75 (0.55, 1.03)
No	151	274 ± 229	Reference	27.2	Reference
P		0.40	0.56	0.19	0.07
Number of household assets[7]					
0-4	90	223 ± 167	Reference	18.9	Reference
5-7	183	260 ± 170	37 (-10, 85)	29.0	1.34 (0.85, 2.09)
8-9	92	303 ± 268	78 (5, 151)	22.8	0.96 (0.57, 1.63)
10-12	113	255 ± 186	55 (-10, 121)	16.8	1.05 (0.59, 1.86)
P, trend		0.21	0.05	0.30	0.55
Household income					
Lower <25%	94	251 ± 172	Reference	21.3	Reference
Medium 25-75%	215	273 ± 208	15 (-30, 61)	25.6	1.05 (0.69, 1.59)
Higher >75%	159	249 ± 196	-9 (-56, 39)	21.4	0.98 (0.63, 1.54)
P, trend		0.77	0.61	0.87	0.88
Food security					
No insecurity	149	251 ± 207	Reference	21.5	Reference
Mild insecurity	127	274 ± 193	-7 (-55, 40)	22.1	0.82 (0.54, 1.25)
Moderate insecurity	117	239 ± 177	0 (-47, 48)	22.2	1.21 (0.76, 1.94)
Severe insecurity	83	287 ± 208	10 (-55, 76)	28.9	1.14 (0.69, 1.89)
P, trend		0.56	0.75	0.35	0.37
Country of origin					

Characteristics	N[1]	Urinary iodine (µg/d)	Adjusted mean differences (95% CI)[2]	Iodine concentrations[3] ≥300 µg/L N = 110	Adjusted PR (95% CI)[4]
Guatemala	58	175 ± 88	Reference	10.3	Reference
El Salvador	58	325 ± 229	138 (76, 201)	19.0	1.93 (0.80, 4.65)
Dominican Republic	58	427 ± 223	234 (166, 302)	67.2	6.63 (3.19, 13.81)
Honduras	59	324 ± 218	134 (69, 199)	35.6	3.64 (1.67, 7.93)
Nicaragua	62	235 ± 137	44 (-7, 95)	14.5	1.34 (0.51, 3.52)
Panama	46	141 ± 117	-51 (-99, -4)	8.7	0.85 (0.28, 2.65)
Costa Rica	52	216 ± 106	25 (-16, 67)	3.9	0.38 (0.08, 1.70)
Mexico	59	241 ± 240	55 (-11, 121)	28.8	2.84 (1.22, 6.66)
Belize	26	181 ± 93	2 (-48, 51)	3.9	0.42 (0.06, 3.11)
P		0.002	0.0003	0.02	0.02

Footnotes to Supplemental Table 3.4

[1]N may be less than 478 due to missing values.

[2]From linear regression models. All models were adjusted for age, sex, height, and country of origin.

[3]Urinary iodine concentrations ≥300 μg/L correspond to excessive iodine intake[33].

[4]From Poisson regression models. All models were adjusted for age, sex, and country of origin.

[5]χ^2 score statistic with urinary iodine or excessive iodine intake from linear o Poisson regression models, respectively as the outcome, and indicator variables for each level of the characteristics as predictors.

[6]Test for linear trend with urinary iodine or excessive iodine intake as the outcome from linear or Poisson regression models, respectively, and a variable representing ordinal categories of each characteristic introduced as a continuous predictor. An independent covariance structure was specified in all models to account for clustering by family membership.

[7]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

Supplemental Table 3.5. β coefficients[1] of the stepwise regression algorithm for the main dietary determinants of urinary sodium among children and adults from Mesoamerica

Food item	β	β	β	β	β	β	β
			Children				
Cereal[2]	-0.07	-0.08	-0.07	-0.07	-0.06	-0.06	-0.05
Tomato sauce[3]		0.06	0.07	0.07	0.08	0.07	0.07
Coated sandwich cookies[4]			-0.06	-0.07	-0.06	-0.07	-0.07
Corn products[5]				0.07	0.07	0.06	0.06
American cheese[6]					-0.05	-0.06	-0.06
Added butter[7]						0.05	0.05
Chorizo[8]							-0.06
R^2	3.3	5.6	7.7	9.9	11.4	12.9	14.2
			Adults				
Rice[9]	0.09	0.12	0.11	0.11	0.11	0.11	0.09
Fried chicken[4]		-0.08	-0.07	-0.08	-0.07	-0.08	-0.08
Boiled chicken[4]			-0.07	-0.07	-0.07	-0.06	-0.06
Corn products[5]				0.06	0.06	0.06	0.06
Atole[10]					-0.06	-0.06	-0.06
Hard cheese[11]						0.04	0.04
Salami[4]							0.04
R^2	3.3	5.2	6.7	8.1	9.4	10.7	11.7

Footnotes to Supplemental Table 3.5

[1]From stepwise regression algorithm using linear regression models with log-transformed urinary sodium concentrations as the outcome and log-transformed frequency intake weights as predictors.

[2]One cup.

[3]1/4 cup.

[4]One portion.

[5]One portion. Includes arepas, empanadas, and tamales.

[6]One slice

[7]One tablespoon. Added to bread or other prepared foods.

[8]One unit.

[9]2/3 cup.

[10]1/2 cup. Hot thin gruel made from corn, oats, barley or plantains.

[11]Two pieces.

Supplemental Table 3.6. β coefficients[1] of the stepwise regression algorithm for the main dietary determinants of iodine intake among children and adults from Mesoamerica

Food item	β	β	β	β	β	β
		Children				
Sodium intake[2]	0.89	0.90				
Cream[3]		-0.07				
R^2	40.0	41.7				
		Adults				
Sodium intake	0.73	0.71	0.70	0.69	0.68	0.68
Salami[4]		0.06	0.07	0.06	0.06	0.05
Fried chicken[4]			-0.07	-0.07	-0.07	-0.07
Cream cheese[3]				-0.05	-0.05	-0.05
Sausage[5]					-0.05	-0.05
Beef or pork as side dish[4]						0.05
R^2	36.3	37.6	38.7	39.6	40.4	41.0

Footnotes to Supplemental Table 3.6

[1]From stepwise regression algorithm using linear regression models with log-transformed urinary concentrations as the outcome and log-transformed frequency intake weights as predictors.

[2]Sodium excretion was used as a proxy for sodium intake.

[3]One tablespoon.

[4]One portion.

[5]One unit.

Chapter 4 . Trace minerals and metabolic syndrome in children and their adult parents

Abstract

Background and aims: The trace minerals zinc, manganese, and copper may have beneficial roles on metabolic syndrome (MetS), but their impact remains debated. We evaluated the associations between these minerals with MetS among Mesoamerican children and their adult parents.

Methods: We conducted a cross-sectional study among 198 children and 378 parents from the capitals of Belize, Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Chiapas State in Mexico. Exposures were plasma zinc, manganese, and copper. In children, the outcome was a continuous metabolic risk score calculated through sex- and age-standardization of waist circumference, the homeostatic model assessment for insulin resistance (HOMA-IR), mean arterial pressure (MAP), serum high-density lipoprotein cholesterol (HDL-C), and serum triglycerides. In parents, the outcome was the prevalence of MetS according to the Adult Treatment Panel (ATP) III criteria[1]. 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.

Results: Among children, there was no association between plasma zinc with the metabolic score or any of the components. Plasma manganese was inversely associated with the metabolic score, waist circumference, and HOMA-IR scores. Compared with children in the lowest manganese quartile, children in the highest quartile had an adjusted 0.09 units lower

metabolic score (95% CI: -0.19, -0.01; P, trend=0.04. In contrast, plasma copper was not associated with the metabolic score but was positively associated with the waist circumference score. In adults, the trace minerals concentrations were not associated with MetS; only plasma copper was positively associated with abdominal obesity.

Conclusions: Manganese was the only trace mineral associated with the metabolic risk score in children. In adults, there were no associations between the trace minerals with MetS. Longitudinal evidence could help further elucidate the role of the trace minerals on MetS.

Keywords: Metabolic syndrome, plasma zinc, plasma manganese, plasma copper.

Introduction

Metabolic syndrome (MetS) is a cluster of independent risk factors associated with an increased risk of cardiovascular disease, type 2 diabetes mellitus, and mortality that include abdominal obesity, insulin resistance, hypertension, and dyslipidemia[1]. MetS is on the rise in many world regions, and in Latin America, the prevalence ranges from 19% to 43%[2,3], which is similar to the prevalence in the US[4] and Europe[5]. MetS components can start from childhood[6] and track into adulthood[7], increasing the risk for cardiovascular disease[7]. Because MetS is mostly preventable, identifying potentially modifiable risk factors is a research priority.

Zinc has multiple functions as a catalyst and structural cofactor associated with insulin, lipid, and reactive oxygen species (ROS) metabolism, which could help prevent the development or progression of MetS and type 2 diabetes mellitus[8]. In low- and middle-income countries, zinc deficiency prevalence is high and could have a deleterious metabolic impact, especially during childhood[9]. In Latin America, the prevalence ranges from 20% to 56%, and it is higher among rural, indigenous populations, where diet is predominantly plant-based[10]. Previous evidence in children found that zinc supplementation improved MetS components in 6 to 10-year-old Iranian children[11], but in prenatal supplementation trials there was no benefit in MetS components among 5-year-old Peruvian[12], and 6 to 8-year-old Nepali children[13]. Evidence from observational studies is also mixed and varies by sex. A study in 12 to 18-year-old Iranian girls found no association between zinc and MetS components[14]. Others found an inverse association with MetS, fasting glucose, and body mass index (BMI) in Colombian boys 11 to 16 years[15], Australian children 8 and 15 years[16], and US children 6 to 19 years[17], respectively. In contrast, a study in school-aged Brazilian children found a positive association between zinc and insulin resistance[18]. As in children, the evidence in adults is mixed. A meta-analysis by Zhang &

Zhang[19] found a positive association between zinc and MetS when evaluating two longitudinal studies conducted in Finnish and French adults, but when the authors evaluated cross-sectional studies in different adult populations, there was no association between zinc and MetS.

Manganese is involved in amino acid, cholesterol, and carbohydrate metabolism[20] and therefore could have a beneficial impact on MetS development. In children, previous studies have only evaluated the association between manganese and some of the MetS components. A previous study in 8 to 13-year-old Spanish girls found an inverse association between insulin levels and resistance markers[21], whereas a different study in US children and adolescents found a positive association with obesity[17]. In adults, prior evidence in Chinese adults found an inverse association between manganese and MetS[22], whereas others among Korean[23] and US[24] adults found no association with MetS. Animal studies found a potential beneficial role of manganese in MetS; in manganese-deficient rats, there was impaired glucose tolerance and pancreatic insulin release[25], and lower insulin-stimulated glucose oxidation in adipose tissue[26], when compared to non-deficient rats. This previous evidence highlight a potential association between MetS but evidence in children is scant and no studies have evaluated the Mesoamerican region.

Copper acts a catalyzer in multiple redox reactions and may help promote antioxidant activity and therefore, has been hypothesized to be beneficial for MetS[20]. However, previous evidence in children about the association between copper and MetS is mixed. Studies in Colombian[15] and Iranian[14,27] children found no significant association between copper and MetS[15,27] or MetS components[14]. However, a study in US children and adolescents found a positive association between copper and obesity[17]. In adults, the evidence is also mixed. Investigations in Chinese[22,28], US[24], Croatian[29], and European[30] adults found no association between copper and MetS, whereas, in postmenopausal Korean women[31] and Chinese[32] adults,

there was an inverse association with MetS. Moreover, another study in Chinese adults found a positive association between copper and MetS[33].

The bulk of the current evidence regarding the association of the previous trace minerals and MetS comes from developed countries, and thus there is a need to assess their status in populations facing the epidemiological transition like Mesoamerica, where various forms of malnutrition are likely to occur[34]. Additionally, there is scant evidence about the association between manganese and copper with MetS, especially in children. We aimed to study the association between trace minerals with MetS in children and their adult parents from Mesoamerica.

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 Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, Belize, and Chiapas State in Mexico. Details of the study have been described elsewhere[2]. Briefly, we recruited 267 family units comprising a school-aged child and both biological parents. Eligibility criteria included child's age from 7 to 12 years, living with both biological parents, not being pregnant or having a pregnant mother, and not having a sibling already participating in the study. The study protocol and procedures were approved by the Institutional Review Boards (IRB) of collaborating institutions in each country and by the University of Michigan Health and Behavioral Sciences IRB. We obtained written parental informed consent and children's assent to participate before enrollment.

