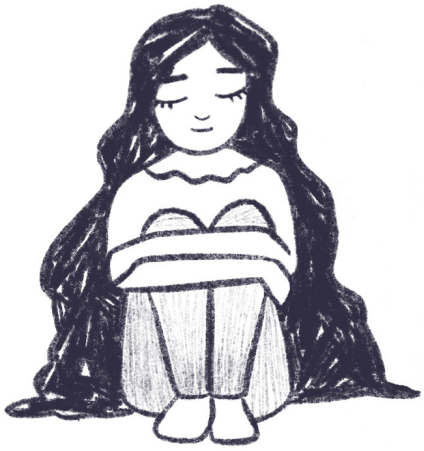
A time of change



Iron deficiency and depression among Mexican adolescent girls

Arli G Zarate Ortiz

Propositions

1. In Mexico, a plant-based diet is not synonymous with a healthy diet.
   (this thesis)

2. Iron deficiency and anemia threaten the mental health of Mexican adolescent girls.
   (this thesis)

3. Hyperspectral imagery in food recognition is not an environmentally sustainable alternative for food composition analysis.

4. The strategic decision of some journals not to publish findings from cross-sectional studies leads to the segregation of researchers with tight budgets or early careers.

5. The public health agenda in Mexico should include reducing interpersonal violence and recruitment of youth by organized crime.

6. Doing a Ph.D. is good therapy for individuals who like to be in control.

7. The university should cater its courses in accordance with its principles.

Propositions belonging to the thesis, entitled

A time of change: Iron Deficiency and Depression among Mexican Adolescent Girls.

Arli Guadalupe Zarate Ortiz
Wageningen, 16 May 2022

A time of change:

Iron Deficiency and Depression among Mexican Adolescent Girls

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A time of change:
Iron Deficiency and Depression among Mexican Adolescent Girls

Arli Guadalupe Zarate-Ortiz

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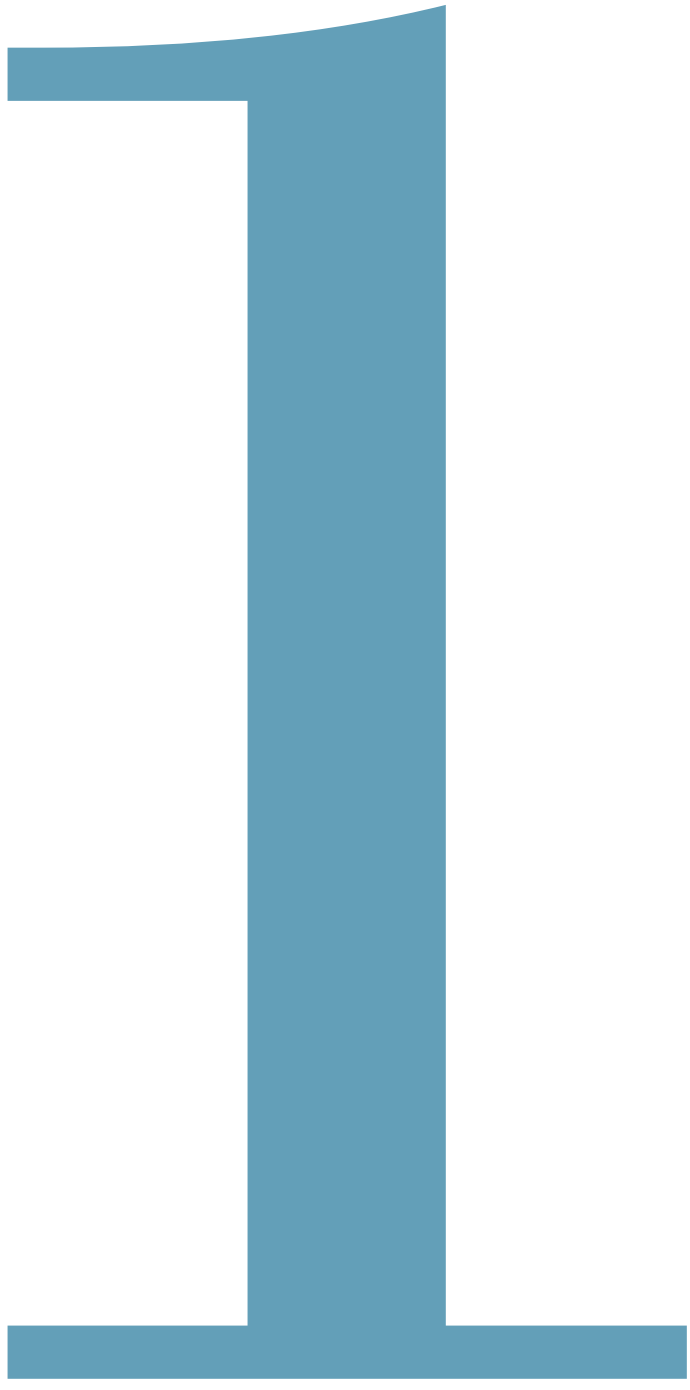

Chapter 1

General Introduction

Adolescents are the future workforce and bearers of the next generation. Therefore, improving their health and development through nutritional, social, and economic pathways is a crucial opportunity to shape the health and wellbeing of this generation and the next. With this motivation, we started the preparations for the Ten2Twenty project in 2015. The overall Ten2Twenty project was conducted in a range of low- and middle-income countries, including Mexico, and aimed to improve understanding of the interactions and interrelationships between nutritional, sociological, and economic trajectories of optimizing adolescent nutrition. This chapter provides an overview of adolescents' nutrition and health globally and in Mexico specifically. The chapter ends with the rationale and aims of this thesis.

Adolescence: the transition from childhood to adulthood.

Adolescence is the second intensive developmental period during the life-course, after childhood and is defined by the World Health Organization (WHO) as the period in life between 10 to 19 years old [1]. The term comes from the Latin verb “adolescere“, which means “growing up”. During this stage, numerous biological and psychosocial changes prompt the transition from childhood to adult life. In addition, adolescence is a period of hormonal changes; the production of adrenal androgens increases, the growth hormone, the insulin--like growth factor and thyroid axes mature, and the regulation of oxytocin and vasopressin changes [2].

Hormonal changes during the adolescence period also lead to dramatic changes in body composition, such as increased bone and muscle mass, expansion of blood volume, and increase in body weight and height [3]. During adolescence, 50% of the adult body weight, and 15-25% of final height are gained [3,4]. In girls, final height is achieved usually one year after menarche. Adolescence is a period of rapid growth; consequently, the energy and nutrient requirements increase [3]. In addition, dietary patterns, physical activity, and eating behavior during adolescence are heavily influenced by internal factors (such as attitudes, beliefs, perceived barriers, food preferences, self-efficacy, and biological change), external factors (family, friends, fast food outlets, and social and cultural norms), and macrosystems (such as food availability, food production, distribution systems, mass media, and advertising) [3]. Thus, adolescents are nutritionally vulnerable because of the increased nutritional demands alongside the social adaptation to adulthood.

Simultaneous to rapid physical growth, large adjustments in the brain occur during the transition from childhood to adulthood. The human brain undergoes a developmental pattern of extremely rapid growth in infancy, slower sustained growth in childhood, and a final burst of growth and reorganization during the second decade of life initiated by puberty [5]. The structural reorganization of the brain during adolescence is called brain remodeling. Brain remodeling includes increased myelination and decreased grey matter volume in cortical areas, synaptic elaboration and subsequent pruning in the striatum and prefrontal cortex, cell death in the primary visual cortex, and changes in connectivity in the amygdala and prefrontal cortex [6]. This brain remodeling links to changes in cognitive, affective, and social processing. However more research is needed in order to understand the influence of nutrition and other lifestyle factors on the remodeling of the adolescent brain.

Puberty: the alarm bell for maturation

The onset of puberty is considered the starting point of adolescence [2]. Puberty is a highly programmed and biologically driven process that triggers sexual maturation and affects behavior, emotional wellbeing, and health in a complex way [7]. Sexual maturation comprises two distinct but overlapping processes, adrenarche, and gonadarche. Adrenarche starts around the age of 6-8 years and is marked by the dramatic rise of adrenal steroid hormones [8,9]. The physical manifestation of adrenarche includes skeletal maturation, increased skin oil, pubic hair growth, and adult apocrine odor [9]. Gonadarche follows adrenarche approximately 1-2 years later and is a gradual and lengthy process that starts with the pulsatile nocturnal release of gonadotropin-releasing hormone (GnRH), which leads to the pituitary release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [7]. The resulting gonadal growth and production of gonadal sex steroids are responsible for the development of secondary sexual characteristics [8,9]. The changes associated with gonadarche include an increase in height velocity, thelarche and menarche in girls, and testicle growth in boys [9].

The onset of puberty varies 4 to 5 years between individuals and is influenced by neurotransmitters and neuropeptides, ethnicity, and other environmental factors. Approximately 70-80% of the variance in pubertal timing can be explained by heritable factors (genetic component), and the rest by environmental components like nutritional status, socioeconomic conditions, light exposure, climate, and other stressors such as chronic illness, high performance sports and psychological stress [9,10].

Secular trends to decreasing ages at menarche (AAM) were first detected among European populations in the early nineteenth century. Decreasing ages at menarche have been widely observed, but the timing and pace have varied among populations. Decreases have proceeded more swiftly where they have occurred more recently, and appear to plateau around a median age of 12.5–13.5 years. A widespread acceleration in the onset of breast development (thelarche) contributes to perceptions that age at puberty continues to decrease, but it appears to be dissociated from the hypothalamic-pituitary-gonadal (HPG) axis activation and menarche, and may respond to an environmental disruptor [5,11]. In the United States, early pubertal timing is more common in African American and Latino girls, youth from low socioeconomic classes, and youth with history of life adversities [9]. The precise pathways by which hereditable and environmental factors influences pubertal timing remain unclear.

Adolescent health and nutrition worldwide at a glance

With a worldwide population of 1.2 billion, adolescents represent the largest cohort of young people in the history. Globally, around 1 million adolescents died in 2015 from HIV, road injuries, interpersonal violence, and self-harm as the leading causes of death [10]. Despite being the largest population group in the world, adolescents have been neglected from the public health agenda [12]. There is a clear need to address sexual, reproductive and mental health, substance use and violence. However, addressing nutrition is equally important, since malnutrition is one of the leading contributing factors to the global burden of disease [10,13].

Stunting

Stunting in adolescents reflects an inadequacy in the environment (such as suboptimal nutrition, inadequate care, repeated infections, and environmental stress) to which children have been exposed [10]. There is limited data regarding stunting prevalence in adolescents, but the prevalence of stunting in adolescent girls may range from 6% (in Brazil) to 52% (in Guatemala) [10]. In 2019, the mean height of 19-year-old adolescents differed approximately 20 cm between countries with the tallest population (mean height 183.8 cm for boys and 170.4 cm for girls) in The Netherlands, and the shortest boys living in Timor-Leste (160.1 cm), and girls (150.9 cm) in Guatemala. For example, 19-year old boys in Asia, Latin America, and sub-Saharan Africa have the same mean height as their Dutch peers aged 13 years. In some Middle Eastern and Latin American countries, children had an optimal height at 5 years of age, but by

the time they reached 19 years of age, their height was lower than the median of the WHO standards [14].

Underweight and overweight

The global prevalence of underweight among children and adolescents has remained stable in recent decades. In 2016, 8.4% of girls and 12.4% of boys presented with underweight [10]. Obesity, on the other hand, has increased in all regions of the world; one in every four adolescents are overweight or obese in most of the countries in the Eastern Mediterranean, Western Pacific, and the American regions [10]. In 2019, the difference between the highest and lowest mean BMI in 19-year-olds was approximately 9-10 kg/m^2, which is equivalent to ~25 kg of weight. The unhealthiest changes in anthropometric status (lowest gains in height and highest gain in weight) occurred in sub-Saharan Africa, the United States of America, Malaysia (for boys), and Mexico (for girls) [14].

Iron deficiency and anemia

Micronutrient deficiencies are a leading, underlying risk factor contributing to the global burden of disease. Iron deficiency and iron-deficiency anemia account for the majority of disability-adjusted life years (DALYs) associated with micronutrient deficiencies [15]. In 2016, the prevalence of anemia in girls aged 10-14 years in LMICs ranged from 20-30%, whereas in the upper-middle- and high-income countries, the prevalence was below 10%. [13]. Iron deficiency and iron-deficiency anemia contribute more than 2500 DALYs per 100,000 adolescent girls [16].

Anemia is commonly a result of iron and other micronutrient deficiencies and is, therefore, a useful indicator of nutrient status. In younger adolescents, anemia is the leading cause of disease burden [13]. Consistent with rapid growth and the onset of menstruation, the prevalence of anemia rises for adolescent girls during puberty and then stabilizes throughout the remaining reproductive years [15].

Non-communicable diseases (NCDs)

Several non-communicable diseases already arise during adolescence. In 2017, approximately 20-30% of young people lived with a chronic illness, particularly diabetes mellitus type 1 and 2 [16]. The burden of diabetes mellitus is higher among adolescents in the Middle East, North- and Latin- America, and the Caribbean region [10]. Along with the growing prevalence of obesity worldwide, the prevalence of metabolic syndrome (MetS) has increased in adolescents.

