Polyunsaturated Fatty Acids and Early-Life Cardiometabolic Disease Risk

by

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Abstract

Background: Polyunsaturated fatty acid (PUFA) intake is low throughout Latin America, but sociodemographic patterning and dietary sources of PUFA status in the region are poorly characterized. PUFA may be related to the development of early-life risk factors for cardiometabolic diseases, which have reached epidemic proportions in Latin America. However, the relations between these nutrients and cardiometabolic risk in children are poorly understood.

Objectives: To identify sociodemographic, anthropometric, and dietary correlates of PUFA status in Mesoamerica (aim 1), and to examine the relations of n-3 and n-6 PUFA with metabolic syndrome (MetS) (aim 2) and development of adiposity in children (aim 3).

Methods: Aims 1 and 2 were completed using data from the Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMeS) study, a cross-sectional investigation of school-age children and their parents from the capital cities of Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Belize, Panama, the Dominican Republic, and the city of Tuxtla Gutíerrez in Chiapas, Mexico. Researchers collected information on sociodemographic characteristics; food intake and the type of cooking oil used in the home were measured by food frequency questionnaire. PUFA concentrations were quantified in adipose tissue by gas chromatography.

In aim 1, we assessed correlates of adipose tissue PUFA biomarkers by estimating percent mean differences in each PUFA between levels of predictors using multivariable-adjusted linear regression models.

In aim 2, we examined associations between PUFA and MetS in parents, and between PUFA and a continuous metabolic risk score in children. We estimated prevalence ratios of MetS in adults and mean differences in metabolic score in children across quartiles of PUFA using multivariable-adjusted Poisson and linear regression models, respectively.

Aim 3 was conducted in the context of a cohort of children from Santiago, Chile who were recruited in infancy and followed through adolescence. PUFA were quantified in serum at 5 and 10 y of age. Body mass index (BMI) was measured at 5, 10, and 16 y. We compared the change in BMI-for-age Z scores through 16 y of age between PUFA quartiles at 5 or 10 y and between quartiles of PUFA change from 5 to 10 y by fitting growth curves from multivariable linear mixed models with restricted cubic splines.

Results: Country of origin was the strongest predictor of all essential and long-chain PUFA biomarkers. The type of cooking oil used in the home was the strongest dietary correlate of PUFA status. Among adults, MetS prevalence was inversely associated with adipose tissue alpha-linolenic acid (ALA) and gamma-linolenic acid (GLA), and positively associated with eicosapentaenoic acid (EPA), dihomo-gamma-linolenic acid (DGLA), and the Δ6-desaturase activity index. Among children, metabolic risk score was positively associated with docosapentaenoic acid (DPA). Serum concentrations of some long-chain n-3 PUFA in middle childhood were associated with less weight gain through adolescence whereas the n-6 PUFA arachidonic acid (AA) and estimated Δ5-desaturase activity are related to increased weight gain.

Conclusions and significance: PUFA status within Latin America is heterogeneous and is related to the type of vegetable oil used for cooking. ALA is inversely associated with MetS among adults but long-chain n-3 PUFA do not appear to be protective against MetS in children or adults. However, they may be protective against development of adiposity in middle

childhood. These results suggest that PUFA are related to cardiometabolic risk in early life and could serve as modifiable targets for intervention.

Chapter 1. Introduction

Overview

Polyunsaturated fatty acids (PUFA) are vital nutrients with roles in many different physiological processes. In Latin America, information on the state of PUFA nutrition is sparse. However, available evidence suggests that intake of n-3 and n-6 PUFA is inadequate (1,2).

Cardiometabolic diseases such as cardiovascular disease (CVD) and diabetes have become some of the leading causes of death in Latin America (3). Risk factors for these conditions can develop in childhood (4) and persist to adulthood (5), underscoring the importance of early prevention. The prevalence of some of these early-life risk factors including childhood obesity and metabolic syndrome (MetS) in Latin American countries is alarmingly high (6). Nutrition in childhood is related to the development of cardiometabolic risk (7) and potentially modifiable, yet the relations between early-life PUFA nutrition and these risk factors are poorly understood.

Specific PUFA are related to lower risk of CVD (8,9) and diabetes (10,11). Among adults, PUFA are also associated with risk factors for these diseases such as MetS and its components (12,13) and obesity (14), although not all studies are consistent. Investigations of these associations in children are very limited, and the results are inconclusive (15–19). In addition, very few studies have been conducted in Latin American populations and results from high-income countries may not be generalizable to this region. This dissertation seeks to address gaps in knowledge regarding PUFA and cardiometabolic risk in Latin America, with a particular focus on children.

Specific aims

This work aims to describe sociodemographic and dietary correlates of PUFA status in Mesoamerica, and to characterize associations between specific PUFA and cardiometabolic risk factors in early life.

Aim 1. To identify sociodemographic, anthropometric, and dietary factors that are associated with adipose tissue PUFA concentrations among school-age children and their parents from Mesoamerica. We will examine characteristics such as sex, age, height, body mass index (BMI), socioeconomic status indicators, and country of origin. Potential dietary correlates include the type of cooking oil used in the home and frequency of intake of foods high in PUFA.

Aim 2. To examine the relations between PUFA in adipose tissue and MetS among Mesoamerican parents and their children. As a secondary aim, we will also examine the associations of PUFA biomarkers with the individual MetS components: abdominal obesity, high fasting glucose, hypertension, low high-density lipoprotein (HDL) cholesterol, and high serum triglycerides.

Aim 3. To investigate associations between serum PUFA concentrations at 5 and 10 y of age and change in BMI-for-age-and-sex Z scores through adolescence in a cohort of children from Santiago, Chile.

Polyunsaturated fatty acids in Latin America

Overview of polyunsaturated fatty acids

Fatty acids are the basic molecular components of fat. In the diet, they serve both as a source of energy and as nutrients with specific physiological roles. Polyunsaturated fatty acids (PUFA) are those that contain more than one double bond in their aliphatic chain, and they are classified primarily into the n-3 and n-6 families. Each of these families contains a precursor fatty acid considered essential because it can only be obtained from the diet. Endogenous conversion of essential fatty acids into downstream long-chain PUFA occurs through the action of desaturase and elongase enzymes. The efficiency of this conversion is, however, highly variable and generally low (20).

The n-3 essential fatty acid is alpha-linolenic acid (ALA, 18:3 n-3), and long-chain n-3 PUFA include eicosapentaenoic acid (EPA; 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3), and docosahexaenoic acid (DHA; 22:6 n-3). ALA can be obtained from vegetable oils made from soyabean, rapeseed (canola), and flaxseed. EPA and DHA are found preformed in fatty fish and other marine sources, in addition to their endogenous conversion from ALA. In the n-6 family, the essential fatty acid is linoleic acid (LA; 18:2 n-6) and its products include gamma-linolenic acid (GLA; 18:3 n-6), dihomo-gamma-linolenic acid (DGLA; 20:3 n-6), and arachidonic acid (AA; 20:4 n-6). LA is found in vegetable oils, particularly safflower, sunflower, corn, and cottonseed oils. In addition to endogenous production from LA, some AA is obtained in the diet from animal products such as meat and eggs. N-3 and n-6 PUFA are crucial to health and development through their roles as structural components of cell membranes and as precursors for the synthesis of eicosanoids, a class of local signaling molecules that regulate gene expression, cardiovascular function, and immune response (21).

Polyunsaturated fatty acid nutrition in Latin America

Despite the physiologic importance of PUFA, little is known about fatty acid nutrition and status in Latin American countries.

In a meta-analysis of national nutrition surveys, the estimated average intakes of n-6 PUFA in percentage of total energy (%E) among adults in 2010 in central, Andean, tropical, and southern Latin America were 5.5, 4.7, 6.9, and 6.2%E, respectively (2). While these averages are in line with the World Health Organization's (WHO) Acceptable Macronutrient Distribution Range (AMDR) for LA of 2.5-9%E (22), there is evidence that a much higher intake of 12%E from n-6 PUFA is optimal for prevention of chronic disease and mortality, and that 10% of all coronary heart disease deaths in Latin America were attributable to n-6 PUFA intake below this level in 2010 (23). Intakes of seafood-based long-chain n-3 PUFA in central, Andean, tropical, and southern Latin America (in mg/d) were estimated to be 62, 94, 57, and 150 mg/d, and intakes of plant-based n-3 PUFA were 552, 824, 1742, and 1288 mg/d (2). The lower limit of the AMDR set by the WHO is 250 mg/d for long-chain n-3 PUFA and 0.5%E for ALA, equivalent to 1100 mg/d in a 2000 cal/d diet (22); however, optimal intake of ALA for disease prevention is estimated to be 1-2%E (24). None of the estimated intakes of long-chain n-3 PUFA achieve this minimum, and only the estimated intakes in tropical and southern Latin America meet the minimum for ALA. Furthermore, while these results generally suggest low PUFA intake in many parts of Latin America, the only national surveys available for analysis were from Mexico, Colombia, Brazil, and Argentina. Intake for all other countries was estimated based on data from these and other data sources; the true intake levels are unknown. Another analysis using food balance sheets from the Food and Agriculture Organization also concluded that fish availability

was low throughout Latin America, and that availability of vegetable oils high in ALA was low in some countries as well (1). Information on PUFA intake among children specifically is generally consistent with studies of adults. Data from the Mexican National Health and Nutrition Survey from 2006 indicate a mean intake of 4.5%E and 0.3%E from n-6 and n-3 PUFA among school-age children, and 5.5%E and 0.3%E among adolescents (25). In a study of Costa Rican adolescents in 2006, mean intakes of LA and ALA were 7.8%E and 0.9%E, respectively (26). Mean intake of EPA+DHA was 82 mg/d, having decreased from 120 mg/d a decade earlier. In a group of Guatemalan children, mean intakes of LA, ALA, and EPA+DHA were 5.3%E, 0.5%E, and 42 mg/d among children of high socioeconomic status, and 5.9%E, 0.5%E, and 40 mg/d among children of low socioeconomic status (27).

Countries in Latin America are at varying stages of the nutrition transition (28). This transition describes the process through which countries undergoing economic development experience a shift from undernutrition-related conditions towards obesity-related chronic disease as diets become Westernized (29). This Western diet is characterized in part by a high intake of fat, but with an increase in the proportion of saturated relative to unsaturated fats. The transition typically first affects the better-off who can afford access to Westernized diets, and then shifts toward poorer people as access to these lifestyle patterns becomes widespread. The nutrition transition may be contributing to higher intakes of saturated fat at the expense of PUFA among specific demographic and socioeconomic populations within Latin American (30), but current data are lacking. Information on the dietary sources of PUFA in Latin American contexts is also limited. Evidence from Costa Rica and Colombia suggests that vegetable oils used for cooking are the primary source of dietary PUFA (26,31) and are influential in determining PUFA status (32). Based on data from Costa Rica (26), Mexico (25), and Guatemala (27), other dietary

sources of n-3 and n-6 PUFA include beans, baked goods, fast food, dairy, chicken, fish, and eggs.

Limited available evidence suggests that PUFA intake is generally inadequate throughout Latin America. While this potentially represents a significant public health concern, it is difficult to design and implement effective interventions aimed at increasing dietary PUFA without better information about the current state of PUFA status and its sociodemographic and dietary correlates.

Cardiometabolic disease in Latin America

Cardiometabolic diseases including CVD and diabetes are among the leading causes of death in Latin America (3). Although these conditions typically manifest later in life, their risk factors can develop during childhood (4) and persist into adulthood (5,33,34). In conjunction with the high burden of cardiometabolic disease, the prevalence of related risk factors is high among children and adolescents in Latin America (6).

Risk factors among children

Childhood obesity is perhaps the strongest and most prevalent early-life risk factor for chronic diseases in adulthood, including type 2 diabetes, hypertension, and coronary heart disease (5). Childhood obesity is highly prevalent in Latin America and has been increasing in many countries over the past decade (35). Based on data from the WHO Global Database on Child Growth and Malnutrition (36), the prevalences of overweight and obesity in children under 5 years of age in 1990 in Central and South America were 5.1% and 7.3% respectively, and in 2011 they were 6.4% and 7.4% (35). Among school-age children, the prevalences of overweight/obesity estimated from recent national surveys (2009 onwards) in Brazil, Colombia, and Mexico were 33.5%, 18.9%, and 34.5%, with increases of about 1% per year between 2005 and 2010 in Colombia and between 1999 and 2006 in Mexico (35).

Metabolic syndrome (MetS) is a cluster of typically co-occurring cardiovascular risk factors including abdominal obesity, high fasting glucose, hypertension, low HDL cholesterol, and high serum triglycerides (37). These risk factors can be present in childhood and track into adulthood (38,39), when they are associated with high risk of CVD or diabetes (40) and CVD-related mortality (41). Adverse metabolic profiles in childhood predict the development of cardiometabolic outcomes in adulthood, including type 2 diabetes and atherosclerosis (38,42).

Information on the prevalence of pediatric MetS in Latin America is scarce, and assessment is complicated by the fact that there is no widely agreed-upon definition of the syndrome in children. Evidence from some low- and middle-income countries suggests that metabolic abnormalities are highly prevalent among children (43). One study of 61 Bolivian children and adolescents defined MetS using a modified version of the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) definition and found a prevalence of 36% (44). The Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMeS) Study, a cross-sectional study of 267 children and their parents from urban areas in Mesoamerica, used a continuous metabolic score rather than a dichotomous definition of metabolic syndrome (45). Higher metabolic scores were associated with having parents with metabolic syndrome. The prevalence of metabolic syndrome among parents was high (37.9% in mothers and 35.3% in fathers).

Risk factors among adults

Prevalence of cardiometabolic risk factors is also high among Latin American adults. The Latin American Consortium of Studies on Obesity combined data on obesity from 11 studies conducted between 2000 and 2008 in urban and rural populations from 8 countries (46). The overall prevalence of obesity was 16.1%; abdominal obesity, 35.8%; hypertension, 20.2%; low HDL, 53.3%; and high triglycerides, 26.5% (47). The CARMELA study of adults in 7 Latin American cities found similar results: the prevalence of obesity was 23%; hypertension, 18%; and MetS, 20% (48). In the NiMeCoMeS study, the prevalence of obesity was 34.2% overall; in women and men respectively, the prevalence of abdominal obesity was 70.0% and 24.1%; high fasting glucose, 9.8% and 8.1%; hypertension, 15.4% and 29.4%; low HDL, 83.3% and 78.9%; high triglycerides, 39.8% and 63.2%; and MetS, 37.9% and 35.3% (45).

Taken together, these findings indicate that cardiometabolic disease and early development of cardiometabolic risk factors are of great public health concern in Latin America. Risk factors in childhood represent a key point of intervention against these conditions, when the cumulative impact on health is still limited. Childhood nutrition, which is related to early development of cardiometabolic risk factors (7), is a potentially modifiable and influential target for intervention.

Polyunsaturated fatty acids and cardiometabolic risk factors

PUFA may influence the development of cardiometabolic risk factors in adults (12,14,49), although studies conducted among Latin American populations are limited (50,51). Intervening on PUFA nutrition could be a promising means of reducing the burden of childhood obesity and MetS, but associations between PUFA and these conditions in children are poorly understood.

PUFA and development of adiposity

In vitro and animal studies indicate that n-6 PUFA promote adipogenesis, while n-3 PUFA inhibit it (52). Prostacyclin, an eicosanoid product of AA, promotes adipocyte differentiation from preadipocytes (53) through up-regulation of transcription factors that promote expression of peroxisome proliferation-activated receptor gamma (PPARγ), a necessary receptor for adipocyte formation (52,54). Long-chain n-3 fatty acids increase satiety and thus decrease total calorie intake (55). They also regulate lipid metabolism by promoting expression of genes involved in lipolysis and β-oxidation and inhibiting lipogenesis, thus reducing fat deposition (56).

In children, most research on PUFA and adiposity is cross-sectional in nature (16,17,57–64). These studies have examined PUFA biomarkers mainly in blood components in relation to BMI or to obesity, and have generally not found consistent associations between specific PUFA and adiposity. DGLA was positively associated with either BMI or obesity in several (17,60,62,64) but not all studies (58,61,63). Results related to all other PUFA were inconclusive.

Two longitudinal studies have examined associations between adiposity and PUFA. In one, ALA was inversely associated with estimated BMI-for-age Z score gain between ages 6 and 14 y among 668 children from Bogotá, Colombia who were 5-12 y old at baseline and followed

for a median of 30 months (15). In the other, 3 y changes in plasma DHA and DGLA were each positively associated with concomitant change in standardized weight, whereas change in LA was inversely associated with weight change among 77 Japanese children who were 10 y old at baseline (18).

PUFA and development of MetS and its components

There are several mechanisms by which PUFA may affect development of metabolic syndrome. Generally, the eicosanoid products of n-6 PUFA such as AA are pro-inflammatory, whereas eicosanoids derived from n-3 PUFA are anti-inflammatory (65,66), and displacement of AA in cell membranes by EPA and DHA or vice versa alters the levels of production of these different classes of eicosanoids and therefore affects inflammatory processes. N-3 PUFA may also reduce inflammation through other mechanisms including conversion into pro-resolving mediators such as resolvin that end acute inflammatory response, and down-regulation of pro-inflammatory transcription factors (66). N-3 PUFA alter lipid profiles; the mechanisms include up-regulation of triglyceride oxidation rather than storage, and lowered secretion of very low density lipoprotein (VLDL) (67). They also promote vasodilation through activation of nitric oxide synthase, and reduce production of vasoconstrictive prostaglandins, which are synthesized from AA (66). N-3 PUFA may reduce insulin resistance through lowered inflammation, up-regulation of the insulin-sensitizing hormones leptin and adiponectin, and regulation of gene expression for several proteins involved in carbohydrate metabolism (68).

Several cross-sectional studies have examined PUFA and pediatric MetS or its components (16,17,63,69,70). Circulating ALA biomarkers were not associated with MetS in these studies (17,63,70), but results for other PUFA were inconsistent. In one longitudinal study of 1267 2-10 y old European children, ALA and EPA at baseline were inversely associated with

blood pressure at follow up among normal weight children, but positively associated among those with overweight or obesity (71). GLA and AA were each positively associated with blood pressure. A few trials of long-chain n-3 PUFA supplementation have been conducted among children. Of these, some found a protective effect of EPA, DHA, or both on blood pressure, HDL cholesterol (72,73), triglycerides (73,74), or measures of insulin sensitivity (73–75); two of these studies were conducted in Mexican children (73,75). One additional trial of EPA+DHA+GLA found no effect on any MetS components (76). Finally, in a review of trials and observational studies of PUFA during pregnancy, lactation, and infancy, there were inconsistent associations between these exposures and MetS components during early childhood and adolescence (19).

Summary

The literature on PUFA and cardiometabolic risk factors in childhood has many limitations. The majority of existing studies are cross-sectional, which prevents causal inference and allows for the possibility of reverse causation if adiposity and metabolic dysregulation affect PUFA levels. Many of these studies are also small, and several did not conduct analyses adjusted for confounders. Most studies relied on PUFA concentrations measured in blood components; only one (62) used adipose tissue biomarkers, which are the gold standard biomarker of fatty acid status because they reflect long-term intake (77). Finally, very few studies have been conducted in Latin American populations, and results may not be generalizable to this region. More research is needed in order to clarify the relation of PUFA status to cardiometabolic risk in childhood.

Summary of chapters

This dissertation seeks to generate knowledge regarding the associations between PUFA status and cardiometabolic risk in Latin American contexts, particularly among children. The sociodemographic patterning and dietary sources of PUFA in Latin America is largely unknown, and more information is needed in order to design effective interventions aimed at increasing PUFA intake. To this end, chapter 2 identifies sociodemographic, anthropometric, and dietary correlates of adipose tissue PUFA biomarkers among a group of school-age children and their parents from Mesoamerica. Next, the work in chapters 3 and 4 focuses on the relations between specific PUFA and cardiometabolic risk factors in children. In chapter 3, we examine associations between adipose tissue PUFA and MetS among the Mesoamerican families. In chapter 4 we assess whether serum PUFA in middle childhood are related to BMI-for-age Z score trajectories through adolescence. Finally, chapter 5 summarizes the main findings from previous chapters and provides a discussion of the significance of the work for public health.

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Chapter 2. Sociodemographic, anthropometric, and dietary predictors of polyunsaturated fatty acids in adipose tissue among Mesoamerican children and their parents

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Abstract

Objective: To characterize sociodemographic, anthropometric, and dietary predictors of polyunsaturated fatty acid (PUFA) status biomarkers in adipose tissue among children and their parents from Mesoamerica.

Design: Cross-sectional study. Concentrations of linoleic acid (LA), total long-chain n-6 PUFA (n-6 LCPUFA), alpha-linolenic acid (ALA), and total long-chain n-3 PUFA (n-3 LCPUFA) from gluteal adipose tissue were quantified with the use of gas chromatography. Dietary information was measured with a food frequency questionnaire. We estimated percent mean differences in each PUFA between levels of predictors using multivariable-adjusted linear regression models.

Setting: Capitals of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Belize, and Tuxtla Gutiérrez in Mexico.

Subjects: Children (n=220) aged 7-12 y and 471 parents.

Results: Country was the strongest predictor of each PUFA while BMI was positively associated with n-6 LCPUFA in children and adults. Cooking primarily with soyabean oil was positively associated with LA and ALA concentrations, whereas cooking with canola oil was related to n-6

and n-3 LCPUFA concentrations. Adipose tissue ALA concentrations were 25.1% (95% CI: 0.4%, 55.9%) higher among children in the 90th percentile of intake frequency of cream, compared to those in the 10th percentile. N-3 LCPUFA concentrations among adults in the 90th percentile of fish and canned fish intake frequency were 12.1% (95% CI: -4.0%, 30.9%) and 16.1% (95% CI: -1.3%, 36.5%) higher, respectively, than among those in the 10th percentiles. **Conclusions:** Adipose tissue PUFA status in Mesoamerica is associated with country of origin, the type of oil used for cooking, and intake of specific foods.

Introduction

Polyunsaturated fatty acids (PUFA) are nutrients that are required for a wide range of physiologic functions and that exert many important health effects. The n-6 PUFA linoleic acid (18:2 n-6; LA) and the n-3 PUFA alpha-linolenic acid (18:3 n-3; ALA) are essential because they cannot be synthesized in the human body and must be obtained through diet, primarily from plant oils. Long-chain n-6 and n-3 PUFA (n-6 and n-3 LCPUFA) are produced through endogenous metabolism of LA and ALA (1), and can also be obtained preformed in the diet from meat or eggs (n-6 LCPUFA) and fatty fish (n-3 LCPUFA). LCPUFA serve as precursors for eicosanoids, a class of signaling molecules that regulate immune and cardiovascular function. Many of the eicosanoids derived from n-6 LCPUFA are pro-inflammatory, while those derived from n-3 LCPUFA are generally anti-inflammatory (1).

In adults, n-3 LCPUFA intake is associated with reduced risk of cardiovascular disease, some cancers, and mood or cognitive disorders (2). ALA may also be cardioprotective (3). Evidence for the effects of LA and n-6 LCPUFA on cardiovascular disease and its risk factors is less conclusive (4). PUFA intake has also been related to cardiometabolic risk factors in children (5,6).

In Latin America, available evidence suggests that PUFA intake in adults and children may be inadequate (7–9). Fish and vegetable oils high in ALA are scarce in many countries (10). Low PUFA intake in this region is of particular concern because the rates of cardiovascular disease (11), childhood obesity (12), and childhood metabolic dysregulation (13) are high. Improving PUFA status through dietary interventions might be a useful public health measure against chronic disease (14). However, current and detailed information about PUFA status in Latin American populations is lacking. It is unclear whether PUFA status varies according to

demographic, socioeconomic, anthropometric, or dietary characteristics. The type of oil used for cooking is an important predictor of PUFA status (7,14,15), and most dietary PUFA is in the form of essential fatty acids while intake of preformed LCPUFA is low (7–9). Many previous studies of PUFA status relied on dietary methods that may be subject to errors due to recall or inaccurate estimation of the fatty acid content of foods. Moreover, these measures may not accurately reflect physiological PUFA status especially with respect to LCPUFA which can be endogenously metabolized. Adipose tissue fatty acids are the gold standard biomarkers of fatty acid status because they reflect intake over several years (16); nevertheless, they are seldom ascertained in population studies.

We conducted a cross-sectional study to examine sociodemographic, anthropometric, and dietary correlates of adipose tissue PUFA concentrations among school-aged children and their parents in nine Mesoamerican countries.

Methods

Study design and population

We conducted a cross-sectional investigation of school children and their parents in the context of the Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMeS) Study. Details of the study design have been described previously (13). In brief, between July 2011 and November 2013 we identified families from the capital cities of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Belize, and the city of Tuxtla Gutiérrez in Chiapas, Mexico, with use of public primary school enrollment lists in each city. From these lists, study teams randomly selected potentially eligible students who were 7-12 y of age. Additional eligibility criteria were assessed at school visits; these included that the child lived with both biological parents, that neither the child nor their mother was pregnant, and that the child did not have any siblings already invited to participate. Parents of children who met all inclusion criteria were offered enrollment. The final sample comprised 267 families (Guatemala, 31; El Salvador, 30; the Dominican Republic, 30; Honduras, 30; Nicaragua, 31; Panama, 26; Costa Rica, 27; Belize, 31; and Mexico, 31).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects/patients were approved by the Institutional Review Boards (IRB) of collaborating institutions in each of the nine countries and by the University of Michigan Health and Behavioral Sciences IRB. All parents provided written informed consent to participate for themselves and their children. Assent to participate was confirmed from the children before enrollment.

Data collection

All data collection procedures took place at home or at a family visit to a health center. Participants were asked to fast for at least 6 hours before the appointment. At this visit, mothers completed a background questionnaire that included questions on each participant's age, education level, and smoking status, as well as socioeconomic status (SES) indicators. Food security was assessed with the Latin American and Caribbean Food Security Scale, an instrument that has been validated for use in this region (17). The scale consists of 16 yes/no questions about experiences of food insecurity over the previous 3 months.

Trained research assistants administered a 97-item food frequency questionnaire (FFQ) to each family member, adapted closely from an FFQ that was validated for use among Costa Rican adults (18). The FFQ incorporated appropriate country-specific adjustments of food names. It inquired about habitual intake frequency over the previous 12 months of foods and food groups commonly eaten in the region, including dairy, fruit, vegetables, eggs and meat, bread, flour, cereal, juice, caffeinated and alcoholic beverages, fast food, desserts, dressings and sauces, and cooking fats. There were also questions on the brand of butter, margarine, lard/shortening, or vegetable oil used if participants indicated that they consumed any of these fats, and a question about the composition of the vegetable oil most frequently used for cooking, from a list that included soyabean, sunflower, corn, palm, olive, canola, mixed oils, or other. Intake frequency options were >6 times/d, 4-5 times/d, 2-3 times/d, 1 time/d, 5-6 times/wk, 2-4 times/wk, 1 time/wk, 1-3 times/mo, and <1 time/mo or never. Portion sizes were described in natural units or with the use of typical measures of volume.

Anthropometric measures were obtained from each participant with use of standardized procedures and calibrated instruments. Height was measured without shoes to the nearest

millimeter with the use of portable Seca stadiometers (Seca, Hamburg, Germany). Weight was measured in light clothing to the nearest 100 g with use of Tanita scales (Tanita, Tokyo, Japan). All measures were obtained in triplicate.

At the end of the visit, researchers obtained samples of subcutaneous adipose tissue from each family member. After numbing the upper external area of the left gluteus with an ice pack and a local anesthetic spray, a 16-gauge needle was inserted at a 45˚ angle and adipose tissue was aspirated into the syringe (19). Samples were transported on ice to laboratories in each country where they were stored in a 3:2 hexane:isopropanol solution in amber vials at -70˚C. The samples were transported frozen from their respective countries to the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City, and from there to the University of Michigan for fatty acid analysis.

Laboratory methods

Analysis of adipose tissue fatty acids was completed at the University of Michigan Regional Comprehensive Metabolomics Resource Core. Total lipids were extracted from 20-25 mg of adipose tissue according to the method described by Bligh and Dyer (20), with 10 μL of 4 mM nonadecanoic acid (C19:0) added as an internal standard. The fatty acid components of the total lipids were then derivatized into their methyl esters using boron trifluoride-methanol (21). The methyl esters were extracted with a 2:1 hexane-water mixture and centrifugation. The hexane layer was removed and dried and the methyl esters were re-dissolved in 100-200 μL of hexane, depending on the volume of the original sample. Fatty acids were analyzed by gas chromatography (GC). One to two μL of sample was injected via auto sampler onto an Agilent 6890N gas chromatograph (Agilent, Santa Clara CA, USA) equipped with a flame ionization detector, a 100 m x 0.25 mm x 0.2 μm SP-2560 column (Sigma-Aldrich, Bellefonte PA, USA),

and Chemstation software for data analysis. Fatty acids were quantified using a calibration curve prepared from known amounts of C19:0 (the internal standard) and other authentic methyl esters. The authentic methyl esters were also used to identify the fatty acids in samples by comparing retention times. The coefficient of variation for GC analyses was between 2.5-3.6%.

Data analyses

Adipose tissue samples were available in 220 children and 471 parents; these constituted the analytic sample. The primary outcomes were four adipose tissue biomarkers of polyunsaturated fatty acids, expressed as mass percentages of the total fatty acids measured in a sample: LA, n-6 LCPUFA (sum of eicosadieneoic acid, dihomogamma-linolenic acid, arachidonic acid, and adrenic acid), ALA, and n-3 LCPUFA (sum of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid).

Sociodemographic exposures included sex, age, education level, smoking status, number of household assets, food security, and country of origin. Parental education was categorized according to the number of completed school years (incomplete elementary, 1–5; complete elementary, 6; incomplete secondary, 7–11; complete secondary, 12; or post-secondary, ≥13). Adults were classified as never, past, or current smokers. We determined the number of assets each family had from a list that included a car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color TV, sound set, computer, and internet. We created a household food insecurity score as the sum of all affirmative answers to the survey and categorized it as none (0), mild (1-5), moderate (6-10), or severe (11-16) (22). Anthropometric exposures included height and body mass index (BMI, calculated as kg/m^2). We estimated the median of the three replicate measures obtained (23). In the adults, height was categorized into sex-specific quartiles and BMI was categorized as <25, 25 - <30, or ≥30 (24). For children, we

considered sex, age, height, BMI, the mother's age at the child's birth, maternal height, maternal BMI, maternal education level, parental smoking history (the number of parents who ever smoked: none, 1, or 2), number of household assets, food security, and country of origin. Children's height and BMI were converted into age-and-sex-specific Z scores using the World Health Organization Growth Reference for children ages 5-19 y (25).

The main dietary exposure was the composition of the household's primary cooking oil (soyabean, canola, corn, sunflower, palm, vegetable mixtures, or other) as reported in the FFQ. Oil composition was derived from manufacturers' information about the reported brand of oil in 40% of participants and from directly reported oil composition in the rest.

We also examined intake frequencies of specific foods from the FFQ as additional dietary exposures. In order to select these foods, we matched each item on the FFQ to its closest corresponding item in a set of nutrient composition tables of Costa Rican foods (26). We then used the food composition tables to identify those foods from the FFQ with the highest concentration of each PUFA (LA, n-6 LCPUFA, ALA, and n-3 LCPUFA) per 100 g. Some items (e.g. fish) were more broadly defined in the FFQ than they were in the food composition tables (e.g. tilapia, haddock, etc.). In those cases, we calculated the mean PUFA concentrations across all matches in the food composition tables. In addition to the foods identified from the composition tables, we included others that have previously been reported as important dietary sources of PUFA in Mesoamerican populations (7–9). The final list of foods is presented in Supplemental Table 2. We estimated intake frequencies in servings/d using as weights the average frequency corresponding to each categorical response on the FFQ. Weights for <1/mo and ≥6/d were 0 and 6, respectively.

We conducted analyses separately among children and adults. In bivariate analyses, we compared the distribution of each outcome between categories of sociodemographic, anthropometric, and cooking oil type variables with the use of means ± SD. For ordinal predictors, we performed tests for linear trend by introducing a variable representing categories of the predictor as a continuous covariate in a linear regression model. For nominal predictors, we conducted χ^2 score tests. For analyses of intake frequencies as exposures, we first log-transformed the PUFA outcomes because their distributions were skewed, and then estimated percent differences in mean PUFA concentrations with 95% confidence intervals comparing the 90^{th} and 10^{th} percentiles of intake frequency in servings/d of each food item using linear regression.

In multivariable analyses we first fitted linear regression models to estimate adjusted percent mean differences in each outcome by levels of sociodemographic and anthropometric characteristics. All models included sex, age, and country of origin, plus additional predictors that were relevant to each outcome per bivariate analyses. Next, we estimated percent mean differences in each outcome by cooking oil type and food intake frequency adjusted for sex, age, education level (adults) or maternal education level (children), and other sociodemographic characteristics that were relevant for the outcome in multivariable analyses. Foods were retained in the model for each outcome when they remained statistically significant at $P < 0.05$ or were considered important from a theoretical viewpoint. In the main analyses, country was excluded from these models because it is likely a substantial source of true variability in the exposures; nevertheless, in supplemental analyses we also fit models in which country was included. BMI was also excluded because it could be on the causal path between cooking oil or food intake frequency and PUFA in adipose tissue. In additional supplemental analyses, we examined the

associations of cooking oil type with Δ6-desaturase activity, one of the enzymes involved in endogenous conversion of essential fatty acids into LCPUFA. The outcome of these models was the ratio of dihomogamma-linolenic acid to LA; because Δ6-desaturase is the rate-limiting step in converting LA to dihomogamma-linolenic acid, a higher ratio should indicate higher enzyme activity. Predictors included cooking oil type, age, sex, and education or maternal education. All regression models were fitted using generalized estimating equations and empirical standard errors, which are robust to heteroskedasticity and non-normality (27). An exchangeable correlation structure was specified in all models for adults to account for clustering by family membership.

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

Results

Children. The mean (±SD) age of participating children was 10.0 ± 1.2 y and 52.3% were girls. Unadjusted means (±SD) of adipose tissue PUFA in children varied by sociodemographic and anthropometric characteristics (**Table 1**), cooking oil type (**Supplemental Table 1**), and food intake frequency (**Supplemental Table 2**). The proportion of households using each type of cooking oil differed by country (**Supplemental Figure 1**), and intake frequency of specific foods varied between children and adults (**Supplemental Table 5**).

Linoleic acid. Mean (±SD) LA weight percentage was 14.02 ± 4.84. In adjusted analysis, LA was positively associated with female sex and age (**Table 2**). Children from the Dominican Republic and Panama had the highest LA concentrations while children from El Salvador and Honduras had the lowest (P<0.0001). LA was highest among children whose families used soyabean or other oils and lowest among those whose families used palm oil (P=0.001) (**Figure 1A**). Simultaneous adjustment for country and cooking oil type attenuated the associations with cooking oil, while the associations with country remained similar. LA was positively associated with intake frequencies of corn tortillas, butter/margarine added to food, dressing/mayonnaise, cake, and fried chicken, and inversely associated with that of hamburger, cream, and fish (**Figure 1A**).

Total long-chain n-6 PUFA. After adjustment, n-6 LCPUFA was positively associated with height- and BMI-for-age Z scores (**Table 2**). Children from Costa Rica and the Dominican Republic had the highest n-6 LCPUFA concentrations, while children from Belize and Honduras had the lowest (P<0.0001). N-6 LCPUFA concentrations were highest in children whose families used mainly canola oil and lowest in those whose families used palm oil (P=0.08) (**Figure 1B**). Cooking oil type was not associated with Δ6-desaturase activity. When country and cooking oil

type were included in the model together, the associations with cooking oil type were attenuated. N-6 LCPUFA in adipose tissue was positively associated with intake frequencies of butter/margarine added to food and cake and inversely associated with frequency of hamburger intake (**Figure 1B**).

Alpha-linolenic acid. Mean (±SD) ALA weight percentage was 0.74 ± 0.35. ALA was inversely associated with the mother's age at the child's birth after multivariable adjustment (**Table 2**). ALA was highest among children from the Dominican Republic and Mexico and lowest among children from El Salvador and Costa Rica ($P<0.0001$). ALA was highest among children whose families used other oils and lowest among those whose families used palm oil ($P=0.04$) (**Figure 1C**). Inclusion of country in the model attenuated the associations with oil type, but did not appreciably change the associations with country. ALA was positively associated with intake frequencies of cream, dressing/mayonnaise, butter/margarine added to food, cake, and corn tacos/tostadas, and inversely associated with those of white bread, hamburger, avocado, refried beans, and fish (**Figure 1C**).

Total long-chain n-3 PUFA. In adjusted analysis, n-3 LCPUFA was positively associated with age and height-for-age Z score (**Table 2**). Children from the Dominican Republic and Mexico had the highest n-3 LCPUFA concentrations whereas children from Honduras and Costa Rica had the lowest ($P<0.0001$). Children whose families used primarily canola oil had the highest n-3 LCPUFA concentrations while those whose families used corn oil had the lowest ($P=0.004$) (**Figure 1D**). When country and oil type were included in the model simultaneously, the associations with oil type were attenuated. Adipose tissue n-3 LCPUFA was positively associated with intake frequencies of dressing/mayonnaise and hot dogs and inversely associated with intake frequencies of refried beans (**Figure 1D**).

Adults. Mean (±SD) age of adult participants was 38.3 ± 7.3 y and 52% were women. Unadjusted means (±SD) of adipose tissue PUFA varied by sociodemographic characteristics (**Table 3**), cooking oil type (**Supplemental Table 3**), and food intake (**Supplemental Table 4**).

Linoleic acid. Mean (±SD) LA weight percentage was 14.78 ± 4.82 in men and 15.26 ± 4.95 in women. In adjusted analysis, age and smoking were inversely associated with adipose tissue LA (**Table 4**). LA concentrations were highest among participants from the Dominican Republic and Costa Rica and lowest among those from Honduras and El Salvador ($P<0.0001$). Adipose tissue LA was highest among participants who used soyabean oil as their primary cooking oil and lowest among those who used palm oil ($P<0.0001$) (**Figure 2A**). When country and cooking oil type were included in the model simultaneously, the associations with cooking oil were attenuated while the associations with country remained mostly unchanged. LA was positively associated with intake frequencies of butter/margarine added to food, canned tuna/sardines, white bread, and viscera, and inversely associated with intake frequencies of cream, corn products, and fresh cheese, after multivariable adjustment (**Figure 2A**).

Total long-chain n-6 PUFA. After adjustment, adipose tissue n-6 LCPUFA was lower in men compared with women and positively associated with BMI (**Table 4**). N-6 LCPUFA concentrations were highest among participants from Costa Rica and Guatemala, and lowest among those from Honduras and El Salvador ($P<0.0001$). People who used canola oil had the highest n-6 LCPUFA concentrations whereas those who primarily used palm oil had the lowest ($P=0.002$) (**Figure 2B**). Cooking oil type was not associated with Δ6-desaturase activity. Including both country and cooking oil type in the model attenuated the associations with cooking oil type. N-6 LCPUFA was positively associated with intake frequencies of

butter/margarine added to food and hamburger, and inversely associated with those of cream, chorizo, fried chicken, and corn products (**Figure 2B**).

Alpha-linolenic acid. Mean (±SD) ALA weight percent was 0.75 ± 0.34 in men and 0.75 ± 0.33 in women. ALA was inversely associated with BMI in multivariable analysis (**Table 4**). Dominican and Nicaraguan participants had the highest concentrations while those from El Salvador and Honduras had the lowest (P<0.0001). Users of soyabean oil had the highest ALA concentrations whereas users of palm oil had the lowest (P<0.0001) (**Figure 2C**). Inclusion of country in the model attenuated the associations with cooking oil type. Intake frequency of salami was positively associated with ALA concentrations, while those of cream, corn tortillas, corn products, and sausage were inversely associated (**Figure 2C**).

Total long-chain n-3 PUFA. N-3 LCPUFA concentration in adipose tissue was lower in men compared with women in multivariable analysis (**Table 4**). N-3 LCPUFA was highest among participants from Costa Rica and Guatemala and lowest among those from Honduras and Nicaragua (P<0.0001). Concentrations were highest in canola oil users and lowest among users of palm or corn oils (P=0.03) (**Figure 2D**). When country was included in the model, the associations with oil type were attenuated. N-3 LCPUFA was positively associated with intake frequency of corn tortillas and inversely associated with intake frequencies of hard cheese, cream, beef/pork in soups or stews, and cooked eggs (**Figure 2D**).

Discussion

In this cross-sectional study of Mesoamerican families, adipose tissue concentrations of LA, n-6 LCPUFA, ALA, and n-3 LCPUFA were associated with demographic and anthropometric characteristics, the main type of oil used for cooking in the home, and intake frequencies of specific foods.