Data collection

Data were collected during a single home or health center visit. Participants fasted for at least 6 hours before the visit. At the visit, participants responded to a questionnaire on sociodemographic 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)[35], a 16 yes/no item survey about food security experiences during the past three months. Researchers measured height, weight, and waist circumference using standardized 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 taken in triplicate, and the median of the three values was used[36]. Blood pressure was measured while seated, using Omron HEM-712C digital blood pressure monitors (Omron Healthcare, Inc., Lake Forest, IL, USA). Three measurements were obtained, separated by at least one minute, and the value of blood pressure used was the average of the second and third measures. At the end of the visit, phlebotomists obtained 7.5 ml of blood through antecubital venipuncture. Samples were placed in refrigerated containers and transported to each country's collaborating laboratories on the day of collection where the serum and plasma were separated, aliquoted, and cryostored at −20°C. Frozen stored samples from all countries were transported to the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City.

Laboratory methods

Quantifications for insulin, glucose, and lipids were conducted at INCAP. Plasma insulin was measured using a chemiluminescent immunoassay on an Immulite 1000 system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Plasma glucose and serum lipids (total and HDL-cholesterol and triglycerides) were quantified on an automated chemistry analyzer (Cobas c111 system; Roche Diagnostics, Mannheim, Germany).

Samples for the trace minerals measurements were processed and analyzed in a Class 100 ultraclean room at the University of Michigan Air Quality Laboratory. For each sample, 300 µL of plasma was transferred to an acid-cleaned Teflon vial using a capillary tip, and 0.9 mL of ultrapure grade nitric acid (HNO3 67-69%, Optima) was added. When a full 300 µL of plasma was not available, less volume (e.g., 100 µL) and a proportionately reduced quantity of nitric acid were used. The solution was then digested in a 90ºC muffle oven for one hour. 0.2 mL of each sample was then transferred to an acid-cleaned 4 mL vial, and 3 mL of Type 1 water was

added. Extracts were then analyzed for target trace elements using high-resolution inductively coupled plasma-mass spectrometry (ELEMENT2, Thermo Finnigan). This analysis method for biological samples incorporates daily quality assurance and quality control measures including blanks, Type I water blanks, replicate analyses and external standards as described elsewhere[37,38]. The accuracies (% differences between the certified values and the measured values) using the National Institute of Standards and Technology (NIST) Standard Reference Material (1640A) were 5%, 3%, and 4% for zinc, manganese, and copper, respectively. The precisions (% differences in standard deviation between the certified values and the measured values) were 7%, 4%, and 3% for zinc, manganese, and copper, respectively.

Definition of outcomes

Children. We calculated a metabolic risk score using MetS components that are consistent with the definition in adults: waist circumference, the homeostatic model assessment for insulin resistance (HOMA-IR)[39], mean arterial pressure (MAP,) serum HDL-cholesterol, and serum triglycerides. We calculated MAP as [(2 x diastolic blood pressure) + systolic blood pressure]/3. The metabolic risk score was created by regressing each log-transformed component on sex and log-transformed age using linear regression models to obtain the standardized regression residuals. The average of the residuals for the five components was used to create the score, with the residuals for HDL-cholesterol multiplied by -1 beforehand. Higher scores indicate a worse metabolic profile.

Adults. We defined MetS according to the Adult Treatment Panel (ATP) III criteria[1] as having any 3 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 triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides.

Definition of exposures

Because zinc deficiency (<700 µg/L)[20] was low (n= 2 among children and 19 among adults), we used quartiles of the distribution as the exposure. Manganese and copper were evaluated as quartiles of the distribution because no specific cutpoints exist for deficiency or toxicity.

Covariates

Children. Height-for-age and BMI-for-age Z scores were calculated using the WHO reference[40]. We used quartiles of parental height and categories of parental BMI according to the WHO classification. Household education was the maximum number of schooling years achieved by either parent and was categorized as presented in Supplemental Table 1. Household food insecurity was categorized by the number of affirmative responses in the ELCSA survey (no insecurity, 0; mild insecurity, 1-5; moderate insecurity, 6-10; severe insecurity, ≥11). The number of household assets was the sum of a car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color television, sound set, computer, or Internet, with a potential maximum number of assets of 12. Maternal parity was categorized as 1, 2, 3, or ≥ 4 births.

Adults. Height, BMI, education level, home ownership, number of household assets, and household food security were categorized as presented in Supplemental Tables 2. Parental smoking was categorized as never, past, or current. Household income was categorized into country-specific quartiles.

Data analysis

For analyses of zinc and manganese, the final analytic samples comprised participants with information available on both elements and MetS; 149 children and 270 adults. For plasma copper, the analytic sample consisted of 198 children and 378 adults.

Children. Children included in analyses of zinc and manganese differed from those excluded in that they were more likely to be girls, had higher height-for-age and BMI-for-age Z scores, higher parental smoking, and had overall better socioeconomic status (higher home ownership and income, and lower food insecurity scores) (**Supplemental Table 7**). Children included analyses of copper differed from those excluded in that they were less likely to be girls, had lower height-for-age Z scores, higher parental smoking exposure and home ownership, and higher food insecurity scores.

In bivariate analysis, we compared the distribution of MetS and its components by quartiles of the exposures using means and SD. We estimated tests for trend by introducing a variable representing the ordinal categories of each quartile as a continuous predictor in a linear regression model with MetS or each component as the outcome.

In multivariable analyses, we estimated adjusted differences with 95% CI in mean metabolic scores between quartiles of exposure using linear regression models with robust variances. Adjustment covariates were independent predictors of the outcome including maternal BMI category, parental smoking history, highest parental education level, and country of origin. Since the metabolic risk score is standardized for the age and sex distributions of the study population, the inclusion of these variables in multivariable models is unnecessary.

Adults. Parents with information on plasma zinc and manganese concentrations were more likely to be females and had fewer years of smoking, higher income, and lower food

insecurity scores than those excluded from these analyses. (**Supplemental Table 8)**. Compared to adults without information on copper, those included in the analyses were more likely to be female and had fewer years of education and less home ownership.

In bivariate analysis, we compared unadjusted MetS prevalences by quartiles of the exposures. We conducted tests for trend by introducing a variable representing ordinal categories of the quartiles as a continuous predictor into a generalized estimation equation (GEE) model using the Poisson distribution. In multivariable analysis, we estimated prevalence ratios with 95% CI between exposure levels from GEE models using an analogous approach to adjustment as described for children. The adjustment covariates were age category, sex, education level, smoking status, and country of origin. Robust variances were specified in all models to account for within-household correlations.

Results

Children

Zinc. Mean (± SD) plasma zinc concentration was 1273 ± 484 µg/L; 1.3% of children had zinc deficiency (<700 µg/L)[20]. Country of origin was a significant predictor of plasma zinc concentrations **(Supplemental Table 1)**. Nicaragua had the highest mean concentration, whereas Costa Rica had the lowest. There was no association between plasma zinc with the metabolic score or any of the components (**Table 1**).

Manganese. Mean (± SD) plasma manganese was 3.66 ± 9.23 µg/L. Maternal BMI was positively associated with plasma manganese concentrations (**Supplemental Table 2**). We found an inverse association between plasma manganese and the metabolic risk score (**Table 2**). Compared with children in the lowest quartile, children in the highest quartile had an adjusted 0.09 units lower score (95% CI: -0.19, -0.01; P, trend=0.04). Manganese was also inversely associated with the waist circumference and HOMA-IR scores (**Table 2**). Children in the highest quartile had an adjusted 0.07 units lower waist circumference score (95% CI: -0.14, 0.00; P, trend=0.03) and an adjusted 0.29 units lower HOMA-IR score (95% CI: -0.59, 0.00; P, trend=0.03) compared to children in the first quartile.

Copper. Mean (± SD) plasma copper concentration was 1070 ± 296 µg/L. Concentrations varied significantly by country; the Dominican Republic had the highest and Belize the lowest (**Supplemental Table 3**). Plasma copper was not associated with the metabolic risk score overall, but it was positively associated with the waist circumference component (**Table 3**). The waist circumference score was 0.05 adjusted units higher in children in the highest quartile compared to those in the lowest quartile (95% CI: -0.01, 0.10; P, trend=0.05).

Adults

Zinc. Mean (± SD) plasma zinc concentration was 1093 ± 321 µg/L; 7.0% of adults had zinc deficiency (<700 µg/L)[20]. Honduras had the highest whereas Mexico had the lowest mean concentrations of plasma zinc. Height was inversely associated with plasma zinc concentrations. **(Supplemental Table 4)**. Plasma zinc was not associated with MetS or any of its components (**Table 4**).

Manganese. Mean (± SD) plasma manganese was 3.48 ± 4.29 µg/L. Manganese concentrations were not significantly different by country. Age was positively associated with plasma manganese concentrations (**Supplemental Table 5**). Plasma manganese was not associated with MetS or any of its components (**Table 5**).

Copper. Mean (± SD) plasma copper concentration was 988 ± 323 µg/L. Country of origin was a significant predictor of concentrations; Honduras had the highest whereas Mexico had the lowest mean concentrations. Female sex and BMI were positively associated with serum copper concentrations (**Supplemental Table 6**). Copper was not associated with MetS prevalence; however, it was positively related to abdominal obesity (**Table 6**). Adults in the highest quartiles had a 72% higher prevalence of abdominal obesity compared to those in the lowest quartile (95% CI: 1.18, 2.51; P, trend=0.01).

Discussion

In children, we found that plasma manganese concentrations were inversely associated with the metabolic risk score, waist circumference, and HOMAR-IR scores. In contrast, plasma copper was positively associated with the waist circumference component. In adults, we found a positive association between plasma copper and abdominal obesity.

We found no association between plasma zinc with MetS or any of the components in either children or adults. Some previous evidence is consistent with our results. Two randomized trials in Peruvian[12] and Nepali[13] children found no association between prenatal zinc supplementation with cardiometabolic risk at ages 4.5[12] and 6 to 8[13] years, respectively. However, one randomized crossover trial of zinc compared to placebo conducted among obese school-aged Iranian children found a significant improvement of MetS components after zinc supplementation[11]. The discrepancy in findings could be due to the difference in supplementation timing and populations used. Cross-sectional studies also found mixed evidence. One study conducted in Iranian girls 12 to 18 years found no association between serum zinc and MetS components[14]. In contrast, another study in Colombian children 11 to 16 years found an inverse association between zinc intake and MetS only in boys[15].

Evidence in adults is also mixed. A supplementation trial with vitamin C and E, β-carotene, and zinc for 7.5 years did not affect the risk of developing MetS compared to placebo[41]. Similarly, a nested case-control study[28] and cross-sectional studies found no association of MetS with serum zinc in Korean[42], Chinese[28], and elderly Croatian[29] adults, plasma zinc in European adults[30], and zinc intake in Chinese adults[22]. In contrast, a longitudinal study conducted in Finnish adults[43] and cross-sectional studies in Iranian men[44], and US[24] and Brazilian[45] adults, found a positive association between serum[24,43,44], and erythrocyte[45] zinc with

MetS, respectively. Moreover, a cross-sectional study in Saudi adults found an inverse association between zinc intake and MetS[46]. From the previous evidence, the direction of the association between zinc and MetS is not consistent, but trials have failed to show a beneficial effect of zinc supplementation. Comparisons are difficult due to different supplement and doses used, and different confounding structures among the populations studied. Another issue is that the different biomarkers used to measure zinc status are not exchangeable, and dietary intake correlates weakly with most of them[9]. Moreover, in the observational studies, the designs and populations involved vary significantly, making direct comparisons and conclusions difficult.

In children, plasma manganese was inversely associated with the metabolic risk score, possibly through the waist circumference and HOMA-IR components. In contrast, in adults, there was no association between plasma manganese with MetS or any of the components. In children, there is no previous evidence about the association between plasma manganese and MetS. Contrarily to our results in children, a previous cross-sectional study in US children and adolescents aged 6 to 19 years found a positive association between serum manganese concentrations and odds of obesity[17].