In American adolescents for example MetS prevalence ranges from 4.2% to 8.4%, depending on the MetS classification criteria used [16]. Several lifestyle habits (i.e., smoking, sedentary lifestyle) associated with NCDs are developed during adolescence. Therefore, NCDs can be prevented if appropriate interventions are provided as early as possible during adolescence.

Depression

Depression is characterized by low mood or loss of interest and often accompanied by feelings of guilt or low self-worth, tiredness, poor concentration, hopelessness, and changes in appetite and sleep. Depression significantly impairs the ability to function and to cope with daily life [17]. After pubertal onset, prevalence of depression in girls is approximately twice that of boys [18,19]. Pubertal development may be the mechanism that underlies the gender difference in rates of depression. Clinical and epidemiological evidence suggests that fluctuations in hormonal concentrations, particularly in estrogens, may influence the regulation of the hypothalamic pituitary adrenal (HPA) axis and this may alter the neurotransmitter systems [20]. Depression in adolescents can impair academic performance and the attainment of important development milestones, such as healthy autonomy and independence [19]. Depression is also a major risk factor for suicide. Globally, suicide ranks number three among causes of death in adolescents [1]. Depression in adolescence predicts depression and anxiety in adulthood, and most depressive episodes occur for the first time during this stage of life [19].

Zooming in on adolescent nutrition and health in Mexico

In the second trimester of 2021, the Mexican population consisted of around 22.7 million persons between 10 to 19 years old, which makes up 17.8 % of the total population in Mexico [21]. At the time of initiation of this study, information on the pubertal development, nutritional status, and mental health of Mexican adolescents was limited. Since then, more knowledge has been generated. Here, we will review the most updated information.

AAM is the most common indicator of pubertal timing in women. In Mexico, AAM has declined from 13.3 years in women born before the 1940s to 12.5 years in those born in the 1980s. Women in urban areas experienced AAM 3.7 months earlier than their counterparts living in rural areas. The differences in AAM among socioeconomic classes, ethnicity, and living area narrowed in the cohort of women born in the 1980s [22]. According to a survey

conducted in Mexico City and Xalapa at public schools, the mean AAM in females born in the 1990s was 11.4 years and 11.3 years, respectively [23].

Mexico faces the double burden of malnutrition characterized by the coexistence of nutrient deficiencies and non-communicable diseases, and adolescents are not an exception. In 2012, the combined prevalence of overweight and obesity was 35.8% and 34.1% for female and male adolescents, respectively, with a higher prevalence in adolescents living in urban areas and in the highest quintile of the household living condition index [24]. From 1988 to 2012, the combined prevalence of overweight and obesity in adolescent girls increased with 24.7%, with the most dramatic increase occurring between 1988 and 1999 [24]. In recent years, the increase in overweight and obesity has been more stable. In 2018, the combined prevalence of overweight and obesity in adolescents reached 37.8% and 35.8% for girls and boys, respectively [25]. Alongside, the prevalence of anemia in 2006 was 11.5%, with no differences by sex [26]. By 2016, anemia prevalence had decreased to 9.6%, but it was higher for adolescent girls (12.0%) than for boys (7.3%) [27]. The most recent information on iron deficiency in adolescents comes from the ENSANUT 2006, which indicates that iron deficiency occurred in 13.4% and 9.3% of adolescent girls and boys, respectively [26].

Regarding dietary habits, data from ENSANUT 2018-19 shows that adolescents consumed less than 90 g of fruit and 50 g of vegetables per day which is only one third of the recommended. In contrast, more than 50% of the adolescents consumed > 35 g of snacks, candies and desserts, and > 485 g of sweetened beverages per day [28]. In addition, data from ENSANUT 2016 shows that the highest prevalence of inadequate nutrient intake was observed for calcium, vitamin A, magnesium, vitamin B12, folate, vitamin C, riboflavin and thiamine, whereas total dietary iron intake exceeded the requirement (136%), although most consisted of non-heme iron [29]. Inevitably, the link between micronutrients and adolescent development is strong. Micronutrient deficiencies during adolescence can compromise growth, decrease cognitive function and depress immune function [12].

Risky eating behaviors (REB) are behaviors and attitudes associated with the desire to achieve or maintain a thin body, and are associated with the subsequent onset of eating disorders From 2006 to 2018, REB have increased among adolescent girls living in rural and urban areas and in boys across all BMI classifications. However, the increment is more evident in female adolescents from high socioeconomic classes living in urban areas. Normative REB (concern about gaining weight, exercising excessively, and dieting to lose weight) increased from 11.9%

to 15.7%, and non-normative REB (binge eating, induced vomiting, use of diuretics, etc.) increased from 12.1% to 21.9% [30]. Thus, REB represents a threat for the physical and mental health of adolescents.

In a survey among Mexican high school students in 2007, depressive symptoms were reported by 34% and 18% of female and male students, respectively [31]. In addition, results from ENSANUT 2018-19 show that 6.1% of Mexican adolescents suffered from depressive symptoms. The inconsistencies in the prevalence of depressive symptoms might be explained by the difference in sampling design and the tool used to evaluate depressive symptoms. The presence of depressive symptoms increased the probability of suicidal ideation 6.0 times and the probability of suicidal attempt 6.5 times [32]. In 2017, suicide was the second cause of death among Mexicans aged 15-29 years [33]. Although depression in adolescents is a major public health concern in Mexico, the available data on contributing factors for depression at the national level is scarce.

Problem statement: iron deficiency and depression

In summary, iron deficiency and anemia remain as persistent nutritional problems in Mexican adolescents. The most recent national surveys focus on overweight and obesity and neglect micronutrient deficiencies. In addition, from previous research, we know that obese women and children have a higher risk for iron deficiency than normal-weight individuals with similar dietary iron intake [34]. Previous studies have also reported an association between iron deficiency and depressive symptoms in children and adolescents [41].

Mental health is also a relevant topic for adolescent health and nutrition. Suicide was the second cause of death among young Mexicans (15-29 years), and depression is one of the most common causes of self-harm behavior and suicide. Nonetheless, there is limited information on the prevalence and determinants of depression among adolescents in Mexico, and the potential link between iron deficiency, anemia and depression is not completely elucidated

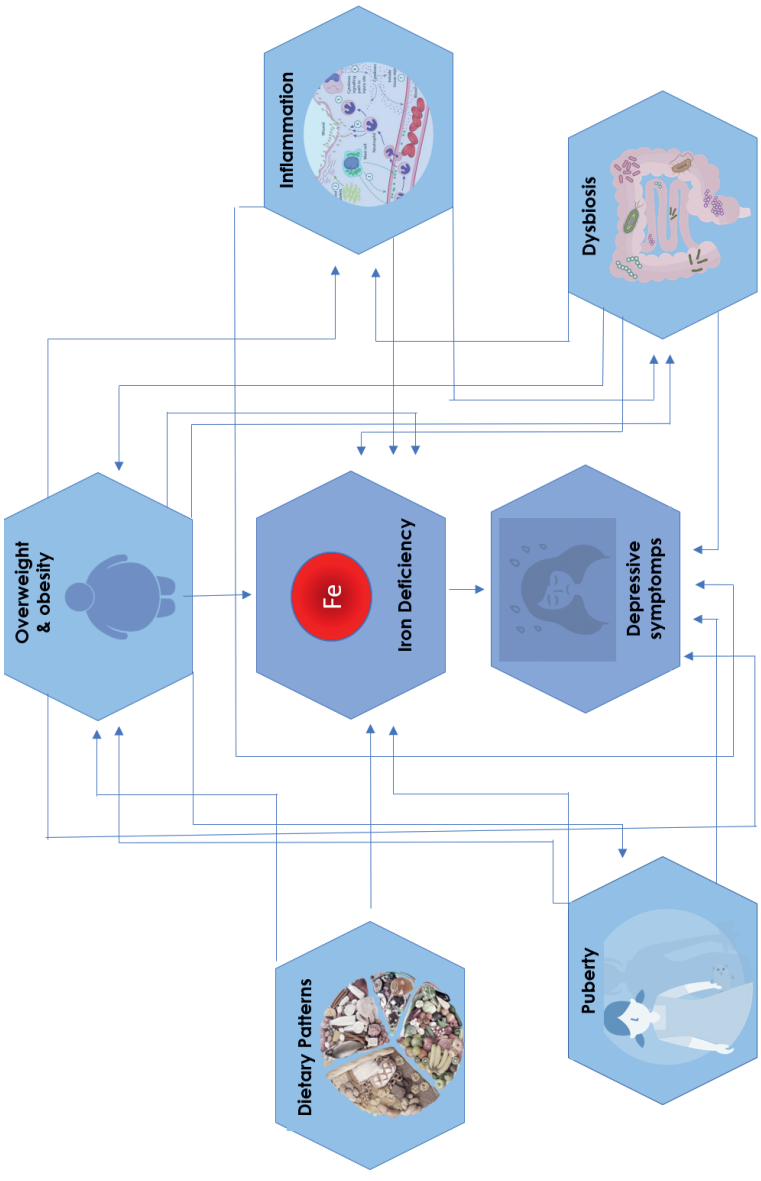

Figure 1.1. Factors associated to iron deficiency and depressive symptoms.

Iron homeostasis

Iron requirements increase during adolescence to support growth, and for adolescent girls also to replace iron lost during menstruation [3,35]. Iron is an important component of hemoglobin in red cells and myoglobin. It is also needed for various cellular mechanisms, including enzymatic processes, DNA synthesis, mitochondrial energy generation, and brain remodeling during adolescence [36,37]. Iron losses occur daily due to menstruation or other forms of bleeding, sweating, skin desquamation, and urinary excretion. However, iron does not have a regulatory pathway for excretion, and therefore dietary intake, intestinal absorption, and iron recycling have to be finely regulated [36,38].

Iron absorption and distribution are closely regulated, both locally in the intestine and systemically through hormonal signals. Dietary iron reaching the digestive tract is mostly absorbed in the duodenum after reduction from the ferric state (Fe3+) to the ferrous state (Fe2+) by the enzyme duodenal cytochrome b. Ferroportin transfers iron through the basolateral membrane of enterocytes to meet the peripheral needs and simultaneously oxidizes it to generate ferric iron Fe3+. In the bloodstream, Fe3+ is captured and distributed to all cells in the body by transferrin via transferrin receptor 1 (TfR1) [38].

The peptide hepcidin regulates systemic iron homeostasis. Hepcidin is mainly secreted by hepatocytes and secondarily by other cells and organs, such as macrophages, adipocytes, dendritic cells, the heart, and the kidney [39–41]. Hepcidin binds to and induces the degradation of ferroportin, thereby reducing iron transport. Iron concentrations in plasma regulate hepcidin production, so more hepcidin is produced when iron is abundant, limiting iron absorption [38,42]. In addition to iron concentrations, hepcidin is regulated by the erythropoietic requirement for iron. During active erythropoiesis, hepcidin is suppressed, making iron available for hemoglobin synthesis. The expression of hepcidin is also upregulated by inflammation through IL-6 and high-performance physical activity, and downregulated by hypoxia and endocrine signals like testosterone, estrogen, and growth factors [38,43].

The link between inflammation and iron deficiency

Iron deficiency adversely affects growth and immune function and may cause anemia. This deficiency can occur in two forms. Absolute iron deficiency arises when iron stores are low or exhausted; functional iron deficiency is a disorder in which total body iron stores are normal or increased, but the iron supply to the bone marrow is insufficient. This form of iron deficiency

can be present in chronic inflammatory states, and the hormone hepcidin has a key role. Both forms of iron deficiency can coexist in the same person [38].

Inflammatory state, characterized by the acute-phase response, can directly affect the concentration of most biomarkers of iron status. Both acute inflammation (due to infection or injury) and chronic inflammation (due to metabolic disturbances) can affect iron trafficking, in part through their effect on the regulation and synthesis of hepatic acute-phase proteins, such as ferritin [44].

Individuals with overweight or obesity are at higher risk of functional and absolute iron deficiency than individuals with normal weight. Potential explanations for this increased risk include dilutional hypoferremia, poor dietary iron intake, increased iron requirements, and/or impaired iron absorption. Recent evidence suggests that obesity-induced inflammation may play a central role through regulation of hepcidin. Hepcidin levels are higher in obese individuals and are related to subclinical inflammation; this may reduce iron absorption and, for example, blunt the effects of iron supplementation/fortification [45]. Thus, low iron status in overweight individuals may result from a combination of nutritional (reduced absorption) and functional iron deficiency [45,46].

Previous research with stable-isotope labeled wheat-based test meals with and without ascorbic acid shows that iron absorption and the enhancing effect of ascorbic acid on iron absorption was lower in women with obesity than in women with normal weight [47]. A prospective study showed a decrease in body fat, IL-6 and hepcidin concentrations six months after bariatric surgery in iron-deficient and non-iron deficient participants, and an increase of 28% in iron absorption in the group with iron deficiency [48].