The overall means of adipose tissue LA and ALA in our study are similar to those from a meta-analysis of 19 studies conducted in healthy adult populations, primarily from Europe and the United States (16). In that review, mean mole percentages of LA and ALA in adipose tissue were 13.9% and 0.8%, respectively, compared with 15.0% and 0.8% in adults and 14.0% and 0.7% in children from Mesoamerica. However, LA and ALA concentrations in some countries in our study, including El Salvador and Honduras, were much lower than those reported in other populations. This suggests that intake of essential fatty acids is low in parts of Mesoamerica as compared to high-income countries, and that there is heterogeneity within the region. The only estimates previously available for Mesoamerica are from a study of Costa Rican adults evaluated between 1994 and 1998. In that study, mean LA and ALA weight percentages were 13.37% and 0.55%, respectively (28); compared with 17.60% and 0.86% in Costa Rican adults from our study. The higher values measured in our study may indicate differences in diet by area of residence or an increase in PUFA intake over the previous decades.

BMI was positively associated with n-6 LCPUFA in all participants, and inversely associated with ALA in adults. Several previous studies of PUFA biomarkers have found positive associations between BMI and specific n-6 LCPUFA, but associations between BMI and ALA are inconsistent (6,29–31). Intake of ALA may inhibit the promotion of adipogenesis by n-6 LCPUFA (32), which could explain both of our findings. However, these associations may also

be attributable to reverse causation as a result of altered PUFA metabolism in adipose tissue of persons with obesity (33).

Country of origin was the strongest predictor of all PUFA in both children and adults. This is consistent with a European study in which region was the strongest predictor of plasma PUFA concentrations (34). Differences by country likely mainly reflect differences in lifestyle and diet, especially in the case of adipose tissue LA and ALA, which do not appear to be genetically determined (35). Country-specific distributions of cooking oil usage were consistent with some differences in PUFA by country: soyabean oil use was highest in Dominican households, corresponding to high LA and ALA among Dominican participants, and palm oil usage was highest in Honduras and El Salvador, corresponding to low levels of all PUFA among participants from these countries. Adjustment for cooking oil type did not appreciably change the associations of any PUFA with country, while the associations with cooking oil type were attenuated. We believe the reason for the attenuation is that country is the main source of variability in type of oil used. Previous studies in Costa Rica suggest that cooking oil is a significant predictor of PUFA intake (7,15), and we cannot rule out that usage of different types of oils explains at least in part the differences in PUFA by country in the present study. These associations may also reflect differences in intake of specific foods or variation in the PUFA content of oils and foods according to the country in which they are produced (36).

The primary type of oil used for cooking in the home was a significant predictor of essential n-6 and n-3 PUFA in children and adults. Some of these associations are expected according to the fatty acid composition of cooking oils in Latin American countries (37–39). Soyabean and canola oils are high in ALA, whereas palm oil is low in all n-6 and n-3 PUFA; correspondingly, in our study soyabean and canola oil users had among the highest ALA levels,

whereas palm oil users had among the lowest levels of all PUFA. LA content is highest in corn and sunflower oils (37–39), but we observed the highest adipose LA concentrations among users of soyabean oil. This is consistent with previous findings from Costa Rica, where use of soyabean oil low in trans fatty acids was associated with higher adipose tissue LA concentrations (15).

We also found that cooking oil usage predicted LCPUFA status, despite the low levels of preformed LCPUFA typically found in vegetable oils (37–39). This is in line with the results of an intervention study among Colombian families in which soyabean or sunflower oil increased whole blood LCPUFA concentrations in school-age children (14). In our study, canola oil users had among the highest levels of both n-6 and n-3 LCPUFA, despite not having the highest levels of LA and ALA. The conversion of essential fatty acids into LCPUFA by desaturase enzymes is inhibited by high essential fatty acid intake (40), and total essential fatty acid content is higher in soyabean and corn oils than in canola oil (37–39). However, Δ6-desaturase activity did not differ significantly between users of different oil types; thus, these findings cannot be explained by higher Δ6-desaturase activity among canola oil users as a result of lower total essential fatty acid intake. Canola oil is a rich source of ALA, which could explain the higher levels of n-3 LCPUFA, but has relatively less LA compared to the other oil types. The reason for higher levels of n-6 LCPUFA among canola users is unclear.

The specific foods associated with each PUFA differed somewhat between children and adults, possibly as a result of different dietary patterns or preparations. LA and ALA were each associated with a mix of plant- and animal-source foods; however, for almost all foods, the difference associated with nearly the entire range of intake frequency in the population was smaller than the range of essential fatty acid levels associated with different oil types. Two of the

strongest positive associations were between ALA and milk and cream among children, which is consistent with a study that found whole milk to be one of the main dietary sources of total n-3 PUFA among Guatemalan children (9). Even at the low frequency of intake in this population, fish intake was positively associated with n-3 LCPUFA among adults and children, and intake of canned tuna/sardines was positively associated among adults. Unexpectedly, we found that canned tuna/sardine intake was inversely associated with n-3 LCPUFA in children, despite a similar range of intake as that among adults. Habitual intake of this food may be measured with greater error in children than adults, or the association may be due to chance.

We did not find significant associations between SES indicators and adipose tissue PUFA. This could be because social determinants of PUFA intake, such as access to or preference for certain cooking oils among people of different SES, vary substantially by country (38,41). Our sample sizes per country precluded within-country analyses.

Our study has several strengths. We examined associations that have not been well-characterized in Mesoamerican populations, and that are relevant to public health concerns in the region, including the low intake of dietary sources of n-3 LCPUFA (10). PUFA were measured in adipose tissue, the gold standard biomarker of fatty acid intake. We had available measures of sociodemographic, anthropometric, and dietary characteristics, which allowed us to assess a wide range of correlates of PUFA status and control for many confounders. Finally, the family design provided an opportunity to investigate associations separately for adults and children.

The primary limitation of this study is its cross-sectional design, which prevents causal inference. Reverse causation could explain some of the results, including the association between BMI and n-6 LCPUFA. The study population was not intended to be representative of any

particular group, which may limit the generalizability of our findings. In addition, the small sample sizes within each country prevented us from fully assessing country-specific associations.

In conclusion, we found that country of origin, type of oil used for cooking, and intake frequency of specific foods are important correlates of adipose tissue PUFA concentrations in Mesoamerican adults and children. Our results suggest that cooking oil type is a predictor not only of essential fatty acid status but of LCPUFA as well, and that some dietary correlates of PUFA status differ between adults and children. Future studies should assess whether and which dietary interventions could improve the PUFA status of this population.

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Table 2.1. Mean (± SD) of adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by sociodemographic characteristics among children from Mesoamerica

Characteristics	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Overall	220	14.02 ± 4.84	0.94 ± 0.73	0.74 ± 0.35	0.18 ± 0.17
Sex					
Female	115	14.65 ± 4.98	0.90 ± 0.40	0.76 ± 0.34	0.15 ± 0.12
Male	105	13.33 ± 4.61	0.99 ± 0.97	0.71 ± 0.36	0.20 ± 0.21
P[b]		0.04	0.40	0.29	0.05
Age (years)					
<9	67	13.27 ± 4.20	0.87 ± 0.36	0.70 ± 0.25	0.16 ± 0.19
9 - <11	79	14.27 ± 4.74	0.96 ± 1.04	0.77 ± 0.40	0.16 ± 0.16
≥11	74	14.43 ± 5.45	1.00 ± 0.54	0.74 ± 0.37	0.21 ± 0.16
P, trend[a]		0.16	0.10	0.44	0.09
Height-for-age Z score					
<-2	11	13.39 ± 7.21	0.81 ± 0.50	0.65 ± 0.44	0.17 ± 0.19
-2 - <-1	41	14.25 ± 4.48	0.85 ± 0.46	0.72 ± 0.31	0.15 ± 0.15
-1 - <0	80	13.05 ± 4.32	0.93 ± 1.03	0.72 ± 0.36	0.17 ± 0.18
0 - <1	56	15.01 ± 5.33	0.95 ± 0.46	0.80 ± 0.35	0.18 ± 0.13
≥1	32	14.62 ± 4.51	1.14 ± 0.49	0.73 ± 0.35	0.22 ± 0.23
P, trend		0.23	0.01	0.30	0.15
BMI-for-age Z score					
<-1	34	13.65 ± 4.47	0.75 ± 0.35	0.73 ± 0.34	0.18 ± 0.19
-1 - <0	44	13.93 ± 5.85	0.81 ± 0.47	0.76 ± 0.40	0.15 ± 0.15
0 - <1	66	13.40 ± 4.72	1.07 ± 1.17	0.73 ± 0.36	0.20 ± 0.21
≥1	76	14.77 ± 4.46	1.00 ± 0.37	0.75 ± 0.32	0.17 ± 0.13
P, trend		0.24	0.0001	0.91	0.79
Maternal age at child's birth (years)					
<20	29	14.75 ± 5.83	1.05 ± 1.60	0.93 ± 0.49	0.22 ± 0.21
20 - <25	54	14.19 ± 5.17	0.92 ± 0.47	0.73 ± 0.32	0.19 ± 0.19
25 - <30	75	13.42 ± 4.48	0.90 ± 0.50	0.68 ± 0.33	0.15 ± 0.12
30 - <35	41	15.16 ± 4.44	0.97 ± 0.37	0.79 ± 0.30	0.14 ± 0.13
≥35	21	12.47 ± 4.20	0.98 ± 0.63	0.60 ± 0.24	0.22 ± 0.26
P, trend		0.41	0.89	0.02	0.32
Maternal height (cm)					
140.6 - 151.9	58	13.30 ± 4.99	0.89 ± 0.48	0.70 ± 0.34	0.20 ± 0.24
152.0 - 155.7	50	13.89 ± 4.59	1.11 ± 1.28	0.78 ± 0.39	0.16 ± 0.17
155.8 - 159.2	58	13.98 ± 4.37	0.88 ± 0.43	0.70 ± 0.27	0.16 ± 0.11
159.3 - 181.9	54	14.95 ± 5.35	0.92 ± 0.44	0.79 ± 0.40	0.18 ± 0.13
P, trend		0.10	0.73	0.43	0.68

Table 2.1. Mean (± SD) of adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by sociodemographic characteristics among children from Mesoamerica

Characteristics	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Maternal body mass index (kg/m^2)					
<25	54	14.49 ± 3.94	0.92 ± 0.36	0.73 ± 0.25	0.16 ± 0.16
25 - <30	79	14.55 ± 5.65	0.95 ± 1.04	0.81 ± 0.44	0.16 ± 0.15
≥30	87	13.25 ± 4.50	0.95 ± 0.54	0.68 ± 0.30	0.20 ± 0.19
P, trend		0.06	0.69	0.19	0.24
Maternal education level					
Incomplete elementary	40	13.71 ± 6.11	0.85 ± 0.47	0.72 ± 0.40	0.19 ± 0.17
Complete elementary	26	12.61 ± 3.69	1.07 ± 0.61	0.64 ± 0.23	0.23 ± 0.29
Incomplete secondary	56	13.68 ± 4.70	1.00 ± 1.23	0.76 ± 0.41	0.17 ± 0.17
Complete secondary	39	14.14 ± 4.29	0.88 ± 0.36	0.78 ± 0.26	0.15 ± 0.11
Post secondary	53	15.14 ± 4.72	0.96 ± 0.40	0.76 ± 0.37	0.17 ± 0.13
P, trend		0.09	0.71	0.30	0.24
Parental smoking history					
Neither parent ever smoked	84	15.08 ± 5.97	0.92 ± 0.42	0.82 ± 0.40	0.19 ± 0.17
One parent ever smoked	99	13.48 ± 3.98	1.02 ± 0.98	0.71 ± 0.31	0.18 ± 0.18
Both parents ever smoked	31	12.77 ± 3.04	0.81 ± 0.37	0.61 ± 0.25	0.15 ± 0.15
P		0.03	0.18	0.008	0.48
Number of household assets[c]					
0-4	42	15.50 ± 5.89	0.86 ± 0.33	0.82 ± 0.44	0.16 ± 0.12
5-7	87	12.75 ± 4.46	0.93 ± 1.03	0.68 ± 0.33	0.21 ± 0.22
8-9	40	14.89 ± 4.64	0.94 ± 0.50	0.76 ± 0.36	0.13 ± 0.12
10-12	51	14.28 ± 4.24	1.04 ± 0.44	0.75 ± 0.28	0.16 ± 0.13
P, trend		0.99	0.08	0.76	0.21
Food insecurity					
No insecurity	65	14.11 ± 4.99	1.00 ± 0.46	0.75 ± 0.34	0.15 ± 0.15
Mild insecurity	58	13.36 ± 4.09	0.89 ± 0.44	0.69 ± 0.30	0.19 ± 0.19
Moderate insecurity	56	13.48 ± 4.52	0.90 ± 1.18	0.73 ± 0.36	0.18 ± 0.19
Severe insecurity	40	15.63 ± 5.81	1.02 ± 0.58	0.82 ± 0.42	0.19 ± 0.16
P, trend		0.28	>0.99	0.41	0.25

Table 2.1. Mean (± SD) of adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by sociodemographic characteristics among children from Mesoamerica

Characteristics	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Country of origin					
Guatemala	27	15.18 ± 3.75	0.94 ± 0.30	0.66 ± 0.16	0.19 ± 0.20
El Salvador	30	10.56 ± 2.34	1.20 ± 1.69	0.50 ± 0.39	0.15 ± 0.20
Dominican Republic	28	22.04 ± 4.83	1.03 ± 0.21	1.23 ± 0.41	0.25 ± 0.11
Honduras	27	10.87 ± 1.95	0.72 ± 0.27	0.76 ± 0.19	0.06 ± 0.07
Nicaragua	27	12.64 ± 3.02	0.75 ± 0.27	0.66 ± 0.22	0.15 ± 0.11
Panama	20	15.49 ± 2.00	0.84 ± 0.32	0.71 ± 0.19	0.17 ± 0.11
Costa Rica	16	12.44 ± 4.55	1.56 ± 0.65	0.58 ± 0.35	0.15 ± 0.22
Mexico	19	14.80 ± 3.36	0.99 ± 0.39	0.82 ± 0.25	0.29 ± 0.25
Belize	26	12.15 ± 3.79	0.67 ± 0.29	0.71 ± 0.29	0.19 ± 0.15
P		<0.0001	<0.0001	<0.0001	<0.0001

[a]Wald test from linear regression models with each fatty acid as the outcome and a variable representing ordinal categories of each characteristic introduced as a continuous predictor. An exchangeable covariance structure was specified in all models to account for clustering by family membership.

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

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

Table 2.2. Adjusted percent differences (95% CI)[a] in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by sociodemographic characteristics among children from Mesoamerica

Characteristics	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Sex				
Female	Reference	Reference	Reference	Reference
Male	-8.2 (-14.6, -1.3)	3.6 (-7.2, 15.5)	-4.6 (-15.2, 7.3)	13.0 (-6.5, 36.5)
P[b]	0.02	0.53	0.43	0.21
Age				
Per 1 year	3.0 (0.6, 5.5)	2.7 (-0.7, 6.2)	0.7 (-2.3, 3.9)	10.4 (4.0, 17.2)
P[b]	0.01	0.12	0.64	0.001
Height-for-age Z score				
Per 1 Z score	2.3 (-0.9, 5.6)	9.1 (3.5, 14.9)	1.7 (-3.7, 7.4)	10.3 (0.9, 20.7)
P[b]	0.16	0.001	0.55	0.03
BMI-for-age Z score				
Per 1 Z score	2.1 (-0.1, 4.3)	5.4 (1.4, 9.6)	0.8 (-2.8, 4.5)	0.4 (-6.9, 8.2)
P[b]	0.06	0.007	0.68	0.92
Maternal age at child's birth				
Per 1 year	-0.1 (-0.6, 0.5)	0.3 (-0.8, 1.4)	-0.9 (-1.7, -0.1)	-0.5 (-2.2, 1.3)
P[b]	0.80	0.59	0.03	0.60
Maternal education level				
Per 1 year	0.6 (-0.2, 1.3)	-0.4 (-1.8, 1.1)	-0.5 (-1.9, 1.0)	-1.9 (-4.0, 0.3)
P[b]	0.13	0.62	0.51	0.09
Country of origin				
Guatemala	Reference	Reference	Reference	Reference
El Salvador	-29.9 (-38.4, -20.2)	-15.1 (-36.2, 13.1)	-32.2 (-45.1, -16.3)	-24.8 (-50.7, 14.7)
Dominican Republic	44.5 (27.2, 64.1)	1.8 (-12.2, 18.0)	77.1 (51.8, 106.6)	50.3 (2.8, 119.8)
Honduras	-27.7 (-36.1, -18.2)	-28.5 (-40.2, -14.4)	11.1 (-9.2, 35.9)	-53.2 (-67.9, -31.8)
Nicaragua	-16.9 (-27.6, -4.7)	-26.8 (-38.9, -12.4)	-5.4 (-19.3, 10.7)	-10.8 (-40.1, 33.0)
Panama	5.8 (-5.7, 18.7)	-21.3 (-37.1, -1.5)	7.1 (-7.6, 24.1)	-4.5 (-37.4, 45.8)
Costa Rica	-25.2 (-41.5, -4.4)	45.5 (15.5, 83.4)	-32.0 (-56.7, 6.6)	-37.0 (-65.3, 14.3)
Mexico	-3.0 (-16.5, 12.8)	-4.0 (-20.8, 16.4)	19.6 (0.8, 41.8)	56.7 (2.9, 138.8)
Belize	-21.7 (-33.9, -7.3)	-35.8 (-47.2, -22.0)	1.9 (-14.5, 21.4)	4.2 (-32.1, 59.8)
P[c]	<0.0001	<0.0001	<0.0001	<0.0001

[a]From multivariable linear regression models with the log-transformed concentration of each fatty acid as the outcome. Estimates are adjusted for age, sex, and country of origin. In addition, the model for long-chain n-6 was adjusted for height-for-age Z score, the model for ALA was adjusted for maternal age at the child's birth, and the model for long-chain n-3 was adjusted for height-for-age Z score.

[b]Wald test from multivariable linear regression models with each fatty acid as the outcome.

[c]χ^2 score statistic from multivariable linear regression models with each fatty acid as the outcome and indicator variables for each level of the characteristic as predictors.

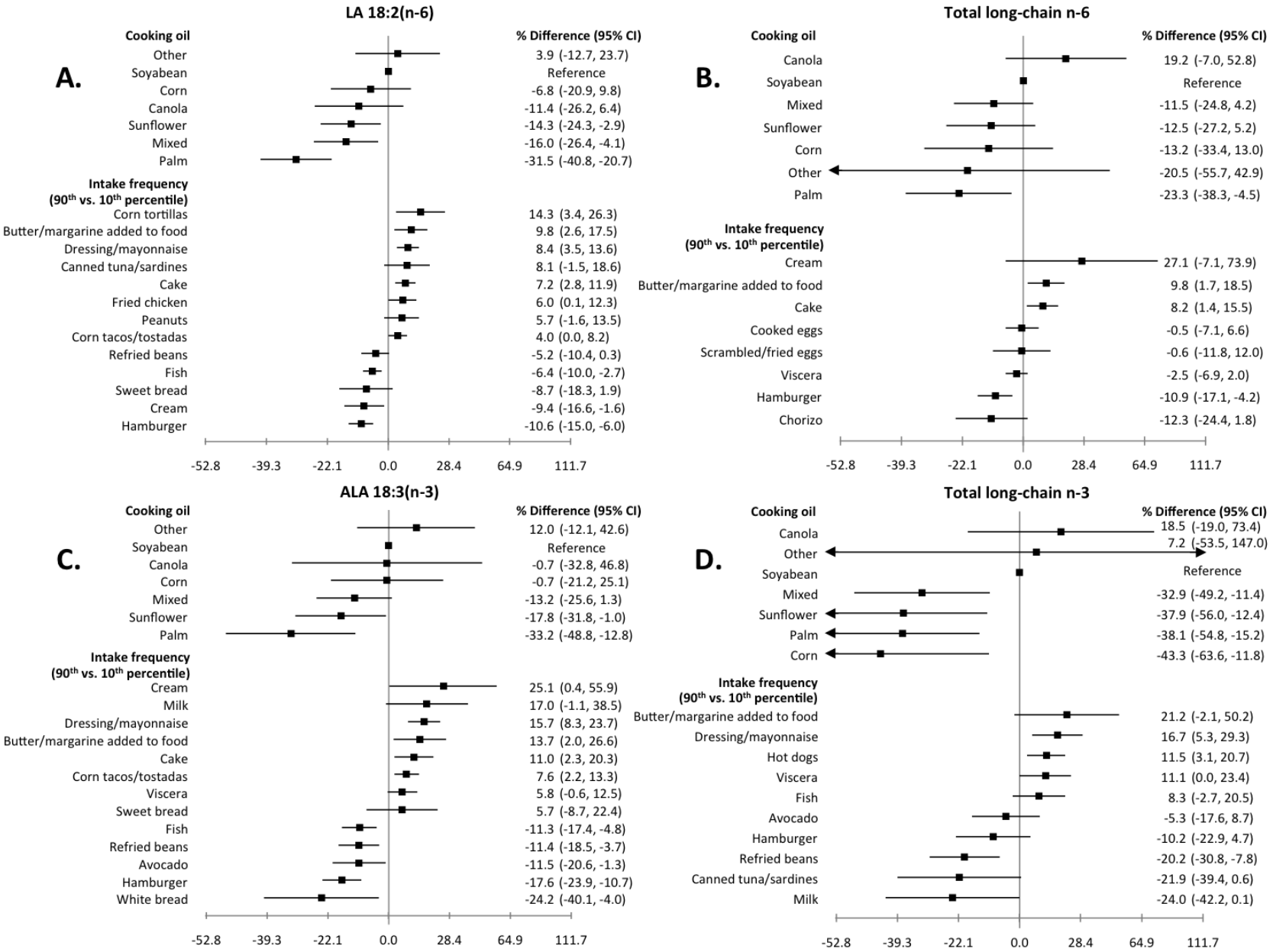

A.
LA 18:2(n-6)
Cooking oil
% Difference (95% CI)
Other 3.9 (-12.7, 23.7)
Soyabean Reference
Corn -6.8 (-20.9, 9.8)
Canola -11.4 (-26.2, 6.4)
Sunflower -14.3 (-24.3, -2.9)
Mixed -16.0 (-26.4, -4.1)
Palm -31.5 (-40.8, -20.7)
Intake frequency (90th vs. 10th percentile)
Corn tortillas 14.3 (3.4, 26.3)
Butter/margarine added to food 9.8 (2.6, 17.5)
Dressing/mayonnaise 8.4 (3.5, 13.6)
Canned tuna/sardines 8.1 (-1.5, 18.6)
Cake 7.2 (2.8, 11.9)
Fried chicken 6.0 (0.1, 12.3)
Peanuts 5.7 (-1.6, 13.5)
Corn tacos/tostadas 4.0 (0.0, 8.2)
Refried beans -5.2 (-10.4, 0.3)
Fish -6.4 (-10.0, -2.7)
Sweet bread -8.7 (-18.3, 1.9)
Cream -9.4 (-16.6, -1.6)
Hamburger -10.6 (-15.0, -6.0)
-52.8 -39.3 -22.1 0.0 28.4 64.9 111.7
B.
Total long-chain n-6
Cooking oil
% Difference (95% CI)
Canola 19.2 (-7.0, 52.8)
Soyabean Reference
Mixed -11.5 (-24.8, 4.2)
Sunflower -12.5 (-27.2, 5.2)
Corn -13.2 (-33.4, 13.0)
Other -20.5 (-55.7, 42.9)
Palm -23.3 (-38.3, -4.5)
Intake frequency (90th vs. 10th percentile)
Cream 27.1 (-7.1, 73.9)
Butter/margarine added to food 9.8 (1.7, 18.5)
Cake 8.2 (1.4, 15.5)
Cooked eggs -0.5 (-7.1, 6.6)
Scrambled/fried eggs -0.6 (-11.8, 12.0)
Viscera -2.5 (-6.9, 2.0)
Hamburger -10.9 (-17.1, -4.2)
Chorizo -12.3 (-24.4, 1.8)
-52.8 -39.3 -22.1 0.0 28.4 64.9 111.7
C.
ALA 18:3(n-3)
Cooking oil
% Difference (95% CI)
Other 12.0 (-12.1, 42.6)
Soyabean Reference
Canola -0.7 (-32.8, 46.8)
Corn -0.7 (-21.2, 25.1)
Mixed -13.2 (-25.6, 1.3)
Sunflower -17.8 (-31.8, -1.0)
Palm -33.2 (-48.8, -12.8)
Intake frequency (90th vs. 10th percentile)
Cream 25.1 (0.4, 55.9)
Milk 17.0 (-1.1, 38.5)
Dressing/mayonnaise 15.7 (8.3, 23.7)
Butter/margarine added to food 13.7 (2.0, 26.6)
Cake 11.0 (2.3, 20.3)
Corn tacos/tostadas 7.6 (2.2, 13.3)
Viscera 5.8 (-0.6, 12.5)
Sweet bread 5.7 (-8.7, 22.4)
Fish -11.3 (-17.4, -4.8)
Refried beans -11.4 (-18.5, -3.7)
Avocado -11.5 (-20.6, -1.3)
Hamburger -17.6 (-23.9, -10.7)
White bread -24.2 (-40.1, -4.0)
-52.8 -39.3 -22.1 0.0 28.4 64.9 111.7
D.
Total long-chain n-3
Cooking oil
% Difference (95% CI)
Canola 18.5 (-19.0, 73.4)
Other 7.2 (-53.5, 147.0)
Soyabean Reference
Mixed -32.9 (-49.2, -11.4)
Sunflower -37.9 (-56.0, -12.4)
Palm -38.1 (-54.8, -15.2)
Corn -43.3 (-63.6, -11.8)
Intake frequency (90th vs. 10th percentile)
Butter/margarine added to food 21.2 (-2.1, 50.2)
Dressing/mayonnaise 16.7 (5.3, 29.3)
Hot dogs 11.5 (3.1, 20.7)
Viscera 11.1 (0.0, 23.4)
Fish 8.3 (-2.7, 20.5)
Avocado -5.3 (-17.6, 8.7)
Hamburger -10.2 (-22.9, 4.7)
Refried beans -20.2 (-30.8, -7.8)
Canned tuna/sardines -21.9 (-39.4, 0.6)
Milk -24.0 (-42.2, 0.1)
-52.8 -39.3 -22.1 0.0 28.4 64.9 111.7

Figure 2.1. Adjusted percent differences (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acid concentrations by main oil type used at home for cooking and by food intake frequency (90^{th} vs. 10^{th} percentiles of servings/d) among children from Mesoamerica.

Estimates and confidence intervals are plotted on the log scale. Estimates are from multivariable linear regression models with log-transformed fatty acids as the outcome. For each fatty acid, cooking oil type and all foods for which estimates are presented are included in the model as predictors simultaneously. In addition, all estimates are adjusted for age (continuous), sex, and maternal education (continuous). Additionally, the model for long-chain n-6 is adjusted for height-for-age Z score (continuous), the model for ALA is adjusted for maternal age at child's birth (continuous), and the model for long-chain n-3 is adjusted for height-for-age Z score (continuous). Serving sizes: cream, dressing/mayonnaise, butter/margarine added to food: 1 tbs; chorizo, cooked eggs, fried/scrambled eggs, sausage, corn tortillas: 1 unit; sweet bread, white bread, canned tuna/sardines, fried chicken, hamburger, cake, fish, corn tacos/tostadas, hot dog: 1 portion; peanuts: 30g or 1 tbs; refried beans: 1/3 cup; viscera: ½ cup; avocado: ¼ unit; milk, 1 glass. N in each cooking oil category: soyabean, 63; sunflower, 17; corn, 20; palm, 24; canola, 15; mixed, 70; other, 3.

Table 2.3. Mean (± SD) adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by sociodemographic characteristics among adults from Mesoamerica

Characteristics	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Overall	471	15.03 ± 4.89	1.16 ± 0.51	0.75 ± 0.34	0.24 ± 0.21
Sex					
Female	245	15.26 ± 4.95	1.21 ± 0.50	0.75 ± 0.33	0.25 ± 0.17
Male	226	14.78 ± 4.82	1.12 ± 0.50	0.75 ± 0.34	0.22 ± 0.25
P[b]		0.06	0.03	0.78	0.18
Age (years)					
<30	54	15.45 ± 4.87	1.08 ± 0.31	0.78 ± 0.32	0.22 ± 0.10
30 - <35	100	15.66 ± 5.13	1.12 ± 0.48	0.83 ± 0.38	0.23 ± 0.23
35 - <40	145	14.91 ± 4.56	1.16 ± 0.48	0.73 ± 0.30	0.24 ± 0.20
40 - <45	97	14.99 ± 5.03	1.23 ± 0.64	0.73 ± 0.36	0.22 ± 0.15
45 - <55	62	14.31 ± 5.02	1.23 ± 0.50	0.70 ± 0.33	0.25 ± 0.15
55+	12	12.88 ± 4.66	1.09 ± 0.54	0.68 ± 0.24	0.39 ± 0.70
P, trend[a]		0.07	0.21	0.08	0.34
Height quartile (mothers/fathers medians, cm)					
Q1 (148.9/159.0)	115	14.77 ± 4.51	1.19 ± 0.47	0.69 ± 0.31	0.26 ± 0.30
Q2 (153.1/164.9)	115	14.25 ± 4.99	1.13 ± 0.50	0.70 ± 0.34	0.22 ± 0.15
Q3 (157.1/169.7)	127	15.16 ± 4.66	1.15 ± 0.59	0.77 ± 0.33	0.23 ± 0.16
Q4 (162.8/176.4)	114	15.94 ± 5.30	1.18 ± 0.44	0.84 ± 0.35	0.23 ± 0.21
P, trend		0.44	0.72	0.002	0.50
Body mass index (kg/m^2)					
<25	118	15.95 ± 5.38	1.11 ± 0.51	0.78 ± 0.35	0.26 ± 0.28
25-<30	190	14.91 ± 4.92	1.17 ± 0.48	0.74 ± 0.34	0.22 ± 0.20
≥30	163	14.51 ± 4.40	1.20 ± 0.53	0.74 ± 0.33	0.23 ± 0.16
P, trend		0.09	0.11	0.21	0.37
Education level					
Incomplete elementary	65	15.31 ± 5.72	1.14 ± 0.40	0.75 ± 0.37	0.22 ± 0.13
Complete elementary	63	14.49 ± 3.73	1.22 ± 0.72	0.72 ± 0.28	0.23 ± 0.27
Incomplete secondary	137	15.45 ± 4.89	1.16 ± 0.51	0.81 ± 0.35	0.22 ± 0.14
Complete secondary	74	14.17 ± 4.98	1.09 ± 0.42	0.71 ± 0.35	0.24 ± 0.19
Post secondary	122	15.28 ± 4.96	1.21 ± 0.48	0.74 ± 0.33	0.26 ± 0.28
P, trend		0.97	0.80	0.82	0.20

Table 2.3. Mean (± SD) adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by sociodemographic characteristics among adults from Mesoamerica

Characteristics	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Smoking status					
Never	301	15.81 ± 5.15	1.19 ± 0.50	0.78 ± 0.35	0.24 ± 0.18
Past	129	13.92 ± 4.08	1.15 ± 0.54	0.71 ± 0.31	0.24 ± 0.29
Current	39	12.38 ± 3.45	0.98 ± 0.36	0.70 ± 0.31	0.18 ± 0.13
P		0.0009	0.03	0.29	0.05
Number of household assets[c]					
0-4	84	14.90 ± 5.24	1.14 ± 0.46	0.76 ± 0.35	0.22 ± 0.12
5-7	179	14.89 ± 5.06	1.08 ± 0.37	0.74 ± 0.34	0.22 ± 0.18
8-9	93	15.58 ± 5.05	1.11 ± 0.44	0.78 ± 0.34	0.20 ± 0.12
10-12	115	14.90 ± 4.21	1.36 ± 0.68	0.74 ± 0.32	0.31 ± 0.32
P, trend		0.83	0.004	0.78	0.008
Food security					
No insecurity	147	15.19 ± 4.63	1.30 ± 0.61	0.75 ± 0.32	0.28 ± 0.29
Mild insecurity	127	14.62 ± 4.98	1.10 ± 0.44	0.74 ± 0.34	0.22 ± 0.13
Moderate insecurity	111	14.69 ± 5.04	1.10 ± 0.47	0.74 ± 0.35	0.21 ± 0.21
Severe insecurity	84	15.80 ± 5.03	1.11 ± 0.40	0.79 ± 0.36	0.22 ± 0.13
P, trend		0.62	0.007	0.47	0.04
Country of origin					
Guatemala	55	16.77 ± 2.74	1.23 ± 0.39	0.65 ± 0.15	0.30 ± 0.26
El Salvador	59	11.41 ± 2.21	0.90 ± 0.41	0.44 ± 0.13	0.18 ± 0.15
Dominican Republic	59	22.40 ± 4.93	1.22 ± 0.39	1.16 ± 0.36	0.24 ± 0.10
Honduras	58	10.08 ± 2.61	0.97 ± 0.29	0.60 ± 0.32	0.16 ± 0.13
Nicaragua	56	13.31 ± 2.92	1.02 ± 0.32	0.88 ± 0.29	0.15 ± 0.09
Panama	46	15.72 ± 3.31	1.13 ± 0.34	0.69 ± 0.22	0.23 ± 0.16
Costa Rica	43	17.60 ± 3.89	1.86 ± 0.92	0.86 ± 0.32	0.44 ± 0.46
Mexico	40	15.05 ± 3.25	1.10 ± 0.23	0.82 ± 0.27	0.22 ± 0.07
Belize	55	13.67 ± 3.33	1.21 ± 0.46	0.70 ± 0.29	0.24 ± 0.09
P		<0.0001	<0.0001	<0.0001	<0.0001

[a]Wald test from linear regression models with each fatty acid as the outcome and a variable representing ordinal categories of each characteristic introduced as a continuous predictor. An exchangeable covariance structure was specified in all models to account for clustering by family membership.

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

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

Table 2.4. Adjusted percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by sociodemographic characteristics among adults from Mesoamerica

Characteristics	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Sex				
Female	Reference	Reference	Reference	Reference
Male	0.5 (-4.3, 5.4)	-9.5 (-14.7, -3.9)	-0.8 (-7.9, 6.9)	-17.5 (-25.4, -8.7)
P[b]	0.85	0.001	0.84	0.0002
Age (years)				
Per 1 year	-0.4 (-0.8, -0.1)	0.3 (-0.3, 0.8)	-0.3 (-0.8, 0.2)	0.1 (-0.8, 0.9)
P[b]	0.02	0.33	0.18	0.87
Height				
Per 1 cm	-0.1 (-0.4, 0.3)	0.0 (-0.5, 0.4)	0.4 (-0.1, 1.0)	-0.5 (-1.4, 0.5)
P[b]	0.72	0.88	0.15	0.33
BMI				
Per 1 kg/m^2	-0.2 (-0.6, 0.1)	0.8 (0.2, 1.4)	-0.7 (-1.2, -0.1)	0.0 (-0.9, 1.0)
P[b]	0.16	0.01	0.02	0.94
Education level				
Per 1 year	0.0 (-0.5, 0.5)	0.5 (-0.2, 1.2)	-0.2 (-0.9, 0.6)	1.0 (-0.1, 2.1)
P[b]	0.97	0.13	0.68	0.07
Smoking status				
Never	Reference	Reference	Reference	Reference
Past	-2.2 (-7.5, 3.5)	2.0 (-6.0, 10.7)	-0.5 (-8.7, 8.5)	9.2 (-4.0, 24.4)
Current	-14.1 (-22.9, -4.2)	-5.0 (-15.2, 6.4)	-8.7 (-21.5, 6.2)	2.0 (-16.2, 24.2)
P[c]	0.04	0.49	0.49	0.43
Country of origin				
Guatemala	Reference	Reference	Reference	Reference
El Salvador	-31.6 (-35.8, -27.0)	-28.8 (-36.1, -20.6)	-32.8 (-38.6, -26.5)	-39.3 (-50.9, -25.0)
Dominican Republic	29.4 (19.7, 39.9)	-1.8 (-12.0, 9.7)	75.0 (57.2, 94.7)	-13.9 (-28.2, 3.1)
Honduras	-40.6 (-45.5, -35.3)	-22.6 (-30.9, -13.2)	-13.9 (-24.6, -1.8)	-44.6 (-54.4, -32.6)
Nicaragua	-20.7 (-27.0, -13.9)	-17.1 (-26.6, -6.2)	29.9 (17.4, 43.9)	-45.6 (-54.6, -34.9)
Panama	-7.9 (-14.7, -0.6)	-8.9 (-19.2, 2.7)	4.0 (-7.3, 16.6)	-23.7 (-39.0, -4.7)
Costa Rica	3.3 (-5.2, 12.4)	38.6 (17.6, 63.2)	27.0 (7.7, 49.8)	18.5 (-8.1, 52.6)
Mexico	-12.4 (-20.5, -3.3)	-8.2 (-16.3, 0.6)	22.0 (6.9, 39.3)	-17.5 (-30.9, -1.4)
Belize	-21.4 (-27.9, -14.3)	-6.0 (-18.0, 7.7)	1.8 (-12.5, 18.4)	-11.2 (-25.1, 5.3)
P[c]	<0.0001	<0.0001	<0.0001	<0.0001

[a]From multivariable linear regression models of log-transformed fatty acids. Estimates are adjusted for age, sex, country of origin, and in the case of LA, smoking status.

[b]Wald test from multivariable linear regression models with a given fatty acid as the outcome.

[c]Type III F test of a categorical predictor from multivariable linear regression models with a given fatty acid as the outcome.