Similar to our findings, a cross-sectional study in Spanish girls aged 8 to 13 years found that while all girls fulfill the manganese adequate intake (AI), those below the 100% of AI had higher levels of insulin and HOMA-IR compared to those above 100%[21]. Likewise, two cross-sectional studies in Korean[47] and Italian[48] adults found an inverse association between blood manganese and diabetes mellitus. Results from animal studies suggest a potential role of manganese in insulin metabolism. Rats feed a low manganese diet had impaired glucose tolerance and pancreatic insulin release when compared to those with a normal manganese diet[25]. Moreover, a similar experiment found that compared to adipose cells from non-deficient rats,

adipose cells from manganese-deficient rats had impaired insulin-stimulated glucose oxidation[26]. The potential role of manganese on insulin metabolism could explain our findings in children. In adults, previous cross-sectional studies in Korean[23,31] and elderly Croatian adults[29] found no association between manganese intake[31] or serum[23,29] levels with MetS, which are consistent with our results. In contrast, other cross-sectional studies found an inverse association between manganese intake with MetS in Korean men[49] and Chinese adults[22], and with abdominal obesity in Korean men[49]. These discrepancies could be related to the different exposure measurements used.

In both children and adults, we did not find an association between serum copper and MetS, but we found a positive association with the abdominal obesity component. Previous evidence is consistent with our findings. Cross-sectional studies in Colombian children 11 to 16 years[15], and Iranian children 10 to 18 years[27] found no association between copper intake and MetS. Additionally, a cross-sectional study in US children and adolescents ages 6 to 19 years found that children in the highest quartile of serum copper had higher odds of obesity compared to those in the lowest quartile[17]. In contrast, several cross-sectional studies found lower mean serum copper concentrations in obese children compared to those with healthy weight in Egyptian[50] children 5 to 10 years and Turkish children 7 to 11[51] and 6 to 17 years[52]. This difference could be due to confounding factors like age and sex, not accounted for in the later studies describing unadjusted mean concentrations.

In adults, one nested case-control study conducted among Chinese adults 40 years and older found no association between serum copper and MetS[28]. Similarly, cross-sectional studies found no association with MetS and plasma copper in European adults[30], serum copper in elderly Croatian adults[29], whole blood copper in US adults[24], and copper intake in Chinese adults[22].

Contrastingly, a cross-sectional study found an inverse association between copper intake with MetS in postmenopausal Korean women[31] and Chinese adults[32]. These discrepancies could be due to the different exposure measurements used, because blood measurements of copper do not correlate well with intake levels[20], and therefore are not directly comparable. Additionally, the populations, sample size, and confounding structures could vary considerably. In adults, cross-sectional studies in US[24], Thai[53], and Tunisian[54] adults found higher serum copper concentrations in overweight or obese compared to healthy weight adults. On the contrary, one cross-sectional study in Spanish adults found decreasing serum copper concentrations with increasing BMI categories[55]. The different findings in mean serum concentrations according to weight status could be due to confounding factors since the authors did not perform adjusted analysis.

Our study has several strengths. Data collected on both parents and children allowed adjustment for parental characteristics that could be considered potential confounders for the associations in children. Our study adds to the current body of literature on the associations between trace minerals and MetS, especially among children, in an understudied population like Mesoamerica.

Due to its cross-sectional design, causal inference is limited in our study. Comparisons of the metabolic risk with other populations are limited because we constructed the score based on the specific distribution of the different components. Due to laboratory constraints, this study was conducted in a subsample of the original, and the comparison analysis indicated potential selection bias. The most likely impact was an attenuation of the associations, especially for zinc; because the original sample was on an urban non-indigenous population, and the subsample had a better socioeconomic status, zinc deficiency was very low compared to similar estimated in the region[10]. Of note, selection bias in the original sample cannot be ruled out entirely since we used

convenience rather than random sampling. Finally, due to small sample size, country-specific analyses were not possible.

In conclusion, we found that in children, plasma manganese was inversely associated with the metabolic risk score, waist circumference, and the HOMA-IR scores. In contrast, plasma copper was positively associated with the waist circumference score and abdominal obesity, in children and adults, respectively. Longitudinal studies are needed to further elucidate the association between manganese and MetS, since our results support a potential beneficial effect. Additionally, copper could have a potential role in obesity development and further evaluation of the mechanism involved is warranted.

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Table 4.1. Means and adjusted mean differences in metabolic syndrome score[1] and its components[2] by plasma concentrations of zinc among children from Mesoamerica

	Q1	Q2	Q3	Q4	
Median plasma zinc, μg/L	891 (n=37)	1090 (n=37)	1289 (n=38)	1664 (n=37)	P-trend[2]
Overall metabolic score					
Mean ± SD	0.03 ± 0.20	0.01 ± 0.23	-0.02 ± 0.25	0.03 ± 0.21	
Unadjusted difference (95% CI)	Reference	-0.02 (-0.12, 0.08)	-0.05 (-0.15, 0.05)	0.01 (-0.09, 0.10)	0.99
Adjusted difference (95% CI)[4]	Reference	-0.03 (-0.13, 0.08)	-0.05 (-0.14, 0.05)	-0.01 (-0.10, 0.09)	0.82
Waist circumference score					
Mean ± SD	0.01 ± 0.13	0.02 ± 0.17	0.00 ± 0.17	0.01 ± 0.13	
Unadjusted difference (95% CI)	Reference	0.01 (-0.06, 0.08)	-0.01 (-0.08, 0.06)	0.00 (-0.06, 0.06)	0.81
Adjusted difference (95% CI)	Reference	0.01 (-0.06, 0.08)	-0.02 (-0.08, 0.05)	-0.01 (-0.08, 0.05)	0.51
HOMA-IR score					
Mean ± SD	0.07 ± 0.58	0.04 ± 0.60	-0.13 ± 0.65	0.01 ± 0.61	
Unadjusted difference (95% CI)	Reference	-0.03 (-0.30, 0.23)	-0.20 (-0.48, 0.07)	-0.06 (-0.33, 0.20)	0.40
Adjusted difference (95% CI)	Reference	-0.03 (-0.30, 0.23)	-0.15 (-0.43, 0.12)	-0.02 (-0.29, 0.26)	0.70
Mean arterial pressure (MAP) score					
Mean ± SD	-0.01 ± 0.13	0.03 ± 0.17	0.01 ± 0.16	0.03 ± 0.15	
Unadjusted difference (95% CI)	Reference	0.03 (-0.04, 0.10)	0.02 (-0.04, 0.08)	0.04 (-0.03, 0.10)	0.33
Adjusted difference (95% CI)	Reference	0.03 (-0.04, 0.10)	0.02 (-0.05, 0.08)	0.02 (-0.05, 0.10)	0.68
Serum HDL-cholesterol score					
Mean ± SD	-0.05 ± 0.24	-0.03 ± 0.22	-0.01 ± 0.32	-0.05 ± 0.30	
Unadjusted difference (95% CI)	Reference	0.02 (-0.08, 0.13)	0.05 (-0.08, 0.17)	0.00 (-0.12, 0.12)	0.89
Adjusted difference (95% CI)	Reference	0.02 (-0.09, 0.12)	0.05 (-0.06, 0.15)	0.04 (-0.07, 0.15)	0.42
Serum triglycerides score					
Mean ± SD	0.00 ± 0.33	-0.08 ± 0.47	0.01 ± 0.40	0.07 ± 0.37	
Unadjusted difference (95% CI)	Reference	-0.08 (-0.26, 0.10)	0.01 (-0.15, 0.17)	0.07 (-0.09, 0.23)	0.25
Adjusted difference (95% CI)	Reference	-0.12 (-0.31, 0.07)	-0.03 (-0.20, 0.14)	0.01 (-0.15, 0.18)	0.56

Footnotes to Table 4.1

[1]The overall score was calculated as the average of the five component scores after the HDL cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Test for linear trend from linear regression models with the metabolic score or each component score as the outcome and a variable representing ordinal categories of plasma zinc introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[4]From linear regression models. Models were adjusted for maternal BMI category, parental smoking history, highest parental education level, and country of origin.

Table 4.2. Means and adjusted mean differences in metabolic syndrome score[1] and its components[2] by plasma concentrations of manganese among children from Mesoamerica

	Q1	Q2	Q3	Q4	
Median plasma manganese, µg/L	1.08 (n=37)	1.63 (n=37)	2.49 (n=38)	5.03 (n=37)	P-trend[2]
Overall metabolic score					
Mean ± SD	0.07 ± 0.22	0.03 ± 0.27	-0.03 ± 0.17	-0.02 ± 0.21	
Unadjusted difference (95% CI)	Reference	-0.04 (-0.15, 0.07)	-0.11 (-0.19, -0.02)	-0.09 (-0.19, 0.01)	0.03
Adjusted difference (95% CI)[4]	Reference	-0.05 (-0.15, 0.06)	-0.12 (-0.21, -0.03)	-0.09 (-0.19, 0.01)	0.04
Waist circumference score					
Mean ± SD	0.08 ± 0.15	-0.01 ± 0.15	-0.04 ± 0.14	0.00 ± 0.13	
Unadjusted difference (95% CI)	Reference	-0.09 (-0.16, -0.02)	-0.12 (-0.19, -0.06)	-0.08 (-0.14, -0.01)	0.01
Adjusted difference (95% CI)	Reference	-0.08 (-0.15, -0.01)	-0.13 (-0.20, -0.06)	-0.07 (-0.14, 0.00)	0.03
HOMA-IR score					
Mean ± SD	0.08 ± 0.58	0.05 ± 0.69	-0.09 ± 0.56	-0.06 ± 0.61	
Unadjusted difference (95% CI)	Reference	-0.03 (-0.31, 0.26)	-0.17 (-0.42, 0.09)	-0.13 (-0.40, 0.14)	0.22
Adjusted difference (95% CI)	Reference	-0.14 (-0.40, 0.13)	-0.32 (-0.60, -0.04)	-0.29 (-0.59, 0.00)	0.03
Mean arterial pressure (MAP) score					
Mean ± SD	0.00 ± 0.12	0.02 ± 0.14	0.05 ± 0.18	0.01 ± 0.15	
Unadjusted difference (95% CI)	Reference	0.02 (-0.04, 0.08)	0.05 (-0.02, 0.12)	0.01 (-0.05, 0.07)	0.54
Adjusted difference (95% CI)	Reference	0.02 (-0.04, 0.07)	0.04 (-0.03, 0.12)	0.02 (-0.06, 0.09)	0.53
Serum HDL-cholesterol score					
Mean ± SD	-0.12 ± 0.31	-0.08 ± 0.30	0.04 ± 0.21	0.02 ± 0.24	
Unadjusted difference (95% CI)	Reference	0.04 (-0.10, 0.18)	0.15 (0.04, 0.27)	0.13 (0.01, 0.26)	0.01
Adjusted difference (95% CI)	Reference	-0.04 (-0.15, 0.07)	0.07 (-0.03, 0.16)	0.02 (-0.10, 0.13)	0.41
Serum triglycerides score					
Mean ± SD	0.08 ± 0.41	0.02 ± 0.45	-0.05 ± 0.33	-0.04 ± 0.37	
Unadjusted difference (95% CI)	Reference	-0.07 (-0.26, 0.13)	-0.13 (-0.30, 0.03)	-0.12 (-0.30, 0.05)	0.12
Adjusted difference (95% CI)	Reference	-0.08 (-0.28, 0.12)	-0.13 (-0.29, 0.03)	-0.09 (-0.28, 0.10)	0.29

Footnotes to Table 4.2

[1]The overall score was calculated as the average of the five component scores after the HDL cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Test for linear trend from linear regression models with the metabolic score or each component score as the outcome and a variable representing ordinal categories of plasma manganese introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[4]From linear regression models. Models were adjusted for maternal BMI category, parental smoking history, highest parental education level, and country of origin.

