Nutrition in Middle Childhood and Externalizing and Internalizing Problems in Adolescence: Results from the Bogota School Children Cohort

by

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Glossary of Acronyms

25(OH)D	25-hydroxy vitamin D
AA	Arachidonic acid
AdA	Adrenic acid
ALA	Alpha-linolenic acid
BMI	Body mass index
BoSCCo	Bogotá School Children Cohort
CI	Confidence interval
CRP	C-reactive protein
D5D	Δ5-desaturase
D6D	Δ6-desaturase
DBP	Vitamin D binding protein
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EDA	Eicosadienoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
GLA	Gamma-linolenic acid
HPA	Hypothalamic–pituitary–adrenal
ID	Iron deficiency
LA	Linoleic acid
N-3	Omega-3
N-6	Omega-6
PUFA	Polyunsaturated fatty acids
SD	Standard deviation
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
VDD	Vitamin D deficiency
YSR	Youth Self Report

Abstract

Background. An optimal diet is essential for adequate neurodevelopment. Behavior problems during adolescence are highly prevalent and result in adverse health outcomes in the short- and long-term. The role of middle childhood diet on adolescent behavior problems is uncertain.

Objectives. We examined the associations of the following dietary exposures in middle childhood with externalizing and internalizing behavior problems in adolescence: 1) iron deficiency (ID) and anemia, 2) vitamin D deficiency (VDD) and vitamin D binding protein (DBP) concentrations, and 3) serum n-3 and n-6 polyunsaturated fatty acid (PUFA) concentrations.

Methods. These studies take place in the context of the Bogota School Children Cohort (BoSCCo), a longitudinal investigation of nutrition and health in Bogota, Colombia. Three thousand two hundred and two schoolchildren, aged 5-12 y, were recruited into BoSCCo from primary public schools in February, 2006. Upon recruitment, parents completed a background survey on family sociodemographic information and the health habits of their child. Research assistants obtained a baseline fasting blood sample from 88% of the children. After a median 6 y, total externalizing and internalizing behavior problems and their subscales (aggressive behavior, rule breaking behavior, anxious/depressed, withdrawn/depressed, and somatic complaints) were assessed with use of the Youth Self Report in a random sample of approximately one-third of the cohort.

We assessed whether ID (ferritin <15 µg/L when C-reactive protein ≤10 mg/L), anemia (hemoglobin <12.7 g/dL) or blood concentrations of zinc, vitamins A and B-12, and erythrocyte folate in middle childhood were associated with behavior problems in adolescence in 1,042 schoolchildren. The associations between middle childhood VDD (25-hydroxy vitamin D <50 nmol/L) or low DBP concentration (<2497 nmol/L) and adolescent behavior problems were examined in 278 schoolchildren. Finally, we investigated the relations between PUFA concentrations in middle childhood and behavior problems in adolescence among 444 schoolchildren.

In bivariate analysis, behavior problems score distributions were compared across levels of potential confounding variables measured at baseline. Then, the continuous distributions of behavior problems scores were compared across categories of each of the nutritional exposures of interest. Mean differences in behavior problems scores were estimated by categories of nutritional exposures with the use of multivariable linear regression.

Results. After controlling for potential confounding variables, ID, anemia, and low vitamin B-12 in middle childhood were each positively related to total externalizing problems scores among boys. In addition, ID was positively related to total internalizing problems scores among boys. Middle childhood VDD was positively associated with total externalizing problems scores while low DBP was positively related to aggressive behavior and anxious/depressed scores. Docosapentaenoic acid was inversely associated with total externalizing problems scores whereas docosahexaenoic acid was positively associated with this outcome. In addition, the Δ5-desaturase enzyme activity index was inversely related to total externalizing problems scores. Alpha-linolenic acid was positively associated with total internalizing problems scores whereas adrenic acid was inversely related to this outcome.

Conclusions and significance. Nutrition in middle childhood was associated with behavior problems in adolescence. Adolescents with externalizing and internalizing problems are at high risk for psychiatric disorders in adulthood. In a country that has experienced over 50 years of civil war, the long-term consequences of behavior problems may be of critical public health concern. These studies could provide a basis for the planning of nutritional intervention studies.

Chapter 1. Introduction

Overview

Psychiatric disorders are the leading cause of years lived with disability worldwide (1). As life expectancy increases, the disease burden attributable to psychiatric disorders is expected to increase. Externalizing (e.g., conduct and attention deficit hyperactivity disorders) and internalizing disorders (e.g., depressive and anxiety disorders) alone account for 107 million years lived with disability / disability adjusted life years globally (1). In adolescence, the prevalence of externalizing or internalizing disorders is greater than 10% (2). An externalizing or internalizing disorder in adolescence can indicate onset of lifelong psychiatric disorders (3). These disorders lie at the extreme end of a spectrum of externalizing and internalizing behavior problems, which are also highly predictive of mental health disorders later in life (4).

Prenatal and infant nutrition play a key role in the development of behavior problems throughout childhood (5). Specific nutrients in the prenatal or infancy period, including iron (6, 7), vitamin D (8, 9), and polyunsaturated fatty acids (PUFA) (10-12), alter monoamine neurotransmitter metabolism or the formation of neuronal networks in the developing brain. Much less is known about the potential effects of diet in middle childhood (from approximately 6 to 11 years of age). Middle childhood is a period of changes in functional activity in the central nervous system (13) that may be responsive to environmental exposures. Identifying modifiable factors that predict externalizing and internalizing problems in middle childhood could provide the basis for cost-effective school-based interventions.

Specific Aims

This work aims to investigate the relation between nutrition in middle childhood and externalizing and internalizing problems in adolescence within the context of the Bogotá School Children Cohort, a prospective study of nutrition and health in Bogotá, Colombia.

Aim 1. To examine the relations between iron deficiency (ID) and anemia in middle childhood and externalizing and internalizing problems in adolescence independent of other micronutrients that are relevant for neurodevelopment, such as zinc, vitamin A, folate, and vitamin B-12. A secondary aim is to assess the associations of middle childhood sociodemographic characteristics and health habits of children and their families with behavior problems in adolescence.

Aim 2. To evaluate whether vitamin D serostatus in middle childhood is associated with externalizing and internalizing problems in adolescence. As an exploratory, hypothesis generating aim, I will examine the associations of vitamin D binding protein (DBP) concentrations in middle childhood with externalizing and internalizing problems in adolescence.

Aim 3. To investigate the relations of serum concentrations of n-3 and n-6 polyunsaturated fatty acids (PUFA) in middle childhood with externalizing and internalizing problems in adolescence. Since endogenous conversion of alpha-linolenic acid and linoleic acid into long-chain PUFA depends on desaturase enzyme activity, as an exploratory aim I will assess the associations of the Δ6-desaturase (D6D) and Δ5-desaturase (D5D) enzyme activity indices with adolescent behavior problems.

This research will contribute new knowledge on the role of middle childhood diet on adolescent behavior problems.

Overview of Externalizing and Internalizing Problems

Definition and Burden of Disease

The central feature of externalizing problems is defiant, aggressive, or irregular behavior. Externalizing disorders typically include conduct and attention deficit hyperactivity disorder. On the other hand, internalizing problems are characterized by disordered mood or emotions and encompass depressive and anxiety disorders. The distributions of externalizing and internalizing disorders vary by age and sex. Externalizing disorders are often diagnosed in middle childhood whereas the prevalence of internalizing disorders increases in adolescence, especially among girls (2). Externalizing disorders are more prevalent in boys while the prevalence of internalizing disorders is higher in girls (14).

Externalizing and internalizing problems in childhood or adolescence are predictive of adult psychiatric disorders (4) and substance use disorders (15). Internalizing problems in childhood are associated with having a major depressive episode, bipolar disorder, or dysthymia in adulthood whereas childhood externalizing problems are related to anxiety disorders in adulthood (4). In one study, the odds of having cannabis use disorder in young adulthood were 2.6 times higher among adolescents with externalizing problems compared with adolescents without externalizing problems (15). Individuals with psychiatric disorders may find day-to-day tasks difficult to manage and some, including those with bipolar (16) and obsessive-compulsive disorder (17), can be constantly plagued by self-doubt. These disorders can negatively impact their relationships with friends and family as well. In addition to being caustic to an individual's mental wellbeing and their close relations, these disorders are at higher risk of adverse physical and economic outcomes. For example, depressive disorders are positively related to cardiovascular disease (18), suicide (19), and increased health care costs (20).

The prevalence and burden of psychiatric disorders vary by country (1, 21). Culture, urbanicity, and economic and conflict status are each associated with the prevalence of psychiatric disorders in a country (21). Globally, exposure to war, revolution, or persecution in a country has been associated with a 60% higher prevalence of anxiety disorders on the country level compared with countries which were not exposed to such conflicts (21). Although high income countries have higher reported prevalence of psychiatric disorders, psychiatric disorders' contribution to years lived with disability and disability adjusted life-years is increasing in low- and middle-income countries (1) which may not have the infrastructure or resources to treat these disorders adequately (22).

Potential Biologic Mechanisms of Externalizing and Internalizing Problems

Complex biologic mechanisms govern mood and behavior in individuals. Altered connectivity between the prefrontal cortex and the basal ganglia, areas of the brain responsible for decision making, memory formation, and emotion regulation, is associated with behavior problems (23). Prenatal environmental factors, including nutrition and environmental toxicants, influence dendritic arborization and synaptic connectivity (7, 10, 11, 24). The metabolism of the monoamine neurotransmitters dopamine and serotonin are also regulated by environmental factors such as iron, vitamin D, and pesticide exposure (8, 9, 25, 26). Among other functions, dopamine regulates higher order cognitive and emotional processes (27), while serotonin plays a key role in governing mood, sleep, and the stress response (28). Thus, alterations of these monoamine metabolisms may play a role in the development of behavior problems.

Another relevant system is the hypothalamic-pituitary-adrenal (HPA) axis, a neuroendocrine system involved in the production, release, and regulation of cortisol, a glucocorticoid involved in the stress response. Altered cortisol levels can impair learning and

memory, reduce neurogenesis, and lead to cell apoptosis. In addition, high levels of cortisol reduce the mRNA expression and alter the binding function of the post-synaptic serotonergic receptors 5-HT_{1A} in animal studies (29). These alteration in the serotonergic metabolism may further dysregulate the HPA axis function (29). Increased production of cortisol occurs in adults with major depressive disorders, however, it is unknown if this is a consequence or a cause of the disorder (30). In children, dysregulation of the HPA axis and higher cortisol levels have been associated with externalizing and internalizing problems (31).

Predictors of Externalizing and Internalizing Problems

Genetic factors account for approximately 50-60% of the variation in externalizing and internalizing problems (32, 33). Environmental modifications also influence the development of behavior problems. During the prenatal and perinatal periods, maternal exposure to alcohol intake (34), nutritional deficiencies (35), heavy metals (36), and endocrine disruptors (37) affect fetal brain development and are associated with more behavior problems in children and adolescents. Studies of the relations between childhood exposures and behavior problems have focused on childhood habits, parenting strategies, and markers of socioeconomic status. For example, physical activity is associated with fewer behavior problems in youth in experimental studies (38). Time spent watching television or playing video games is related to more internalizing, hyperactivity, and inattention problems (39). Child maltreatment and parental substance use disorders are positively associated with childhood behavior problems (40, 41). By contrast, parenting styles that provide children with learning opportunities and cognitively stimulating experiences are associated with more self-confidence and positive affect in children (42). Indicators of low socioeconomic status, such as household food insecurity (43), lower family income and parental education (44), and neighborhood disadvantage (45), are associated with more externalizing and internalizing problems in children.

The brain continues to develop into the third decade of life. Exposures that are hypothesized to affect brain development in the prenatal or infancy period may influence middle childhood brain maturation, depending on the particular aspects of central nervous system development that mature late. Most central nervous system development occurs before the age of 2 y, however, many gains in higher-order cognitive abilities occur in middle childhood (46, 47). Metabolic rates for glucose are higher in children ages 3-16 years than in adults, indicating

greater energy metabolism in the brain during this period, possibly due to increased energy requirements for development of brain tissue (47). Both progressive (involving tissue growth) and regressive (involving tissue loss) alterations occur in the brain after birth (47). After age 4 y, higher order cortical regions like the prefrontal cortex and the temporal association areas experience the most alterations compared to other regions of the brain (13). Between the ages of 4 and 18 y, hippocampal volume increases in girls while amygdala volume increases in boys (47, 48). These areas of the brain are involved in complex decision making, personality determination, memory formation, and sensory processing (13). Nutrients hypothesized to affect brain development in the intrauterine period, including iron, vitamin D, and PUFA, may also influence middle childhood brain development. However, few studies have examined the associations of childhood nutrition with behavior problems.

Iron is an essential nutrient with multiple functions in the circulatory, immune, and neurological systems. Although the most commonly recognized function of iron is enabling oxygen transport in erythrocytes, iron also plays a role in myelination, dendritic growth, synaptogenesis, and neurotransmitter metabolism in the central nervous system (49). Iron is a cofactor in tyrosine hydroxylase, an enzyme involved in the formation of the monoamine neurotransmitters dopamine, serotonin, and norepinephrine (49). In addition, prenatal iron may alter the metabolic pathways of the monoamine neurotransmitters, glutamate, and GABA (25, 50, 51). For example, dopamine receptors are downregulated in the presence of ID in the striatum and nucleus accumbens (51). In animal experiments, animals who were iron deficient (ID) in utero exhibit less exploratory and more anxiety-related behaviors (52, 53). Prospective studies have found an association between chronic iron deficiency in infancy and behavior problems later in childhood (54-57). The prospective association between ID or anemia in

middle childhood and later behavior problems has not been investigated. Mechanisms governing a potential association between middle childhood ID and behavior are speculative. As with prenatal ID, middle childhood ID may alter the expression of brain-derived neurotrophic factor (BDNF) (58), which influences synaptic plasticity (59) and thus may be associated with psychiatric disorders (60).

Vitamin D deficiency (VDD) in infancy or childhood may be related to emotional, cognitive, or developmental problems later in life. Although vitamin D is best known for its role in bone metabolism, in recent years the vitamin has been investigated as a neurosteroid (61). Vitamin D enhances the expression of tyrosine hydroxylase (62, 63), an enzyme involved in the formation of dopaminergic neurons and the function of dopamine, norepinephrine, and epinephrine, suggesting a role on behavior. In animal models, developmental VDD is associated with morphological alterations in the offspring's brain stereology, including smaller lateral ventricles, smaller hippocampi, and larger striatum compared with controls (64). Animals with developmental VDD also exhibit behavioral alterations such as hyperlocomotion (65). In humans, the effects of prenatal VDD may be dependent on the timing of the deficiency. VDD in the first trimester of pregnancy is related to behavior problems in childhood (66, 67), whereas VDD in the latter half of pregnancy is not associated with these outcomes (68-71). Only one study has investigated the association of VDD in middle childhood with later behavior problems, higher vitamin D concentrations at 10 y were associated with more prosocial behavior 2 years later (72). The mechanisms by which VDD in middle childhood impact later behavior may differ from those in the prenatal period. In middle childhood, vitamin D enhances the expression of glial cell line-derived neurotrophic factor, which promotes the survival of dopaminergic neurons (73, 74).

Polyunsaturated fatty acids (PUFA), fatty acids that contain more than one double bond, have also been hypothesized to relate to behavior problems. There are two main groups of PUFA: omega-3 (n-3) and omega-6 (n-6). Their nomenclature depends on the location of double bonds; n-3 fatty acids (FA) have their first double bond on the third carbon, whereas n-6 FA have their first double bond on the sixth carbon. The n-3 and n-6 PUFA docosahexaenoic acid (22:6 n-3, DHA), arachidonic acid (20:4 n-6, AA), and adrenic acid (22:4 n-6, AdA) concentrate in the brain during the third trimester of pregnancy. DHA continues to accumulate in the brain throughout childhood (75). Precursors to these PUFA, alpha-linolenic acid (18:3 n-3, ALA) and linoleic acid (18:2 n-6, LA), cannot be synthesized in humans and are therefore considered essential as they must be obtained in diet. These precursors are metabolized into longer chain n-3 and n-6 PUFA through a series of elongation and desaturation reactions. However, this process is inefficient. Longer chain PUFA are also obtained in the diet. N-3 PUFA may influence behavioral outcomes through three primary pathways (76). First, PUFA that have accumulated in the brain are incorporated into the plasma membranes of neuronal and glial cells (77). The FA composition of the cell membrane influences membrane protein function and fluidity. Second, n-3 PUFA are precursors of anti-inflammatory eicosanoids which may exhibit neuroprotective effects (76). Finally, n-3 PUFA may influence transcription factors regulating neuroinflammation (76). N-6 PUFA also promote and regulate neuroinflammation. However, n-6 PUFA metabolize into pro-inflammatory eicosanoids (78), which may dysregulate the HPA axis (79) and damage neurons (78). Longitudinal observational studies that examine the associations between maternal or infant PUFA and behavior problems in childhood have mixed results (80-86). No study of prenatal exposure extends into adolescence when many behavior problems develop. In short-term supplementation trials of n-3 PUFA during adolescence, the supplemented group had

decreased aggression or disruptive behavior compared with the control group after 3-12 months (87, 88). No study has examined the association between middle childhood PUFA exposure and adolescent behavior problems after an extended follow-up. A potential mechanism of PUFA in middle childhood is that PUFA concentrations may influence synaptic plasticity through the regulation of long-term potentiation (89, 90).

The previous lack of focus on middle childhood exposures points towards the need to improve understanding of how dietary factors influence behavior throughout the lifespan. As the majority of children are in schools during middle childhood, interventions at this time are relatively cost-effective and easy to implement. This question is especially salient within the context of Colombia, where the prevalence of childhood behavior problems may have increased (91) because of the more than 50-year civil war and resulting "culture of violence" that has developed in reaction to the unrest (92). Thus, studying modifiable determinants of behavior problems may be particularly important to public health in Colombia.

Summary of Chapters

This dissertation contributes new knowledge on modifiable nutritional factors that predict adolescent behavior problems in middle childhood. Middle childhood is a period highly amenable to intervention and brain development continues in this period. However, predictors of later behavior problems are understudied.

The following chapters of this dissertation describe the relations between middle childhood nutrition and self-reported adolescent externalizing and internalizing problems in a sample of schoolchildren from Bogotá, Colombia. Chapter 2 focuses on middle childhood iron deficiency and anemia as exposures. Chapter 2 also examines micronutrients related to neurodevelopment that may be associated with iron intake or anemia, including zinc, vitamin A, folate, and vitamin B-12. Chapter 3 evaluates the associations of middle childhood vitamin D serostatus and vitamin D binding protein with behavior problems. Chapter 4 examines n-3 and n-6 PUFA as exposures. Finally, a summary of the dissertation's main findings and public health importance are discussed in Chapter 5.

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Chapter 2. Iron Deficiency, Anemia, and Low Vitamin B-12 Serostatus in Middle Childhood are Associated with Behavior Problems in Adolescent Boys

Abstract

Iron deficiency (ID) in infancy is related to subsequent behavior problems. The effects of micronutrient status in middle childhood are uncertain. The aim of the study was to examine the associations of micronutrient status biomarkers in middle childhood with externalizing and internalizing behavior problems in adolescence. We assessed whether ID (ferritin <15 μg/L when C-reactive protein ≤10 mg/L), anemia (hemoglobin <12.7 g/dL), or blood concentrations of zinc, vitamins A and B-12, and folate at ages 5-12 y were associated with externalizing or internalizing behavior problems in adolescence in 1,042 schoolchildren from Bogotá, Colombia. Behavior problems were assessed with the Youth Self Report questionnaire after a median 6.2 y of follow-up. Mean problem score differences with 95% confidence intervals (CI) were estimated between categories of micronutrient status biomarkers with use of multivariable linear regression. Mean ± standard deviation externalizing and internalizing problems scores were 52.6 ± 9.6 and 53.8 ± 9.9, respectively. Among boys, middle childhood ID, anemia, and low plasma vitamin B-12 were associated with 5.9 (95% CI: 1.0, 10.7), 6.6 (95% CI: 1.9, 11.3), and 2.7 (95% CI: 0.4, 4.9) units higher mean externalizing problems scores in adolescence, respectively; after adjustment for baseline age, time spent watching television/playing video games, mother's height, and socioeconomic status. Also in boys, ID was related to an adjusted 6.4 (95% CI: 1.2, 11.6) units higher mean internalizing problems scores. There were no associations among girls.

Other micronutrient status biomarkers were not associated with behavior problems. ID, anemia, and low vitamin B-12 in middle childhood are related to behavior problems in adolescent boys.

Introduction

Mental health problems affect 10-20% of children and adolescents worldwide (1) and are associated with adverse health outcomes in the short- and long-term (2). Among these problems, externalizing and internalizing behavior disorders, including conduct, attention-deficit hyperactivity, depressive, and anxiety disorders, pose a particularly hefty burden accounting for >100 million disability-adjusted life years globally (3). These disorders are at the extreme end of a spectrum of more subtle, yet also highly relevant, behavior problems that are predictive of impaired mental (4) and physical (5) health in adulthood.

Nutrition plays an important role in the development of behavior from infancy through adolescence (6), but the effects of individual nutrients throughout the life cycle are not well characterized. Most research has focused on micronutrient status in infancy. For example, iron deficiency (ID) in infancy is associated with lower positive affect in infancy (7) and middle childhood (8), externalizing (9-11) and internalizing (9, 10, 12) behavior problems in adolescence, and lower self-rated emotional health in young adulthood (13). Nevertheless, the effects of exposure to iron or other micronutrient deficiencies later in childhood have not been studied in prospective investigations. Structural changes in areas of the brain that may be important in the development of behavior problems, including the basal ganglia, hippocampus, amygdala, and prefrontal cortex, occur throughout childhood (14). Rodent experiments indicate that exposure to gestational, perinatal, and post-weaning iron deficiency (15-17), post-weaning zinc (18) or vitamin A (19) deficiencies, low gestational vitamin B-12 (20), or gestational folate deficiency (21) can disrupt the normal development of these regions, but data in humans are scant.

The objective of this study was to investigate the associations between micronutrient status in middle childhood and externalizing and internalizing behavior problems in adolescence in a cohort of schoolchildren from Bogotá, Colombia. We hypothesized that low concentrations of micronutrient status biomarkers (ferritin, hemoglobin, zinc, vitamins A and B-12, and folate) in middle childhood would be related to increased externalizing and internalizing problems in adolescence.

Methods

Study design and population

We conducted a prospective study in the context of the Bogotá School Children Cohort, a longitudinal investigation of nutrition and health in Bogotá, Colombia. Details on the cohort design have been previously reported (22). Briefly, beginning in February 2006, we recruited 3,202 randomly selected children aged 5-12 years (y) from primary public schools. A majority of children in the public school system in Bogotá are from low- and middle-income socioeconomic backgrounds. Therefore, our sample pertains to these groups.

Baseline information

At the time of enrollment we collected information on child, parental, and household characteristics with the use of a survey that was sent to the children's homes. The questionnaire inquired about children's background and habits, including the time usually spent watching television/playing video games or playing outdoors. The survey also included questions on parental age, marital status, and education level and on maternal parity, height, and weight. Household characteristics involved the local government's socioeconomic status (SES) classification and the level of food insecurity according to a validated version of the United States Department of Agriculture Household Food Security Survey module (23).

During the weeks following enrollment, trained research assistants scheduled data and sample collection school visits, after contacting the primary caregivers requesting that the child fast overnight before the visit day. At these visits, height was measured without shoes to the nearest 1 mm using a wall-mounted portable Seca 202 stadiometer (Seca, Hanover, MD) and weight was measured in light clothing to the nearest 0.1 kg using Tanita H5301 electronic scales (Tanita, Arlington Heights, IL). Height and weight were also measured among the children's mothers

who were present at schools (37%). At the same visits, the research assistants obtained fasting blood samples through antecubital venipuncture in 88% of the children. Twelve percent of children were unwilling to provide a blood sample. One aliquot was collected in an EDTA-coated tube and a second one in a metal-free polypropylene tube without anticoagulant for separation of serum. The samples were protected from sunlight and transported in refrigerated coolers on the day of collection to the Colombian National Institute of Health, where they were processed and cryostored for future analyses.

Follow-up

Between 2011-2015 we conducted an in-person follow-up assessment in a random sample of approximately one-third of cohort members (n = 1,139). Adolescents were assessed at school or home if absent from school. At this assessment, we ascertained child behavior with the use of the Spanish language version of the Youth Self Report (YSR) questionnaire (24), a widely used method to assess behavioral and emotional problems in adolescents. The YSR is a self-administered questionnaire consisting of 112 statements addressing behaviors or feelings that children rate as false, sometimes true, or very/often true. From responses to these questions, an Assessment Data Manager (ADM) software (25) calculates continuous scores for 8 behavior problem subscales: aggressive behavior, rule breaking behavior, anxious/depressed, withdrawn/depressed, somatic complaints, attention problems, social problems, and thought problems. The sum of the aggressive and rule breaking behavior subscale scores constitute the total externalizing problems score, whereas the sum of anxious/depressed, withdrawn/depressed, and somatic complaints scores comprise the total internalizing problems score (26). The ADM software standardizes the scores by age and sex to a reference population derived from data collected periodically in United States national surveys (25). The YSR has been validated for use

in adolescents ages 11-18 y from English-speaking populations (27), has high reliability (24), and is generalizable to Spanish-speaking populations (28). It has been utilized in studies of Chilean (12), Costa Rican (9), and Puerto Rican (24) adolescents. A general questionnaire was also administered to primary care givers at the follow-up visit to update information on mother's marital status, education, parity, and BMI, and household food security and socioeconomic status.

The parents or primary caregivers of all children gave written informed consent prior to enrollment into the study and before the follow-up assessment. Children gave written assent to participate. The study protocol was approved by the Ethics Committee of the National University of Colombia Medical School. The Institutional Review Board at the University of Michigan approved the use of data from the study.

Laboratory methods

All analyses took place at the Colombian National Institute of Health. Plasma ferritin concentration was measured using a competitive chemiluminescent immunoassay in an ADVIA Centaur analyzer (BayerDiagnostics, Tarrytown, NY, USA). Serum C-reactive protein (CRP) level was measured using a turbidimetric immunoassay on an ACS180 analyzer (Bayer Diagnostics, Tarrytown, NY). Hemoglobin concentrations were determined by the hemiglobincyanide method. Serum zinc concentrations were determined using an atomic absorption technique (29) on a Shimadzu AA6300 spectrophotometer. Plasma retinol was measured with high performance liquid chromatography on a Waters 600 System. Plasma vitamin B-12 and erythrocyte folate were also quantified using a competitive chemiluminescent immunoassay in an ADVIA Centaur analyzer.