The link between inflammation and depression

Major Depressive Disorder (MDD) is a heterogeneous disease that can be subdivided into melancholic and atypical depression, based on distinct symptomatic profiles. Melancholic depression is characterized by anhedonia without mood reactivity, psychomotor disturbances, and vegetative symptoms, while atypical depression shows mood reactivity. Many factors play a role in the development of MDD, and recent evidence points towards a dysregulated immune system and an increased inflammatory response, although this does not seem to be true for all subtypes of MDD [49,50]. For instance, higher concentrations of cytokines and higher BMI have been observed in patients with atypical depression as compared to patients with melancholic depression. On the other hand, patients with melancholic depression show a

hyperactive hypothalamic–pituitary–adrenal (HPA) axis and hypercortisolism with subsequent vegetative symptoms like changes in sleep and appetite patterns [50].

A meta-analysis found elevated concentrations of C-reactive protein (CRP), interleukin-1 (IL-1) and interleukin-6 (IL-6) in a cohort of adults with depression [51]. Also, significantly higher concentrations of tumor necrosis factor-α (TNF-α) and IL-6 have been found in depressed patients compared to controls [49]. From this, it may be concluded that certain cytokines play a role in the etiology of MDD. Cytokines operate through activation of their receptors expressed in different regions of the central nervous system (CNS). Additionally, cytokines can activate microglial cells, which can produce pro-inflammatory factors impacting neurotransmitter systems. Activated microglial cells interfere with the synthesis of serotonin and dopamine [50].

The gut microbiota and depression

The gut microbiota has an essential role in human health and nutrition. Disruption of the gut microbiota homeostasis is known as dysbiosis, which has been associated with many health complications [52]. Lower microbial diversity or an increase in the proportion of harmful bacteria is associated with the appearance of diseases, indicating that an intestinal ecosystem with high richness and diversity of bacteria is more robust against environmental influences [53,54]. Consequently, diversity seems to be a good indicator of a "healthy gut". When there is dysbiosis, the intestinal barrier is more exposed to the attack of harmful bacteria, viruses, or other pathogens. In response to this, the intestinal permeability increases, causing the so called "leaky gut". A dysfunctional intestinal barrier can allow undesirable particles to translocate, and subsequently induce a systemic inflammatory response [55].

In the last decade, clinical research has focused on the biochemical signaling between the gastrointestinal (GI) tract and the CNS, referred to as the "gut-brain axis". This bidirectional communication network includes endocrine, neural, immune, and metabolic pathways, supported by several mediators, including cytokines, short-chain fatty acids (SCFA), and neurotransmitters [56,57]. Recent literature indicates that gut microbes may influence the neurological features of depression, like the activation of a low-grade inflammatory state by the hypothalamic-pituitary-adrenocortical (HPA) axis, and the production of GABA, serotonin, or tryptophan, among others [58,59]. The HPA axis coordinates the adaptive stress response in the body. Dysregulation of this axis increases the production of cortisol and inflammatory mediators, boosting a sustained pro-inflammatory state [60,61]. Excessive levels of circulating cortisol and inflammatory mediators increase intestinal permeability, thus, allowing gram-

negative bacteria to translocate into the bloodstream, which may induce chronic inflammation of the CNS [60,62]. Hence, evidence shows that the gut microbiota plays an important role in the physiological pathways that may contribute to changes in the brain and that it can be involved in the regulations of emotions, behaviors, and higher cognitive functions through the gut-brain axis [58,63,64]. This potential link between the gut microbiota and depression suggests a potential target for novel antidepressant treatments.

Aim of this study

This thesis aims to investigate the interrelationships between nutritional exposure and health outcomes among adolescent girls in Mexico, with specific focus on iron deficiency and depression. Therefore, the following specific objectives have been addressed:

1. To assess whether a time trend in declining age at menarche is associated with chronic disease risk in Mexican women.
2. To describe the dietary patterns (DPs) of Mexican adolescents and to examine their association with nutritional status, including anemia, using data from adolescents aged 12–19 years.
3. To examine the association between iron status and depressive symptoms in Mexican adolescent girls and to explore whether depressive symptoms are associated with hemoglobin concentration, body weight and pubertal onset.
4. To investigate the relationship between intestinal microbiota composition and the presence of depressive symptoms in Mexican adolescent girls.
5. To compare the commonly used and the alternative methods to assess inflammation-adjusted iron status, and to identify the best approach to determine iron deficiency in Mexican adolescent girls in a setting with a mild burden of inflammation.

Thesis outline

This thesis is divided into two parts. Part I, chapters 2 and 3 consist of secondary data analysis that contributes to the literature on pubertal onset and adolescent nutrition in Mexico; since we used nationally representative data, the conclusions can be translated to the entire Mexican population. **Chapter 2** investigates the time trends in age at menarche during the 20th century in Mexico and tests the association between age at menarche and the risk of non-communicable diseases in adult life, using data from the Mexican National Health Survey (ENSA-2000).

Chapter 3 explores the existing dietary patterns among Mexican adolescents and examines their association with nutritional status. In this chapter we used data from the National Survey of Health and Nutrition (ENSANUT-2006).

Part II, chapters 4-6, results from a cross-sectional study conducted in Santa Catarina and Monterrey, Northern Mexico, from September 2018 to January 2019. **Chapter 4** studies the subtypes of depressive symptoms and their relation with iron status, body weight, and pubertal onset. **Chapter 5** investigates if gut microbiota composition in girls with depressive symptoms differs from that of girls without depressive symptoms. **Chapter 6** examines the existing methods to adjust biomarkers of iron status for inflammation and proposes an alternative method to account for inflammation when assessing iron status. Finally, **Chapter 7** summarizes the main findings and integrates them to fill in some of the research gaps mentioned in this introductory chapter.

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Part I
Secondary Analysis

Chapter 2

Time Trends in Age at Menarche and Related Non-Communicable Disease Risk during the 20th Century in Mexico

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Abstract

Developed countries have shown a time trend towards a younger age at menarche (AAM), which is associated with increased risk of later obesity and non-communicable diseases. This study aimed to assess whether a time trend in AAM is associated with disease risk in Mexican women (n = 30,628), using data from the Mexican National Health Survey (ENSA-2000). Linear and log binomial regression was used for nutritional and disease outcomes, while Welch-ANOVA was used to test for a time trend. AAM (in years) decreased over time ($p < 0.001$), with a maximal difference of 0.99 years between the 1920s (13.6 years) and 1980s (12.6 years). AAM was negatively associated with weight (β = -1.01 kg; 95% CI -1.006, -1.004) and body mass index (BMI) (β = -1.01 kg/m²; -1.007, -1.006), and positively with height (β = 0.18 cm; 0.112, 0.231). AAM was associated with diabetes (RR = 0.95; 0.93, 0.98) and hypercholesterolemia (RR = 0.93; 0.90, 0.95), but not with hypertension, breast cancer or arthritis. In Mexico, AAM decreased significantly during the 20th century. AAM was inversely associated with adult weight and BMI, and positively with height. Women with a later AAM had a lower risk of diabetes and hypercholesterolemia.

Introduction

It is well known that infancy is a critical period for obesity development, but a growing body of evidence also suggests a relevant role of adolescence due to changes in the amount and distribution of body fat [1–4]. Moreover, age at menarche (AAM) is a substantial and important period in the health of adolescent girls and has a significant impact on their health in the upcoming years.

AAM is dependent on several intrinsic and extrinsic factors. Low body weight may delay the onset of menstruation [5], while overweight and obesity are closely linked to an earlier pubertal onset and a younger AAM [6,7]. Extrinsic factors such as living conditions, nutrition, overall health, physical activity, and socioeconomic status are important and modifiable aspects in the timing of menarche [8]. As a result, mean AAM differs between developing and developed countries. In developed countries, AAM is low (ranging from 12 years in Greece to 13.5 years in Ireland [8]) and has been constantly decreasing in the last 100 years [9]. In developing and emerging countries, much higher AAMs can be seen (ranging from 12.3 years in Thailand to 16.1 years in Senegal [8]). Here, the trend towards a younger AAM has only become visible in recent decades and at a slower rate [10–12].

Among the intrinsic factors that influence sexual maturation, the hormonal signals of the adipose tissue (leptin), pancreas (insulin), and gastrointestinal tract (ghrelin) play an important role in the transmission of metabolic information to the central nervous system to stimulate or delay the onset of puberty [5,13,14]. In line with a recent publication by Reinehr et al., this link between metabolic signals and pubertal onset suggests that pubertal timing may have influence on metabolic health in adult life [4].

Studies suggest a link between AAM and adult weight, body mass index (BMI), and height [15]. A meta-analysis of 48 studies found an inverse association between AAM under the age of 12 years and BMI [16]. Similar results were found in both the Nurses' Health Study I and II (NSH I and NSHII) [17]. In addition, early AAM was found to be associated with a decreased adult height [15,18], suggesting that AAM is not only influencing weight gain but also linear growth. This is important considering that low height is related to the development of respiratory diseases, lung growth, and respiratory obstruction [18–20] as well as non-communicable diseases, including cardiovascular diseases and diabetes [21–23]. Moreover, early AAM has also been linked with an increased risk of the development of chronic diseases,

such as rheumatoid arthritis and cancer, particularly breast cancer [24,25]. A meta-analysis of 9 studies reported a 3% lower risk of cardiovascular (CV) related death with each one-year delay in menarche [26]. Additionally, several studies have shown an inverse correlation between age at menarche and risk of diabetes and pre-diabetes [17,27,28]. However, smaller sample size studies have failed to reproduce these findings [29]. Further, AAM is suggested to be linked to increased blood triglyceride concentrations [30].

In Mexico, similar to many other countries, a secular trend towards a younger AAM has been observed. Studies conducted in the cities of Xalapa and Mexico City [31] and a rural region of southern Mexico [32] showed a significant decline in the mean AAM in recent decades. To the best of our knowledge, a nationwide research assessing the trend in AAM and its implications for women's health in Mexico has not been performed. Therefore, in this study, we assessed the presence of a nationwide secular trend of age at menarche from 1902 until 1980. Moreover, we examined possible associations between AAM and nutrition and health outcomes later in life among Mexican women aged between 20 and 98 years.

Materials and Methods

Design and Study Population

We conducted a secondary cross-sectional study based on data obtained during the Mexican

National Health Survey (Encuesta Nacional de Salud-ENSA). Data collection took place from September 1999 until March 2000 (details can be consulted elsewhere) [33]. The number of households was allocated proportionally per urban and rural area. Per state, 14 municipalities were selected with a proportional number of households [33]. The initial sample consisted of 30,628 women aged >20 years having the required information. Menarche at less than seven years of age or above 19 years was considered to have a pathological reason; therefore, we treated them as missing values [8]. All disease outcomes were checked on plausibility, and any cases exceeding the chosen cut-off points were handled as missing. The cut-off points for the variables weight, height, and BMI were based on an study carried out on the same data by Hernández-Cordero et al. [34], blood pressure measurements were regarded as implausible when being classified as high as a hypertensive crisis, and a cut-off of >5 standard deviations was chosen for blood glucose measurements. After eliminating implausible anthropometric,

AAM, and disease outcome data, the final study population comprised 19,215 women. An overview of the complete data cleaning process can be found in Figure 1. The survey protocol was approved by the Ethics Commission of the National Institute of Public Health (INSP by its Spanish acronym), and informed consent was obtained from each participant [33].

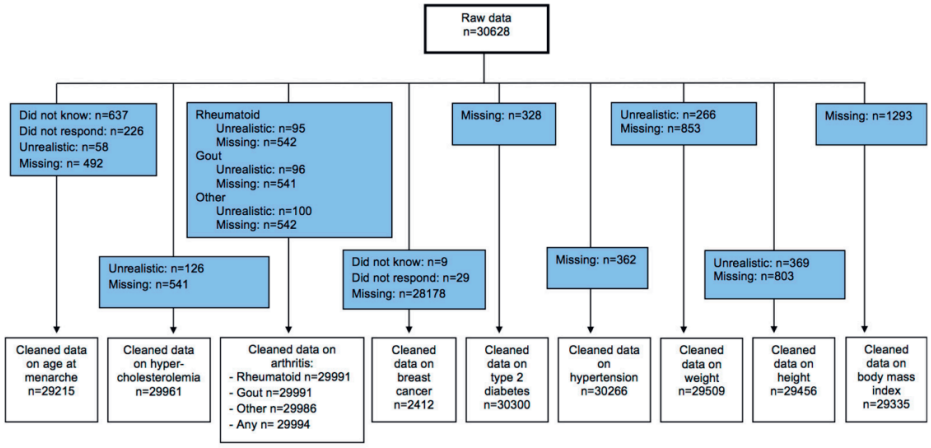

Figure 2.1. Overview of data cleaning

Data Collection and Variable Construction

Interviews on current health status took place at home and were led by trained professionals with a background in nursing. Information collected included questions on the prevalence of diabetes, hypertension, arthritis, and breast cancer, as well as on family history of diabetes and hypertension. On average, each house was visited three times to achieve the required quality and response rate. Next to the questionnaire, weight, height, and blood pressure were measured. Both capillary and venous blood samples (≈19 mL) were taken by trained staff. All blood samples collected to assess blood glucose levels were concentrated and centrifuged in the field laboratory before being sent back to the INSP laboratory [33].