Table 4.3. Means and adjusted mean differences in metabolic syndrome score[1] and its components[2] by plasma concentrations of copper among children from Mesoamerica

	Q1	Q2	Q3	Q4	
Median plasma copper, µg/L	773 (n=49)	957 (n=50)	1125 (n=50)	1339 (n=49)	P-trend[2]
Overall metabolic score					
Mean ± SD	-0.01 ± 0.23	-0.02 ± 0.23	0.04 ± 0.23	0.04 ±0.22	
Unadjusted difference (95% CI)	Reference	-0.01 (-0.10, 0.07)	0.05 (-0.04, 0.14)	0.05 (-0.04, 0.14)	0.13
Adjusted difference (95% CI)[4]	Reference	0.03 (-0.06, 0.12)	0.07 (-0.03, 0.16)	0.07 (-0.02, 0.16)	0.11
Waist circumference score					
Mean ± SD	-0.02 ± 0.12	-0.01 ± 0.15	0.02 ± 0.15	0.02 ± 0.15	
Unadjusted difference (95% CI)	Reference	0.01 (-0.04, 0.07)	0.04 (-0.01, 0.09)	0.05 (-0.01, 0.10)	0.06
Adjusted difference (95% CI)	Reference	0.03 (-0.03, 0.09)	0.05 (0.00, 0.11)	0.05 (-0.01, 0.10)	0.05
HOMA-IR score					
Mean ± SD	0.05 ± 0.52	-0.04 ± 0.59	0.02 ± 0.66	0.03 ± 0.64	
Unadjusted difference (95% CI)	Reference	-0.09 (-0.31, 0.13)	-0.03 (-0.26, 0.20)	-0.02 (-0.25, 0.20)	0.98
Adjusted difference (95% CI)	Reference	0.04 (-0.20, 0.27)	0.04 (-0.20, 0.28)	0.10 (-0.13, 0.34)	0.40
Mean arterial pressure (MAP) score					
Mean ± SD	-0.02 ± 0.14	-0.03 ± 0.15	0.01 ± 0.15	0.02 ± 0.15	
Unadjusted difference (95% CI)	Reference	-0.01 (-0.06, 0.05)	0.03 (-0.03, 0.09)	0.05 (-0.01, 0.10)	0.06
Adjusted difference (95% CI)	Reference	0.01 (-0.05, 0.07)	0.04 (-0.02, 0.09)	0.04 (-0.02, 0.10)	0.16
Serum HDL-cholesterol score					
Mean ± SD	0.03 ± 0.26	0.04 ± 0.26	-0.07 ± 0.24	-0.07 ± 0.30	
Unadjusted difference (95% CI)	Reference	0.01 (-0.10, 0.11)	-0.10 (-0.20, 0.00)	-0.10 (-0.21, 0.01)	0.02
Adjusted difference (95% CI)	Reference	0.00 (-0.09, 0.09)	-0.09 (-0.19, 0.00)	-0.05 (-0.15, 0.04)	0.10
Serum triglycerides score					
Mean ± SD	-0.02 ± 0.43	-0.01 ± 0.40	0.08 ± 0.44	0.06 ± 0.46	
Unadjusted difference (95% CI)	Reference	0.02 (-0.15, 0.18)	0.10 (-0.07, 0.27)	0.09 (-0.09, 0.26)	0.22
Adjusted difference (95% CI)	Reference	0.09 (-0.09, 0.26)	0.11 (-0.08, 0.29)	0.10 (-0.09, 0.29)	0.31

Footnotes to Table 4.3

[1]The overall score was calculated as the average of the five component scores after the HDL cholesterol score was multiplied by -1.

[2]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were computed by regressing each log-transformed component on sex and log-transformed age in linear regression models to obtain standardized residuals.

[3]Test for linear trend from linear regression models with the metabolic score or each component score as the outcome and a variable representing ordinal categories of plasma copper introduced as continuous. An independent covariance structure was indicated in all models to obtain empirical variances.

[4]From linear regression models. Models were adjusted for maternal BMI category, parental smoking history, highest parental education level, and country of origin.

Table 4.4. Prevalence and adjusted prevalence ratios (PR) of metabolic syndrome[1] and its components by plasma concentrations of zinc among adults from Mesoamerica

	Q1		Q2		Q3		Q4	
Median plasma zinc, µg/L	800 (n=67)		962 (n=68)		1133 (n=67)		1425 (n=68)	P-trend[2]
Metabolic Syndrome							.	
Prevalence (%)	47.8		35.3		40.3		41.2	
Unadjusted PR (95% CI)	Reference	0.74	(0.49, 1.12)	0.84	(0.58, 1.22)	0.86	(0.60, 1.24)	0.57
Adjusted PR (95% CI)[3]	Reference	0.62	(0.41, 0.94)	0.78	(0.54, 1.13)	0.71	(0.48, 1.04)	0.20
Abdominal obesity[4]								
Prevalence (%)	52.2		52.9		46.3		50.0	
Unadjusted PR (95% CI)	Reference	1.01	(0.73, 1.41)	0.89	(0.62, 1.27)	0.96	(0.69, 1.33)	0.62
Adjusted PR (95% CI)	Reference	0.92	(0.70, 1.23)	0.82	(0.59, 1.13)	0.91	(0.66, 1.26)	0.46
High fasting blood glucose[5]								
Prevalence (%)	4.5		11.8		13.4		5.9	
Unadjusted PR (95% CI)	Reference	2.63	(0.73, 9.44)	3.00	(0.93, 9.63)	1.31	(0.31, 5.62)	0.64
Adjusted PR (95% CI)	Reference	2.34	(0.62, 8.83)	3.70	(1.15, 11.92)	1.71	(0.36, 8.08)	0.22
High blood pressure[6]								
Prevalence (%)	26.9		22.4		19.4		27.9	
Unadjusted PR (95% CI)	Reference	0.83	(0.46, 1.52)	0.72	(0.38, 1.38)	1.04	(0.60, 1.80)	0.99
Adjusted PR (95% CI)	Reference	0.56	(0.30, 1.04)	0.61	(0.31, 1.19)	0.76	(0.44, 1.32)	0.49
Low serum HDL-cholesterol[7]								
Prevalence (%)	89.6		77.9		89.6		92.7	
Unadjusted PR (95% CI)	Reference	0.87	(0.74, 1.02)	1.00	(0.90, 1.11)	1.03	(0.93, 1.15)	0.20
Adjusted PR (95% CI)	Reference	0.87	(0.73, 1.03)	1.00	(0.90, 1.10)	1.05	(0.91, 1.20)	0.21
High serum triglycerides[8]								
Prevalence (%)	49.3		48.5		52.2		52.9	
Unadjusted PR (95% CI)	Reference	0.99	(0.71, 1.37)	1.06	(0.77, 1.46)	1.07	(0.78, 1.49)	0.59
Adjusted PR (95% CI)	Reference	0.85	(0.61, 1.18)	1.03	(0.76, 1.41)	0.89	(0.63, 1.27)	0.83

Footnotes to Table 4.4

[1]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 triglycerides.

[2]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing ordinal categories of plasma zinc introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[3]From Poisson regression models. Models were adjusted for age category, sex, education level, smoking status, and country of origin.

[4]Waist circumference >102 cm in men and >88 cm in women.

[5]Fasting blood glucose ≥100 mg/dL

[6]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[7]HDL-C <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL cholesterol.

[8]TG ≥150 mg/dL or receiving treatment for hyperlipidemia.

Table 4.5. Prevalence and adjusted prevalence ratios (PR) of metabolic syndrome[1] and its components by plasma concentrations of manganese among adults from Mesoamerica

	Q1	Q2	Q3	Q4	
Median plasma manganese, µg/L	1.12 (n=67)	1.94 (n=68)	3.20 (n=68)	5.67 (n=67)	P-trend[2]
Metabolic Syndrome					
Prevalence (%)	34.3	47.1	35.3	47.8	
Unadjusted PR (95% CI)	Reference	1.37 (0.92, 2.04)	1.03 (0.65, 1.64)	1.39 (0.92, 2.11)	0.29
Adjusted PR (95% CI)[3]	Reference	1.30 (0.85, 1.98)	0.96 (0.59, 1.56)	1.12 (0.71, 1.78)	0.91
Abdominal obesity[4]					
Prevalence (%)	46.3	60.3	41.2	53.7	
Unadjusted PR (95% CI)	Reference	1.30 (0.94, 1.80)	0.89 (0.60, 1.31)	1.16 (0.81, 1.68)	0.92
Adjusted PR (95% CI)	Reference	1.30 (0.91, 1.87)	0.89 (0.60, 1.33)	1.11 (0.75, 1.66)	0.97
High fasting blood glucose[5]					
Prevalence (%)	10.5	2.9	8.8	13.4	
Unadjusted PR (95% CI)	Reference	0.28 (0.06, 1.34)	0.84 (0.29, 2.47)	1.29 (0.51, 3.23)	0.39
Adjusted PR (95% CI)	Reference	0.11 (0.01, 0.89)	0.77 (0.28, 2.14)	0.85 (0.30, 2.44)	0.72
High blood pressure[6]					
Prevalence (%)	20.9	28.4	22.1	25.4	
Unadjusted PR (95% CI)	Reference	1.36 (0.79, 2.33)	1.06 (0.55, 2.03)	1.21 (0.64, 2.32)	0.77
Adjusted PR (95% CI)	Reference	1.33 (0.80, 2.20)	1.18 (0.62, 2.25)	1.34 (0.70, 2.56)	0.44
Low serum HDL-cholesterol[7]					
Prevalence (%)	88.1	86.8	88.2	86.6	
Unadjusted PR (95% CI)	Reference	0.99 (0.86, 1.13)	1.00 (0.90, 1.12)	0.98 (0.86, 1.12)	0.87
Adjusted PR (95% CI)	Reference	0.98 (0.85, 1.12)	0.97 (0.85, 1.10)	0.95 (0.81, 1.10)	0.47
High serum triglycerides[8]					
Prevalence (%)	40.3	61.8	45.6	55.2	
Unadjusted PR (95% CI)	Reference	1.53 (1.11, 2.12)	1.13 (0.76, 1.68)	1.37 (0.97, 1.94)	0.30
Adjusted PR (95% CI)	Reference	1.43 (1.04, 1.95)	1.21 (0.81, 1.80)	1.20 (0.82, 1.77)	0.56

Footnotes to Table 4.5

[1]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 triglycerides.

[2]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing ordinal categories of plasma manganese introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[3]From Poisson regression models. Models were adjusted for age category, sex, education level, smoking status, and country of origin.

[4]Waist circumference >102 cm in men and >88 cm in women.

[5]Fasting blood glucose ≥100 mg/dL

[6]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[7]HDL-C <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL cholesterol.

[8]TG ≥150 mg/dL or receiving treatment for hyperlipidemia.

Table 4.6. Prevalence and adjusted prevalence ratios (PR) of metabolic syndrome[1] and its components by plasma concentrations of copper among adults from Mesoamerica

	Q1	Q2	Q3	Q4	
Median plasma copper, µg/L	688 (n=94)	877 (n=95)	1026 (n=95)	1275 (n=94)	P-trend[2]
Metabolic Syndrome					
Prevalence (%)	37.2	39.0	34.7	50.0	
Unadjusted PR (95% CI)	Reference	1.05 (0.74, 1.48)	0.93 (0.65, 1.34)	1.34 (0.99, 1.83)	0.11
Adjusted PR (95% CI)[3]	Reference	1.06 (0.73, 1.54)	0.99 (0.65, 1.50)	1.37 (0.96, 1.96)	0.09
Abdominal obesity[4]					
Prevalence (%)	25.5	49.5	50.5	71.3	
Unadjusted PR (95% CI)	Reference	1.94 (1.31, 2.86)	1.98 (1.33, 2.94)	2.79 (1.97, 3.96)	<0.0001
Adjusted PR (95% CI)	Reference	1.51 (1.02, 2.25)	1.33 (0.88, 2.00)	1.72 (1.18, 2.51)	0.01
High fasting blood glucose[5]					
Prevalence (%)	6.4	9.5	6.3	14.9	
Unadjusted PR (95% CI)	Reference	1.48 (0.55, 4.03)	0.99 (0.33, 2.95)	2.33 (1.04, 5.24)	0.07
Adjusted PR (95% CI)	Reference	1.18 (0.46, 3.04)	0.81 (0.28, 2.34)	2.28 (1.00, 5.17)	0.06
High blood pressure[6]					
Prevalence (%)	27.7	14.7	21.1	24.7	
Unadjusted PR (95% CI)	Reference	0.53 (0.29, 0.96)	0.76 (0.46, 1.27)	0.89 (0.56, 1.42)	0.90
Adjusted PR (95% CI)	Reference	0.62 (0.35, 1.11)	1.07 (0.62, 1.85)	1.32 (0.78, 2.21)	0.20
Low serum HDL-cholesterol[7]					
Prevalence (%)	81.9	85.3	84.2	87.2	
Unadjusted PR (95% CI)	Reference	1.04 (0.92, 1.18)	1.03 (0.90, 1.17)	1.06 (0.95, 1.19)	0.34
Adjusted PR (95% CI)	Reference	1.01 (0.88, 1.15)	1.05 (0.91, 1.21)	1.04 (0.91, 1.20)	0.48
High serum triglycerides[8]					
Prevalence (%)	58.5	52.6	51.6	50.0	
Unadjusted PR (95% CI)	Reference	0.90 (0.70, 1.15)	0.88 (0.68, 1.14)	0.85 (0.66, 1.11)	0.25
Adjusted PR (95% CI)	Reference	0.95 (0.74, 1.23)	1.00 (0.76, 1.32)	1.00 (0.76, 1.32)	0.94

Footnotes to Table 4.6

[1]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 triglycerides.