Data analysis

The YSR was completed by 1,097 of the 1,139 cohort members who participated in the follow-up assessment; 13 forms could not be processed by the ADM software due to an excess of missing values. Forty-two children who were younger than 11 y or older than 18 y were excluded from the analysis, because the YSR was developed and validated for use in 11- to 18 year-olds (30); thus, the final sample consisted of 1,042 children. The sample size had been calculated to provide >85% statistical power to detect differences in mean behavior scores >10% between extreme quartiles of exposure, assuming a type I error of 5% and mean ± SD scores of 50.0 ± 10.0 in the unexposed. Power would be >95% to detect linear trends in multivariable analyses with as many as 10 covariates. Compared with cohort participants who were not included in the analysis (i.e., did not participate in the follow-up assessment, did not complete the YSR, or were not between the ages of 11-18 y), children in the analytic sample spent more time watching television/playing video games, had better educated mothers, were of higher SES, and the boys had lower prevalence of anemia at baseline (**Table 1**). They did not differ with regard to the distributions of other micronutrient status biomarkers.

The primary outcomes of interest were the continuous distributions of total externalizing and internalizing problems scores. Secondary endpoints were the subscales of these composite scores: aggressive and rule breaking behavior for externalizing problems and anxious/depressed, withdrawn/depressed, and somatic complaints for internalizing problems. In supplemental analyses we considered attention, social, and thought problems subscales.

We considered as exposures biomarkers for micronutrients that are relevant to neurobehavioral development. These included iron, zinc, vitamin A, vitamin B-12, and folate. ID was defined as plasma ferritin <15 μg/L (31). Thirteen children with serum CRP >10 mg/L were excluded from the analysis of ID. Anemia was defined as hemoglobin <12.7 g/dL after

adjustment for the altitude of Bogotá (32). Vitamin A status was categorized as deficient (plasma retinol <20 μg/dL), low (20 to <30 μg/dL), or adequate (≥30 μg/dL) (33). Serum zinc, plasma vitamin B-12, and erythrocyte folate were categorized into sex-specific quartiles since the prevalence of these micronutrient deficiencies according to conventional cut-points was low (<2%).

Covariates included sociodemographic, anthropometric, and health-related characteristics measured at baseline. Children's height- and body mass index (BMI)-for-age Z scores were calculated according to the World Health Organization (WHO) growth reference for children and adolescents (34). Maternal BMI was calculated as kg/m^2 from objectively measured height and weight in 37% of mothers and from self-reported data in the rest. Correlations between objectively measured and reported values were 0.79 (P<0.0001) for height and 0.81 (P<0.0001) for BMI. Covariates were categorized as presented in **Table 2**.

All analyses were conducted separately by sex. First, to identify independent predictors of the outcomes, we compared the distributions of total externalizing and internalizing problems scores across categories of baseline characteristics using means ± standard deviation (SD). Next, we examined the distributions of these outcomes by levels of micronutrient status indicators. For ordinal exposures in which levels have a hierarchical relation with each other, we conducted tests for linear trend by fitting linear regression models with the behavior problems scale as the outcome and a variable representing ordinal categories of each predictor as a continuous covariate. This is a conventional method to examine linearity of associations for nutritional exposures that are categorized into quantiles (35). For ID and anemia, we used the χ^2 score statistic. In all models, an independent correlation matrix was used to account for clustering by sibship, since there were 107 siblings in the sample. Empirical estimates of the variance were

specified to overcome potential deviations from the multivariate normality assumption. We estimated mean adjusted differences and 95% confidence intervals (CI) for total externalizing or internalizing problems scores between categories of sociodemographic and nutritional predictors with the use of multivariable linear regression. In each model, adjustment variables included independent predictors of the outcome. Child's age at baseline was included in all final models as it was considered important from a mechanistic viewpoint. Other covariates were retained in the final model when they remained statistically significant ($P<0.05$). We examined the associations of micronutrient status biomarkers with scores on each subscale following an analogous approach.

To further understand if changes in sociodemographic factors from middle childhood through adolescence that could represent underlying changes in micronutrient status were related to the outcomes, we examined differences in total externalizing and internalizing problems by changes in maternal marital status, education, parity, and BMI, and household food security and socioeconomic status.

All analyses were performed using the Statistical Analyses System version 9.4 (SAS Institute Inc.).

Results

Mean ± SD age at enrollment was 8.5 ± 1.6 y; 56.1% of children were girls. Prevalence of ID, anemia, and vitamin A deficiency was 3.2%, 3.0%, and 14.8%, respectively. None of the anemic children had ID, 32.1% of the anemic children had vitamin A deficiency, and 14.3% of the non-anemic children had vitamin A deficiency. Among children with and without ID, the prevalence of vitamin A deficiency was 13.8% and 14.9%, respectively. Mean ± SD age at the time of follow-up assessment was 14.7 ± 1.7 y. Children were followed for a median of 6.2 y.

Total externalizing problems. Mean ± SD total externalizing problems scores were 51.9 ± 9.6 in boys and 53.1 ± 9.6 in girls. In bivariate analysis, age at follow-up assessment and time spent watching television/playing video games at baseline were positively associated with total externalizing problems scores in boys and girls (**Table 2**). In girls, baseline age was positively related to total externalizing problems scores, whereas maternal height and low socioeconomic status were inversely associated with this outcome (**Table 2**). ID, anemia, and low vitamin B-12 concentrations were each related to higher total externalizing problems scores among boys (**Table 3**). In girls, anemia was related to lower total externalizing problems scores.

In multivariable analysis, ID, anemia, and low vitamin B-12 serostatus were positively associated with total externalizing problems scores in boys after adjustment for age at baseline, time spent watching television/playing video games, maternal height, and socioeconomic status. Boys with ID had an adjusted 5.9 units (95% CI: 1.0, 10.7) higher mean total externalizing problems scores compared with iron-sufficient boys (**Table 4**). Compared with non-anemic boys, boys with anemia had an adjusted 6.6 units (95% CI: 1.9, 11.3) higher total externalizing problems scores. Boys with plasma vitamin B-12 in the lowest quartile had adjusted mean total externalizing problems scores 2.7 units (95% CI: 0.4, 4.9) higher than did boys with higher

concentrations. Anemia was not associated with total externalizing problems in girls after adjustment for these covariates.

Externalizing problems subscales. The distributions of aggressive and rule breaking behavior subscale scores varied significantly by sociodemographic characteristics and micronutrient status biomarkers (**Supplemental Table 1**). In multivariable analysis among boys, vitamin B-12 concentrations in the lowest quartile were related to a 2.3 units higher mean aggressive behavior score (95% CI: 0.4, 2.3) compared with higher concentrations (**Supplemental Figure 1**). Among girls, anemia was associated with a 2.5 units lower mean rule breaking behavior score (95% CI: -3.7, -1.3) (**Supplemental Figure 1**).

Total internalizing problems. In boys and girls, mean total internalizing problems scores were 53.4 ± 9.7 and 54.1 ± 10.1, respectively. In bivariate analysis among girls, baseline age, BMI-for-age Z score, and maternal BMI were positively related to total internalizing problems scores, whereas maternal education, severe food insecurity, and low socioeconomic status were inversely related to this outcome (**Table 5**). ID among boys and vitamin A status among girls were positively associated with total internalizing problems scores (**Table 6**).

In multivariable analysis, ID was related to total internalizing problems scores in boys. After adjustment for child's age and BMI-for-age Z score, maternal education, and household food insecurity and socioeconomic status, boys with ID had 6.4 units (95% CI: 1.2, 11.6) higher mean total internalizing problems scores than did iron-sufficient boys (**Table 7**). Vitamin A status was not significantly associated with total internalizing problems scores in girls after adjustment for these covariates.

Internalizing problems subscales. The distributions of anxious/depressed, withdrawn/depressed, and somatic complaints subscale scores varied by sociodemographic

characteristics and micronutrient status biomarkers (**Supplemental Table 2**). In multivariable analyses among boys, anxious/depressed scores were positively related to ID and anemia (**Supplemental Figure 2**). Among girls, ID was inversely related to withdrawn/depressed scores (**Supplemental Figure 2**). Somatic complaints scores were positively associated with ID, anemia, and vitamin B-12 concentrations in the lowest quartile among boys (**Supplemental Figure 2**).

Attention, social, and thought problems subscales. None of the nutritional status biomarkers examined were associated with attention or social problems scores. Thought problems scores among boys were positively related to ID and anemia (**Supplemental Figure 3**).

Changes in sociodemographic factors. Single motherhood, maternal education level, parity, and BMI, and the percent of households with food security were higher at the follow-up assessment during adolescence than at recruitment in middle childhood, whereas the percent of households in the lowest socioeconomic strata did not change (**Supplemental Table 3**). None of the changes in sociodemographic characteristics from middle childhood to adolescence was related to total externalizing or internalizing problems scores among boys or girls (**Supplemental Table 4**).

Discussion

In this longitudinal study of low- and middle-income Colombian schoolchildren, ID, anemia, and low plasma vitamin B-12 in middle childhood were associated with increased total externalizing behavior problems scores in adolescence among boys. In addition, boys with ID in middle childhood had higher total internalizing problems scores in adolescence than did iron-sufficient boys. These associations were independent of other micronutrient status indicators and child, parental, and household characteristics. After adjustment for potential confounding variables, there were no statistically significant associations between the micronutrients examined and total externalizing or internalizing problems scores in girls.

The nutritional causes of behavior problems in adolescence are poorly understood. Previous longitudinal studies primarily focused on the effects of early-life iron status on cognitive and behavioral development. ID in infancy was related to total externalizing (9) and internalizing problems (9, 10) during early adolescence in Costa Rica. In Chile, iron deficiency anemia in infancy was associated with more rule breaking behavior, an externalizing problem, at 15 y of age (11); whereas ID was related to total internalizing problems at age 10 y (12). However, the potential effect of exposure to ID during middle childhood on subsequent behavior problems was not investigated.

The results of our study may not be comparable with those from previous investigations, because the mechanisms operating in infancy could differ from those in middle childhood. The mechanisms through which ID in infancy may result in behavior problems during adolescence could involve developmental alterations in myelination throughout the brain (16) as well as diminished hippocampal oligodendrocyte function and dendritic arborization (15), according to rodent studies. Further, ID is associated with lower D1 and D2 receptor densities (17), elevated

levels of extracellular dopamine (36), and lower dopamine transporter density (37) in the basal ganglia. Dopamine is essential in the regulation of emotion, reward, motivation, and motor control; thus, dopaminergic dysfunction may be associated with behavior problems. In addition, ID may alter the metabolism of serotonin, norepinephrine, and gamma-aminobutyric acid, which could relate to emotional or behavioral development (38). Whether the same mechanisms could explain the effects of exposure to ID in middle childhood is speculative. Iron gradually concentrates in the basal ganglia (38); activity in this region during a cognitive task in children and adolescents aged 6-20 y is associated with future working memory capacity, a marker of cognitive and behavioral development (39). It is also possible that ID measured in middle childhood was already present in infancy. In this case, our findings could represent the cumulative effect of ID on behavioral development. Of note, in our study ID in middle childhood was associated with externalizing and internalizing problems in boys only. Some rodent studies suggest that males may be more sensitive to the effects of ID than females (17, 40); however, previous epidemiologic studies did not examine sex-specific associations.

Anemia in middle childhood was also associated with higher total externalizing problems scores in adolescence among boys. In this population, anemia was not due to ID and was generally uncorrelated with biomarkers of micronutrients that are relevant for hemoglobin metabolism, including zinc, folate, and vitamins B-12 and A (22). Other causes of anemia, such as parasitic infections, sickle cell disease, and thalassemia, are relatively infrequent in children from Bogotá. Thus, the nature of the association between anemia and externalizing behavior problems is uncertain. It could reflect low intake of nutrients we did not measure, such as riboflavin or vitamin C.

Among boys, low plasma vitamin B-12 was associated with higher externalizing problems scores, possibly due to increased aggressive behavior. Growing evidence suggests that vitamin B-12 status is associated with cognition in childhood (41). However, evidence related to potential effects on behavior is limited. Three cross-sectional studies examined the association between vitamin B-12 intake and externalizing and internalizing problems (42) or depressive symptoms (43, 44) in adolescence. None found an association, which could be due to lack of variability in the exposure, reverse causation bias, measurement error, or confounding. The mechanisms to explain a potential effect of vitamin B-12 on behavior could be related to its role in the metabolism of S-adenosylmethionine (SAM), a methyl donor involved in the synthesis of dopamine, serotonin, and norepinephrine. SAM may improve mood among adults with depressive disorders (45), especially in males.

Although the micronutrient status biomarkers studied were not related to the primary outcomes in girls, anemia and ID were related to decreased scores in the rule breaking behavior and withdrawn/depressed subscales, respectively. In rodent models, ID among females was associated with higher serotonin transporter density in several brain regions whereas among males ID was related to lower serotonin transporter density (40). This may help explain why anemia would be associated with higher behavior problems in boys but lower behavior problems in girls.

Family level socioeconomic status indicators, including mother's height, mother's education, and household food security, were inversely related to internalizing problems among girls. Low maternal education level and household food insecurity may impact parental mental and physical health (46, 47), while mother's height is a marker of intergenerational socioeconomic status (48). Parental wellbeing and socioeconomic status have previously been

associated with the development of behavior problems (49). On the other hand, household socioeconomic status, as measured through a neighborhood level indicator, was positively associated with externalizing and internalizing problems in girls. The positive association with neighborhood socioeconomic status is contrary to results from studies in the United States (50) and the Netherlands (51). In Colombia, neighborhoods with low socioeconomic status may have higher social cohesion which can modify the association between neighborhood socioeconomic status and behavior problems (52). Changes in sociodemographic factors that could represent underlying changes in micronutrient status from middle childhood to adolescence were unrelated to the outcomes of interest. This suggests that a potential effect of micronutrients in middle childhood might be independent of these factors.

This study has several strengths. First, its longitudinal nature minimizes the potential for reverse causation bias. Prospective collection of outcome information reduces misclassification. We used objectively measured biomarkers of micronutrient status as the exposures, which precludes recall bias. The YSR questionnaire is valid in populations similar to ours (28). Finally, we controlled for many potential confounders of the association between micronutrient status and behavior problems.

There are limitations as well. First, we did not have a baseline measurement of behavior problems in middle childhood. If behavior problems in adolescence were already present at the time of exposure assessment, reverse causation cannot be disregarded as an explanation of some of these results. Second, we lacked an assessment of micronutrient status during adolescence. If micronutrient status in middle childhood was correlated with that during adolescence, then the results may reflect exposure in adolescence rather than exposure in middle childhood. Third, some of the biomarkers we used may result in misclassification of micronutrient status. For

example, plasma vitamin B-12 represents both intake and stores of the vitamin, not necessarily the vitamin available in tissues. Since plasma vitamin B-12 concentrations are maintained while depletion occurs in the tissues, low plasma vitamin B-12 may not capture developing deficiencies. Further, low concentrations may reflect long-term marginal intake or absorption abnormalities rather than deficiency (53). Concentrations of vitamin B-12 metabolites, methylmalonic acid or homocysteine, may be more sensitive to capturing deficiency than plasma vitamin B-12 concentrations alone (53), but we did not have the means to quantify these analytes. Fourth, boys included in the analysis were less likely to be anemic than those who were not included. If the anemic children who were not included had less total externalizing problems than those included, we may have overestimated the association of anemia with externalizing behavior problems. Fifth, not all children had an available measurement for all micronutrients examined. Sixth, unmeasured independent predictors of behavior problems that may be associated with micronutrient status indicators, such as blood lead levels, history of child behavior problems, history of parental psychiatric disorders, or exposure to violence, could have resulted in residual confounding. Another limitation is that we did not have objectively measured anthropometric data in all mothers. Measurement error could have obscured the associations of these covariates with the outcomes. The prevalence of ID and anemia are low, which limits the public health significance of our findings since improvement of these conditions would only benefit a few children. Finally, our results might not be generalizable to other populations, especially high-income children. Nevertheless, in a recent national nutrition survey in Colombia, prevalence of ID, anemia, and vitamin B-12 deficiency among children of the highest socioeconomic strata were comparable to those of children in our study (54).

In conclusion, ID in middle childhood was strongly related to both externalizing and internalizing behavior problems in male adolescents. Anemia and low vitamin B-12 serostatus in boys were each related to increased externalizing behavior problems. Intervention studies are warranted to test whether improving status of these micronutrients in middle childhood enhances behavioral development through adolescence.

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EV designed the research; CM, HO, and MMP conducted the research; BJR and BL provided essential materials; BL provided expertise in outcome assessment and data interpretation; SLR analyzed the data; SLR and EV wrote the paper and have primary responsibility for final content. All authors read and approved the final manuscript.

None of the authors has conflicts of interest in relation to this manuscript.

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Table 2.1. Sociodemographic characteristics in middle childhood among children included vs. not included in the analysis

Characteristic	Boys		Girls	
	Included (*n*=458) Mean ± SD[1]	Not Included (*n*=1109) Mean ± SD	Included (*n*=584) Mean ± SD	Not included (*n*=1051) Mean ± SD
Child's age at baseline, y	8.4 ± 1.6	8.8 ± 1.9	8.5 ± 1.7	8.9 ± 1.8
Height-for-age Z score[2] at baseline	-0.77 ± 0.94	-0.82 ± 0.95	-0.76 ± 0.97	-0.75 ± 1.05
BMI-for-age Z score[2] at baseline	0.22 ± 1.09	0.20 ± 1.05	0.07 ± 0.96	0.09 ± 0.92
Time spent watching television / playing video games, h/wk	21.8 ± 17.9	18.0 ± 14.9	20.8 ± 17.6	18.4 ± 14.1
Time playing outdoors, h/wk	8.4 ± 9.1	8.5 ± 10.1	6.7 ± 8.9	7.4 ± 9.8
Mother's education, y	9.2 ± 3.2	8.5 ± 3.3	8.8 ± 3.3	8.5 ± 3.5
Mother's parity	2.7 ± 1.1	2.7 ± 1.1	2.7 ± 1.1	2.8 ± 1.1
Mother's height, cm	157.6 ± 6.5	157.8 ± 6.3	157.7 ± 6.3	157.9 ± 6.5
Mother's BMI, kg/m^2	24.1 ± 3.8	24.2 ± 3.9	24.1 ± 3.6	24.0 ± 3.7
Food insecure, %	74.0	75.1	76.8	76.4
Socioeconomic status, %				
1	5.9	6.9	5.7	6.8
2	29.9	34.6	30.8	33.0
3	56.3	51.4	56.9	53.0
4	7.9	7.0	6.7	7.3
Plasma ferritin, µg/L	41.9 ± 22.9	41.1 ± 23.5	43.4 ± 24.1	42.8 ± 22.7
Iron deficiency[3], %	3.4	3.2	3.0	3.3
Hemoglobin, g/dL	14.5 ± 1.3	14.5 ± 1.2	14.6 ± 1.1	14.5 ± 1.1
Anemia[4], %	2.4	4.8	3.5	3.1
Serum zinc, µmol/L	21.7 ± 6.7	21.4 ± 6.3	21.3 ± 6.4	21.4 ± 6.1
Vitamin A, µg/dL	29.3 ± 10.2	29.9 ± 9.8	29.4 ± 9.8	29.9 ± 10.0
Plasma vitamin B-12, pmol/L	322 ± 104	317 ± 103	339 ± 105	333 ± 110
Erythrocyte folate, nmol/L	861 ± 223	875 ± 294	822 ± 227	857 ± 239

Footnotes to Table 2.1

[1] Values are mean ± SD unless noted otherwise.

[2] According to the World Health Organization growth reference for children and adolescents (34).

[3] Plasma ferritin concentration <15 µg/L. 47 children with CRP >10 mg/L were excluded from the analysis.

[4] Hemoglobin <12.7 g/dL

Table 2.2. Sociodemographic characteristics in middle childhood and total externalizing problems score at 11-18 years of age in the Bogotá School Children Cohort

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	*n*	Mean ± SD
Overall	458	51.9 ± 9.6	584	53.1 ± 9.6
Child's age at baseline, y				
5-6	100	50.3 ± 11.7	122	49.5 ± 10.7
7-8	168	51.9 ± 8.7	216	52.8 ± 9.8
9-10	176	53.0 ± 9.0	221	54.9 ± 8.2
11-12	14	50.4 ± 9.2	25	56.8 ± 8.1
P-trend[2]		0.10		<0.0001
Child's age at assessment, y				
<12	41	50.1 ± 12.0	47	47.3 ± 10.7
12-13	103	48.7 ± 10.2	137	50.1 ± 10.6
14-15	195	53.0 ± 8.8	248	54.4 ± 8.8
>15	119	53.4 ± 8.8	152	55.3 ± 8.1
P-trend		0.001		<0.0001
Height-for-age Z score[3] at baseline				
<-2.0	40	51.3 ± 11.0	58	52.1 ± 9.2
-2.0 to <-1.0	145	51.9 ± 9.0	186	53.1 ± 9.4
-1.0 to <0.0	166	52.4 ± 10.1	210	52.6 ± 9.7
≥0.0	92	51.7 ± 9.2	122	54.3 ± 10.1
P-trend		0.83		0.22
BMI-for-age Z score[3] at baseline				
<-1.0	58	50.9 ± 8.8	76	51.5 ± 9.4
-1.0 to <0.0	137	52.3 ± 9.3	200	52.9 ± 9.7
0.0 to <1.0	152	52.6 ± 10.6	202	53.7 ± 9.5
≥1.0	95	51.1 ± 9.2	97	53.2 ± 9.9
P-trend		0.99		0.19
Time spent watching television / playing video games, h/wk				
<10	124	50.5 ± 9.6	155	52.2 ± 9.1
10 to <20	108	52.4 ± 9.9	171	52.6 ± 10.1
20 to <30	110	50.9 ± 9.0	125	53.2 ± 9.5
≥30	104	54.2 ± 9.6	116	54.9 ± 9.4
P-trend		0.02		0.02

Table 2.2 (continued).

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	*n*	Mean ± SD
Time playing outdoors, h/wk				
<1.5	53	53.7 ± 10.8	109	53.4 ± 9.8
1.5 to <4.5	82	50.1 ± 9.4	113	53.4 ± 9.7
4.5 to <10	97	50.6 ± 8.4	94	52.1 ± 9.9
≥10	96	52.5 ± 10.1	86	52.7 ± 9.4
P-trend		0.94		0.44
Mother's education, y				
Incomplete primary, 1-4	24	53.7 ± 9.2	34	52.7 ± 8.2
Complete primary, 5	76	51.1 ± 10.3	109	54.4 ± 9.0
Incomplete secondary, 6-10	114	52.0 ± 9.0	135	52.5 ± 9.5
Complete secondary, 11	190	51.8 ± 10.1	242	53.1 ± 10.1
University, >11	42	51.9 ± 8.2	41	51.8 ± 9.4
P-trend		0.89		0.31
Mother's parity				
1	47	50.7 ± 11.2	69	52.8 ± 9.7
2	182	51.4 ± 9.7	198	52.5 ± 9.7
3	132	52.2 ± 9.6	184	53.4 ± 9.8
4	45	51.4 ± 5.6	66	53.2 ± 9.4
≥5	40	54.8 ± 10.4	50	54.1 ± 8.2
P-trend		0.08		0.26
Mother's height quartile (median), cm				
Q1, (150)	113	50.7 ± 9.6	140	54.4 ± 10.4
Q2, (155)	121	52.5 ± 9.3	160	53.1 ± 9.7
Q3, (160)	101	52.0 ± 9.8	126	53.4 ± 9.3
Q4, (165)	113	52.3 ± 9.8	144	51.5 ± 8.8
P-trend		0.31		0.02
Mother's BMI, kg/m^2				
<18.5	19	54.4 ± 11.0	16	50.6 ± 7.4
18.5 to <25.0	261	52.2 ± 9.5	364	52.9 ± 9.9
25.0 to <30.0	135	50.8 ± 9.4	146	53.3 ± 8.9
≥30.0	30	52.9 ± 10.8	39	55.1 ± 9.5
P-trend		0.36		0.12

Table 2.2 (continued).

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	n	Mean ± SD
Food insecurity in the household				
None	119	50.6 ± 9.4	135	53.8 ± 9.5
Insecure – no hunger	222	52.5 ± 9.5	283	52.6 ± 9.9
Insecure – moderate hunger	78	51.8 ± 10.4	98	54.1 ± 8.9
Insecure – severe hunger	38	52.9 ± 9.1	66	52.0 ± 9.4
P-trend		0.21		0.53
Socioeconomic status				
1 (lowest)	27	52.1 ± 11.8	33	47.6 ± 10.0
2	137	53.0 ± 9.0	180	54.5 ± 9.1
3	258	51.4 ± 9.7	332	53.0 ± 9.7
4	36	51.1 ± 9.7	39	51.4 ± 8.9
P-trend		0.24		0.83

Footnotes to Table 2.2

[1] Sums may be less than the total due to missing values in covariates.

[2] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous covariate. Empirical estimates of the variance were used in all models.

[3] According to the World Health Organization growth reference for children and adolescents (34).

Table 2.3. Micronutrient status in middle childhood and total externalizing problems score at 11-18 years of age in the Bogotá School Children Cohort

Micronutrient status indicator	Boys		Girls	
	n[1]	Mean ± SD	*n*	Mean ± SD
Iron deficiency[2]				
Yes	14	57.3 ± 8.2	15	52.1 ± 9.9
No	400	51.9 ± 9.7	489	53.1 ± 9.6
P[3]		0.01		0.71
Anemia[4]				
Yes	10	57.5 ± 6.8	18	48.1 ± 9.2
No	413	51.9 ± 9.7	498	53.4 ± 9.6
P		0.008		0.01
Serum zinc quartile (median boys/girls), µmol/L				
Q1, (15.3/15.1)	106	51.7 ± 10.2	127	53.6 ± 10.0
Q2, (18.6/18.2)	104	51.9 ± 9.4	128	53.8 ± 9.1
Q3, (22.1/22.3)	104	51.8 ± 10.1	127	52.1 ± 10.2
Q4, (30.7/29.6)	107	53.0 ± 9.0	128	53.1 ± 9.2
P-trend[5]		0.37		0.40
Vitamin A, µg/dL				
<20	63	53.5 ± 10.6	76	52.7 ± 10.4
20-29.9	188	51.9 ± 9.3	211	52.1 ± 10.0
≥30	173	51.7 ± 9.6	228	54.2 ± 8.8
P-trend		0.31		0.07
Plasma vitamin B-12 quartile (median boys/girls), pmol/L				
Q1, (204/218)	105	54.2 ± 9.4	123	53.5 ± 10.0
Q2, (278/303)	100	51.2 ± 10.0	122	53.6 ± 9.9
Q3, (345/363)	104	52.0 ± 9.8	123	52.4 ± 9.1
Q4, (450/452)	103	51.4 ± 9.2	124	53.4 ± 9.2
P-trend		0.06		0.68
Erythrocyte folate quartile (median boys/girls), nmol/L				
Q1, (633/573)	100	51.4 ± 8.3	123	53.5 ± 9.5
Q2, (759/735)	102	51.5 ± 10.6	124	52.1 ± 10.0
Q3, (898/874)	101	53.0 ± 10.0	124	53.4 ± 9.8
Q4, (1122/1062)	101	51.9 ± 9.5	124	53.8 ± 9.4
P-trend		0.46		0.60

Footnotes to Table 2.3

[1] Sums may be less than the total due to missing values in covariates.