The health needs addressed in ENSA 2000 included variables on health care usage, perceived health, and measurements on non-communicable diseases [33]. The nutritional status of the participants is reflected in the variables height (cm) [18], weight (kg), and BMI (kg/m2) [16]. Weight was assessed with a solar scale, calibrated with 10 kg tare, and height was measured by a flexometer and a squad. We selected these variables as relevant outcome variables of timing of menarche based on data availability and literature search. Next to that, occurrence of

hypertension [35], diabetes [28], arthritis [25], breast cancer [36], and hypercholesterolemia [35] were considered to be related to timing of menarche and were thus selected for analysis.

The age, sex, area of residence, and socioeconomic status (SES) of the sample women were obtained by means of a household questionnaire. Area of residence was defined as rural for localities with <2500 inhabitants and urban for localities with ≥2500 inhabitants. An SES index was constructed based on housing conditions (flooring and roofing materials); ownership of home appliances (refrigerator, stove, washing machine, television, radio, video player, telephone, and computer); and the number of rooms in the house.

Presence of hypertension was assessed by questionnaire and blood pressure measurements and was defined as systolic blood pressure (sBP >140 mmHg) or diastolic blood pressure (dBP >90 mmHg). The instrument that was used to measure blood pressure was a mercury column sphygmomanometer, model TXJ-10. A subject was considered as diabetic if diagnosed by a doctor or if random blood glucose levels exceeded 200 mg/dL. In order to present descriptive data for different groups of AAM, three categories were formed. Based on the data distribution and in line with literature studied on timing of menarche [16,26,28], "early" menarche was defined as reaching first menstruation before the age of 12 years, while ages of 12–14 years were considered as "normal" and first menstruation after the age of 14 years was defined as "late".

Missing data were not considered as missing completely at random. Thus, five iterations of multiple imputations were performed for the variables AAM, age, weight, and height (to calculate BMI), and family history of diseases. The main independent variables and all dependent variables of the two study questions were treated as predictors in the imputation model. Imputed variables were only chosen for when used as predictors, not as outcomes.

Statistical Analysis

Prior to analysis, we cleaned the dataset by removing outliers. To describe our analytic sample, we stratified women by AAM (early, normal, late). For all continuous outcomes, we calculated means and standard deviations. We present binary outcomes as frequencies and percentages. We considered a p-value of <0.05 to be statistically significant. We used multiple linear regressions to analyze the relationship of the nutritional indicators weight, height, BMI, and AAM. The variables of weight and BMI violated the assumption of normality of residuals, and we consequently log transformed them for further analysis. As we expected to find a decrease of AAM in younger individuals, we adjusted the crude model for current age. We used linear

regression models to analyze the association of nutritional status and AAM. The crude model was stepwise adjusted for the confounding variables age, BMI and, if possible, for family history of the disease. As most disease outcomes were expected to violate the rare disease assumption, they were analyzed using log binomial regression with complementary log-log link, in order to obtain relative risks (RR). The models used for log binomial regression included only those variables that showed to add to the crude model significantly. Normality of AAM per decade of birth group was visually assessed and all distributions were considered as normal. To study whether AAM differed between the decade groups, we used Welch–ANOVA, because the data failed the assumption of equal variances. The Games–Howell post-Hoc test [37] was performed to further investigate which decade groups differed in statistically significant manner. We performed all of the analyses using IBM SPSS Statistics 23 software (2015. Armonk, NY, USA: IBM Corp).

Results

Sample Characteristics

Mean AAM of the total study population was 13 years. A total of 4073 women (14 %) were classified as having an "early" AAM, 70% had a "normal" AAM, and 16% were considered "late". The "early AAM" group was characterized by having the lowest mean current age (37 years), while having the highest BMI (28.8 kg/m2) and weight (67.6 kg). The "late AAM" group had the highest mean age (45 years) and the lowest weight (64.2 kg) and BMI (27.3 kg/m2). The "late AAM" group had the highest percentage of diseased women for each disease outcome, except for hypercholesterolemia and breast cancer. Hypercholesterolemia prevalence was highest (10%) amongst the "early AAM" group, while breast cancer prevalence was highest in the "early AAM" and "normal AAM" group (4%; **Table 2.1**).

Table 2.1. Descriptive characteristics of study population per age at menarche group.

	Age at Menarche		
	Early	**Normal**	**Late**
	(<12 years)	**(12-14 years)**	**(>14 years)**
Observed *n*	4328	21333	5055
Observed %	14	70	16
Age (years) [§]	37 (14.0) [a]	40 (15.1) [b]	46 (16.3) [c]
Height (cm) [§]	153.3 (7.0) [a]	152.9 (7.0) [b]	153.1 (7.2) [ab]
Weight (kg) [§]	67.6 (14.1) [a]	64.9 (13.4) [b]	64.2 (13.8) [c]
BMI (kg/m^2) [§]	28.8 (5.7) [a]	27.7 (5.3) [b]	27.3 (5.3) [c]
Hypertension [ǂ]	732 (18%) [a]	3587 (18%) [a]	1030 (22%) [b]
Diabetes [ǂ]	337 (8%) [ab]	1559 (8%) [a]	432 (9%) [ab]
Arthritis [ǂ]	201 (5%) [a]	1122 (6%) [a]	319 (7%) [b]
Hypercholesterolemia [ǂ]	385 (10%) [a]	1454 (7%) [b]	374 (8%) [b]
Breast cancer [ǂ]	15 (4%) [a]	70 (4%) [a]	12 (3%) [ab]

[§] Results are shown as mean (standard deviations—SDs), [ǂ] Results are shown as frequency (% of total), [abc] Significant differences between groups, non-significant differences are indicated by the same letter.

The number of subjects per decade group differed from only 53 (1900s) to 8823 in the 1970s. The corresponding standard deviations of the decade groups of 1910s–1980s, however, were comparable. Women born in the 1900s and 1920s had the highest age when reaching menarche, while women born in the 1980s showed the youngest AAM (**Figure 2.2**).

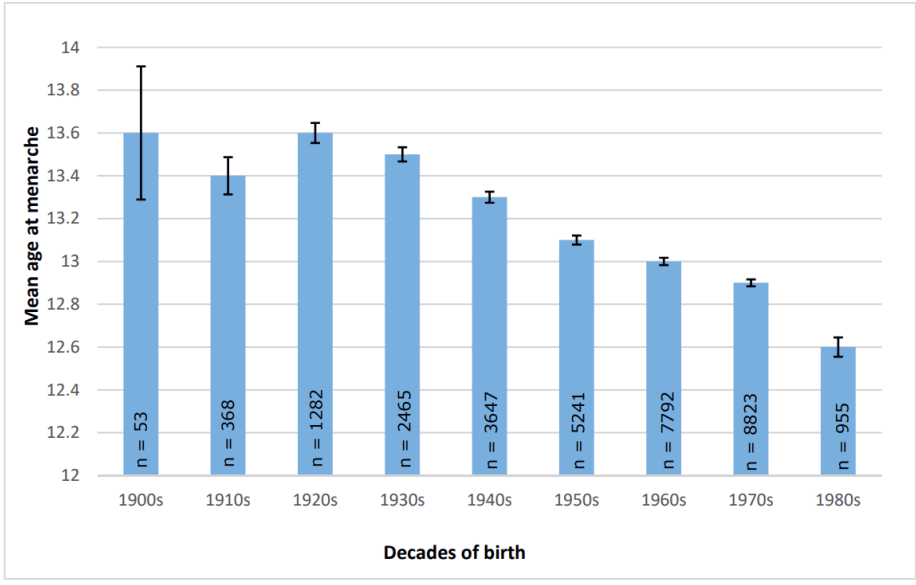

Figure 2.2. Mean age at menarche per decade of birth (1900s–1980s).

Association of Age at Menarche and Nutritional Status

AAM was negatively associated with BMI scores and weight, both before and after adjustment for current age (see Table 2.2). In the adjusted model, AAM significantly predicted BMI (β= −1.01; 95% CI −1.007, −1.006). The results indicate that the used model explained 4.0% of the variance. Weight was significantly negatively associated with an older AAM (β = −1.01; 95% CI −1.006, −1.004). The combined predictors AAM and current age explained 0.9% of the model variance. In contrast to that, final attained height was positively associated with AAM and current age (β = 0.18; 95% CI 0.112, 0.231). The model explained 6.3% of the variance

Table 2.2. Association between age at menarche and nutritional status.

Outcome variable	β Estimates	95% CI	R^2
Crude model: age at menarche			
BMI (kg/m^2)	-1.00 *	-1.005, -1.004	0.007
Height (cm)	-0.02 *	-0.077, -0.034	0.000
Weight (kg)	-1.01 *	-1.005, -1.004	0.006
Adjusted model: age at menarche + current age			
BMI (kg/m^2)	-1.01 *	-1.007, -1.006	0.040
Height (cm)	0.18 *	-0.231, -0.112	0.063
Weight (kg)	-1.01 *	-1.006, -1.004	0.009

*p-value <0.001

Association of Age at Menarche and Disease Status

Univariable predictor testing proved all possible confounding variables (BMI, age, BMI*age interaction, and family history) to be significantly associated with each disease variable, with the exception of breast cancer. Consequently, the crude models were adjusted for all co-variables mentioned above. After adjustment, diabetes and hypercholesterolemia were significantly inversely related to AAM with relative risks (RRs) of 0.95 (95% CI 0.93, 0.98) and 0.93 (95% CI 0.90, 0.95), respectively, see **Table 2.3.**

The adjusted analysis of AAM and hypertension, breast cancer, gout arthritis, and other arthritis showed the hypothesized inverse trend. However, these associations were not statistically significant ($p > 0.05$). AAM was associated with higher disease risk for gout arthritis (RR 1.05); however, this result was not statistically significant ($p > 0.05$) either.

Table 2.3. Relative risks of the adjusted model.

Disease Outcome	RR[1]	95%CI[1]
Diabetes	0.95 *	0.93, 0.98
Hypertension	1.00	0.98, 1.02
Hypercholesterolemia	0.93 *	0.90, 0.95
Breast cancer	0.95	0.98, 1.02
Arthritis	1.00	0.97, 1.03
Gout arthritis	1.05	0.94, 1.18
Rheumatoid arthritis	1.00	0.99, 1.02
Other arthritis	0.93	0.93, 1.07

[1]Adjusted for BMI, age, BMI*age interaction, and family history of disease, *p-value <0.001, §Unadjusted.

Time Trend of Age at Menarche

Analysis of variances for the association of decade of birth and AAM showed a statistically significant difference between groups (Welch $F = 99.42$, $p < 0.001$). The largest statistically significant reduction in AAM was seen between the decades of the 1920s and 1980s, in which the mean age of menarche decreased by 0.99 years (see Table 2.4). A significant downwards trend in AAM between the decades of the 1930s and 1980s was observed.

Table 2.4. Mean differences of age at menarche among different decades of birth.

Decade	1900s	1910s	1920s	1930s	1940s	1950s	1960s	1970s	1980s
1900s	1	0.10	0.14	-0.04	-0.20	-0.40	-0.49	-0.69*	-0.86*
1910s		1	0.24	-0.13	-0.11	-0.30*	-0.39**	-0.59**	-0.76**
1920s			1	-0.10	-0.34**	-0.53**	-0.63**	-0.83**	-0.99**
1930s				1	-0.24**	-0.43**	-0.53**	-0.72**	-0.89**
1940s					1	-0.19**	-0.29**	-0.49**	-0.65**
1950s						1	-0.10*	-0.29**	-0.46**
1960s							1	-0.20**	-0.37**
1970s								1	-0.17**
1980s									1

*Mean difference significant at the p-value <0.005, **Mean difference significant at the p-value <0.001.

Discussion

We observed a trend towards a younger age at menarche and related non-communicable disease risk between the decades of the 1920s and 1980s. We found that earlier AAM was associated with lower adult height, higher adult weight and BMI, and risk of diabetes and hypercholesterolemia.

Our study showed a secular trend towards a younger AAM in Mexico from 1900 to 1980. Mean AAM significantly decreased compared to each previous decade group from the 1930s onwards. Largest total decrease in AAM was between the 1920s and 1980s, with a decline of mean AAM by almost a full year. Marván et al. assessed the declining AAM in the cities of Xalapa and Mexico City. In their study, AAM decreased by 1.6 years between the 1940s (and earlier) and the 1990s [31]. Similar to that, in a rural community in Southern Mexico, a larger decline of 1.8 years took place over the previous 23 years [32]. Thus, our findings indicate a nationwide trend towards a younger AAM, yet there is no consensus on the extent of the decrease. Possible explanations can be the difference in the designs of the studies. Both Marván et al. and Malina et al. used predominately the status quo method to assess AAM, while data in our study were obtained retrospectively. This increased the chance of recall bias and may have decreased the accuracy of our study. Yet, compared to these studies, the study population used in our study was considerably larger and the validity of our results was thus high. Another possible explanation for the difference at hand is the setting in which the studies were

conducted. While our study represented the nationwide average of AAM, Marván et al. and Malina et al. focused their research on specific areas, which are geographically and culturally different. Overweight prevalence and poverty rate, as a marker of socioeconomic status, differ between the study locations [38,39]. It is thus suggested that the magnitude of the decrease in AAM differs per location and its specific environmental conditions.