[2]Test for linear trend from Poisson regression models with metabolic syndrome or each component as the outcome and a variable representing ordinal categories of plasma manganese introduced as continuous. An independent covariance structure was specified in all models to account for clustering by family membership.

[3]From Poisson regression models. Models were adjusted for age category, sex, education level, smoking status, and country of origin.

[4]Waist circumference >102 cm in men and >88 cm in women.

[5]Fasting blood glucose ≥100 mg/dL

[6]Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or receiving antihypertensive medication.

[7]HDL-C <40 mg/dL in men and <50 mg/dL in women or receiving treatment for low HDL cholesterol.

[8]TG ≥150 mg/dL or receiving treatment for hyperlipidemia.

Supplemental Table 4.1. Mean (± SD) plasma concentrations of zinc according to sociodemographic characteristics among children from Mesoamerica

Characteristics	N[1]	Plasma zinc (μg/L)	Adjusted mean difference (95% CI)[2]
Sex			
Female	81	1251 ± 514	Reference
Male	68	1299 ± 448	58 (-88, 205)
P[3]		0.39	0.44
Age (years)			
<9	53	1397 ± 639	Reference
9 - <11	49	1212 ± 419	-129 (-324, 65)
≥11	47	1196 ± 285	-156 (-343, 30)
P, trend[4]		0.06	0.19
Height-for-age Z score			
<-1	26	1257 ± 357	Reference
-1 - <0	54	1300 ± 581	-80 (-260, 100)
0 - <1	45	1285 ± 510	-61 (-255, 133)
≥1	24	1207 ± 300	-142 (-322, 39)
P, trend		0.63	0.29
BMI-for-age Z score			
<-1	20	1228 ± 287	Reference
-1 - <0	29	1442 ± 728	246 (-32, 523)
0 - <1	44	1267 ± 388	64 (-105, 232)
≥1	56	1206 ± 442	12 (-145, 170)
P, trend		0.17	0.23
Maternal age (years)			
<30	25	1322 ± 501	Reference
30 - <35	30	1171 ± 426	-175 (-423, 74)
35 - <40	53	1327 ± 548	13 (-229, 255)
40 - <45	26	1334 ± 487	111 (-140, 361)
≥45	15	1097 ± 242	-166 (-381, 49)
P, trend		0.69	0.77
Maternal height (cm)			
Q1 (149)	31	1182 ± 345	Reference
Q2 (153)	36	1305 ± 451	56 (-155, 266)
Q3 (157)	39	1391 ± 588	157 (-44, 359)
Q4 (162)	43	1205 ± 485	-30 (-223, 164)
P, trend		0.98	0.74
Maternal body mass index (kg/m^2)			
<25	24	1187 ± 331	Reference
25 - <30	59	1352 ± 619	192 (-14, 399)
≥30	66	1233 ± 378	51 (-97, 200)
P, trend		0.92	0.99
Maternal parity			

Characteristics	N[1]	Plasma zinc (μg/L)	Adjusted mean difference (95% CI)[2]
1	11	1203 ± 324	Reference
2	43	1226 ± 454	193 (-61, 446)
3	54	1383 ± 461	255 (-7, 518)
≥4	40	1199 ± 571	152 (-197, 500)
P, trend		0.98	0.84
Paternal age			
<35	39	1199 ± 362	Reference
35 - <40	40	1262 ± 382	166 (-26, 357)
40 - <45	33	1362 ± 550	239 (-8, 485)
45 - <55	25	1403 ± 708	303 (-42, 647)
≥55	9	1076 ± 296	23 (-149, 194)
P, trend		0.48	0.19
Paternal height (cm)			
Q1 (158)	36	1236 ± 437	Reference
Q2 (165)	38	1361 ± 724	67 (-180, 313)
Q3 (170)	37	1277 ± 326	-29 (-199, 142)
Q4 (177)	36	1232 ± 345	-50 (-221, 121)
P, trend		0.99	0.68
Paternal body mass index (kg/m^2)			
<25	34	1248 ± 377	Reference
25 - <30	70	1301 ± 564	40 (-133, 213)
≥30	43	1264 ± 429	-40 (-217, 137)
P, trend		0.94	0.63
Parental smoking history			
Neither parent ever smoked	62	1265 ± 409	Reference
One parent ever smoked	63	1303 ± 558	12 (-135, 160)
Both parents ever smoked	21	1256 ± 486	-152 (-464, 160)
P		0.94	0.36
Parental metabolic syndrome			
No parent	54	1228 ± 381	Reference
Mother only	32	1324 ± 659	75 (-158, 308)
Father only	36	1270 ± 461	-10 (-188, 167)
Both parents	23	1337 ± 480	83 (-108, 273)
P, trend		0.43	0.68
Highest parental education level			
Incomplete elementary	12	1309 ± 675	Reference
Complete elementary	13	1034 ± 201	-313 (-703, 78)
Incomplete secondary	44	1346 ± 584	-43 (-445, 358)
Complete secondary	24	1232 ± 298	-185 (-554, 185)
Post secondary	56	1281 ± 457	-114 (-486, 257)
P, trend		0.48	0.90
Home ownership			

Characteristics	N[1]	Plasma zinc (μg/L)	Adjusted mean difference (95% CI)[2]
Yes	108	1254 ± 436	-101 (-293, 92)
No	41	1324 ± 597	Reference
P		0.48	0.22
Number of household assets[5]			
0-4	26	1379 ± 718	Reference
5-7	51	1254 ± 383	-61 (-356, 234)
8-9	31	1198 ± 429	-82 (-374, 209)
10-12	41	1285 ± 461	73 (-242, 388)
P, trend		0.56	0.42
Household income			
Lower <25%	24	1314 ± 513	Reference
Medium 25-75%	66	1284 ± 559	-49 (-315, 217)
Higher >75%	56	1252 ± 383	-117 (-364, 129)
P, trend		0.66	0.34
Food insecurity			
No insecurity	59	1194 ± 424	Reference
Mild insecurity	40	1359 ± 480	116 (-42, 275)
Moderate insecurity	27	1282 ± 665	40 (-207, 287)
Severe insecurity	23	1313 ± 375	-42 (-284, 201)
P, trend		0.15	0.79
Country of origin[6]			
Dominican Republic	23	1344 ± 399	Reference
Honduras	26	1425 ± 450	87 (-142, 317)
Nicaragua	31	1441 ± 716	96 (-198, 389)
Costa Rica	20	1066 ± 268	-232 (-438, -25)
Mexico	26	1188 ± 456	-121 (-381, 140)
Belize	23	1080 ± 182	-260 (-450, -71)
P		0.0001	0.0003

Footnotes to Supplemental Table 4.1

[1]Total may be less than 149 due to missing values because only samples with acid digestion during laboratory analysis were used.

[2]From linear regression models. All models were adjusted for age, sex, and country of origin.

[3]χ^2 score statistic from linear regression models with plasma concentrations of zinc as the outcome and indicator variables of the characteristic as predictors.

[4]Test for linear trend from linear regression models with plasma concentrations of zinc as the outcome and a variable representing ordinal categories of each characteristic as a continuous predictor.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

[6]Information for Guatemala and El Salvador is not available because only samples with acid digestion during laboratory analysis were used. Information for Panama was not available for any of the trace minerals measurements.

Supplemental Table 4.2. Mean (± SD) plasma concentrations of manganese according to sociodemographic characteristics among children from Mesoamerica

Characteristics	N[1]	Plasma manganese (µg/L)	Adjusted mean difference (95% CI)[2]
Sex			
Female	81	3.22 ± 3.94	Reference
Male	68	4.18 ± 13.0	0.87 (-2.57, 4.31)
P[3]		0.97	0.62
Age (years)			
<9	53	5.02 ± 14.75	Reference
9 - <11	49	3.39 ± 4.05	-1.31 (-4.70, 2.09)
≥11	47	2.40 ± 2.64	-2.37 (-5.94, 1.19)
P, trend[4]		0.28	0.27
Height-for-age Z score			
<-1	26	2.16 ± 1.06	Reference
-1 - <0	54	3.12 ± 2.85	-0.01 (-1.82, 1.80)
0 - <1	45	5.77 ± 16.34	3.17 (-1.02, 7.35)
≥1	24	2.55 ± 1.96	0.22 (-1.03, 1.46)
P, trend		0.35	0.12
BMI-for-age Z score			
<-1	20	3.15 ± 2.40	Reference
-1 - <0	29	3.33 ± 4.40	0.27 (-2.20, 2.73)
0 - <1	44	2.80 ± 2.63	-0.52 (-2.53, 1.49)
≥1	56	4.69 ± 14.49	1.94 (-1.93, 5.81)
P, trend		0.88	0.65
Maternal age (years)			
<30	25	2.64 ± 2.29	Reference
30 - <35	30	5.96 ± 19.49	3.57 (-3.32, 10.47)
35 - <40	53	2.97 ± 2.88	1.00 (-0.84, 2.84)
40 - <45	26	2.81 ± 1.95	1.29 (-0.90, 3.48)
≥45	15	4.68 ± 6.99	2.88 (-1.23, 6.99)
P, trend		0.41	0.29
Maternal height (cm)			
Q1 (148)	31	2.91 ± 2.30	Reference
Q2 (153)	36	3.04 ± 3.51	0.31 (-1.55, 2.17)
Q3 (157)	39	2.66 ± 1.56	-0.46 (-1.98, 1.07)
Q4 (163)	43	5.62 ± 16.68	3.24 (-1.57, 8.04)
P, trend		0.73	0.75
Maternal body mass index (kg/m^2)			
<25	24	3.15 ± 4.83	Reference
25 - <30	59	2.40 ± 2.04	-0.66 (-2.91, 1.59)
≥30	66	4.97 ± 13.36	1.28 (-1.87, 4.43)
P, trend		0.006	0.04
Maternal parity			

Characteristics	N[1]	Plasma manganese (µg/L)	Adjusted mean difference (95% CI)[2]
1	11	2.95 ± 2.92	Reference
2	43	2.36 ± 2.66	1.05 (-1.93, 4.03)
3	54	5.35 ± 14.84	3.16 (-1.85, 8.17)
≥4	40	3.00 ± 2.73	1.17 (-1.21, 3.55)
P, trend		0.98	0.68
Paternal age			
<35	39	2.47 ± 1.87	Reference
35 - <40	40	2.55 ± 1.96	1.45 (-0.93, 3.83)
40 - <45	33	6.46 ± 18.68	6.46 (-2.71, 15.62)
45 - <55	25	3.67 ± 4.77	3.12 (-0.75, 6.98)
≥55	9	3.55 ± 5.40	2.01 (-2.26, 6.29)
P, trend		0.49	0.19
Paternal height (cm)			
Q1 (158)	36	3.34 ± 3.50	Reference
Q2 (165)	38	5.31 ± 17.31	2.02 (-3.55, 7.59)
Q3 (170)	37	3.01 ± 2.98	-0.42 (-2.08, 1.25)
Q4 (177)	36	2.88 ± 4.16	-0.38 (-2.20, 1.44)
P, trend		0.34	0.56
Paternal body mass index (kg/m^2)			
<25	34	2.31 ± 1.33	Reference
25 - <30	70	4.69 ± 13.19	2.30 (-0.64, 5.23)
≥30	43	3.04 ± 2.96	0.24 (-1.09, 1.56)
P, trend		0.63	0.88
Parental smoking history			
Neither parent ever smoked	62	2.60 ± 2.82	Reference
One parent ever smoked	63	4.34 ± 13.50	0.80 (-1.84, 3.44)
Both parents ever smoked	21	4.78 ± 5.78	-0.20 (-5.32, 4.91)
P		0.004	0.51
Parental metabolic syndrome			
No parent	54	2.39 ± 2.07	Reference
Mother only	32	3.36 ± 2.99	-0.19 (-2.46, 2.09)
Father only	36	3.29 ± 4.18	0.63 (-1.24, 2.50)
Both parents	23	7.75 ± 22.34	5.45 (-2.41, 13.32)
P, trend		0.23	0.06
Highest parental education level			
Incomplete elementary	12	10.88 ± 30.81	Reference
Complete elementary	13	3.66 ± 3.83	-8.74 (-27.27, 9.80)
Incomplete secondary	44	3.43 ± 2.99	-9.74 (-29.33, 9.85)
Complete secondary	24	2.38 ± 2.67	-9.88 (-27.01, 7.25)
Post secondary	56	2.84 ± 3.63	-9.45 (-27.34, 8.44)
P, trend		0.02	0.09
Home ownership			