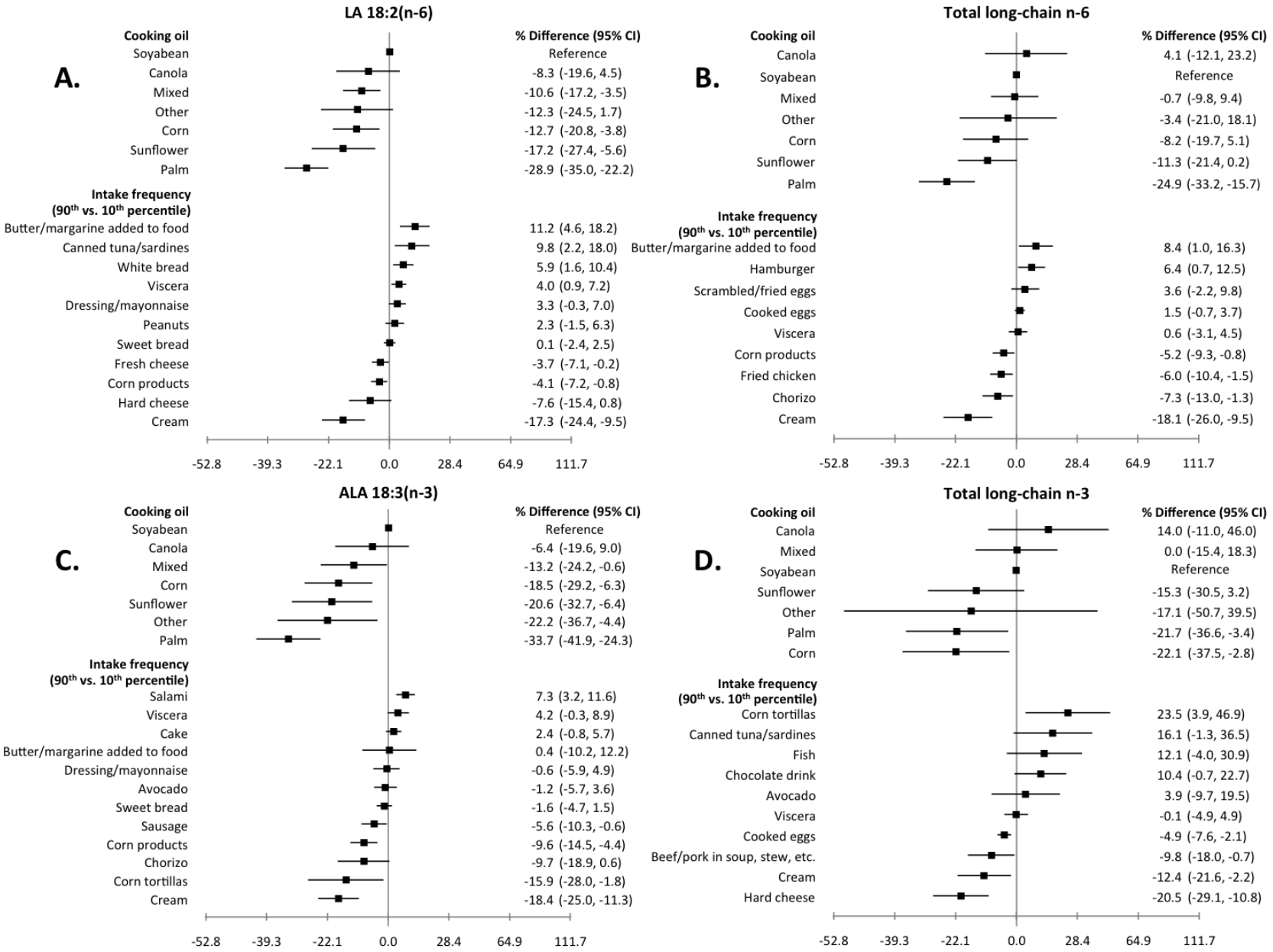

A.
LA 18:2(n-6)
Cooking oil
% Difference (95% CI)
Soyabean
Reference
Canola
-8.3 (-19.6, 4.5)
Mixed
-10.6 (-17.2, -3.5)
Other
-12.3 (-24.5, 1.7)
Corn
-12.7 (-20.8, -3.8)
Sunflower
-17.2 (-27.4, -5.6)
Palm
-28.9 (-35.0, -22.2)
Intake frequency
(90th vs. 10th percentile)
Butter/margarine added to food
11.2 (4.6, 18.2)
Canned tuna/sardines
9.8 (2.2, 18.0)
White bread
5.9 (1.6, 10.4)
Viscera
4.0 (0.9, 7.2)
Dressing/mayonnaise
3.3 (-0.3, 7.0)
Peanuts
2.3 (-1.5, 6.3)
Sweet bread
0.1 (-2.4, 2.5)
Fresh cheese
-3.7 (-7.1, -0.2)
Corn products
-4.1 (-7.2, -0.8)
Hard cheese
-7.6 (-15.4, 0.8)
Cream
-17.3 (-24.4, -9.5)
-52.8
-39.3
-22.1
0.0
28.4
64.9
111.7
B.
Total long-chain n-6
Cooking oil
% Difference (95% CI)
Canola
4.1 (-12.1, 23.2)
Soyabean
Reference
Mixed
-0.7 (-9.8, 9.4)
Other
-3.4 (-21.0, 18.1)
Corn
-8.2 (-19.7, 5.1)
Sunflower
-11.3 (-21.4, 0.2)
Palm
-24.9 (-33.2, -15.7)
Intake frequency
(90th vs. 10th percentile)
Butter/margarine added to food
8.4 (1.0, 16.3)
Hamburger
6.4 (0.7, 12.5)
Scrambled/fried eggs
3.6 (-2.2, 9.8)
Cooked eggs
1.5 (-0.7, 3.7)
Viscera
0.6 (-3.1, 4.5)
Corn products
-5.2 (-9.3, -0.8)
Fried chicken
-6.0 (-10.4, -1.5)
Chorizo
-7.3 (-13.0, -1.3)
Cream
-18.1 (-26.0, -9.5)
-52.8
-39.3
-22.1
0.0
28.4
64.9
111.7
C.
ALA 18:3(n-3)
Cooking oil
% Difference (95% CI)
Soyabean
Reference
Canola
-6.4 (-19.6, 9.0)
Mixed
-13.2 (-24.2, -0.6)
Corn
-18.5 (-29.2, -6.3)
Sunflower
-20.6 (-32.7, -6.4)
Other
-22.2 (-36.7, -4.4)
Palm
-33.7 (-41.9, -24.3)
Intake frequency
(90th vs. 10th percentile)
Salami
7.3 (3.2, 11.6)
Viscera
4.2 (-0.3, 8.9)
Cake
2.4 (-0.8, 5.7)
Butter/margarine added to food
0.4 (-10.2, 12.2)
Dressing/mayonnaise
-0.6 (-5.9, 4.9)
Avocado
-1.2 (-5.7, 3.6)
Sweet bread
-1.6 (-4.7, 1.5)
Sausage
-5.6 (-10.3, -0.6)
Corn products
-9.6 (-14.5, -4.4)
Chorizo
-9.7 (-18.9, 0.6)
Corn tortillas
-15.9 (-28.0, -1.8)
Cream
-18.4 (-25.0, -11.3)
-52.8
-39.3
-22.1
0.0
28.4
64.9
111.7
D.
Total long-chain n-3
Cooking oil
% Difference (95% CI)
Canola
14.0 (-11.0, 46.0)
Mixed
0.0 (-15.4, 18.3)
Soyabean
Reference
Sunflower
-15.3 (-30.5, 3.2)
Other
-17.1 (-50.7, 39.5)
Palm
-21.7 (-36.6, -3.4)
Corn
-22.1 (-37.5, -2.8)
Intake frequency
(90th vs. 10th percentile)
Corn tortillas
23.5 (3.9, 46.9)
Canned tuna/sardines
16.1 (-1.3, 36.5)
Fish
12.1 (-4.0, 30.9)
Chocolate drink
10.4 (-0.7, 22.7)
Avocado
3.9 (-9.7, 19.5)
Viscera
-0.1 (-4.9, 4.9)
Cooked eggs
-4.9 (-7.6, -2.1)
Beef/pork in soup, stew, etc.
-9.8 (-18.0, -0.7)
Cream
-12.4 (-21.6, -2.2)
Hard cheese
-20.5 (-29.1, -10.8)
-52.8
-39.3
-22.1
0.0
28.4
64.9
111.7

Figure 2.2. Adjusted percent differences (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acid concentrations by main oil type used at home for cooking and by food intake frequency (90^{th} vs. 10^{th} percentiles of servings/d) among adults from Mesoamerica.

Estimates and confidence intervals are plotted on the log scale. Estimates are from multivariable linear regression models with log-transformed fatty acids as the outcome. For each fatty acid, cooking oil type and all foods for which estimates are presented are included in the model as predictors simultaneously. In addition, all estimates are adjusted for age (continuous), sex, education (continuous), and for LA, smoking status (never, former, or current). Corn products include foods such as empanadas, pupusas, chorreadas, arepas, tamal asado, and others. Serving sizes: cream, dressing/mayonnaise, butter/margarine added to food: 1 tbs; corn products, chorizo, cooked eggs, fried/scrambled eggs, sausage, corn tortillas: 1 unit; fresh cheese, hard cheese, salami: 1 piece; sweet bread, white bread, canned tuna/sardines, fried chicken, hamburger, beef tortitas, cake, fish, beef/pork in soup or stew: 1 portion; peanuts, cashews: 30g or 1 tbs; viscera: ½ cup; avocado: ¼ unit; chocolate drink: 1 cup. N in each cooking oil category: soyabean, 140; sunflower, 33; corn, 43; palm, 50; canola, 34; mixed, 148; other, 10.

Supplemental Table 2.1. Means (±SD) of adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by primary type of cooking oil among children from Mesoamerica

Oil type	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Soyabean	63	16.54 ± 6.02	0.97 ± 0.33	0.87 ± 0.42	0.23 ± 0.19
Sunflower	17	13.96 ± 3.43	0.79 ± 0.28	0.70 ± 0.27	0.12 ± 0.11
Corn	20	13.22 ± 5.23	0.87 ± 0.58	0.66 ± 0.39	0.12 ± 0.13
Palm	24	10.63 ± 2.16	0.83 ± 0.62	0.57 ± 0.25	0.11 ± 0.15
Canola	15	14.96 ± 2.79	1.24 ± 0.59	0.84 ± 0.27	0.22 ± 0.16
Mixed oils	70	13.24 ± 3.89	0.95 ± 1.10	0.70 ± 0.31	0.18 ± 0.18
Other	3	14.39 ± 2.89	0.86 ± 0.50	0.67 ± 0.26	0.24 ± 0.19
P[a]		<0.0001	0.17	0.007	0.01

[a] χ^2 score statistic from linear regression models with each fatty acid as the outcome and indicator variables for each oil type as predictors.

Supplemental Table 2.2. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among children from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Butter/margarine added to food[d]				
Unadjusted	16.8 (9.0, 25.2)	8.9 (0.9, 17.5)	19.2 (6.9, 33.0)	9.9 (-7.5, 30.6)
Adjusted, without country[b]	16.0 (7.1, 25.6)	9.0 (0.5, 18.2)	20.8 (6.5, 36.9)	16.0 (-1.3, 36.3)
Adjusted, with country[c]	6.3 (0.2, 12.7)	0.4 (-7.5, 8.9)	14.3 (3.0, 26.9)	10.6 (-4.5, 28.2)
Dressing/mayonnaise[d]				
Unadjusted	12.9 (6.8, 19.4)	4.6 (-3.1, 12.9)	21.2 (12.9, 30.0)	20.1 (6.0, 36.1)
Adjusted, without country	12.7 (6.5, 19.2)	3.7 (-3.3, 11.2)	22.0 (13.6, 30.9)	19.9 (5.9, 35.7)
Adjusted, with country	4.4 (-0.4, 9.5)	0.3 (-5.5, 6.6)	9.6 (1.7, 18.0)	14.3 (1.3, 29.0)
Avocado[e]				
Unadjusted	-1.5 (-8.2, 5.8)	-2.2 (-10.0, 6.3)	-5.3 (-13.6, 3.7)	1.9 (-12.0, 17.9)
Adjusted, without country	-3.3 (-11.7, 6.0)	-5.6 (-14.6, 4.3)	-7.4 (-17.4, 3.8)	-4.4 (-20.6, 15.1)
Adjusted, with country	-5.5 (-10.6, -0.2)	-12.5 (-20.6, -3.6)	-9.9 (-17.2, -2.0)	-4.1 (-17.7, 11.9)
Peanuts[f]				
Unadjusted	3.7 (-7.9, 16.8)	-9.8 (-21.6, 3.9)	0.3 (-19.6, 25.2)	1.2 (-15.5, 21.2)
Adjusted, without country	3.1 (-8.6, 16.3)	-10.8 (-22.5, 2.6)	-1.6 (-21.7, 23.8)	-0.4 (-15.6, 17.6)
Adjusted, with country	2.1 (-3.2, 7.8)	-7.8 (-15.6, 0.7)	-9.7 (-26.3, 10.7)	1.1 (-10.5, 14.3)
Cashews[f]				
Unadjusted	-2.3 (-6.0, 1.4)	-2.8 (-8.9, 3.7)	-2.9 (-7.6, 2.0)	-1.7 (-9.3, 6.7)
Adjusted, without country	-2.9 (-6.1, 0.5)	-3.1 (-9.5, 3.8)	-3.3 (-8.1, 1.7)	0.2 (-7.9, 9.0)
Adjusted, with country	-0.4 (-3.9, 3.2)	-0.5 (-6.5, 5.8)	0.1 (-4.5, 4.9)	2.7 (-6.0, 12.2)
Refried beans[g]				
Unadjusted	-9.8 (-15.0, -4.3)	-7.5 (-15.3, 1.0)	-9.7 (-16.9, -1.8)	-18.7 (-28.8, -7.1)
Adjusted, without country	-8.2 (-13.7, -2.4)	-7.2 (-14.5, 0.6)	-10.0 (-17.2, -2.2)	-21.6 (-31.6, -10.1)
Adjusted, with country	3.1 (-2.4, 8.9)	2.9 (-5.9, 12.4)	1.7 (-6.1, 10.1)	-16.5 (-29.4, -1.2)
Corn tortillas[h]				
Unadjusted	-6.1 (-13.2, 1.7)	2.1 (-9.0, 14.6)	-5.6 (-14.8, 4.6)	-9.1 (-24.1, 8.8)
Adjusted, without country	-6.0 (-13.9, 2.6)	3.1 (-9.0, 16.9)	-5.5 (-16.1, 6.5)	-14.6 (-29.8, 4.0)
Adjusted, with country	4.5 (-3.8, 13.6)	4.8 (-16.5, 31.4)	5.7 (-10.2, 24.3)	-17.5 (-41.1, 15.6)
Corn products[h,n]				
Unadjusted	-1.9 (-4.1, 0.4)	-0.5 (-7.5, 7.1)	-3.8 (-7.0, -0.4)	-2.3 (-7.2, 2.7)
Adjusted, without country	-1.7 (-4.0, 0.7)	-0.7 (-7.4, 6.6)	-3.5 (-6.9, 0.1)	-3.5 (-7.8, 1.0)
Adjusted, with country	0.2 (-1.9, 2.3)	-0.5 (-8.0, 7.6)	-0.2 (-3.5, 3.2)	-4.3 (-9.9, 1.6)
Corn tostadas/tacos[i]				
Unadjusted	1.0 (-2.2, 4.3)	0.2 (-5.0, 5.8)	2.4 (-2.4, 7.4)	7.1 (-2.2, 17.3)
Adjusted, without country	1.0 (-2.2, 4.4)	-0.2 (-5.7, 5.6)	2.5 (-2.3, 7.7)	5.6 (-3.9, 16.0)
Adjusted, with country	3.5 (-0.1, 7.2)	3.9 (-1.2, 9.4)	3.5 (-1.6, 8.8)	3.7 (-6.4, 14.8)

Supplemental Table 2.2. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among children from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
White bread[i]				
Unadjusted	-3.8 (-15.1, 8.9)	6.1 (-17.3, 36.1)	-23.6 (-39.3, -3.8)	4.5 (-23.6, 43.0)
Adjusted, without country	-4.8 (-16.0, 7.8)	4.0 (-19.2, 33.9)	-23.1 (-40.0, -1.5)	-2.0 (-28.1, 33.8)
Adjusted, with country	-6.7 (-17.3, 5.4)	-13.9 (-34.1, 12.4)	-13.4 (-34.7, 14.9)	-5.0 (-38.8, 47.5)
Sweet bread[i]				
Unadjusted	-11.1 (-22.2, 1.6)	6.1 (-11.6, 27.3)	-11.9 (-24.8, 3.2)	-16.6 (-35.9, 8.6)
Adjusted, without country	-12.1 (-23.5, 1.1)	8.0 (-10.6, 30.4)	-12.9 (-27.3, 4.2)	-26.1 (-43.8, -2.9)
Adjusted, with country	-2.0 (-10.0, 6.8)	18.2 (-2.3, 43.0)	1.1 (-15.2, 20.4)	-13.7 (-40.2, 24.6)
Cake[i]				
Unadjusted	9.9 (3.6, 16.7)	5.2 (-1.4, 12.3)	14.4 (7.3, 21.9)	11.5 (-6.8, 33.4)
Adjusted, without country	10.1 (3.9, 16.7)	5.9 (-1.0, 13.3)	13.2 (6.1, 20.8)	14.0 (-1.3, 31.6)
Adjusted, with country	0.7 (-4.0, 5.6)	3.0 (-3.4, 9.8)	1.3 (-5.3, 8.3)	7.7 (-5.7, 23.0)
Coated sandwich cookies[i]				
Unadjusted	0.1 (-4.6, 5.0)	1.1 (-7.6, 10.7)	-3.0 (-10.7, 5.3)	-2.9 (-17.5, 14.2)
Adjusted, without country	2.1 (-3.3, 7.8)	0.6 (-7.2, 9.1)	-3.1 (-11.4, 6.1)	-4.6 (-18.3, 11.3)
Adjusted, with country	2.5 (-2.6, 8.0)	-1.2 (-9.1, 7.4)	2.6 (-5.2, 11.0)	-4.9 (-18.2, 10.4)
Chocolate drink[j]				
Unadjusted	13.5 (1.1, 27.5)	1.5 (-13.1, 18.5)	10.0 (-9.6, 33.9)	1.6 (-24.8, 37.1)
Adjusted, without country	9.5 (-2.9, 23.6)	-3.8 (-17.7, 12.4)	8.4 (-10.2, 30.9)	-3.2 (-28.9, 31.8)
Adjusted, with country	1.5 (-7.3, 11.2)	-9.6 (-21.8, 4.5)	-7.7 (-23.5, 11.3)	-3.2 (-25.4, 25.6)
Milk[k]				
Unadjusted	9.1 (-1.8, 21.3)	-3.5 (-18.1, 13.7)	13.3 (-3.2, 32.7)	-27.5 (-45.8, -3.0)
Adjusted, without country	10.9 (-1.9, 25.4)	0.4 (-16.8, 21.1)	15.6 (-3.8, 38.9)	-16.1 (-36.4, 10.5)
Adjusted, with country	13.8 (2.8, 26.0)	-3.9 (-19.7, 15.1)	28.6 (8.2, 52.9)	-10.9 (-32.4, 17.4)
Fresh cheese[l]				
Unadjusted	-5.7 (-12.6, 1.8)	0.8 (-10.4, 13.3)	1.7 (-9.1, 13.7)	0.0 (-18.1, 22)
Adjusted, without country	-6.5 (-13.4, 1.0)	1.0 (-10.0, 13.3)	0.8 (-10.1, 13.1)	0.4 (-16.4, 20.5)
Adjusted, with country	3.1 (-3.3, 9.9)	2.1 (-8.7, 14.3)	13.1 (1.0, 26.5)	20.9 (1.3, 44.4)
Hard cheese[l]				
Unadjusted	-11.6 (-23.9, 2.8)	-3.4 (-19.4, 15.9)	-4.9 (-22.1, 16.1)	-16.3 (-29.9, 0.0)
Adjusted, without country	-11.4 (-23.1, 2.0)	-2.7 (-18.8, 16.5)	-6.0 (-22.4, 13.8)	-14.6 (-28.5, 1.9)
Adjusted, with country	-2.7 (-7.8, 2.6)	3.5 (-14.9, 25.9)	1.9 (-13.1, 19.4)	-3.8 (-29.9, 31.9)

Supplemental Table 2.2. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among children from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Cream cheese[d]				
Unadjusted	-0.3 (-3.3, 2.8)	-2.9 (-6.9, 1.3)	-1.3 (-7.3, 5.0)	-7.4 (-11.2, -3.4)
Adjusted, without country	-0.7 (-3.6, 2.3)	-2.6 (-6.4, 1.3)	-1.8 (-7.1, 3.9)	-6.8 (-10.7, -2.7)
Adjusted, with country	0.3 (-1.3, 1.9)	-4.0 (-8.0, 0.2)	0.0 (-2.7, 2.7)	-9.9 (-14.4, -5.2)
Cream[d]				
Unadjusted	-21.6 (-30.3, -11.9)	15.3 (-14.5, 55.6)	-3.1 (-22, 20.4)	-9.3 (-40.8, 39.0)
Adjusted, without country	-19.8 (-28.2, -10.4)	16.5 (-12.8, 55.6)	-3.9 (-22, 18.4)	-9.0 (-39.2, 36.3)
Adjusted, with country	3.1 (-7.3, 14.6)	28.4 (-6.7, 76.7)	37.2 (8.1, 74.1)	41.5 (1.1, 97.9)
Cooked eggs[h]				
Unadjusted	3.6 (-1.8, 9.4)	-1.9 (-8.0, 4.6)	6.8 (-0.6, 14.8)	12.3 (-2.8, 29.8)
Adjusted, without country	6.5 (-0.6, 14.3)	0.1 (-6.9, 7.6)	7.6 (-1.8, 17.9)	13.7 (-4.2, 34.9)
Adjusted, with country	-3.9 (-8.3, 0.8)	-1.5 (-7.5, 4.9)	-5.3 (-11.8, 1.7)	1.5 (-12.9, 18.3)
Scrambled/fried eggs[h]				
Unadjusted	1.4 (-7.4, 10.9)	3.5 (-7.7, 16.1)	4.7 (-8.4, 19.8)	-1.0 (-21.5, 24.9)
Adjusted, without country	-1.1 (-9.6, 8.2)	0.4 (-11.2, 13.4)	4.5 (-10.0, 21.4)	-8.2 (-27.6, 16.4)
Adjusted, with country	5.6 (-1.0, 12.6)	-2.1 (-13.0, 10.3)	13.3 (-0.3, 28.8)	1.3 (-20.4, 28.9)
Fish[i]				
Unadjusted	-4.2 (-8.1, -0.2)	-1.9 (-8.4, 5.1)	-7.7 (-14.0, -1.0)	0.8 (-8.9, 11.4)
Adjusted, without country	-5.1 (-8.8, -1.2)	-2.1 (-8.8, 5.0)	-9.0 (-15.4, -2.2)	3.9 (-5.4, 14.1)
Adjusted, with country	-2.2 (-5.4, 1.2)	-2.6 (-8.3, 3.5)	-5.0 (-11.1, 1.6)	9.8 (0.4, 20.2)
Canned tuna/sardines[i]				
Unadjusted	11.8 (-0.2, 25.2)	0.8 (-15.2, 19.8)	5.5 (-7.2, 20.0)	6.0 (-19.9, 40.1)
Adjusted, without country	9.4 (-1.0, 20.8)	-3.6 (-19.0, 14.7)	1.0 (-10.4, 13.7)	-0.5 (-24.9, 31.8)
Adjusted, with country	-0.6 (-8.0, 7.3)	-9.0 (-22.0, 6.2)	-6.5 (-17.3, 5.8)	-8.8 (-28.1, 15.8)
Fried chicken[i]				
Unadjusted	3.6 (-3.5, 11.2)	-9.4 (-18.7, 1.0)	3.6 (-6.2, 14.4)	-0.1 (-19.1, 23.2)
Adjusted, without country	5.2 (-1.4, 12.2)	-8.2 (-16.9, 1.4)	2.7 (-6.6, 12.9)	2.0 (-15.7, 23.3)
Adjusted, with country	-0.3 (-4.7, 4.3)	-5.2 (-15.5, 6.4)	-1.0 (-7.5, 6.0)	-5.5 (-20.6, 12.3)
Chicken (cooked/roasted)[i]				
Unadjusted	7.0 (0.2, 14.3)	0.3 (-7.9, 9.3)	6.8 (-2.5, 16.9)	12.1 (-4.0, 30.9)
Adjusted, without country	5.1 (-1.8, 12.5)	-0.4 (-9.1, 9.1)	5.6 (-4.0, 16.1)	11.4 (-3.7, 29.0)
Adjusted, with country	-1.1 (-6.2, 4.3)	3.6 (-4.8, 12.9)	-1.2 (-9.0, 7.3)	4.0 (-10.0, 20.2)
Beef/pork as main dish[i]				
Unadjusted	-5.0 (-13.3, 4.0)	-2.2 (-9.5, 5.7)	-10.7 (-22.7, 3.2)	2.6 (-12.8, 20.7)
Adjusted, without country	-6.5 (-14.9, 2.7)	-4.8 (-12.0, 3.0)	-12.1 (-24.1, 1.8)	-0.6 (-16.1, 17.8)
Adjusted, with country	-1.8 (-6.5, 3.1)	-8.9 (-15.9, -1.4)	-3.5 (-12.7, 6.6)	6.3 (-9.2, 24.5)

Supplemental Table 2.2. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among children from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Beef/pork in soups, stews, etc.[i]				
Unadjusted	7.6 (-3.1, 19.5)	2.4 (-8.1, 14.1)	4.8 (-8.6, 20.1)	20.4 (-2.0, 48.0)
Adjusted, without country	5.9 (-4.9, 18.0)	-0.5 (-10.8, 10.9)	4.4 (-8.6, 19.3)	18.2 (-3.7, 45.2)
Adjusted, with country	-1.5 (-8.2, 5.7)	-12.6 (-20.7, -3.7)	1.0 (-9.4, 12.6)	13.3 (-6.9, 37.9)
Beef tortitas[i]				
Unadjusted	-2.1 (-6.5, 2.4)	1.1 (-3.0, 5.4)	-1.5 (-5.7, 2.8)	1.5 (-8.0, 11.9)
Adjusted, without country	-1.9 (-6.4, 2.8)	0.5 (-3.8, 5.0)	-1.0 (-5.3, 3.5)	0.0 (-9.9, 11.0)
Adjusted, with country	0.8 (-1.8, 3.5)	2.2 (-2.6, 7.3)	2.8 (-1.4, 7.2)	9.3 (-1.5, 21.3)
Hamburger[i]				
Unadjusted	-5.6 (-10.2, -0.7)	-8.4 (-14.6, -1.7)	-9.6 (-15.6, -3.1)	-8.4 (-20.8, 6.0)
Adjusted, without country	-6.4 (-11.0, -1.5)	-8.6 (-14.9, -1.8)	-10.8 (-16.9, -4.1)	-7.4 (-21.2, 8.8)
Adjusted, with country	-4.4 (-8.4, -0.2)	-6.5 (-12.7, 0.1)	-7.6 (-13.3, -1.5)	-7.3 (-19.9, 7.3)
Hot dog[i]				
Unadjusted	3.7 (-1.0, 8.7)	1.3 (-3.0, 5.8)	3.2 (-2.2, 8.9)	12.0 (3.6, 21.1)
Adjusted, without country	3.5 (-0.6, 7.8)	0.5 (-3.7, 4.8)	2.6 (-2.8, 8.3)	12.0 (3.7, 21.0)
Adjusted, with country	0.4 (-2.6, 3.6)	3.7 (-0.2, 7.8)	-0.5 (-5.1, 4.2)	8.5 (1.4, 16.1)
Salami[l]				
Unadjusted	5.3 (1.8, 8.9)	3.3 (0.6, 6.2)	5.9 (0.3, 11.8)	8.8 (2.2, 15.8)
Adjusted, without country	4.6 (0.9, 8.5)	2.7 (-0.5, 6.0)	5.3 (-0.4, 11.2)	9.0 (1.1, 17.6)
Adjusted, with country	-4.4 (-6.7, -2.1)	0.4 (-2.5, 3.4)	-5.5 (-9.6, -1.2)	2.5 (-5.6, 11.4)
Chorizo[h]				
Unadjusted	5.9 (-7.8, 21.7)	-11.2 (-22.9, 2.2)	11.3 (-7.4, 33.7)	8.3 (-16.9, 41.1)
Adjusted, without country	6.8 (-6.0, 21.4)	-13.8 (-25.4, -0.5)	11.3 (-6.9, 33.1)	5.9 (-18.7, 37.8)
Adjusted, with country	-8.2 (-15.2, -0.7)	-14.7 (-25.8, -2.0)	-6.2 (-17.4, 6.4)	-4.5 (-23.0, 18.4)
Sausage[h]				
Unadjusted	0.8 (-8.3, 10.8)	4.3 (-8.1, 18.5)	-2.3 (-14.0, 10.9)	16.5 (-6.9, 45.9)
Adjusted, without country	-0.9 (-10.2, 9.3)	5.9 (-7.3, 20.9)	-3.8 (-16.4, 10.6)	23.7 (-0.5, 53.8)
Adjusted, with country	-1.8 (-10.0, 7.3)	12.0 (-1.6, 27.5)	2.7 (-9.8, 16.9)	22.3 (-1.9, 52.5)
Viscera[m]				
Unadjusted	6.4 (0.2, 13.0)	-2.9 (-8.6, 3.1)	7.2 (0.0, 14.8)	8.7 (-2.9, 21.8)
Adjusted, without country	5.4 (-1.3, 12.5)	-3.6 (-9.2, 2.3)	6.6 (-1.2, 14.9)	9.7 (-2.1, 22.9)
Adjusted, with country	0.6 (-3.6, 5.1)	-4.7 (-9.4, 0.1)	1.5 (-2.6, 5.8)	3.1 (-6.9, 14.1)

Footnotes to Supplemental Table 2.2

[a]From linear regression models with log-transformed fatty acid variables as outcomes and a continuous predictor representing intake frequency of a given food in servings per day.

[b]All models adjusted for age (continuous), sex, maternal education (continuous), and the following: long-chain n-6, height-for-age Z score (continuous); ALA, maternal age at child's birth (continuous); long-chain n-3, height-for-age Z score (continuous).

[c]Adjusted for all of the above and country of origin.

[d]1 tbs

[e]1/4 unit

[f]30g or 2 tbs

[g]1/3 cup

[h]1 unit

[i]1 portion

[j]1 cup

[k]1 glass

[l]1 piece

[m]1/2 cup

[n]Empanadas, arepas, pupusas, etc.

Supplemental Table 2.3. Means (±SD) of adipose tissue n-6 and n-3 polyunsaturated fatty acid weight percent concentrations by primary type of cooking oil among adults from Mesoamerica

Oil type	N	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Soyabean	140	18.03 ± 5.62	1.26 ± 0.55	0.95 ± 0.36	0.26 ± 0.28
Sunflower	33	14.39 ± 4.57	1.06 ± 0.32	0.70 ± 0.30	0.19 ± 0.10
Corn	43	13.96 ± 4.21	1.11 ± 0.48	0.65 ± 0.26	0.19 ± 0.14
Palm	50	10.50 ± 1.93	0.86 ± 0.26	0.51 ± 0.19	0.16 ± 0.13
Canola	34	15.07 ± 3.80	1.32 ± 0.72	0.79 ± 0.27	0.29 ± 0.18
Mixed oils	148	14.63 ± 3.68	1.18 ± 0.45	0.70 ± 0.31	0.25 ± 0.20
Other	10	13.56 ± 3.05	1.22 ± 0.42	0.69 ± 0.33	0.18 ± 0.14
P[a]		<0.0001	0.0004	<0.0001	0.002

[a] χ^2 score statistic from linear regression models with each fatty acid as the outcome and indicator variables for each oil type as predictors.

Supplemental Table 2.4. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among adults from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Butter/margarine added to food[d]				
Unadjusted	18.8 (8.8, 29.7)	10.3 (2.6, 18.6)	12.2 (-1.4, 27.8)	0.1 (-12.8, 14.9)
Adjusted, without country[b]	17.7 (8.3, 28.0)	8.8 (1.2, 17.0)	14.1 (0.4, 29.7)	-1.3 (-14.1, 13.4)
Adjusted, with country[c]	2.2 (-2.0, 6.5)	-0.1 (-6.6, 6.8)	-3.0 (-9.8, 4.3)	-13.7 (-25.6, 0.1)
Dressing/mayonnaise[d]				
Unadjusted	5.3 (0.4, 10.5)	1.9 (-1.6, 5.6)	4.1 (-2.4, 11.0)	3.9 (-2.9, 11.2)
Adjusted, without country	4.9 (0.1, 9.8)	1.1 (-2.5, 4.8)	4.3 (-2.5, 11.5)	3.3 (-3.8, 10.9)
Adjusted, with country	0.2 (-2.3, 2.9)	-1.6 (-5.6, 2.6)	-4.3 (-9.1, 0.7)	-0.2 (-6.7, 6.7)
Avocado[e]				
Unadjusted	0.7 (-3.7, 5.3)	-1.9 (-8.2, 4.9)	0.7 (-6.1, 8.0)	1.8 (-10.5, 15.8)
Adjusted, without country	-0.1 (-4.8, 4.9)	-0.7 (-8.8, 8.3)	-0.1 (-7.2, 7.6)	2.5 (-11.8, 19.1)
Adjusted, with country	-0.1 (-3.8, 3.7)	-1.2 (-8.8, 6.9)	-1.1 (-5.3, 3.3)	5.2 (-7.1, 19.0)
Peanuts[f]				
Unadjusted	0.5 (-6.4, 8.0)	-1.4 (-5.9, 3.4)	0.5 (-9.6, 11.7)	-3.6 (-10.4, 3.6)
Adjusted, without country	1.6 (-4.6, 8.2)	-0.7 (-5.2, 4.0)	1.7 (-7.6, 12.0)	-1.7 (-8.5, 5.5)
Adjusted, with country	2.8 (-1.1, 6.8)	0.0 (-5.5, 5.8)	1.8 (-2.6, 6.5)	-1.6 (-8.1, 5.4)
Cashews[f]				
Unadjusted	0.1 (-1.6, 1.9)	0.7 (-1.6, 3.0)	-2.5 (-5.8, 1.0)	0.0 (-3.7, 3.8)
Adjusted, without country	0.1 (-1.6, 1.8)	0.4 (-1.8, 2.5)	-2.3 (-5.8, 1.3)	-0.3 (-3.8, 3.4)
Adjusted, with country	0.4 (-1.3, 2.1)	0.3 (-1.7, 2.3)	-2.1 (-4.9, 0.8)	-0.5 (-4.3, 3.5)
Refried beans[g]				
Unadjusted	-5.7 (-10.0, -1.1)	-5.3 (-9.7, -0.6)	-5.4 (-11.0, 0.5)	-11.0 (-18.5, -2.9)
Adjusted, without country	-5.4 (-9.8, -0.7)	-4.8 (-9.4, 0.0)	-6.2 (-11.6, -0.5)	-11.6 (-19.2, -3.4)
Adjusted, with country	-0.9 (-3.8, 2.0)	-2.2 (-7.6, 3.6)	-2.1 (-6.0, 2.0)	-5.3 (-13.2, 3.3)
Corn tortillas[h]				
Unadjusted	-13.0 (-20.4, -4.9)	-13.4 (-22.0, -3.8)	-34.0 (-41.1, -26.1)	-0.6 (-16.2, 18.0)
Adjusted, without country	-12.3 (-19.8, -4.0)	-12.0 (-21.5, -1.3)	-36.2 (-43.4, -28.2)	4.7 (-12.6, 25.3)
Adjusted, with country	-4.1 (-15.2, 8.4)	-2.7 (-17.2, 14.4)	-8.2 (-23.2, 9.7)	12.3 (-17.5, 52.8)
Corn products[h,n]				
Unadjusted	-4.7 (-8.2, -1.1)	-4.8 (-9.0, -0.4)	-12.0 (-16.1, -7.7)	-8.9 (-14.2, -3.4)
Adjusted, without country	-5.2 (-8.7, -1.6)	-4.6 (-8.8, -0.2)	-11.8 (-16.1, -7.3)	-8.3 (-13.5, -2.7)
Adjusted, with country	-1.9 (-4.7, 0.9)	0.6 (-4.3, 5.7)	-3.5 (-7.0, 0.1)	-6.1 (-11.2, -0.6)
Corn tostadas/tacos[i]				
Unadjusted	0.3 (-3.3, 4.0)	2.1 (-2.1, 6.4)	-2.4 (-7.5, 2.9)	2.8 (-3.5, 9.6)
Adjusted, without country	0.0 (-3.6, 3.8)	2.9 (-1.7, 7.7)	-0.2 (-5.5, 5.4)	3.6 (-3.4, 11.2)
Adjusted, with country	2.0 (-1.0, 5.0)	2.2 (-2.2, 6.8)	0.5 (-4.9, 6.3)	-1.5 (-8.3, 5.9)

Supplemental Table 2.4. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among adults from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
White bread[i]				
Unadjusted	0.1 (-4.4, 4.8)	-5.1 (-10.8, 0.9)	-8.1 (-12.9, -3.0)	-1.2 (-10.9, 9.6)
Adjusted, without country	1.7 (-3.1, 6.7)	-4.2 (-10.3, 2.4)	-7.5 (-12.8, -1.9)	0.9 (-9.3, 12.1)
Adjusted, with country	-0.9 (-4.4, 2.9)	-2.0 (-8.0, 4.5)	-0.3 (-5.0, 4.6)	0.6 (-10.2, 12.7)
Sweet bread[i]				
Unadjusted	-2.8 (-5.9, 0.4)	-0.9 (-4.1, 2.5)	-7.5 (-11.2, -3.7)	0.9 (-3.6, 5.6)
Adjusted, without country	-2.1 (-5.2, 1.1)	-0.5 (-3.8, 3.0)	-7.9 (-11.8, -3.8)	0.9 (-4.1, 6.1)
Adjusted, with country	-0.4 (-2.4, 1.6)	1.0 (-1.9, 3.9)	-1.5 (-4.3, 1.3)	-0.8 (-5.3, 4.0)
Cake[i]				
Unadjusted	0.2 (-1.9, 2.4)	-1.4 (-3.7, 1.0)	0.3 (-3.4, 4.1)	-3.2 (-7.4, 1.3)
Adjusted, without country	0.3 (-1.9, 2.5)	-1.6 (-3.9, 0.6)	0.2 (-3.5, 4.0)	-3.3 (-7.5, 1.1)
Adjusted, with country	0.6 (-0.8, 2.0)	-0.8 (-3.2, 1.7)	0.1 (-2.4, 2.6)	-1.3 (-5.2, 2.9)
Coated sandwich cookies[i]				
Unadjusted	0.2 (-3.1, 3.5)	-0.6 (-5.3, 4.3)	0.6 (-4.1, 5.6)	-3.8 (-9.8, 2.7)
Adjusted, without country	0.8 (-2.1, 3.7)	-0.1 (-4.6, 4.6)	0.0 (-4.9, 5.2)	-3.3 (-8.8, 2.7)
Adjusted, with country	0.3 (-2.0, 2.7)	-0.4 (-5.2, 4.7)	0.7 (-3.1, 4.7)	-3.0 (-9.9, 4.6)
Chocolate drink[j]				
Unadjusted	5.3 (-3.5, 14.9)	-1.3 (-9.4, 7.5)	7.2 (-4.2, 19.9)	13.0 (2.4, 24.7)
Adjusted, without country	4.2 (-4.1, 13.2)	-2.6 (-10.7, 6.2)	7.9 (-3.6, 20.9)	13.2 (1.8, 25.8)
Adjusted, with country	-6.0 (-10.3, -1.3)	-5.8 (-14.2, 3.5)	-8.2 (-14.9, -1.0)	8.7 (-4.2, 23.5)
Milk[k]				
Unadjusted	4.4 (-0.9, 10.0)	7.0 (1.4, 12.8)	4.1 (-3.2, 11.9)	6.5 (-2.1, 15.9)
Adjusted, without country	3.2 (-1.8, 8.4)	6.4 (0.6, 12.6)	4.4 (-3.0, 12.3)	5.0 (-3.9, 14.9)
Adjusted, with country	0.0 (-4.1, 4.3)	2.2 (-3.1, 7.8)	1.1 (-5.3, 7.8)	-1.3 (-9.4, 7.5)
Fresh cheese[l]				
Unadjusted	-5.6 (-8.9, -2.2)	1.3 (-7.0, 10.4)	-3.5 (-9.0, 2.4)	-0.2 (-7.6, 7.9)
Adjusted, without country	-5.6 (-8.8, -2.2)	1.4 (-7.3, 10.9)	-3.1 (-8.6, 2.7)	0.3 (-7.4, 8.7)
Adjusted, with country	-0.7 (-4.1, 2.8)	3.7 (-4.6, 12.6)	3.9 (-1.0, 9.0)	5.0 (-2.6, 13.2)
Hard cheese[l]				
Unadjusted	-15.4 (-23.3, -6.7)	-11.6 (-19.0, -3.6)	-17.7 (-27.3, -6.7)	-27.3 (-35.0, -18.7)
Adjusted, without country	-15.3 (-23.1, -6.7)	-11.6 (-18.8, -3.7)	-18.1 (-27.4, -7.6)	-27.5 (-34.7, -19.4)
Adjusted, with country	-1.9 (-8.3, 4.9)	1.6 (-5.5, 9.2)	-3.2 (-12.4, 7.0)	-10.9 (-20.5, -0.2)
Cream cheese[d]				
Unadjusted	0.0 (-4.7, 5.0)	4.6 (-3.4, 13.3)	-3.8 (-11.1, 4.0)	5.6 (-3.2, 15.2)
Adjusted, without country	-0.5 (-5.4, 4.6)	3.3 (-5.2, 12.6)	-5.2 (-11.9, 2.1)	2.9 (-6.3, 13.0)
Adjusted, with country	0.7 (-2.8, 4.5)	2.0 (-4.4, 8.8)	-2.3 (-8.0, 3.8)	1.0 (-7.1, 9.8)