[2] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[3] From linear regression with externalizing problems score as the continuous outcome and the nutrient biomarker as the categorical predictor. Empirical estimates of the variance were used in all models.

[4] Hemoglobin <12.7 g/dL.

[5] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

Table 2.4. Adjusted mean differences and 95% confidence intervals (CI)[1] in total externalizing problems score at 11-18 years of age according to iron deficiency, anemia, and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort

	Boys Difference (95% CI)	Girls Difference (95% CI)
Iron deficiency[2], yes vs. no	5.9 (1.0, 10.7)	0.0 (-4.4, 4.4)
Anemia[3], yes vs. no	6.6 (1.9, 11.3)	-3.3 (-7.7, 1.1)
Plasma vitamin B-12, quartile 1 vs. >1	2.7 (0.4, 4.9)	1.0 (-0.8, 2.9)
Child's age at baseline, per 1 year	0.7 (0.0, 1.4)	1.4 (0.9, 1.9)
Time spent watching television / playing video games, ≥30 h/wk vs. <30 h/wk	3.7 (1.0, 6.4)	1.8 (-0.3, 3.9)
Mother's height quartile (median), cm		
Q1, (150)	-1.2 (-4.0, 1.6)	2.8 (0.4, 5.2)
Q2, (155)	1.0 (-1.8, 3.7)	1.1 (-1.1, 3.3)
Q3, (160)	-0.4 (-3.5, 2.6)	1.0 (-1.3, 3.3)
Q4, (165)	Reference	Reference
P-trend[4]	0.64	0.03
Socioeconomic status, 1 (lowest) vs. >1	-1.3 (-6.8, 4.2)	-5.5 (-9.8, -1.1)

Footnotes to Table 2.4

[1] Adjusted mean difference and 95% CI from a linear regression model with total externalizing problems score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[2] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[3] Hemoglobin <12.7 g/dL.

[4] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

Table 2.5. Sociodemographic characteristics in middle childhood and total internalizing problems score at 11-18 years of age in the Bogotá School Children Cohort

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	*n*	Mean ± SD
Overall	458	53.4 ± 9.7	584	54.1 ± 10.1
Child's age at baseline, y				
5-6	100	53.2 ± 11.0	122	51.6 ± 10.9
7-8	168	53.4 ± 9.7	216	54.2 ± 10.3
9-10	176	53.8 ± 8.8	221	54.9 ± 9.1
11-12	14	49.0 ± 8.4	25	58.9 ± 10.1
P-trend[2]		0.88		0.0005
Child's age at assessment, y				
<12	41	54.0 ± 11.7	47	51.4 ± 10.7
12-13	103	51.2 ± 9.8	137	50.9 ± 10.5
14-15	195	53.8 ± 9.5	248	55.3 ± 9.4
>15	119	54.5 ± 9.0	152	56.0 ± 9.8
P-trend		0.12		<0.0001
Height-for-age Z score[3] at baseline				
<-2.0	40	52.4 ± 10.5	58	52.8 ± 9.6
-2.0 to <-1.0	145	53.4 ± 9.8	186	53.5 ± 10.1
-1.0 to <0.0	166	53.1 ± 9.9	210	54.7 ± 10.3
≥0.0	92	54.6 ± 8.7	122	54.8 ± 10.1
P-trend		0.28		0.10
BMI-for-age Z score[3] at baseline				
<-1.0	58	53.0 ± 9.9	76	51.5 ± 9.1
-1.0 to <0.0	137	53.9 ± 9.4	200	53.8 ± 10.6
0.0 to <1.0	152	53.0 ± 10.2	202	55.4 ± 9.5
≥1.0	95	53.5 ± 9.2	97	54.4 ± 10.9
P-trend		0.95		0.02
Time spent watching television / playing video games, h/wk				
<10	124	54.3 ± 8.9	155	53.5 ± 8.9
10 to <20	108	52.2 ± 9.7	171	53.7 ± 10.7
20 to <30	110	53.3 ± 10.6	125	53.7 ± 10.7
≥30	104	53.9 ± 9.4	116	55.5 ± 9.5
P-trend		0.94		0.12

Table 2.5 (continued).

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	n	Mean ± SD
Time playing outdoors, h/wk				
<1.5	53	53.1 ± 9.4	109	55.7 ± 9.7
1.5 to <4.5	82	53.3 ± 10.6	113	53.0 ± 10.4
4.5 to <10	97	54.0 ± 8.4	94	53.5 ± 10.0
≥10	96	52.3 ± 9.9	86	53.3 ± 10.3
P-trend		0.64		0.14
Mother's education, y				
Incomplete primary, 1-4	24	55.4 ± 9.2	34	55.8 ± 10.3
Complete primary, 5	76	52.6 ± 9.3	109	55.1 ± 9.9
Incomplete secondary, 6-10	114	53.7 ± 9.1	135	54.5 ± 9.9
Complete secondary, 11	190	53.5 ± 10.5	242	53.7 ± 10.4
University, >11	42	53.1 ± 8.5	41	51.9 ± 8.4
P-trend		0.78		0.04
Mother's parity				
1	47	54.5 ± 10.7	69	55.2 ± 10.3
2	182	53.3 ± 9.6	198	53.5 ± 10.4
3	132	53.7 ± 9.9	184	54.4 ± 9.9
4	45	51.9 ± 8.0	66	55.3 ± 9.9
≥5	40	53.8 ± 10.1	50	53.4 ± 9.3
P-trend		0.59		0.99
Mother's height quartile (median), cm				
Q1, (150)	113	53.4 ± 9.7	140	55.1 ± 11.3
Q2, (155)	121	54.4 ± 10.1	160	53.4 ± 10.4
Q3, (160)	101	53.2 ± 9.1	126	54.9 ± 9.1
Q4, (165)	113	53.0 ± 9.8	144	53.0 ± 9.3
P-trend		0.54		0.20
Mother's BMI, kg/m^2				
<18.5	19	52.9 ± 12.9	16	51.3 ± 6.5
18.5 to <25.0	261	53.5 ± 10.0	364	53.8 ± 10.5
25.0 to <30.0	135	53.4 ± 8.9	146	54.1 ± 9.4
≥30.0	30	54.4 ± 8.4	39	57.4 ± 9.7
P-trend		0.68		0.03

Table 2.5 (continued).

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	n	Mean ± SD
Food insecurity in the household				
None	119	52.4 ± 9.8	135	55.3 ± 10.5
Insecure – no hunger	222	53.7 ± 9.5	283	54.0 ± 10.3
Insecure – moderate hunger	78	54.1 ± 10.4	98	54.9 ± 8.8
Insecure – severe hunger	38	52.9 ± 8.9	66	51.5 ± 9.9
P-trend		0.45		0.04
Socioeconomic status				
1 (lowest)	27	55.1 ± 11.1	33	50.7 ± 9.1
2	137	54.2 ± 10.7	180	54.4 ± 10.0
3	258	52.9 ± 9.1	332	54.2 ± 10.3
4	36	52.7 ± 8.7	39	55.4 ± 8.7
P-trend		0.16		0.15

Footnotes to Table 2.5

[1] Sums may be less than the total due to missing values in covariates.

[2] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous covariate. Empirical estimates of the variance were used in all models.

[3] According to the World Health Organization growth reference for children and adolescents (34).

Table 2.6. Micronutrient status in middle childhood and total internalizing problems score at 11-18 years of age in the Bogotá School Children Cohort

Micronutrient status indicator	Boys		Girls	
	n[1]	Mean ± SD	*n*	Mean ± SD
Iron deficiency[2]				
Yes	14	60.1 ± 9.3	15	53.6 ± 7.9
No	400	53.3 ± 9.6	489	54.3 ± 10.1
P[3]		0.005		0.74
Anemia[4]				
Yes	10	58.0 ± 11.6	18	52.1 ± 10.2
No	413	53.3 ± 9.7	498	54.4 ± 10.0
P		0.18		0.32
Serum zinc quartile (median boys/girls), µmol/L				
Q1, (15.3/15.1)	106	54.1 ± 8.7	127	54.8 ± 10.1
Q2, (18.6/18.2)	104	52.0 ± 10.1	128	54.4 ± 9.5
Q3, (22.1/22.3)	104	53.9 ± 10.3	127	54.3 ± 10.9
Q4, (30.7/29.6)	107	53.9 ± 9.6	128	53.7 ± 9.7
P-trend[5]		0.72		0.41
Vitamin A, µg/dL				
<20	63	55.3 ± 10.0	76	53.3 ± 9.7
20-29.9	188	53.3 ± 9.9	211	53.6 ± 11.0
≥30	173	52.9 ± 9.4	228	55.3 ± 9.2
P-trend		0.14		0.05
Plasma vitamin B-12 quartile (median boys/girls), pmol/L				
Q1, (204/218)	105	54.3 ± 10.4	123	54.4 ± 9.5
Q2, (278/303)	100	52.3 ± 9.3	122	55.0 ± 10.1
Q3, (345/363)	104	53.5 ± 9.8	123	52.9 ± 11.2
Q4, (450/452)	103	53.8 ± 9.5	124	55.1 ± 8.8
P-trend		0.95		0.98
Erythrocyte folate quartile (median boys/girls), nmol/L				
Q1, (633/573)	100	52.2 ± 9.2	123	54.3 ± 10.7
Q2, (759/735)	102	53.8 ± 9.9	124	53.4 ± 9.6
Q3, (898/874)	101	53.9 ± 9.5	124	55.2 ± 10.1
Q4, (1122/1062)	101	54.0 ± 10.0	124	54.6 ± 9.6
P-trend		0.20		0.54

Footnotes to Table 2.6

[1] Sums may be less than the total due to missing values in covariates.

[2] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[3] From linear regression with internalizing problems score as the continuous outcome and the nutrient biomarker as the categorical predictor. Empirical estimates of the variance were used in all models.

[4] Hemoglobin <12.7 g/dL.

[5] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

Table 2.7. Adjusted mean differences and 95% confidence intervals (CI)[1] in total internalizing problems score at 11-18 years of age according to iron deficiency and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort

	Boys Difference (95% CI)	Girls Difference (95% CI)
Iron deficiency[2], yes vs. no	6.4 (1.2, 11.6)	0.9 (-2.6, 4.4)
Child's age at baseline, per 1 year	0.1 (-0.6, 0.7)	1.1 (0.5, 1.7)
BMI-for-age Z score[3] at baseline, <-1.0 vs. ≥-1.0	-0.7 (-3.8, 2.4)	-3.7 (-6.0, -1.4)
Mother's education, y		
Incomplete primary, 1-4	Reference	Reference
Complete primary, 5	-2.3 (-6.7, 2.2)	-1.9 (-5.8, 2.0)
Incomplete secondary, 6-10	-1.5 (-5.9, 2.8)	-1.9 (-5.7, 1.9)
Complete secondary, 11	-1.8 (-6.1, 2.5)	-3.7 (-7.4, 0.0)
University, >11	-2.3 (-7.2, 2.7)	-6.3 (-10.9, -1.6)
P-trend[4]	0.70	0.005
Food insecurity, severe hunger vs. no severe hunger	-1.2 (-4.2, 1.8)	-3.3 (-6.0, -0.6)
Socioeconomic status, 1 (lowest) vs. >1	1.9 (-3.2, 7.0)	-3.6 (-7.2, -0.1)

Footnotes to Table 2.7

[1] Adjusted mean difference and 95% CI from a linear regression model with total internalizing problems score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[2] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[3] According to the World Health Organization growth reference for children and adolescents (34).

[4] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

Supplemental Table 2.1. Sociodemographic characteristics and micronutrient status in middle childhood and externalizing problems subscales score at 11-18 years of age in the Bogotá School Children Cohort

	Boys			Girls		
	n[1]	Aggressive Behavior[2]	Rule Breaking Behavior[2]	n	Aggressive Behavior	Rule Breaking Behavior
Overall	458	55.6 ± 7.1	54.2 ± 5.1	584	56.7 ± 7.4	54.4 ± 4.7
Sociodemographic characteristic						
Child's age at baseline, y						
5-6	100	55.6 ± 8.2	54.2 ± 5.7	122	55.5 ± 7.4	52.8 ± 3.5
7-8	168	55.2 ± 6.8	53.9 ± 4.9	216	56.5 ± 7.5	54.4 ± 5.0
9-10	176	56.0 ± 6.9	54.6 ± 5.1	221	57.3 ± 7.1	55.1 ± 4.7
11-12	14	54.6 ± 6.3	53.0 ± 4.0	25	58.8 ± 8.6	56.4 ± 5.1
P-trend[3]		0.70	0.66		0.01	<0.0001
Child's age at assessment, y						
<12	41	55.6 ± 8.0	54.2 ± 5.8	47	54.6 ± 6.7	51.9 ± 2.9
12-13	103	54.3 ± 7.0	52.9 ± 4.2	137	55.2 ± 7.5	53.8 ± 4.8
14-15	195	55.8 ± 7.2	54.7 ± 5.3	248	57.2 ± 7.6	54.9 ± 4.7
>15	119	56.5 ± 6.8	54.6 ± 5.2	152	57.9 ± 7.0	55.0 ± 4.6
P-trend		0.08	0.07		0.0001	<0.0001
Height-for-age Z score[4] at baseline						
<-2.0	40	55.6 ± 7.5	54.6 ± 4.7	58	56.3 ± 8.0	53.2 ± 3.7
-2.0 to <-1.0	145	55.4 ± 7.0	54.0 ± 5.1	186	56.7 ± 7.0	54.3 ± 4.4
-1.0 to <0.0	166	56.0 ± 7.3	54.7 ± 5.7	210	56.1 ± 7.4	54.5 ± 4.8
≥0.0	92	55.4 ± 7.3	54.1 ± 4.7	122	57.9 ± 7.9	55.0 ± 5.2
P-trend		0.93	0.95		0.28	0.02
BMI-for-age Z score[4] at baseline						
<-1.0	58	54.7 ± 6.3	53.6 ± 4.0	76	55.5 ± 6.8	53.9 ± 4.7
-1.0 to <0.0	137	55.7 ± 7.4	54.3 ± 5.2	200	56.5 ± 7.3	54.4 ± 4.5
0.0 to <1.0	152	56.4 ± 7.9	54.8 ± 5.7	202	57.3 ± 7.8	54.5 ± 4.7
≥1.0	95	55.0 ± 6.2	53.9 ± 5.1	97	56.8 ± 7.4	54.6 ± 4.9
P-trend		0.73	0.67		0.17	0.34
Time spent watching television / playing video games, h/wk						
<10	124	54.7 ± 6.4	53.7 ± 4.8	155	55.7 ± 6.6	53.9 ± 4.3
10 to <20	108	55.9 ± 7.3	54.8 ± 5.4	171	56.3 ± 7.4	54.6 ± 4.7
20 to <30	110	54.8 ± 6.2	53.5 ± 5.0	125	57.0 ± 8.0	54.3 ± 4.6
≥30	104	57.2 ± 8.5	55.2 ± 5.3	116	58.0 ± 7.8	55.2 ± 5.1
P-trend		0.04	0.12		0.008	0.04
Time playing outdoors, h/wk						
<1.5	53	56.9 ± 8.6	55.6 ± 6.2	109	56.9 ± 7.6	54.9 ± 5.2
1.5 to <4.5	82	54.1 ± 5.9	53.8 ± 5.1	113	56.9 ± 7.6	54.4 ± 4.6
4.5 to <10	97	54.8 ± 6.0	53.1 ± 3.8	94	56.4 ± 6.8	53.8 ± 4.3
≥10	96	56.3 ± 8.1	54.3 ± 4.9	86	56.1 ± 7.4	54.4 ± 4.9
P-trend		0.79	0.26		0.40	0.30

	Boys			Girls		
	n[1]	Aggressive Behavior[2]	Rule Breaking Behavior[2]	n	Aggressive Behavior	Rule Breaking Behavior
Mother's education, y						
Incomplete primary, 1-4	24	57.5 ± 8.5	53.6 ± 4.9	34	55.3 ± 6.0	54.9 ± 4.6
Complete primary, 5	76	55.0 ± 7.0	54.7 ± 5.4	109	57.1 ± 7.1	55.1 ± 5.1
Incomplete secondary, 6-10	114	55.6 ± 7.1	54.1 ± 4.6	135	56.3 ± 7.3	53.9 ± 4.5
Complete secondary, 11	190	55.8 ± 7.3	54.3 ± 5.4	242	57.1 ± 7.9	54.4 ± 4.7
University, >11	42	54.9 ± 6.3	53.6 ± 4.9	41	55.5 ± 6.6	53.9 ± 3.8
P-trend		0.69	0.75		0.80	0.16
Mother's parity						
1	47	56.0 ± 8.2	53.6 ± 4.9	69	57.0 ± 7.1	53.7 ± 4.5
2	182	55.3 ± 6.9	54.1 ± 5.3	198	56.0 ± 7.3	54.5 ± 5.0
3	132	55.9 ± 7.5	54.4 ± 5.1	184	57.1 ± 7.5	54.5 ± 4.6
4	45	54.0 ± 4.2	52.9 ± 3.7	66	56.6 ± 8.2	54.6 ± 4.4
≥5	40	57.4 ± 8.5	56.1 ± 5.6	50	57.1 ± 6.9	54.4 ± 4.2
P-trend		0.60	0.17		0.51	0.45
Mother's height quartile (median), cm						
Q1, (150)	113	54.9 ± 6.7	53.9 ± 4.7	140	58.3 ± 8.4	55.0 ± 5.2
Q2, (155)	121	56.1 ± 7.1	54.3 ± 4.8	160	56.7 ± 7.1	54.4 ± 4.9
Q3, (160)	101	55.9 ± 7.7	54.0 ± 5.4	126	56.8 ± 7.4	54.4 ± 4.4
Q4, (165)	113	55.6 ± 7.3	54.7 ± 5.7	144	55.1 ± 6.2	53.7 ± 4.0
P-trend		0.56	0.39		0.0005	0.02
Mother's BMI, kg/m^2						
<18.5	19	57.1 ± 8.5	56.1 ± 6.7	16	53.7 ± 3.0	52.8 ± 2.4
18.5 to <25.0	261	55.7 ± 7.4	54.3 ± 5.2	364	56.8 ± 7.7	54.5 ± 4.8
25.0 to <30.0	135	54.9 ± 6.4	53.8 ± 4.6	146	56.7 ± 6.9	54.1 ± 4.0
≥30.0	30	57.0 ± 8.3	54.8 ± 5.8	39	58.1 ± 7.7	55.6 ± 5.9
P-trend		0.71	0.39		0.20	0.31
Food insecurity						
None	119	54.8 ± 6.4	53.3 ± 4.6	135	57.4 ± 8.2	54.4 ± 4.6
Insecure – no hunger	222	55.8 ± 7.5	54.6 ± 5.4	283	56.3 ± 7.4	54.5 ± 4.9
Insecure – moderate hunger	78	56.0 ± 7.2	54.4 ± 5.2	98	57.3 ± 7.1	54.4 ± 4.4
Insecure – severe hunger	38	55.9 ± 6.8	54.8 ± 5.1	66	55.8 ± 6.4	54.1 ± 4.4
P-trend		0.24	0.07		0.33	0.61
Socioeconomic status						
1 (lowest)	27	56.5 ± 8.4	55.3 ± 5.4	33	54.2 ± 5.6	52.2 ± 2.9
2	137	56.5 ± 7.7	54.1 ± 5.1	180	57.5 ± 7.4	55.1 ± 4.8
3	258	55.1 ± 6.7	54.3 ± 5.1	332	56.7 ± 7.7	54.3 ± 4.6
4	36	55.0 ± 6.4	53.8 ± 5.1	39	54.9 ± 5.7	54.2 ± 4.8
P-trend		0.09	0.52		0.71	0.81
Micronutrient status						
Iron deficiency[5]						
Yes	14	59.4 ± 9.9	56.0 ± 4.0	15	56.5 ± 8.3	53.5 ± 5.7
No	400	55.6 ± 7.2	54.3 ± 5.3	489	56.7 ± 7.4	54.5 ± 4.6
P[6]		0.14	0.11		0.93	0.48

	Boys			Girls		
	n[1]	Aggressive Behavior[2]	Rule Breaking Behavior[2]	*n*	Aggressive Behavior	Rule Breaking Behavior
Anemia[7]						
Yes	10	59.9 ± 7.0	55.6 ± 4.1	18	53.8 ± 6.9	51.7 ± 2.2
No	413	55.6 ± 7.3	54.3 ± 5.2	498	56.8 ± 7.5	54.6 ± 4.8
P		0.04	0.30		0.06	<0.0001
Serum zinc quartile (median boys/girls), µmol/L						
Q1, (15.3/15.1)	106	55.7 ± 6.9	54.3 ± 5.7	127	57.3 ± 8.2	54.8 ± 5.1
Q2, (18.6/18.2)	104	55.6 ± 6.8	54.3 ± 5.0	128	56.8 ± 7.1	54.7 ± 4.6
Q3, (22.1/22.3)	104	55.5 ± 8.0	54.4 ± 5.2	127	56.4 ± 7.3	54.1 ± 4.4
Q4, (30.7/29.6)	107	56.1 ± 7.5	54.4 ± 5.1	128	56.4 ± 7.6	54.5 ± 4.7
P-trend		0.67	0.84		0.29	0.47
Vitamin A, µg/dL						
<20	63	57.0 ± 8.6	55.3 ± 5.9	76	56.6 ± 7.6	54.7 ± 4.6
20-29.9	188	55.6 ± 6.8	53.9 ± 4.8	211	56.2 ± 7.5	54.3 ± 4.7
≥30	173	55.4 ± 7.2	54.4 ± 5.3	228	57.2 ± 7.5	54.7 ± 4.7
P-trend		0.20	0.56		0.30	0.75
Plasma vitamin B-12 quartile (median boys/girls), pmol/L						
Q1, (204/218)	105	57.6 ± 8.3	54.7 ± 5.4	123	57.1 ± 8.3	54.7 ± 5.0
Q2, (278/303)	100	55.1 ± 7.3	54.2 ± 5.3	122	57.3 ± 8.0	54.7 ± 4.8
Q3, (345/363)	104	55.2 ± 6.6	55.1 ± 5.6	123	55.6 ± 6.1	54.4 ± 4.8
Q4, (450/452)	103	55.3 ± 6.7	53.6 ± 4.6	124	56.9 ± 7.4	54.3 ± 4.1
P-trend		0.03	0.26		0.46	0.38
Erythrocyte folate quartile (median boys/girls), nmol/L						
Q1, (633/573)	100	54.8 ± 6.0	53.7 ± 4.5	123	57.3 ± 7.5	54.3 ± 4.5
Q2, (759/735)	102	55.9 ± 8.3	54.4 ± 5.1	124	56.3 ± 7.7	54.2 ± 4.8
Q3, (898/874)	101	56.3 ± 7.7	54.9 ± 6.0	124	56.8 ± 8.0	54.8 ± 4.7
Q4, (1122/1062)	101	55.6 ± 6.7	54.1 ± 4.9	124	56.9 ± 7.2	54.8 ± 4.9
P-trend		0.33	0.43		0.85	0.26

Footnotes to Supplemental Table 2.1

[1] Sums may be less than the total due to missing values in covariates.

[2] Mean ± SD

[3] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous predictor. Empirical estimates of the variance were used in all models.

[4] According to the World Health Organization growth reference for children and adolescents (34).

[5] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[6] From linear regression with externalizing problems subscales score as the continuous outcome and the nutrient biomarker as the categorical predictor.

[7] Hemoglobin <12.7 g/dL.