We showed that each one-year delay in menarche was associated with a 0.18 cm increase in final attained height. Our results are consistent with other studies. In the UK, the impact of AAM was greater, with an increase of 0.59 cm in final height per one year of delayed menarche [18]. The EPIC study (European Prospective Investigation into Cancer and Nutrition) assessed the impact of AAM on final height in several European countries in women born before and after 1945. In women born before 1945, there is evidence of an increase in height in France, the Netherlands, and Spain, with 0.41 cm, 0.19 cm, and 0.21 cm, respectively. Interestingly, AAM had a higher impact on women born after 1945 [40]. Pubertal sex hormones and growth hormones generally increase simultaneously, leading to skeletal growth. Height velocity decreases after menarche. On average, final height is attained one year after menarche [41]. Thus, a younger AAM can be linked to an overall shorter stature, which may lead to impaired health outcomes [42,43].

We found adulthood BMI to be significantly reduced with increasing AAM; each one-year delay in menarche was associated with a reduction of 1.01 kg/m2. The large US Nurses' Health Studies (NSH I/NSH II) assessed the years 1980 and 2005 and found a smaller impact of AAM. In the NSH I, a decrease of 0.2 kg/m2 was found with each one-year increase in AAM, while in NSH II, a slightly larger reduction (0.26 kg/m2) was seen [17]. In Scotland, an earlier study found a decrease in BMI of 0.64 kg/m2 [44]. All these findings are in line with our results. However, it is remarkable that the extent to which BMI decreases with increasing AAM in our study is almost twice as high as the findings from the Scottish study [44] . Compared to the NSH I, reduction in BMI in our study is five times as high as the decrease found in the US. A higher BMI increases the risk of cardiovascular diseases, diabetes, osteoarthritis, and some cancers [45] and thus represents a large public health burden. Since childhood BMI is closely related to AAM [6,7], the association might be based on reversed causality. Studies suggest that the inverse association of AAM and BMI is not explained by confounding of childhood BMI [44] while in other studies, minor attenuation of results was found [16]. For future studies, we therefore recommend taking childhood BMI into account in the association of AAM and BMI.

Of the non-communicable diseases we assessed, diabetes and hypercholesterolemia were significantly associated with AAM. The risk for diabetes was slightly lower when AAM increased (RR 0.95). This is in line with findings of previous studies that indicated a decreased risk of diabetes with later AAM [17,28,46]. Stöckl and colleagues found an inverse association with an RR of 0.88, which is comparable to the findings of Lakshman et al. who found an OR of 0.91. Data from the NSH II study suggested an RR of 0.97 per one-year delay of menarche [17]. Thus, our findings support the hypothesis of an inverse association of AAM and diabetes risk, found in other studies. Studies on the association of AAM and hypercholesterolemia are rare. However, an association of younger AAM with higher triglyceride concentrations has been observed [30]. No such effect was found in the studies of Stöckl D et al. [28] and Frontini et al. [47]. Thus, the specific influence of a younger AAM on hypercholesterolemia has not been investigated intensely, while studies on the effect on triglyceride concentrations are inconsistent. Our findings add to the hypothesis of an inverse association between AAM and hypercholesterolemia; however, these results should be validated by more cohort studies investigating hypercholesterolemia directly.

We did not observe a significant association between AAM and risk of hypertension, arthritis or breast cancer. Studies among British [27] and Korean [48] cohorts showed that risk of hypertension decreased with increasing age at menarche, although a more recent study could not reproduce these findings [49]. The lack of association between AAM and risk of hypertension in our population could be explained by the presence of other confounders that were not evaluated in the survey, like physical activity, smoking, alcohol consumption, and educational level. Similarly, the evidence of the effect of early AAM on arthritis has been inconsistent. The NSH I study found a higher risk of rheumatoid arthritis among women with an early AAM (RR: 1.3) [25]. Yet, other studies suggest no association [50,51] or a higher risk with later AAM [52,53]. We observed a decreased risk for breast cancer with later AAM. Although our results were not significant, the observed trend is consistent with another study that showed an increased risk for breast cancer with decreasing AAM [24]. Based on our findings and the current state of the literature, it remains uncertain if AAM is associated with risk of hypertension, breast cancer or arthritis in adulthood.

The strengths of the current study include the large sample size and selection of participants. All participants were chosen randomly and women of all states, covering urban and rural living environments, were included in this study. As a result, the representativeness of our study is expected to be high. Several limitations should also be acknowledged. First, the lack of more

sociodemographic and lifestyle data that could influence the results. BMI and related non-communicable disease risk are closely associated with a person's socioeconomic status [54,55] and lifestyle factors such as physical activity and nutritional behavior [56,57]. Socioeconomic status [58], nutrition [59], and physical activity [60] are associated with AAM as well and they thus might act as confounding factors in the studied association. Thus, including these factors in the model might have changed the final results of this study. Lastly, when studying the secular trend of AAM over time, one should consider the differences in sample size. While the decade group of the 1970s consisted of 8823 women, data of only 53 women were available in the decade of the 1900s. Even though standard deviations between all groups are comparable, this difference might explain the non-significant differences of the decades of the 1900s and 1910s. Yet, the overall trend towards a younger age at menarche is not affected by that.

Finally, results cannot be interpreted as causality, because of the transversal nature of our study. In parallel with the obesity epidemic in Mexico, the prevalence of type 2 diabetes mellitus has increased rapidly during the last several decades. It has been shown that by the time type 2 diabetes is diagnosed, some individuals have already developed serious complications. Therefore, it has become increasingly important to identify persons at risk in early life so they may benefit from early interventions. Our findings stress the importance of assessing age at menarche from clinical and public health perspective. Age at menarche can be an indicator of nutritional status and normal development of adolescent girls, and earlier AAM can be an independent risk factor of non-communicable diseases. Because recall bias can be present in retrospective studies, longitudinal and cross-sectional studies in adolescents may help to understand the association between pubertal onset and health. It is recommended to monitor AAM on a regular basis by making it a permanent variable of national health surveys.

Conclusions

Our research showed a trend towards a younger AAM and related non-communicable disease risk during the 20th century in Mexico. Additionally, we found that younger AAM is associated with lower adult height, higher adult weight and BMI, and higher risk for type 2 diabetes and hypercholesterolemia in adulthood. Thus, early age at menarche might represent a risk factor for early identification of women who are at increased risk of being overweight or obese and

of developing type 2 diabetes in adulthood. Yet, in order to confirm these findings, additional research which includes data on sociodemographic and lifestyle factors is needed.

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Chapter 3

Dietary Patterns and the Double Burden of Malnutrition in Mexican Adolescents: Results from ENSANUT-2006

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Abstract

Mexico is facing the double burden of malnutrition, and adolescents are not an exception. Diet plays an important role, both in causing overweight and undernutrition. This study aimed to describe the dietary patterns (DPs) of Mexican adolescents and to examine its association with nutritional status using data from adolescents aged 12–19 years (n = 7380) from the National Survey of Health and Nutrition (ENSANUT-2006). Principal component analysis was used to derive the DPs. Associations between DP and nutritional status were determined by prevalence ratio (PR). Four DPs were identified: nontraditional and breakfast-type, Western, plant-based, and protein-rich. The prevalence of overweight and obesity was higher in adolescents who scored high on the Western pattern (PR: 1.15, 95% CI: 1.08–1.21) or on the plant-based pattern (PR: 1.09, 95% CI:1.03–1.17). The Western pattern was positively associated with anemia in girls (PR: 1.18, 95% CI:1.03–1.35), while the nontraditional and breakfast-type pattern was inversely associated with anemia in adolescents aged 12–15 years (PR: 0.87, 95% CI: 0.76–0.99) and in girls (PR: 0.84, 95% CI: 0.75–0.97). The Western and plant-based patterns were simultaneously associated with overweight–obesity and at least one indicator of undernutrition. In the context of the double burden of malnutrition, dietary advice must consider malnutrition in all its forms.

Introduction

After childhood, adolescence is the second important developmental period in the life course [1]. Complex interactions of physiological, sexual, neurological, and behavioral factors prompt the transition from childhood to adult life [1,2]. Adolescence is a period characterized by rapid growth and changes in body composition, leading to increased energy and micronutrient requirements [1].

Eating behavior of adolescents may be influenced by sociodemographic characteristics, such as socioeconomic level, education level, ethnicity, and gender [3]. Dietary habits established during adolescence tend to persist during adulthood. Moreover, dietary patterns adopted during this stage may contribute to health outcomes later in life [1,4]. Failure to comply with nutritional demands during adolescence can lead to growth retardation, impaired organ remodeling, and micronutrient deficiencies [1]. In addition, evidence suggests that, in adolescents, a Western-type dietary pattern (i.e., an industrialized diet, mainly characterized by high energy, fat, and sugar content) is associated with the prevalence of obesity [5,6], metabolic risk [7], and depression [8,9].

The double burden of malnutrition is characterized by the coexistence of micronutrient deficiencies and diet-related chronic conditions. This problem can be present at individual, household, and/or national levels [10]. In Mexico, micronutrient deficiency, anemia, and stunting are still prevalent among adolescents [11,12], while the prevalence of overweight and obesity has increased dramatically during the last 13 years. In 2012, more than 30% of Mexican adolescents were overweight or obese [13]. Furthermore, other metabolic disorders related to poor dietary quality, such as type 2 diabetes and metabolic syndrome, have started to emerge in this age group [14,15].

Up until now, adolescents' dietary intake at a national scale in Mexico has only been examined in terms of glycemic index and intake of single nutrients and food group consumption [16–18]. However, the single nutrient approach has some limitations when the aim is to demonstrate associations between diet and health outcomes. People do not eat isolated foods or nutrients but rather consume meals consisting of a variety of foods and nutrients [5]. Dietary patterns present a broader picture of a combination of foods and nutrients, including the antagonist,

additive, and synergetic effect of the food matrix [19]. Thus, the dietary pattern approach is a better alternative to study the interactions between diet and health. Therefore, the primary aim of this study was to describe dietary patterns of Mexican adolescents and to associate these with overweight–obesity, anemia, and stunting.

Materials and Methods

Study Design and Study Population

This study used data from the National Health and Nutrition Survey (ENSANUT-2006). ENSANUT-2006 is a probabilistic, cross-sectional, stratified cluster sample study representative at national, regional, and state levels and that of urban and rural areas in Mexico. The survey was conducted from October 2005 to May 2006. The description of sampling procedures is detailed in Palma et al. [20]. ENSANUT-2006 aimed to characterize the health and nutritional status of different age groups in the Mexican population. For the purposes of this study, we used the adolescents' subsample, which includes 8768 boys and girls aged 12 to 19 years. A flow chart of the selection process of the study population is shown in Figure 3.1. We excluded 69 adolescent girls that were pregnant, lactating, or both because nutritional requirements and body composition change during these stages. Secondly, we excluded subjects with missing or implausible values for anthropometric information (n = 549) and with implausible reported energy intake (n = 549). For exclusion criteria, we considered a plausible body mass index (BMI) to be > 10 and < 58 kg/m2 [13]. In addition, we used the energy intake/basal metabolic rate ratio (EI:BMR) cutoffs > 0.5 and below 3 standard deviations (SD) to assess plausible reported energy intake [21,22]. After exclusions, 7380 subjects with detailed information of diet and anthropometry remained, representing around 7,078,490 adolescents. Out of the 7380 adolescents, a subsample of 7080 (representing 6,868,671 adolescents) had data on hemoglobin concentrations. To facilitate the interpretation of the results, we classified the study subjects into two adolescent stages: 12–15 and 16–19 years.

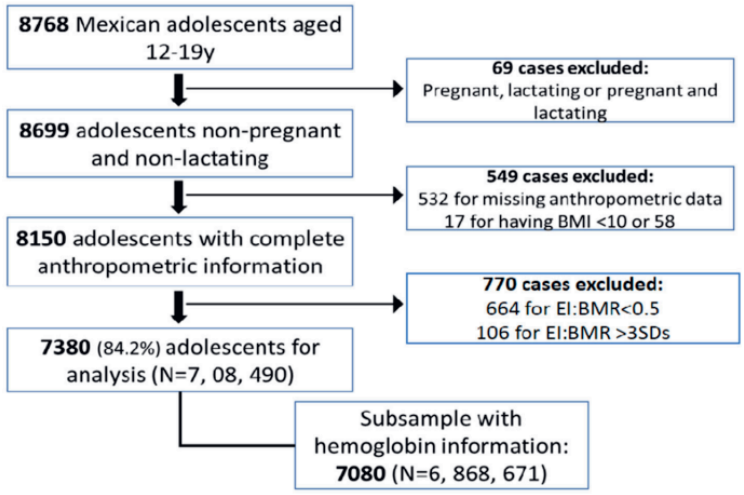

Figure 3.1. Selection process of study population. "N" represents the expanded sample. The sample of 7380 subjects represented 7,078,490 adolescents, while the subsample of 7080 subjects represented 6,868,671 adolescents. y = year; BMI = body mass index; EI:BMR = energy intake/basal metabolic rate ratio.