Supplemental Table 2.4. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among adults from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Cream[d]				
Unadjusted	-23.2 (-30.8, -14.7)	-20.1 (-27.4, -12.1)	-22.7 (-30.9, -13.5)	-17.7 (-27.0, -7.3)
Adjusted, without country	-22.3 (-29.8, -14.0)	-20.3 (-27.7, -12.1)	-24.1 (-32.1, -15.1)	-18.8 (-28.2, -8.3)
Adjusted, with country	-7.5 (-15.9, 1.8)	-12.6 (-21.7, -2.4)	-4.8 (-12.4, 3.4)	-2.5 (-13.0, 9.2)
Cooked eggs[h]				
Unadjusted	0.0 (-2.6, 2.8)	-0.2 (-2.5, 2.2)	-0.9 (-3.9, 2.2)	-6.4 (-10.3, -2.3)
Adjusted, without country	0.4 (-2.5, 3.4)	0.1 (-2.2, 2.5)	-0.4 (-4.0, 3.2)	-6.1 (-10.3, -1.7)
Adjusted, with country	-0.4 (-2.4, 1.6)	0.9 (-1.1, 2.8)	-0.3 (-3.0, 2.6)	-5.6 (-8.9, -2.3)
Scrambled/fried eggs[h]				
Unadjusted	-2.6 (-6.7, 1.7)	-2.8 (-9.4, 4.3)	-4.2 (-11.0, 3.0)	-2.0 (-10.4, 7.3)
Adjusted, without country	-0.1 (-4.0, 3.9)	-1.0 (-8.0, 6.6)	-2.9 (-9.5, 4.1)	1.8 (-7.4, 12.0)
Adjusted, with country	-0.6 (-3.7, 2.7)	-0.8 (-5.9, 4.5)	-1.4 (-5.8, 3.3)	3.1 (-5.1, 11.9)
Fish[i]				
Unadjusted	0.8 (-5.5, 7.6)	1.2 (-9.2, 12.8)	1.1 (-10.6, 14.4)	7.4 (-9.2, 27.1)
Adjusted, without country	0.3 (-6.2, 7.2)	0.5 (-10.0, 12.1)	-0.7 (-11.6, 11.6)	7.0 (-10.0, 27.3)
Adjusted, with country	-1.4 (-6.8, 4.3)	1.4 (-7.0, 10.6)	-4.5 (-12.5, 4.2)	13.9 (-2.4, 32.9)
Canned tuna/sardines[i]				
Unadjusted	20.1 (9.8, 31.4)	15.0 (2.7, 28.8)	19.9 (4.9, 37.0)	25.3 (7.4, 46.3)
Adjusted, without country	20.4 (10.1, 31.8)	14.2 (2.0, 27.9)	17.8 (3.0, 34.8)	23.8 (6.1, 44.5)
Adjusted, with country	6.5 (0.5, 12.9)	4.5 (-5.5, 15.5)	-2.8 (-12.4, 7.7)	13.0 (-1.1, 29.0)
Fried chicken[i]				
Unadjusted	-0.6 (-5.7, 4.8)	-5.5 (-10.1, -0.7)	6.0 (-2.3, 15.0)	-9.7 (-17.7, -0.9)
Adjusted, without country	-0.3 (-5.0, 4.6)	-4.1 (-8.8, 0.9)	4.3 (-3.8, 13.1)	-9.2 (-17.0, -0.5)
Adjusted, with country	-2.3 (-5.7, 1.1)	-2.4 (-6.8, 2.3)	0.7 (-4.6, 6.3)	-6.8 (-13.7, 0.7)
Chicken (cooked/roasted)[i]				
Unadjusted	2.6 (-0.5, 5.8)	3.3 (0.6, 6.0)	0.0 (-4.3, 4.4)	3.1 (-2.2, 8.6)
Adjusted, without country	3.2 (0.4, 6.1)	3.1 (0.4, 6.0)	0.3 (-4.2, 4.9)	3.3 (-2.2, 9.1)
Adjusted, with country	3.1 (0.8, 5.5)	4.2 (1.2, 7.2)	1.6 (-2.3, 5.6)	4.6 (-0.8, 10.2)
Beef/pork as main dish[i]				
Unadjusted	0.0 (-4.1, 4.4)	2.9 (-1.6, 7.6)	-6.9 (-12.6, -0.8)	-3.0 (-14.9, 10.5)
Adjusted, without country	-0.1 (-4.1, 4.0)	2.8 (-1.5, 7.4)	-7.4 (-13.1, -1.4)	-3.5 (-15.3, 9.9)
Adjusted, with country	0.6 (-2.7, 4.1)	2.9 (-2.1, 8.3)	-1.9 (-6.2, 2.7)	-2.4 (-13.5, 10.1)
Beef/pork in soups, stews, etc.[i]				
Unadjusted	0.8 (-4.9, 6.8)	2.7 (-1.9, 7.6)	1.6 (-6.2, 10.0)	-6.6 (-14.6, 2.2)
Adjusted, without country	0.3 (-5.4, 6.3)	3.4 (-1.1, 8.1)	1.0 (-6.6, 9.1)	-5.4 (-13.7, 3.7)
Adjusted, with country	-3.3 (-7.3, 0.8)	0.9 (-3.5, 5.5)	-4.4 (-9.6, 1.1)	-10.2 (-18.7, -0.9)

Supplemental Table 2.4. Percent differences[a] (95% CI) in means of adipose tissue n-6 and n-3 polyunsaturated fatty acids by food intake frequency (90th vs. 10th percentiles of servings/d) among adults from Mesoamerica

Foods	LA 18:2(n-6)	Total long-chain n-6	ALA 18:3(n-3)	Total long-chain n-3
Beef tortitas[i]				
Unadjusted	-4.3 (-10.2, 2.0)	-0.6 (-4.5, 3.4)	-5.4 (-11.4, 0.9)	-7.1 (-14.5, 0.9)
Adjusted, without country	-3.6 (-8.9, 2.1)	-0.7 (-4.7, 3.4)	-5.6 (-11.4, 0.6)	-7.2 (-14.7, 0.8)
Adjusted, with country	-0.5 (-4.3, 3.5)	1.5 (-1.9, 4.9)	-1.4 (-6.1, 3.6)	-3.7 (-9.3, 2.1)
Hamburger[i]				
Unadjusted	1.5 (-2.2, 5.3)	4.4 (-1.2, 10.2)	4.7 (-2.4, 12.3)	-1.4 (-11.4, 9.8)
Adjusted, without country	2.4 (-1.4, 6.4)	5.5 (0.0, 11.2)	3.7 (-3.6, 11.6)	-0.2 (-10.2, 11.0)
Adjusted, with country	3.2 (-0.2, 6.6)	3.8 (-0.9, 8.8)	2.9 (-3.6, 9.7)	-1.0 (-10.2, 9.1)
Hot dog[i]				
Unadjusted	-0.4 (-3.5, 2.8)	-1.1 (-5.6, 3.5)	1.1 (-5.0, 7.7)	0.3 (-6.8, 7.8)
Adjusted, without country	-0.2 (-3.5, 3.2)	-0.3 (-4.6, 4.3)	0.2 (-6.0, 6.7)	1.5 (-5.8, 9.4)
Adjusted, with country	-0.2 (-3.7, 3.5)	0.4 (-4.2, 5.2)	-1.5 (-7.7, 5.1)	1.7 (-4.8, 8.7)
Salami[l]				
Unadjusted	6.0 (2.6, 9.5)	0.4 (-2.4, 3.2)	11.6 (6.8, 16.5)	-2.3 (-8.3, 4.1)
Adjusted, without country	5.3 (2.2, 8.6)	0.5 (-2.2, 3.4)	11.0 (6.6, 15.7)	-2.0 (-8.2, 4.7)
Adjusted, with country	0.8 (-1.9, 3.6)	-0.9 (-4.4, 2.8)	1.6 (-2.3, 5.5)	-3.6 (-11.2, 4.8)
Chorizo[h]				
Unadjusted	-0.5 (-8.3, 8.0)	-5.1 (-10.7, 0.9)	-6.5 (-15.6, 3.6)	-6.1 (-14.9, 3.6)
Adjusted, without country	-0.4 (-8.4, 8.3)	-5.0 (-10.8, 1.3)	-7.8 (-16.1, 1.4)	-6.2 (-14.9, 3.5)
Adjusted, with country	-3.5 (-9.2, 2.4)	-3.6 (-10.0, 3.4)	-9.8 (-15.9, -3.3)	-3.9 (-12.3, 5.3)
Sausage[h]				
Unadjusted	0.1 (-3.3, 3.7)	1.3 (-2.0, 4.8)	-6.0 (-10.5, -1.3)	0.2 (-6.6, 7.5)
Adjusted, without country	0.2 (-3.4, 3.8)	1.3 (-2.1, 4.8)	-6.3 (-10.6, -1.7)	-0.1 (-6.9, 7.2)
Adjusted, with country	0.1 (-2.7, 3.1)	1.9 (-1.8, 5.8)	-3.0 (-7.1, 1.4)	-0.8 (-6.3, 5.0)
Viscera[m]				
Unadjusted	5.0 (1.2, 8.9)	1.1 (-3.0, 5.4)	5.4 (-0.1, 11.2)	-1.0 (-6.1, 4.5)
Adjusted, without country	5.5 (1.8, 9.4)	1.5 (-2.5, 5.8)	5.6 (0.0, 11.5)	-0.4 (-5.8, 5.4)
Adjusted, with country	1.4 (-1.1, 4.1)	1.5 (-2.1, 5.3)	0.3 (-3.2, 4.0)	-0.5 (-5.7, 4.9)

Footnotes to Supplemental Table 2.4.

[a]From linear regression models with log-transformed fatty acid variables as outcomes and a continuous predictor representing intake frequency of a given food in servings per day.

[b]All models adjusted for age (continuous), sex, education (continuous), and the following: LA, smoking status (never, former, or current).

[c]Adjusted for all of the above and country of origin.

[d]1 tbs

[e]1/4 unit

[f]30g or 2 tbs

[g]1/3 cup

[h]1 unit

[i]1 portion

[j]1 cup

[k]1 glass

[l]1 piece

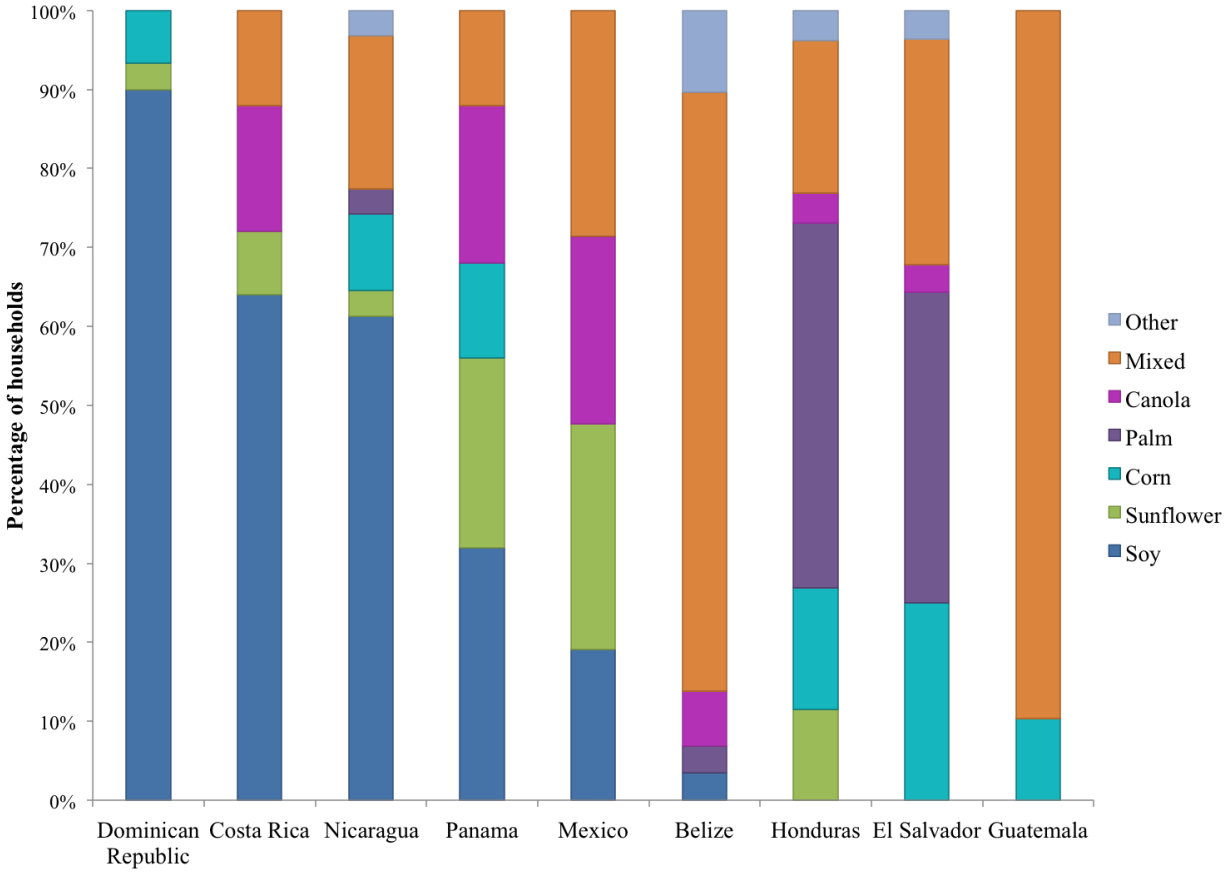
[m]1/2 cup

[n]Empanadas, arepas, pupusas, etc.

Supplemental Table 2.5. Median (10th, 90th percentile) intake frequency of specific foods among adults and children from Mesoamerica

Foods	Adults median (10th, 90th percentile) intake frequency, servings/d		Children median (10th, 90th percentile) intake frequency, servings/d		P[a]
Butter/margarine added to food[d]	0.07	(0.00, 1.00)	0.14	(0.00, 1.00)	0.46
Dressing/mayonnaise[d]	0.03	(0.00, 0.43)	0.07	(0.00, 0.43)	0.29
Avocado[e]	0.14	(0.00, 1.00)	0.14	(0.00, 0.79)	<0.0001
Peanuts[f]	0.00	(0.00, 0.43)	0.00	(0.00, 0.43)	0.41
Cashews[f]	0.00	(0.00, 0.07)	0.00	(0.00, 0.07)	0.18
Refried beans[g]	0.14	(0.00, 1.00)	0.14	(0.00, 1.00)	0.34
Corn tortillas[h]	1.00	(0.00, 6.00)	1.00	(0.00, 2.50)	<0.0001
Corn products[h,n]	0.07	(0.00, 0.43)	0.07	(0.00, 0.43)	0.08
Corn tacos/tostadas[i]	0.00	(0.00, 0.14)	0.00	(0.00, 0.14)	0.75
White bread[i]	0.79	(0.07, 2.50)	1.00	(0.07, 4.50)	0.0004
Sweet bread[i]	0.14	(0.00, 1.00)	0.43	(0.00, 2.50)	0.02
Cake[i]	0.00	(0.00, 0.14)	0.07	(0.00, 0.43)	0.002
Coated sandwich cookies[i]	0.00	(0.00, 0.43)	0.14	(0.00, 1.00)	<0.0001
Chocolate drink[j]	0.00	(0.00, 0.43)	0.07	(0.00, 0.79)	<0.0001
Milk[k]	0.14	(0.00, 1.00)	0.43	(0.07, 2.50)	<0.0001
Fresh cheese[l]	0.14	(0.00, 0.43)	0.07	(0.00, 0.43)	0.006
Hard cheese[l]	0.10	(0.00, 1.00)	0.07	(0.00, 1.00)	0.14
Cream cheese[d]	0.00	(0.00, 0.43)	0.00	(0.00, 0.43)	0.69
Cream[d]	0.07	(0.00, 1.00)	0.10	(0.00, 1.00)	0.21
Cooked eggs[h]	0.14	(0.00, 0.43)	0.14	(0.00, 0.43)	0.58
Scrambled/fried eggs[h]	0.43	(0.07, 1.00)	0.43	(0.07, 1.00)	0.62
Fish[i]	0.07	(0.00, 0.43)	0.07	(0.00, 0.14)	0.24
Canned tuna/sardines[i]	0.07	(0.00, 0.43)	0.07	(0.00, 0.43)	0.40
Fried chicken[i]	0.14	(0.00, 0.43)	0.14	(0.00, 0.43)	0.58
Chicken (cooked/roasted)[i]	0.43	(0.07, 0.43)	0.43	(0.07, 0.43)	0.28
Beef/pork as main dish[i]	0.14	(0.00, 0.43)	0.14	(0.00, 0.43)	0.87
Beef/pork in soups, stews, etc.[i]	0.14	(0.00, 0.43)	0.14	(0.00, 0.43)	0.58
Beef tortitas[i]	0.07	(0.00, 0.14)	0.07	(0.00, 0.14)	0.08
Hamburger[i]	0.00	(0.00, 0.14)	0.07	(0.00, 0.14)	0.02
Hot dog[i]	0.00	(0.00, 0.14)	0.00	(0.00, 0.14)	0.01
Salami[l]	0.00	(0.00, 0.43)	0.07	(0.00, 0.43)	0.03
Chorizo[h]	0.07	(0.00, 0.43)	0.07	(0.00, 0.43)	0.10
Sausage[h]	0.14	(0.00, 0.43)	0.14	(0.00, 0.43)	0.17
Viscera[m]	0.00	(0.00, 0.14)	0.00	(0.00, 0.14)	0.006

[a]From a Wilcoxon signed rank test.
[d]1 tbs
[e]1/4 unit
[f]30g or 2 tbs
[g]1/3 cup
[h]1 unit
[i]1 portion
[j]1 cup
[k]1 glass
[l]1 piece
[m]1/2 cup
[n]Empanadas, arepas, pupusas, etc.



Supplemental Figure 2.1. Country-specific proportions of primary cooking oil usage among Mesoamerican households. The figure includes all households for which information was available on primary cooking oil type.

N for each country: Dominican Republic, 30; Costa Rica, 25; Nicaragua, 31; Panama, 25; Mexico, 21; Belize, 29; Honduras, 26; El Salvador, 28; Guatemala, 29.

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Chapter 3. Adipose tissue polyunsaturated fatty acids and metabolic syndrome among adult parents and their children in Mesoamerica

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Abstract

Objective: The aim of the study was to examine the associations of adipose tissue n-3 and n-6 polyunsaturated fatty acid (PUFA) biomarkers with metabolic syndrome (MetS) among adult parents and their school-age children in 9 Mesoamerican countries.

Design: In this cross-sectional study, we measured concentrations of n-3 and n-6 PUFA in gluteal adipose tissue by gas chromatography. In adults, MetS was defined according to the National Cholesterol Education Program's Adult Treatment Panel III definition (three or more of the following: abdominal obesity, high fasting glucose, hypertension, low serum high-density lipoprotein (HDL) cholesterol, and high triglycerides). In children, we created an age- and sex-standardized metabolic risk score using abdominal circumference, the homeostasis model of insulin resistance, blood pressure, serum HDL cholesterol, and triglycerides. We estimated prevalence ratios of MetS in adults and mean differences in metabolic score in children across quartiles of PUFA using multivariable-adjusted Poisson and linear regression models, respectively.

Setting: Capitals of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Belize, and Tuxtla Gutiérrez in Mexico.

Subjects: 468 parents and 201 children aged 7-12 y.

Results: Among adults, MetS was associated with low alpha-linolenic acid (ALA; *P*=0.004), high eicosapentaenoic acid (EPA; *P*=0.04), and low gamma-linolenic acid (GLA; *P*=0.01) in adipose tissue. It was linearly, positively associated with dihomogamma-linolenic acid (DGLA; *P*=0.0004) concentrations and with a Δ6-desaturase activity index (*P*=0.04). There was a significant interaction between ALA and linoleic acid (LA; *P*=0.03); the lowest MetS prevalence was among adults with high ALA and low LA. Among children, the metabolic score was positively associated with docosapentaenoic acid (DPA; *P*, trend=0.01); no n-6 PUFA were significantly associated with the metabolic score after adjustment.

Conclusions: Among Mesoamerican adults, MetS prevalence is inversely associated with adipose tissue ALA and GLA, and positively associated with EPA, DGLA, and the Δ6-desaturase index. Among children, metabolic risk score is positively associated with DPA.

Keywords: polyunsaturated fatty acids; metabolic syndrome; children; Mesoamerica

Introduction

The metabolic syndrome (MetS) is a clustering of risk factors for chronic disease including abdominal obesity, high fasting glucose, hypertension, low HDL cholesterol, and high serum triglycerides (1). It involves the development of insulin resistance following abdominal obesity (2) and is associated with increased risk of cardiovascular disease, diabetes, and mortality (3). MetS component risk factors can track from childhood into adulthood (4,5), and adverse metabolic profiles in childhood predict the development of cardiometabolic outcomes in adulthood, including type II diabetes and atherosclerosis (4,6). Therefore, the identification of modifiable exposures leading to metabolic disregulation in early life is crucial in order to prevent the development of chronic disease.

Polyunsaturated fatty acids (PUFA) are nutritional factors that could play a role in the development of MetS. The main classes of PUFA are the n-3 and n-6 families. Each consists of one essential fatty acid (alpha-linolenic acid [18:3 n-3; ALA] and linoleic acid [18:2 n-6; LA], respectively) that can only be obtained from diet, and several long-chain PUFA that can be produced through endogenous elongation and desaturation of the essential fatty acids. Intake of long-chain n-3 PUFA including eicosapentaenoic acid (20:5 n-3; EPA) and docosahexaenoic acid (22:6 n-3; DHA) has been inversely related to metabolic characteristics including visceral adiposity and serum triglycerides, and positively associated with high-density lipoprotein (HDL) cholesterol in adults (7). A recent meta-analysis of cross-sectional and case-control studies of adults and children found that circulating biomarkers of EPA, DHA, and docosapentaenoic acid (22:5 n-3; DPA), another n-3 PUFA, were all inversely associated with MetS (8). However, studies using adipose tissue concentrations, which best reflect long-term intake (9), have found positive associations between these PUFA and MetS or its insulin resistance component in adults

(10,11). In addition, other meta-analyses have not found protective associations between long-chain n-3 PUFA and type 2 diabetes (12) or cardiovascular disease (13). Evidence concerning other PUFA is more limited. Some (10,14–17) but not all (8,18) studies have found that ALA and LA biomarkers and intake are inversely associated with MetS or its components, whereas each has been inversely associated with risk of type 2 diabetes (12,19).

The relation between PUFA status and cardiometabolic health in childhood is uncertain. Supplementation trials and observational studies with circulating biomarkers in pregnancy, infancy, or early childhood have found inconsistent associations of PUFA with measures of blood pressure, insulin resistance, and blood lipids throughout childhood and adolescence (20). Long-chain n-3 PUFA supplementation during middle childhood and adolescence has resulted in decreases in blood pressure, insulin resistance, and serum triglycerides in some (21–24) but not all (25) trials. Associations of blood biomarkers of ALA, LA, or long-chain n-6 PUFA with MetS components in middle childhood and adolescence are inconsistent (26–29).

The majority of observational studies of the relation between PUFA and MetS have relied on dietary assessment or blood biomarkers for ascertainment of exposure status. Dietary assessment is subject to measurement error and blood concentrations of PUFA may not reflect long-term intake. Furthermore, evidence in children is scant, especially in low- and middle-income countries. We conducted a cross-sectional study among families from nine countries in Mesoamerica, a region experiencing a large and growing burden of MetS and cardiometabolic disease (30,31). We ascertained PUFA status using adipose tissue concentrations, which are the gold standard biomarkers of long-term fatty acid intake (9). The aim of the study was to examine associations of these biomarkers with MetS in adult parents and their school-aged children.

Methods

Study design and population

This study was conducted in the context of the Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMeS) Study, a cross-sectional investigation of school-aged children and their parents. Details of the study have been described previously (30). Briefly, participants were recruited between July 2011 and November 2013 from the capital cities of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Belize, and the city of Tuxtla Gutiérrez in Chiapas, Mexico. Using enrollment lists from public primary schools in each city, study personnel randomly selected potentially eligible students who were 7-12 years of age. Researchers visited the schools to assess additional eligibility criteria, including that the child lived with both biological parents, neither the child nor the mother was pregnant, and that the child did not have any siblings already invited to participate. If children met all criteria, their parents received an explanation of the study and its procedures, and were invited to enroll. The final sample consisted of 267 families (Guatemala, 31; El Salvador, 30; the Dominican Republic, 30; Honduras, 30; Nicaragua, 31; Panama, 26; Costa Rica, 27; Belize, 31; and Mexico, 31).

All study procedures were approved by the Institutional Review Boards (IRB) of collaborating institutions in each of the nine countries and by the University of Michigan Health and Behavioral Sciences IRB. All adults provided written informed consent to participate. Parents provided written informed consent for their children. Assent to participate was confirmed from the children before enrollment.

Data collection

Data collection took place at home or at a family visit to a health center. All participants were asked to fast for at least 6 hours before the appointment. At the visit, participants completed questionnaires that inquired on sociodemographic characteristics, including their age, education level, smoking status, and socioeconomic status (SES) indicators.

Trained study personnel obtained anthropometric measurements on each participant using standardized procedures and calibrated instruments. Height was measured without shoes to the nearest millimeter with the use of portable Seca stadiometers (Seca, Hamburg, Germany). Weight was measured in light clothing to the nearest 100 g with use of Tanita scales (Tanita, Tokyo, Japan). Waist circumference was measured to the nearest millimeter at the end of an unforced exhalation using an inelastic measuring tape. In adults, it was measured at the midpoint of the lower end of the ribcage and the iliac crest; in children, it was measured above the uppermost lateral border of the right ilium. All anthropometric measures were obtained in triplicate. Blood pressure was measured using Omron HEM-712C digital blood pressure monitors (Omron Healthcare, Inc., Lake Forest, IL, USA) while the participant was seated. It was measured in triplicate with at least one minute between each reading, and the final blood pressure value was calculated as the average of the second and third readings.

Finally, researchers obtained biological samples from all participants. A fasting blood sample was obtained by venipuncture of the antecubital vein. A gluteal adipose tissue sample was obtained by numbing the upper area of the left gluteus with an anesthetic spray and an ice pack, inserting a 16-gauge needle at a 45˚ angle, and aspirating tissue into the syringe (32). All samples were transported on ice to laboratories in the country in which they were collected. There, plasma and serum were separated and stored at -20˚C, and adipose tissue samples were

stored in a 3:2 hexane:isopropanol solution in amber vials at -70˚C. Samples were then transported frozen to the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City, Guatemala, where biochemical analysis of plasma and serum took place. Adipose tissue samples were subsequently transported to the University of Michigan for fatty acids analysis.

Laboratory methods

Serum insulin was measured by chemiluminescent immunoassay on an Immulite 1000 system (Siemens Healthcare Diagnostics Products, Tarrytown, NY, USA). Plasma glucose concentrations were measured using the Cobas c111 automated chemistry analyzer system (Roche Diagnostics, Manneheim, Germany), and serum lipid profiles were assessed by enzymatic colorimetric assays on the same system.

Adipose tissue fatty acid analysis was conducted at the University of Michigan Regional Comprehensive Metabolomics Resource Core. Using the Bligh and Dyer method (33), total lipids were extracted from 20-25 mg of adipose tissue with 10 μL of 4 mM nonadecanoic acid (C19:0) added as an internal standard. The fatty acid portions of the total lipids were derivatized into methyl esters using boron trifluoride-methanol as previously described (34). These were then extracted using a 2:1 hexane-water mixture and centrifugation. The hexane layer containing the methyl esters was separated from the aqueous layer and dried, and the esters were re-dissolved in 100-200 μL hexane, depending on the original sample volume. Fatty acids were analyzed by gas chromatography (GC). Using an autosampler, 1-2 μL of sample was injected onto an Agilent 680N GC (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector, a 100 m x 0.25 mm x 0.2 μm SP-2560 column (Sigma-Aldrich, Bellefonte, PA, USA), and Chemstation software for data analysis. A calibration curve was created from known

amounts of C19:0 (the internal standard) and other authentic methyl esters in order to quantify fatty acids. The authentic methyl ester mixture was also used to identify fatty acids in samples based on retention times. The coefficient of variation for GC analyses was between 2.5-3.6%.

Definition of outcomes

In adults, the primary outcome was the presence of MetS defined according to the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) as having three or more of the following five conditions: abdominal obesity, high fasting glucose, high blood pressure, low serum HDL cholesterol, and high serum triglycerides (1). Abdominal obesity was defined as waist circumference >102 cm in men and >88 cm in women; high fasting glucose as plasma glucose ≥100 mg/dL; high blood pressure as either systolic blood pressure ≥130 mm Hg, diastolic blood pressure ≥85 mm Hg, or treatment with antihypertensive medication; low HDL cholesterol as serum HDL <40 mg/dL in men or <50 mg/dL in women, or treatment for low HDL cholesterol levels; and high serum triglycerides as concentrations ≥150 mg/dL or treatment with medication for high triglyceride levels.

There is not a conventional definition of MetS in children under 10 years of age. For this reason, the primary outcome in children was a continuous metabolic score, as has been previously suggested (35). This score was based on the same five components as MetS in adults, including waist circumference, insulin resistance measured using the homeostasis model of insulin resistance (HOMA-IR) (36), mean arterial pressure, serum HDL cholesterol, and serum triglycerides. Sex- and age-standardized component scores were created by regressing each log-transformed component on sex and log-transformed age using linear regression, and obtaining standardized residuals. After multiplying the HDL cholesterol score by -1, we calculated the

mean of the five component scores in order to create a single overall metabolic score. Higher values of this score represent poorer metabolic health.

In supplemental analyses, we also considered as outcomes the five MetS or metabolic score components in adults and children, respectively.

Definition of exposures

In both adults and children, the primary exposures were adipose tissue biomarkers of n-3 and n-6 PUFA, expressed as mass percentages of total fatty acids measured in a sample. N-3 PUFA included ALA, EPA, DPA, and DHA. Among n-6 PUFA, we examined LA, gamma-linolenic acid (18:3 n-6; GLA), dihomo-gamma-linolenic acid (20:3 n-6; DGLA), and arachidonic acid (20:4 n-6; AA). We also considered as exposures the DGLA/LA and AA/DGLA ratios, as measures of Δ6-desaturase (D6D) and Δ5-desaturase (D5D) enzymatic activity, respectively.

Exposures were categorized into quartiles to allow for non-linear associations with the outcomes. Since EPA and GLA concentrations in adipose tissue are typically low and many samples had no detectable amount, we categorized EPA into tertiles for adults and children, and categorized GLA into tertiles for children.

Covariates

We considered as covariates known independent predictors of MetS (30), including sociodemographic and anthropometric characteristics as well as other adipose tissue fatty acid biomarkers. In adults, education level was defined as the number of completed years of school and categorized as incomplete elementary (1-5), complete elementary (6), incomplete secondary (7-11), complete secondary (12), or post-secondary (≥13). For anthropometric measures, we used the median of the three replicates in all analyses (37). Body mass index (BMI) was calculated as

kg/m^2. In children, we calculated sex- and age-standardized Z scores for height and BMI using the World Health Organization Growth Reference for children ages 5-19 y (38).

Data analysis

The final analytic sample consisted of 468 parents and 201 children in whom an adipose tissue sample and assessment of MetS components were both available.

Adults

In bivariate analysis, we estimated prevalences of MetS and its components by quartiles of fatty acid concentrations. We performed tests for linear trends using Poisson regression models with MetS or a component as the dichotomous outcome and a variable representing medians of each fatty acid quartile introduced as a continuous predictor. In multivariable analysis, we estimated adjusted prevalence ratios and 95% confidence intervals (CI) using Poisson regression models. All models were adjusted for known independent predictors of MetS (30) including sex, age, height, education level, country of origin, total adipose tissue *trans* fatty acids, and adipose tissue palmitoleic acid (16:1), a product of de novo lipogenesis that is associated with carbohydrate intake (39). Further adjustment for total energy intake as measured through a food frequency questionnaire, previously described in detail (40), did not affect the results; thus, it was excluded from the final models. Models for each PUFA were adjusted for other PUFA that could confound the association with the main exposure, but that would not be intermediates on the possible causal pathway from the main PUFA exposure to MetS. ALA and LA were adjusted for each other; long-chain n-3 PUFA were adjusted for LA, total long-chain n-6 PUFA, and their precursor n-3 PUFA; and long-chain n-6 PUFA were adjusted for ALA, total long-chain n-3 PUFA, and their precursor n-6 PUFA. For long-chain n-3 PUFA, we also fitted models that included the D6D index. To control for potential residual confounding arising from

non-linearity of the fatty acid-MetS associations, covariate fatty acids were entered into the model as linear and non-linear terms obtained from restricted cubic spline models (41) with knots placed at the 5th, 50th, and 95th percentiles of their respective distributions.

Supplemental analyses of MetS components followed an analogous approach. Models with high fasting glucose, high blood pressure, low HDL cholesterol, or low serum triglycerides as outcomes were additionally adjusted for BMI. To assess whether associations between PUFA and MetS differed by underlying health status, we fitted additional models of MetS components other than high fasting glucose only among adults without high fasting glucose/diabetes diagnosis. We were unable to examine associations among those with high fasting glucose/diabetes diagnosis because of the small sample size (N=54).

Finally, we assessed the presence of interaction between the precursor fatty acids of each path, ALA and LA. We cross-classified participants according to whether their adipose tissue concentrations of these fatty acids were low or high. Low concentrations were defined as being below the median of all adults in the study (ALA: 0.71%, LA: 15.9%), and high concentrations were defined as being at or above the median. We calculated the prevalence of MetS in each of the four categories of this cross-classification, and tested for interaction using a multivariable-adjusted Poisson regression model with indicator variables for high concentrations of ALA and LA, and a term for their cross-product as predictors.

All models were fitted using generalized estimating equations and empirical standard errors, which are robust to heteroskedasticity and non-normality (42). An exchangeable correlation structure was specified to account for clustering by family membership.

Children

In bivariate analysis, we compared the distribution of the overall and component-specific metabolic scores across quartiles of each fatty acid exposure using means ± SD. We performed tests for linear trends using linear regression models with the metabolic score as the outcome and a variable representing medians of each fatty acid quartile introduced as a continuous predictor.

In multivariable analysis, we estimated adjusted mean differences in the overall or component-specific metabolic scores and 95% CI using linear regression models. These models were adjusted for previously reported predictors of the metabolic score (30) including the child's height-for-age Z score, maternal height, the number of parents with MetS, country of origin, total adipose tissue *trans* fatty acids, and adipose tissue palmitoleic acid. Models with HOMA-IR, mean arterial pressure, serum HDL cholesterol, or serum triglyceride scores as outcomes were adjusted for BMI-for-age Z scores. In addition, models for each PUFA were adjusted for other PUFA as described for adults. Because the prevalence of insulin resistance (HOMA-IR ≥3.2) among children in our study was only 2%, we were unable to examine interactions between insulin resistance and PUFA on the score components.

We assessed interaction between ALA and LA by cross-classifying children according to whether they had high or low concentrations of these PUFA. Low and high levels were defined as being below or at or above the median values among children (ALA: 0.69%, LA: 13.59%), respectively. We tested for interaction using a linear regression model with indicator variables for high ALA and high LA, and a term for their cross-product. All models were fitted using generalized estimating equations and robust standard errors.

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

Results

Adults. Mean (±SD) age among adult participants was 38.3 ± 7.3 y; 51.9% were women. The overall prevalence of MetS was 36.8%.

N-3 PUFA. In bivariate analysis, the prevalence of MetS was not associated with any n-3 PUFA (**Table 1**). In multivariable analysis, low ALA and high EPA concentrations were each positively associated with MetS in non-linear manners. The prevalence among participants in the lowest quartile of ALA was 68% higher than that among adults in quartiles 2, 3, or 4 (95% CI: 18%, 138%; *P*=0.004). Participants in the highest tertile of EPA had a 38% higher prevalence than did those in the lowest two tertiles (95% CI: 1%, 88%; *P*=0.04). Adjustment for the D6D activity index did not change this association.

ALA was inversely related to fasting glucose and serum triglycerides. Adjusted prevalences of high fasting glucose and high serum triglycerides among people in the lowest quartile of ALA were 158% higher (95% CI: 8%, 515%; *P*=0.03) and 34% higher (95% CI: 7%, 69%; *P*=0.01), respectively, than were those among participants in the highest three quartiles (**Supplemental Table 1**). EPA was positively associated with high blood pressure. Associations between n-3 PUFA and MetS components other than high fasting glucose were similar among the entire sample and among adults without high fasting glucose/diabetes diagnosis (**Supplemental Table 3**).

N-6 PUFA. In unadjusted analysis, DGLA, AA, and the D6D activity index were positively associated with prevalence of MetS, while the D5D activity index was inversely associated with this outcome (**Table 2**). After adjustment, high GLA was inversely related to MetS in a non-linear fashion. The prevalence in the highest quartile was 38% lower than the prevalence in quartiles 1, 2, or 3 (95% CI: -58%, -11%; *P*=0.01). DGLA remained positively

related to MetS; every 1 SD (0.16%) difference in adipose tissue DGLA was associated with a 28% greater prevalence of MetS (95% CI: 12%, 46%; *P*=0.0002). In addition, every 1 SD (0.01) difference in the D6D activity index was associated with an 18% greater prevalence (95% CI: 1%, 38%; *P*=0.04). AA and the D5D activity index were not related to MetS after adjustment.

GLA was inversely associated with fasting glucose (**Supplemental Table 2**). DGLA and the D6D activity index were both positively associated with all components of MetS, most strongly with high fasting glucose. Every 1 SD difference in DGLA and the D6D activity index was associated with an 86% (95% CI: 16%, 198%; *P*=0.01) and a 42% (95% CI: 8%, 86%; *P*=0.01) higher prevalence of high fasting glucose, respectively. The D5D activity index was inversely related to fasting glucose. Associations between n-6 PUFA and MetS components other than high fasting glucose were similar in the subset of adults without high fasting glucose/diabetes diagnosis (**Supplemental Table 4**).

Interaction between ALA and LA. The prevalence of MetS was highest among adults with low ALA/low LA (38.8% of 183) or high ALA/high LA (38.8% of 183), intermediate among adults with low ALA/high LA (29.4% of 51), and lowest among those with high ALA/low LA (25.5% of 51) (*P*, test for interaction=0.04). After adjustment, prevalence ratios comparing each group to those with low ALA/low LA were: for high ALA/high LA, 0.85 (95% CI: 0.62, 1.15; *P*=0.29); for low ALA/high LA, 0.66 (95% CI: 0.39, 1.11; *P*=0.12); and for high ALA/low LA, 0.56 (95% CI: 0.34, 0.94; *P*=0.03) (*P*, test for interaction=0.03).

Children. Mean age of children was 10.0 ± 1.5 y; 54.2% were girls.

N-3 PUFA. In bivariate analysis, DPA and DHA were each positively associated with the metabolic score (**Table 3**). After adjustment, children in the highest quartile of DPA had a mean metabolic score 0.09 units higher than did children in the lowest quartile (95% CI: 0.02, 0.17;

P=0.02); adjustment for the D6D activity index did not change the association. After adjustment, the association with DHA was attenuated and became non-statistically significant.

DPA was positively associated with HOMA-IR and with the serum triglyceride score in a non-linear manner (**Supplemental Table 3**). Children in the lowest quartile of DPA had a mean serum triglyceride score 0.17 units lower than did children in quartiles 2, 3, or 4 (95% CI: -0.31, -0.03; *P*=0.02). DHA was inversely associated with serum HDL cholesterol.

N-6 PUFA. In bivariate analysis, LA, DGLA, AA, and the D6D activity index were each positively associated with the metabolic score (**Table 4**). After adjustment, these positive associations remained but were attenuated and became non-statistically significant.

LA and DGLA were each positively associated with waist circumference after adjustment (**Supplemental Table 4**). AA was positively associated with waist circumference and inversely associated with serum triglycerides. The D6D and D5D activity indices were each positively related to waist circumference.

Interaction between ALA and LA. Children with low ALA/low LA (N=76), low ALA/ high LA (N=24), and high ALA/high LA (N=77) had similar mean metabolic scores (-0.01 ± 0.23, 0.03 ± 0.20, and 0.04 ± 0.21, respectively). Children with high ALA/low LA (N=24) had the lowest mean score (-0.11 ± 0.26). However, the interaction was not statistically significant (*P*=0.19).

Discussion

In this cross-sectional study of Mesoamerican families we found that, among adults, MetS prevalence was inversely associated with adipose tissue ALA but positively related to EPA. In addition, MetS was inversely related to GLA but positively to DGLA and the D6D activity index. In children, DPA was positively associated with an overall metabolic risk score.

The inverse association between ALA and MetS among Mesoamerican adults is in line with results from previous studies that suggested a protective association (14,43). In particular, of three cross-sectional investigations of adipose tissue ALA and MetS or its components, two found inverse associations with insulin resistance (11,15) and one found inverse relations with MetS, high waist circumference, and fasting glucose (10). By contrast, a meta-analysis of cross-sectional and case-control studies of blood biomarkers or intake of ALA found no significant association with MetS (8). Some of the studies included in the meta-analysis were conducted among children and several did not adjust for any confounders, which may explain the discrepancy with our results in adults. In addition, blood biomarkers may not reflect long-term intake as adipose tissue does. A protective effect of ALA could be explained through its incorporation into cell membranes (44) and the resulting enhancement of membrane fluidity and glucose transport, which is also consistent with the inverse association we found between ALA and high fasting glucose. Although ALA can be endogenously metabolized into EPA, DPA, and DHA, and some of these long chain PUFA have been related to cardiometabolic health (7), our results do not support the view that a potential protective effect of ALA may operate through these PUFA because EPA was related to MetS in the opposite direction as ALA; in addition, conversion is generally inefficient (45).

We did not find an association between LA and MetS in adults. Research on LA and MetS is limited, but available evidence generally suggests a potential protective role. Some (16,17) but not all (18) studies have found inverse associations between LA concentrations in blood and incident MetS. Circulating LA is also associated with lower serum triglycerides and higher HDL cholesterol (46), and inversely associated with insulin resistance (47) and risk of type 2 diabetes (19). Our results may not be consistent because of differences in the level of LA intake in our study population as compared to others, the use of adipose tissue biomarkers to ascertain exposure status, or unmeasured confounding by other aspects of diet.