Supplemental Table 2.2. Sociodemographic characteristics and micronutrient status in middle childhood and internalizing problems subscales score at 11-18 years of age in the Bogotá School Children Cohort

	Boys				Girls			
	n[1]	Anxious/ Depressed[2]	Withdrawn /Depressed[2]	Somatic Complaints[2]	*n*	Anxious/ Depressed	Withdrawn /Depressed	Somatic Complaints
Overall	458	55.7 ± 6.5	55.4 ± 6.1	56.1 ± 6.6	584	55.7 ± 7.0	55.8 ± 6.7	57.6 ± 7.8
Sociodemographic characteristic								
Child's age at baseline, y								
5-6	100	56.4 ± 7.4	55.4 ± 6.9	56.3 ± 6.5	122	54.6 ± 6.3	54.6 ± 6.0	56.9 ± 7.6
7-8	168	55.8 ± 6.8	55.2 ± 5.9	56.2 ± 7.0	216	56.1 ± 7.6	55.8 ± 6.4	57.3 ± 7.6
9-10	176	55.5 ± 5.7	55.7 ± 5.9	56.0 ± 6.3	221	55.6 ± 6.4	56.1 ± 7.0	58.1 ± 8.0
11-12	14	52.4 ± 3.7	52.9 ± 3.7	54.9 ± 6.1	25	59.1 ± 8.2	59.5 ± 7.8	59.1 ± 8.7
P-trend[3]		0.07	0.90	0.53		0.04	0.003	0.10
Child's age at assessment, y								
<12	41	57.7 ± 8.4	55.3 ± 6.4	56.7 ± 7.1	47	54.6 ± 5.7	54.3 ± 5.2	56.6 ± 6.9
12-13	103	54.5 ± 6.3	54.2 ± 6.4	55.4 ± 6.2	137	54.2 ± 6.5	54.1 ± 5.1	56.3 ± 7.8
14-15	195	55.5 ± 6.3	55.7 ± 5.7	56.4 ± 6.7	248	56.0 ± 7.1	56.4 ± 7.2	58.1 ± 7.6
>15	119	56.4 ± 6.0	55.8 ± 6.3	56.0 ± 6.6	152	56.9 ± 7.2	57.0 ± 7.0	58.1 ± 8.4
P-trend		0.77	0.17	0.96		0.0004	<0.0001	0.04
Height-for-age Z score[4] at baseline								
<-2.0	40	55.6 ± 6.1	54.9 ± 6.1	56.0 ± 7.7	58	55.4 ± 6.6	55.7 ± 7.3	55.8 ± 6.3
-2.0 to <-1.0	145	56.0 ± 6.8	55.4 ± 6.3	55.7 ± 6.2	186	55.1 ± 6.5	55.3 ± 6.2	57.6 ± 7.9
-1.0 to <0.0	166	55.5 ± 6.6	55.2 ± 5.6	56.3 ± 6.6	210	55.9 ± 7.0	56.2 ± 7.0	58.3 ± 8.4
≥0.0	92	55.6 ± 6.3	56.0 ± 6.6	56.7 ± 6.9	122	56.5 ± 7.6	56.2 ± 6.6	57.4 ± 7.3
P-trend		0.74	0.42	0.32		0.11	0.28	0.23
BMI-for-age Z score[4] at baseline								
<-1.0	58	55.5 ± 6.6	55.0 ± 5.1	55.9 ± 6.6	76	54.1 ± 4.6	54.7 ± 6.1	55.5 ± 6.0
-1.0 to <0.0	137	56.1 ± 7.2	55.4 ± 6.1	56.3 ± 6.6	200	55.8 ± 7.5	55.7 ± 6.9	57.4 ± 7.9
0.0 to <1.0	152	55.4 ± 6.2	55.7 ± 6.6	56.1 ± 7.0	202	56.3 ± 7.1	56.3 ± 6.5	58.2 ± 8.1
≥1.0	95	55.5 ± 5.9	55.2 ± 6.0	56.1 ± 6.2	97	55.7 ± 7.0	56.2 ± 7.1	58.5 ± 8.3
P-trend		0.64	0.82	0.98		0.08	0.09	0.008

	Boys				Girls			
	n[1]	Anxious/ Depressed[2]	Withdrawn /Depressed[2]	Somatic Complaints[2]	*n*	Anxious/ Depressed	Withdrawn /Depressed	Somatic Complaints
Time spent watching television / playing video games, h/wk								
<10	124	56.0 ± 6.9	55.1 ± 5.8	56.8 ± 7.2	155	55.0 ± 5.5	55.4 ± 6.1	56.8 ± 7.0
10 to <20	108	55.0 ± 6.0	55.1 ± 5.2	55.2 ± 6.4	171	55.6 ± 7.4	55.7 ± 6.8	57.5 ± 8.3
20 to <30	110	55.7 ± 6.8	55.7 ± 6.7	56.7 ± 6.8	125	56.0 ± 7.5	55.4 ± 6.4	57.5 ± 7.7
≥30	104	56.0 ± 6.2	55.6 ± 6.5	55.9 ± 5.8	116	56.0 ± 6.8	56.3 ± 6.7	58.7 ± 8.3
P-trend		0.84	0.36	0.66		0.14	0.36	0.07
Time playing outdoors, h/wk								
<1.5	53	55.5 ± 6.1	55.0 ± 5.8	55.8 ± 6.1	109	56.4 ± 7.0	56.5 ± 6.9	58.5 ± 8.1
1.5 to <4.5	82	56.2 ± 7.6	55.2 ± 5.6	56.1 ± 6.4	113	55.7 ± 7.0	55.6 ± 6.0	56.4 ± 7.2
4.5 to <10	97	55.3 ± 6.5	55.3 ± 5.8	56.6 ± 6.7	94	55.7 ± 7.7	55.1 ± 6.3	57.2 ± 7.3
≥10	96	55.1 ± 5.7	54.5 ± 5.9	55.9 ± 7.1	86	55.2 ± 6.7	55.2 ± 5.8	57.1 ± 8.0
P-trend		0.40	0.58	0.90		0.24	0.11	0.34
Mother's education, y								
Incomplete primary, 1-4	24	56.4 ± 6.4	56.4 ± 5.8	56.7 ± 6.0	34	56.2 ± 7.4	57.2 ± 8.2	59.7 ± 8.5
Complete primary, 5	76	55.0 ± 6.4	54.5 ± 5.1	56.0 ± 6.7	109	55.8 ± 6.6	56.4 ± 6.7	58.1 ± 8.4
Incomplete secondary, 6-10	114	55.5 ± 6.2	55.5 ± 6.1	56.2 ± 6.8	135	56.2 ± 7.2	55.8 ± 6.5	57.5 ± 7.6
Complete secondary, 11	190	56.2 ± 6.9	55.6 ± 6.6	56.2 ± 6.7	242	55.6 ± 7.2	55.6 ± 6.8	57.5 ± 7.9
University, >11	42	54.9 ± 5.3	54.8 ± 5.3	56.1 ± 6.2	41	53.7 ± 5.0	54.9 ± 5.7	55.3 ± 4.9
P-trend		0.80	0.88	0.91		0.17	0.09	0.03
Mother's parity								
1	47	56.4 ± 7.3	56.5 ± 7.1	56.5 ± 7.0	69	56.5 ± 8.3	56.9 ± 8.3	57.6 ± 6.9
2	182	55.6 ± 6.1	55.4 ± 6.1	56.3 ± 6.5	198	55.3 ± 6.9	55.5 ± 6.7	57.4 ± 7.2
3	132	56.0 ± 7.0	55.4 ± 5.8	56.2 ± 7.0	184	55.9 ± 6.6	56.0 ± 6.1	57.6 ± 8.3
4	45	54.2 ± 5.3	54.4 ± 5.3	55.1 ± 5.1	66	55.9 ± 7.3	56.3 ± 6.7	59.1 ± 9.0
≥5	40	56.1 ± 6.9	55.0 ± 6.1	56.7 ± 7.1	50	55.5 ± 6.1	54.9 ± 6.3	56.4 ± 7.9
P-trend		0.59	0.20	0.73		0.84	0.46	0.96

	Boys				Girls			
	n[1]	Anxious/ Depressed[2]	Withdrawn /Depressed[2]	Somatic Complaints[2]	*n*	Anxious/ Depressed	Withdrawn /Depressed	Somatic Complaints
Mother's height quartile (median), cm								
Q1, (150)	113	56.0 ± 6.5	54.9 ± 5.6	56.0 ± 6.2	140	56.6 ± 7.7	56.7 ± 7.8	58.6 ± 9.2
Q2, (155)	121	56.2 ± 7.4	56.2 ± 6.9	56.7 ± 6.7	160	55.4 ± 6.5	55.9 ± 6.4	56.9 ± 7.5
Q3, (160)	101	55.2 ± 5.5	55.3 ± 6.0	56.2 ± 6.8	126	55.7 ± 6.4	56.1 ± 6.7	57.8 ± 7.8
Q4, (165)	113	55.6 ± 6.3	55.1 ± 5.7	55.7 ± 6.8	144	54.9 ± 6.8	54.6 ± 5.3	56.8 ± 6.6
P-trend		0.48	0.87	0.61		0.08	0.02	0.13
Mother's BMI, kg/m^2								
<18.5	19	57.0 ± 6.0	55.4 ± 5.2	56.5 ± 8.2	16	52.8 ± 2.4	53.6 ± 3.9	54.8 ± 5.3
18.5 to <25.0	261	55.9 ± 6.8	55.5 ± 6.2	56.2 ± 6.9	364	55.7 ± 7.1	55.8 ± 6.7	57.4 ± 7.8
25.0 to <30.0	135	55.4 ± 6.3	55.4 ± 6.2	56.0 ± 5.9	146	55.4 ± 6.6	55.8 ± 6.7	57.3 ± 7.0
≥30.0	30	55.8 ± 5.5	55.2 ± 5.3	56.3 ± 6.8	39	57.2 ± 6.7	56.8 ± 6.8	60.6 ± 10.2
P-trend		0.42	0.83	0.88		0.20	0.27	0.06
Food insecurity								
None	119	55.1 ± 6.0	54.9 ± 5.5	55.5 ± 6.0	135	56.6 ± 8.0	56.3 ± 6.8	58.3 ± 8.1
Insecure – no hunger	222	55.7 ± 6.7	55.4 ± 6.0	56.6 ± 6.8	283	55.5 ± 7.0	55.8 ± 6.9	57.8 ± 8.2
Insecure – moderate hunger	78	56.4 ± 6.7	56.4 ± 7.5	56.2 ± 6.9	98	55.9 ± 6.5	55.9 ± 6.9	57.3 ± 6.4
Insecure – severe hunger	38	55.8 ± 5.9	54.2 ± 4.7	55.1 ± 6.1	66	54.5 ± 5.0	54.7 ± 5.1	55.8 ± 7.3
P-trend		0.26	0.69	0.95		0.08	0.11	0.03
Socioeconomic status								
1 (lowest)	27	58.5 ± 9.7	55.5 ± 7.2	56.4 ± 6.5	33	53.6 ± 5.7	53.3 ± 4.5	56.4 ± 6.9
2	137	56.3 ± 7.0	55.8 ± 6.0	57.2 ± 7.6	180	55.9 ± 6.9	56.4 ± 7.1	57.5 ± 7.8
3	258	55.2 ± 5.8	55.2 ± 5.9	55.6 ± 6.1	332	55.7 ± 7.0	55.8 ± 6.6	57.8 ± 8.0
4	36	55.1 ± 6.1	54.7 ± 6.5	55.3 ± 5.0	39	56.6 ± 8.0	55.5 ± 6.2	57.4 ± 6.8
P-trend		0.03	0.35	0.04		0.21	0.58	0.50
Micronutrient status								
Iron deficiency[5]								
Yes	14	60.4 ± 8.6	56.6 ± 6.8	61.4 ± 8.5	15	54.3 ± 6.9	52.9 ± 2.9	58.3 ± 6.9
No	400	55.6 ± 6.5	55.4 ± 6.1	56.1 ± 6.6	489	55.9 ± 7.0	55.9 ± 6.6	57.6 ± 8.0
P[6]		0.03	0.49	0.02		0.37	0.0002	0.67

	Boys				Girls			
	n[1]	Anxious/ Depressed[2]	Withdrawn /Depressed[2]	Somatic Complaints[2]	*n*	Anxious/ Depressed	Withdrawn /Depressed	Somatic Complaints
Anemia[7]								
Yes	10	59.8 ± 6.2	55.1 ± 5.2	61.0 ± 8.0	18	55.1 ± 8.6	54.1 ± 6.2	57.4 ± 6.4
No	413	55.6 ± 6.6	55.4 ± 6.1	56.1 ± 6.6	498	55.9 ± 7.0	55.9 ± 6.5	57.7 ± 8.0
P		0.03	0.87	0.04		0.68	0.21	0.83
Serum zinc quartile (median boys/girls), µmol/L								
Q1, (15.3/15.1)	106	56.1 ± 5.8	55.3 ± 6.4	56.3 ± 7.1	127	56.2 ± 7.1	55.8 ± 6.4	58.3 ± 8.4
Q2, (18.6/18.2)	104	55.2 ± 6.1	55.0 ± 5.2	55.1 ± 5.6	128	55.5 ± 6.5	55.4 ± 5.7	57.9 ± 8.6
Q3, (22.1/22.3)	104	56.1 ± 7.7	55.9 ± 7.4	56.4 ± 6.9	127	56.3 ± 7.3	56.4 ± 7.4	57.3 ± 7.6
Q4, (30.7/29.6)	107	55.7 ± 6.8	55.3 ± 5.3	56.9 ± 7.1	128	55.3 ± 6.9	55.6 ± 6.6	57.3 ± 7.3
P-trend		0.98	0.71	0.31		0.53	0.88	0.26
Vitamin A, µg/dL								
<20	63	56.9 ± 7.7	56.7 ± 7.0	57.3 ± 7.1	76	55.5 ± 7.2	55.0 ± 6.4	56.8 ± 7.0
20-29.9	188	55.8 ± 6.7	55.4 ± 6.3	56.1 ± 6.1	211	55.5 ± 7.1	55.9 ± 6.8	57.7 ± 8.4
≥30	173	55.3 ± 6.0	54.9 ± 5.5	56.0 ± 7.1	228	56.3 ± 6.9	56.0 ± 6.3	58.0 ± 8.0
P-trend		0.13	0.06	0.28		0.26	0.26	0.28
Plasma vitamin B-12 quartile (median boys/girls), pmol/L								
Q1, (204/218)	105	56.6 ± 7.9	55.2 ± 7.1	57.3 ± 7.8	123	55.5 ± 6.5	55.9 ± 6.7	57.8 ± 7.7
Q2, (278/303)	100	55.1 ± 5.8	55.4 ± 5.7	54.9 ± 5.8	122	56.5 ± 7.1	56.5 ± 6.8	57.6 ± 8.2
Q3, (345/363)	104	55.8 ± 6.3	55.4 ± 5.8	56.4 ± 6.6	123	55.4 ± 7.7	55.4 ± 6.5	57.2 ± 8.2
Q4, (450/452)	103	55.7 ± 6.3	55.7 ± 6.0	56.3 ± 6.4	124	55.9 ± 6.4	55.7 ± 6.2	58.0 ± 7.8
P-trend		0.49	0.61	0.60		0.99	0.47	0.97
Erythrocyte folate quartile (median boys/girls), nmol/L								
Q1, (633/573)	100	54.6 ± 6.0	55.1 ± 6.2	55.4 ± 6.1	123	56.4 ± 7.9	55.9 ± 7.1	57.5 ± 8.5
Q2, (759/735)	102	56.2 ± 7.3	54.8 ± 6.4	57.0 ± 7.4	124	54.7 ± 6.0	55.4 ± 6.0	57.2 ± 7.4
Q3, (898/874)	101	56.0 ± 6.5	55.4 ± 5.8	56.4 ± 7.2	124	56.7 ± 7.5	56.0 ± 6.3	58.1 ± 8.0
Q4, (1122/1062)	101	55.9 ± 6.1	56.3 ± 6.2	56.0 ± 5.9	124	55.5 ± 6.4	55.9 ± 6.7	58.1 ± 8.1
P-trend		0.19	0.14	0.62		0.80	0.86	0.42

Footnotes to Supplemental Table 2.2

[1] Sums may be less than the total due to missing values in covariates.

[2] Mean ± SD

[3] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous predictor. Empirical estimates of the variance were used in all models.

[4] According to the World Health Organization growth reference for children and adolescents (34).

[5] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[6] From linear regression with internalizing problems subscales score as the continuous outcome and the nutrient biomarker as the categorical predictor.

[7] Hemoglobin <12.7 g/dL.

Supplemental Table 2.3. Sociodemographic characteristics in middle childhood and in adolescence in the Bogotá School Children Cohort

Characteristic	*n*	Middle childhood	Adolescence	*P*[1]
Single mother, %	902	25.5	28.1	0.05
Mother's education, y, mean ± SD	882	9.0 ± 3.2	9.5 ± 3.5	<0.0001
Mother's parity, mean ± SD	917	2.7 ± 1.1	2.8 ± 1.1	<0.0001
Mother's BMI, kg/m^2, mean ± SD	836	24.1 ± 3.7	25.6 ± 4.1	<0.0001
Food secure, %	983	24.8	51.0	<0.0001
Low socioeconomic status (strata 1), %	966	5.6	5.7	0.84

Footnotes to Supplemental Table 2.3

[1] From an intercept-only linear regression model with change in the covariate as the outcome. Empirical estimates of the variance and an independent correlation matrix were specified in all models.

Supplemental Table 2.4. Change in sociodemographic characteristics from middle childhood to adolescence and behavior problems in adolescence in the Bogotá School Children Cohort

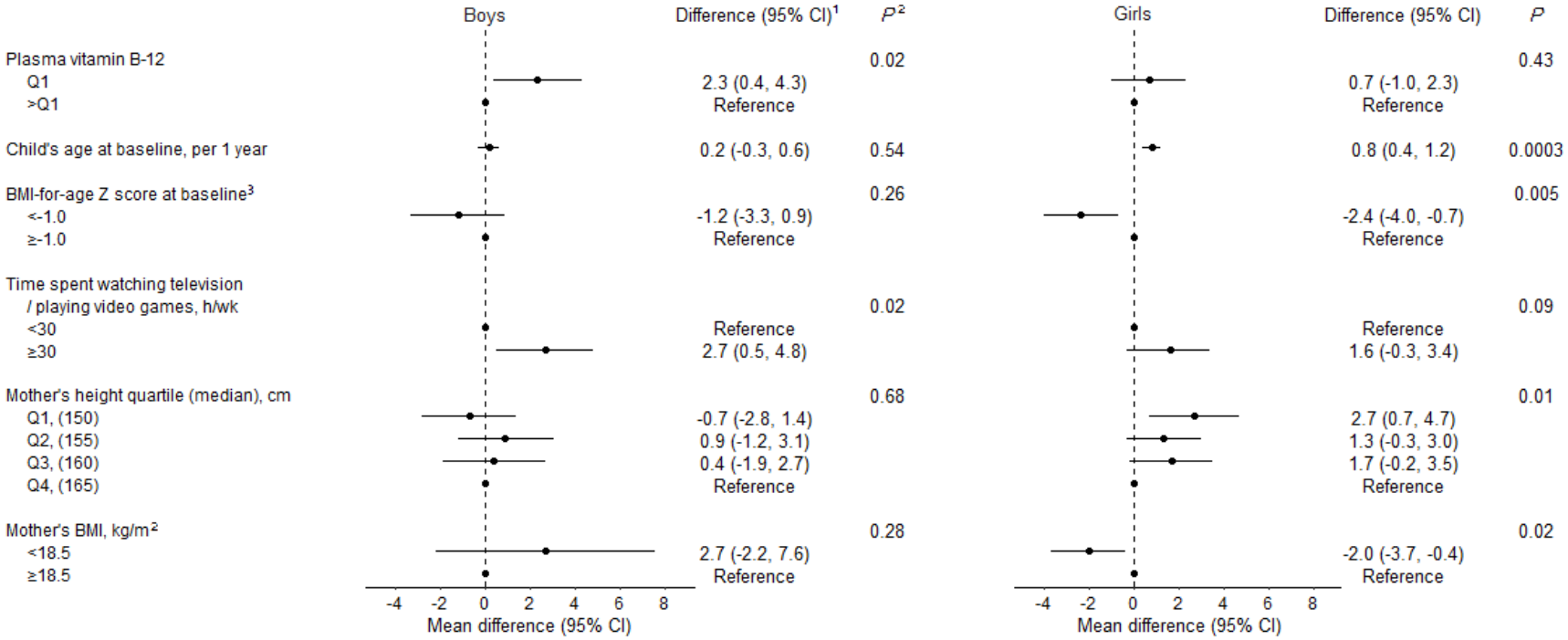
Characteristic	Total externalizing problems	Total internalizing problems
	Mean difference (95% CI)[1]	Mean difference (95% CI) [1]
Mother became single, yes vs. no		
Boys	-1.3 (-4.6, 2.0)	-0.7 (-3.7, 2.3)
Girls	0.0 (-3.3, 3.2)	-0.9 (-3.7, 2.0)
Change in mother's education, per 1 year		
Boys	0.0 (-0.4, 0.4)	0.0 (-0.5, 0.4)
Girls	-0.3 (-0.7, 0.1)	-0.4 (-0.8, 0.0)
Change in mother's parity, per 1 child		
Boys	1.5 (-0.4, 3.4)	0.8 (-0.8, 2.4)
Girls	-0.2 (-2.3, 2.0)	0.2 (-2.3, 2.6)
Change in mother's BMI, per kg/m^2		
Boys	0.3 (-0.1, 0.6)	0.0 (-0.4, 0.3)
Girls	-0.2 (-0.6, 0.1)	0.0 (-0.3, 0.3)
Household became food secure, yes vs. no		
Boys	-1.0 (-2.9, 1.0)	-0.6 (-2.5, 1.4)
Girls	-0.5 (-2.2, 1.3)	0.6 (-1.2, 2.4)

Footnotes to Supplemental Table 2.4

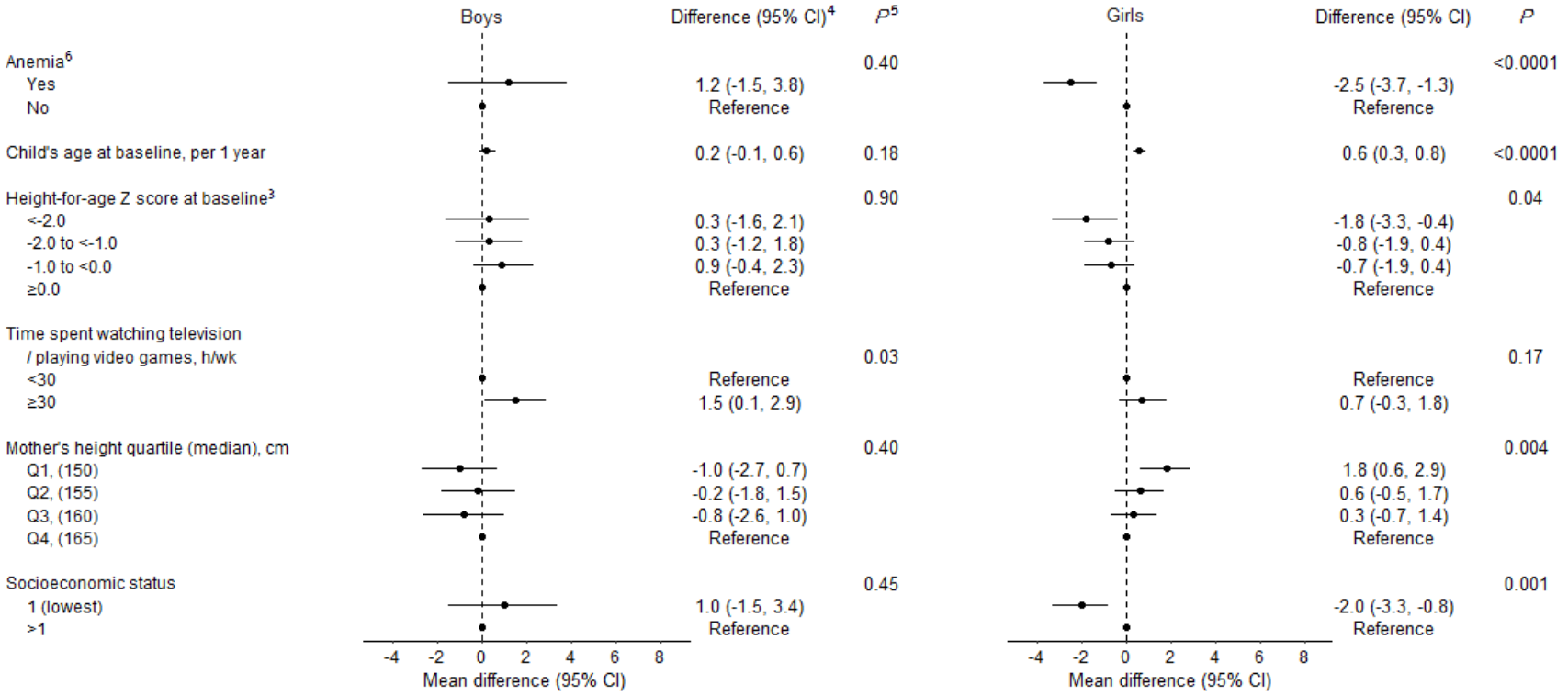
[1] From a linear regression model with behavior problems score as the continuous outcome and change in the covariate as the exposure. Empirical estimates of the variance and an independent correlation matrix were specified in all models.

Supplemental Figure 2.1. Adjusted mean differences and 95% confidence intervals (CI) in externalizing problems subscale score (A. aggressive behavior scores and B. rule breaking behavior scores) at 11-18 years of age according to micronutrient status and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort.

A. Aggressive behavior scores



B. Rule breaking behavior scores



Footnotes to Supplemental Figure 2.1

[1] Adjusted mean difference and 95% CI from a linear regression model with aggressive behavior score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[2] χ^2 score statistic for plasma vitamin B-12, child's age at baseline, BMI-for-age Z score at baseline, time spent watching television/playing video games, and mother's BMI. Test for linear trend for mother's height quartile.

[3] According to the World Health Organization growth reference for children and adolescents (34).

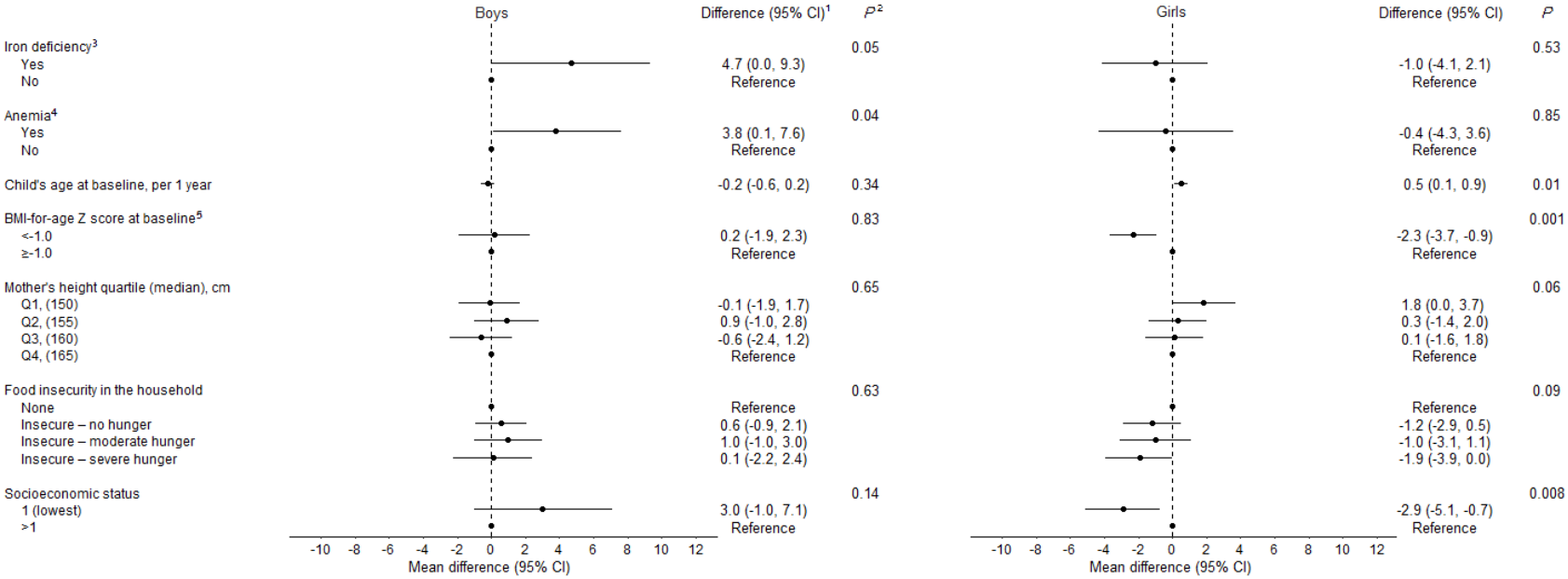
[4] Adjusted mean difference and 95% CI from a linear regression model with rule breaking behavior score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[5] χ^2 score statistic for anemia, child's age at baseline, time spent watching television/playing video games, and socioeconomic status. Test for linear trend for height-for-age Z score at baseline and mother's height quartile.