Population Characteristics

Information on several sociodemographic characteristics was obtained. The country was divided into three geographic regions that have similar characteristics: north, center, and south. The living area was defined as the type of locality, i.e., (1) urban for a population of ≥ 2500 inhabitants and (2) rural for a population of < 2500 inhabitants. Socioeconomic level was classified into tertiles: (1) low, (2) medium, and (3) high. The classification was based on household characteristics, goods, and availability of basic services [20].

For nutritional status data, staff with standardized training measured the weight and height; details of the anthropometric techniques and materials are described elsewhere [23]. The indicators height-for-age Z-score (HAZ) and BMI-for-age Z-score (BAZ) were used to classify nutritional status according to the World Health Organization (WHO) standards [24]. Stunting was defined as HAZ below 2 SD, and overweight/obesity was defined as BAZ above 1 SD. Hemoglobin concentrations were adjusted for residential altitude [25]. Anemia was defined according to the WHO cutoff points per gender and age group as follows: boys aged 12–14 years and all girls, hemoglobin concentrations <120 g/L; boys aged >15 years, hemoglobin concentrations <130 g/L [25].

Dietary Data

Trained staff administered a seven-day food frequency questionnaire (FFQ). This FFQ includes 101 food items and is based on a previously validated FFQ [26]. For the current analysis, we classified the 101 food items into 30 food groups according to their nutritional profile and cultural relevance. For deriving the dietary patterns, we excluded those food groups consumed by less than 10% of the adolescents (alcoholic drinks, drinks without energy, and nutritional supplements). **Supplementary Table S3.1** displays examples of each food group and the percentage of consumers.

To identify the dietary patterns, principal component analysis (PCA) was used. We estimated mean intake per food group (g/day) for each subject and standardized the values with Z-scores. Then, the 26 food groups were consolidated into principal components and rotated orthogonally (varimax rotation) to facilitate interpretability. The number of patterns to be retained was chosen on the basis of the scree plot and eigenvalue > 1.0. Four dietary patterns were retained, which accounted for a cumulative variation of 28.0% in food intake. Food groups with factor loadings ≥ 0.30 were considered as key contributors to the dietary patterns and used to label them.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics 22. PCA provided a factor score for each subject for each of the four dietary patterns. We categorized the factor scores of each dietary pattern into quartiles. Energy and nutrient intake across the four dietary patterns were calculated and compared. Population characteristics and prevalence ratios (PR) were calculated using CSPLAN for complex samples. Basic statistics were performed to describe the sociodemographic characteristics of our study population. In order to identify possible confounders, we compared sociodemographic characteristics (urban/rural, geographic region, and socioeconomic level) and nutritional status (BMI/age, anemia, and stunting) among adolescent age and gender groups using the F-statistic (corrected and weighted chi-square).

Next, we investigated the association between nutritional status (overweight–obesity, anemia, and stunting) and dietary patterns by Cox regression as it is considered an alternative for logistic regression in cross-sectional studies when the outcome of interest is not rare [27]. PR for overweight–obesity and stunting were calculated by adjusting for the sociodemographic characteristics. EI:BMR ratio and BAZ were inversely correlated, suggesting underreporting in obese subjects. Thus, we additionally adjusted for EI:BMR ratio in order to control for

misreporting (PRs unadjusted for EI:BMR ratio are shown in **Supplementary Table S3.2**). The PR for anemia was adjusted for sociodemographic characteristics and BAZ because evidence suggests a positive association between BMI and iron deficiency [28]. Cox regression with constant survival time gives a good approximation of the standard error of the prevalence ratio when robust variance estimates are used [27], but because this procedure was not present in SPSS, we calculated the confidence intervals using the Wald test result from logistic regression of the same model and the beta coefficient from the Cox regression analysis. The analysis of the association between nutritional indicators and dietary patterns was also conducted separately for adolescents' age and sex groups because major changes in body composition and growth spurt occur during early adolescence (10–15 years), and differences in nutritional requirements between girls and boys are notable during adolescence [1].

Because energy and nutrient intakes were not normally distributed, Kruskal–Wallis H-test was used to assess differences in energy and nutrient intake across the quartiles in each pattern. Subsequently, we used Spearman correlation coefficients between intake of energy, macro- and micronutrients, and each dietary pattern.

Results

Sociodemographic characteristics and nutritional status of Mexican adolescents from ENSANUT-2006 are described by sex and age groups in **Table 3.1**. The sample characteristics are comparable to the Mexican population characteristics reported in 2006 [13,17]. Anemia was significantly higher in the group of boys aged 12–15 years compared to boys aged 16–19 years. In contrast, for adolescent girls, weight status and anemia were not significantly different between age groups. From the total number of subjects with anemia, about 30% presented overweight or obesity simultaneously.

Table 3.1. Sociodemographic characteristics and nutritional status of Mexican adolescents from ENSANUT-2006.

	Girls			Boys		
	12-15 years (*n* = 2222)	16-19 years (*n* = 1630)	*p*-value	12-15 years (*n* = 2377)	16-19 years (*n* = 1377)	*p*-value
Age median (range)	13.5 (12.0–15.0)	17.5 (16.0–19.0)		13.5 (12.0–15.0)	17.5 (16.0–19.0)	
Socioeconomic level (%)						
Low	48.9	48.1	0.38	48.5	43.2	0.08
Medium	32.9	31.2		31.0	31.8	
High	18.2	20.8		20.5	25.0	
Area of living (%)						
Urban	56.5	59.9	0.12	57.1	61.8	0.07
Region (%)						
North	13.1	11.9	0.27	11.3	15.2	0.06
Centre	45.5	48.7		48.6	46.3	
South	48.6	39.4		40.1	38.6	
Weight status (%)						
Thinness	1.4	1.8	0.86	2.5	2.1	0.015
Normal weight	66.6	67.2		67.2	71.8	
Overweight/obese	31.9	31.1		30.3	26.2	
Stunting (%)	13.8	21.1	< 0.001	14.3	19.2	0.012
Stunted subjects with overweight–obesity (%) [ǂ]	25.0	31.2		19.2	25.2	
Anemia (%) [§]	10.2	10.8	0.67	6.3	3.6	0.05
Anemic subjects with overweight–obesity (%) [§]	40.2	30.2		33.5	24.0	

Sample size: 7380; expansion factor: 7,078,490; [§]Sample size: 7080; expansion factor: 6,868,671. Anemia was estimated with hemoglobin concentration adjusted for geographic altitude; [ǂ]Proportion of overweight–obesity among stunted subjects; [§]Proportion of overweight–obesity among subjects with anemia.

Dietary Patterns

Four dietary patterns were identified: (1) nontraditional and breakfast-type, (2) Western, (3) plant-based, (4) protein-rich. In total, the four patterns accounted for 28% of the variance in the dietary variables. Details of the mean intake (g/day) and factor loading for food groups in each dietary pattern are listed in **Table 3.2**, and the correlation between nutritional content and the dietary pattern scores is shown in **Table 3.3**. The nontraditional and breakfast-type pattern was characterized by a high intake of milk, breakfast cereals, dairy, sweets, and sandwiches

and a low intake of tortilla (Table 3.2). The nontraditional and breakfast-type pattern was positively correlated with energy from protein and fat and inversely correlated with fiber and EI:BMR ratio (Table 3.3). This pattern accounted for 12.0% of the variance in the diet. The Western pattern was characterized by high intake of industrialized sweet drinks, salty snacks, charcuterie, saturated fat, sandwiches, fast-food, and cereals/tubers. The Western pattern showed a positive correlation with the intake of total energy as well as percentage of energy from fat and sugar and contributed to 6.3% of the variance in the diet. The plant-based pattern was represented by high consumption of fruit, non-industrialized sweet drinks, vegetables, avocado and nuts, maize-based food, fried vegetarian dishes, and sweet bakery. The plant-based pattern was positively correlated with total energy and intake of micronutrients, such as calcium, iron, zinc, vitamin C, vitamin A, and folate, and aggregated 5.2% of the variance in the diet. The protein-rich pattern was represented by a high intake of legumes, pasta and rice, eggs, soup, and poultry and red meat. The protein-rich pattern was highly correlated with total percentage of energy from protein as well as intake of iron and zinc and accounted for 4.5% of the diet variance. The content of energy, macronutrients, and micronutrients across the quartiles of the four dietary patterns is presented in **Supplementary Table S3.3**.

Table 3.2. Factor loading matrix for the four dietary patterns and their food groups.

Food group	Average intake (g/d)	Non-traditional and breakfast-type	Western	Plant-based	Protein-rich
Tortilla	194.5	−0.51			
Other cereals	29.6		0.33		
Breakfast cereals	5.0	0.63			
Maize-based food	50.2			0.36	
Fast-food	5.1		0.34		
Legumes	74.3				0.58
Fruit	186.2			0.54	
Vegetables	63.8			0.50	
Poultry and red meat	47.1				0.31
Fish and seafood	8.3				
Charcuterie	8.3		0.49		
Milk *	166.8	0.66			
Eggs	31.4				0.51
Fat	1.9		0.40		
Sweets	24.7	0.35			
Cookies	202.7				
Salty snacks	9.0		0.55		
Industrialized sweet drinks*	262.6		0.63		
Non-industrialized sweet drinks*	256.2			0.53	
Dairy	33.5	0.37			
Sandwich	21.6	0.30	0.36		
Fried vegetarian dishes	6.6			0.36	
Sweet bakery	27.3			0.31	
Soup	66.3				0.50
Pasta and rice	23.7				0.57
Nuts and avocado	7.5			0.40	

Values from −0.30 through to 0.30 were excluded for simplicity in the interpretation. Average intake was calculated for each food item as the mean in grams per day. *(mL/d).

Table 3.3 Correlation coefficients between dietary patterns and nutritional content.

	Non-traditional and breakfast-type	Western	Plant-based	Protein-rich
EI:BMR ratio	−0.04*	0.29*	0.48*	0.29*
Energy (kcal)	−0.04*	0.36*	0.47*	0.33*
Protein (% energy)	0.38*	−0.05*	−0.09*	0.27*
Fat (% energy)	0.32*	0.43*	−0.03*	0.13*
CHO's (% energy)	−0.37*	−0.36*	0.05*	−0.18*
Fiber (g)	−0.30*	−0.02*	0.41*	0.37*
Sugar (g)	0.15*	0.38*	0.23*	−0.01
Ca (mg)	0.15*	0.06*	0.38*	0.20*
Fe (mg)	−0.06*	0.26*	0.37*	0.35*
Zn (mg)	0.11*	0.31*	0.38*	0.39*
Vit C (mg)	0.18*	−0.03*	0.51*	0.16*
Vit A (IU)	0.25*	−0.04*	0.49*	0.23*
Vit B12 (mg)	0.40*	0.33*	0.26*	0.23*

*Significant at p-value 0.01.

Association of Dietary Patterns with Overweight and Obesity

The prevalence of overweight and obesity was higher in adolescents who scored high on the Western pattern (PR: 1.15, 95% CI: 1.08–1.21) and on the plant-based pattern (PR: 1.10, 95%: CI 1.03–1.17) after taking into account the potential confounders of age, sex, living area, socioeconomic status, and region (**Table 3.4**), indicating an increase of 15% and 10%, respectively, per quartile of dietary pattern score. This increase was more pronounced in the younger adolescents (12–15 years) and in boys. A positive association was also seen for the plant-based pattern in the younger adolescents and in girls. The positive association between nontraditional and breakfast-type pattern and overweight/obesity in younger adolescents depended on inclusion of EI:BMR ratio in the model; after adjusting for EI:BMR

Table 3.4. Adjusted prevalence ratios of overweight and obesity for dietary patterns among Mexican adolescents

Dietary pattern	Total* (*n* = 7380)	12–15 years § (*n* = 4451)	16–19 years § (*n* = 2929)	Boys ǂ (*n* = 3610)	Girls ǂ (*n* = 3770)
Non-traditional and breakfast-type	1.03 (0.97–1.10)	0.98 (0.97–1.00)	0.91 (0.83–1.00)	1.01 (0.93–1.11)	1.05 (0.97–1.14)
Western	1.15 (1.08–1.21)	1.14 (1.06–1.22)	1.05 (0.95–1.16)	1.23 (1.12–1.34)	1.08 (0.99–1.18)
Plant-based	1.10 (1.03–1.17)	1.20 (1.11–1.30)	1.04 (0.92–1.17)	1.07 (0.97–1.18)	1.12 (1.04–1.22)
Protein-rich	1.02 (0.95–1.09)	0.97 (0.96–1.03)	1.05 (0.95–1.16)	1.06 (0.98–1.15)	0.98 (0.91–1.05)

All prevalence ratios were calculated per quartile of dietary pattern scores. *Model adjusted for sex, living area, socioeconomic status, region, age, and EI:BMR ratio. §Model adjusted for sex, living area, socioeconomic status, region, and EI:BMR ratio. ǂModel adjusted for living area, socioeconomic status, region, age, and EI:BMR ratio.