MetS prevalence was lowest among adults with high adipose tissue concentrations of ALA and low concentrations of LA. Two studies of PUFA intake did not find evidence for an interaction between ALA and LA with respect to MetS or coronary heart disease incidence (48,49). Our use of adipose tissue biomarkers may explain this discrepancy. A commonly proposed mechanism by which ALA and LA might interact is through competition for desaturase and elongase enzymes, which means that endogenous production of long chain n-3 PUFA from ALA could depend on intake levels of LA (46). However, our results do not support this explanation since long chain n-3 PUFA were not associated with improved metabolic health. Instead, the possible protective effects of ALA may be influenced by LA. For example, ALA incorporation into cell membranes may be reduced in the presence of high dietary LA. The interaction may also be spurious.

Unexpectedly, we found that EPA, a long chain n-3 PUFA, was positively associated with MetS among adults. This is consistent with a cross-sectional study of MetS and adipose tissue PUFA that was conducted in Costa Rica (10) but in contrast with other evidence (7,8). Two additional cross-sectional studies found no association between adipose tissue EPA (11) or

EPA+DHA (15) and insulin resistance in elderly Swedish men and adults from North America, respectively. It is possible that adipose tissue concentrations of EPA may not reflect levels in plasma membranes, which could have a direct influence on metabolic outcomes (50). Alternatively, our results might be specific to Mesoamerican populations, which are underrepresented in existing literature. In support of this possibility, the association between intake or circulating biomarkers of EPA and type 2 diabetes differs by region (12). In addition, variation in the genes encoding for desaturase enzymes modify the association between dietary PUFA and MetS components (51). Finally, although adjustment for the D6D activity index did not change any of the associations between these PUFA and MetS or its components, residual confounding by D6D activity could remain (51).

Among adults, DGLA and the D6D activity index were strongly positively associated with MetS and all its components. DGLA accumulation in adipose tissue could be a result of higher D6D activity, but independent effects of DGLA and D6D on MetS cannot be ruled out. The positive association of DGLA with MetS is consistent with many (17,18,52,53) but not all (16,54) previous studies. Although DGLA is a precursor for anti-inflammatory eicosanoids (55), in one study plasma DGLA was positively associated with C-reactive protein (56), a marker of inflammation. Higher D6D activity has been associated with MetS, insulin resistance, and risk of diabetes (57). Furthermore, variations in the FADS2 gene that encodes D6D are associated with MetS and its components in adults (51). D6D is the rate-limiting step in production of long chain n-6 PUFA from LA, so the adverse metabolic consequences of D6D activity could be related to increased production of pro-inflammatory and vasoconstrictive eicosanoids from long-chain n-6 PUFA.

Among children, ALA was not associated with the metabolic score. This is consistent with the few cross-sectional studies that have previously addressed this question (26–28). The fact that there was an inverse association in adults but not among children could reflect differences in the effect of ALA by age. Alternatively, the effects of ALA may be cumulative from childhood and only apparent in adult life. Also, ALA may only have an effect on extreme metabolic dysregulation which may not be apparent when comparing the continuous distributions of a metabolic score. Because there were fewer children than adults in our study, we may have also lacked statistical power to detect an association.

We found no association between LA and the metabolic score in children, consistent with results from two previous investigations of blood biomarkers of LA and MetS (27) or blood pressure (29). Two other studies found and inverse association between LA in blood and MetS (26,28). These studies used varying definitions of MetS, rather than a continuous score as we did. Discrepancies may also be related to the use of adipose tissue biomarkers to measure PUFA status in our study, which had not been performed before in pediatric MetS research.

We found that DPA was positively associated with metabolic score among children, while DHA and EPA were not significantly related to this outcome in multivariable analysis. The only previous study that examined this question in children found a positive but non-statistically significant association between plasma DPA and MetS (27). Another study found that a pattern of high plasma EPA, DPA, and DHA in pregnancy was associated with higher HDL cholesterol and lower serum triglycerides at 6 years (58). Studies of EPA and DHA supplementation in pregnancy, infancy, and early childhood have produced inconsistent results related to MetS components (20). In middle childhood and adolescence, supplementation has been protective in some (21–24) but not all (25) trials. Observational studies of EPA and DHA among children are

also inconsistent (26–28,59,60). Overall, our results and those of previous studies do not consistently support a protective effect of long-chain n-3 PUFA on MetS in school-age children.

We did not find an association between D6D activity and metabolic score in children, in contrast with previous studies of blood PUFA or FADS gene variants (28,61–63). The specific gene variants in FADS2 present in this population may not be associated with lipid and glucose metabolism in childhood, or we may have lacked sufficient statistical power to detect these association. LA, DGLA, AA, and the D6D and D5D activity indices were each positively associated with waist circumference, which may reflect upregulation of adipogenesis by n-6 PUFA (64). Because abdominal adiposity is central to the etiology of MetS (2), these associations may suggest that children with high n-6 PUFA status might develop MetS in the future.

The primary limitation of this study is its cross-sectional design, which prevents making causal inference. For example, reverse causation could explain the findings with long chain PUFA and enzyme activity indices if metabolic dysregulation alters desaturase activity. Because biomarkers can reflect both dietary intake and endogenous metabolism of long chain PUFA, it is unclear to what extent our results are related to each of these factors. Finally, the relatively small number of children may have limited statistical power. The main strength of the study is the use of adipose tissue biomarkers of PUFA, which is the gold standard for assessing long-term fatty acid intake (9). Adipose tissue biomarkers have rarely been employed among children. Another strength is that the study provides information on the associations between fatty acid status and MetS in very under-studied populations. The family design allowed us to examine associations among both children and adults. We were also able to adjust for a number of important confounders in the analyses.

In conclusion, MetS prevalence is inversely associated with adipose tissue ALA and GLA, and positively associated with EPA, DGLA, and the D6D activity index among adults. Among children, metabolic risk score is positively associated with DPA in adipose tissue. Future studies should assess these associations using longitudinal designs. A potential protective effect of ALA against MetS warrants further investigation, since ALA status can be easily enhanced through relatively simple dietary interventions (65).

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Table 3.1. Prevalence and adjusted prevalence ratios of metabolic syndrome[a] by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among adults from Mesoamerica

Fatty acid[b] category (median, weight % of total FA)	N	Unadjusted prevalence (%)	Adjusted PR (95% CI)[c]
Overall	468	36.3	
ALA 18:3(n-3)			
Q1 (0.40)	117	40.2	1.00
Q2 (0.62)	117	33.3	0.61 (0.42, 0.89)
Q3 (0.80)	117	35.0	0.57 (0.37, 0.86)
Q4 (1.15)	117	36.8	0.62 (0.37, 1.02)
P, trend[d]		0.71	0.15
EPA 20:5(n-3)			
Q1 (0.00)	320	35.6	1.00
Q2 (0.03)	74	32.4	0.88 (0.61, 1.27)
Q3 (0.07)	74	43.2	1.35 (0.98, 1.85)
P, trend		0.31	0.14
DPA 22:5(n-3)			
Q1 (0.00)	117	31.6	1.00
Q2 (0.06)	117	40.2	1.36 (0.96, 1.93)
Q3 (0.10)	117	40.2	1.15 (0.78, 1.69)
Q4 (0.17)	117	33.3	1.08 (0.72, 1.62)
P, trend		0.68	0.78
DHA 22:6(n-3)			
Q1 (0.00)	117	32.5	1.00
Q2 (0.08)	117	46.2	1.35 (0.94, 1.93)
Q3 (0.12)	117	30.8	0.78 (0.50, 1.20)
Q4 (0.18)	117	35.9	0.99 (0.64, 1.54)
P, trend		0.81	0.59

Footnotes to Table 3.1.

[a]Defined according to the National Cholesterol Education Program's Adult Treatment Panel III 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.

[b]ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

[c]From Poisson regression models. All models are adjusted for sex, age, height, education level, country of origin, total trans fatty acids, and palmitoleic acid. Additionally, estimates for ALA are adjusted for LA. Estimates for specific long-chain n-3 PUFA are adjusted for LA, total long-chain n-6 PUFA, and their precursor PUFA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[d]Wald test from Poisson regression models with a metabolic syndrome as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Table 3.2. Prevalence and adjusted prevalence ratios of metabolic syndrome[a] by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among adults from Mesoamerica

Fatty acid[b] category (median, weight % of total FA)	N	Unadjusted prevalence (%)	Adjusted PR (95% CI)[c]
LA 18:2(n-6)			
Q1 (9.84)	117	35.0	1.00
Q2 (12.67)	117	36.8	1.06 (0.70, 1.59)
Q3 (16.17)	117	39.3	1.15 (0.74, 1.81)
Q4 (20.58)	117	34.2	1.26 (0.71, 2.25)
P, trend[d]		0.93	0.40
GLA 18:3(n-6)			
Q1 (0.00)	153	37.9	1.00
Q2 (0.05)	105	39.0	1.01 (0.74, 1.40)
Q3 (0.08)	105	40.0	0.96 (0.70, 1.32)
Q4 (0.15)	105	27.6	0.61 (0.41, 0.91)
P, trend		0.06	0.02
DGLA 20:3(n-6)			
Q1 (0.15)	117	23.9	1.00
Q2 (0.21)	117	29.9	1.37 (0.89, 2.11)
Q3 (0.29)	117	41.9	1.94 (1.34, 2.80)
Q4 (0.41)	117	49.6	2.39 (1.62, 3.54)
P, trend		<0.0001	<0.0001
AA 20:4(n-6)			
Q1 (0.25)	117	26.5	1.00
Q2 (0.34)	117	33.3	1.18 (0.78, 1.78)
Q3 (0.46)	117	41.9	1.25 (0.81, 1.92)
Q4 (0.65)	117	43.6	1.22 (0.74, 2.02)
P, trend		0.003	0.51
Δ6-Desaturase index (DGLA/LA)			
Q1 (0.011)	117	24.8	1.00
Q2 (0.015)	117	27.4	1.22 (0.80, 1.84)
Q3 (0.020)	117	41.9	1.79 (1.21, 2.64)
Q4 (0.028)	117	51.3	2.57 (1.71, 3.88)
P, trend		<0.0001	<0.0001
Δ5-Desaturase index (AA/DGLA)			
Q1 (1.13)	117	44.4	1.00
Q2 (1.46)	117	39.3	0.96 (0.69, 1.32)
Q3 (1.77)	117	31.6	0.80 (0.57, 1.13)
Q4 (2.41)	117	29.9	0.96 (0.64, 1.44)
P, trend		0.02	0.74

Footnotes to Table 3.2.

[a]Defined according to the National Cholesterol Education Program's Adult Treatment Panel III 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.

[b]LA, linoleic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid.

[c]From Poisson regression models. All models are adjusted for sex, age, height, education level, country of origin, total trans fatty acids, and palmitoleic acid. Additionally, estimates for LA are adjusted for ALA. Estimates for specific long-chain n-6 PUFA are adjusted for ALA, total long-chain n-3 PUFA, and their precursor PUFA. The Δ6-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and GLA. The Δ5-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and DGLA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[d]Wald test from Poisson regression models with a metabolic syndrome as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Table 3.3. Means and adjusted mean differences in metabolic syndrome score[a] by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among children from Mesoamerica

Fatty acid[b] category (median, weight % of total FA)	N	Mean Z score (±SD)	Adjusted mean difference (95% CI)[c]
ALA 18:3(n-3)			
Q1 (0.40)	50	-0.04 ± 0.22	Reference
Q2 (0.61)	50	0.03 ± 0.22	0.01 (-0.07, 0.09)
Q3 (0.76)	51	-0.01 ± 0.23	0.02 (-0.06, 0.11)
Q4 (1.09)	50	0.02 ± 0.22	0.01 (-0.08, 0.11)
P, trend[d]		0.38	0.81
EPA 20:5(n-3)			
Q1 (0.00)	170	0.00 ± 0.23	Reference
Q2 (0.04)	15	0.03 ± 0.17	-0.08 (-0.17, 0.02)
Q3 (0.10)	16	0.03 ± 0.19	0.01 (-0.07, 0.10)
P, trend		0.39	0.90
DPA 22:5(n-3)			
Q1 (0.00)	73	-0.05 ± 0.22	Reference
Q2 (0.05)	42	-0.01 ± 0.19	0.01 (-0.06, 0.09)
Q3 (0.08)	43	0.06 ± 0.22	0.09 (0.00, 0.17)
Q4 (0.15)	43	0.05 ± 0.25	0.09 (0.02, 0.17)
P, trend		0.01	0.01
DHA 22:6(n-3)			
Q1 (0.00)	51	-0.07 ± 0.23	Reference
Q2 (0.06)	50	-0.02 ± 0.18	-0.04 (-0.12, 0.03)
Q3 (0.10)	50	0.06 ± 0.23	0.00 (-0.08, 0.08)
Q4 (0.18)	50	0.03 ± 0.25	0.06 (-0.02, 0.15)
P, trend		0.01	0.06

Footnotes to Table 3.3.

[a]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were created by regressing each log-transformed component on sex and log-transformed age in linear regression models and obtaining standardized residuals. The overall score was calculated as the average of the five component scores, after the HDL cholesterol score was multplied by -1.

[b]ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

[c]From linear regression models. All models are adjusted for the child's height-for-age Z score, maternal height, country of origin, total trans fatty acids, and palmitoleic acid. In addition, estimates for ALA are adjusted for LA. Estimates for specific long-chain n-3 PUFA are adjusted for LA, total long-chain n-6 PUFA, and their precursor PUFA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[d]Wald test from linear regression models with metabolic score as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Table 3.4. Means and adjusted mean differences in metabolic syndrome score[a] by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among children from Mesoamerica

Fatty acid[b] category (median, weight % of total FA)	N	Mean Z score (±SD)	Adjusted mean difference (95% CI)[c]
LA 18:2(n-6)			
Q1 (9.20)	50	-0.04 ± 0.23	Reference
Q2 (12.04)	50	-0.03 ± 0.25	0.00 (-0.08, 0.08)
Q3 (14.85)	51	0.03 ± 0.19	0.05 (-0.04, 0.14)
Q4 (19.82)	50	0.05 ± 0.22	0.05 (-0.06, 0.17)
P, trend[d]		0.02	0.37
GLA 18:3(n-6)			
Q1 (0.00)	87	-0.02 ± 0.23	Reference
Q2 (0.06)	57	0.03 ± 0.20	-0.01 (-0.08, 0.06)
Q3 (0.15)	57	0.00 ± 0.25	-0.01 (-0.10, 0.08)
P, trend		0.59	0.77
DGLA 20:3(n-6)			
Q1 (0.09)	50	-0.07 ± 0.18	Reference
Q2 (0.14)	50	-0.02 ± 0.21	-0.01 (-0.08, 0.06)
Q3 (0.19)	51	0.01 ± 0.25	0.02 (-0.06, 0.09)
Q4 (0.29)	50	0.08 ± 0.24	0.05 (-0.04, 0.13)
P, trend		0.0002	0.23
AA 20:4(n-6)			
Q1 (0.17)	50	-0.07 ± 0.20	Reference
Q2 (0.28)	50	-0.01 ± 0.20	0.00 (-0.07, 0.07)
Q3 (0.36)	51	0.04 ± 0.25	0.01 (-0.06, 0.09)
Q4 (0.55)	50	0.05 ± 0.23	0.01 (-0.07, 0.09)
P, trend		0.004	0.77
Δ6-Desaturase index (DGLA/LA)			
Q1 (0.007)	50	-0.07 ± 0.17	Reference
Q2 (0.011)	50	0.00 ± 0.19	-0.03 (-0.10, 0.03)
Q3 (0.014)	51	-0.02 ± 0.23	-0.03 (-0.10, 0.05)
Q4 (0.019)	50	0.09 ± 0.28	0.07 (-0.01, 0.14)
P, trend		0.002	0.06
Δ5-Desaturase index (AA/DGLA)			
Q1 (1.27)	50	0.00 ± 0.24	Reference
Q2 (1.68)	50	0.04 ± 0.23	0.05 (-0.02, 0.12)
Q3 (1.97)	51	0.00 ± 0.23	0.01 (-0.06, 0.07)
Q4 (3.16)	50	-0.03 ± 0.20	0.03 (-0.05, 0.11)
P, trend		0.25	0.63

Footnotes to Table 3.4.

[a]Component scores (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were created by regressing each log-transformed component on sex and log-transformed age in linear regression models and obtaining standardized residuals. The overall score was calculated as the average of the five component scores, after the HDL cholesterol score was multplied by -1.

[b]LA, linoleic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid.

[c]From linear regression models. All models are adjusted for the child's height-for-age Z score, maternal height, country of origin, total trans fatty acids, and palmitoleic acid. Additionally, estimates for LA are adjusted for ALA. Estimates for specific long-chain n-6 PUFA are adjusted for ALA, total long-chain n-3 PUFA, and their precursor PUFA. The Δ6-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and GLA. The Δ5-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and DGLA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[d]Wald test from linear regression models with metabolic score as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Supplemental Table 3.1. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among adults from Mesoamerica

	Abdominal obesity[a]		High fasting glucose[b]		High blood pressure[c]		Low serum HDL cholesterol[d]		High serum triglycerides[e]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[f]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
Overall	47.6		8.8		21.6		81.8		51.5	
ALA 18:3(n-3)										
Q1 (0.40)	49.6	1.00	16.2	1.00	14.5	1.00	77.8	1.00	63.2	1.00
Q2 (0.62)	46.2	0.84 (0.62, 1.14)	9.4	0.54 (0.24, 1.19)	22.4	1.02 (0.56, 1.88)	78.6	0.91 (0.79, 1.05)	50.4	0.77 (0.61, 0.99)
Q3 (0.80)	53.8	0.91 (0.65, 1.28)	3.4	0.15 (0.05, 0.45)	23.9	1.19 (0.60, 2.37)	81.2	0.85 (0.73, 1.00)	46.2	0.70 (0.52, 0.93)
Q4 (1.15)	41.0	0.80 (0.51, 1.26)	6.0	0.41 (0.13, 1.30)	25.6	0.98 (0.39, 2.42)	89.7	0.93 (0.78, 1.11)	46.2	0.76 (0.54, 1.08)
P, trend	0.24	0.44	0.02	0.13	0.04	0.91	0.005	0.56	0.005	0.16
EPA 20:5(n-3)										
Q1 (0.00)	46.6	1.00	9.4	1.00	19.7	1.00	82.8	1.00	53.1	1.00
Q2 (0.03)	48.6	0.89 (0.70, 1.14)	5.4	0.77 (0.29, 2.07)	23.0	1.46 (0.89, 2.40)	79.7	0.94 (0.84, 1.06)	41.9	0.82 (0.62, 1.08)
Q3 (0.07)	51.4	1.08 (0.84, 1.37)	9.5	1.12 (0.49, 2.56)	28.4	1.46 (0.95, 2.25)	79.7	1.03 (0.90, 1.17)	54.1	1.13 (0.88, 1.43)
P, trend	0.45	0.74	0.83	0.91	0.09	0.05	0.48	0.88	0.7	0.65
DPA 22:5(n-3)										
Q1 (0.00)	38.5	1.00	8.5	1.00	20.5	1.00	74.4	1.00	53.0	1.00
Q2 (0.06)	45.3	0.96 (0.74, 1.25)	8.5	1.49 (0.60, 3.69)	21.4	1.28 (0.76, 2.15)	86.3	1.14 (1.00, 1.28)	55.6	1.22 (0.98, 1.53)
Q3 (0.10)	60.7	0.99 (0.75, 1.29)	11.1	1.41 (0.53, 3.74)	20.5	0.99 (0.59, 1.66)	81.2	0.99 (0.86, 1.14)	47.9	1.05 (0.81, 1.37)
Q4 (0.17)	46.2	0.90 (0.66, 1.21)	6.8	0.76 (0.26, 2.16)	24.1	1.04 (0.59, 1.82)	85.5	1.06 (0.92, 1.22)	49.6	1.02 (0.78, 1.33)
P, trend	0.30	0.51	0.70	0.64	0.53	0.92	0.06	0.64	0.42	0.93

Supplemental Table 3.1. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among adults from Mesoamerica

	Abdominal obesity[a]		High fasting glucose[b]		High blood pressure[c]		Low serum HDL cholesterol[d]		High serum triglycerides[e]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[f]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
DHA 22:6(n-3)										
Q1 (0.00)	40.2	1.00	7.7	1.00	20.5	1.00	76.9	1.00	53.8	1.00
Q2 (0.08)	54.7	1.11 (0.84, 1.46)	10.3	1.47 (0.53, 4.03)	27.4	1.86 (1.08, 3.21)	83.8	0.99 (0.87, 1.14)	52.1	1.12 (0.87, 1.44)
Q3 (0.12)	59.0	1.05 (0.79, 1.41)	6.8	0.81 (0.22, 3.00)	14.5	1.06 (0.53, 2.11)	83.8	0.92 (0.79, 1.07)	46.2	0.94 (0.72, 1.22)
Q4 (0.18)	36.8	0.80 (0.57, 1.11)	10.3	1.56 (0.46, 5.23)	24.1	1.44 (0.73, 2.84)	82.9	0.95 (0.81, 1.11)	53.8	1.04 (0.79, 1.37)
P, trend	0.63	0.15	0.62	0.59	0.82	0.44	0.22	0.40	0.82	0.96

Footnotes to Supplemental Table 3.1.

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

[b]≥100 mg/dL

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

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

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

[f]From Poisson regression models. All models are adjusted for sex, age, height, education level, country of origin, total trans fatty acids, and palmitoleic acid. Models for all risk factors other than waist circumference are adjusted for BMI. Additionally, estimates for ALA are adjusted for LA. Estimates for specific long-chain n-3 PUFA are adjusted for LA, total long-chain n-6 PUFA, and their precursor PUFA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[g]Wald test from Poisson regression models with each metabolic characteristic as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Supplemental Table 3.2. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among adults from Mesoamerica

	Abdominal obesity[a]		High fasting glucose[b]		High blood pressure[c]		Low serum HDL cholesterol[d]		High serum triglycerides[e]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[f]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
LA 18:2(n-6)										
Q1 (9.84)	46.2	1.00	12.0	1.00	17.1	1.00	74.4	1.00	54.7	1.00
Q2 (12.67)	54.7	1.05 (0.82, 1.34)	11.1	0.81 (0.37, 1.77)	14.7	0.84 (0.43, 1.67)	80.3	1.10 (0.95, 1.28)	55.6	1.20 (0.91, 1.58)
Q3 (16.17)	43.6	0.91 (0.67, 1.24)	6.0	0.45 (0.14, 1.47)	27.4	1.62 (0.82, 3.21)	88.0	1.22 (1.05, 1.42)	54.7	1.29 (0.93, 1.81)
Q4 (20.58)	46.2	1.11 (0.72, 1.73)	6.0	0.75 (0.17, 3.22)	27.4	1.80 (0.76, 4.26)	84.6	1.18 (0.97, 1.45)	41.0	1.17 (0.77, 1.76)
P, trend[g]	0.45	0.78	0.06	0.59	0.008	0.07	0.02	0.10	0.02	0.52
GLA 18:3(n-6)										
Q1 (0.00)	44.4	1.00	13.1	1.00	21.7	1.00	77.8	1.00	54.9	1.00
Q2 (0.05)	51.4	0.94 (0.73, 1.21)	9.5	0.80 (0.38, 1.70)	21.0	1.29 (0.76, 2.18)	82.9	1.12 (0.99, 1.27)	53.3	1.09 (0.85, 1.39)
Q3 (0.08)	55.2	0.92 (0.73, 1.15)	7.6	0.68 (0.30, 1.53)	21.0	1.01 (0.59, 1.74)	83.8	1.13 (1.00, 1.27)	56.2	1.23 (0.95, 1.59)
Q4 (0.15)	41.0	0.94 (0.67, 1.33)	2.9	0.24 (0.07, 0.87)	22.9	0.76 (0.47, 1.24)	84.8	1.02 (0.90, 1.16)	40.0	0.91 (0.68, 1.21)
P, trend	0.85	0.62	0.003	0.01	0.83	0.32	0.14	0.57	0.03	0.83
DGLA 20:3(n-6)										
Q1 (0.15)	36.8	1.00	3.4	1.00	17.1	1.00	73.5	1.00	46.2	1.00
Q2 (0.21)	35.9	0.89 (0.64, 1.23)	6.8	2.36 (0.72, 7.82)	17.9	1.02 (0.57, 1.82)	80.3	1.04 (0.91, 1.19)	50.4	1.31 (1.01, 1.70)
Q3 (0.29)	59.0	1.26 (0.98, 1.63)	13.7	4.12 (1.34, 12.67)	23.1	1.57 (0.89, 2.77)	82.9	1.09 (0.96, 1.25)	50.4	1.43 (1.11, 1.85)
Q4 (0.41)	59.0	1.31 (1.00, 1.73)	11.1	4.90 (1.52, 15.81)	28.4	1.68 (0.95, 2.94)	90.6	1.22 (1.07, 1.39)	59.0	1.73 (1.32, 2.28)
P, trend	0.0001	0.007	0.01	0.002	0.01	0.04	0.0003	0.001	0.04	0.0001

Supplemental Table 3.2. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among adults from Mesoamerica

	Abdominal obesity[a]		High fasting glucose[b]		High blood pressure[c]		Low serum HDL cholesterol[d]		High serum triglycerides[e]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[f]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
AA 20:4(n-6)										
Q1 (0.25)	35.9	1.00	6.0	1.00	16.2	1.00	77.8	1.00	50.4	1.00
Q2 (0.34)	46.2	1.21 (0.90, 1.61)	6.8	0.92 (0.35, 2.41)	19.7	1.08 (0.62, 1.88)	82.9	1.00 (0.88, 1.14)	52.1	1.05 (0.80, 1.37)
Q3 (0.46)	52.1	1.27 (0.95, 1.71)	12.0	1.31 (0.49, 3.48)	25.0	1.20 (0.67, 2.15)	80.3	0.91 (0.79, 1.05)	48.7	0.87 (0.65, 1.18)
Q4 (0.65)	56.4	1.41 (1.01, 1.96)	10.3	0.71 (0.26, 1.95)	25.6	0.97 (0.55, 1.71)	86.3	0.96 (0.83, 1.11)	54.7	0.94 (0.68, 1.31)
P, trend	0.006	0.06	0.15	0.42	0.05	0.82	0.13	0.49	0.61	0.55
Δ6-Desaturase index (DGLA/LA)										
Q1 (0.011)	28.2	1.00	2.6	1.00	20.5	1.00	78.6	1.00	39.3	1.00
Q2 (0.015)	49.6	1.47 (1.08, 1.99)	4.3	1.50 (0.38, 5.97)	17.1	1.02 (0.61, 1.70)	77.8	1.03 (0.91, 1.16)	45.3	1.12 (0.83, 1.49)
Q3 (0.020)	55.6	1.56 (1.12, 2.15)	9.4	2.48 (0.69, 8.96)	24.8	1.52 (0.89, 2.60)	82.9	1.09 (0.96, 1.24)	57.3	1.36 (1.03, 1.80)
Q4 (0.028)	57.3	1.62 (1.16, 2.27)	18.8	6.69 (1.73, 25.88)	24.1	1.77 (1.03, 3.04)	88.0	1.20 (1.04, 1.38)	64.1	1.73 (1.29, 2.33)
P, trend	<0.0001	0.01	<0.0001	0.0001	0.27	0.02	0.02	0.006	<0.0001	<0.0001
Δ5-Desaturase index (AA/DGLA)										
Q1 (1.13)	53.0	1.00	12.0	1.00	21.6	1.00	85.5	1.00	60.7	1.00
Q2 (1.46)	52.1	1.05 (0.85, 1.31)	10.3	1.24 (0.64, 2.41)	23.1	1.03 (0.61, 1.71)	86.3	0.94 (0.85, 1.05)	52.1	0.89 (0.70, 1.13)
Q3 (1.77)	46.2	1.11 (0.86, 1.42)	12.0	1.27 (0.60, 2.70)	17.9	0.82 (0.45, 1.49)	77.8	0.86 (0.76, 0.98)	45.3	0.76 (0.59, 0.98)
Q4 (2.41)	39.3	1.14 (0.84, 1.53)	0.9	0.15 (0.02, 1.18)	23.9	1.13 (0.64, 1.97)	77.8	0.90 (0.78, 1.03)	47.9	0.82 (0.63, 1.08)
P, trend	0.03	0.39	0.0001	0.05	0.76	0.67	0.06	0.12	0.04	0.15

Footnotes to Supplemental Table 3.2.

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

[b]≥100 mg/dL

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

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

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

[f]From Poisson regression models. All models are adjusted for sex, age, height, education level, country of origin, total trans fatty acids, and palmitoleic acid. Models of all risk factors other than waist circumference are adjusted for BMI. Additionally, estimates for LA are adjusted for ALA. Estimates for specific long-chain n-6 PUFA are adjusted for ALA, total long-chain n-3 PUFA, and their precursor PUFA. The Δ6-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and GLA. The Δ5-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and DGLA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[g]Wald test from Poisson regression models with each metabolic characteristic as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Supplemental Table 3.3. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among adults from Mesoamerica without high fasting glucose or diagnosed diabetes

	Abdominal obesity[a]		High blood pressure[b]		Low serum HDL cholesterol[c]		High serum triglycerides[d]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[e]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
Overall	45.4		21.7		81.4		49.0	
ALA 18:3(n-3)								
Q1 (0.40)	47.3	1.00	17.2	1.00	77.4	1.00	58.1	1.00
Q2 (0.62)	42.7	0.83 (0.58, 1.17)	20.4	0.78 (0.41, 1.50)	78.6	0.88 (0.75, 1.04)	48.5	0.82 (0.61, 1.09)
Q3 (0.80)	52.7	0.93 (0.63, 1.38)	22.3	1.04 (0.52, 2.07)	80.4	0.81 (0.68, 0.96)	46.4	0.80 (0.58, 1.11)
Q4 (1.15)	38.7	0.83 (0.50, 1.37)	26.4	0.99 (0.40, 2.43)	88.7	0.88 (0.73, 1.07)	44.3	0.84 (0.56, 1.25)
P, trend[f]	0.30	0.62	0.11	0.88	0.01	0.35	0.04	0.48
EPA 20:5(n-3)								
Q1 (0.00)	43.4	1.00	19.6	1.00	82.6	1.00	50.9	1.00
Q2 (0.03)	50.0	0.92 (0.71, 1.18)	23.5	1.68 (1.01, 2.81)	77.9	0.91 (0.80, 1.03)	38.2	0.79 (0.57, 1.08)
Q3 (0.07)	49.2	1.03 (0.78, 1.36)	29.2	1.46 (0.92, 2.31)	80.0	1.03 (0.89, 1.18)	52.3	1.10 (0.84, 1.44)
P, trend	0.31	0.96	0.07	0.05	0.50	>0.99	0.78	0.84
DPA 22:5(n-3)								
Q1 (0.00)	37.7	1.00	20.8	1.00	72.6	1.00	51.9	1.00
Q2 (0.06)	42.7	0.90 (0.67, 1.20)	23.3	1.37 (0.79, 2.36)	86.4	1.16 (1.01, 1.32)	53.4	1.17 (0.91, 1.50)
Q3 (0.10)	58.0	0.98 (0.73, 1.32)	20.0	0.96 (0.54, 1.70)	82.0	1.05 (0.90, 1.22)	43.0	0.97 (0.73, 1.30)
Q4 (0.17)	43.8	0.85 (0.59, 1.22)	22.9	1.07 (0.59, 1.95)	84.8	1.08 (0.92, 1.27)	47.6	1.01 (0.75, 1.38)
P, trend	0.33	0.43	0.81	0.89	0.05	0.42	0.38	0.98

Supplemental Table 3.3. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among adults from Mesoamerica without high fasting glucose or diagnosed diabetes

	Abdominal obesity[a]		High blood pressure[b]		Low serum HDL cholesterol[c]		High serum triglycerides[d]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[e]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
DHA 22:6(n-3)								
Q1 (0.00)	38.1	1.00	21.9	1.00	75.2	1.00	53.3	1.00
Q2 (0.08)	51.9	1.11 (0.82, 1.51)	28.8	1.82 (1.03, 3.22)	84.6	1.00 (0.87, 1.15)	50.0	1.05 (0.80, 1.39)
Q3 (0.12)	54.8	1.03 (0.73, 1.44)	13.5	0.87 (0.41, 1.83)	84.6	0.94 (0.80, 1.10)	43.3	0.88 (0.65, 1.19)
Q4 (0.18)	36.6	0.82 (0.56, 1.20)	22.8	1.31 (0.63, 2.74)	81.2	0.93 (0.79, 1.11)	49.5	0.95 (0.69, 1.30)
P, trend	0.94	0.25	0.71	0.64	0.23	0.36	0.42	0.62

Footnotes to Supplemental Table 3.3.

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

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

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

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

[e]From Poisson regression models. All models are adjusted for sex, age, height, education level, country of origin, total trans fatty acids, and palmitoleic acid. Models for all risk factors other than waist circumference are adjusted for BMI. Additionally, estimates for ALA are adjusted for LA. Estimates for specific long-chain n-3 PUFA are adjusted for LA, total long-chain n-6 PUFA, and their precursor PUFA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[f]Wald test from Poisson regression models with each metabolic characteristic as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Supplemental Table 3.4. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among adults from Mesoamerica without high fasting glucose or diagnosed diabetes

	Abdominal obesity[a]		High blood pressure[b]		Low serum HDL cholesterol[c]		High serum triglycerides[d]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[e]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
LA 18:2(n-6)								
Q1 (9.84)	43.4	1.00	19.2	1.00	75.8	1.00	51.5	1.00
Q2 (12.67)	51.5	1.04 (0.78, 1.38)	14.6	0.72 (0.34, 1.56)	77.7	1.07 (0.92, 1.24)	51.5	1.22 (0.88, 1.71)
Q3 (16.17)	41.0	0.87 (0.62, 1.23)	26.7	1.54 (0.73, 3.25)	87.6	1.22 (1.05, 1.41)	53.3	1.41 (0.96, 2.08)
Q4 (20.58)	45.8	1.05 (0.65, 1.71)	26.2	1.55 (0.60, 3.98)	84.1	1.19 (0.98, 1.46)	40.2	1.28 (0.80, 2.06)
P, trend[f]	0.78	0.99	0.06	0.14	0.05	0.06	0.10	0.32
GLA 18:3(n-6)								
Q1 (0.00)	39.7	1.00	20.6	1.00	76.3	1.00	51.1	1.00
Q2 (0.05)	51.1	0.94 (0.71, 1.24)	21.7	1.44 (0.82, 2.53)	82.6	1.10 (0.97, 1.25)	51.1	1.09 (0.83, 1.43)
Q3 (0.08)	53.8	0.92 (0.71, 1.19)	22.6	1.16 (0.66, 2.02)	84.9	1.13 (0.99, 1.29)	54.8	1.22 (0.91, 1.63)
Q4 (0.15)	39.8	0.94 (0.65, 1.36)	22.4	0.80 (0.49, 1.30)	83.7	1.01 (0.88, 1.16)	38.8	0.89 (0.64, 1.24)
P, trend	0.82	0.66	0.72	0.46	0.15	0.65	0.10	0.77
DGLA 20:3(n-6)								
Q1 (0.15)	36.0	1.00	18.0	1.00	73.9	1.00	45.9	1.00
Q2 (0.21)	32.4	0.82 (0.57, 1.17)	19.0	1.15 (0.65, 2.04)	80.0	1.06 (0.92, 1.21)	49.5	1.29 (0.97, 1.70)
Q3 (0.29)	57.7	1.26 (0.96, 1.65)	21.6	1.52 (0.84, 2.75)	83.5	1.11 (0.96, 1.27)	44.3	1.33 (0.99, 1.79)
Q4 (0.41)	57.4	1.33 (0.99, 1.78)	28.7	1.63 (0.92, 2.89)	89.1	1.21 (1.05, 1.38)	56.4	1.72 (1.26, 2.35)
P, trend	0.0001	0.006	0.04	0.08	0.002	0.004	0.13	0.0009

Supplemental Table 3.4. Prevalence and adjusted prevalence ratios of metabolic syndrome components by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among adults from Mesoamerica without high fasting glucose or diagnosed diabetes

	Abdominal obesity[a]		High blood pressure[b]		Low serum HDL cholesterol[c]		High serum triglycerides[d]	
Fatty acid category (median, weight % of total FA)	Unadjusted prevalence (%)	Adjusted PR (95% CI)[e]	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)	Unadjusted prevalence (%)	Adjusted PR (95% CI)
AA 20:4(n-6)								
Q1 (0.25)	35.8	1.00	17.4	1.00	78.0	1.00	49.5	1.00
Q2 (0.34)	44.2	1.19 (0.87, 1.64)	20.2	1.06 (0.59, 1.88)	82.7	0.98 (0.86, 1.11)	49.0	1.03 (0.76, 1.40)
Q3 (0.46)	49.0	1.32 (0.95, 1.84)	23.5	1.14 (0.61, 2.12)	79.6	0.90 (0.78, 1.04)	43.9	0.87 (0.63, 1.20)
Q4 (0.65)	53.4	1.41 (0.98, 2.02)	26.2	0.99 (0.55, 1.81)	85.4	0.94 (0.80, 1.10)	53.4	1.01 (0.70, 1.46)
P, trend	0.02	0.08	0.09	0.94	0.22	0.37	0.63	0.90
Δ6-Desaturase index (DGLA/LA)								
Q1 (0.011)	26.8	1.00	20.5	1.00	77.7	1.00	39.3	1.00
Q2 (0.015)	50.0	1.53 (1.11, 2.10)	17.6	1.04 (0.61, 1.79)	79.6	1.07 (0.95, 1.21)	45.4	1.17 (0.88, 1.57)
Q3 (0.020)	52.4	1.52 (1.07, 2.17)	24.3	1.52 (0.88, 2.62)	81.6	1.08 (0.95, 1.24)	53.4	1.33 (0.99, 1.79)
Q4 (0.028)	54.9	1.74 (1.22, 2.47)	25.3	1.64 (0.92, 2.94)	87.9	1.20 (1.03, 1.39)	60.4	1.72 (1.23, 2.40)
P, trend	0.0002	0.006	0.23	0.05	0.05	0.02	0.0006	0.0005
Δ5-Desaturase index (AA/DGLA)								
Q1 (1.13)	53.5	1.00	20.8	1.00	84.2	1.00	56.4	1.00
Q2 (1.46)	48.0	0.98 (0.76, 1.26)	23.5	1.03 (0.58, 1.82)	86.7	0.97 (0.86, 1.10)	48.0	0.82 (0.61, 1.10)
Q3 (1.77)	42.6	1.09 (0.82, 1.45)	18.8	0.95 (0.50, 1.77)	78.2	0.89 (0.77, 1.02)	43.6	0.81 (0.60, 1.08)
Q4 (2.41)	38.6	1.11 (0.80, 1.53)	23.7	1.12 (0.64, 1.97)	77.2	0.90 (0.78, 1.03)	48.2	0.86 (0.65, 1.14)
P, trend	0.04	0.47	0.71	0.65	0.09	0.13	0.21	0.43

Footnotes to Supplemental Table 3.4.