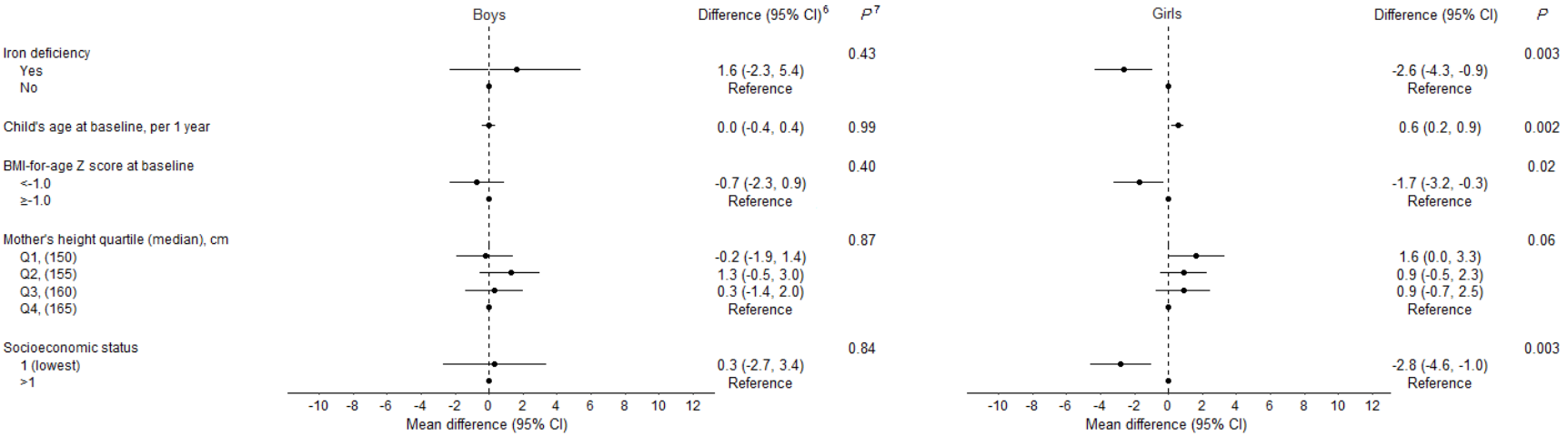
[6] Hemoglobin <12.7 g/dL.

Supplemental Figure 2.2. Adjusted mean differences and 95% confidence intervals (CI) in internalizing problems subscale score (A. anxious/depressed scores, B. withdrawn/depressed scores, and C. somatic complaints scores) at 11-18 years of age according to micronutrient status and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort.

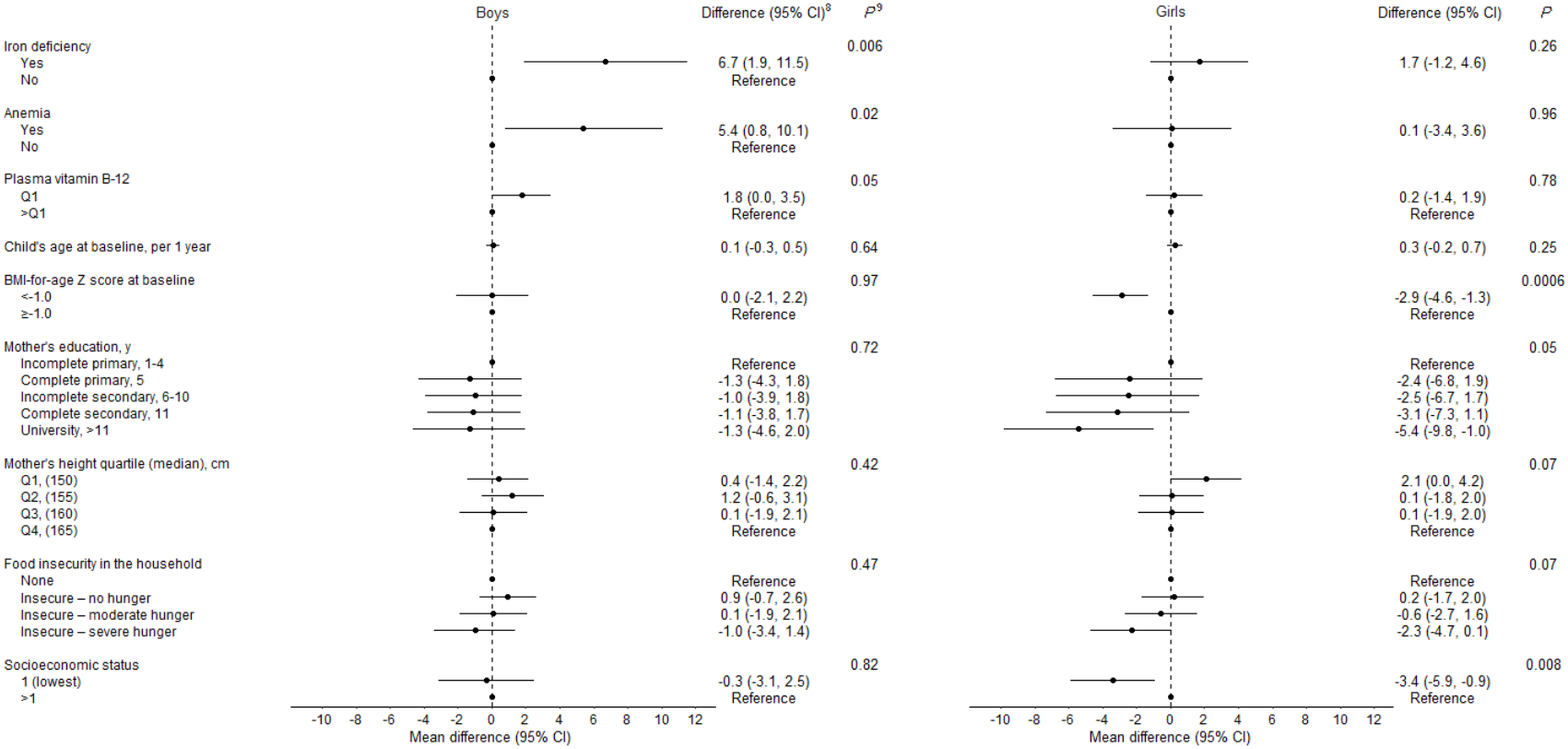
A. Anxious/depressed scores



B. Withdrawn/depressed scores



C. Somatic complaints scores



Footnotes to Supplemental Table 2.2

[1] Adjusted mean difference and 95% CI from a linear regression model with anxious/depressed behavior score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[2] χ^2 score statistic for iron deficiency, anemia, child's age at baseline, BMI-for-age Z score at baseline, and socioeconomic status. Test for linear trend for mother's height quartile and food insecurity in the household.

[3] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[4] Hemoglobin <12.7 g/dL.

[5] According to the World Health Organization growth reference for children and adolescents (34).

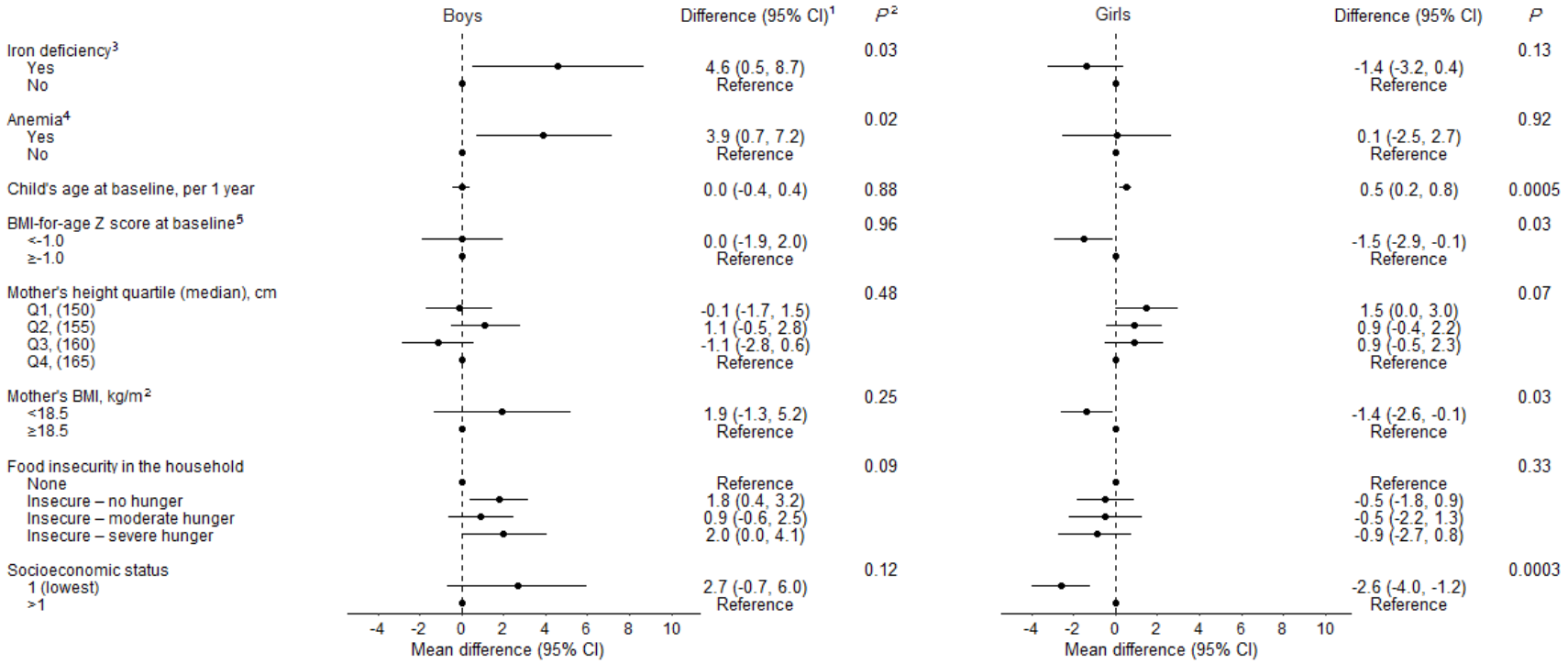
[6] Adjusted mean difference and 95% CI from a linear regression model with withdrawn/depressed score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[7] χ^2 score statistic for iron deficiency, child's age at baseline, BMI-for-age Z score at baseline, and socioeconomic status. Test for linear trend for mother's height quartile.

[8] Adjusted mean difference and 95% CI from a linear regression model with somatic complaints score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[9] χ^2 score statistic for iron deficiency, anemia, plasma vitamin B-12, child's age at baseline, BMI-for-age Z score at baseline, and socioeconomic status. Test for linear trend for mother's education, mother's height quartile, and food insecurity in the household.

Supplemental Figure 2.3. Adjusted mean differences and 95% confidence intervals (CI) in though problems subscale score at 11-18 years of age according to micronutrient status and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort



Footnotes to Supplemental Table 2.3

[1] Adjusted mean difference and 95% CI from a linear regression model with thought problems score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.

[2] χ^2 score statistic for iron deficiency, anemia, child's age at baseline, BMI-for-age Z score at baseline, mother's BMI, and socioeconomic status. Test for linear trend for mother's height quartile and food insecurity in the household.

[3] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[4] Hemoglobin <12.7 g/dL.

[5] According to the World Health Organization growth reference for children and adolescents (34).

Chapter 3. Vitamin D Deficiency and Vitamin D Binding Protein in Middle Childhood and Behavior Problems in Adolescence

Abstract

Vitamin D deficiency (VDD) is associated with adverse psychiatric outcomes in adults. Vitamin D binding protein (DBP) transports vitamin D to target tissues and may influence vitamin D status. It is uncertain whether VDD or DBP in middle childhood are associated with later behavior problems. We examined whether VDD (25-hydroxy vitamin D <50 nmol/L) or low DBP concentration (<2497 nmol/L) at ages 5-12 y were associated with total externalizing and internalizing behavior problems and their subscales, after a median 6 y among 278 schoolchildren from Bogotá, Colombia. Behavior problems were assessed with the Youth Self-Report. We estimated mean problems score differences and 95% CIs between exposure categories with the use of multivariable linear regression. The percent of the associations between DBP and behavior problems mediated through VDD was assessed under the assumptions of a counterfactual frame. Mean ± SD externalizing and internalizing problems scores were 53.2 ± 9.5 and 53.7 ± 9.8, respectively. Prevalence of VDD was 10.3%. VDD was associated with an adjusted 3.8 (95% CI 0.7, 7.0; *P*=0.02) units higher total externalizing problems. Low DBP concentration was related to an adjusted 1.8 (95% CI 0.1, 3.6; *P*=0.04) and 2.1 (95% CI 0.6, 3.6; *P*=0.006) units higher aggressive behavior and anxious/depressed scores, respectively. The associations between low DBP and behavior problems were not mediated

through VDD. VDD and low DBP in middle childhood are related to behavior problems in adolescence.

Introduction

Psychiatric disorders are the leading cause of years lived with disability worldwide. Vitamin D deficiency (VDD) is associated with depression in adults (1), but little is known about VDD during development. Externalizing and internalizing behavior problems in childhood and adolescence are related to psychiatric disorders later in life. Results of prospective studies on vitamin D in pregnancy and behavior problems of the offspring are inconsistent (2-4). Although the brain develops throughout childhood, only one study has investigated the relation of middle childhood vitamin D on behavior problems in adolescence. Among 2267 children in England, serum 25-hydroxy vitamin D [25(OH)D] was inversely associated with prosocial problems (5). Little else is known about vitamin D's role in the development of adolescent behavior problems, which may predict adverse psychiatric outcomes in adults.

Vitamin D binding protein (DBP) binds and transports vitamin D to target tissues. Extra-hepatic synthesis of DBP has been demonstrated in hypothalamic regions that produce neurotransmitters involved in the stress response (6), which suggests a potential role of DBP on behavior. However, this association has not been investigated.

The objective of this study was to examine the associations of VDD and circulating DBP in middle childhood with externalizing and internalizing behavior problems in adolescence. We hypothesized that VDD and low DBP concentration would be positively associated with behavior problems in adolescence.

Methods

Study design and population

We conducted this longitudinal investigation as part of the Bogota School Children Cohort, which has been detailed previously (7). In brief, in February 2006 we recruited 3202 children aged 5-12 y through random selection from primary public schools in Bogotá, Colombia. At baseline, we obtained information on sociodemographic characteristics and health habits of children and their parents with the use of a parental self-administered survey. Trained research assistants measured the children's height and weight and obtained fasting blood samples through antecubital venipuncture. Samples were protected from sunlight and transported on the day of collection to the Colombian National Institute of Health, where they were cyrostored.

Between 2011-2015, we conducted an in-person follow-up assessment for a random sample of 1139 participants. During this assessment, we ascertained behavior problems with the Youth Self-Report (YSR) of the Child Behavior Checklist series. The YSR, a self-administered questionnaire validated for adolescents aged 11-18 y (8), consists of 112 questions on behaviors or feelings that adolescents may experience. It has been widely used in other Latin American settings (9). From the participants' responses to the questionnaire, we computed sex- and age-standardized continuous scores for 8 behavior problems subscales (aggressive and rule breaking behavior, anxious/depressed, withdrawn/depressed, somatic complaints, and attention, social, and thought problems) using the Assessment Data Manager software. The software then computed scores for total externalizing and internalizing problems composite scales, which are comprised of the aggressive and rule breaking behavior, and the anxious/depressed, withdrawn/depressed, and somatic complaints subscales, respectively.

Primary caregivers gave written informed consent. Youth gave written assent to participate. The Ethics Committee of the National University of Colombia Medical School and the Institutional Review Board at the University of Michigan (UM) approved the study.

Laboratory methods

Plasma 25(OH)D was quantified in a random subset of the samples collected at recruitment at Children's Hospital Boston by an enzyme immunoassay (Immunodiagnostic Systems Inc.) with a competitive binding technique (10). This validated method has a sensitivity of 2.5 ng/mL, and an inter- and intra-assay CV of 11.2% and 8.1%. Plasma DBP was measured with a Quantikine ELISA kit (R&D Systems, Inc.) that uses a monoclonal antibody specific to DBP at the Center for Chemical Genomics, UM. The mean CV for replicate measures was 13.21%; individual sample CVs ranged from 0.02% to 33.05%.

Data analysis

278 children in the vitamin D subset completed the YSR. Five children who were not 11-18 y old were excluded from the analyses; thus, the final sample consisted of 273 children. The primary outcomes were the continuous scores for total externalizing and internalizing problems. Secondary outcomes were the 8 behavior problems subscales scores. Primary exposures were VDD defined as plasma 25(OH)D <50 nmol/L, a cutpoint recommended by the United States Endocrine Society (11), and low plasma DBP defined as DBP below the population median (2497 nmol/L), since there are no conventionally accepted cutpoints.

In bivariate analysis, we compared the distributions of behavior problems scores across categories of VDD and low DBP with the use of the Wilcoxon rank-sum test. We then estimated mean adjusted differences and 95% CIs in behavior problems scores between exposure categories with the use of multivariable linear regression with empirical estimates of variance.

All models were adjusted for baseline age, sex, and time spent watching television/playing video games, which are independent predictors of behavior problems in this population (Robinson et al., accepted J Nutr). Estimates by VDD categories were additionally adjusted for low DBP, since DBP may influence 25(OH)D concentrations (12). In supplemental analyses, we calculated the proportion of the associations of low DBP with the outcomes that was mediated through VDD, assuming no unmeasured confounding and no effect of the exposure on confounders of the mediator-outcome relation, with use of the %mediation macro (13) for the Statistical Analysis System version 9.4 (SAS Institute Inc.).

Results

Mean ± SD age at enrollment was 8.6 ± 1.6 y; 53.5% of children were girls. Mean ± SD age at the follow-up assessment was 14.7 ± 1.7 y. Mean ± SD externalizing and internalizing problems scores were 53.2 ± 9.5 and 53.7 ± 9.8, respectively.

Vitamin D. Mean ± SD plasma 25(OH)D was 74.1 ± 25.8 nmol/L. VDD prevalence was 10.3%. In bivariate analysis, VDD was positively associated with total externalizing problems, aggressive behavior, and rule breaking behavior scores (**Table 1**). After multivariable adjustment, children with VDD had 3.8 units (P=0.02) higher total externalizing problems scores compared with children without VDD. Total internalizing problems and subscale scores were higher in children with versus without VDD, but these differences were not statistically significant after adjustment.

DBP. Mean ± SD DBP concentration was 2660 ± 1131 nmol/L. Low DBP concentration was associated with higher aggressive behavior scores in bivariate analysis (**Table 2**). In multivariable analysis, low DBP concentration was associated with higher aggressive behavior (P=0.04) and anxious/depressed (P=0.006) scores (**Table 2).**

Mediation. Low DBP and VDD were weakly positively associated with each other; the prevalence of VDD in low and high DBP categories was 11.0% and 9.6%, respectively. VDD did not mediate the association between low DBP and total externalizing problems (% mediated=0.8) or aggressive behavior (% mediated=0.4) scores.

Discussion

In this longitudinal study, VDD in middle childhood was associated with higher total externalizing problems scores in adolescence. In addition, low DBP concentration was associated with higher aggressive behavior and anxious/depressed scores.

Previous studies focused on the potential effects of vitamin D status in pregnancy on behavior problems of the offspring. Early pregnancy 25(OH)D concentrations were inversely associated with externalizing problems in childhood (2), whereas late pregnancy 25(OH)D concentrations were not associated with behavior problems (3, 4). The potential effects of early pregnancy VDD on behavior could relate to altered brain morphology and neurotransmitter metabolism, which may impact inhibitory and social responses, according to studies in rodents (14). Only one previous investigation addressed the relation of vitamin D status in middle childhood and later behavior. Among 2267 English children, serum 25(OH)D at ages 7-9 y was inversely associated with prosocial problems at age 11 y (5). Follow-up was short; externalizing and internalizing problems often develop later in adolescence. Mechanisms by which VDD in middle childhood influences behavioral development may differ from those related to intrauterine exposure. During middle childhood, dopamine receptor density decreases, which may indicate synaptic pruning. VDD could influence this process by modifying the expression of glial cell line-derived neurotrophic factor, which promotes the survival of dopaminergic neurons. Dopamine regulates emotions and motivation, and thus alterations in the dopaminergic system may have long lasting impacts on behavior.

In our study, low DBP concentration was associated with higher aggressive behavior and anxious/depressed scores. This is contrary to results from two case-control studies in adults in which major depressive disorder (MDD) was positively related to DBP concentration (15, 16).

Results may not be comparable due to reverse causation; MDD alters the inflammatory response, which may be associated with higher concentrations of DBP. Further, exposure in adulthood could have different effects than in middle childhood. Mechanisms that might explain this association are speculative as this association was not mediated by vitamin D. The functions of DBP in the innate immune response as a chemotaxic enhancer, a macrophage-activating factor, and through binding extracellular actin (17) could relate to behavior problems.

The strengths of our study include its prospective nature, which minimizes reverse causation bias. Further, the vitamin D biomarker we used integrates dietary and sunlight sources of the vitamin. We also tested a novel hypothesis on the potential role of DBP on behavioral development. Finally, we used a validated questionnaire to measure adolescent behavior problems (8).

There are limitations as well. We lacked a baseline measurement of behavior. Moreover, 25(OH)D and DBP concentrations may reflect long-term exposure, rather than middle childhood exposure specifically. This limits our ability to identify target ages for intervention. Plasma DBP concentrations vary by isoform of the protein, determined by genetic variants in the *GC* gene. Among Hispanics, the predominant isoform is 1f/1f (34-35%), followed by 1s/2 (16-18%), 1s/1s (14-18%), 1f/2 (15-16%), 1f/1f (13-14%) and 2/2 (3-4%) (12, 18). If isoform distribution is related to behavior outcomes, our results may be confounded by genetics. Finally, the findings may not be generalizable to settings with higher or lower income populations or different nutritional profiles.

In conclusion, VDD and low DBP concentration in middle childhood were associated with behavior problems in adolescence. Additional studies in other populations with different distributions of vitamin D status and DBP are warranted.

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Table 3.1. Youth Self-Report behavior scores at 11-18 y of age according to plasma 25-hydroxy vitamin D [25(OH)D] concentrations in middle childhood among children from Bogotá, Colombia

Behavior problems[1]	Plasma 25(OH)D		*P*[2]	Adjusted difference (95% CI)[3]
	≥50 nmol/L n=245	<50 nmol/L n=28		
Externalizing problems				
Total	52.7 ± 9.6	57.4 ± 7.7	0.009	3.8 (0.7, 7.0)
Aggressive behavior	56.1 ± 7.5	58.7 ± 7.9	0.03	2.2 (-0.9, 5.3)
Rule breaking behavior	54.8 ± 5.4	56.6 ± 5.4	0.03	1.8 (-0.4, 3.9)
Internalizing problems				
Total	53.4 ± 9.9	56.0 ± 8.3	0.22	2.7 (-0.5, 6.0)
Anxious/depressed	55.5 ± 6.5	55.9 ± 7.1	0.80	0.6 (-2.2, 3.4)
Withdrawn/depressed	55.3 ± 6.0	56.5 ± 7.1	0.32	1.4 (-1.4, 4.1)
Somatic complaints	56.9 ± 7.6	58.5 ± 8.7	0.14	1.7 (-1.7, 5.2)
Other problems				
Attention problems	52.4 ± 4.1	51.9 ± 2.6	0.83	-0.9 (-2.0, 0.2)
Social problems	56.4 ± 6.9	56.2 ± 7.0	0.81	-0.4 (-3.0, 2.3)
Thought problems	54.7 ± 5.8	55.5 ± 5.6	0.32	0.7 (-1.6, 3.0)

Footnotes to Table 3.1.

[1] Mean ± SD.

[2] From Wilcoxon rank-sum tests.

[3] From linear regression models with the behavior problems score as the continuous outcome. Predictors included indicator variables for VDD, sex, and low DBP, and baseline age and usual weekly hours spent watching television/playing video games as continuous. Empirical variances were specified in each model.

Table 3.2. Youth Self-Report behavior scores at 11-18 y of age according to plasma vitamin D binding protein (DBP) concentrations in middle childhood among children from Bogotá, Colombia

Behavior problems[1]	Plasma DBP		*P*[2]	Adjusted difference (95% CI)[3]
	≥2497 nmol/L n=136	<2497 nmol/L n=137		
Externalizing problems				
Total	52.3 ± 9.1	54.0 ± 10.0	0.10	1.6 (-0.6, 3.8)
Aggressive behavior	55.3 ± 6.9	57.3 ± 8.0	0.02	1.8 (0.1, 3.6)
Rule breaking behavior	54.8 ± 5.3	55.2 ± 5.5	0.67	0.4 (-0.9, 1.7)
Internalizing problems				
Total	53.1 ± 8.6	54.3 ± 10.9	0.16	1.5 (-0.8, 3.8)
Anxious/depressed	54.6 ± 5.6	56.5 ± 7.2	0.07	2.1 (0.6, 3.6)
Withdrawn/depressed	55.4 ± 5.8	55.5 ± 6.5	0.91	0.2 (-1.3, 1.6)
Somatic complaints	56.4 ± 7.5	57.6 ± 7.9	0.21	1.3 (-0.5, 3.2)
Other problems				
Attention problems	52.2 ± 3.9	52.6 ± 3.9	0.15	0.3 (-0.6, 1.3)
Social problems	55.8 ± 6.0	57.0 ± 7.7	0.49	1.3 (-0.3, 2.9)
Thought problems	54.2 ± 4.9	55.4 ± 6.5	0.23	1.3 (0.0, 2.7)

Footnotes to Table 3.2

[1] Mean ± SD.

[2] From Wilcoxon rank-sum tests.

[3] From linear regression models with the behavior problems score as the continuous outcome. Predictors included indicator variables for low DBP and sex, and baseline age and usual weekly hours spent watching television/playing video games as continuous. Empirical variances were specified in each model.

Chapter 4. Polyunsaturated Fatty Acids in Middle Childhood and Externalizing and Internalizing Behavior Problems in Adolescence

Abstract

Long-chain omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFA) are essential for brain structural and functional development. It is unclear whether PUFA status in childhood could be related to neurobehavioral outcomes later in life. We sought to determine the associations of n-3 and n-6 PUFA in middle childhood with externalizing and internalizing behavior problems in adolescence. We quantified n-3 and n-6 PUFA in serum samples of 444 Colombian schoolchildren who were 5-12 y of age at the time of enrollment into a cohort study, using gas-liquid chromatography. After a median 6 y follow-up, adolescent externalizing and internalizing behavior problems were determined with the Youth Self Report questionnaire. We estimated adjusted mean behavior problems score differences with 95% confidence intervals (CI) between quartiles of each PUFA, with the use of multivariable linear regression. We also considered as exposures the Δ6-desaturase (D6D) and Δ5-desaturase (D5D) enzyme activity indices. Docosapentaenoic acid (DPA) was inversely associated with externalizing problems in a linear manner. Every standard deviation (SD) of DPA concentration was related to an adjusted 0.9 unit lower externalizing problems score (95% CI: -1.7, 0.0). By contrast, every SD of the docosahexaenoic acid (DHA) distribution was associated with 1 adjusted unit higher externalizing problems score (95% CI: 0.1, 1.9). The D5D enzyme activity index was inversely related to externalizing problems. Alpha-linolenic acid (ALA) concentration was positively associated with internalizing problems, whereas adrenic acid (AdA) was inversely related to this

outcome. DPA in middle childhood is inversely related to externalizing problems in adolescence whereas DHA is positively associated with this endpoint. ALA and AdA are related to internalizing problems in opposite directions. Some of these associations might reflect the role of D5D enzyme activity.

Introduction

One in five adolescents worldwide has an externalizing or internalizing disorders such as attention deficit hyperactivity, bipolar, depressive, or anxiety disorder (1). These disorders lie at the extreme end of a continuum of behavior problems in adolescence. Externalizing and internalizing problems in youth are relevant to adult health and social status because they are associated with low educational attainment (2), substance use (3), psychiatric disorders (4), and criminality (5) later in life.

Omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFA) are essential for adequate neurodevelopment. The precursors of each family, alpha-linolenic acid (18:3 n-3, ALA) and linoleic acid (18:2 n-6, LA), respectively, cannot be synthesized in humans and are therefore considered essential as they must be obtained from the diet. ALA and LA are metabolized into longer chain PUFA through a series of elongation and desaturation reactions. Conversion of ALA and LA to longer chain PUFA is considered biologically inefficient and efficiency varies from person-to-person depending on the Δ6-desaturase (D6D) and Δ5-desaturase (D5D) enzyme activity. These long-chain PUFA can also be obtained directly from diet.

Different mechanisms explain the roles of PUFA on brain function and development. The long-chain PUFA docosahexaenoic acid (22:6 n-3, DHA), arachidonic acid (20:4 n-6, AA), and adrenic acid (22:4 n-6, AdA) accumulate in the fetal brain (6) and DHA accretion continues throughout childhood (7). PUFA incorporate into neuronal and glial cell membranes where they impact membrane protein function and fluidity, neuronal arborization, and synaptogenesis (8). N-3 PUFA, such as DHA, influence monoamine neurotransmitter transportation and metabolism (9, 10) and long-chain n-3 PUFA are precursors to anti-inflammatory eicosanoids, which exert antidepressant effects in rodent models (11). On the other hand, long-chain n-6 PUFA, such as

AA and AdA, metabolize into oxidative, pro-inflammatory eicosanoids which promote neuroinflammation, (12) and thus may play an etiologic role in the development of psychiatric disorders (13).

Epidemiologic research on the role of PUFA on behavior has focused primarily on maternal or infant DHA and AA PUFA status in relation to behavior problems in early or middle childhood. DHA concentration has been inversely associated with internalizing problems in some (14-16) but not all (17, 18) studies. Results for AA are similarly mixed (14-18). Although approximately half of externalizing or internalizing disorders develop in adolescence (19), very few studies have included this age group. In Australia, n-3 PUFA intake in adolescence was not associated with depressive symptoms three years later (20). In a short-term trial of adolescents from the United Kingdom (UK), supplementation with n-3 PUFA, vitamins, and minerals resulted in improved behavior after 12 weeks but the effect cannot be necessarily attributed to PUFA (21). Among Mauritian children 8-16 y of age, n-3 PUFA supplementation for 6 months decreased aggression after one year (22). No study has examined the relations of PUFA status in middle childhood and behavior problems in adolescence, after an extended follow-up.

The aim of this study was to investigate the associations between serum PUFA concentrations at ages 5-12 y and externalizing and internalizing problems in adolescence among schoolchildren from Bogotá, Colombia. We hypothesized that high concentrations of n-3 PUFA would be inversely related to externalizing and internalizing problems in adolescence whereas high concentrations of n-6 PUFA would be positively related to these outcomes. Because endogenous conversion of essential fatty acids (FA) into long-chain PUFA depends on desaturase enzymes, as an exploratory aim we examined the associations of desaturase enzymatic activity indices with behavioral outcomes.

Methods

Study design and population

We conducted this study in the context of the Bogotá School Children Cohort, a prospective investigation of nutrition and health in Bogotá, Colombia. Details on the cohort design have been previously published (23). In brief, in February 2006, we randomly sampled 3202 primary schoolchildren aged 5-12 y from public schools in Bogotá and enrolled them into the study. Since the majority of children in the public school system in Bogotá are from low- and middle-income socioeconomic backgrounds, our sample pertains to these groups. The parents or primary caregivers of all children gave written informed consent prior to enrollment into the study and before the follow-up assessment. Children gave written assent to participate. The study protocol was approved by the Ethics Committee of the National University of Colombia Medical School. The Institutional Review Board at the University of Michigan approved the use of data from the study.

Baseline information

At the time of enrollment, we obtained information on sociodemographic characteristics of children and their families through a parental self-administered questionnaire. The survey inquired about child characteristics and health habits, maternal age, education level, height, and weight, and the household's socioeconomic status (SES) per the classification by the local government for tax and planning purposes. The level of household food insecurity was assessed with a Spanish-language version of the United States Department of Agriculture Household Food Security Survey module (24) that has been validated for use in this setting (25).

During the weeks following enrollment, trained research assistants visited schools to collect anthropometric data and blood samples from the children. At these visits, height was

measured without shoes to the nearest 1 mm with a wall-mounted portable Seca 202 stadiometer (Seca, Hanover, MD) and weight was measured in light clothing to the nearest 0.1 kg with Tanita H5301 electronic scales (Tanita, Arlington Heights, IL). Research assistants also measured height and weight among mothers who were present at schools. At the end of the visit, investigators collected fasting blood samples through antecubital venipuncture in 88% of children. One aliquot was collected in a metal-free polypropylene tube without anticoagulant for separation of serum. The samples were protected from sunlight and transported on dry ice to the Colombian National Institute of Health on the day of collection, where they were processed and cryostored. Samples were then transported to the United States for analyses.

Follow-up

Between 2011-2015 we conducted an in-person follow-up assessment on a random sample of 1139 cohort participants. At this assessment, we ascertained adolescent behavior problems with the Spanish language version of the Youth Self Report (YSR) (26). The YSR is a self-administered questionnaire designed for use in adolescents aged 11-18 y (27). The questionnaire consists of 112 statements of behaviors or feelings that the adolescents rate as never/false, sometimes true, or very/often true. From responses to these questions, we calculated continuous scores for 8 behavior problems subscales using software provided by the test developer (28): aggressive behavior, rule breaking behavior, anxious/depressed, withdrawn/depressed, somatic complaints, attention problems, social problems, and thought problems. We then computed scores for total externalizing and internalizing problems scores. The total externalizing problems score is calculated as the sum of the aggressive and rule breaking behavior subscale scores while the sum of anxious/depressed, withdrawn/depressed, and somatic complaints subscale scores comprise the total internalizing problems score. The

scores are standardized by age and sex to a reference population derived from data collected periodically in the United States (28). The YSR has been widely used in other Latin American settings (26, 29, 30).

Laboratory methods

Quantification of serum FA took place at the University of Michigan Metabolomics and Obesity Research Center. Lipids were extracted from baseline serum samples in a random subset of approximately 20% of participants. We prepared FA methyl esters of total lipids with BF3-methanol (31). Methyl esters were extracted from a thin-layer chromatography plate, and the solvents were dried and resuspended in hexane. Approximately 2 ml of sample was injected via an autosampler and analyzed on a gas–liquid chromatography machine using a 100 m SP-2560 column with optimum conditions for separation (Model 6890 N, Agilent, Santa Clara, CA). Eluted peaks were analyzed with the use of the Chemstation software (Agilent). The concentration of each FA was determined using a calibration curve with C17:0 methyl ester as the standard. Serum concentrations of n-3 ALA, eicosapentaenoic acid (20:5 n-3, EPA), docosapentaenoic acid (22:5 n-3, DPA) and DHA; and n-6 LA, gamma-linolenic acid (18:3 n-6, GLA), eicosadienoic acid (20:2 n-6, EDA), dihomo-gamma-linolenic acid (20:3 n-6, DGLA), AA, and AdA were each expressed as percentage weight concentrations of total FA. Inter-assay coefficients of variation ranged from 1.1% to 2.3% for all PUFA.

Data analysis

Four-hundred forty-four children in the subset selected for quantification of FA completed the YSR at 11-18 years of age. The primary outcomes of interest were the continuous total externalizing and internalizing problems scores. Secondary outcomes were scores on the 5 behavior problems subscales that comprise total externalizing and internalizing problems. The

primary exposures of interest were the percentage of total weight concentrations for serum n-3 (ALA, EPA, DPA, and DHA) and n-6 (LA, GLA, EDA, DGLA, AA, and AdA) PUFA. We also examined as exposures the ratios of GLA to LA and AA to DGLA as indices of enzymatic activity for Δ6-desaturase (D6D) and Δ5-desaturase (D5D), respectively.

Covariates included sociodemographic and anthropometric characteristics as well as health habits measured at baseline. Children's height-for-age Z scores were calculated according to the World Health Organization (WHO) growth reference for children and adolescents (32). Maternal BMI was calculated as kg/m^2 from objectively measured height and weight in 34.7% of mothers and from self-reported data in the rest. Covariates were categorized as presented in **Table 1**.

We first compared the distributions of total externalizing and internalizing problems scores across categories of baseline characteristics using means and standard deviation (SD). For ordinal exposures, we conducted tests for linear trend by fitting linear regression models with the behavior problems score as the continuous outcome and a variable representing ordinal categories of each predictor as a continuous covariate. For sex, we used the χ^2 score statistic.

We then compared the distributions of total externalizing and internalizing problems scores across quartiles of serum FA concentrations. Tests for linear trend were conducted by introducing a variable representing the median of each quartile into a linear regression model as a continuous predictor. In adjusted analyses, we estimated mean differences with 95% confidence intervals (CI) for total externalizing and internalizing problems scores between quartiles of FA. In addition, we estimated unadjusted and adjusted mean differences with 95% CI in behavior problems scores per 1 SD of serum FA concentrations when the associations seemed linear. In adjusted models, we included as covariates baseline characteristics which were

associated with total externalizing or internalizing problems in bivariate analysis ($P<0.05$). Child's sex and age at baseline were included in all models as they were considered important from a mechanistic viewpoint. We deliberately avoided adjusting for child's BMI-for-age Z score because PUFA may influence the development of adiposity (33). N-3 (ALA, EPA, DPA, and DHA) and n-6 PUFA (LA, GLA, EDA, DGLA, AA, and AdA) were adjusted for LA and ALA, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s). Enzyme activity indices were adjusted in the same manner as their n-6 products; the D6D activity index was adjusted for ALA and LA, whereas the D5D activity index was adjusted for ALA, GLA, and EDA. Empirical estimates of the variance were specified in all models to overcome potential deviations from the multivariate normality assumption.

In supplemental analyses, we examined the associations of n-3 and n-6 PUFA with scores on the component subscales for total externalizing and internalizing problems following an analogous approach to that used for the primary outcomes.

All analyses were performed with the Statistical Analyses System version 9.4 (SAS Institute Inc.).

Results

Mean ± SD age at enrollment was 8.6 ± 1.6 y; 57.4% of children were girls. Mean ± SD age at the time of follow-up assessment was 14.4 ± 1.6 y. In adolescence, mean ± SD total externalizing and internalizing problems scores were 52.0 ± 9.5 and 53.2 ± 9.7, respectively. Child's age at baseline and time spent watching television / playing video games were positively associated with total externalizing problems scores (**Table 1**). Child's BMI-for-age Z score and mother's BMI at baseline were positively related to total externalizing and internalizing problems scores, whereas mother's education level was inversely associated with these outcomes (**Table 1**).

Total externalizing problems. In bivariate analysis, EPA and DPA concentrations and the D5D activity index were inversely associated with total externalizing problems scores whereas DHA concentration was positively related to this outcome (**Table 2**). In multivariable analysis, every SD of DPA or DHA concentrations was related to a -0.9 (P=0.05) and a 1.0 (P=0.03) unit difference in total externalizing problems scores, respectively. Total externalizing problems were 3.2 units (P=0.02) lower in children with a D5D activity index in the highest quartile compared with those in the lowest quartile (**Table 2**).

Externalizing problems subscales. In multivariable analysis, DPA concentration and the D5D activity index were inversely related to aggressive behavior scores whereas DHA concentration was positively associated with this outcome (**Supplemental Table 2**). DPA and AA concentrations and the D5D activity index were inversely associated with rule breaking behavior scores (**Supplemental Table 3**). In addition, rule breaking behavior scores were higher in children with DGLA concentration in the highest quartile compared with those in the lowest quartile (**Supplemental Table 3**).

Total internalizing problems. In bivariate analysis, ALA concentration was positively associated with total internalizing problems scores, whereas AdA concentration was inversely associated with these scores (**Table 3**). After adjustment for potential confounders, every SD of ALA concentration was related to a 1.2 (P=0.01) unit difference in total internalizing problems scores (**Table 3**). The adjusted difference in total internalizing problems scores between the highest and lowest quartile of AdA concentration was -3.2 units (P= 0.01) (**Table 3**).

Internalizing problems subscales. In multivariable analysis, ALA concentration was positively associated with anxious/depressed scores whereas DPA and AdA concentrations and the D5D activity index were inversely related to this outcome (**Supplemental Table 4**). DPA concentration was inversely related to withdrawn/depressed scores in a nonlinear manner (**Supplemental Table 5**). AA concentration was also inversely associated with this outcome (**Supplemental Table 5**). There were no associations between PUFA concentrations and the somatic complaints scores (**Supplemental Table 6**).

Discussion

In this prospective study of schoolchildren from Bogotá, Colombia, serum concentrations of DPA in middle childhood were related to decreased total externalizing problems scores in adolescence, whereas DHA concentrations were associated with increased scores. The D5D enzyme activity index was inversely related to externalizing problems. Alpha-linolenic acid concentration was positively associated with internalizing problems, whereas AdA was inversely related to this outcome. These associations were independent of baseline child, parental, and household characteristics.

The inverse associations of DPA with total externalizing problems and the internalizing problems subscales anxious/depressed and withdrawn/depressed had not been previously reported among children or adolescents. Nevertheless, DPA in blood has been inversely associated with depressive symptoms in pregnant (34) and postmenopausal women (35). Evidence from animal experiments offers insights into potential explanatory mechanisms. Rodents supplemented with DPA perform better on the forced swim test (36), an animal model of depressive behavior. DPA could enhance hippocampal long-term potentiation (37), thereby countering stress- or glucocorticoid-induced damage which may be associated with psychiatric disorders (38).

The positive association between serum DHA and total externalizing problems scores in adolescence was against our initial hypothesis. The majority of previous longitudinal studies have focused on the potential effects of DHA and other long-chain n-3 PUFA during the prenatal or infancy periods on early or middle childhood behavior problems. In all (14, 15, 17, 18) but one (16) studies DHA biomarkers during pregnancy have not been associated with childhood externalizing problems. In addition, maternal or early childhood intake of fatty fish, a major

source of DHA, has not been related to behavior problems (39, 40). DHA supplementation during the second half of pregnancy resulted in an increase of total problems at ages 4 and 7 years among Australian children (41, 42). Only a handful of investigations have addressed the potential effect of DHA on behavior problems during adolescence. DHA supplementation of schoolchildren in Mauritius and the UK was related to decreased aggressive and disruptive behavior, respectively (21, 22). The apparent discrepancy between the positive association we found and the protective effect of supplementation trials might be related to other PUFA present in the supplement. An adverse effect of DHA in middle childhood could be explained through a number of mechanisms. Fish oil supplementation has been associated with delayed neurodevelopment in rodents, potentially due to reduced myelination in the brainstem (43). High DHA concentration has also been associated with behavioral delays in rodents when there is an imbalance of n-3 and n-6 PUFA (44). Some methodological limitations of observational studies could also explain these findings. For example, the association of DHA and behavior problems in our study may be confounded by mercury or lead since these neurotoxic heavy metals are readily found in fish consumed in Bogotá (45) and fish intake is an important source of preformed DHA.

The D5D activity index in middle childhood was related to lower total externalizing problems scores in adolescence, possibly through lower aggressive and rule breaking behavior. Although no prior study has examined the association between the D5D activity index and behavior problems, some investigations have examined whether single nucleotide polymorphisms (SNPs) that influence D5D activity are related to child development. SNPs which encode for lower D5D activity were positively associated with infant psychomotor development (46) or child cognition (47), but it is unknown whether they are related to behavioral outcomes.

Serum ALA was positively related to total internalizing problems scores. These results are in line with those from a study of 10 year-old German children in whom prenatal ALA concentration was positively associated with peer relationship problems, an internalizing problem (18). The nature of this association is unclear. Lifelong ALA supplementation has been associated with more depressive-like behaviors in rodents, potentially attributable to alterations in the endocannabinoid system (48).

AdA, a product of AA elongation, was inversely related to total internalizing problems scores, possibly through decreased anxious/depressed feelings. AA was inversely related to problems scores in some subscales. Epidemiologic evidence of the potential effects of n-6 PUFA on behavior is limited and conflicting. Studies to date have only examined the association of prenatal or infant AA concentration with behavior problems; three studies found no relation (14, 15, 17), one found an inverse association (18), and another found a positive relation (16). Mechanisms underlying these associations are speculative; AA may enhance long-term potentiation of synapses in areas of the brain involved in reward (49), learning, and memory, (50) while AdA could be important in myelination (51). Of note, the D5D activity index and AA concentration were associated with rule breaking behavior in the same direction. It is plausible that the associations with AA represent underlying genetic polymorphisms related to D5D activity.

Our study has several strengths. First, the prospective design limits bias due to reverse causation. While previous studies primarily focused on DHA and AA, we examined the associations of other PUFA biomarkers that may be biologically relevant. Further, serum biomarkers of several FA are highly correlated with measures of long-term PUFA intake (52). The YSR is a validated measure of externalizing and internalizing problems in adolescence (53).

Finally, we controlled for many potential confounders of the associations between PUFA concentration and behavior problems, including FA precursors.

There are limitations as well. We do not have a baseline measurement of behavior problems and thus cannot preclude reverse causation as an explanation for our findings if behavior problems developed before middle childhood. Our ability to identify a time at which PUFA may exert a potential effect on behavior is limited since PUFA concentrations in middle childhood may be correlated with concentrations at other periods in development. We were unable to measure heavy metals which may share dietary sources with long-chain n-3 PUFA, especially EPA and DHA; thus, residual confounding may have attenuated or changed the direction of the associations. Finally, our results may not be generalizable to children from the highest socioeconomic status since they do not attend public schools in Bogotá.

In conclusion, DPA concentration was inversely associated with externalizing problems whereas DHA concentration was positively related to this outcome. The D5D activity index was related to decreased externalizing problems. ALA concentration was positively associated with internalizing problems whereas AdA was inversely related to this outcome. Additional studies in other populations with different distributions of PUFA are warranted.

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Table 4.1. Total behavior problems scores at 11-18 y of age according to sociodemographic characteristics in middle childhood among children from Bogota, Colombia

Characteristic	n[1]	Externalizing problems Mean ± SD	Internalizing problems Mean ± SD
Sex			
Boys	189	51.4 ± 9.5	52.4 ± 9.2
Girls	255	52.4 ± 9.4	53.8 ± 10.1
P[2]		0.27	0.13
Child's age at baseline, y			
5-6	78	48.5 ± 10.5	52.6 ± 11.5
7-8	171	51.1 ± 9.1	52.5 ± 9.2
9-10	170	53.7 ± 8.6	53.8 ± 9.2
11-12	25	56.5 ± 9.8	56.1 ± 11.0
P-trend[3]		<0.0001	0.12
Height-for-age Z score[3] at baseline			
<-2.0	35	51.4 ± 9.8	52.7 ± 9.4
-2.0 to <-1.0	135	51.0 ± 9.7	52.4 ± 10.1
-1.0 to <0.0	164	52.1 ± 9.2	53.8 ± 9.7
≥0.0	97	53.4 ± 9.4	53.8 ± 9.6
P-trend		0.08	0.24
BMI-for-age Z score[3] at baseline			
<-1.0	61	50.4 ± 8.9	51.2 ± 8.5
-1.0 to <0.0	145	51.0 ± 9.5	52.4 ± 10.3
0.0 to <1.0	152	53.0 ± 9.7	54.2 ± 9.5
≥1.0	73	53.2 ± 9.0	54.8 ± 10.0
P-trend		0.02	0.008
Time spent watching television / playing video games, h/wk			
<10	140	51.1 ± 9.3	53.9 ± 8.7
10 to <20	106	51.7 ± 9.9	52.4 ± 10.7
20 to <30	115	51.5 ± 8.9	52.8 ± 10.0
≥30	76	54.7 ± 9.7	54.2 ± 9.8
P-trend		0.02	0.98

Characteristic	n[1]	Externalizing problems Mean ± SD	Internalizing problems Mean ± SD
Mother's education, y			
Incomplete primary, 1-4	29	53.9 ± 8.5	56.4 ± 9.1
Complete primary, 5	82	53.3 ± 8.8	54.2 ± 10.0
Incomplete secondary, 6-10	105	51.9 ± 9.4	53.8 ± 9.8
Complete secondary, 11	189	51.4 ± 10.2	52.3 ± 9.7
University, >11	29	50.3 ± 8.1	50.8 ± 9.4
P-trend		0.03	0.005
Mother's height quartile (median), cm			
Q1, (150)	108	52.6 ± 9.8	54.8 ± 10.0
Q2, (155)	112	51.3 ± 10.0	52.0 ± 10.2
Q3, (160)	101	51.6 ± 8.9	53.8 ± 9.3
Q4, (165)	111	52.2 ± 9.3	52.4 ± 9.4
P-trend		0.79	0.20
Mother's BMI, kg/m^2			
<18.5	11	49.0 ± 8.9	47.2 ± 11.1
18.5 to <25.0	259	51.5 ± 9.3	52.9 ± 9.7
25.0 to <30.0	126	52.2 ± 9.8	53.4 ± 9.4
≥30.0	32	55.9 ± 9.5	57.6 ± 9.2
P-trend		0.02	0.007
Food insecurity			
Secure	108	51.3 ± 9.0	53.4 ± 9.6
Insecure – no hunger	222	51.9 ± 9.9	53.1 ± 10.0
Insecure – moderate hunger	61	53.2 ± 9.1	54.8 ± 9.1
Insecure – severe hunger	52	52.1 ± 9.3	51.8 ± 9.7
P-trend		0.35	0.66
Socioeconomic status			
1 (lowest)	33	49.7 ± 10.4	53.9 ± 10.8
2	101	51.9 ± 8.9	52.2 ± 9.9
3	250	52.6 ± 9.6	53.5 ± 9.7
4	60	50.6 ± 9.1	53.4 ± 9.1
P-trend		0.65	0.67

Footnotes to Table 4.1

[1] Sums may be less than the total due to missing values in covariates.

[2] χ^2 score statistic from linear regression with the behavior problems score as the continuous outcome and sex as the categorical predictor. Empirical estimates of the variance were used in all models.

[3] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous covariate.

[4] According to the World Health Organization growth reference for children and adolescents (31).

Table 4.2. Total externalizing problems scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Total externalizing problems scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	P^1	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	51.4 ± 8.8	51.7 ± 8.8	52.5 ± 10.4	52.3 ± 9.8	0.40	0.4 (-0.4, 1.3)
Adjusted differences[4]	Reference	0.4 (-2.0, 2.8)	0.3 (-2.2, 2.8)	0.9 (-1.5, 3.4)	0.48	0.4 (-0.4, 1.3)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	53.0 ± 9.4	52.6 ± 9.7	51.4 ± 10.2	50.8 ± 8.4	0.05	-0.8 (-1.5, 0.0)
Adjusted differences	Reference	0.0 (-2.5, 2.4)	-0.1 (-2.6, 2.4)	-1.0 (-3.4, 1.3)	0.42	-0.3 (-1.1, 0.5)
22:5n-3 docosapentaenoic acid						
Mean ± SD	52.6 ± 10.0	52.3 ± 9.1	53.0 ± 9.3	49.8 ± 9.1	0.02	-1.0 (-1.8, -0.2)
Adjusted differences	Reference	-1.3 (-3.8, 1.2)	-0.2 (-2.7, 2.4)	-3.0 (-5.7, -0.3)	0.05	-0.9 (-1.7, 0.0)
22:6n-3 docosahexaenoic acid						
Mean ± SD	51.3 ± 9.7	51.7 ± 9.3	51.5 ± 9.9	53.3 ± 8.9	0.11	1.0 (0.1, 1.9)
Adjusted differences	Reference	0.9 (-1.5, 3.3)	-0.5 (-3.1, 2.1)	1.7 (-0.7, 4.1)	0.22	1.0 (0.1, 1.9)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	51.2 ± 9.7	51.6 ± 9.6	53.5 ± 9.6	51.6 ± 8.8	0.49	0.1 (-0.8, 0.9)
Adjusted differences	Reference	0.9 (-1.6, 3.4)	2.8 (0.3, 5.3)	0.6 (-1.8, 2.9)	0.41	0.2 (-0.7, 1.0)
18:3n-6 γ-linolenic acid						
Mean ± SD	51.7 ± 9.3	52.2 ± 9.9	51.8 ± 9.5	52.1 ± 9.3	0.80	-0.1 (-1.0, 0.7)
Adjusted differences	Reference	-0.1 (-2.7, 2.4)	-0.5 (-3.0, 2.0)	-0.1 (-2.6, 2.5)	0.95	-0.1 (-1.0, 0.8)
20:2n-6 eicosadienoic acid						
Mean ± SD	52.2 ± 9.1	53.2 ± 10.2	51.4 ± 9.4	51.1 ± 9.0	0.23	-0.2 (-1.0, 0.6)
Adjusted differences	Reference	1.5 (-1.0, 4.1)	-0.9 (-3.3, 1.6)	-0.5 (-3.2, 2.1)	0.40	-0.1 (-1.0, 0.7)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	50.5 ± 9.1	52.9 ± 10.0	52.0 ± 9.9	52.4 ± 8.8	0.20	0.3 (-0.6, 1.3)
Adjusted differences	Reference	2.4 (-0.1, 4.9)	1.1 (-1.4, 3.7)	2.6 (0.0, 5.3)	0.14	0.6 (-0.5, 1.7)

Total externalizing problems scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	52.9 ± 9.7	52.3 ± 9.5	51.8 ± 9.1	50.8 ± 9.5	0.09	-0.9 (-1.8, 0.0)
Adjusted differences	Reference	0.2 (-2.4, 2.7)	-0.3 (-2.7, 2.1)	-1.8 (-4.3, 0.8)	0.15	-0.8 (-1.7, 0.1)
22:4n-6 adrenic acid						
Mean ± SD	52.8 ± 9.5	51.8 ± 9.0	51.8 ± 10.3	51.5 ± 9.1	0.28	-0.5 (-1.3, 0.4)
Adjusted differences	Reference	-1.6 (-3.9, 0.8)	-0.9 (-3.3, 1.6)	-1.6 (-4.0, 0.8)	0.25	-0.5 (-1.4, 0.3)
Enzyme activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	52.3 ± 9.3	51.3 ± 9.2	52.2 ± 10.0	52.1 ± 9.3	0.89	-0.2 (-1.0, 0.7)
Adjusted differences	Reference	-1.5 (-3.9, 0.9)	-0.4 (-2.9, 2.2)	-0.5 (-3.2, 2.2)	0.97	-0.2 (-1.2, 0.8)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	53.2 ± 9.7	51.1 ± 9.3	53.5 ± 9.4	50.0 ± 9.2	0.05	-0.8 (-1.8, 0.2)
Adjusted differences	Reference	-1.6 (-4.1, 0.8)	0.7 (-1.8, 3.2)	-3.2 (-5.8, -0.6)	0.05	-0.7 (-1.8, 0.3)