Characteristics	N[1]	Plasma manganese (µg/L)	Adjusted mean difference (95% CI)[2]
Yes	108	4.03 ± 10.75	1.27 (-1.57, 4.11)
No	41	2.68 ± 2.12	Reference
P		0.84	0.32
Number of household assets[5]			
0-4	26	3.15 ± 2.91	Reference
5-7	51	4.88 ± 15.06	3.31 (-3.53, 10.15)
8-9	31	2.03 ± 1.34	1.36 (-3.37, 6.08)
10-12	41	3.69 ± 4.54	3.67 (-1.85, 9.19)
P, trend		0.66	0.29
Household income			
Lower <25%	24	2.39 ± 1.32	Reference
Medium 25-75%	66	3.16 ± 3.42	0.64 (-0.63, 1.91)
Higher >75%	56	4.82 ± 14.56	1.84 (-1.81, 5.50)
P, trend		0.92	0.52
Food insecurity			
No insecurity	59	3.08 ± 4.07	Reference
Mild insecurity	40	5.79 ± 16.98	1.74 (-2.32, 5.79)
Moderate insecurity	27	2.66 ± 1.74	-2.51 (-5.78, 0.77)
Severe insecurity	23	2.62 ± 1.68	-3.20 (-8.74, 2.35)
P, trend		0.73	0.89
Country of origin[6]			
Dominican Republic	23	1.67 ± 1.62	Reference
Honduras	26	4.14 ± 5.12	2.51 (0.33, 4.68)
Nicaragua	31	6.60 ± 19.00	4.93 (-1.50, 11.37)
Costa Rica	20	1.56 ± 0.63	0.53 (-0.97, 2.04)
Mexico	26	3.46 ± 3.93	2.24 (0.43, 4.05)
Belize	23	3.18 ± 3.00	1.45 (-0.12, 3.01)
P		0.13	0.10

Footnotes to Supplemental Table 4.2

[1]Total may be less than 149 due to missing values because only samples with acid digestion during laboratory analysis were used.

[2]From linear regression models. All models were adjusted for age, sex, and country of origin.

[3]χ^2 score statistic from linear regression models with plasma concentrations of manganese as the outcome and indicator variables of the characteristic as predictors.

[4]Test for linear trend from linear regression models with plasma concentrations of manganese as the outcome and a variable representing ordinal categories of each characteristic as a continuous predictor.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

[6]Information for Guatemala and El Salvador is not available because only samples with acid digestion during laboratory analysis were used. Information for Panama was not available for any of the trace minerals measurements.

Supplemental Table 4.3. Mean (± SD) plasma concentrations of copper according to sociodemographic characteristics among children from Mesoamerica

Characteristics	N[1]	Plasma copper (μg/L)	Adjusted mean difference (95% CI)[2]
Sex			
Female	103	1051 ± 316	62 (-15, 140)
Male	95	1092 ± 274	Reference
P[3]		0.18	0.11
Age (years)			
<9	67	1111 ± 291	Reference
9 - <11	69	1041 ± 318	-28 (-127, 70)
≥11	62	1060 ± 277	-21 (-113, 70)
P, trend[4]		0.27	0.59
Height-for-age Z score			
<-1	46	1075 ± 284	Reference
-1 - <0	78	1084 ± 301	-43 (-140, 54)
0 - <1	48	1058 ± 342	-82 (-204, 40)
≥1	26	1046 ± 214	-108 (-226, 10)
P, trend		0.55	0.10
BMI-for-age Z score			
<-1	27	1073 ± 273	Reference
-1 - <0	40	1141 ± 420	86 (-67, 238)
0 - <1	64	1027 ± 276	-15 (-125, 94)
≥1	67	1069 ± 227	12 (-87, 112)
P, trend		0.67	0.82
Maternal age (years)			
<30	34	1105 ± 288	Reference
30 - <35	40	1040 ± 266	-79 (-198, 40)
35 - <40	65	1101 ± 359	-8 (-138, 122)
40 - <45	34	1043 ± 251	-11 (-127, 106)
≥45	25	1030 ± 231	-28 (-148, 92)
P, trend		0.43	0.96
Maternal height (cm)			
Q1 (148)	52	1013 ± 246	Reference
Q2 (153)	52	1069 ± 237	11 (-87, 110)
Q3 (157)	47	1166 ± 386	113 (-7, 233)
Q4 (163)	47	1039 ± 290	-50 (-164, 65)
P, trend		0.43	0.82
Maternal body mass index (kg/m^2)			
<25	45	998 ± 223	Reference
25 - <30	77	1123 ± 338	135 (39, 230)
≥30	76	1060 ± 283	75 (-14, 164)
P, trend		0.49	0.33
Maternal parity			

Characteristics	N[1]	Plasma copper (μg/L)	Adjusted mean difference (95% CI)[2]
1	13	1051 ± 338	Reference
2	61	1045 ± 321	57 (-131, 245)
3	68	1116 ± 258	83 (-97, 264)
≥4	55	1049 ± 307	64 (-136, 264)
P, trend		0.61	0.39
Paternal age			
<35	52	1034 ± 245	Reference
35 - <40	53	1095 ± 319	120 (10, 230)
40 - <45	41	1099 ± 314	74 (-43, 190)
45 - <55	34	1077 ± 364	86 (-66, 238)
≥55	13	999 ± 185	67 (-47, 182)
P, trend		0.92	0.48
Paternal height (cm)			
Q1 (158)	56	1040 ± 221	Reference
Q2 (165)	49	1032 ± 333	-60 (-162, 43)
Q3 (169)	49	1110 ± 263	9 (-74, 93)
Q4 (177)	40	1107 ± 383	3 (-116, 123)
P, trend		0.30	0.93
Paternal body mass index (kg/m^2)			
<25	52	1040 ± 260	Reference
25 - <30	87	1088 ± 343	40 (-51, 132)
≥30	55	1067 ± 259	13 (-83, 108)
P, trend		0.60	0.76
Parental smoking history			
Neither parent ever smoked	75	1119 ± 297	Reference
One parent ever smoked	93	1019 ± 295	-77 (-157, 3)
Both parents ever smoked	25	1111 ± 308	-35 (-195, 125)
P		0.32	0.34
Parental metabolic syndrome			
No parent	73	1044 ± 276	Reference
Mother only	47	1100 ± 327	76 (-26, 179)
Father only	39	1049 ± 246	-56 (-156, 44)
Both parents	32	1118 ± 369	48 (-76, 171)
P, trend		0.48	0.95
Highest parental education level			
Incomplete elementary	17	1147 ± 299	Reference
Complete elementary	25	960 ± 152	-200 (-333, -67)
Incomplete secondary	58	1071 ± 332	-100 (-245, 45)
Complete secondary	32	1042 ± 232	-144 (-284, -4)
Post secondary	66	1106 ± 325	-95 (-248, 57)
P, trend		0.58	0.71
Home ownership			

Characteristics	N[1]	Plasma copper (μg/L)	Adjusted mean difference (95% CI)[2]
Yes	139	1049 ± 254	-82 (-179, 15)
No	59	1122 ± 375	Reference
P		0.21	0.10
Number of household assets[5]			
0-4	38	1104 ± 398	Reference
5-7	80	1061 ± 239	-10 (-135, 115)
8-9	36	1049 ± 289	-38 (-187, 111)
10-12	44	1075 ± 303	26 (-130, 181)
P, trend		0.79	0.64
Household income			
Lower <25%	38	1027 ± 268	Reference
Medium 25-75%	89	1079 ± 305	53 (-51, 158)
Higher >75%	66	1079 ± 303	26 (-80, 133)
P, trend		0.48	0.74
Food insecurity			
No insecurity	65	1037 ± 303	Reference
Mild insecurity	51	1093 ± 272	36 (-68, 139)
Moderate insecurity	44	1072 ± 307	22 (-110, 153)
Severe insecurity	37	1089 ± 311	-29 (-173, 115)
P, trend		0.33	0.87
Country of origin[6]			
Guatemala	28	1046 ± 223	Reference
El Salvador	21	1004 ± 198	-51 (-166, 65)
Dominican Republic	23	1242 ± 349	197 (27, 367)
Honduras	26	1177 ± 267	128 (-2, 259)
Nicaragua	31	1163 ± 406	116 (-44, 276)
Costa Rica	20	1008 ± 322	-25 (-188, 139)
Mexico	26	953 ± 210	-92 (-206, 22)
Belize	23	931 ± 147	-123 (-221, -25)
P		0.004	0.005

Footnotes to Supplemental Table 4.3

[1]Total may be less than 198 due to missing values.

[2]From linear regression models. All models were adjusted for age, sex, and country of origin.

[3]χ^2 score statistic from linear regression models with plasma concentrations of copper as the outcome and indicator variables of the characteristic as predictors.

[4]Test for linear trend from linear regression models with plasma concentrations of copper as the outcome and a variable representing ordinal categories of each characteristic as a continuous predictor.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

[6]Information for Panama was not available for any of the trace minerals measurements.

Supplemental Table 4.4. Mean (± SD) plasma concentrations of zinc according to sociodemographic characteristics among adults from Mesoamerica

Characteristics	N[1]	Plasma zinc (μg/L)	Adjusted mean difference (95% CI)[2]
Sex			
Female	150	1082 ± 290	Reference
Male	120	1106 ± 357	14 (-62, 89)
P[3]		0.52	0.72
Age (years)			
<30	29	1076 ± 248	Reference
30 - <35	59	1082 ± 386	-31 (-157, 96)
35 - <40	91	1073 ± 317	-29 (-134, 76)
40 - <45	49	1105 ± 309	5 (-122, 131)
45 - <5	31	1179 ± 272	50 (-70, 171)
55+	11	1057 ± 351	22 (-166, 210)
P, trend[4]		0.33	0.33
Height quartile (mothers/fathers medians, cm)			
Q1 (148/158)	57	1137 ± 374	Reference
Q2 (153/165)	68	1104 ± 310	-78 (-192, 35)
Q3 (157/170)	73	1101 ± 287	-84 (-193, 25)
Q4 (163/177)	72	1038 ± 319	-137 (-253, -21)
P, trend		0.12	0.02
Body mass index (kg/m^2)			
<25	53	1074 ± 289	Reference
25-<30	116	1119 ± 337	21 (-85, 128)
≥30	101	1072 ± 320	-2 (-102, 98)
P, trend		0.80	0.87
Education level			
Incomplete elementary	30	1092 ± 278	Reference
Complete elementary	35	1118 ± 413	28 (-144, 201)
Incomplete secondary	95	1064 ± 333	-13 (-139, 113)
Complete secondary	37	1050 ± 220	-93 (-220, 33)
Post secondary	69	1126 ± 281	-9 (-129, 111)
P, trend		0.70	0.51
Smoking status			
Never	177	1096 ± 318	Reference
Past	68	1105 ± 331	-38 (-139, 63)
Current	24	1037 ± 330	-131 (-294, 33)
P		0.59	0.12
Home ownership			
Yes	188	1075 ± 311	-94 (-192, 4)
No	82	1132 ± 342	Reference
P		0.21	0.06
Number of household assets[5]			

Characteristics	N[1]	Plasma zinc (μg/L)	Adjusted mean difference (95% CI)[2]
0-4	45	1101 ± 323	Reference
5-7	93	1095 ± 391	40 (-105, 185)
8-9	52	1034 ± 218	-37 (-164, 90)
10-12	80	1123 ± 285	65 (-89, 218)
P, trend		0.80	0.66
Household income			
Lower <25%	45	1158 ± 350	Reference
Medium 25-75%	122	1073 ± 314	-53 (-168, 62)
Higher >75%	98	1082 ± 317	-78 (-194, 38)
P, trend		0.35	0.19
Food security			
No insecurity	112	1061 ± 300	Reference
Mild insecurity	70	1132 ± 361	58 (-43, 159)
Moderate insecurity	45	1090 ± 343	-21 (-139, 98)
Severe insecurity	43	1113 ± 282	3 (-128, 135)
P, trend		0.38	0.90
Country of origin[6]			
Dominican Republic	44	1084 ± 273	Reference
Honduras	33	1301 ± 243	208 (89, 326)
Nicaragua	58	1153 ± 411	75 (-70, 221)
Costa Rica	53	1060 ± 223	-22 (-128, 84)
Mexico	60	976 ± 330	-113 (-245, 19)
Belize	22	1035 ± 260	-52 (-192, 87)
P		0.0007	0.0008

Footnotes to Supplemental Table 4.4

[1]N may be less than 274 due to missing values, only samples with acid digestion during laboratory analysis were used.