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

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

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

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

[e]From Poisson regression models. All models are adjusted for sex, age, height, education level, country of origin, total trans fatty acids, and palmitoleic acid. Models of all risk factors other than waist circumference are adjusted for BMI. Additionally, estimates for LA are adjusted for ALA. Estimates for specific long-chain n-6 PUFA are adjusted for ALA, total long-chain n-3 PUFA, and their precursor PUFA. The Δ6-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and GLA. The Δ5-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and DGLA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[f]Wald test from Poisson regression models with each metabolic characteristic as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Supplemental Table 3.5. Means and adjusted mean differences in metabolic syndrome component scores[a] by adipose tissue biomarkers of n-3 polyunsaturated fatty acid intake among children from Mesoamerica

	Waist circumference		HOMA-IR		Mean arterial pressure		Serum HDL cholesterol		Serum triglycerides	
Fatty acid category (median, weight % of total FA)	Mean Z score (±SD)	Adjusted mean difference (95% CI)[b]	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)
ALA 18:3(n-3)										
Q1 (0.40)	-0.02 ± 0.16	Reference	-0.08 ± 0.57	Reference	-0.04 ± 0.13	Reference	0.03 ± 0.23	Reference	-0.02 ± 0.45	Reference
Q2 (0.61)	0.02 ± 0.13	-0.02 (-0.06, 0.03)	0.07 ± 0.58	0.08 (-0.14, 0.31)	0.00 ± 0.12	0.00 (-0.05, 0.04)	0.04 ± 0.27	0.05 (-0.06, 0.15)	0.11 ± 0.47	0.07 (-0.12, 0.26)
Q3 (0.76)	0.00 ± 0.16	0.00 (-0.04, 0.05)	0.04 ± 0.56	0.17 (-0.07, 0.40)	0.00 ± 0.15	-0.01 (-0.07, 0.05)	0.05 ± 0.28	0.05 (-0.05, 0.16)	-0.02 ± 0.45	-0.03 (-0.24, 0.18)
Q4 (1.09)	0.02 ± 0.17	-0.01 (-0.06, 0.04)	-0.07 ± 0.65	0.11 (-0.19, 0.41)	0.01 ± 0.13	-0.02 (-0.08, 0.04)	-0.17 ± 0.34	0.00 (-0.14, 0.13)	-0.06 ± 0.35	-0.01 (-0.24, 0.21)
P, trend	0.39	0.77	0.90	0.54	0.05	0.53	0.0002	0.81	0.29	0.72
EPA 20:5(n-3)										
Q1 (0.00)	0.00 ± 0.15	Reference	-0.02 ± 0.60	Reference	-0.01 ± 0.13	Reference	-0.01 ± 0.30	Reference	0.00 ± 0.45	Reference
Q2 (0.04)	0.07 ± 0.18	-0.05 (-0.11, 0.01)	0.00 ± 0.46	-0.10 (-0.38, 0.18)	0.03 ± 0.14	0.01 (-0.06, 0.08)	0.00 ± 0.38	0.11 (-0.03, 0.25)	0.05 ± 0.32	0.03 (-0.16, 0.22)
Q3 (0.10)	0.01 ± 0.18	-0.01 (-0.07, 0.06)	0.14 ± 0.61	0.12 (-0.13, 0.37)	0.00 ± 0.12	0.01 (-0.05, 0.07)	-0.02 ± 0.19	-0.01 (-0.11, 0.10)	-0.02 ± 0.44	-0.02 (-0.24, 0.20)
P, trend	0.47	0.60	0.30	0.53	0.53	0.70	0.97	0.69	0.99	0.92
DPA 22:5(n-3)										
Q1 (0.00)	-0.04 ± 0.12	Reference	-0.10 ± 0.57	Reference	-0.02 ± 0.14	Reference	0.06 ± 0.25	Reference	-0.04 ± 0.45	Reference
Q2 (0.05)	0.01 ± 0.15	0.00 (-0.05, 0.04)	-0.02 ± 0.53	0.09 (-0.13, 0.31)	-0.04 ± 0.14	-0.04 (-0.09, 0.01)	0.03 ± 0.29	-0.02 (-0.12, 0.08)	0.04 ± 0.41	0.15 (-0.03, 0.33)
Q3 (0.08)	0.06 ± 0.17	0.03 (-0.02, 0.08)	0.06 ± 0.64	0.14 (-0.05, 0.34)	0.05 ± 0.10	0.04 (-0.02, 0.09)	-0.09 ± 0.31	-0.02 (-0.11, 0.08)	0.02 ± 0.46	0.20 (0.02, 0.37)
Q4 (0.15)	0.02 ± 0.17	0.01 (-0.03, 0.06)	0.08 ± 0.63	0.20 (0.01, 0.38)	0.01 ± 0.13	0.01 (-0.03, 0.06)	-0.09 ± 0.32	-0.06 (-0.15, 0.03)	0.03 ± 0.41	0.17 (0.01, 0.32)
P, trend	0.01	0.40	0.09	0.04	0.07	0.36	0.002	0.18	0.37	0.04
DHA 22:6(n-3)										
Q1 (0.00)	-0.05 ± 0.12	Reference	-0.13 ± 0.60	Reference	-0.01 ± 0.15	Reference	0.08 ± 0.25	Reference	-0.08 ± 0.42	Reference
Q2 (0.06)	0.02 ± 0.15	-0.05 (-0.09, 0.00)	-0.01 ± 0.55	0.12 (-0.12, 0.36)	-0.02 ± 0.13	-0.04 (-0.10, 0.01)	0.08 ± 0.23	0.03 (-0.08, 0.13)	0.01 ± 0.29	0.08 (-0.10, 0.26)
Q3 (0.10)	0.04 ± 0.18	-0.04 (-0.08, 0.01)	0.13 ± 0.64	0.15 (-0.07, 0.36)	0.03 ± 0.11	-0.01 (-0.06, 0.05)	-0.08 ± 0.30	-0.06 (-0.18, 0.06)	0.01 ± 0.52	0.07 (-0.14, 0.28)
Q4 (0.18)	0.01 ± 0.15	0.01 (-0.04, 0.05)	-0.02 ± 0.56	0.15 (-0.04, 0.34)	-0.03 ± 0.14	-0.04 (-0.09, 0.02)	-0.14 ± 0.33	-0.14 (-0.24, -0.04)	0.08 ± 0.48	0.19 (-0.02, 0.41)
P, trend	0.05	0.43	0.24	0.16	0.66	0.30	<0.0001	0.001	0.10	0.08

Footnotes to Supplemental Table 3.5.

[a]Scores for metabolic syndrome components (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were created by regressing each log-transformed component on sex and log-transformed age in linear regression models and obtaining standardized residuals.

[b]From linear regression models. All models are adjusted for the child's height-for-age Z score, maternal height, country of origin, total trans fatty acids, and palmitoleic acid. Models for all risk factors except waist circumference are adjusted for BMI-for-age Z score. In addition, estimates for ALA are adjusted for LA. Estimates for specific long-chain n-3 PUFA are adjusted for LA, total long-chain n-6 PUFA, and their precursor PUFA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[c]Wald test from linear regression models with each metabolic score component as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

Supplemental Table 3.6. Means and adjusted mean differences in metabolic syndrome component scores[a] by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among children from Mesoamerica

	Waist circumference		HOMA-IR		Mean arterial pressure		Serum HDL cholesterol		Serum triglycerides	
Fatty acid category (median, weight % of total FA)	Mean Z score (±SD)	Adjusted mean difference (95% CI)[b]	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)
LA 18:2(n-6)										
Q1 (9.20)	-0.03 ± 0.14	Reference	-0.10 ± 0.61	Reference	-0.05 ± 0.11	Reference	0.06 ± 0.24	Reference	0.01 ± 0.44	Reference
Q2 (12.04)	-0.01 ± 0.16	0.01 (-0.03, 0.04)	-0.07 ± 0.64	-0.02 (-0.21, 0.17)	0.00 ± 0.14	0.03 (-0.02, 0.07)	0.07 ± 0.25	0.01 (-0.08, 0.11)	0.00 ± 0.48	-0.05 (-0.24, 0.13)
Q3 (14.85)	0.00 ± 0.14	0.03 (-0.01, 0.08)	0.16 ± 0.54	0.20 (-0.05, 0.45)	0.00 ± 0.14	-0.02 (-0.08, 0.05)	0.04 ± 0.24	0.03 (-0.09, 0.16)	0.00 ± 0.38	-0.15 (-0.38, 0.07)
Q4 (19.82)	0.05 ± 0.16	0.09 (0.03, 0.15)	-0.02 ± 0.54	-0.08 (-0.44, 0.28)	0.02 ± 0.14	-0.02 (-0.09, 0.05)	-0.21 ± 0.35	-0.03 (-0.20, 0.13)	-0.01 ± 0.45	-0.23 (-0.51, 0.05)
P, trend[c]	0.008	0.002	0.32	0.54	0.02	0.48	<0.0001	0.59	0.83	0.10
GLA 18:3(n-6)										
Q1 (0.00)	-0.02 ± 0.14	Reference	-0.02 ± 0.60	Reference	-0.01 ± 0.13	Reference	0.03 ± 0.23	Reference	-0.02 ± 0.43	Reference
Q2 (0.06)	0.04 ± 0.16	-0.01 (-0.05, 0.03)	0.10 ± 0.48	0.00 (-0.17, 0.18)	0.01 ± 0.14	0.02 (-0.02, 0.06)	0.04 ± 0.30	0.11 (0.03, 0.19)	0.06 ± 0.45	0.05 (-0.10, 0.20)
Q3 (0.15)	0.00 ± 0.16	-0.02 (-0.07, 0.02)	-0.10 ± 0.66	0.00 (-0.23, 0.22)	-0.01 ± 0.14	0.01 (-0.05, 0.06)	-0.12 ± 0.34	-0.01 (-0.12, 0.10)	-0.02 ± 0.44	0.03 (-0.16, 0.22)
P, trend	0.41	0.33	0.51	0.99	0.85	0.73	0.004	0.83	0.93	0.72
DGLA 20:3(n-6)										
Q1 (0.09)	-0.06 ± 0.14	Reference	-0.17 ± 0.49	Reference	-0.04 ± 0.13	Reference	0.08 ± 0.24	Reference	-0.01 ± 0.42	Reference
Q2 (0.14)	-0.02 ± 0.13	0.00 (-0.05, 0.04)	-0.08 ± 0.57	-0.06 (-0.27, 0.16)	0.00 ± 0.13	0.04 (-0.01, 0.09)	0.00 ± 0.28	-0.04 (-0.14, 0.06)	-0.02 ± 0.46	-0.11 (-0.28, 0.07)
Q3 (0.19)	0.02 ± 0.14	0.03 (-0.02, 0.07)	0.06 ± 0.66	-0.01 (-0.24, 0.22)	-0.01 ± 0.13	0.02 (-0.03, 0.08)	-0.02 ± 0.29	0.00 (-0.11, 0.11)	-0.01 ± 0.43	-0.16 (-0.35, 0.03)
Q4 (0.29)	0.08 ± 0.17	0.06 (0.01, 0.11)	0.16 ± 0.60	0.02 (-0.22, 0.26)	0.02 ± 0.14	0.01 (-0.04, 0.06)	-0.11 ± 0.34	0.01 (-0.11, 0.14)	0.05 ± 0.44	-0.15 (-0.34, 0.04)
P, trend	<0.0001	0.005	0.001	0.71	0.03	0.98	0.001	0.61	0.50	0.16
AA 20:4(n-6)										
Q1 (0.17)	-0.07 ± 0.15	Reference	-0.14 ± 0.58	Reference	-0.05 ± 0.11	Reference	0.09 ± 0.22	Reference	-0.03 ± 0.40	Reference
Q2 (0.28)	-0.03 ± 0.13	0.02 (-0.03, 0.06)	-0.06 ± 0.57	-0.10 (-0.29, 0.10)	-0.02 ± 0.13	-0.02 (-0.06, 0.03)	-0.04 ± 0.29	-0.06 (-0.15, 0.03)	0.01 ± 0.46	-0.05 (-0.21, 0.11)
Q3 (0.36)	0.04 ± 0.15	0.05 (0.00, 0.10)	0.03 ± 0.63	-0.14 (-0.34, 0.07)	0.02 ± 0.15	0.01 (-0.04, 0.06)	-0.07 ± 0.34	0.01 (-0.08, 0.10)	0.03 ± 0.48	-0.13 (-0.29, 0.03)
Q4 (0.55)	0.08 ± 0.16	0.06 (0.01, 0.11)	0.13 ± 0.57	-0.08 (-0.30, 0.14)	0.02 ± 0.14	-0.03 (-0.08, 0.03)	-0.02 ± 0.31	0.08 (-0.03, 0.19)	0.01 ± 0.41	-0.18 (-0.35, -0.02)
P, trend	<0.0001	0.02	0.01	0.57	0.005	0.35	0.09	0.08	0.66	0.03
Δ6-Desaturase index (DGLA/LA)										
Q1 (0.007)	-0.06 ± 0.13	Reference	-0.21 ± 0.53	Reference	-0.02 ± 0.12	Reference	-0.02 ± 0.32	Reference	-0.06 ± 0.39	Reference
Q2 (0.011)	-0.01 ± 0.13	-0.01 (-0.05, 0.04)	0.00 ± 0.50	-0.12 (-0.32, 0.07)	-0.01 ± 0.12	-0.01 (-0.06, 0.03)	-0.01 ± 0.32	0.02 (-0.07, 0.12)	0.03 ± 0.42	-0.09 (-0.26, 0.07)
Q3 (0.014)	0.03 ± 0.14	0.03 (-0.02, 0.07)	-0.02 ± 0.57	-0.14 (-0.36, 0.07)	-0.01 ± 0.14	-0.01 (-0.06, 0.05)	0.01 ± 0.25	0.00 (-0.09, 0.10)	-0.07 ± 0.48	-0.18 (-0.35, -0.01)
Q4 (0.019)	0.06 ± 0.18	0.06 (0.02, 0.11)	0.20 ± 0.69	0.01 (-0.19, 0.22)	0.03 ± 0.15	0.01 (-0.03, 0.06)	-0.03 ± 0.30	-0.01 (-0.11, 0.09)	0.10 ± 0.44	-0.05 (-0.22, 0.12)
P, trend	<0.0001	0.002	0.001	0.76	0.06	0.49	0.84	0.80	0.10	0.57

Supplemental Table 3.6. Means and adjusted mean differences in metabolic syndrome component scores[a] by adipose tissue biomarkers of n-6 polyunsaturated fatty acid intake among children from Mesoamerica

	Waist circumference		HOMA-IR		Mean arterial pressure		Serum HDL cholesterol		Serum triglycerides	
Fatty acid category (median, weight % of total FA)	Mean Z score (±SD)	Adjusted mean difference (95% CI)[b]	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)	Mean Z score (±SD)	Adjusted mean difference (95% CI)
Δ5-Desaturase index (AA/DGLA)										
Q1 (1.27)	-0.01 ± 0.19	Reference	-0.02 ± 0.67	Reference	-0.03 ± 0.12	Reference	-0.03 ± 0.29	Reference	0.03 ± 0.42	Reference
Q2 (1.68)	0.01 ± 0.14	0.02 (-0.02, 0.06)	0.07 ± 0.53	0.04 (-0.15, 0.24)	0.00 ± 0.13	0.02 (-0.02, 0.07)	-0.05 ± 0.33	0.03 (-0.07, 0.12)	0.07 ± 0.48	0.03 (-0.13, 0.19)
Q3 (1.97)	0.04 ± 0.14	0.05 (0.01, 0.09)	-0.01 ± 0.63	-0.13 (-0.32, 0.07)	0.02 ± 0.16	0.02 (-0.03, 0.07)	0.03 ± 0.28	0.08 (-0.01, 0.17)	-0.03 ± 0.41	-0.09 (-0.25, 0.06)
Q4 (3.16)	-0.01 ± 0.14	0.05 (0.01, 0.10)	-0.07 ± 0.53	-0.05 (-0.26, 0.16)	-0.01 ± 0.13	0.02 (-0.03, 0.06)	0.01 ± 0.27	0.06 (-0.03, 0.16)	-0.06 ± 0.44	-0.14 (-0.33, 0.05)
P, trend	0.78	0.01	0.45	0.52	0.67	0.68	0.39	0.22	0.19	0.10

Footnotes to Supplemental Table 3.6.

[a]Scores for metabolic syndrome components (waist circumference, HOMA-IR, mean arterial pressure, serum HDL cholesterol, and serum triglycerides) were created by regressing each log-transformed component on sex and log-transformed age in linear regression models and obtaining standardized residuals.

[b]From linear regression models. All models are adjusted for the child's height-for-age Z score, maternal height, country of origin, total trans fatty acids, and palmitoleic acid. Models for all risk factors except waist circumference are adjusted for BMI-for-age Z score. Additionally, estimates for LA are adjusted for ALA. Estimates for specific long-chain n-6 PUFA are adjusted for ALA, total long-chain n-3 PUFA, and their precursor PUFA. The Δ6-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and GLA. The Δ5-desaturase index is adjusted for ALA, total long-chain n-3 PUFA, and DGLA. All covariate fatty acids were entered into the model as restricted cubic splines with knots placed at the 5th, 50th, and 95th percentiles of observed values.

[c]Wald test from linear regression models with each metabolic score component as the outcome and a variable representing medians of ordinal categories of the predictor introduced as continuous.

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Chapter 4. Serum polyunsaturated fatty acids in middle childhood and body mass index change through adolescence

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Abstract

Objective: The aim of this study was to assess associations of serum biomarkers of n-3 and n-6 polyunsaturated fatty acids (PUFA) at 5 and 10 y of age with change in body mass index (BMI)-for-age Z scores (BAZ) through age 16 y.

Design: In this longitudinal study, we quantified serum PUFA by gas chromatography in samples collected at 5 y and 10 y of age. BMI was measured at 5, 10, and 16 y. We compared the change in BAZ through 16 y of age between PUFA quartiles at 5 or 10 y and between quartiles of PUFA change from 5 to 10 y by fitting growth curves from multivariable linear mixed models with restricted cubic splines.

Setting: Santiago, Chile.

Population: 418 children.

Results: At 5 y of age, serum docosahexaenoic acid (DHA) was inversely associated with BAZ change from ages 5 to 16 y. Among children in the highest quartile of DHA, mean BAZ change was 0.51 Z scores lower (95% CI: -0.88, -0.13; P=0.008) than among children in the lowest quartile. At 10 y of age, arachidonic acid (AA) was positively related to BAZ change from ages 10 to 16 y. Children in the lowest quartile of AA had a mean change in BAZ 0.17 Z scores lower

(95% CI: -0.34, -0.01; P=0.04) compared with children in the highest three quartiles. Change in eicosapentaenoic acid (EPA) between ages 5 and 10 y was inversely associated with change in BAZ from ages 10 to 16 y (P, trend=0.04). Change in AA (P, trend=0.03) and the Δ5-desaturase (D5D) activity index (P, trend=0.003) were each positively associated with BAZ change.

Conclusions: Serum concentrations of some long-chain n-3 PUFA in middle childhood are associated with less weight gain through adolescence whereas the n-6 PUFA AA and D5D activity are related to increased weight gain.

Keywords: polyunsaturated fatty acids, children, BMI, Chile, growth

Introduction

Childhood obesity is perhaps the strongest and most prevalent early life risk factor for the development of chronic diseases, including type 2 diabetes, hypertension, and coronary heart disease (1). Childhood obesity has increased globally throughout recent decades (2) and represents a significant public health concern. The identification of potentially modifiable exposures that influence the development of adiposity in early life is crucial in order to address this epidemic. Nutrition in childhood can affect long-term cardiometabolic health (3), and is potentially amenable to cost-effective interventions; thus, it represents a promising means of preventing or reducing childhood obesity.

Polyunsaturated fatty acids (PUFA), the basic constituents of dietary fat, are potentially relevant nutritional factors in the etiology of chronic disease. These are fatty acids with more than one double bond in their carbon chain, classified primarily into the n-3 and n-6 families. Alpha-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6) are the essential fatty acids in each of these families and can only be obtained from diet. Through a series of enzymatic desaturation and elongation reactions, ALA and LA are converted endogenously into long-chain PUFA, which have a wide range of physiological roles in child development and in the regulation of cardiovascular function and inflammation (4).

In vitro and animal studies suggest that PUFA can influence body composition. The long-chain n-3 PUFA eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaneoic acid (DHA; 22:6 n-3) reduce adiposity and inhibit weight gain (5), while n-6 PUFA promote adipogenesis (6). However, results from studies in humans are mixed (7), and most investigations have been conducted in adults. Because PUFA are necessary for fetal development and are present in breast milk, a number of studies have focused on maternal PUFA intake during pregnancy or lactation,

or PUFA intake in infancy (8). These studies have found positive, inverse, and null associations between specific PUFA and adiposity throughout childhood and adolescence in both intervention and observational studies. Fewer studies have focused on PUFA in middle childhood. Of these, most are cross-sectional (9–18), and have produced inconclusive results regarding specific PUFA and measures of adiposity. One longitudinal study of 77 Japanese children found that changes in plasma DHA and dihomo-gamma-linolenic acid (DGLA; 20:3 n-6), a long-chain n-6 PUFA, from ages 10 to 13 y were positively associated with concomitant changes in height-standardized weight, while change in LA was inversely associated with weight change (19). In another study of Colombian children who were 5-12 y at baseline, serum ALA was inversely associated with estimated change in BMI-for-age Z scores between 6 and 14 y (20).

Middle childhood comprises a number of important developmental changes in body composition and adiposity (21,22), and this period is also the time during which the number of adipocytes in the body is determined (23). Therefore, middle childhood represents a potentially sensitive period during which diet quality might influence the development of obesity. Few studies have been conducted in low- and middle-income countries where childhood obesity is increasing rapidly (2), and the PUFA supply in diet is limited (24). Findings from higher-income settings may not be generalizable to these populations. In Latin America in particular, the public health burden of childhood obesity and chronic disease is high (25,26).

We conducted a longitudinal study of serum PUFA biomarkers and BMI change in a cohort of children from Santiago, Chile, who were recruited during the beginning of the country's nutrition transition. The aim of the study was to investigate associations between PUFA status at 5 and 10 y of age and change in BMI-for-age Z scores through adolescence.

Methods

Study design and population

This longitudinal investigation was conducted in a cohort of Chilean children who were followed from infancy through adolescence as participants of studies on the effects of iron status on neurodevelopment. Details of the original studies have been described previously (27,28). Briefly, participants were infants from low- and middle-income families living in Santiago, Chile, who were recruited between 1991 and 1996 when they were 4 to 6 months old. Eligible infants were singleton, born at term weighing at least 3 kg through uncomplicated, vaginal births, and did not have major health complications. Infants without iron deficiency anemia at recruitment were enrolled in a supplementation trial; they were randomly assigned to receive high or low- dose iron supplementation (either through drops or infant formula) or usual nutrition until they were 12 months old. Infants who were anemic at recruitment were treated with iron and enrolled in an observational neurodevelopment study, along with the next non-anemic infant. Of the 1798 infants originally enrolled, 1657 participants completed the trial and 135 completed the neurodevelopment study. Follow up continued during childhood; 888 children were assessed at age 5 y and 1127 children were examined at 10 y of age. A subset of 679 participants were assessed at 16 y of age as part of a study of obesity and cardiovascular risk. This group did not differ from the original cohort with respect to sociodemographic and nutritional characteristics including socioeconomic status, birth weight, or breastfeeding. Data used in the current investigation are from participants in the study at 16 y of age who had available serum samples at one or both of the 5 and 10 y time points.

Study procedures were approved by institutional review boards at the University of Chile Institute of Nutrition and Food Technology (INTA), the University of Michigan, and the

University of California, San Diego. Parents provided written informed consent to participate for their children and written assent was obtained from children beginning at age 10 y.

Data collection

Birth weight in g, birth length in cm, and gestational age in weeks were obtained from hospital records. Gestational age was estimated from the date of the mother's last menstrual period. At enrollment, study nutritionists collected information from parents on household socioeconomic indicators. Information on the date of the first bottle feeding and last breastfeeding was collected prospectively from mothers at weekly study visits between enrollment and 12 months of age. When infants had already been bottle fed at enrollment, mothers were asked to recall the date of the first bottle. Because feeding breast milk by bottle was rare in this population, bottle feeding was equivalent to providing infant formula or cow's milk.

Anthropometric measurements were collected at INTA at all time points by trained study personnel using standardized techniques. At 5, 10, and 16 y of age weight was measured to the nearest 100 g using a Seca scale (Seca, Hamburg, Germany) and height was measured to the nearest millimeter using a Holtain stadiometer (Holtain, Crymych, UK). Measures were obtained in duplicate and a third measurement was obtained if the first two differed by more than 0.3 kg or 0.5 cm.

Researchers obtained a blood sample by venipuncture at the 5 and 10 y assessments. Blood components were separated and serum samples were transported to the University of Michigan where they were cryostored at -80˚C.

Fatty acid analysis

Serum fatty acids were quantified at the University of Michigan Regional Comprehensive Metabolomics Resource Core. Total lipids were extracted from 200 □l of serum using the method described by Bligh and Dyer (29). 10 μL of 4 mM nonadecanoic acid (C19:0) was added as an internal standard. Boron trifluoride-methanol was used to derivatize the fatty acid portion of the total lipids into their methyl esters as previously described (30). To extract the methyl esters, a 2:1 hexane-water mixture was added and the sample was centrifuged. The hexane layer containing the methyl esters was removed from the aqueous layer and dried, and the methyl esters were re-suspended in 100-200 μL of hexane, depending on the volume of the original sample. Fatty acids were analyzed using gas chromatography (GC). 1-2 μL of sample was injected by autosampler onto an Agilent 680N GC (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector, a 100 m x 0.25 mm x 0.2 μm SP-2560 column (Sigma-Aldrich, Bellefonte, PA, USA), and Chemstation software for data analysis. To quantify fatty acids, known amounts of C19:0 and other authentic methyl esters were used to create a calibration curve. The authentic methyl esters were also used to identify fatty acids in samples based on their retention times. The coefficient of variation for GC analyses of specific fatty acids ranged between 2.5-3.6%.

Definition of exposures

The main exposures were serum biomarkers of n-3 and n-6 PUFA expressed as weight percentages of total fatty acids. We examined serum PUFA concentrations measured at the 5 y and 10 y assessments, and change in serum PUFA concentrations between the two time points. Among n-3 PUFA, we considered ALA, EPA, docosapentaenoic acid (22:5 n-3; DPA), and DHA. The n-6 PUFA were LA, gamma-linolenic acid (18:3 n-3; GLA), DGLA, and arachidonic

acid (20:4 n-6; AA). We also considered the GLA/LA and DGLA/AA ratios, as activity indices of the Δ6-desaturase (D6D) and Δ5-desaturase (D5D) enzymes, respectively. We categorized all exposures into quartiles to allow for non-linear associations with the outcomes.

Definition of outcomes

The primary outcomes were changes in body mass index (BMI), expressed as age- and sex-standardized Z scores (BAZ). We calculated BMI as kg/m^2, and converted it to BAZ using the World Health Organization Growth Reference for children ages 5-19 y (31). Using the 5 y PUFA biomarkers as exposures, we considered BAZ change from 5 to 16 y as the outcome. In the analyses of 10 y PUFA and 5-10 y change in PUFA concentrations, the outcome was change in BAZ between 10 and 16 y.

Covariates

Covariates were sociodemographic and anthropometric characteristics measured in infancy, and other serum fatty acids measured at 5 and 10 y. Birth length and birth weight were categorized as average or large for gestational age using the INTERGROWTH 21^{st} standards for newborn size (32). We defined large for gestational age as being $\geq 90^{th}$ percentile. Because birth weight ≥3 kg was one of the inclusion criteria into the study, there were no children $\leq 10^{th}$ percentile of weight for gestational age and thus we did not consider a category of small for gestational age. Seven children who were $\leq 10^{th}$ percentile for length were included in the average for gestational age category. Breastfeeding was categorized as <6 months (last breastfeeding before 180 days old), ≥6 months mixed breastfeeding (last breastfeeding on or after 180 days old and first bottle feeding on or before 180 days old), and ≥6 months exclusive breastfeeding (last breastfeeding on or after 180 days old and first bottle feeding after 180 days old or never). Iron supplementation from the original study was categorized as any vs. none by

combining the high and low dose supplement groups. Socioeconomic status (SES) in infancy was measured using a modified Graffar index (33). This index comprises 13 items related to family structure; education and employment of the head of household; crowding and physical condition of the home; and ownership of the home, car, and household appliances. Each item is scored from 0 to 5 for a possible score of 0 to 65 on the total index, with higher values indicating lower SES. We divided the index into quintiles. We also considered as covariates serum total *trans* fatty acids which have been related to weight gain among adults (34) and serum palmitoleic acid (16:1 n-7), which is associated with carbohydrate intake (35).

Statistical analysis

The final analytic samples consisted of children with serum PUFA and BAZ measured at 5 y and at least one subsequent BAZ measurement at 10 or 16 y of age (N=239), and children with PUFA measured at 10 y and BAZ measured at 10 and 16 y (N=418). The analysis of change in PUFA concentrations from 5 to 10 y of age in relation to change in BAZ from ages 10 to 16 y included 141 children with PUFA measurements at both 5 and 10 y and BAZ at 10 and 16 y of age.

Predictors of PUFA at 5 and 10 y of age

We first examined the distribution of PUFA biomarkers at ages 5 and 10 y by categories of covariates using means ± SD, to identify potential confounders of the PUFA-BAZ change associations. For dichotomous covariates we tested the statistical significance of the associations with use of Wald tests from linear regression models with each PUFA as the outcome and an indicator for the characteristic as a predictor. For categorical variables, we used a χ^2 score statistic. For ordinal covariates, we conducted tests for linear trend by introducing into the model a variable representing category-specific medians into the model as a continuous predictor.

PUFA at 5 y of age and BAZ change from ages 5 to 16 y

In bivariate analysis, we estimated mean ± SE BAZ at ages 5 and 16 y and BAZ change from 5 to 16 y of age by quartiles of PUFA biomarkers measured at age 5 y. These estimates were from growth curves calculated separately among children in each PUFA quartile with the use of mixed effects linear regression models as previously described (36). The outcome in the models was BAZ, and age was represented as a predictor using restricted cubic splines (37). These spline functions consist of piecewise cubic polynomials that are smoothly joined at the knots and constrained to be linear in the tails. They allow for flexible, smoothed modeling of non-linear changes in BAZ through childhood. We fitted these models using data from the 5, 10, and 16 y of age assessments, and placed the three knots at the median ages of children at each assessment. Predictors included indicators for each PUFA quartile, linear and spline terms for age, and interaction terms between the quartiles and age terms. The models also included random intercepts and age slopes for each child to account for within-child correlation of the BAZ measurements over time. Because these models do not require an equal number of observations per child, we included all available measurements in the model using the exact age of the children at each visit, in decimal years. From these models, we estimated the mean BAZ ± SE at 5 and 16 y, and the mean ± SE BAZ change between 5 and 16 y among children in each PUFA quartile.

In multivariable analysis, we obtained adjusted mean differences and 95% confidence intervals (CI) in BAZ change from ages 5 to 16 y between PUFA quartiles using adjusted mixed effects regression models. We included as adjustment variables the covariates that were associated with PUFA biomarkers in bivariate analysis or that are known predictors of BAZ change. The final models included sex, birth weight, breastfeeding, Graffar index, total serum

trans fatty acids, and palmitoleic acid measured at 5 y. In addition, we adjusted each PUFA for its measured concentration in infancy, and for other PUFA measured at 5 y that could confound the association with BAZ change but would not be intermediates on the causal pathway. All n-3 PUFA were adjusted for LA and total long-chain n-6 PUFA, while all n-6 PUFA were adjusted for ALA and total long-chain n-3 PUFA. Long chain PUFA were also adjusted for their immediate precursor. All covariate fatty acids were represented using restricted cubic splines with knots placed at the 5^{th}, 50^{th}, and 95^{th} percentiles of their distribution in order to account for possible non-linearity of their associations with the outcome. All models were fitted using empirical variance estimates, which are robust to heteroskedasticity and deviations from normality (38).

In supplemental analyses, we determined whether associations between PUFA and BAZ change differed by baseline weight status by stratifying the analyses between normal-weight participants (BAZ ≤1) and children with overweight/obesity (BAZ >1) at the age 5 y assessment. We tested the statistical significance of interaction terms between baseline weight status and PUFA predictors with the use of three-way cross-product terms between PUFA, age terms, and weight status.

PUFA at 10 y of age and BAZ change from ages 10 to 16 y

In bivariate analysis, we estimated mean ± SE BAZ at ages 10 and 16 y and BAZ changes from 10 to 16 y of age by quartiles of PUFA biomarkers measured at the age 10 y assessment. These estimates were from mixed effects linear regression models fitted using data from the ages 10 and 16 y assessments. The outcome was BAZ and predictors included indicator variables for PUFA quartiles, a linear term for age, and interaction terms between each quartile and age. Models also included random intercepts for each child.

In multivariable analysis, we obtained adjusted mean differences with 95% CI in BAZ change from 10 to 16 y of age between PUFA quartiles using the first quartile as reference. Models were adjusted for sex, birth weight, breastfeeding, Graffar index, the main exposure PUFA measured in infancy, and serum *trans* fatty acids and palmitoleic acid measured at 10 y of age. Additionally, we included other PUFA biomarkers assessed at age 10 y as described for the models with age 5 y PUFA as the main exposures. We did not adjust for any PUFA measured at 5 y because 277 children lacked this information. We conducted supplemental analyses stratified by weight status at 10 y by including an indicator variable for overweight/obesity and interaction terms between this variable and PUFA exposures in the models.

Change in PUFA concentrations from ages 5 to 10 y and change in BAZ from ages 10 to 16 y

In the subset of children with data on serum PUFA at both 5 and 10 y of age (N=141), we calculated the change in serum concentrations of each PUFA by subtracting the measurements at the 5 y assessment from those at the 10 y assessment. Next, we estimated mean ± SE BAZ at ages 10 and 16 y and BAZ change from ages 10 to 16 y by quartiles of change in PUFA following an analogous approach to that described for age 10 y PUFA as main exposures. In addition to baseline characteristics, mean differences in BAZ change were further adjusted for change in total *trans* fatty acids, palmitoleic acid, and other PUFA between ages 5 and 10 y.

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

Results

PUFA at 5 y of age. Among children included in the analysis of 5 y serum PUFA (N=239), 11, 33, and 195 had measurements at ages 10 y only, 16 y only, and both 10 and 16 y, respectively. Fifty-three percent were boys. Mean ± SD BAZ at 5, 10, and 16 y of age were, respectively, 0.94 ± 1.17, 1.00 ± 1.14, and 0.67 ± 1.18. Forty-four percent had overweight or obesity at 5 y of age.

Predictors of PUFA at 5 y of age. Serum ALA concentrations were higher among children who had been breastfed <6 months compared with those who had been breastfed ≥6 months (**Table 1**). Socioeconomic status in infancy was positively associated with EPA and DGLA; whereas iron supplementation was related to higher DGLA. Serum AA was higher among children born tall for gestational age.

BAZ change from ages 5 to 16 y. In bivariate analysis, DPA and DHA at age 5 y were each inversely associated with BAZ change (**Table 2**). After adjustment, the association with DPA was not statistically significant; however, DHA remained inversely associated with BAZ change (P, trend=0.03). Compared with children in the lowest quartile of serum DHA, BAZ change among those in the highest quartile was 0.51 Z scores lower (95% CI: -0.88, -0.13; P=0.008). This association did not differ significantly between normal weight children and children with overweight or obesity (P, interaction=0.07) (**Supplemental Table 1**). None of the n-6 PUFA were associated with BAZ change.

LA was inversely associated with BAZ change among children with overweight or obesity only (P, trend=0.02) (P, interaction=0.004) (**Supplemental Table 1**).

PUFA at 10 y of age. Among children included in the analysis of 10 y PUFA (N=418), 53% were boys. Mean ± SD BAZ at 10 and 16 y of age were 1.03 ± 1.15 and 0.70 ± 1.12; 55% had overweight or obesity at 10 y of age.

Predictors of PUFA at 10 y of age. At 10 y, ALA was lower among boys compared with girls (**Table 3**). EPA was inversely associated with birth weight, whereas DPA was positively associated with iron supplementation. LA was lower among boys than girls. GLA and the D6D activity index were each positively associated with birth length and weight.

BAZ change from ages 10 to 16 y. None of the n-3 PUFA were significantly associated with change in BAZ (**Table 4**). In bivariate analysis, AA was positively associated with BAZ change (P, trend=0.02), as was the D5D activity index (P, trend=0.03). After adjustment, children in the lowest quartile of AA had a mean change in BAZ 0.17 Z scores lower (95% CI: -0.34, -0.01; P=0.04) than did children in the highest three quartiles. The association with D5D was attenuated and became non-statistically significant. None of the associations differed by 10 y weight status at age 10 y (**Supplemental Table 2**).

Change in PUFA concentrations between 5 and 10 y of age. In bivariate analysis, no changes in n-3 PUFA were significantly associated with BAZ change from 10 to 16 y of age (**Table 5**). After adjustment, change in EPA was inversely associated with change in BAZ. Children in the highest quartile of EPA change had a 0.32 Z score lower change in BAZ (95% CI: -0.64, 0.00; P, trend=0.04).

In bivariate analysis of n-6 PUFA, change in the D5D activity index was positively associated with 10-16 y BAZ change. After adjustment, children in the highest quartile of D5D activity change had a mean change in BAZ 0.64 Z scores higher than children in the lowest quartile (95% CI: 0.27, 1.02; P=0.001). Change in AA was also positively associated with

change in BAZ after adjustment. Mean BAZ change among children in the highest quartile was 0.51 Z scores higher than among those in the lowest quartile (95% CI: 0.11, 0.90; P=0.01).

Discussion

In this longitudinal study of Chilean children, serum DPA and DHA at 5 y of age were inversely associated with BAZ change between 5 and 16 y of age, while serum AA at 10 y of age or change in AA from 5 to 10 y were positively associated with BAZ from 10 to 16 y. Increased EPA from 5 to 10 y was related to less change in BAZ between 10 and 16 y, while increased D5D activity was associated with higher BAZ change.

BAZ in this population was generally high, likely as a result of the ongoing nutrition transition in Chile at the time of measurement (39). Nearly half and over half of children had overweight or obesity at 5 and 10 y of age, respectively, according to World Health Organization cut points (40). At high percentiles, BMI in childhood is strongly correlated with fat mass (41), and the World Health Organization BAZ cut points have a high specificity for diagnosing overweight (42). Thus, in the context of this study, BAZ likely mainly reflects adiposity rather than fat-free mass, and greater changes in BAZ represent increases in adiposity above levels that are already associated with adverse health outcomes such as high serum triglycerides and insulin resistance (43).

We found that when children were 5 y old, DPA and DHA were inversely associated with change in BAZ through 16 y of age. Most previous studies of these PUFA and BMI in middle childhood are cross-sectional in design. Of these, three (10–12) found an inverse association between plasma or serum DHA and either BMI or obesity, one found a positive association between DHA in adipose tissue and overweight or obesity (15), and seven others (9,13,14,16–19) found no association between DHA in plasma, erythrocytes, or whole blood and BMI or obesity. In one study of 77 Japanese children, a 3-year change in plasma DHA was positively associated with concomitant change in height-standardized weight among boys only (19). In a

longitudinal study of 668 Colombian children, serum DHA at baseline (when the children were 5-12 y old) was not associated with change in BAZ estimated between 6 and 14 y of age (20). Finally, two small randomized trials of supplementation with either EPA+DHA for 1 month (44) or DHA alone for 6 months (45) found no effect on BMI. The relation of DPA in plasma or erythrocytes and BMI among children was studied mostly in a few cross-sectional studies. Of these, all three (13,14,17) found no association. There are several potential explanations for discrepancies between our results and those of previous investigations. Many of the cross-sectional studies are small and in some the authors did not adjust for potential confounders in their analyses. Moreover, the findings from these studies could be biased by reverse causation, since adiposity itself could influence PUFA levels through effects on the activity of desaturase enzymes (46). The age ranges of children in these studies were either entirely older than 5 y or included 5-y-old children only as the very youngest participants, so discrepancies may reflect true differences in the associations at different points of middle childhood. Consistent with this notion, we found no association between DHA at 10 y of age and BAZ change. A difference in participants' ages may explain the discrepancy between our findings and those of the study in Colombia, since most of the participating children were older than 5 y at baseline when serum PUFA concentrations were measured. Finally, the two randomized interventions may have not had effects because the intervention periods were too short to effect changes in BMI.

There are several mechanisms that could explain a protective effect of DHA against the development of adiposity. Long-chain n-3 fatty acids may reduce total caloric intake by increasing feelings of satiety (47). They may also regulate lipid metabolism by promoting lipolysis and β-oxidation and inhibiting lipogenesis, thus reducing fat deposition in adipose tissue (48). Our finding that DHA was associated with BAZ change when measured at 5 y of age

but not at 10 y may reflect a greater influence of this PUFA in earlier childhood on long-term body composition. The adiposity rebound, an inflexion point in growth when BMI begins to increase, starts at 5 y of age, on average, and is followed approximately 2 years later by an increase in fat mass (21). The timing of the adiposity rebound is related to risk of adult obesity (49); thus, a reduction in adiposity by DHA at this developmental stage may be particularly influential on adiposity trajectories. However, we cannot rule out selection bias as a possible explanation for the findings, since 277 children with information on PUFA at 10 y of age lacked measurements at 5 y of age.

We found an inverse association between change in EPA from 5 to 10 y of age and BAZ change from 10 to 16 y. Most previous investigations have found no association (10,11,14–16,18,20) or a positive (9,19) relation between EPA and BMI or obesity in children. Results from our study are consistent with the proposed mechanism of inhibition of adipose tissue deposition by long-chain n-3 PUFA, but it is unclear why the associations of PUFA at age 5 y and PUFA change between 5 and 10 y of age are inconsistent. It is possible that EPA and DHA are associated with BAZ change through different mechanisms that operate during different periods of development. The association with EPA may also be spurious.