Footnotes to Table 4.2.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with total externalizing problems score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Table 4.3. Total internalizing problems scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Total internalizing problems scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	52.3 ± 10.0	52.2 ± 9.4	53.5 ± 9.8	54.9 ± 9.6	0.03	1.0 (0.1, 2.0)
Adjusted differences[3]	Reference	0.0 (-2.7, 2.6)	1.1 (-1.6, 3.8)	2.8 (0.1, 5.5)	0.02	1.2 (0.3, 2.2)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	52.5 ± 10.0	53.4 ± 9.6	53.7 ± 9.8	53.4 ± 9.5	0.47	0.2 (-0.7, 1.0)
Adjusted differences	Reference	1.1 (-1.4, 3.7)	1.7 (-0.9, 4.3)	1.5 (-1.1, 4.1)	0.22	0.4 (-0.4, 1.3)
22:5n-3 docosapentaenoic acid						
Mean ± SD	54.7 ± 11.2	53.3 ± 9.5	52.4 ± 9.1	52.5 ± 9.0	0.12	-0.4 (-1.3, 0.4)
Adjusted differences	Reference	-1.8 (-4.6, 1.0)	-2.6 (-5.3, 0.2)	-2.0 (-4.8, 0.9)	0.21	-0.2 (-1.1, 0.6)
22:6n-3 docosahexaenoic acid						
Mean ± SD	53.8 ± 10.5	53.2 ± 10.1	52.8 ± 9.1	53.1 ± 9.3	0.60	0.2 (-0.7, 1.2)
Adjusted differences	Reference	0.0 (-2.7, 2.7)	-0.9 (-3.5, 1.8)	-0.4 (-3.0, 2.2)	0.69	0.4 (-0.6, 1.3)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	52.3 ± 8.9	53.3 ± 9.9	54.1 ± 10.0	53.3 ± 10.1	0.37	0.3 (-0.6, 1.2)
Adjusted differences	Reference	0.7 (-1.8, 3.2)	1.2 (-1.3, 3.8)	0.7 (-1.8, 3.1)	0.54	0.2 (-0.6, 1.1)
18:3n-6 γ-linolenic acid						
Mean ± SD	53.4 ± 10.0	53.4 ± 9.9	53.2 ± 9.5	52.9 ± 9.7	0.64	-0.3 (-1.2, 0.7)
Adjusted differences	Reference	-0.3 (-3.0, 2.3)	-0.4 (-3.1, 2.2)	-0.3 (-3.0, 2.4)	0.84	0.0 (-1.0, 1.0)
20:2n-6 eicosadienoic acid						
Mean ± SD	52.3 ± 9.8	54.5 ± 10.5	54.1 ± 9.7	52.1 ± 8.8	0.71	0.2 (-0.6, 1.1)
Adjusted differences	Reference	2.0 (-0.8, 4.8)	0.9 (-1.7, 3.5)	-0.5 (-3.2, 2.3)	0.58	0.2 (-0.8, 1.1)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	52.4 ± 9.7	53.6 ± 10.1	53.9 ± 10.0	53.1 ± 9.2	0.57	0.0 (-0.9, 0.8)
Adjusted differences	Reference	1.1 (-1.6, 3.9)	1.2 (-1.6, 3.9)	0.9 (-2.0, 3.8)	0.60	-0.1 (-1.1, 0.9)

Total internalizing problems scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	54.2 ± 9.7	53.4 ± 10.3	53.7 ± 9.4	51.8 ± 9.5	0.08	-0.8 (-1.6, 0.1)
Adjusted differences	Reference	-0.7 (-3.5, 2.0)	-0.3 (-2.8, 2.2)	-2.0 (-4.6, 0.5)	0.16	-0.6 (-1.5, 0.3)
22:4n-6 adrenic acid						
Mean ± SD	55.2 ± 10.3	52.4 ± 9.3	54.1 ± 9.8	51.3 ± 9.2	0.01	-1.1 (-2.1, -0.2)
Adjusted differences	Reference	-3.1 (-5.6, -0.6)	-0.3 (-2.9, 2.3)	-3.2 (-5.8, -0.7)	0.06	-0.8 (-1.7, 0.0)
Enzymatic activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	54.1 ± 10.3	52.7 ± 9.6	53.5 ± 9.3	52.6 ± 9.8	0.40	-0.3 (-1.2, 0.5)
Adjusted differences	Reference	-1.1 (-3.8, 1.5)	-0.6 (-3.2, 2.1)	-0.9 (-3.8, 2.0)	0.67	-0.1 (-1.1, 0.9)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	53.7 ± 9.2	52.8 ± 10.2	55.1 ± 10.8	51.3 ± 8.4	0.14	-0.4 (-1.4, 0.6)
Adjusted differences	Reference	-0.4 (-3.0, 2.2)	1.8 (-0.8, 4.5)	-1.9 (-4.4, 0.5)	0.22	-0.2 (-1.1, 0.7)

Footnotes to Table 4.3.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with total internalizing problems score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Supplemental Table 4.1 Distribution of serum fatty acid (FA) percentage weight concentrations

Serum FA percentage weight concentration	Mean ± SD
n-3 polyunsaturated FA (PUFA) (%)	
18:3n-3 α-linolenic acid	0.48 ± 0.14
20:5n-3 eicosapentaenoic acid	0.23 ± 0.13
22:5n-3 docosapentaenoic acid	0.46 ± 0.19
22:6n-3 docosahexaenoic acid	2.24 ± 0.92
Total long-chain n-3 PUFA[1]	2.93 ± 0.96
Total n-3 PUFA	3.47 ± 0.97
n-6 PUFA (%)	
18:2n-6 linoleic acid	30.62 ± 3.00
18:3n-6 γ-linolenic acid	0.30 ± 0.16
20:2n-6 eicosadienoic acid	0.29 ± 0.08
20:3n-6 dihomo-γ-linolenic acid	1.59 ± 0.36
20:4n-6 arachidonic acid	6.04 ± 1.17
22:4n-6 adrenic acid	3.15 ± 1.20
Total long-chain n-6 PUFA[2]	11.07 ± 1.76
Total n-6 PUFA	41.99 ± 3.31
Enzymatic activity	
Δ6-desaturase 18:3n-6/18:2n-6	0.01 ± 0.01
Δ5-desaturase 20:4n-6/20:3n-6	4.01 ± 1.49

Footnotes to Supplemental Table 4.1.

[1] Sum of 20:5n-3 eicosapentaenoic acid, 22:5n-3 docosapentaenoic acid, and 22:6n-3 docosahexaenoic acid.

[2] Sum of 20:2n-6 eicosadienoic acid, 20:3n-6 dihomo-γ-linolenic acid, 20:4n-6 arachidonic acid, and 22:4n-6 adrenic acid.

Supplemental Table 4.2 Aggressive behavior scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Aggressive behavior scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	54.8 ± 6.1	55.1 ± 6.4	56.8 ± 8.7	55.8 ± 6.8	0.13	0.5 (-0.1, 1.1)
Adjusted differences[4]	Reference	0.3 (-1.4, 2.0)	1.4 (-0.5, 3.3)	1.1 (-0.7, 2.8)	0.15	0.5 (-0.1, 1.1)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	56.3 ± 7.5	56.3 ± 8	55.6 ± 7.3	54.3 ± 5.1	0.02	-0.6 (-1.2, -0.1)
Adjusted differences	Reference	0.3 (-1.7, 2.3)	0.5 (-1.5, 2.4)	-1.3 (-3.0, 0.4)	0.19	-0.3 (-0.9, 0.2)
22:5n-3 docosapentaenoic acid						
Mean ± SD	56.6 ± 8.0	55.4 ± 6.8	56.3 ± 7.2	54.3 ± 6.0	0.02	-0.8 (-1.4, -0.2)
Adjusted differences	Reference	-1.7 (-3.7, 0.2)	-0.5 (-2.5, 1.6)	-2.1 (-4.1, 0.0)	0.12	-0.6 (-1.2, 0.0)
22:6n-3 docosahexaenoic acid						
Mean ± SD	55.3 ± 7.3	55.6 ± 7.3	55.4 ± 7.2	56.3 ± 6.7	0.29	0.8 (0.1, 1.6)
Adjusted differences	Reference	0.7 (-1.2, 2.6)	-0.3 (-2.2, 1.7)	0.9 (-0.9, 2.8)	0.42	0.9 (0.1, 1.6)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	55.0 ± 6.7	55.6 ± 7.0	56.5 ± 7.6	55.4 ± 7.0	0.46	0.1 (-0.6, 0.8)
Adjusted differences	Reference	1.0 (-0.7, 2.8)	1.9 (0.0, 3.8)	0.6 (-1.1, 2.4)	0.38	0.2 (-0.5, 0.9)
18:3n-6 γ-linolenic acid						
Mean ± SD	55.3 ± 6.8	55.9 ± 7.9	55.7 ± 7.1	55.6 ± 6.5	0.92	-0.1 (-0.7, 0.5)
Adjusted differences	Reference	0.1 (-1.8, 2.1)	-0.1 (-1.9, 1.8)	0.1 (-1.8, 1.9)	0.99	0.0 (-0.7, 0.6)
20:2n-6 eicosadienoic acid						
Mean ± SD	55.4 ± 7.3	56.7 ± 8.3	55.6 ± 6.8	54.8 ± 5.7	0.31	-0.1 (-0.7, 0.5)
Adjusted differences	Reference	1.5 (-0.6, 3.5)	0.3 (-1.5, 2.2)	-0.6 (-2.5, 1.4)	0.39	-0.1 (-0.7, 0.6)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	54.5 ± 5.9	56.3 ± 8.3	56.2 ± 7.7	55.5 ± 6.2	0.36	0.2 (-0.4, 0.7)
Adjusted differences	Reference	1.8 (-0.2, 3.8)	1.3 (-0.5, 3.2)	1.4 (-0.6, 3.3)	0.30	0.3 (-0.3, 1.0)

Aggressive behavior scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	56.1 ± 7.4	55.9 ± 7.3	55.8 ± 7.2	54.6 ± 6.4	0.11	-0.7 (-1.3, -0.1)
Adjusted differences	Reference	0.4 (-1.5, 2.4)	0.4 (-1.5, 2.3)	-1.3 (-3.1, 0.6)	0.18	-0.6 (-1.3, 0.0)
22:4n-6 adrenic acid						
Mean ± SD	56.4 ± 7.3	54.9 ± 6.5	56.1 ± 8.2	55.1 ± 6.2	0.31	-0.3 (-0.9, 0.3)
Adjusted differences	Reference	-1.8 (-3.7, 0.0)	-0.2 (-2.1, 1.8)	-1.4 (-3.2, 0.4)	0.28	-0.3 (-0.9, 0.3)
Enzyme activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	55.7 ± 7.2	55.3 ± 7.0	55.9 ± 7.7	55.6 ± 6.5	0.86	-0.1 (-0.7, 0.5)
Adjusted differences	Reference	-0.7 (-2.5, 1.2)	0.2 (-1.7, 2.2)	0.0 (-2.0, 2.0)	0.76	0.0 (-0.7, 0.7)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	56.5 ± 6.9	55.2 ± 7.2	56.6 ± 8.1	54.2 ± 5.7	0.04	-0.5 (-1.2, 0.1)
Adjusted differences	Reference	-0.9 (-2.8, 0.9)	0.5 (-1.5, 2.5)	-2.2 (-3.9, -0.4)	0.05	-0.5 (-1.2, 0.2)

Footnotes to Supplemental Table 4.2.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with aggressive behavior score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Supplemental Table 4.3. Rule breaking behavior scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Rule breaking behavior scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	P^1	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	53.9 ± 4.3	54.1 ± 4.5	54.3 ± 5.3	54.6 ± 5.4	0.33	0.2 (-0.3, 0.6)
Adjusted differences[4]	Reference	0.2 (-1.0, 1.3)	0.0 (-1.2, 1.3)	0.7 (-0.6, 2.0)	0.31	0.2 (-0.2, 0.7)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	54.6 ± 4.9	54.4 ± 5.4	54.0 ± 4.8	54.0 ± 4.5	0.29	-0.3 (-0.7, 0.1)
Adjusted differences	Reference	0.1 (-1.2, 1.5)	0.1 (-1.1, 1.3)	-0.1 (-1.3, 1.2)	0.93	-0.1 (-0.5, 0.3)
22:5n-3 docosapentaenoic acid						
Mean ± SD	54.5 ± 5.3	54.5 ± 5.0	54.7 ± 4.8	53.3 ± 4.4	0.05	-0.4 (-0.8, 0.0)
Adjusted differences	Reference	-0.5 (-1.8, 0.9)	-0.1 (-1.5, 1.2)	-1.5 (-2.9, -0.1)	0.04	-0.4 (-0.8, 0.1)
22:6n-3 docosahexaenoic acid						
Mean ± SD	54.1 ± 4.7	53.7 ± 4.7	54.4 ± 5.1	54.8 ± 5.0	0.18	0.4 (-0.1, 0.9)
Adjusted differences	Reference	-0.1 (-1.3, 1.2)	0.3 (-1.0, 1.6)	0.7 (-0.6, 2.0)	0.22	0.4 (0.0, 0.9)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	54.4 ± 5.0	53.9 ± 4.8	55.1 ± 5.4	53.5 ± 4.1	0.31	-0.3 (-0.8, 0.1)
Adjusted differences	Reference	-0.5 (-1.7, 0.8)	0.8 (-0.6, 2.1)	-1.0 (-2.2, 0.2)	0.27	-0.3 (-0.8, 0.1)
18:3n-6 γ-linolenic acid						
Mean ± SD	54.1 ± 4.9	54.5 ± 5.0	54.1 ± 4.9	54.2 ± 4.8	0.90	-0.1 (-0.6, 0.4)
Adjusted differences	Reference	0.0 (-1.3, 1.4)	-0.6 (-1.9, 0.7)	-0.5 (-1.9, 0.9)	0.36	-0.3 (-0.8, 0.3)
20:2n-6 eicosadienoic acid						
Mean ± SD	54.3 ± 4.6	55.1 ± 5.4	53.5 ± 4.2	53.9 ± 5.1	0.23	-0.2 (-0.7, 0.2)
Adjusted differences	Reference	0.7 (-0.7, 2.1)	-1.0 (-2.2, 0.2)	-0.4 (-1.8, 1.0)	0.25	-0.3 (-0.7, 0.2)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	53.6 ± 4.5	54.8 ± 5.4	53.9 ± 4.5	54.5 ± 5.0	0.31	0.2 (-0.3, 0.7)
Adjusted differences	Reference	1.2 (-0.1, 2.5)	0.1 (-1.1, 1.3)	1.4 (0.1, 2.8)	0.15	0.4 (-0.1, 0.9)

Rule breaking behavior scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	55.2 ± 5.6	54.2 ± 4.9	53.6 ± 4.2	53.9 ± 4.7	0.04	-0.6 (-1.1, -0.1)
Adjusted differences	Reference	-0.6 (-2.0, 0.8)	-1.2 (-2.6, 0.1)	-1.3 (-2.7, 0.1)	0.05	-0.6 (-1.1, -0.1)
22:4n-6 adrenic acid						
Mean ± SD	54.3 ± 4.9	54.4 ± 5.1	54.2 ± 4.9	54.0 ± 4.7	0.63	-0.1 (-0.5, 0.4)
Adjusted differences	Reference	0.1 (-1.2, 1.4)	0.1 (-1.2, 1.3)	-0.1 (-1.4, 1.1)	0.86	0.0 (-0.5, 0.4)
Enzyme activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	54.4 ± 5.1	53.8 ± 4.3	54.5 ± 5.3	54.2 ± 4.9	0.99	-0.1 (-0.5, 0.3)
Adjusted differences	Reference	-0.9 (-2.1, 0.4)	-0.4 (-1.8, 0.9)	-0.9 (-2.4, 0.6)	0.39	-0.3 (-0.9, 0.2)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	55.2 ± 5.4	53.6 ± 4.5	54.6 ± 5.3	53.5 ± 4.1	0.04	-0.5 (-0.9, -0.1)
Adjusted differences	Reference	-1.4 (-2.8, -0.1)	-0.4 (-1.8, 1.0)	-1.9 (-3.2, -0.6)	0.02	-0.6 (-1.0, -0.1)

Footnotes to Supplemental Table 4.3.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with rule breaking behavior score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Supplemental Table 4.4 Anxious/depressed scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Anxious/depressed scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	54.7 ± 6.1	54.7 ± 5.8	55.8 ± 7.2	56.0 ± 7.0	0.08	0.5 (-0.1, 1.1)
Adjusted differences[4]	Reference	-0.1 (-1.7, 1.5)	1.2 (-0.6, 3.0)	1.5 (-0.3, 3.4)	0.05	0.7 (0.0, 1.3)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	54.7 ± 6.4	55.1 ± 6.8	55.6 ± 6.0	55.7 ± 7.0	0.21	0.2 (-0.4, 0.8)
Adjusted differences	Reference	0.6 (-1.1, 2.4)	1.3 (-0.4, 3.0)	1.3 (-0.5, 3.1)	0.11	0.3 (-0.3, 0.9)
22:5n-3 docosapentaenoic acid						
Mean ± SD	56.9 ± 7.4	55.4 ± 6.7	54.5 ± 5.7	54.2 ± 6.0	0.002	-0.8 (-1.3, -0.2)
Adjusted differences	Reference	-1.6 (-3.5, 0.3)	-2.6 (-4.3, -0.8)	-3.2 (-5.1, -1.3)	0.001	-0.8 (-1.4, -0.2)
22:6n-3 docosahexaenoic acid						
Mean ± SD	56.2 ± 7.1	55.8 ± 7.2	54.1 ± 6.0	55.0 ± 5.7	0.12	-0.2 (-0.8, 0.5)
Adjusted differences	Reference	0.1 (-1.8, 2.0)	-1.7 (-3.6, 0.1)	-0.8 (-2.5, 0.9)	0.24	0.0 (-0.6, 0.7)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	54.4 ± 5.3	55.5 ± 6.8	55.7 ± 7.3	55.5 ± 6.7	0.18	0.4 (-0.2, 1.0)
Adjusted differences	Reference	0.7 (-1.0, 2.3)	0.8 (-0.9, 2.5)	0.6 (-1.0, 2.2)	0.49	0.3 (-0.3, 0.9)
18:3n-6 γ-linolenic acid						
Mean ± SD	55.2 ± 6.7	55.1 ± 6.1	55.6 ± 7.0	55.1 ± 6.4	0.97	0.0 (-0.6, 0.6)
Adjusted differences	Reference	-0.4 (-2.1, 1.4)	-0.1 (-1.9, 1.7)	0.0 (-1.8, 1.9)	0.87	0.1 (-0.6, 0.8)
20:2n-6 eicosadienoic acid						
Mean ± SD	54.9 ± 7.1	55.6 ± 6.3	56.2 ± 7.0	54.5 ± 5.7	0.66	0.0 (-0.5, 0.6)
Adjusted differences	Reference	0.3 (-1.6, 2.2)	0.8 (-1.2, 2.7)	-0.6 (-2.5, 1.3)	0.61	0.0 (-0.6, 0.7)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	54.5 ± 5.7	55.7 ± 7.4	55.7 ± 7.0	55.2 ± 5.9	0.53	0.0 (-0.6, 0.5)
Adjusted differences	Reference	1.0 (-0.8, 2.9)	0.9 (-0.9, 2.6)	0.8 (-1.0, 2.7)	0.51	0.0 (-0.6, 0.7)

Anxious/depressed scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	55.5 ± 6.4	55.8 ± 7.1	55.9 ± 6.9	54.0 ± 5.6	0.08	-0.5 (-1.1, 0.0)
Adjusted differences	Reference	0.5 (-1.4, 2.4)	0.6 (-1.2, 2.4)	-1.3 (-3.0, 0.3)	0.13	-0.5 (-1.0, 0.1)
22:4n-6 adrenic acid						
Mean ± SD	56.9 ± 7.8	54.3 ± 5.6	55.5 ± 6.8	54.3 ± 5.5	0.01	-0.9 (-1.5, -0.2)
Adjusted differences	Reference	-2.8 (-4.6, -1.0)	-1.0 (-2.9, 0.9)	-2.4 (-4.2, -0.7)	0.03	-0.7 (-1.3, -0.1)
Enzyme activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	55.5 ± 6.9	55.1 ± 6.4	55.4 ± 6.4	55.2 ± 6.5	0.82	-0.1 (-0.7, 0.5)
Adjusted differences	Reference	-0.3 (-2.2, 1.5)	-0.3 (-2.0, 1.5)	0.2 (-1.8, 2.2)	0.77	0.1 (-0.6, 0.8)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	55.4 ± 6.1	55.3 ± 6.8	56.7 ± 7.8	53.8 ± 4.9	0.08	-0.2 (-0.9, 0.5)
Adjusted differences	Reference	0.3 (-1.5, 2.0)	1.4 (-0.5, 3.3)	-1.6 (-3.1, 0.0)	0.07	-0.2 (-0.9, 0.6)

Footnotes to Supplemental Table 4.4.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with anxious/depressed score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Supplemental Table 4.5. Withdrawn/depressed scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Withdrawn/depressed scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	55.0 ± 5.9	54.3 ± 5.1	54.9 ± 6.1	56.0 ± 6.7	0.14	0.4 (-0.2, 1.0)
Adjusted differences[4]	Reference	-0.8 (-2.3, 0.7)	-0.3 (-1.9, 1.3)	1.1 (-0.6, 2.8)	0.15	0.4 (-0.2, 1.0)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	55.2 ± 6.7	55.6 ± 6.2	54.7 ± 5.8	54.8 ± 5.3	0.44	-0.3 (-0.9, 0.2)
Adjusted differences	Reference	0.6 (-1.1, 2.3)	-0.2 (-1.8, 1.4)	-0.2 (-1.8, 1.4)	0.66	-0.2 (-0.8, 0.3)
22:5n-3 docosapentaenoic acid						
Mean ± SD	56.5 ± 6.6	54.5 ± 5.0	54.5 ± 6.0	54.8 ± 6.1	0.14	-0.3 (-0.8, 0.2)
Adjusted differences	Reference	-2.4 (-3.9, -0.8)	-2.2 (-3.9, -0.5)	-1.5 (-3.4, 0.4)	0.28	-0.2 (-0.8, 0.3)
22:6n-3 docosahexaenoic acid						
Mean ± SD	55.1 ± 6.0	55.1 ± 5.7	55.1 ± 6.3	55.0 ± 6.0	0.85	0.1 (-0.5, 0.7)
Adjusted differences	Reference	0.3 (-1.2, 1.9)	0.1 (-1.6, 1.8)	-0.1 (-1.8, 1.5)	0.77	0.2 (-0.4, 0.8)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	54.8 ± 5.7	55.7 ± 6.8	55.1 ± 5.6	54.8 ± 5.9	0.81	-0.1 (-0.6, 0.4)
Adjusted differences	Reference	0.9 (-0.7, 2.6)	0.2 (-1.3, 1.8)	0.0 (-1.5, 1.5)	0.79	-0.1 (-0.6, 0.4)
18:3n-6 γ-linolenic acid						
Mean ± SD	55.8 ± 6.6	54.9 ± 6.0	54.7 ± 5.7	54.9 ± 5.7	0.33	-0.2 (-0.9, 0.4)
Adjusted differences	Reference	-1.2 (-2.9, 0.4)	-1.5 (-3.2, 0.1)	-1.3 (-3.0, 0.4)	0.21	-0.3 (-1.0, 0.4)
20:2n-6 eicosadienoic acid						
Mean ± SD	54.5 ± 5.7	56.0 ± 7.0	55.5 ± 6.1	54.3 ± 4.9	0.56	0.0 (-0.5, 0.5)
Adjusted differences	Reference	1.2 (-0.6, 3.0)	0.4 (-1.2, 2.0)	-0.6 (-2.3, 1.0)	0.31	-0.1 (-0.7, 0.5)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	55.2 ± 6.4	55.2 ± 5.9	55.3 ± 6.3	54.6 ± 5.4	0.41	-0.3 (-0.8, 0.2)
Adjusted differences	Reference	-0.1 (-1.8, 1.7)	-0.1 (-1.9, 1.7)	-0.5 (-2.3, 1.3)	0.60	-0.2 (-0.8, 0.4)

Withdrawn/depressed scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	55.8 ± 6.0	55.7 ± 6.8	54.6 ± 5.5	54.2 ± 5.5	0.01	-0.7 (-1.2, -0.1)
Adjusted differences	Reference	-0.1 (-1.8, 1.7)	-1.3 (-2.9, 0.2)	-1.5 (-3.1, 0.1)	0.03	-0.6 (-1.1, 0.0)
22:4n-6 adrenic acid						
Mean ± SD	55.7 ± 7.0	54.6 ± 5.5	55.8 ± 6.1	54.1 ± 5.2	0.15	-0.5 (-1.0, 0.1)
Adjusted differences	Reference	-1.4 (-3.1, 0.2)	0.3 (-1.4, 2.0)	-1.5 (-3.0, 0.1)	0.25	-0.4 (-0.9, 0.2)
Enzyme activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	55.9 ± 6.6	54.8 ± 5.7	54.7 ± 5.8	54.8 ± 5.8	0.26	-0.2 (-0.8, 0.4)
Adjusted differences	Reference	-1.2 (-2.8, 0.5)	-1.6 (-3.2, 0.1)	-1.5 (-3.4, 0.3)	0.14	-0.3 (-1.1, 0.4)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	54.8 ± 5.6	55.2 ± 6.1	56.0 ± 6.7	54.2 ± 5.4	0.48	-0.3 (-0.8, 0.2)
Adjusted differences	Reference	0.4 (-1.2, 2.1)	1.1 (-0.6, 2.8)	-0.6 (-2.1, 0.9)	0.46	-0.3 (-0.8, 0.2)

Footnotes to Supplemental Table 4.5.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with withdrawn/depressed score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Supplemental Table 4 6. Somatic complaints scores at 11-18 y of age according to serum fatty acid (FA) percentage weight concentration in middle childhood among children from Bogota, Colombia