Association of Dietary Patterns with Anemia

None of the four dietary patterns were independently associated with the prevalence of anemia in the total study population. However, girls who scored high on the Western pattern presented higher prevalence of anemia (PR: 1.18, 95% CI: 1.03–1.35) (**Table 3.5**). In contrast, the nontraditional and breakfast-type pattern was inversely associated with anemia in younger adolescents (12–15 years) (PR: 0.88, 95% CI: 0.76–0.99), in boys (PR: 0.87, 95% CI: 0.76–0.99), and in girls (PR: 0.84, 95% CI: 0.75–0.97). These results were not affected by additional adjustment for total energy intake or EI:BMR ratio.

Table 3.5. Prevalence ratios of anemia among Mexican adolescents across dietary patterns.

Dietary pattern	Total* (*n* = 7080)	12–15 years § (*n* = 4288)	16–19 years § (*n* = 2792)	Boys ǂ (*n* = 3447)	Girls ǂ (*n* = 3633)
Non-traditional and breakfast-type	0.91 (0.81–1.01)	0.87 (0.76–0.99)	0.96 (0.80–1.16)	0.87 (0.76–0.99)	0.83 (0.73–0.96)
Western	1.11 (0.98–1.26)	1.11 (0.94–1.30)	1.13 (0.92–1.38)	1.11 (0.94–1.30)	1.24 (1.06–1.45)
Plant-based	0.99 (0.88–1.11)	1.04 (0.89–1.22)	0.87 (0.76–1.01)	1.04 (0.89–1.22)	0.96 (0.84–1.09)
Protein-rich	1.02 (0.92–1.12)	1.09 (0.95–1.25)	0.92 (0.79–1.06)	1.09 (0.95–1.25)	0.98 (0.83–1.05)

A subsample of 7080 subjects counted with hemoglobin data. Hemoglobin concentrations were adjusted for geographic altitude. All prevalence ratios were calculated per quartile of dietary pattern scores. *Model adjusted for sex, living area, socioeconomic status, region, age, and BMI-for-age Z-score (BAZ). §Model adjusted for sex, living area, socioeconomic status, region, and BAZ. ǂModel adjusted for living area, socioeconomic status, region, age, and BAZ.

Association of Dietary Patterns with Stunting

Adolescents who scored high on the nontraditional and breakfast-type (PR: 0.88, 95% CI: 0.81–0.95), Western (PR: 0.86, 95% CI: 0.80–0.92), and plant-based (PR: 0.85, 95% CI: 0.79–0.92) diets were less likely to be stunted (**Table 3.6**). With adjustment for EI:BMR ratio and stratifying by age and sex, the association between nontraditional and breakfast-type pattern was only significant for the age group 16–19 years and for boys. High scores on the Western pattern were inversely associated with stunting for the two age groups and for boys but not for girls. Finally, high scores on the plant-based pattern were significantly associated with lower prevalence of stunting for the 12–15 year age group, for boys, and for girls

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Table 3.6. Adjusted prevalence ratios of stunting among Mexican adolescents across dietary patterns.

Dietary pattern	Total* (*n* = 7380)	12–15 years § (*n* = 4451)	16–19 years § (*n* = 2929)	Boys ǂ (*n* = 3610)	Girls ǂ (*n* = 3770)
Non-traditional and breakfast-type	0.88 (0.81–0.95)	0.90 (0.80–1.00)	0.85 (0.77–0.95)	0.89 (0.81–0.97)	0.86 (0.77–1.03)
Western	0.85 (0.80–0.92)	0.88 (0.80–0.97)	0.85 (0.76–0.96)	0.81 (0.74–0.89)	0.93 (0.83–1.03)
Plant-based	0.85 (0.79–0.92)	0.80 (0.71–0.90)	0.90 (0.80–1.02)	0.80 (0.71–0.90)	0.80 (0.72–0.89)
Protein-rich	0.96 (0.90–1.02)	0.92 (0.84–1.01)	1.00 (0.91–1.10)	0.92 (0.84–1.01)	0.98 (0.90–1.07)

All prevalence ratios were calculated per quartile of dietary pattern scores. *Model adjusted for sex, living area, socioeconomic status, region, age, and EI:BMR ratio. §Model adjusted for sex, living area, socioeconomic status, region, and EI:BMR ratio. ǂModel adjusted for living area, socioeconomic status, region, age, and EI:BMR ratio.

Discussion

In this study, four dietary patterns were identified, reflecting both Westernized/modern patterns (nontraditional and breakfast-type and Western) and traditional/transitioning patterns (plant-based and protein-rich). These findings are consistent with the nutritional transition in Mexico and with results reported in previous studies [7,29] and parallel the double burden of malnutrition. Kroker-Lobos et al. (2014) showed the coexistence of anemia, stunting, and overweight–obesity at individual, household, and national levels in Mexican children and women [30]. In addition, an association between mid-to-moderate household food insecurity and the co-occurrence of anemia and overweight–obesity in Mexican women has been reported [31]. Our results show that the double burden of malnutrition is also present at national and individual levels in Mexican adolescents. From 2006 to the present, the prevalence of overweight–obesity has increased, while the prevalence of anemia has decreased in Mexican adolescents. However, we do not have current dietary pattern data [12,13]. To our knowledge, this is the first study to link dietary patterns and indicators of the double burden of malnutrition at the individual level.

In the present study, the Western pattern was associated with malnutrition (overnutrition and undernutrition). Adolescents who scored high on the Western pattern were more likely to be overweight–obese and anemic. Similarly, high scores on the plant-based pattern were associated with a higher prevalence of overweight–obesity, but no association with anemia was observed. The Western and plant-based patterns had the highest correlation with total energy

intake (0.36 and 0.47, $p < 0.01$) and sugar (0.38 and 0.23, $p < 0.01$). The correlation between the plant-based pattern and sugar can be explained by some of the food items that characterize this patter, such as non-industrialized sweet drinks and sweet bakery. Both high energy and sugar intake have been associated with overweight and obesity [32–35]. Other studies conducted among adolescents from different countries have also shown that a Westernized dietary pattern increases the odds of being overweight or obese [7,36–39].

Anemia has been recognized as one of the three main causes of disability-adjusted life years among adolescents worldwide [40,41]. A systematic analysis of national surveys showed that the proportion of anemia associated with iron deficiency in women of reproductive age in Latin America was 59% [42]. Girls with a high intake of the Western pattern were more likely to be anemic. Although the Western pattern was positively correlated with iron intake (0.26, $p < 0.01$), adolescent girls have higher requirements of iron compared to boys [1]. Our results suggest that the content of iron in this pattern was not enough to cover the nutritional demands of adolescent girls. In addition, the bioavailability of iron in the Mexican diet has been shown to be low [11]. The associations between the nontraditional and breakfast-type pattern and lower prevalence of anemia in adolescents aged 12–15 years, both boys and girls, may be explained by nutrient interactions. This pattern was positively correlated with vitamin C (0.18, $p < 0.01$), which is an enhancer of iron absorption, and inversely correlated with fiber content (-0.30, $p < 0.01$), which inhibits iron absorption. However, anemia is not caused exclusively by iron deficiency. Other nutritional deficiencies, inflammatory status, and presence of parasites are factors that can also contribute to the development of anemia [42–44]. However, these data were not available in our study.

Stunting is an indicator of chronic undernutrition, which not only reflects poor nutrition but also the inadequacy of the environment to which a person has been exposed for a long period [45–47]. Therefore, the interpretation of a cross-sectional association between dietary patterns and stunting must be done with care. Stunting is mainly studied in children under five years old. However, adolescence is the second period of rapid linear growth after infancy, during which adolescents have a second opportunity to catch up on height [1,48]. Hence, we considered it important to study the association between dietary patterns and stunting in Mexican adolescents. We found that the prevalence of stunting was higher in the older age group of adolescents compared to the younger age group, which may be explained by a generation effect associated with the economic and nutritional transition. This would imply that adolescents in the older age group were more exposed to undernutrition during early

childhood than in the younger group [49]. However, it may also reflect an early growth spurt with decelerating growth in later adolescence. The prevalence of stunting was lower in adolescents who scored high on the nontraditional and breakfast-type, Western, and plant-based patterns. Probably, adolescents in the fourth quartile of these three patterns have maintained a high caloric intake throughout their lives, which has allowed them to meet the caloric requirements to reach an optimal height for their age. In addition to energy intake, protein intake is essential for linear growth. In this study, the protein intake across all the quartiles of the four patterns fell into the recommended values for the age groups (34–52 g/day) [1]. Notwithstanding, the correlations between these patterns and energy (−0.04, 0.36, and 0.47, $p < 0.01$) as well as percentage of energy from protein (0.38, −0.05, −0.09, $p < 0.01$), iron (−0.06, 0.26, 0.27, $p < 0.01$), and zinc (0.11, 0.31, 0.38, $p < 0.01$) were inconsistent. Thus, drawing conclusions on the association between the actual dietary intake and prevalence of stunting is not straightforward.

This study has several limitations. Firstly, as with all cross-sectional studies, we could not determine causality between the adherence to a dietary pattern and nutritional status. Secondly, a seven-day FFQ was employed to collect dietary information. This FFQ provides an overview of what the participants ate the previous week and does not fully reflect habitual intake. Thirdly, as with all dietary intake assessments, this study was prone to measurement error and underreporting of energy-dense food. To overcome the last point, we used the EI:BMR ratio to adjust the analyses [50,51]. After adjusting for EI:BMR ratio, the direction of the associations between dietary patterns and overweight–obesity and stunting radically changed. Given the inverse correlation between BAZ and EI:BMR ratio (−0.13, $p < 0.01$), this may be due to underreporting of energy intake by subjects with high BAZ, which has also been suggested by a previous study in Mexican adolescents [52].

This study also has several strengths. We used PCA to derive dietary patterns, a methodology that involves several subjective decisions but provides a summary measure of eating habits, diet quality, and potential interactions between nutrients. Another strength is that our large sample size is representative at national, regional, and state levels. Therefore, our results are generalizable to the entire population of Mexican adolescents.

Conclusions

In conclusion, this assessment of the association between the main dietary patterns in Mexican adolescents in 2006 and indicators of malnutrition provides insight on how dietary patterns may increase the risk of one or more indicators of malnutrition. Although longitudinal studies are needed to assess causal associations, our results add to the existent literature that higher adherence to the Western pattern is associated with the double burden of malnutrition. The Western pattern was positively associated with overweight–obesity among all the adolescents and with anemia in girls. In the context of the double burden of malnutrition, dietary advice must consider malnutrition in all its forms. Given that adolescence is a crucial stage from a life-cycle perspective, efforts to improve nutritional habits during this stage can have a positive impact on the health of adolescents, their future adulthood, and for the next generation.

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SUPPLEMENTAL TABLE S3.1: Food groups, examples of food items and percentage of consumers per food group.

Food group	Examples	% consumers
1. Tortilla	Maize tortilla	90.2
2. Cereals (except corn)	Potatoes, bread, wheat tortilla, salty cookie	63.6
3. Breakfast cereals	Breakfast cereals with low and high fiber content	30.1
4. Maize based food	Tamales, pozole, taco, tlacoyo, sope, atole (maize-drink)	49.1
5. Fast food	Hotdog, pizza, hamburger	13.1
6. Alcoholic drinks	Beer, wine, spirits	4.2
7. Legumes	Beans, lentils, chick peas, soy	90.0
8. Fruit	Apple, banana, grapes, grapefruit, kiwi, lemon, melon, papaya, pear, pineapple, strawberry, watermelon, others.	93.3
9. Vegetables	Bell pepper, carrots, chili pepper, col, corn, cucumber, dark green vegetables, jicama, lettuce, tomato, zucchini, others.	97.6
10. Poultry and red meat	Chicken, beef, pork, lamb, entrails	86.4
11. Fish and sea food	Tuna, sardines, dry fish, fresh fish, sea food	34.4
12. Processed meat	Ham, sausages, bacon, chorizo	45.5
13. Milk	Fat free and low fat milk, whole milk	68.5
14. Eggs		75.8
15. Saturated fat	Oil, butter, margarine, mayonnaise, cream	34.2
16. Sugar and sweets	Sugar, candies, chocolate, jelly	74.3
17. Cookies	Sweet cookies, granola bars	32.6
18. Salty snacks	Corn snacks and chips	51.0
19. Industrialized sweet-drinks	All types of soda and industrialized juice	84.9
20. Non-industrialized sweet-drinks	Natural juice, water with sugar, coffee or tea with sugar	83.3
21. Milk with added sugar	Milk with sugar, honey or chocolate, Yakult	5.9
22. Drinks without energy	Coffee or tea without sugar, water, diet sodas	8.8
23. Dairy products	Yogurt, cheese	70.5
24. Sandwich	Sandwich and torta (baguette type)	36.5
25. Fried dishes (plant based)	Breaded or capped vegetables, fried banana, fried potatoes, potato-tortita	28.5
26. Sweet-bakery	Cakes, sweet bread, doughnut, churros, muffin	60.5
27. Supplements	NUTRISANO and NUTRIVIDA (dietary supplements administrated by the health ministry)	0.3
28. Soups	Vegetable soup, vegetables cream, fideo soup	77.3
29. Dry soups	Rice, pasta	68.6
30. Avocado and nuts	Avocado and oily seeds (nuts, almond, soy)	39.0