Serum AA at 10 y of age and change in AA between ages 5 and 10 y were each positively associated with BAZ change between 10 and 16 y. AA may promote adiposity through production of the eicosanoid prostacyclin, which promotes differentiation of preadipocytes into adipocytes (50). Adipose tissue AA was positively associated with obesity among 10- to 12-y-old children in one previous study (15). However, plasma, serum, or erythrocyte AA was not associated with BMI or obesity in most previous cross-sectional studies (9–11,13,14,18,19,51), or with change in standardized weight among Japanese children (19) or BAZ change in the

longitudinal study of Colombian children (20). Some of the discrepancies between our results and previous studies may be related to the fact that some did not adjust for potential confounders, or because of differences in the range of AA levels present in different study populations. Mean serum AA weight percentages ranged from 6.0 among the Colombian children to 8.3-9.1 in the Japanese compared with 3.2 among the Chilean children at 10 y of age. This may reflect differences in intake or endogenous metabolism of AA that also affect the association with BAZ change.

Change in the D5D activity index between 5 and 10 y of age was positively associated with BAZ change from 10 to 16 y. Few previous studies have assessed this relation in children, and the results have been inconclusive (12,14,18,19,51,52). In two longitudinal studies, change in D5D activity over 3 y was inversely associated with simultaneous change in height-standardized weight among Japanese children (19), and baseline D5D activity was inversely associated with BMI 2 y later in a European cohort (52). Moreover, higher D5D activity in adults is inversely associated with a number of other cardiovascular risk factors including triglyceride levels and insulin resistance, and with incident diabetes and cardiovascular disease (53). We noted that change in the D5D activity index was inversely associated with BAZ at 10 y of age, but independent of BAZ at age 16 y. It is possible that D5D activity is inversely related to BAZ but only in the short term.

We did not find overall associations between ALA or LA and BAZ changes, although LA at 5 y of age was inversely associated with BAZ change among overweight and obese children. In previous cross-sectional studies, biomarkers of ALA have been either positively associated (9,13,14,17,18) or not associated (10,11,15,19) with measures of adiposity, while associations with LA have been either inverse (9,13,18) or null (10,11,14,15,19). Three year change in

plasma ALA was not associated with concomitant change in weight among Japanese children, while there was an inverse association between LA and weight changes (19). Mean ALA and LA concentrations among the Japanese children were much lower than those in our study population, which may explain the discrepancy. The Japanese children may also have been generally heavier than children in our study, which would be consistent with our finding that LA was only inversely associated with BAZ change among overweight/obese children. However, because the authors did not report BMI or BAZ in the Japanese study but rather a different measure of standardized weight, it is difficult to compare the relative levels of adiposity in the two populations. This study also assessed simultaneous changes in PUFA and weight, which may reflect reverse causation or short-term effects of ALA and LA that differ from associations with longer-term adiposity. Among Colombian children, serum ALA was inversely associated with 6-14 y BAZ change (20). Levels of these PUFA present in the Colombian population were comparable to those among children in the present study, and we did not find that associations between ALA and BAZ change differed by baseline BAZ. There may be other aspects of dietary context that differ between the two populations and modify or confound the ALA-BAZ change.

One of the limitations of this study is that most covariates were only measured in infancy, rather than concurrently with the exposures, so there may be residual confounding by sociodemographic characteristics or diet in childhood. Some of our results may be driven in part by associations between PUFA and BAZ at baseline, which could be the result of reverse causation; thus the true associations with BAZ change may be attenuated compared with those we report. Nevertheless, we chose not to adjust for baseline BAZ in our primary analyses of BAZ change, since this adjustment itself can induce bias (54). For this reason, the results of our analyses stratified by baseline weight status should be interpreted with caution.

One of the primary strengths of this study is the prospective design, which greatly limits the possibility of reverse causation compared to previous cross-sectional studies. Compared with dietary measures, the use of PUFA biomarkers is not subject to recall bias or systematic errors from the use of food composition tables. Our study provides information on a topic of great public health importance that has not been frequently studied, especially in populations undergoing the nutrition transition. Finally, the availability of multiple exposure and outcome measurements during follow up allowed us to assess these associations separately during different developmental periods.

In conclusion, serum concentrations of some long-chain n-3 PUFA in middle childhood are associated with less weight gain through adolescence whereas the n-6 PUFA AA and D5D activity are related to increased weight gain. Future studies should examine whether the observed associations between PUFA in childhood and BAZ change through adolescence persist and predict health outcomes in adulthood. The effect of dietary interventions involving long-chain n-3 PUFA in childhood on the development of adiposity requires investigation in randomized trials.

Acknowledgements

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Table 4.1. Serum biomarkers of polyunsaturated fatty acids[a] at 5 y by categories of sociodemographic characteristics in infancy and other fatty acids at 5 y among children from Santiago, Chile

		N-3				N-6					
Characteristics	N	ALA 18:3(n-3)	EPA 20:5(n-3)	DPA 22:5(n-3)	DHA 22:6(n-3)	LA 18:2(n-6)	GLA 18:3(n-6)	DGLA 20:3(n-6)	AA 20:4(n-6)	Δ6-desaturase activity index (GLA/LA)	Δ5-desaturase activity index (AA/DGLA)
Overall	239	0.30 ± 0.16	0.27 ± 0.12	0.17 ± 0.09	0.62 ± 0.54	27.14 ± 4.44	0.21 ± 0.18	0.77 ± 0.36	1.78 ± 0.50	0.008 ± 0.008	4.23 ± 6.80
Infancy sociodemographic characteristics											
Sex											
Female	113	0.30 ± 0.15	0.27 ± 0.13	0.17 ± 0.09	0.63 ± 0.74	27.51 ± 4.05	0.20 ± 0.16	0.74 ± 0.31	1.74 ± 0.49	0.007 ± 0.006	4.09 ± 5.82
Male	126	0.30 ± 0.17	0.27 ± 0.12	0.17 ± 0.08	0.61 ± 0.25	26.81 ± 4.75	0.23 ± 0.20	0.80 ± 0.39	1.81 ± 0.50	0.009 ± 0.010	4.36 ± 7.59
P[b]		0.89	0.69	0.80	0.80	0.22	0.24	0.17	0.30	0.07	0.76
Birth length											
Average for gestational age[c]	157	0.30 ± 0.16	0.27 ± 0.12	0.17 ± 0.08	0.62 ± 0.64	26.98 ± 4.57	0.22 ± 0.18	0.78 ± 0.37	1.73 ± 0.48	0.008 ± 0.008	3.79 ± 5.98
Large for gestational age	82	0.29 ± 0.15	0.27 ± 0.13	0.18 ± 0.10	0.63 ± 0.26	27.45 ± 4.19	0.21 ± 0.18	0.75 ± 0.32	1.87 ± 0.52	0.008 ± 0.008	5.07 ± 8.12
P[b]		0.77	0.75	0.19	0.86	0.42	0.73	0.64	0.04	0.90	0.21
Birth weight											
Average for gestational age	168	0.30 ± 0.15	0.27 ± 0.12	0.17 ± 0.07	0.62 ± 0.62	27.08 ± 4.48	0.22 ± 0.18	0.77 ± 0.37	1.75 ± 0.47	0.008 ± 0.008	4.05 ± 6.65
Large for gestational age	71	0.28 ± 0.19	0.27 ± 0.14	0.18 ± 0.11	0.61 ± 0.24	27.29 ± 4.37	0.21 ± 0.18	0.76 ± 0.32	1.84 ± 0.56	0.008 ± 0.008	4.65 ± 7.18
P[b]		0.48	0.98	0.22	0.79	0.73	0.88	0.72	0.22	0.81	0.55
Breastfeeding											
Breastfeeding <6 mo	57	0.35 ± 0.19	0.26 ± 0.12	0.17 ± 0.10	0.74 ± 1.01	26.86 ± 4.34	0.22 ± 0.16	0.80 ± 0.50	1.78 ± 0.50	0.009 ± 0.007	3.80 ± 4.48
Mixed bottle/breastfeeding, ≥6 mo	95	0.28 ± 0.15	0.27 ± 0.13	0.17 ± 0.09	0.56 ± 0.24	27.01 ± 4.69	0.20 ± 0.19	0.74 ± 0.30	1.76 ± 0.51	0.008 ± 0.009	4.67 ± 7.80
Exclusive breastfeeding, ≥6 mo	84	0.29 ± 0.14	0.27 ± 0.12	0.17 ± 0.08	0.60 ± 0.23	27.20 ± 4.03	0.22 ± 0.19	0.77 ± 0.30	1.79 ± 0.49	0.008 ± 0.008	4.10 ± 7.04
P[d]		0.05	0.70	0.99	0.26	0.89	0.76	0.57	0.91	0.91	0.68
Iron supplementation											
None	155	0.31 ± 0.17	0.28 ± 0.13	0.17 ± 0.08	0.59 ± 0.25	27.38 ± 4.09	0.20 ± 0.16	0.80 ± 0.38	1.78 ± 0.51	0.008 ± 0.007	3.91 ± 6.32
Any	84	0.27 ± 0.14	0.26 ± 0.11	0.18 ± 0.10	0.68 ± 0.84	26.69 ± 5.01	0.24 ± 0.21	0.71 ± 0.30	1.78 ± 0.48	0.009 ± 0.010	4.82 ± 7.61
P[b]		0.07	0.22	0.55	0.35	0.28	0.21	0.05	0.93	0.19	0.35

Table 4.1. Serum biomarkers of polyunsaturated fatty acids[a] at 5 y by categories of sociodemographic characteristics in infancy and other fatty acids at 5 y among children from Santiago, Chile

		N-3				N-6					
Characteristics	N	ALA 18:3(n-3)	EPA 20:5(n-3)	DPA 22:5(n-3)	DHA 22:6(n-3)	LA 18:2(n-6)	GLA 18:3(n-6)	DGLA 20:3(n-6)	AA 20:4(n-6)	Δ6-desaturase activity index (GLA/LA)	Δ5-desaturase activity index (AA/DGLA)
Graffar index[e]											
Q1 (high SES)	50	0.29 ± 0.11	0.31 ± 0.14	0.17 ± 0.06	0.60 ± 0.25	27.85 ± 4.02	0.24 ± 0.19	0.90 ± 0.49	1.85 ± 0.56	0.009 ± 0.008	2.90 ± 3.58
Q2	45	0.28 ± 0.14	0.26 ± 0.12	0.16 ± 0.07	0.65 ± 0.27	27.53 ± 3.79	0.22 ± 0.15	0.81 ± 0.30	1.83 ± 0.53	0.008 ± 0.006	3.35 ± 4.17
Q3	57	0.31 ± 0.21	0.26 ± 0.12	0.17 ± 0.09	0.56 ± 0.22	26.50 ± 4.41	0.18 ± 0.17	0.70 ± 0.33	1.73 ± 0.42	0.007 ± 0.008	5.27 ± 8.32
Q4	46	0.29 ± 0.15	0.26 ± 0.12	0.16 ± 0.08	0.74 ± 1.12	27.75 ± 4.78	0.20 ± 0.18	0.74 ± 0.30	1.81 ± 0.58	0.007 ± 0.006	4.32 ± 7.02
Q5 (low SES)	41	0.31 ± 0.15	0.24 ± 0.10	0.19 ± 0.12	0.56 ± 0.25	26.04 ± 5.05	0.24 ± 0.20	0.70 ± 0.30	1.65 ± 0.37	0.010 ± 0.011	5.27 ± 9.05
P, trend[f]		0.44	0.007	0.31	0.86	0.13	0.89	0.01	0.06	0.56	0.12
5 y serum fatty acids (median, weight % of total fatty acids)											
Total *trans* fatty acids											
Q1 (1.58)	59	0.30 ± 0.14	0.28 ± 0.13	0.18 ± 0.08	0.75 ± 0.27	29.04 ± 4.40	0.27 ± 0.22	0.79 ± 0.31	1.95 ± 0.48	0.010 ± 0.010	4.13 ± 6.87
Q2 (2.24)	60	0.31 ± 0.17	0.30 ± 0.14	0.17 ± 0.05	0.71 ± 0.98	28.02 ± 3.78	0.22 ± 0.18	0.83 ± 0.48	1.87 ± 0.56	0.008 ± 0.008	4.70 ± 7.62
Q3 (2.88)	60	0.29 ± 0.15	0.25 ± 0.12	0.18 ± 0.08	0.56 ± 0.21	26.67 ± 4.06	0.18 ± 0.12	0.72 ± 0.34	1.70 ± 0.43	0.007 ± 0.005	5.01 ± 7.74
Q4 (4.06)	60	0.29 ± 0.17	0.24 ± 0.10	0.16 ± 0.12	0.46 ± 0.18	24.86 ± 4.45	0.19 ± 0.17	0.74 ± 0.26	1.59 ± 0.45	0.008 ± 0.008	3.08 ± 4.47
P, trend[f]		0.53	0.008	0.43	<0.0001	<0.0001	0.02	0.13	<0.0001	0.33	0.25
Palmitoleic acid 16:1(n-7)											
Q1 (1.07)	59	0.27 ± 0.15	0.29 ± 0.13	0.17 ± 0.09	0.79 ± 0.99	28.55 ± 4.97	0.23 ± 0.21	0.76 ± 0.47	1.89 ± 0.52	0.009 ± 0.011	4.51 ± 7.15
Q2 (1.38)	60	0.29 ± 0.15	0.29 ± 0.14	0.18 ± 0.07	0.59 ± 0.23	28.31 ± 3.58	0.19 ± 0.17	0.77 ± 0.33	1.80 ± 0.49	0.007 ± 0.006	4.65 ± 7.39
Q3 (1.64)	60	0.29 ± 0.18	0.24 ± 0.10	0.17 ± 0.07	0.57 ± 0.22	27.46 ± 4.12	0.21 ± 0.19	0.73 ± 0.32	1.81 ± 0.49	0.008 ± 0.008	4.51 ± 6.37
Q4 (2.12)	60	0.33 ± 0.16	0.26 ± 0.12	0.18 ± 0.11	0.54 ± 0.22	24.26 ± 3.67	0.22 ± 0.14	0.82 ± 0.28	1.61 ± 0.46	0.009 ± 0.006	3.25 ± 6.30
P, trend[f]		0.05	0.07	0.83	0.05	<0.0001	0.95	0.44	0.001	0.60	0.26

[a]Expressed as percentage of total fatty acids by weight.

[b]Wald test from linear regression models with each fatty acid as the outcome and an indicator variable for the characteristic as a predictor.

[c]Includes 7 children who were small for gestational age according to birth length.

[d]χ^2 score statistic from linear regression models with each fatty acid as the outcome and indicator variables for levels of the characteristic as predictors.

[e]Index of socioeconomic status that includes information on family structure, education and employment of the head of household, crowding and physical condition of the home, and ownership of the home, car, and household appliances (33). Higher values indicate lower socioeconomic status.

[f]Wald test from linear regression models with each fatty acid as the outcome and a variable representing category-specific medians of an ordinal characteristic introduced as a continuous predictor.

Table 4.2. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 5 years of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	5 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	5 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	5 y - 16 y Adjusted difference in change (95% CI)[c]
Overall	239	0.93 ± 0.08	0.70 ± 0.07	-0.23 ± 0.06	
N-3					
ALA (18:3 n-3)					
Q1 (0.12)	59	0.93 ± 0.16	0.68 ± 0.16	-0.25 ± 0.13	Reference
Q2 (0.25)	60	0.64 ± 0.14	0.59 ± 0.12	-0.05 ± 0.13	0.30 (-0.08, 0.67)
Q3 (0.34)	60	1.12 ± 0.16	0.76 ± 0.16	-0.36 ± 0.12	0.06 (-0.29, 0.41)
Q4 (0.45)	60	1.05 ± 0.15	0.78 ± 0.15	-0.26 ± 0.11	0.05 (-0.30, 0.39)
P, trend[d]		0.29	0.54	0.56	0.94
EPA (20:5 n-3)					
Q1 (0.12)	59	0.87 ± 0.14	0.71 ± 0.14	-0.16 ± 0.13	Reference
Q2 (0.22)	60	0.90 ± 0.16	0.60 ± 0.15	-0.30 ± 0.11	-0.16 (-0.50, 0.18)
Q3 (0.30)	60	1.10 ± 0.18	0.87 ± 0.15	-0.23 ± 0.12	-0.09 (-0.43, 0.25)
Q4 (0.41)	60	0.86 ± 0.14	0.63 ± 0.15	-0.23 ± 0.13	-0.06 (-0.44, 0.32)
P, trend		0.84	0.95	0.77	0.84
DPA (22:5 n-3)					
Q1 (0.11)	59	0.91 ± 0.14	0.88 ± 0.14	-0.02 ± 0.12	Reference
Q2 (0.14)	60	0.74 ± 0.15	0.52 ± 0.16	-0.21 ± 0.11	-0.23 (-0.57, 0.10)
Q3 (0.18)	60	0.93 ± 0.14	0.63 ± 0.16	-0.30 ± 0.14	-0.31 (-0.70, 0.08)
Q4 (0.23)	60	1.15 ± 0.18	0.79 ± 0.14	-0.36 ± 0.12	-0.31 (-0.68, 0.07)
P, trend		0.18	0.93	0.05	0.13
DHA (22:6 n-3)					
Q1 (0.32)	59	0.70 ± 0.15	0.72 ± 0.15	0.02 ± 0.12	Reference
Q2 (0.48)	60	0.97 ± 0.15	0.62 ± 0.13	-0.35 ± 0.13	-0.37 (-0.74, -0.01)
Q3 (0.62)	60	0.93 ± 0.15	0.87 ± 0.16	-0.07 ± 0.13	0.00 (-0.36, 0.35)
Q4 (0.90)	60	1.12 ± 0.16	0.60 ± 0.15	-0.51 ± 0.10	-0.51 (-0.88, -0.13)
P, trend		0.09	0.73	0.004	0.03

Table 4.2. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 5 years of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	5 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	5 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	5 y - 16 y Adjusted difference in change (95% CI)[c]
N-6					
LA (18:2 n-6)					
Q1 (21.58)	59	0.92 ± 0.15	0.69 ± 0.15	-0.23 ± 0.10	Reference
Q2 (26.06)	60	1.05 ± 0.15	0.80 ± 0.14	-0.25 ± 0.12	-0.07 (-0.40, 0.25)
Q3 (28.99)	60	1.08 ± 0.16	0.81 ± 0.14	-0.27 ± 0.12	-0.08 (-0.40, 0.23)
Q4 (32.02)	60	0.68 ± 0.15	0.50 ± 0.16	-0.18 ± 0.15	0.03 (-0.38, 0.44)
P, trend		0.40	0.49	0.84	0.95
GLA (18:3 n-6)					
Q1 (0.07)	59	0.65 ± 0.14	0.58 ± 0.14	-0.06 ± 0.13	Reference
Q2 (0.11)	60	0.90 ± 0.16	0.66 ± 0.15	-0.24 ± 0.14	-0.06 (-0.44, 0.32)
Q3 (0.21)	60	0.85 ± 0.14	0.54 ± 0.16	-0.30 ± 0.11	-0.07 (-0.42, 0.29)
Q4 (0.40)	60	1.33 ± 0.16	1.01 ± 0.15	-0.32 ± 0.11	-0.06 (-0.41, 0.29)
P, trend		0.004	0.03	0.20	0.80
DGLA (20:3 n-6)					
Q1 (0.35)	59	0.73 ± 0.15	0.53 ± 0.15	-0.20 ± 0.12	Reference
Q2 (0.71)	60	1.03 ± 0.15	0.77 ± 0.15	-0.26 ± 0.14	-0.06 (-0.41, 0.29)
Q3 (0.88)	60	0.77 ± 0.14	0.62 ± 0.13	-0.15 ± 0.10	0.13 (-0.19, 0.46)
Q4 (1.08)	60	1.20 ± 0.17	0.89 ± 0.16	-0.30 ± 0.13	0.05 (-0.31, 0.41)
P, trend		0.10	0.14	0.71	0.64
AA (20:4 n-6)					
Q1 (1.17)	59	0.81 ± 0.14	0.61 ± 0.15	-0.19 ± 0.13	Reference
Q2 (1.58)	60	0.94 ± 0.15	0.73 ± 0.14	-0.21 ± 0.12	0.00 (-0.41, 0.40)
Q3 (1.93)	60	0.85 ± 0.17	0.60 ± 0.15	-0.25 ± 0.10	0.01 (-0.35, 0.38)
Q4 (2.28)	60	1.13 ± 0.16	0.87 ± 0.16	-0.26 ± 0.14	-0.07 (-0.48, 0.35)
P, trend		0.19	0.35	0.68	0.78
Δ6-Desaturase index (GLA/LA)					
Q1 (0.002)	59	0.74 ± 0.16	0.60 ± 0.14	-0.13 ± 0.14	Reference
Q2 (0.004)	60	0.85 ± 0.14	0.66 ± 0.14	-0.20 ± 0.13	0.01 (-0.37, 0.40)
Q3 (0.008)	60	0.84 ± 0.15	0.55 ± 0.16	-0.29 ± 0.11	-0.02 (-0.39, 0.34)
Q4 (0.016)	60	1.30 ± 0.16	0.99 ± 0.14	-0.31 ± 0.11	-0.02 (-0.38, 0.35)
P, trend		0.009	0.04	0.32	0.87

Table 4.2. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 5 years of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	5 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	5 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	5 y - 16 y Adjusted difference in change (95% CI)[c]
Δ5-Desaturase index (AA/DGLA)					
Q1 (1.54)	59	0.95 ± 0.16	0.66 ± 0.15	-0.29 ± 0.11	Reference
Q2 (1.96)	60	1.02 ± 0.16	0.80 ± 0.14	-0.22 ± 0.14	0.08 (-0.27, 0.43)
Q3 (2.43)	60	0.82 ± 0.15	0.67 ± 0.15	-0.15 ± 0.12	0.08 (-0.27, 0.43)
Q4 (4.64)	60	0.94 ± 0.16	0.68 ± 0.16	-0.26 ± 0.12	-0.01 (-0.34, 0.32)
P, trend		0.94	0.86	0.91	0.72

[a]According to the World Health Organization Growth Reference 2007 (31) for children ages 5-19 y.

[b]Estimates are from growth curves created using mixed effects linear regression models with BMI-for-age Z score as the outcome, and predictors that included indicator variables for each PUFA quartile, linear and cubic spline terms for age, and interaction terms between the quartiles and age terms. All models included random intercepts and age slopes to account for within-child correlation of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs. average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), total serum trans fatty acids, palmitoleic acid, and serum concentrations of the PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA, while all n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. All long-chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[d]From mixed effects linear regression models with Z score as the outcome and a variable representing medians of PUFA quartiles introduced as a continuous predictor.

Table 4.3. Serum biomarkers of polyunsaturated fatty acids[a] at 10 y by categories of sociodemographic characteristics in infancy and other fatty acids at 10 y among children from Santiago, Chile

		N-3				N-6					
Characteristics	N	ALA 18:3(n-3)	EPA 20:5(n-3)	DPA 22:5(n-3)	DHA 22:6(n-3)	LA 18:2(n-6)	GLA 18:3(n-6)	DGLA 20:3(n-6)	AA 20:4(n-6)	Δ6-desaturase activity index (GLA/LA)	Δ5-desaturase activity index (AA/DGLA)
Overall	418	0.42 ± 0.17	0.26 ± 0.14	0.21 ± 0.06	0.65 ± 0.22	30.99 ± 4.26	0.20 ± 0.17	1.18 ± 0.30	3.18 ± 0.88	0.007 ± 0.006	2.92 ± 1.87
Infancy sociodemographic characteristics											
Sex											
Female	198	0.44 ± 0.18	0.25 ± 0.15	0.21 ± 0.06	0.66 ± 0.22	31.46 ± 4.50	0.21 ± 0.18	1.15 ± 0.27	3.10 ± 0.87	0.007 ± 0.006	2.81 ± 0.96
Male	220	0.40 ± 0.16	0.27 ± 0.13	0.22 ± 0.06	0.64 ± 0.22	30.57 ± 4.00	0.19 ± 0.16	1.20 ± 0.32	3.24 ± 0.89	0.006 ± 0.006	3.02 ± 2.41
P[b]		0.05	0.37	0.14	0.48	0.03	0.32	0.08	0.10	0.59	0.23
Birth length											
Average for gestational age[c]	279	0.42 ± 0.17	0.27 ± 0.14	0.21 ± 0.06	0.65 ± 0.21	30.87 ± 4.31	0.18 ± 0.13	1.18 ± 0.30	3.14 ± 0.83	0.006 ± 0.005	2.91 ± 2.15
Large for gestational age	139	0.42 ± 0.17	0.25 ± 0.13	0.22 ± 0.07	0.66 ± 0.24	31.22 ± 4.17	0.24 ± 0.23	1.18 ± 0.31	3.26 ± 0.96	0.008 ± 0.008	2.94 ± 1.14
P[b]		0.78	0.11	0.37	0.61	0.42	0.002	0.99	0.21	0.005	0.87
Birth weight											
Average for gestational age	305	0.42 ± 0.16	0.27 ± 0.14	0.21 ± 0.06	0.65 ± 0.21	31.02 ± 4.17	0.18 ± 0.14	1.17 ± 0.29	3.19 ± 0.86	0.006 ± 0.005	2.97 ± 2.09
Large for gestational age	113	0.43 ± 0.20	0.23 ± 0.11	0.21 ± 0.06	0.64 ± 0.24	30.90 ± 4.52	0.25 ± 0.23	1.21 ± 0.33	3.15 ± 0.92	0.008 ± 0.008	2.78 ± 1.07
P[b]		0.75	0.002	0.81	0.62	0.81	0.001	0.32	0.74	0.001	0.23
Breastfeeding											
Breastfeeding <6 mo	148	0.43 ± 0.17	0.25 ± 0.14	0.21 ± 0.07	0.66 ± 0.22	30.72 ± 4.10	0.22 ± 0.15	1.17 ± 0.31	3.21 ± 0.86	0.007 ± 0.005	3.16 ± 2.87
Mixed bottle/breastfeeding, ≥6 mo	147	0.41 ± 0.15	0.27 ± 0.14	0.22 ± 0.06	0.65 ± 0.20	31.08 ± 4.41	0.19 ± 0.17	1.17 ± 0.30	3.19 ± 0.87	0.006 ± 0.006	2.88 ± 0.97
Exclusive breastfeeding, ≥6 mo	116	0.42 ± 0.19	0.27 ± 0.13	0.21 ± 0.06	0.64 ± 0.23	31.12 ± 4.26	0.20 ± 0.20	1.22 ± 0.30	3.11 ± 0.93	0.006 ± 0.006	2.66 ± 0.90
P[d]		0.69	0.71	0.65	0.78	0.67	0.30	0.30	0.68	0.26	0.05
Iron supplementation											
None	205	0.42 ± 0.18	0.25 ± 0.12	0.21 ± 0.06	0.63 ± 0.22	30.97 ± 4.38	0.19 ± 0.17	1.17 ± 0.29	3.11 ± 0.87	0.006 ± 0.006	2.78 ± 0.98
Any	213	0.42 ± 0.17	0.27 ± 0.15	0.22 ± 0.06	0.67 ± 0.22	31.01 ± 4.16	0.21 ± 0.17	1.19 ± 0.31	3.24 ± 0.89	0.007 ± 0.006	3.05 ± 2.44
P[b]		0.91	0.14	0.03	0.11	0.93	0.20	0.68	0.14	0.25	0.13

Table 4.3. Serum biomarkers of polyunsaturated fatty acids[a] at 10 y by categories of sociodemographic characteristics in infancy and other fatty acids at 10 y among children from Santiago, Chile

		N-3				N-6					
Characteristics	N	ALA 18:3(n-3)	EPA 20:5(n-3)	DPA 22:5(n-3)	DHA 22:6(n-3)	LA 18:2(n-6)	GLA 18:3(n-6)	DGLA 20:3(n-6)	AA 20:4(n-6)	Δ6-desaturase activity index (GLA/LA)	Δ5-desaturase activity index (AA/DGLA)
Graffar index[e]											
Q1 (high SES)	71	0.42 ± 0.16	0.26 ± 0.13	0.21 ± 0.07	0.62 ± 0.21	30.81 ± 3.98	0.22 ± 0.20	1.16 ± 0.33	3.15 ± 0.93	0.008 ± 0.008	3.07 ± 2.07
Q2	82	0.44 ± 0.23	0.28 ± 0.15	0.21 ± 0.07	0.64 ± 0.20	31.27 ± 3.84	0.16 ± 0.12	1.17 ± 0.28	3.16 ± 0.76	0.005 ± 0.004	2.86 ± 1.00
Q3	99	0.44 ± 0.15	0.25 ± 0.13	0.22 ± 0.05	0.70 ± 0.23	31.35 ± 4.05	0.22 ± 0.21	1.22 ± 0.27	3.24 ± 0.93	0.007 ± 0.007	2.74 ± 0.80
Q4	83	0.39 ± 0.13	0.26 ± 0.13	0.21 ± 0.06	0.63 ± 0.20	30.89 ± 4.55	0.20 ± 0.15	1.19 ± 0.32	3.10 ± 0.86	0.007 ± 0.005	2.72 ± 0.83
Q5 (low SES)	83	0.41 ± 0.17	0.25 ± 0.14	0.22 ± 0.07	0.64 ± 0.24	30.52 ± 4.85	0.20 ± 0.15	1.16 ± 0.30	3.22 ± 0.90	0.006 ± 0.005	3.26 ± 3.40
P, trend[f]		0.22	0.41	0.24	0.95	0.42	0.81	0.95	0.78	0.70	0.62
10 y serum fatty acids (median, weight % of total fatty acids)											
Total *trans* fatty acids											
Q1 (1.11)	104	0.46 ± 0.20	0.27 ± 0.14	0.22 ± 0.06	0.72 ± 0.25	32.47 ± 4.08	0.22 ± 0.20	1.22 ± 0.29	3.44 ± 0.95	0.007 ± 0.006	2.93 ± 0.98
Q2 (1.45)	105	0.42 ± 0.16	0.26 ± 0.14	0.22 ± 0.06	0.69 ± 0.22	31.38 ± 4.23	0.19 ± 0.17	1.23 ± 0.33	3.23 ± 0.83	0.006 ± 0.006	3.01 ± 3.01
Q3 (1.82)	105	0.43 ± 0.19	0.26 ± 0.12	0.21 ± 0.06	0.62 ± 0.18	31.26 ± 4.12	0.19 ± 0.17	1.19 ± 0.26	3.09 ± 0.88	0.006 ± 0.006	2.70 ± 0.91
Q4 (2.46)	104	0.37 ± 0.12	0.25 ± 0.14	0.20 ± 0.06	0.57 ± 0.18	28.84 ± 3.85	0.21 ± 0.15	1.08 ± 0.30	2.95 ± 0.79	0.007 ± 0.005	3.04 ± 1.78
P, trend[f]		0.0002	0.48	0.003	<0.0001	<0.0001	0.77	0.0001	<0.0001	0.44	0.80
Palmitoleic acid 16:1(n-7)											
Q1 (0.97)	104	0.39 ± 0.18	0.29 ± 0.14	0.21 ± 0.06	0.67 ± 0.22	33.33 ± 4.38	0.21 ± 0.18	1.09 ± 0.30	3.49 ± 0.99	0.007 ± 0.006	3.35 ± 0.97
Q2 (1.29)	105	0.41 ± 0.20	0.26 ± 0.13	0.20 ± 0.06	0.70 ± 0.24	32.09 ± 3.62	0.18 ± 0.15	1.10 ± 0.28	3.23 ± 0.88	0.006 ± 0.005	3.27 ± 3.01
Q3 (1.67)	105	0.42 ± 0.14	0.28 ± 0.16	0.22 ± 0.07	0.65 ± 0.20	30.82 ± 3.24	0.20 ± 0.17	1.23 ± 0.31	3.18 ± 0.81	0.006 ± 0.006	2.83 ± 1.69
Q4 (2.29)	104	0.46 ± 0.16	0.22 ± 0.10	0.22 ± 0.06	0.57 ± 0.20	27.70 ± 3.60	0.21 ± 0.18	1.31 ± 0.26	2.81 ± 0.69	0.008 ± 0.007	2.22 ± 0.65
P, trend[f]		0.002	0.0001	0.39	<0.0001	<0.0001	0.62	<0.0001	<0.0001	0.05	<0.0001

[a]Expressed as percentage of total fatty acids by weight.

[b]Wald test from linear regression models with each fatty acid as the outcome and an indicator variable for the characteristic as a predictor.

[c]Includes 7 children who were small for gestational age according to birth length.

[d]χ^2 score statistic from linear regression models with each fatty acid as the outcome and indicator variables for levels of the characteristic as predictors.

[e]Index of socioeconomic status that includes information on family structure, education and employment of the head of household, crowding and physical condition of the home, and ownership of the home, car, and household appliances (33). Higher values indicate lower socioeconomic status.

[f]Wald test from linear regression models with each fatty acid as the outcome and a variable representing category-specific medians of an ordinal characteristic introduced as a continuous predictor.

Table 4.4. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 10 years of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	10 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	10 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	10 y - 16 y Adjusted difference in change (95% CI)[c]
Overall	418	1.03 ± 0.06	0.73 ± 0.05	-0.31 ± 0.03	
N-3					
ALA (18:3 n-3)					
Q1 (0.27)	104	0.64 ± 0.11	0.46 ± 0.09	-0.18 ± 0.07	Reference
Q2 (0.37)	105	1.01 ± 0.10	0.65 ± 0.10	-0.36 ± 0.08	-0.14 (-0.32, 0.04)
Q3 (0.44)	105	0.96 ± 0.11	0.64 ± 0.11	-0.32 ± 0.06	-0.13 (-0.31, 0.05)
Q4 (0.57)	104	1.53 ± 0.11	1.16 ± 0.11	-0.37 ± 0.07	-0.13 (-0.31, 0.05)
P, trend[d]		<0.0001	<0.0001	0.07	0.22
EPA (20:5 n-3)					
Q1 (0.11)	104	0.98 ± 0.12	0.71 ± 0.11	-0.26 ± 0.07	Reference
Q2 (0.21)	105	1.00 ± 0.11	0.61 ± 0.11	-0.39 ± 0.06	-0.12 (-0.30, 0.05)
Q3 (0.28)	105	1.07 ± 0.12	0.80 ± 0.11	-0.27 ± 0.07	0.02 (-0.18, 0.21)
Q4 (0.42)	104	1.09 ± 0.10	0.78 ± 0.09	-0.31 ± 0.07	-0.11 (-0.29, 0.07)
P, trend		0.42	0.43	0.94	0.43
DPA (22:5 n-3)					
Q1 (0.15)	104	0.89 ± 0.10	0.63 ± 0.09	-0.26 ± 0.06	Reference
Q2 (0.19)	105	1.00 ± 0.12	0.64 ± 0.11	-0.36 ± 0.07	-0.06 (-0.24, 0.12)
Q3 (0.23)	105	1.21 ± 0.11	0.91 ± 0.11	-0.30 ± 0.06	-0.04 (-0.22, 0.15)
Q4 (0.29)	104	1.04 ± 0.12	0.73 ± 0.11	-0.31 ± 0.08	-0.11 (-0.32, 0.10)
P, trend		0.25	0.29	0.81	0.36
DHA (22:6 n-3)					
Q1 (0.43)	104	1.13 ± 0.11	0.67 ± 0.11	-0.45 ± 0.07	Reference
Q2 (0.57)	105	0.98 ± 0.10	0.67 ± 0.09	-0.31 ± 0.07	0.06 (-0.14, 0.26)
Q3 (0.68)	105	0.93 ± 0.11	0.80 ± 0.10	-0.13 ± 0.06	0.25 (0.05, 0.45)
Q4 (0.90)	104	1.10 ± 0.12	0.76 ± 0.12	-0.34 ± 0.07	0.11 (-0.11, 0.34)
P, trend		0.95	0.51	0.26	0.31

Table 4.4. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 10 years of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	10 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	10 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	10 y - 16 y Adjusted difference in change (95% CI)[c]
N-6					
LA (18:2 n-6)					
Q1 (25.96)	104	1.09 ± 0.11	0.72 ± 0.10	-0.37 ± 0.08	Reference
Q2 (29.92)	105	1.22 ± 0.11	0.90 ± 0.12	-0.32 ± 0.07	0.07 (-0.14, 0.28)
Q3 (32.59)	105	0.96 ± 0.11	0.68 ± 0.11	-0.27 ± 0.07	0.06 (-0.17, 0.28)
Q4 (35.77)	104	0.87 ± 0.11	0.60 ± 0.10	-0.27 ± 0.06	-0.08 (-0.32, 0.17)
P, trend		0.09	0.28	0.27	0.58
GLA (18:3 n-6)					
Q1 (0.06)	104	1.05 ± 0.10	0.81 ± 0.10	-0.24 ± 0.07	Reference
Q2 (0.11)	105	0.83 ± 0.11	0.51 ± 0.11	-0.33 ± 0.07	-0.08 (-0.27, 0.10)
Q3 (0.20)	105	1.14 ± 0.12	0.78 ± 0.10	-0.37 ± 0.08	-0.12 (-0.31, 0.07)
Q4 (0.37)	104	1.12 ± 0.11	0.81 ± 0.11	-0.31 ± 0.07	-0.09 (-0.27, 0.10)
P, trend		0.23	0.38	0.62	0.46
DGLA (20:3 n-6)					
Q1 (0.85)	104	0.68 ± 0.11	0.43 ± 0.10	-0.25 ± 0.07	Reference
Q2 (1.08)	105	0.94 ± 0.11	0.64 ± 0.11	-0.30 ± 0.06	0.03 (-0.16, 0.21)
Q3 (1.27)	105	1.10 ± 0.11	0.78 ± 0.10	-0.31 ± 0.06	0.04 (-0.14, 0.22)
Q4 (1.55)	104	1.42 ± 0.11	1.05 ± 0.11	-0.37 ± 0.08	0.07 (-0.15, 0.29)
P, trend		<0.0001	<0.0001	0.26	0.53
AA (20:4 n-6)					
Q1 (2.23)	104	1.33 ± 0.11	0.88 ± 0.11	-0.44 ± 0.07	Reference
Q2 (2.81)	105	0.89 ± 0.10	0.59 ± 0.11	-0.30 ± 0.07	0.15 (-0.05, 0.35)
Q3 (3.37)	105	1.03 ± 0.11	0.76 ± 0.10	-0.27 ± 0.07	0.20 (0.01, 0.39)
Q4 (4.19)	104	0.89 ± 0.12	0.67 ± 0.11	-0.22 ± 0.07	0.22 (0.00, 0.44)
P, trend		0.03	0.37	0.02	0.06
Δ6-Desaturase index (GLA/LA)					
Q1 (0.002)	104	1.00 ± 0.10	0.79 ± 0.10	-0.22 ± 0.07	Reference
Q2 (0.004)	105	0.88 ± 0.12	0.54 ± 0.10	-0.34 ± 0.07	-0.13 (-0.32, 0.06)
Q3 (0.006)	105	1.10 ± 0.12	0.75 ± 0.11	-0.35 ± 0.07	-0.14 (-0.32, 0.05)
Q4 (0.012)	104	1.16 ± 0.11	0.84 ± 0.11	-0.32 ± 0.07	-0.11 (-0.30, 0.08)
P, trend		0.11	0.27	0.44	0.44

Table 4.4. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 10 years of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	10 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	10 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	10 y - 16 y Adjusted difference in change (95% CI)[c]
Δ5-Desaturase index (AA/DGLA)					
Q1 (1.79)	104	1.52 ± 0.11	1.14 ± 0.11	-0.39 ± 0.07	Reference
Q2 (2.42)	105	1.13 ± 0.10	0.76 ± 0.10	-0.37 ± 0.07	0.00 (-0.20, 0.20)
Q3 (3.00)	105	0.86 ± 0.11	0.57 ± 0.11	-0.29 ± 0.06	0.04 (-0.15, 0.22)
Q4 (3.80)	104	0.63 ± 0.11	0.44 ± 0.10	-0.19 ± 0.07	0.05 (-0.16, 0.26)
P, trend		<0.0001	<0.0001	0.03	0.56

[a]According the World Health Organization Growth Reference 2007 (31) for children ages 5-19 y

[b]Estimates are from growth curves created using mixed effects linear regression models with BMI-for-age Z score as the outcome, and predictors that included indicator variables for each PUFA quartile, a linear term for age, and interaction terms between the quartiles and age. All models included random intercepts to account for within-child correlations of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs. average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), total serum trans fatty acids, palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA, while all n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[d]From mixed effects linear regression models with Z score as the outcome and a variable representing medians of PUFA quartiles introduced as a continuous predictor.