Somatic complaints scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-3 polyunsaturated FA (PUFA)						
18:3n-3 α-linolenic acid						
Mean ± SD	56.9 ± 7.2	56.5 ± 7.3	56.8 ± 7.8	57.3 ± 7.5	0.61	0.3 (-0.4, 1.1)
Adjusted differences[4]	Reference	-0.2 (-2.1, 1.7)	0.0 (-2.0, 1.9)	0.6 (-1.3, 2.6)	0.49	0.5 (-0.2, 1.3)
20:5n-3 eicosapentaenoic acid						
Mean ± SD	56.8 ± 7.0	56.7 ± 7.1	57.4 ± 8.4	56.6 ± 7.2	0.95	0.1 (-0.5, 0.8)
Adjusted differences	Reference	0.1 (-1.8, 2.0)	0.9 (-1.2, 3.0)	0.4 (-1.5, 2.3)	0.51	0.4 (-0.3, 1.1)
22:5n-3 docosapentaenoic acid						
Mean ± SD	57.4 ± 8.8	57.2 ± 6.7	55.9 ± 6.8	56.9 ± 7.2	0.55	-0.1 (-0.8, 0.6)
Adjusted differences	Reference	-0.3 (-2.4, 1.7)	-1.3 (-3.4, 0.7)	0.3 (-1.9, 2.5)	0.84	0.2 (-0.5, 0.9)
22:6n-3 docosahexaenoic acid						
Mean ± SD	57.4 ± 8.4	56.2 ± 7.1	57.2 ± 7.5	56.7 ± 6.7	0.64	0.3 (-0.6, 1.2)
Adjusted differences	Reference	-0.9 (-2.9, 1.1)	-0.2 (-2.4, 1.9)	-0.5 (-2.5, 1.4)	0.76	0.4 (-0.5, 1.3)
n-6 PUFA						
18:2n-6 linoleic acid						
Mean ± SD	56.2 ± 7.4	56.6 ± 7.4	57.5 ± 7.6	57.2 ± 7.5	0.25	0.3 (-0.4, 1.1)
Adjusted differences	Reference	-0.1 (-2.0, 1.8)	1.0 (-1.0, 3.1)	0.8 (-1.2, 2.8)	0.32	0.4 (-0.5, 1.2)
18:3n-6 γ-linolenic acid						
Mean ± SD	56.8 ± 7.6	57.5 ± 8.5	56.6 ± 6.6	56.5 ± 7.1	0.51	-0.3 (-1.0, 0.4)
Adjusted differences	Reference	0.6 (-1.6, 2.7)	0.0 (-1.9, 1.9)	0.4 (-1.7, 2.5)	0.87	0.1 (-0.7, 0.8)
20:2n-6 eicosadienoic acid						
Mean ± SD	56.4 ± 6.3	58.5 ± 9.6	56.7 ± 7.1	55.9 ± 6	0.25	-0.1 (-0.7, 0.4)
Adjusted differences	Reference	2.0 (-0.2, 4.2)	0.1 (-1.8, 1.9)	-0.5 (-2.4, 1.5)	0.33	-0.1 (-0.7, 0.6)
20:3n-6 dihomo-γ-linolenic acid						
Mean ± SD	56.4 ± 7.5	56.9 ± 7.7	57.3 ± 6.8	56.9 ± 7.8	0.63	0.1 (-0.6, 0.8)
Adjusted differences	Reference	0.6 (-1.5, 2.7)	0.8 (-1.2, 2.8)	1.0 (-1.3, 3.3)	0.39	0.1 (-0.7, 0.9)

Somatic complaints scores	Q1 n=111	Q2 n=111	Q3 n=111	Q4 n=111	*P*[1]	Mean difference per 1 SD[2]
n-6 PUFA, *cont.*						
20:4n-6 arachidonic acid						
Mean ± SD	57.5 ± 8.4	56.5 ± 7.0	56.6 ± 7.3	56.8 ± 7.0	0.55	-0.4 (-1.2, 0.3)
Adjusted differences	Reference	-1.3 (-3.3, 0.8)	-0.9 (-3.0, 1.3)	-0.7 (-2.8, 1.4)	0.64	-0.4 (-1.1, 0.4)
22:4n-6 adrenic acid						
Mean ± SD	57.9 ± 7.7	56.9 ± 7.3	57.3 ± 8.1	55.5 ± 6.4	0.03	-0.7 (-1.3, 0.0)
Adjusted differences	Reference	-1.0 (-2.9, 0.9)	0.1 (-2.0, 2.2)	-1.8 (-3.6, 0.0)	0.14	-0.4 (-1.1, 0.2)
Enzyme activity indices						
Δ6-desaturase 18:3n-6/18:2n-6						
Mean ± SD	57.6 ± 8.3	56.5 ± 6.8	57.2 ± 7.4	56.3 ± 7.1	0.31	-0.4 (-1.1, 0.3)
Adjusted differences	Reference	-0.9 (-2.9, 1.2)	-0.2 (-2.3, 1.9)	-0.3 (-2.6, 2.0)	0.97	0.0 (-0.8, 0.8)
Δ5-desaturase 20:4n-6/20:3n-6						
Mean ± SD	57.5 ± 8.3	56.2 ± 6.8	57.9 ± 8.4	55.9 ± 5.8	0.23	-0.2 (-0.8, 0.3)
Adjusted differences	Reference	-1.2 (-3.2, 0.8)	0.5 (-1.8, 2.8)	-1.8 (-3.8, 0.3)	0.21	-0.3 (-0.8, 0.3)

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Footnotes to Supplemental Table 4.6.

[1] Test for linear trend when a variable representing the median of each quartile was introduced into the linear regression model as a continuous predictor. Empirical estimates of the variance were specified in all models.

[2] From a linear regression model with somatic complaints score as the outcome and % of total serum FA per 1 SD (continuous) as the predictor.

[3] Adjusted for child's sex (dichotomous), child's age at baseline (continuous), weekly hours spent watching television / playing video games (continuous), years of mother's education (continuous) and mother's BMI (continuous). n-3 and n-6 PUFA were adjusted for the continuous % of total serum FA per 1 SD of linoleic acid and α-linolenic acid, respectively. Long-chain FA were additionally adjusted for their immediate FA precursor(s) (continuous). Δ6-desaturase activity was additionally adjusted for α-linolenic acid and linoleic acid (continuous). Δ5-desaturase activity was additionally adjusted for α-linolenic acid, γ-linolenic acid, and eicosadienoic acid (continuous).

Chapter 5 Conclusions

Summary of Findings

This work generates new knowledge on the potential effects of nutritional exposures in middle childhood on externalizing and internalizing problems in adolescence.

In Chapter 2, we found that ID, anemia, and low vitamin B-12 in middle childhood were positively associated with total externalizing problems among boys in Bogotá, Colombia. The association between low vitamin B-12 and total externalizing problems may be explained by higher aggressive behavior scores. In addition, ID was positively associated with total internalizing problems among boys, perhaps due to higher anxious/depressed and somatic complaints scores. These nutritional exposures in middle childhood were not associated with total externalizing or internalizing problems in adolescence among girls. Previous studies focused on iron status in infancy as an exposure. Chronic iron deficiency in infancy has been related to behavior problems in early and middle childhood, adolescence, and young adulthood (1-4). Iron is required for the production of the monoamine neurotransmitters (dopamine, serotonin, and norepinephrine) as well as gamma-aminobutyric acid and glutamate (5). In our population, anemia was not related to biomarkers of nutrients relevant to the hemoglobin metabolism, thus the nature of this association is uncertain. Anemia may be indicative of micronutrient deficiencies that we did not measure such as vitamin C or riboflavin. Vitamin B-12 intake in adolescence has not been associated with behavior problems in cross-sectional studies (6-8). Vitamin B-12 is involved in the formation of S-adenosylmethionine, a precursor to the

monoamine neurotransmitters which govern mood and behavior, which has used to treat clinical depression in adults (9).

In Chapter 3, we focus on vitamin D and its carrier protein, DBP, as exposures. VDD was positively associated with total externalizing problems. In addition, low DBP was related to higher aggressive behavior and anxious/depressed scores. The associations between DBP and behavior problems were not mediated by VDD. Previous findings on the association of vitamin D status and behavior problems have been inconsistent (10-14). Vitamin D deficiency alters dopamine metabolism and thus the vitamin may be related to the regulation of emotions and motivation. In middle childhood, VDD may regulate glial cell line-derived neurotrophic factor (15) which influences dopaminergic neuronal pruning (16). Our finding that low DBP concentration was associated with behavior problems is novel and hypothesis generating. DBP is involved in the innate immune response (17), which could influence behavior problems.

Chapter 4 examines the associations of PUFA concentrations in middle childhood with total externalizing and internalizing problems in adolescence. The n-3 PUFA DPA was inversely associated with total externalizing problems, while DHA was positively related to this outcome. In addition, the D5D activity index was inversely related to total externalizing problems. ALA concentration was positively related to total internalizing problems, whereas AdA was inversely related to this outcome. Although not related to total externalizing or internalizing problems, AA was inversely associated with the behavior problems subscales rule breaking behavior and withdrawn/depressed scores. The positive association of DHA with total externalizing problems was unexpected, though consistent with several previous studies (18-20). A few rodent studies have found that DHA is related to delayed neurodevelopment (21, 22). Alternatively, limitations of observational studies may account for this positive association. For example, the association

between DHA and behavior problems may be confounded by environmental toxicants found in fatty fish, a dietary source of DHA. Mechanisms explaining the inverse associations of DPA, AA, and AdA with behavior problems are speculative. DPA and AA influence long-term potentiation of synapses in the striatum or the hippocampus, areas of the brain involved in behavior and cognition, whereas AdA may be involved in myelination (23). Since AA and the D5D activity index were both inversely related to rule breaking behavior scores, it is plausible that the associations with AA represent individual differences related to D5D activity.

This dissertation has several strengths. The longitudinal study design minimizes the possibility of bias due to reverse causation. We used plasma or serum biomarkers to measure nutrient intake, which is considered an objective measurement method free of recall bias (24). Serum ferritin concentrations are adequately correlated with iron stores in the absence of inflammation (25). We excluded those with CRP >10 mg/L, thereby addressing inflammation. Total circulating 25(OH)D is considered the most complete way to capture vitamin D status, as it integrates dietary and sunlight sources of the vitamin (26). Serum biomarkers of FA adequately correlate with long-term intake of PUFA (27). In addition, the YSR is generalizable to many populations and widely used in epidemiologic research (28). We adjusted for key potential confounding variables collected at baseline including children's BMI-for-age Z scores, time spent watching television or playing video games, maternal BMI, household food insecurity, and household socioeconomic status.

There are some limitations as well. We do not have a baseline measurement of behavior problems in children and therefore do not know whether the problems measured in adolescence developed after the measurement of exposure or were present before. If behavior problems were already present at the time of enrollment and had caused changes in dietary behavior, reverse

causation cannot be ruled out as an explanation of the results. With regard to internalizing problems, reverse causation is unlikely, as average age of onset is during adolescence (29). Laboratory methods to quantify serum and plasma biomarkers may introduce random error. However, the majority of our coefficients of variation were low (<10%), which indicates small amounts of random error. Further, all exposures were assessed only once. However, for many of these biomarkers, a single measurement may adequately represent long-term intake (30, 31), and may therefore be representative of middle childhood exposure. If middle childhood nutrition is correlated with nutrition in other periods of development, then the results may reflect exposure at other ages rather than exposure in middle childhood. Ferritin, vitamin B-12, vitamin D, and PUFA concentrations may be correlated. However, we were unable to control for all dietary biomarker exposures in each Chapter as the study samples in Chapters 2-4 did not coincide. In addition, we were not able to control for several independent predictors of behavior problems, including genetics, parenting styles, and environmental toxicants. If these unmeasured independent predictors of the behavior problems are associated with the nutritional exposures of interest, then our results would be subject to residual confounding. Finally, our results may not be generalizable to children from the highest socioeconomic status, because they were not represented in this sample of children attending public school in Bogotá, Colombia.

In conclusion, nutrition in middle childhood was associated with externalizing and internalizing problems in adolescence. Among boys, ID, anemia, and low vitamin B-12 were positively related to externalizing problems. ID was positively associated with internalizing problems among boys. VDD was associated with externalizing problems among boys and girls. Low DBP was related to high aggressive behavior and anxious/depressed scores. DPA and the D5D activity index were inversely related to externalizing problems whereas DHA was

positively associated with this outcome. ALA and AdA were positively and inversely related to internalizing problems, respectively. These results contribute knowledge on the relation between nutrition in middle childhood and adolescent behavior problems and may eventually form the basis for planning effective interventions in school-aged children.

Public Health Implications

This dissertation represents a relatively comprehensive investigation of middle childhood diet in relation to adolescent behavior problems in the context of the BoSCCo study. Most studies conducted to date have only examined the influence of diet during the prenatal or infancy periods on child behavior problems. Middle childhood is a key period in cognitive development, as the frontal and temporal lobes undergo further changes (32). Many behavior problems first occur in adolescence (29). Thus, it is a critical time period to investigate behavior problem outcomes.

We found that middle childhood ID, anemia, and low vitamin B-12 were associated with adolescent behavior problems among boys. As the association with ID is consistent with studies that have examined ID in infancy, results from this observational investigation could be the basis for the planning of intervention studies aimed at testing the effect of correcting ID or low vitamin B-12 in childhood on behavior problems later in life. In this population, administration of a school snack designed to provide 30, 50, and 40% of the recommended daily intake of energy, iron, and calcium, respectively, increased vitamin B-12 concentrations after approximately 3 months (33). Public schools in Bogotá continue to administer this daily school snack. In addition, a limited number of schools with the necessary infrastructure provide a hot breakfast or lunch for their students. In 1996, Colombia mandated wheat flour fortification of iron and folate, however, vitamin B-12 fortification is not required by law (34). Daily vitamin supplementation or fortification of commonly eaten food sources could be implemented and scaled up at very low cost. School-based dietary interventions can be highly cost-effective, since they can be implemented as part of ongoing school nutrition programs.

The associations observed between middle childhood VDD and DBP concentrations are quite novel and need replication in other populations with different distributions of these nutrients or biomarkers. For example, the prevalence of VDD in Hispanic children in the United States is higher than the prevalence in our study (35, 36). To strengthen causal inference, future observational studies should use various informants to measure adolescent behavior problems. In addition, future studies should measure DBP with a polyclonal assay to account for potential confounding by isoform. If these results are replicated in future observational studies, then the planning of intervention studies would be warranted. Milk fortified with vitamin D increases 25(OH)D concentrations in various populations (37). A school-based intervention in Bogotá could offer vitamin D fortified milk as part of the existing school snack program. DBP cannot be provided as a supplement, and there are few modifiable predictors of DBP concentration. Therefore, it would be difficult to intervene on DBP concentration. Associations between DPB and behavior may represent underlying genetic differences in children. If replicated, this finding could advance the etiologic understanding of adolescent behavior problems.

The associations between PUFA concentrations and behavior problems also need replication in different populations. Future observational studies should investigate the PUFA metabolites DPA and AdA as exposures, since they may be important in the development of behavior problems. In addition, it would be valuable to elucidate the role of the D5D enzyme in the development of behavior problems. The D5D activity is regulated by genetic and environmental factors. Thus, future research could identify modifiable environmental factors that enhance the activity of this enzyme. The positive association between DHA concentrations and behavior problems should be interpreted with caution, since the observed association could be

confounded by intake of heavy metals or environmental pollutants, as these exposures are related to fatty fish intake, a source of preformed DHA.

These studies are especially relevant because they took place among low- and middle-income schoolchildren in Bogotá, Colombia. Mental health is understudied in low- to middle-income countries and often neglected in pediatric populations. Over the next 35 years, the number of children with psychiatric and developmental disorders is expected to increase in low- to middle-income countries (38), which largely do not have the resources to address psychiatric disorders adequately (39) This is particularly relevant in a country like Colombia, which has been subjected to political and social unrest for the past 6 decades in what sociologists and historians call a new "culture of violence" (40) and thus may have a high prevalence of childhood behavior problems.

Psychiatric and substance use disorders are the fifth leading cause of disability adjusted life years worldwide. They are the leading cause of years lived with disability (41). Externalizing problems are related to violent and criminal behavior (42), which may not only affect the immediate environment of children (e.g., family and school) but also adversely impact the dynamics of a society as a whole. Internalizing problems in adolescence are highly correlated with depressive and anxiety disorders in adults (43). Identifying modifiable predictors of externalizing and internalizing problems is the first step in lowering the burden of disease attributable to psychiatric disorders.

In summation, this dissertation research shows that nutrition in middle childhood is associated with externalizing and internalizing problems in adolescence. As research on adolescent behavior problems and psychiatric disorders continues to advance, we anticipate that middle childhood and adolescence will be increasingly considered as critical periods in

development that are responsive to environmental conditions. With additional research on the correlates of nutrition in middle childhood with later behavioral health outcomes, these findings may have implications for planning effective intervention studies among school-aged children.

Future Directions

The work presented in this dissertation may eventually provide scientific rationale for planning school-based dietary interventions. However, several steps should be taken to strengthen causal inference before planning intervention studies.

First, the mechanisms presented throughout this dissertation are speculative. Most animal models focus on the effects of nutrition on central nervous system development in the prenatal period. Mechanisms in middle childhood are unknown. There are a variety of possible mechanisms; nutrition in middle childhood could alter synaptic plasticity, influence neuronal pruning, impact neurotransmitter metabolisms, or alter glial cell functioning. Identifying these mechanisms would provide biologic rationale for a long-term effect of nutrition in middle childhood on behavior. In addition, knowledge of the specific insults of nutritional deficiencies on brain development may allow future researchers to develop targeted interventions to restore optimal brain functioning.

In this work, we were not able to account for nutrition in the prenatal or infancy period and thus cannot determine if the associations found in these studies represent the potential effect of middle childhood nutrition or the cumulative effect of early life and middle childhood nutrition. To isolate the critical period relevant to the development of behavior problems, future observational studies should control for nutrition in the prenatal or infancy period. If the results in this dissertation are replicated in studies which account for earlier nutrition, this would indicate that the association between nutrition in middle childhood and adolescent behavior problems is independent of nutrition earlier in life and strengthen justification for planning intervention studies among school-aged children.

If these results are replicated in future observational studies, then testing the hypotheses examined in this work with the use of intervention studies would be warranted for some exposures examined. Since ID has known negative health effects (44), a randomized controlled trial of iron supplementation among children with ID would not be ethical. Randomized controlled trials of vitamin D fortification (45, 46) and fish oil supplementation (47, 48) conducted in school-aged children have good adherence and high short-term retention. Although resource intensive, an intervention study of vitamin D fortification, or vitamin B-12 or DPA supplementation with long-term follow-up would provide the strongest epidemiologic evidence for causal inference.

Public health interventions would be merited if these results are replicated in intervention studies. In Colombia, fortification of wheat flour with vitamin B-12 or milk with vitamin D is not mandatory. I would recommend the addition of products made with vitamin B-12 fortified wheat flour and vitamin D fortified milk to the existing school snack program in Bogotá. Vitamin D fortification of milk is not required in many Latin American countries (49). Therefore, this recommendation could also apply to other countries in the region. Since the prevalence of ID, anemia, and VDD were low in our study, screening all schoolchildren to identify those with deficiencies or anemia would not be a cost-effective intervention strategy. However, in regions where the prevalence of nutritional deficiencies is higher, schools could partner with local pediatric clinics to detect children deficient in key nutrients through minimally invasive methods and provide supplementation to correct their deficiency.

In summary, investigations of the potential effects of middle childhood nutrition on later behavior problems are scant. Future research using animal models could identify mechanisms by which middle childhood nutrition affects brain development. In tandem, observational

epidemiologic research should work to isolate the effect of middle childhood nutrition on later behavior problems. Intervention studies of supplementation or fortification of commonly eaten foods would strengthen causal inference and could provide the basis for public health interventions at the local or national level.

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Appendix – Associations between Examined Exposures

The biomarker dietary exposures used across the three aims of this dissertation may be correlated. Although it is challenging to properly examine this overlap given that the study samples do not coincide, our objective was to present the associations between exposures where data was available.

Among children with plasma 25-hydroxy vitamin D and vitamin D binding protein measured, 8 and 5 children had ID and anemia, respectively. Among children with ID, 37.5% were VDD compared with 10.3% of children without ID. None of the anemic children were VDD. Among children with low vitamin B-12, 16.7% were VDD; 9.8% of children without low vitamin B-12 were VDD. However, there were no statistically significant associations between VDD or low DBP with ID, anemia, and low vitamin B-12 (**Table 1**).

Among children with PUFA concentration ascertained, 13, 14, and 25 children had ID, anemia, and VDD, respectively. ID and anemia were not associated with n-3 or n-6 PUFA concentrations or with the D6D or D5D enzyme activity indices (**Table 2**). Low vitamin B-12 was positively associated with GLA and DGLA concentrations as well as the D6D activity index and inversely associated with AdA concentrations and the D5D activity index. VDD was positively associated with ALA concentrations and inversely associated with AA concentrations and the D6D and D5D activity indices. DBP was not associated with PUFA concentrations or with the D6D or D5D activity indices (**Table 2**).

Given these associations, some of the results observed in aims 2 and 3 of this dissertation may be confounded by other nutritional exposures. For instance, the inverse association between

D5D and externalizing problems may be confounded by low vitamin B-12 or VDD, which were each positively associated with externalizing problems. If we had been able to control for low vitamin B-12 or VDD, the inverse association between D5D and externalizing problems would likely have been attenuated.

When nutritional exposures share a common food source or enzyme activity may be influenced by nutritional deficiencies, future studies should examine the joint distributions of nutritional status biomarkers to determine whether to control for other nutritional exposures.

Table 6.1. Associations between vitamin D deficiency and low vitamin D binding protein with iron deficiency, anemia, and low vitamin B-12 concentration in middle childhood

Nutritional characteristic	Vitamin D deficiency[1]		Low vitamin D binding protein[2]	
	n	%	*n*	%
Iron deficiency[3]				
Yes	8	37.5	8	62.5
No	213	10.3	213	48.8
P[4]		0.17		0.45
Anemia[5]				
Yes	5	0.0	5	60.0
No	217	11.5	217	49.3
P		0.54		0.64
Low vitamin B-12[6]				
Yes	54	16.7	54	42.6
No	164	9.8	164	52.4
P		0.22		0.21

Footnotes to Table 6.1

[1] Plasma 25(OH)D <50 nmol/L.

[2] Plasma vitamin D binding protein <2497 nmol/L.

[3] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[4] χ^2 score test statistic from Poisson regression with vitamin D deficiency or low vitamin D binding protein as the dichotomous outcome. Empirical estimates were used in all models. Fisher's exact test for anemia and vitamin D deficiency.

[5] Hemoglobin <12.7 g/dL.

[6] Plasma vitamin B-12 in quartile 1 (median boys: 204 pmol/L, girls: 218 pmol/L).

Table 6.2. Polyunsaturated fatty acid serum percentage weight concentration and enzyme activity indices according to categories of micronutrient status indicators among schoolchildren from Bogotá, Colombia

Nutritional characteristic	*n*	n-3 polyunsaturated fatty acid (PUFA)[1]				n-6 PUFA						Enzyme activity indices	
		18:3n-3 ALA	20:5n-3 EPA	22:5n-3 DPA	22:6n-3 DHA	18:2n-6 LA	18:3n-6 GLA	20:2n-6 EDA	20:3n-6 DGLA	20:4n-6 AA	22:4n-6 AdA	GLA/LA Δ6-desaturase	AA/DGLA Δ5-desaturase
Iron deficiency[2]													
Yes	13	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	2.4 ± 1.1	31.1 ± 2.9	0.3 ± 0.1	0.3 ± 0.1	1.6 ± 0.2	5.9 ± 1.0	3.3 ± 1.0	0.008 ± 0.003	3.8 ± 0.8
No	428	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	2.2 ± 0.9	30.6 ± 3.0	0.3 ± 0.2	0.3 ± 0.1	1.6 ± 0.4	6.0 ± 1.2	3.1 ± 1.2	0.01 ± 0.006	4.0 ± 1.5
P[3]		0.59	0.89	0.22	0.54	0.54	0.09	0.71	0.82	0.72	0.63	0.06	0.44
Anemia[4]													
Yes	14	0.5 ± 0.2	0.2 ± 0.2	0.5 ± 0.2	2.6 ± 1.2	31.2 ± 2.4	0.2 ± 0.1	0.3 ± 0.1	1.4 ± 0.4	6.4 ± 1.0	3.4 ± 1.1	0.008 ± 0.004	4.9 ± 2.1
No	430	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	2.2 ± 0.9	30.6 ± 3.0	0.3 ± 0.2	0.3 ± 0.1	1.6 ± 0.4	6.0 ± 1.2	3.1 ± 1.2	0.01 ± 0.006	4.0 ± 1.5
P		0.88	0.34	0.56	0.24	0.36	0.10	0.70	0.20	0.14	0.45	0.08	0.10
Low vitamin B-12[5]													
Yes	121	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	2.1 ± 0.9	30.3 ± 3.0	0.3 ± 0.2	0.3 ± 0.1	1.7 ± 0.4	6.0 ± 1.1	3.0 ± 1.1	0.01 ± 0.007	3.8 ± 1.1
No	310	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	2.3 ± 0.9	30.8 ± 3.0	0.3 ± 0.1	0.3 ± 0.1	1.6 ± 0.4	6.1 ± 1.2	3.2 ± 1.2	0.01 ± 0.006	4.1 ± 1.6
P		0.59	0.08	0.68	0.12	0.12	0.01	0.93	0.04	0.33	0.03	0.01	0.02
Vitamin D deficiency[6]													
Yes	25	0.6 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	2.4 ± 0.8	30.8 ± 3.2	0.3 ± 0.1	0.3 ± 0.1	1.6 ± 0.2	5.3 ± 0.9	3.0 ± 1.2	0.008 ± 0.004	3.5 ± 0.9
No	197	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	2.6 ± 1.1	30.2 ± 2.9	0.3 ± 0.2	0.3 ± 0.1	1.6 ± 0.4	5.9 ± 1.1	3.1 ± 1.0	0.01 ± 0.006	4.0 ± 1.8
P		0.03	0.43	0.58	0.25	0.34	0.09	0.70	0.61	0.01	0.69	0.06	0.03
Low vitamin D binding protein[7]													
Yes	110	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	2.7 ± 1.1	30.5 ± 2.9	0.3 ± 0.1	0.3 ± 0.1	1.6 ± 0.3	5.9 ± 1.2	3.1 ± 1.1	0.01 ± 0.005	4.0 ± 2.2
No	112	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	2.6 ± 0.9	29.9 ± 3.0	0.3 ± 0.2	0.3 ± 0.1	1.6 ± 0.4	5.9 ± 1.1	3.1 ± 0.9	0.01 ± 0.006	3.8 ± 1.1
P		0.91	0.22	0.76	0.64	0.12	0.39	0.12	0.56	0.85	0.66	0.24	0.54

Footnotes to Table 6.2

[1] Mean ± SD of serum fatty acid percentage weight concentration. Abbreviations are as follows: alpha-linolenic acid, ALA; eicosapentaenoic acid, EPA; docosapentaenoic acid, DPA; docosahexaenoic acid, DHA; linoleic acid, LA; gamma-linolenic acid, GLA; eicosadienoic acid, EDA; dihomo-gamma-linolenic acid, DGLA; arachidonic acid, AA; and adrenic acid, AdA.

[2] χ^2 score test statistic from linear regression with PUFA percentage weight concentration as the continuous outcome. Empirical estimates were used in all models.

[3] Plasma ferritin concentration <15 µg/L. 13 children with CRP >10 mg/L were excluded from the analysis.

[4] Hemoglobin <12.7 g/dL.

[5] Plasma vitamin B-12 in quartile 1 (median boys: 204 pmol/L, girls: 218 pmol/L).

[6] Plasma 25(OH)D <50 nmol/L.

[7] Plasma vitamin D binding protein <2497 nmol/L.