[2]From linear regression models. All models were adjusted for sex, age, and country of origin.

[3]χ^2 score statistic from linear regression models with plasma zinc as the outcome and indicator variables for each level of the characteristic as predictors.

[4]Test for linear trend from linear regression models with plasma zinc as the outcome and a variable representing ordinal categories of each characteristic introduced as a continuous predictor.

An independent covariance structure was specified in all models to account for clustering by family membership.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

[6]Information for Guatemala and El Salvador is not available because only samples with acid digestion during laboratory analysis were used. Information for Panama was not available for any of the trace minerals measurements.

Supplemental Table 4.5. Mean (± SD) plasma concentrations of manganese according to sociodemographic characteristics among adults from Mesoamerica

Characteristics	N[1]	Plasma manganese (µg/L)	Adjusted mean difference (95% CI)[2]
Sex			
Female	150	3.48 ± 5.0	Reference
Male	120	3.49 ± 3.21	0.08 (-1.08, 1.24)
P[3]		0.97	0.89
Age (years)			
<30	29	3.35 ± 2.05	Reference
30 - <35	59	2.65 ± 1.94	-0.56 (-1.42, 0.31)
35 - <40	91	3.48 ± 5.38	0.39 (-0.73, 1.50)
40 - <45	49	4.40 ± 5.75	1.36 (-0.41, 3.13)
45 - <5	31	3.59 ± 3.21	0.50 (-0.70, 1.69)
55+	11	3.93 ± 1.76	0.60 (-0.50, 1.70)
P, trend[4]		0.02	0.01
Height quartile (mothers/fathers medians, cm)			
Q1 (148/158)	57	3.16 ± 1.80	Reference
Q2 (153/165)	68	3.07 ± 2.30	0.44 (-0.28, 1.16)
Q3 (157/170)	73	4.51 ± 5.47	1.76 (0.52, 3.00)
Q4 (163/177)	72	3.09 ± 5.52	0.54 (-0.59, 1.68)
P, trend		0.61	0.18
Body mass index (kg/m^2)			
<25	53	3.20 ± 3.35	Reference
25-<30	116	3.24 ± 3.57	-0.05 (-1.12, 1.03)
≥30	101	3.91 ± 5.36	0.51 (-0.79, 1.81)
P, trend		0.27	0.40
Education level			
Incomplete elementary	30	3.90 ± 3.95	Reference
Complete elementary	35	2.74 ± 1.81	-1.10 (-2.66, 0.46)
Incomplete secondary	95	3.92 ± 4.32	0.21 (-1.50, 1.93)
Complete secondary	37	3.07 ± 3.13	-0.92 (-2.66, 0.82)
Post secondary	69	3.24 ± 5.68	-0.56 (-2.63, 1.51)
P, trend		0.61	0.77
Smoking status			
Never	177	3.29 ± 4.24	Reference
Past	68	4.22 ± 5.0	1.03 (-0.61, 2.68)
Current	24	2.91 ± 1.67	-0.04 (-1.31, 1.23)
P		0.56	0.48
Home ownership			
Yes	188	3.62 ± 4.77	0.37 (-0.86, 1.60)
No	82	3.18 ± 2.90	Reference
P		0.34	0.55
Number of household assets[5]			

Characteristics	N[1]	Plasma manganese (µg/L)	Adjusted mean difference (95% CI)[2]
0-4	45	3.75 ± 3.62	Reference
5-7	93	3.25 ± 2.68	-0.51 (-1.88, 0.86)
8-9	52	3.41 ± 3.09	-0.12 (-1.59, 1.35)
10-12	80	3.66 ± 6.38	-0.16 (-1.91, 1.59)
P, trend		0.88	0.87
Household income			
Lower <25%	45	2.72 ± 1.83	Reference
Medium 25-75%	122	3.38 ± 2.99	0.35 (-0.34, 1.03)
Higher >75%	98	3.65 ± 5.40	0.69 (-0.56, 1.94)
P, trend		0.19	0.33
Food security			
No insecurity	112	3.66 ± 5.72	Reference
Mild insecurity	70	3.29 ± 2.06	0.01 (-0.89, 0.92)
Moderate insecurity	45	3.55 ± 3.43	-0.08 (-1.20, 1.04)
Severe insecurity	43	3.29 ± 3.49	-0.10 (-1.42, 1.21)
P, trend		0.67	0.86
Country of origin[6]			
Dominican Republic	44	3.15 ± 3.53	Reference
Honduras	33	3.02 ± 3.27	-0.41 (-1.92, 1.09)
Nicaragua	58	3.44 ± 3.03	0.54 (-0.56, 1.64)
Costa Rica	53	3.44 ± 7.54	0.01 (-2.15, 2.18)
Mexico	60	3.79 ± 2.94	0.52 (-0.63, 1.67)
Belize	22	4.22 ± 1.68	1.01 (-0.23, 2.25)
P		0.12	0.21

Footnotes to Supplemental Table 4.5

[1]N may be less than 274 due to missing values, only samples with acid digestion during laboratory analysis were used.

[2]From linear regression models. All models were adjusted for sex, age, and country of origin.

[3]χ^2 score statistic from linear regression models with plasma manganese as the outcome and indicator variables for each level of the characteristic as predictors.

[4]Test for linear trend from linear regression models with plasma manganese as the outcome and a variable representing ordinal categories of each characteristic introduced as a continuous predictor.

An independent covariance structure was specified in all models to account for clustering by family membership.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

[6]Information for Guatemala and El Salvador is not available because only samples with acid digestion during laboratory analysis were used. Information for Panama was not available for any of the trace minerals measurements.

Supplemental Table 4.6. Mean (± SD) plasma concentrations of copper according to sociodemographic characteristics among adults from Mesoamerica

Characteristics	N[1]	Plasma copper (µg/L)	Adjusted mean difference (95% CI)[2]
Sex			
Female	209	1093 ± 339	Reference
Male	169	858 ± 248	-244 (-304, -184)
P[3]		<0.0001	<0.0001
Age (years)			
<30	42	1045 ± 272	Reference
30 - <35	84	960 ± 386	-34 (-151, 82)
35 - <40	121	1009 ± 346	15 (-94, 124)
40 - <45	66	934 ± 282	-30 (-148, 89)
45 - <5	51	1019 ± 258	53 (-64, 170)
55+	14	943 ± 219	76 (-56, 209)
P, trend[4]		0.50	0.20
Height quartile (mothers/fathers medians, cm)			
Q1 (14/158)	98	999 ± 260	Reference
Q2 (153/165)	104	990 ± 375	-32 (-122, 58)
Q3 (157/170)	97	984 ± 359	-10 (-95, 76)
Q4 (163/177)	79	976 ± 277	-32 (-109, 45)
P, trend		0.55	0.59
Body mass index (kg/m^2)			
<25	93	935 ± 343	Reference
25-<30	155	970 ± 263	50 (-23, 123)
≥30	130	1047 ± 365	105 (28, 181)
P, trend		0.02	0.008
Education level			
Incomplete elementary	54	1044 ± 372	Reference
Complete elementary	55	1029 ± 413	27 (-106, 159)
Incomplete secondary	125	926 ± 292	-61 (-174, 52)
Complete secondary	52	1025 ± 289	-15 (-131, 101)
Post secondary	84	988 ± 287	-11 (-117, 96)
P, trend		0.48	0.71
Smoking status			
Never	243	1022 ± 311	Reference
Past	98	963 ± 367	-10 (-95, 75)
Current	36	821 ± 210	-96 (-188, -3)
P		<0.0001	0.15
Home ownership			
Yes	252	996 ± 351	15 (-45, 74)
No	126	971 ± 261	Reference
P		0.42	0.62
Number of household assets[5]			

Characteristics	N[1]	Plasma copper (µg/L)	Adjusted mean difference (95% CI)[2]
0-4	74	999 ± 291	Reference
5-7	150	983 ± 330	13 (-88, 113)
8-9	66	982 ± 385	23 (-103, 150)
10-12	88	992 ± 291	79 (-34, 193)
P, trend		0.93	0.20
Household income			
Lower <25%	70	1000 ± 391	Reference
Medium 25-75%	180	987 ± 281	-2 (-108, 103)
Higher >75%	118	963 ± 278	-47 (-169, 76)
P, trend		0.48	0.38
Food security			
No insecurity	122	971 ± 274	Reference
Mild insecurity	102	994 ± 303	-8 (-87, 71)
Moderate insecurity	82	968 ± 363	-79 (-171, 13)
Severe insecurity	70	1037 ± 383	3 (-99, 104)
P, trend		0.32	0.70
Country of origin[6]			
Guatemala	54	1013 - 400	Reference
El Salvador	54	1006 - 200	0 (-99, 99)
Dominican Republic	44	994 ± 320	-3 (-112, 107)
Honduras	33	1071 ± 289	60 (-76, 196)
Nicaragua	58	1041 ± 432	40 (-124, 205)
Costa Rica	53	917 ± 276	-76 (-182, 31)
Mexico	60	898 ± 246	-106 (-216, 4)
Belize	22	1026 ± 332	-90 (-260, 80)
P		0.03	0.007

Footnotes to Supplemental Table 4.6

[1]N may be less than 378 due to missing values.

[2]From linear regression models. All models were adjusted for sex, age, and country of origin.

[3]χ^2 score statistic from linear regression models with plasma manganese as the outcome and indicator variables for each level of the characteristic as predictors.

[4]Test for linear trend from linear regression models with plasma manganese as the outcome and a variable representing ordinal categories of each characteristic introduced as a continuous predictor.

An independent covariance structure was specified in all models to account for clustering by family membership.

[5]From a list that included car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet.

[6]Information for Panama was not available for any of the trace minerals measurements.

Supplemental Table 4.7. Means (±SD)[1] of metabolic syndrome score and sociodemographic characteristics in children with and without information on plasma concentrations of zinc, manganese, and copper

Characteristic	With zinc and manganese information (n=149)	Without zinc and manganese information (n=118)	With copper information (n=198)	Without copper information (n=69)
Metabolic syndrome score	0.01 ± 0.22	-0.01 ± 0.23	0.01 ± 0.23	-0.03 ± 0.21
Female sex, %	54.4	44.9	48.0	55.1
Child's age, y	9.9 ± 1.7	9.9 ± 1.7	9.9 ± 1.7	9.7± 1.7
Height-for-age Z score	-0.05 ± 1.07	-0.43 ± 1.17	-0.23 ± 1.07	-0.18 ± 1.31
BMI-for-age Z score	0.66 ± 1.46	0.44 ± 1.42	0.56 ± 1.44	0.58 ± 1.48
Maternal age, y	36.9 ± 6.6	37.1 ± 5.9	37.2 ± 6.6	36.4 ± 5.2
Maternal height, cm	156 ± 6.0	154 ± 6.8	155 ± 6.2	156 ± 6.9
Maternal BMI, kg/m^2	30.1 ± 5.7	28.1 ± 5.2	29.3 ± 5.6	28.8 ± 5.5
Maternal parity	3.2 ± 1.7	3.2 ± 2.2	3.3 ± 2.0	2.8 ± 1.6
Paternal age, y	40.5 ± 8.4	40.4 ± 8.1	40.6 ± 8.7	40.0 ± 6.9
Paternal height, cm	167 ± 7.4	167 ± 7.3	167 ± 7.4	170 ± 6.8
Paternal BMI, kg/m^2	28.1 ± 4.8	27.8 ± 4.6	27.9 ± 4.8	28.3 ± 4.4
Parental smoking history, %				
Neither parent ever smoked	42.5	36.0	38.9	41.8
One parent ever smoked	43.2	54.4	48.2	47.8
Both parents ever smoked	14.4	9.7	13.0	10.4
Parental metabolic syndrome, %				
No parent	37.2	49.1	38.2	54.4
Mother only	22.0	22.8	24.6	16.2
Father only	24.8	14.0	20.4	19.1
Both parents	15.9	14.0	16.8	10.3
Household education, y	11.7 ± 4.8	11.2 ± 4.8	11.2 ± 4.8	12.3 ± 4.6
Home ownership, %	72.5	66.1	70.2	68.1
Household number of assets	7.3 ± 2.8	6.8 ± 2.7	6.9 ± 2.8	7.7 ± 2.7
Household income, %				
Lower <25%	16.4	23.7	19.7	19.4
Medium 25-75%	45.2	46.5	46.1	44.8
Higher >75%	38.4	29.8	34.2	35.8
Household food security score	4.1 ± 4.7	5.8 ± 5.1	5.1 ± 5.1	4.3 ± 4.3
Country of origin, %				
Guatemala	-	26.3	14.1	4.4
El Salvador	-	25.4	10.6	13.0
Dominican Republic	15.4	5.9	11.6	10.1
Honduras	17.5	3.4	13.1	5.8
Nicaragua	20.8	-	15.7	-
Panama	-	22.0	-	37.7
Costa Rica	13.4	5.9	10.1	10.1
Mexico	17.5	4.2	13.1	7.3
Belize	15.4	6.8	11.6	11.6