Table 4.5. Changes in BMI-for-age Z scores[a] according to changes in serum biomarkers of polyunsaturated fatty acids at 5 and 10 years of age among children from Santiago, Chile

Quartile of fatty acid change (median, weight % of total FA)	N	10 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	10 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	10 y - 16 y Adjusted difference in change (95% CI)[c]
Overall	141	0.97 ± 0.10	0.67 ± 0.10	-0.30 ± 0.06	
N-3					
ALA (18:3 n-3)					
Q1 (-0.10)	35	0.69 ± 0.20	0.48 ± 0.18	-0.21 ± 0.13	Reference
Q2 (0.07)	35	0.64 ± 0.17	0.43 ± 0.16	-0.21 ± 0.11	-0.03 (-0.35, 0.29)
Q3 (0.18)	36	1.01 ± 0.19	0.61 ± 0.21	-0.41 ± 0.13	-0.18 (-0.51, 0.16)
Q4 (0.37)	35	1.55 ± 0.18	1.17 ± 0.21	-0.38 ± 0.11	-0.21 (-0.55, 0.14)
P, trend[d]		0.0007	0.01	0.20	0.18
EPA (20:5 n-3)					
Q1 (-0.20)	35	0.96 ± 0.20	0.71 ± 0.20	-0.26 ± 0.10	Reference
Q2 (-0.07)	35	0.83 ± 0.20	0.68 ± 0.22	-0.15 ± 0.11	0.18 (-0.11, 0.46)
Q3 (0.05)	36	1.14 ± 0.19	0.89 ± 0.18	-0.26 ± 0.13	0.00 (-0.32, 0.31)
Q4 (0.16)	35	0.95 ± 0.17	0.41 ± 0.19	-0.53 ± 0.13	-0.32 (-0.64, 0.00)
P, trend		0.75	0.47	0.08	0.04
DPA (22:5 n-3)					
Q1 (-0.07)	35	1.04 ± 0.19	0.79 ± 0.17	-0.25 ± 0.11	Reference
Q2 (0.02)	35	0.91 ± 0.19	0.56 ± 0.18	-0.34 ± 0.14	-0.08 (-0.41, 0.25)
Q3 (0.07)	36	1.02 ± 0.19	0.50 ± 0.21	-0.52 ± 0.11	-0.27 (-0.56, 0.02)
Q4 (0.13)	35	0.93 ± 0.20	0.84 ± 0.23	-0.09 ± 0.10	0.12 (-0.23, 0.47)
P, trend		0.79	0.96	0.70	0.88
DHA (22:6 n-3)					
Q1 (-0.38)	35	0.85 ± 0.20	0.61 ± 0.18	-0.24 ± 0.11	Reference
Q2 (-0.08)	35	1.02 ± 0.20	0.51 ± 0.23	-0.51 ± 0.13	-0.14 (-0.49, 0.21)
Q3 (0.14)	36	0.86 ± 0.19	0.69 ± 0.17	-0.17 ± 0.12	0.24 (-0.11, 0.59)
Q4 (0.41)	35	1.16 ± 0.18	0.87 ± 0.21	-0.29 ± 0.11	-0.07 (-0.43, 0.29)
P, trend		0.34	0.28	0.81	0.87

Table 4.5. Changes in BMI-for-age Z scores[a] according to changes in serum biomarkers of polyunsaturated fatty acids at 5 and 10 years of age among children from Santiago, Chile

Quartile of fatty acid change (median, weight % of total FA)	N	10 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	10 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	10 y - 16 y Adjusted difference in change (95% CI)[c]
N-6					
LA (18:2 n-6)					
Q1 (-1.64)	35	1.05 ± 0.16	0.61 ± 0.18	-0.44 ± 0.13	Reference
Q2 (1.65)	35	1.11 ± 0.19	0.78 ± 0.19	-0.33 ± 0.13	-0.04 (-0.39, 0.31)
Q3 (5.22)	36	0.96 ± 0.19	0.75 ± 0.20	-0.22 ± 0.10	0.04 (-0.33, 0.41)
Q4 (9.67)	35	0.78 ± 0.22	0.55 ± 0.22	-0.23 ± 0.11	-0.03 (-0.43, 0.37)
P, trend		0.26	0.79	0.17	0.98
GLA (18:3 n-6)					
Q1 (-0.19)	35	1.13 ± 0.19	0.83 ± 0.20	-0.29 ± 0.11	Reference
Q2 (-0.04)	35	1.18 ± 0.20	0.81 ± 0.22	-0.37 ± 0.11	-0.20 (-0.52, 0.11)
Q3 (0.04)	36	0.73 ± 0.21	0.44 ± 0.21	-0.29 ± 0.14	-0.12 (-0.45, 0.21)
Q4 (0.15)	35	0.86 ± 0.16	0.61 ± 0.16	-0.26 ± 0.12	-0.07 (-0.37, 0.22)
P, trend		0.15	0.23	0.77	0.67
DGLA (20:3 n-6)					
Q1 (-0.06)	35	0.55 ± 0.20	0.39 ± 0.21	-0.16 ± 0.12	Reference
Q2 (0.24)	35	1.12 ± 0.17	0.83 ± 0.16	-0.28 ± 0.11	-0.10 (-0.41, 0.21)
Q3 (0.46)	36	1.06 ± 0.19	0.77 ± 0.21	-0.30 ± 0.10	-0.06 (-0.35, 0.24)
Q4 (0.93)	35	1.16 ± 0.19	0.69 ± 0.21	-0.47 ± 0.15	-0.29 (-0.62, 0.04)
P, trend		0.06	0.43	0.11	0.09
AA (20:4 n-6)					
Q1 (0.45)	35	1.38 ± 0.20	0.79 ± 0.21	-0.59 ± 0.12	Reference
Q2 (1.17)	35	0.94 ± 0.19	0.81 ± 0.20	-0.13 ± 0.12	0.56 (0.22, 0.90)
Q3 (1.53)	36	0.73 ± 0.16	0.49 ± 0.15	-0.24 ± 0.10	0.40 (0.10, 0.70)
Q4 (2.17)	35	0.85 ± 0.21	0.61 ± 0.23	-0.24 ± 0.13	0.51 (0.11, 0.90)
P, trend		0.05	0.42	0.06	0.03
Δ6-Desaturase index (GLA/LA)					
Q1 (-0.009)	35	1.11 ± 0.18	0.76 ± 0.21	-0.35 ± 0.12	Reference
Q2 (-0.002)	35	1.44 ± 0.22	1.06 ± 0.23	-0.38 ± 0.10	-0.16 (-0.48, 0.15)
Q3 (0.000)	36	0.47 ± 0.16	0.37 ± 0.17	-0.10 ± 0.12	0.05 (-0.28, 0.38)
Q4 (0.005)	35	0.88 ± 0.17	0.51 ± 0.16	-0.38 ± 0.12	-0.16 (-0.47, 0.15)
P, trend		0.14	0.21	0.86	0.39

Table 4.5. Changes in BMI-for-age Z scores[a] according to changes in serum biomarkers of polyunsaturated fatty acids at 5 and 10 years of age among children from Santiago, Chile

Quartile of fatty acid change (median, weight % of total FA)	N	10 y BMI-for-age Z score (mean ± SE)[b]	16 y BMI-for-age Z score (mean ± SE)	10 y - 16 y Change in BMI-for-age Z score (mean ± SE)[b]	10 y - 16 y Adjusted difference in change (95% CI)[c]
Δ5-Desaturase index (AA/DGLA)					
Q1 (-2.08)	35	1.30 ± 0.20	0.76 ± 0.23	-0.54 ± 0.13	Reference
Q2 (0.23)	35	1.24 ± 0.17	0.86 ± 0.19	-0.37 ± 0.13	0.17 (-0.21, 0.54)
Q3 (0.81)	36	0.95 ± 0.18	0.73 ± 0.17	-0.22 ± 0.09	0.33 (0.01, 0.65)
Q4 (1.62)	35	0.40 ± 0.19	0.33 ± 0.19	-0.06 ± 0.11	0.64 (0.27, 1.02)
P, trend		0.005	0.28	0.008	0.003

[a]According to the World Health Organization Growth Reference 1007 (31) for children ages 5-19 y.

[b]Estimates are from growth curves created using mixed effects linear regression models with BMI-for-age Z score as the outcome, and predictors that included indicator variables for each PUFA change quartile, a linear term for age, and interaction terms between the quartiles and age. All models included random intercepts to account for within-child correlations of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs. average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), change in total serum trans fatty acids, change in palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for change in LA and total long-chain n-6 PUFA, while all n-6 PUFA are adjusted for change in ALA and total long-chain n-3 PUFA. All long chain PUFA are adjusted for change in their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[d]From mixed effects linear regression models with Z score as the outcome and a variable representing medians of PUFA change quartiles introduced as a continuous predictor.

Supplemental Table 4.1. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 5 years of age among children from Santiago, Chile, stratified by weight status at 5 years

Fatty acid quartile (median, normal weight/overweight, weight % of total FA)	5 y - 16 y change in BMI-for-age Z score (mean ± SE)[b]				Adjusted difference in 5 y - 16 y change (95% CI)[c]	
	N	Normal weight[d] at 5 y	N	Overweight/ obese[e] at 5 y	Normal weight at 5 y	Overweight/ obese at 5 y
Overall	134	0.07 ± 0.07	105	-0.60 ± 0.09		
N-3						
ALA (18:3 n-3)						
Q1 (0.14/0.11)	33	-0.11 ± 0.11	26	-0.43 ± 0.26	Reference	Reference
Q2 (0.25/0.25)	40	0.36 ± 0.13	20	-0.86 ± 0.18	0.56 (0.22, 0.91)	-0.47 (-1.17, 0.23)
Q3 (0.33/0.35)	30	0.04 ± 0.18	30	-0.74 ± 0.13	0.34 (-0.08, 0.77)	-0.13 (-0.71, 0.44)
Q4 (0.44/0.47)	31	-0.10 ± 0.15	29	-0.43 ± 0.16	0.14 (-0.24, 0.53)	-0.01 (-0.59, 0.56)
P, trend[f]		0.70		>0.99	0.67	0.94
P, interaction[g]		0.84			0.85	
EPA (20:5 n-3)						
Q1 (0.12/0.11)	33	0.11 ± 0.17	26	-0.51 ± 0.18	Reference	Reference
Q2 (0.22/0.22)	33	-0.14 ± 0.13	27	-0.49 ± 0.19	-0.24 (-0.66, 0.18)	0.02 (-0.53, 0.57)
Q3 (0.30/0.30)	33	0.17 ± 0.12	27	-0.72 ± 0.18	0.01 (-0.40, 0.43)	-0.09 (-0.60, 0.42)
Q4 (0.40/0.41)	35	0.12 ± 0.16	25	-0.69 ± 0.20	-0.07 (-0.53, 0.40)	0.14 (-0.42, 0.70)
P, trend		0.68		0.37	0.98	0.71
P, interaction		0.34			0.77	
DPA (22:5 n-3)						
Q1 (0.11/0.11)	34	0.18 ± 0.13	25	-0.31 ± 0.20	Reference	Reference
Q2 (0.14/0.13)	38	-0.07 ± 0.14	22	-0.44 ± 0.16	-0.35 (-0.74, 0.04)	0.21 (-0.38, 0.80)
Q3 (0.18/0.18)	32	-0.02 ± 0.19	28	-0.64 ± 0.20	-0.40 (-0.85, 0.04)	-0.08 (-0.70, 0.54)
Q4 (0.23/0.23)	30	0.20 ± 0.12	30	-0.93 ± 0.16	-0.03 (-0.45, 0.39)	-0.39 (-0.93, 0.16)
P, trend		0.71		0.01	0.96	0.07
P, interaction		0.02			0.17	
DHA (22:6 n-3)						
Q1 (0.34/0.32)	40	0.19 ± 0.14	19	-0.35 ± 0.21	Reference	Reference
Q2 (0.49/0.47)	34	-0.02 ± 0.14	26	-0.77 ± 0.22	-0.27 (-0.70, 0.16)	-0.14 (-0.80, 0.52)
Q3 (0.64/0.60)	32	0.31 ± 0.16	28	-0.49 ± 0.18	0.02 (-0.44, 0.47)	0.21 (-0.28, 0.71)
Q4 (0.92/0.87)	28	-0.29 ± 0.12	32	-0.71 ± 0.14	-0.57 (-1.03, -0.11)	0.01 (-0.58, 0.61)
P, trend		0.03		0.38	0.03	0.63
P, interaction		0.54			0.07	

Supplemental Table 4.1. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 5 years of age among children from Santiago, Chile, stratified by weight status at 5 years

Fatty acid quartile (median, normal weight/overweight, weight % of total FA)	5 y - 16 y change in BMI-for-age Z score (mean ± SE)[b]				Adjusted difference in 5 y - 16 y change (95% CI)[c]	
	N	Normal weight[d] at 5 y	N	Overweight/ obese[e] at 5 y	Normal weight at 5 y	Overweight/ obese at 5 y
N-6						
LA (18:2 n-6)						
Q1 (22.09/21.46)	32	-0.11 ± 0.11	27	-0.36 ± 0.18	Reference	Reference
Q2 (25.84/26.18)	33	0.12 ± 0.15	27	-0.70 ± 0.16	0.31 (-0.11, 0.72)	-0.48 (-0.99, 0.04)
Q3 (28.99/28.96)	30	0.01 ± 0.15	30	-0.53 ± 0.18	0.20 (-0.19, 0.59)	-0.38 (-0.89, 0.13)
Q4 (31.98/32.29)	39	0.21 ± 0.16	21	-0.90 ± 0.22	0.41 (-0.02, 0.84)	-0.85 (-1.55, -0.15)
P, trend		0.16		0.11	0.08	0.02
P, interaction		0.03			0.004	
GLA (18:3 n-6)						
Q1 (0.07/0.06)	41	0.18 ± 0.13	18	-0.63 ± 0.27	Reference	Reference
Q2 (0.11/0.12)	36	0.10 ± 0.15	24	-0.75 ± 0.21	0.02 (-0.33, 0.38)	0.00 (-0.74, 0.74)
Q3 (0.19/0.22)	33	-0.12 ± 0.14	27	-0.51 ± 0.17	-0.18 (-0.61, 0.24)	0.30 (-0.29, 0.90)
Q4 (0.44/0.39)	24	0.05 ± 0.15	36	-0.56 ± 0.14	-0.09 (-0.46, 0.28)	0.38 (-0.25, 1.00)
P, trend		0.42		0.61	0.48	0.08
P, interaction		0.37			0.07	
DGLA (20:3 n-6)						
Q1 (0.50/0.22)	39	-0.03 ± 0.11	20	-0.54 ± 0.25	Reference	Reference
Q2 (0.71/0.70)	34	0.08 ± 0.17	26	-0.70 ± 0.22	0.18 (-0.22, 0.59)	-0.10 (-0.84, 0.64)
Q3 (0.86/0.68)	34	0.13 ± 0.12	26	-0.49 ± 0.16	0.18 (-0.15, 0.51)	0.15 (-0.65, 0.94)
Q4 (1.10/1.05)	27	0.12 ± 0.19	33	-0.66 ± 0.14	0.13 (-0.34, 0.60)	0.05 (-0.63, 0.74)
P, trend		0.40		0.83	0.40	0.75
P, interaction		0.51			0.85	
AA (20:4 n-6)						
Q1 (1.17/1.17)	35	0.03 ± 0.16	24	-0.51 ± 0.21	Reference	Reference
Q2 (1.58/1.57)	34	0.09 ± 0.14	26	-0.60 ± 0.19	0.14 (-0.35, 0.63)	0.02 (-0.60, 0.63)
Q3 (1.91/1.95)	36	0.02 ± 0.10	24	-0.64 ± 0.19	0.11 (-0.30, 0.51)	0.01 (-0.60, 0.62)
Q4 (2.25/2.29)	29	0.15 ± 0.20	31	-0.64 ± 0.17	0.21 (-0.31, 0.73)	-0.08 (-0.76, 0.60)
P, trend		0.76		0.62	0.49	0.83
P, interaction		0.57			0.56	

Supplemental Table 4.1. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 5 years of age among children from Santiago, Chile, stratified by weight status at 5 years

Fatty acid quartile (median, normal weight/overweight, weight % of total FA)	5 y - 16 y change in BMI-for-age Z score (mean ± SE)[b]				Adjusted difference in 5 y - 16 y change (95% CI)[c]	
	N	Normal weight[d] at 5 y	N	Overweight/ obese[e] at 5 y	Normal weight at 5 y	Overweight/ obese at 5 y
Δ6-Desaturase index (GLA/LA)						
Q1 (0.002/0.003)	41	0.17 ± 0.14	18	-0.82 ± 0.29	Reference	Reference
Q2 (0.004/0.004)	35	0.11 ± 0.15	25	-0.61 ± 0.19	0.05 (-0.33, 0.42)	0.35 (-0.39, 1.09)
Q3 (0.007/0.008)	35	-0.10 ± 0.13	25	-0.52 ± 0.18	-0.22 (-0.60, 0.16)	0.58 (-0.15, 1.31)
Q4 (0.018/0.016)	23	0.08 ± 0.15	37	-0.54 ± 0.14	-0.04 (-0.44, 0.37)	0.59 (-0.08, 1.26)
P, trend		0.60		0.47	0.73	0.07
P, interaction		0.38			0.12	
Δ5-Desaturase index (AA/DGLA)						
Q1 (1.60/1.51)	32	-0.06 ± 0.14	27	-0.56 ± 0.17	Reference	Reference
Q2 (2.00/1.95)	31	0.37 ± 0.18	29	-0.82 ± 0.16	0.52 (0.09, 0.95)	-0.38 (-0.85, 0.10)
Q3 (2.45/2.41)	36	0.04 ± 0.13	24	-0.44 ± 0.20	0.06 (-0.31, 0.43)	-0.01 (-0.56, 0.54)
Q4 (3.54/5.74)	35	-0.06 ± 0.13	25	-0.56 ± 0.21	-0.05 (-0.43, 0.33)	-0.14 (-0.71, 0.43)
P, trend		0.34		0.72	0.21	0.94
P, interaction		0.40			0.45	

[a]According the World Health Organization Growth Reference 2007 (31) for children ages 5-19 y.

[b]Estimates are from growth curves created using mixed effects linear regression models with BMI-for-age Z score as the outcome, and predictors that included indicator variables for each PUFA quartile, linear and cubic spline terms for age, and interaction terms between the quartiles and age terms. All models included random intercepts and age slopes to account for within-child correlations of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs. average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), total serum trans fatty acids, palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA, while all n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[d]BMI-for-age Z score at 5 y ≤1.

[e]BMI-for-age Z at 5 y score >1.

[f]From mixed effects linear regression models with Z score as the outcome and a variable representing medians of PUFA quartiles introduced as a continuous predictor.

[g]From mixed effects linear regression models with an indicator for overweight/obesity as a predictor and cross-product terms between the indicator and all other predictors in the model.

Supplemental Table 4.2. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 10 years of age among children from Santiago, Chile, stratified by weight status at 10 years

Fatty acid quartile (median, normal weight/overweight, weight % of total FA)	10 y - 16 y change in BMI-for-age Z score (mean ± SE)[b]				Difference in 10 y -16 y change (95% CI)[c]	
	N	Normal weight[d] at 10 y	N	Overweight/ obese[e] at 10 y	Normal weight at 10 y	Overweight/ obese at 10 y
Overall	190	-0.07 ± 0.05	228	-0.51 ± 0.05		
N-3						
ALA (18:3 n-3)						
Q1 (0.26/0.28)	60	0.03 ± 0.08	44	-0.46 ± 0.09	Reference	Reference
Q2 (0.37/0.37)	48	-0.07 ± 0.09	57	-0.61 ± 0.11	-0.09 (-0.33, 0.14)	-0.15 (-0.41, 0.12)
Q3 (0.43/0.44)	49	-0.14 ± 0.09	56	-0.48 ± 0.08	-0.16 (-0.39, 0.08)	-0.07 (-0.33, 0.19)
Q4 (0.55/0.57)	33	-0.15 ± 0.10	71	-0.48 ± 0.09	-0.11 (-0.36, 0.14)	0.00 (-0.24, 0.24)
P, trend[f]		0.11		0.77	0.27	0.68
P, interaction[g]		0.19			0.28	
EPA (20:5 n-3)						
Q1 (0.11/0.10)	53	-0.11 ± 0.08	51	-0.42 ± 0.10	Reference	Reference
Q2 (0.21/0.20)	48	-0.19 ± 0.09	57	-0.55 ± 0.08	-0.05 (-0.28, 0.17)	-0.10 (-0.36, 0.16)
Q3 (0.28/0.29)	42	0.09 ± 0.09	63	-0.51 ± 0.09	0.22 (-0.02, 0.47)	-0.06 (-0.34, 0.22)
Q4 (0.45/0.39)	47	-0.06 ± 0.10	57	-0.52 ± 0.10	0.05 (-0.19, 0.29)	-0.14 (-0.41, 0.13)
P, trend		0.38		0.64	0.40	0.40
P, interaction		0.35			0.24	
DPA (22:5 n-3)						
Q1 (0.14/0.15)	53	-0.03 ± 0.08	51	-0.51 ± 0.08	Reference	Reference
Q2 (0.19/0.19)	49	-0.12 ± 0.10	56	-0.57 ± 0.10	-0.08 (-0.31, 0.15)	-0.04 (-0.31, 0.23)
Q3 (0.23/0.23)	43	-0.10 ± 0.09	62	-0.43 ± 0.08	-0.08 (-0.34, 0.19)	0.04 (-0.22, 0.29)
Q4 (0.29/0.28)	45	-0.04 ± 0.10	59	-0.51 ± 0.10	-0.04 (-0.33, 0.24)	-0.12 (-0.41, 0.18)
P, trend		0.98		0.80	0.80	0.52
P, interaction		0.87			0.78	
DHA (22:6 n-3)						
Q1 (0.41/0.44)	44	-0.23 ± 0.10	60	-0.62 ± 0.09	Reference	Reference
Q2 (0.58/0.56)	46	-0.04 ± 0.08	59	-0.51 ± 0.09	0.17 (-0.08, 0.43)	-0.01 (-0.31, 0.29)
Q3 (0.68/0.68)	57	0.03 ± 0.08	48	-0.33 ± 0.09	0.23 (-0.05, 0.50)	0.21 (-0.10, 0.52)
Q4 (0.91/0.90)	43	-0.09 ± 0.10	61	-0.52 ± 0.10	0.16 (-0.16, 0.48)	0.08 (-0.24, 0.39)
P, trend		0.40		0.43	0.45	0.61
P, interaction		0.94			0.83	

Supplemental Table 4.2. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 10 years of age among children from Santiago, Chile, stratified by weight status at 10 years

Fatty acid quartile (median, normal weight/overweight, weight % of total FA)	10 y - 16 y change in BMI-for-age Z score (mean ± SE)[b]				Difference in 10 y -16 y change (95% CI)[c]	
	N	Normal weight[d] at 10 y	N	Overweight/ obese[e] at 10 y	Normal weight at 10 y	Overweight/ obese at 10 y
N-6						
LA (18:2 n-6)						
Q1 (26.24/25.66)	47	0.01 ± 0.09	57	-0.68 ± 0.11	Reference	Reference
Q2 (29.98/29.74)	38	-0.18 ± 0.12	67	-0.40 ± 0.08	-0.18 (-0.46, 0.11)	0.40 (0.09, 0.70)
Q3 (32.54/32.66)	53	-0.03 ± 0.08	52	-0.53 ± 0.09	0.01 (-0.26, 0.28)	0.20 (-0.11, 0.51)
Q4 (35.79/35.71)	52	-0.11 ± 0.08	52	-0.42 ± 0.09	-0.25 (-0.55, 0.05)	0.16 (-0.21, 0.54)
P, trend		0.52		0.12	0.20	0.54
P, interaction		0.11			0.19	
GLA (18:3 n-6)						
Q1 (0.06/0.07)	47	-0.04 ± 0.10	57	-0.39 ± 0.09	Reference	Reference
Q2 (0.11/0.11)	56	-0.14 ± 0.08	49	-0.54 ± 0.10	-0.11 (-0.34, 0.12)	-0.16 (-0.45, 0.13)
Q3 (0.21/0.19)	45	0.04 ± 0.10	60	-0.65 ± 0.09	0.02 (-0.24, 0.28)	-0.21 (-0.47, 0.05)
Q4 (0.40/0.34)	42	-0.11 ± 0.08	62	-0.44 ± 0.09	-0.14 (-0.39, 0.10)	-0.04 (-0.30, 0.22)
P, trend		0.85		0.96	0.45	0.93
P, interaction		0.87			0.56	
DGLA (20:3 n-6)						
Q1 (0.85/0.86)	63	-0.06 ± 0.09	41	-0.55 ± 0.09	Reference	Reference
Q2 (1.07/1.08)	50	-0.16 ± 0.09	55	-0.43 ± 0.09	-0.10 (-0.35, 0.15)	0.25 (0.01, 0.50)
Q3 (1.27/1.27)	43	-0.03 ± 0.07	62	-0.51 ± 0.09	0.03 (-0.19, 0.25)	0.20 (-0.07, 0.46)
Q4 (1.53/1.56)	34	-0.01 ± 0.11	70	-0.54 ± 0.10	0.12 (-0.16, 0.39)	0.18 (-0.11, 0.46)
P, trend		0.64		0.80	0.29	0.55
P, interaction		0.61			0.79	
AA (20:4 n-6)						
Q1 (2.20/2.23)	36	-0.14 ± 0.11	68	-0.60 ± 0.07	Reference	Reference
Q2 (2.81/2.80)	53	-0.09 ± 0.08	52	-0.52 ± 0.11	0.10 (-0.17, 0.36)	0.08 (-0.21, 0.38)
Q3 (3.34/3.38)	48	-0.03 ± 0.09	57	-0.47 ± 0.10	0.12 (-0.15, 0.39)	0.22 (-0.05, 0.48)
Q4 (4.28/4.18)	53	-0.04 ± 0.08	51	-0.40 ± 0.10	0.12 (-0.20, 0.44)	0.25 (-0.07, 0.57)
P, trend		0.46		0.10	0.56	0.10
P, interaction		0.54			0.41	

Supplemental Table 4.2. Changes in BMI-for-age Z scores[a] by serum biomarkers of polyunsaturated fatty acids at 10 years of age among children from Santiago, Chile, stratified by weight status at 10 years

Fatty acid quartile (median, normal weight/overweight, weight % of total FA)	10 y - 16 y change in BMI-for-age Z score (mean ± SE)[b]				Difference in 10 y -16 y change (95% CI)[c]	
	N	Normal weight[d] at 10 y	N	Overweight/ obese[e] at 10 y	Normal weight at 10 y	Overweight/ obese at 10 y
Δ6-Desaturase index (GLA/LA)						
Q1 (0.002/0.002)	49	-0.07 ± 0.09	55	-0.35 ± 0.09	Reference	Reference
Q2 (0.004/0.004)	54	-0.05 ± 0.09	51	-0.65 ± 0.10	-0.04 (-0.28, 0.20)	-0.26 (-0.54, 0.03)
Q3 (0.007/0.006)	46	-0.03 ± 0.09	59	-0.59 ± 0.09	-0.03 (-0.27, 0.21)	-0.21 (-0.48, 0.06)
Q4 (0.014/0.012)	41	-0.14 ± 0.10	63	-0.44 ± 0.09	-0.16 (-0.43, 0.10)	-0.04 (-0.32, 0.24)
P, trend		0.53		0.93	0.23	0.67
P, interaction		0.62			0.25	
Δ5-Desaturase index (AA/DGLA)						
Q1 (1.85/1.77)	29	0.09 ± 0.10	75	-0.57 ± 0.08	Reference	Reference
Q2 (2.42/2.39)	40	-0.14 ± 0.09	65	-0.51 ± 0.10	-0.24 (-0.49, 0.01)	0.11 (-0.14, 0.37)
Q3 (2.99/3.02)	57	-0.14 ± 0.09	48	-0.45 ± 0.09	-0.29 (-0.53, -0.04)	0.23 (-0.01, 0.48)
Q4 (3.94/3.72)	64	-0.03 ± 0.08	40	-0.45 ± 0.10	-0.16 (-0.45, 0.13)	0.12 (-0.18, 0.42)
P, trend		0.76		0.30	0.58	0.32
P, interaction		0.34			0.27	

[a]According the World Health Organization Growth Reference 2007 (31) for children ages 5-19 y

[b]Estimates are from growth curves created using mixed effects linear regression models with BMI-for-age Z score as the outcome, and predictors that included indicator variables for each PUFA quartile, a linear term for age, and interaction terms between the quartiles and age. All models included random intercepts to account for within-child correlations of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs. average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), total serum trans fatty acids, palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA, while all n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[d]BMI-for-age Z score at 10 y ≤1

[e]BMI-for-age Z score at 10 y >1

[f]From mixed effects linear regression models with Z score as the outcome and a variable representing medians of PUFA quartiles introduced as a continuous predictor.

[g]From mixed effects linear regression models with an indicator for overweight/obesity as a predictor and cross-product terms between the indicator and all other predictors in the model.

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Chapter 5. Conclusions

Summary of main findings

This dissertation expands current understanding of PUFA nutrition in Latin American settings, and helps to clarify the relations between these nutrients and the development of cardiometabolic disease risk in childhood.

In chapter 2, we identified sociodemographic, anthropometric, and dietary predictors of adipose tissue PUFA concentrations among a group of parents and their school-age children from 9 Mesoamerican countries. Mean adipose LA and ALA weight percentages across participants from all countries were comparable those in populations from mostly high-income countries (1). However, levels among participants from some countries were much lower which may indicate heterogeneity of intake throughout the region. Furthermore, although we determined that characteristics such as height, BMI, and smoking status were related to specific PUFA biomarkers in children and adults, the strongest predictor of all PUFA was country of origin. This is consistent with a study of plasma PUFA in participants from multiple European countries (2) and likely represents differences in diet and lifestyle. We also found that the type of cooking oil used in the home was a significant predictor of both essential and long-chain PUFA, despite low levels of preformed long-chain PUFA in vegetable oils. This is consistent with results from previous studies in Costa Rica (3,4) and a trial of sunflower and soyabean oils in Colombia (5). High adipose ALA among canola and soyabean oil users and low levels of all PUFA in palm oil users are consistent with the PUFA content of these oils in Latin American

countries (6–8). High LA among participants who used soyabean oil are consistent with previous analyses from Costa Rica (3). High long-chain n-3 PUFA among canola oil users is consistent with high ALA levels in canola oil; however, these participants also had among the highest levels of long-chain n-6 PUFA. The reason for this is uncertain. Other dietary correlates of adipose tissue PUFA differed somewhat between adults and children, and were generally less strongly predictive of these biomarkers than cooking oil type.

In chapter 3, we investigated associations between adipose tissue PUFA biomarkers and MetS in the same Mesoamerican population. Among adults, ALA was inversely associated with MetS which is in line with previous studies of adipose tissue PUFA (9–11). ALA could be protective against MetS through enhancement of membrane fluidity and glucose transport as a result of its incorporation into cell membranes (12). Unexpectedly, EPA was positively associated with MetS. This is consistent with a Costa Rican study that measured PUFA in adipose tissue (11) but inconsistent with evidence for protective associations between EPA and multiple MetS components (13,14). Adipose tissue EPA may not reflect levels of this PUFA in plasma membranes which could be directly related to MetS (15). This association may also be specific to Mesoamerican populations. DGLA and the D6D activity index were both positively associated with MetS. The association with DGLA is consistent with many (16–19) but not all (20,21) previous studies, and associations between D6D and MetS have also been described (22,23). The association with D6D activity may be mediated by its effect on DGLA levels in tissue, or D6D and DGLA may exert independent effects. ALA was not associated with metabolic score among children, which is consistent with the small number of existing studies (24–26). The effects of ALA may be cumulative from childhood, or ALA could only be related to extreme metabolic dysregulation and the association was not apparent with a continuous

metabolic score. DPA was positively associated with metabolic score in children, while EPA and DHA were not associated. Our results in conjunction with those of previous investigations (24–33) do not consistently indicate a protective effect of long-chain n-3 PUFA on metabolic health in childhood. Finally, LA, DGLA, AA, and the D6D and D5D activity indices were all positively associated with waist circumference. This may indicate that n-6 PUFA generally have adipogenic effects in childhood (34).

In chapter 4, we assessed the relations between serum PUFA in middle childhood and BAZ change through adolescence in a cohort of Chilean children. DHA at 5 y of age was inversely associated with BAZ change between ages 5 and 16 y, which is consistent with a few cross-sectional studies (24,35,36) but inconsistent with two previous longitudinal studies (37,38) and trials of DHA+EPA (27) or DHA supplements (28). DHA could reduce weight gain by promoting satiety (39) or upregulating oxidation of fatty acids and downregulating lipogenesis (40). Serum AA at 10 y of age and change in AA between ages 5 and 10 y were positively associated with BAZ change from ages 10 to 16 y. AA is the precursor for the eicosanoid prostacyclin, which promotes differentiation of preadipocytes into adipocytes (34) and thus could promote adipogenesis. Most studies have not found similar associations, including two longitudinal investigations (37,38). Discrepancies could be related to differences in AA levels among different populations. Alternatively, AA at age 10 y was inversely associated with BAZ at 10 y, and our finding may reflect this rather than an effect of AA on subsequent BAZ change. Change in the D5D activity index was also positively associated with subsequent BAZ change, despite prior evidence for an inverse association (41). This finding may also have been driven by an inverse association with baseline BAZ. Neither ALA nor LA were associated with BAZ change in the entire sample, though LA at 5 y of age was inversely associated with BAZ change

among children with overweight or obesity. LA (37) and ALA (38) have each been inversely associated with weight change in previous investigations, and discrepancies may be related to differences in intake levels or unmeasured confounding by other aspects of diet.

Our results were generally inconsistent between the analyses of PUFA and MetS and adiposity. In particular, specific long-chain n-3 PUFA were positively associated with MetS but inversely associated with BAZ change; DGLA was strongly associated with MetS among adults but not related to BAZ change, while AA was positively associated with BAZ change but not related to MetS. One possible explanation may be that the results from chapter 3 are biased by reverse causation, whereas those from chapter 4 are not. There may also be effect modification by region of the associations between PUFA and cardiometabolic risk (42), possibly due to differences between the study populations in terms of genetic background or dietary context.

One of the strengths of this dissertation is the use of objective biomarkers to measure PUFA status, which are not subject to recall bias or systematic errors from the determination of nutrient composition of foods. Adipose tissue and serum concentrations of specific PUFA are correlated with dietary measures (1). In particular, adipose tissue fatty acids are the gold standard biomarkers of fatty acid status because they reflect long-term intake (1), and these biomarkers have not been widely used in pediatric research. The family design of the study in chapters 2 and 3 allowed us to examine associations separately in adults and children within the same population. In chapter 4, the prospective design greatly limits the possibility of reverse causation and allowed us to examine associations between PUFA and adiposity at multiple developmental periods. Finally, this work as a whole provides new information on PUFA nutrition and its relation to early-life cardiometabolic risk in populations where these associations have not been

well-characterized, and where both low PUFA intake (43) and chronic disease (44) are of public health concern.

Among the limitations of this dissertation is the cross-sectional design of the Mesoamerican study, which prevents causal inference. Because of this, we cannot rule out the possibility that reverse causation might explain some of the findings in chapter 3 if, for example, metabolic dysregulation affects desaturase activity. The study population was not intended to be representative of any particular group, which may limit the generalizability of our findings. The relatively small number of children in the study population could have limited statistical power. In the Chilean cohort, most covariates were only measured in infancy, so there may be residual confounding of the associations by childhood characteristics. Some of the results may be related in part to cross-sectional associations between serum PUFA and BAZ, which could be subject to reverse causation. Finally, serum fatty acids are responsive to short-term intake, so the use of a single serum PUFA measurement might misclassify individuals with respect to their habitual diet.

Public health implications and future directions

The state of PUFA nutrition in Latin America is poorly characterized, but available evidence suggests that intake of these nutrients is inadequate (43,45,46). In addition, the burden of chronic disease in the region is large and growing (44), in conjunction with a high prevalence of early-life risk factors such as childhood obesity (47) and metabolic dysregulation (48). As the nutrition transition continues in many of these countries, identifying dietary targets for prevention of cardiometabolic diseases is crucial to reduce their impact on population health. Improving PUFA nutrition is a potentially promising avenue for intervention, but little is known about the sociodemographic and dietary patterning of PUFA status in Latin America, or the relations between PUFA and cardiometabolic risk in childhood. This dissertation contributes to knowledge in these areas, which is essential to the design and implementation of adequate interventions.

In chapter 2, we found that country of origin was a substantial source of variation in adipose tissue PUFA concentrations. This suggests that there is heterogeneity in intake of PUFA across different populations within Latin America, and emphasizes the need for further surveillance efforts in order to identify groups who may be at risk of inadequate intake. Our sample size did not allow for extensive within-country analyses; future work in this area could clarify the mechanisms driving the differences between countries and inform the design of policies or interventions targeting the specific causes of low PUFA status in places such as El Salvador and Honduras. This should involve studies with larger sample sizes from specific countries, and potentially measurement of single nucleotide polymorphisms (SNPs) that have previously been identified as being associated with tissue PUFA levels in order to determine the extent to which between-country differences are influenced by genetics and environment. Our

findings corroborate those from previous studies in Latin American contexts (3–5) that suggest that the type of vegetable oil used for cooking in the home is a primary dietary determinant of PUFA intake and status. In a trial of soyabean and sunflower cooking oils conducted in Bogotá, Colombia, the use of high-PUFA oils increased blood levels of long-chain PUFA as well as essential PUFA, and switching to this type of oil was feasible and acceptable for most families (5). Our results and those from this trial suggest that interventions designed to promote replacement of palm oil with canola or soyabean oils high in LA and ALA could be effective at increasing PUFA status in Mesoamerican populations, and warrants studying their effects in future randomized trials. Nevertheless, interventions should be tailored to national dietary and cultural contexts; the selection of adequate replacement oils should take into consideration issues such as accessibility and cost, acceptability, and the *trans* fat content of specific types of oils within different countries.

Our finding that ALA is inversely associated with MetS among Mesoamerican adults should be investigated further. Our results are in agreement with a study conducted in Costa Rica (11) and may indicate that increasing ALA intake in this population would help to combat the development of MetS and resulting cardiometabolic diseases. A protective effect of ALA against coronary heart disease may be greatest when intake of long-chain n-3 PUFA is low (49). This indicate that increasing ALA intake in Latin American populations with low fish consumption could be especially important for cardiometabolic health. Moreover, we did not find that any long-chain n-3 PUFA were protective against MetS among either adults or children; in fact, EPA and DPA were positively associated with MetS and metabolic score, respectively. The mechanisms behind these unexpected associations are speculative, but the results may suggest that long-chain n-3 PUFA would not be effective targets for intervention against MetS among

Mesoamerican adults or children. Nevertheless, our study was cross-sectional and our results could be explained by reverse causation. While our findings provide preliminary evidence that ALA intake is a more important target than long-chain n-3 PUFA for intervening on MetS, confirmation with longitudinal designs would provide stronger evidence for causality. If the association were confirmed in longitudinal studies, it would further underscore the need to conduct trials of interventions designed to increase ALA intake, possibly through choice of cooking oils.

In the Chilean cohort, DHA was inversely associated with BAZ change. This finding is not consistently supported by existing studies, and requires further investigation with longitudinal observational investigations or randomized trials. If future studies also find evidence for a protective effect of DHA on adiposity in childhood, it will be important to determine what types of interventions could feasibly increase DHA status in areas where fish availability is low, and could be sustainable in the long-term given decreasing global fish stocks (50).

The positive association of serum AA and BAZ gain could be consistent with our finding from chapter 3 that LA, AA, and DGLA were positively associated with waist circumference among Mesoamerican children. AA serves as a precursor to prostacyclin, an eicosanoid that promotes differentiation of preadipocytes into adipocytes and thus may promote adipogenesis in early life (34). LA and DGLA are precursors in the synthesis of AA and could operate through this effect of prostacyclin. Most studies of PUFA and measures of adiposity among children have not found similar associations, so our results should be tested through replication of these analyses in other longitudinal studies. However, the possibility of a positive association between n-6 PUFA and adiposity in childhood is worth investigating, particularly given that some populations in Latin America may be at heightened risk of developing insulin resistance as a

result of adiposity (51,52). The effect of n-6 PUFA on obesity in children is important to consider in the design of interventions intended to increase n-3 PUFA consumption, since LA and ALA co-occur in many of their dietary sources. For example, this may suggest that canola oil is a healthier choice than soyabean oil, given its comparatively lower LA content.

In summary, the research in this dissertation indicates that PUFA status of different populations within Latin America is heterogeneous, and is related to the type of vegetable oil used for cooking at home. In children, PUFA status is related to metabolic dysregulation and adiposity, and thus could have effects on long-term cardiometabolic health. Further studies in pediatric populations are needed, both to confirm the associations we found between specific PUFA and cardiometabolic risk factors and to investigate dietary interventions that would affect PUFA status in this region. Nevertheless, our results indicate that nutrition with regard to PUFA is plausibly related to cardiometabolic health in childhood, and that it is worth pursuing as a means of reducing the public health burden of chronic disease.

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