[1]Unless noted otherwise

Supplemental Table 4.8. Means (±SD)[1] of metabolic syndrome score and sociodemographic characteristics in adults with and without information on plasma concentrations of zinc, manganese, and copper

Characteristic	With zinc and manganese information (n=270)	Without zinc and manganese information (n=264)	With copper information (n=378)	Without copper information (n=156)
Metabolic syndrome, %	41.1	31.8	40.2	27.2
Female sex, %	55.6	44.3	55.3	37.2
Age, y	38.4 ± 7.5	39.0 ± 7.6	38.5 ± 7.6	39.2 ± 7.4
Height, cm	161 ± 8.9	161 ± 9.4	160 ± 9.0	164 ± 9.0
BMI, kg/m^2	29.0 ± 5.4	28.1 ± 4.9	28.6 ± 5.2	28.6 ± 5.1
Education, y	10.3 ± 4.5	10.1 ± 5.1	9.8 ± 4.6	11.2 ± 5.2
Smoking, y	3.9 ± 8.3	4.6 ± 8.5	4.4 ± 8.7	3.8 ± 7.4
Home ownership, %	69.6	69.7	66.7	76.9
Household number of assets	7.4 ± 2.8	6.8 ± 2.7	6.9 ± 2.8	7.5 ± 2.7
Household income, %				
Lower <25%	17.0	22.4	19.0	21.1
Medium 25-75%	46.0	45.5	48.9	38.2
Higher >75%	37.0	32.2	32.1	40.8
Household food security score	4.1 ± 4.9	5.6 ± 4.9	5.1 ± 5.1	4.3 ± 4.5
Country of origin, %				
Guatemala	-	23.5	14.3	5.1
El Salvador	-	22.7	14.3	3.9
Dominican Republic	16.3	6.1	11.6	10.3
Honduras	12.2	10.2	8.7	17.3
Nicaragua	21.5	1.5	15.3	2.6
Panama	-	19.7	-	33.3
Costa Rica	19.6	0.4	14.0	0.6
Mexico	22.2	0.8	15.9	1.3
Belize	8.2	15.2	5.8	25.6

[1]Unless noted otherwise

Chapter 5 . Conclusions

Summary of findings

This dissertation provides new knowledge about the association between several micronutrient status biomarkers and MetS in children and adults from Mesoamerica. We used objective measures of the exposures like the biomarkers of status for the B-vitamins, Hcys, and trace minerals, and biomarkers of intake for sodium and iodine. The information on dietary covariates comes from a region-specific and validated food frequency questionnaire. Additionally, our population comes from an understudied region with unique sociodemographic characteristics and nutritional status that can provide new insight into the associations of interest.

In chapter 2 we evaluated the association between the B-vitamins and Hcys with MetS. We found an inverse association between vitamin B6 and MetS only in adults and previous evidence is consistent with our results[1,2]. Low vitamin B6 can be associated with increased inflammation and oxidative stress[3] which could explain our findings[4]. For vitamin B12, we found an inverse association in children and a non-linear positive association in adults. Previous studies in children are consistent with our results[5,6], but in adults, most prior evidence is inconsistent[7–9]. This discrepancy in adults could be related to residual confounding by foods like red meat, that are considered risk factors for cardiovascular disease and are sources of vitamin B12. Additionally, the difference between children and adults could be attributed to the different outcome measures, and differences in dairy consumption, which was higher in children, and it has been inversely associated with MetS[10].

In both children and adults, we found a positive association between folate and MetS. Most evidence in children has described an inverse association with MetS components[6,11], and in adults, there was an inverse association with MetS prevalence[7]. Our results suggest that folate might harm metabolic functions. A potential mechanism is through the increased exposure to folic acid from fortified foods that can manifest as obesity, dyslipidemia, insulin resistance, and MetS development[12]. Finally, Hcys was not associated with MetS in children or adults, which is contrary to the observational evidence that considers Hcys as a risk factor for MetS[13] and some of the components[13,14]. Nonetheless, evidence form B-vitamin supplementation trials resulted in a decrease in Hcys levels, but there were no reductions in adverse cardiovascular outcomes[15], which supports our null findings.

In chapter 3, we evaluated the association between sodium and iodine with MetS. In children, there was no association between sodium and MetS, but in adults, we found a positive association. A previous cross-sectional study in children found a positive association between sodium and MetS prevalence[16]. One potential reason we could not find an association in children was the low variability in sodium consumption which can limit power to detect associations. In adults, high sodium intake is a known risk factor for hypertension[17], stroke, and cardiovascular disease[18], and several studies have described a positive association between sodium and MetS[19–24]. Hence, our findings are consistent with previous evidence, supporting the harmful health impacts of high sodium consumption.

In both children and adults, we found a positive association between iodine and MetS. Previous evidence about iodine and MetS in children and adults is not available, but in adults, mixed associations with some of the MetS components were described[25,26]. The potential impact

of iodine on MetS could be related to its association with thyroid hormone metabolism[27] and because changes in these hormones can result in MetS development[28].

In chapter 4 we evaluated the relation between the trace minerals zinc, manganese, and copper with MetS. In children and adults, there was no association between zinc and MetS. Consistent with our findings, previous antenatal supplementation trials found no effect of zinc on cardiometabolic risk during childhood[29,30], but another trial in obese children found a significant improvement of MetS components after supplementation[31]. Cross-sectional studies also found mixed evidence[32,33]. As in children, zinc supplementation in adults did not affect MetS risk[34], which is also consistent with our findings. Observational studies in adults also indicated mixed evidence, with several studies describing null associations [35–39]. The discrepancy in previous findings with our results could be related to the different biomarkers of zinc used, and the different population evaluated.

In children, we found an inverse association between manganese and MetS in children, but there was no association in adults. In children, there is no previous evidence about the association between manganese and MetS, but inverse associations with insulin resistance were described[72]. Similarly, evidence from animal studies described an inverse association between manganese intake and insulin resistance[42,43], which could explain our findings in children. Previous evidence in adults has described inverse[36,44] and null[38,45,46] associations between manganese and MetS. The use of different biomarkers and timing of exposure could account for the discrepant results. In both children and adults, we found no association between copper and MetS, but there was a positive association with the abdominal obesity component. Previous evidence regarding MetS is consistent with our null findings, in both children[33,47] and adults[36–39,48]. Similarly, consistent previous positive associations between copper and obesity in

children[49–51] and adults [48,52,53] have been described. In general, the evidence about the association between the trace minerals and MetS is scant in the Mesoamerican region. Additionally, there is not an established biomarker for assessing trace mineral status, and dietary intake weakly correlates with most of them[54], which limits comparability among available studies.

Our study has several strengths. First, we used biomarkers to evaluate micronutrient status and intake, which removes recall bias associated with food frequency questionnaires (FFQ) or recall methods and allows estimation of associations between specific cut points and MetS. Because we collected information on both parents and children, we adjusted for parental characteristics that could be considered potential confounders for the associations in children. The specific exposures evaluated are a novel investigation in children and add to the body of literature about the associations with MetS in a Mesoamerican population.

A limitation of our study is its cross-sectional nature, which limits causal inference. Moreover, since all micronutrients are essential and therefore obtained from specific food sources, it is likely that full adjustment of these food sources that act as potential confounders is not possible due to the high correlation between the micronutrients and the food sources. Hence, our results could reflect the associations between the specific food sources and MetS.

Also, we used a metabolic risk score based on the specific distribution of the different MetS components in this population and therefore, comparisons with other populations are limited. Since our study sample was not representative of the entire Mesoamerican population and deficiency prevalences of micronutrient status varied by country, generalizability might be affected. Moreover, due to selection bias related to convenience sampling, this population comes from urban non-indigenous areas which could explain the lower B-vitamin[55], and zinc[56]deficiency prevalence we found compared to rural areas and estimates from nationally-

representative surveys, resulting in a potential attenuation of the associations of interest. Additionally, we conducted the measures of the trace elements in a subset of the original sample, and comparison analysis indicated potential selection bias. This subset of the population has a higher socioeconomic status compared to the original sample, which can result in further attenuation of the associations between the trace minerals and MetS. Lastly, because of the small sample size, country-specific analyses were not possible.

Public health implications and future directions

Our study provides new knowledge regarding the association between the micronutrients and MetS. Additionally, we generated current estimates of micronutrient deficiencies and excess status in Mesoamerican children and their adult parents.

The two oldest food fortification policies in the Mesoamerican are for folate and iodine, which have resulted in a significant decrease of folate and iodine deficiency and are considered a public health success. However, since our results suggest that folate and high iodine intake could have adverse effects on cardiometabolic health, closer monitoring of the food fortification policies in the Mesoamerican region is warranted. Moreover, the transition from an iodine-deficient region to non-deficient region describes the changes that occurred in the region during the past 30 years[57] and the adverse effects of excess iodine are more likely to occur in areas that suffered from iodine deficiency in the past[58].

Mesoamerican countries suffer from rampant government corruption and bureaucracy in public institutions that results in weak health systems infrastructure and underfunding of public health programs restricting policy surveillance, including food fortification programs[59]. Therefore, it is not difficult to consider that a poor monitorization of food fortification policies affects the region and examples of the negative influence of industry and underfunding are available in the literature[59,60]. Additionally, since high sodium consumption is highly prevalent not just in the region but worldwide, efforts to adhere to the sodium consumption recommendations should be promoted, which can only result in a decreased burden of cardiovascular disease, and not to merely avoid a potential adverse effect of excessive iodine intake.

The epidemiological transition is occurring at a slower rate in the Mesoamerican region compared to developed countries like the United States. Some countries have an increased burden of chronic diseases accompanied by a decrease in infectious diseases while others still have notable infectious-related mortality, especially in children under five years[61,62]. Additionally, the region suffers from a dual burden of malnutrition characterized by significant micronutrient deficiencies and stunting with increasing rates of overweight and obesity[63]. This situation is commonly known as the nutrition transition and is closely related to the epidemiological transition. Food consumption patterns in Latin America from the late '90s support this nutrition transition because there was an initial decline in root tubers, legumes, and cereal consumption associated with traditional dietary patterns with an initial increase in meats and sugar consumption[64], characteristic of a Western dietary pattern. Moreover, recent data found that the significant sources of energy intake in Latin America were grains, pasta, and bread, followed by meat and eggs, and oils and fats. Additionally, more than a quarter of energy intake came from food sources rich in sugar and fat, while only 18 % was from food sources rich in fiber and micronutrients[65]. Therefore, a more Western dietary pattern characterizes current food consumption in Latin America. Finally, since our results are cross-sectional it is important to consider that our results could reflect the association between specific food sources and MetS, because the micronutrients can only be obtained from these specific food sources, it is difficult to fully remove the confounding bias introduced by these foods despite our ability to adjust for them.

Our findings could be a result of both the epidemiological and nutrition transition. Notably, the low prevalence of micronutrient deficiencies, high rates of overweight and obesity with the potentially adverse effects of high micronutrient exposure on cardiometabolic health

portray a population that is on the upper end of the transition with high rates of chronic disease and high consumption of processed food derived from fortified flours.

In summary, our results indicate that the Mesoamerican population can have excessive exposure to folate, sodium, and iodine which could have adverse effects on cardiometabolic health. Further studies are needed to replicate these findings and include a nationally representative sample from each country. Moreover, longitudinal studies could help elucidate the role of vitamin B12 on MetS and evaluate the effect of time of exposure. The relation between folate and MetS should be further evaluated by assessing the association differences between folate and folic acid with MetS. Additionally, there is a need to conduct longitudinal studies that can help clarify the inverse association between manganese and MetS, and the potential mechanisms involved in copper metabolism in obesity. Finally, surveillance and evaluation systems of food fortification policies need to improve in the region, along with increased efforts to adhere to the recommended nutrition guidelines, specifically to reduce sodium consumption.

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