SUPPLEMENTAL TABLE S3.2: Prevalence ratios of overweight-obesity and stunting, and dietary patterns among Mexican adolescents. Not adjusted for EI:BMR ratio

Dietary pattern	Total*	12-15 years§ (n=4,451)	16-19 years§ (n=2,929)	Boys‡ (n=3,610)	Girls‡ (n=3,770)
			Overweight and obesity		
Non-traditional & breakfast/lunch-type	1.05 (0.99-1.11)	1.12 (1.03-1.21)	0.93 (0.84-1.02)	1.02 (0.94-1.11)	1.07 (0.99-1.16)
Western	1.05 (0.99-1.10)	1.09 (1.01-1.17)	0.96 (0.88-1.06)	1.13 (1.04-1.24)	0.98 (0.90-1.06)
Plant-based	0.95 (0.90-1.00)	0.96 (0.89-1.04)	0.93 (0.85-1.02)	0.96 (0.88-1.04)	0.95 (0.88-1.02)
Protein-rich	0.94 (0.89-0.99)	0.92 (0.86-0.98)	0.98 (0.88-1.08)	0.99 (0.90-1.09)	0.90 (0.83-0.97)
			Stunting		
Non-traditional & breakfast/lunch-type	0.86 (0.79-0.94)	0.87 (0.78-0.98)	0.85 (0.76-0.94)	0.87 (0.80-0.95)	0.86 (0.75-0.97)
Western	0.91 (0.84-0.98)	0.94 (0.85-1.04)	0.89 (0.79-0.99)	0.90 (0.81-1.01)	1.01 (0.91-1.11)
Plant-based	0.95 (0.90-1.00)	0.96 (0.89-1.04)	0.93 (0.85-1.02)	0.96 (0.88-1.04)	0.90 (0.83-0.98)
Protein-rich	1.01 (0.95-1.07)	0.98 (0.90-1.08)	1.03 (0.95-1.12)	1.05 (0.95-1.16)	1.05 (0.95-1.17)

*Model adjusted for sex, living area, socioeconomic status, region, age §Model adjusted for sex, living area, socioeconomic status, region and ‡Model adjusted for living area, socioeconomic status, region, age. None of the Prevalence Ratios were adjusted for EI:BMR ratio, for adjusted prevalence ratios see Table 4 and 6.

SUPPLEMENTAL TABLE S3.3. Total energy and nutrient intake across quartiles (Q) of the four dietary patterns.

	Quartile of dietary pattern score				
	Q_1	Q_2	Q_3	Q_4	*p-value*
Non-traditional & breakfast/lunch-type					
EI:BMR ratio (SD)	1.08 (0.42) [2,3,4]	0.85 (0.37) [1,4]	0.88 (0.37) [1,4]	1.01 (0.40)[1,2,3]	*<0.0001*
Energy kcal (SD)	2035.1 (805.4)[2,3,4]	1602.8 (688.0) [1,4]	1627.6 (711.1) [1,4]	1935.5 (774.6)[1,2,3]	*<0.0001*
Protein % (SD)	10.4 (1.7) [2,3,4]	10.7 (2.1) [1,3,4]	11.6 (2.2) [1,2,4]	12.6 (2.4) [1,2,3]	*<0.0001*
Fat % (SD)	22.3 (8.2)[2,4]	24.9 (7.7)[1,3,4]	27.7 (7.1)[2,4]	29.6 (6.3)[1;2;3]	*<0.0001*
Carbohydrates % (SD)	67.2 (8.8)[2;3;4]	64.1 (8.6) [1,4]	60.6 (8.2) [1,4]	57.7 (7.7) [1;2;3]	*<0.0001*
Fiber g (SD)	27.3 (12.3)[2,3,4]	19.4 (9.1)[1,3,4]	17.4 (9.4) [1,2]	18.4 (9.5) [1,2]	*<0.0001*
Sugar g (SD)	13.7 (20.0)[3,4]	13.5 (20.2)[3,4]	16.5 (22.8)[1,2,4]	21.2 (23.9)[1,2,3]	*<0.0001*
Ca mg (SD)	885.2 (450.4)[2,3,4]	677.9 (364.6)[1,3,4]	731.3 (388.0)[1,2,4]	1075.7 (472.4)[1,2,3]	*<0.0001*
Fe mg (SD)	13.7 (5.8)[2,3,4]	9.9 (4.9)[1,4]	9.9 (5.2)[1,4]	12.6 (6.2)[1,2,3]	*<0.0001*
Zn mg (SD)	7.6 (3.3)[2,3,4]	6.0 (3.2)[1,3,4]	6.5 (3.1)[1,2,4]	8.6 (3.8)[1,2,3]	*<0.0001*
Vit C mg (SD)	54.7 (78.0) [2,3,4]	60.7 (78.1) [1,3,4]	70.2 (80.1) [1,2,4]	97.0 (93.4))[1,2,3]	*<0.0001*
Vit A	2306.0 (3262.2)[2,3,4]	2642.2 (3414.9) [1,3,4]	3162.8 (3542.6) [1,2,4]	4928.1 (4612.2) [1,2,3]	*<0.0001*
Folate	236.1(132.2)[2,3,4]	192.7 (109.0)[1,4]	197.9 (111.2)[1,3]	249.6(126.5)[1,2,3]	*<0.0001*
Vit B12	1.3 (1.4) [2,3,4]	1.5 (1.5) [1,3,4]	2.0 (1.5) [1,2,4]	3.2 (2.1))[1,2,3]	*<0.0001*
Western					
EI:BMR ratio (SD)	0.85 (0.37)[3,4]	0.85 (0.35)[3,4]	0.95 (0.37)[1,2,4]	1.189 (0.42)[1,2,3]	*<0.0001*
Energy kcal (SD)	1558.5 (677.8)[3,4]	1562.8 (647.4) [3,4]	1796.4 (689.5)[1,2,4]	2301.3 (807.4)[1,2,3]	*<0.0001*
Protein % (SD)	11.2 (2.0) [,4]	11.3 (2.2) [,4]	11.3 (2.4) [,4]	11.1 (2.4) [1,2,3]	*<0.0001*
Fat % (SD)	21.3 (7.4)[2,3,4]	25.0 (7.0) [1,3,4]	28.1 (7.4)[1,2,4]	31.1 (6.6) [1,2,3]	*<0.0001*
Carbohydrates % (SD)	67.2 (8.6) [2,3,4]	63.6 (8.3) [1,3,4]	60.5 (8.4) [1,2,4]	57.6 (8.2) [1,2,3]	*<0.0001*
Fiber g (SD)	21.7 (10.97)[2,3,4]	19.4 (10.3)[1,4]	19.7 (11.0) [1,4]	20.6 (11.2) [1,2,3]	*<0.0001*
Sugar g (SD)	8.2 (13.2) [2,3,4]	13.7 (17.1) [1,3,4]	19.2 (21.0) [1,2,4]	29.3 (27.5) [1,2,3]	*<0.0001*
Ca mg (SD)	837.2 (444.1)[2,4]	762.1 (407.8)[1,3,4]	808.4 (448.1)[2,4]	896.8 (474.8)1,2,3	*<0.0001*
Fe mg (SD)	10.2 (5.4)[3,4]	9.9 (5.1) [3,4]	11.4 (5.4)[1,2,4]	14.3 (6.1)[1,2,3]	*<0.0001*
Zn mg (SD)	6.2 (3.0) [3,4]	6.2 (3.0) [3,4]	7.2 (3.3) [1,2,4]	9.2 (3.8) [1,2,3]	*<0.0001*
Vit C mg(SD)	77.8 (96.2)[2,3]	61.8 (80.2)[1,4]	66.3 (74.5) [1,4]	77.5 (82.3) [2,3]	*<0.0001*
Vit A	3600.3 (4593.7) [2,3]	2961.0 (3604.7) [1,4]	2922.1 (3594.3) [1,4]	3351.3 (3642.3) [2,3]	*<0.0001*
Folate	201.9 (118.8) [3,4]	197.0 (111.4) [3,4]	218.6 (118.5) [1,2,4]	257.0 (132.9) [1,2,3]	*<0.0001*
Vit B12	1.3 (1.5)[2,3,4]	1.6 (1.5)[1,3,4]	2.0 (1.7)[1,2,4]	3.0 (2.0)[1,2,3]	*<0.0001*
Plant-based					
EI:BMR ratio (SD)	0.75 (0.30)[2,3,4]	0.85 (0.33)[1,3,4]	0.99 (0.35)[1,2,4]	1.31 (0.41)[1,2,3]	*<0.0001*
Energy kcal (SD)	1423.2 (589.0) [2,3,4]	1581.0 (608.9)[1,3,4]	1846.3 (656.1)[1,2,4]	2448.2 (796.0)[1,2,3]	*<0.0001*
Protein % (SD)	11.6 (2.5) [2,3,4]	11.3 (2.2) [1,3,4]	11.1 (2.2) [1,2,4]	10.9 (2.2 [1,2,3]	*<0.0001*
Fat % (SD)	27.4 (8.4) [2,3,4]	26.0 (7.8) [1,3,4]	26.2 (7.7) [1,2,4]	26.5 (7.1) [1,2,3]	*<0.0001*
Carbohydrates % (SD)	60.9(9.9) [2,3,4]	62.5 (8.9)[1,3,4]	62.1 (8.8) [1,2,4]	62.5 (8.2) [1,2,3]	*<0.0001*
Fiber g (SD)	15.8 (8.6) [2,3,4]	18.1 (9.2)[1,3,4]	21.0 (9.7) [1,2,4]	28.1 (11.7) [1,2,3]	*<0.0001*
Sugar g (SD)	9.2 (19.5) [2,3,4]	13.7 (20.5) [1,3,4]	17.2 (21.3) [1,2,4]	24.6 (24.2) [1,2,3]	*<0.0001*
Ca mg (SD)	643.5 (356.7) [2,3,4]	744.2 (382.2)[1,3,4]	852.8 (411.0) [1,2,4]	1122.3 (485.2) [1,2,3]	*<0.0001*
Fe mg (SD)	9.5 (4.8) [2,3,4]	10.0 (5.0)[1,3,4]	11.6 (5.2) [1,2,4]	15.2 (6.2) [1,2,3]	*<0.0001*
Zn mg (SD)	5.9 (2.8) [2,3,4]	6.4 (2.9) [1,3,4]	7.2 (3.2) [1,2,4]	9.6 (3.8) [1,2,3]	*<0.0001*
Vit C mg (SD)	35.9 (45.9) [2,3,4]	54.5 (51.2) [1,3,4]	79.8 (69.4) [1,2,41,2,4]	147.3 (104.2) [1,2,3]	*<0.0001*
Vit A	1719.7 (1874.3) [2,3,4]	2500.5 (2606.6) [1,3,4]	3667.1 (3283.1) [1,2,4]	6309.7 (4908.1) [1,2,3]	*<0.0001*
Folate	178.3 (107.8) [2,3,4]	191.0 (102.8) [1,3,4]	218.3 (110.9) [1,2,4]	296.3 (130.7) [1,2,3]	*<0.0001*
Vit B12)	1.6 (1.5) [3,4]	1.7 (1.5) [3,4]	2.0 (1.8) [1,2,4]	1.9 (1.8) [1,2,3]	*<0.0001*
Protein-rich					
EI:BMR ratio	0.83 (0.36)[2,3,4]	0.88 (0.37)[1,3,4]	0.97 (0.39)[1,2,4]	1.17 (0.42)[1,2,3]	*<0.0001*
Energy kcal (SD)	1434.2 (662.0)[2,3,4]	1637.2 (688.2)[1,3,4]	1805.05 (736.4)[1,2,4]	2226.1 (803.3)[1,2,3]	*<0.0001*
Protein % (SD)	10.3 (2.2) [2,3,4]	11.0 (2.2) [1,3,4]	11.5 (2.1) [1,2,4]	12.0 (2.2) [1,2,3]	*<0.0001*
Fat % (SD)	25.3 (8.2) [,3,4]	25.4 (7.7) [,3,4]	27.0 (7.5) [1,2,4]	28.1 (7.3) [1,2,3]	*<0.0001*
Carbohydrates % (SD)	64.1 (9.2) [2,3,4]	63.5 (8.8) [1,3,4]	61.4 (8.6) [1,2,4]	59.7 (8.6) [1,2,3]	*<0.0001*
Fiber g (SD)	15.9 (8.7) [2,3,4]	18.5 (9.1) [1,3,4]	21.2 (10.3) [1,2,4]	27.0 (11.0) [1,2,3]	*<0.0001*
Sugar g (SD)	18.1 (23.1)[2,3]	14.2 (20.5)[4,1]	15.5 (22.1)[4,1]	17.2 (22.3) [2,3]	*<0.0001*
Ca mg (SD)	726.1 (402.2) [2,3,4]	769.3 (427.9) [1,3,4]	834.1 (440.7) [1,2,4]	973.5 (476.5) [1,2,3]	*<0.0001*
Fe mg (SD)	9.3 (5.0) [2,3,4]	10.3 (5.0) [1,3,4]	11.9 (5.3) [1,2,4]	14.8 (6.2) [1,2,3]	*<0.0001*
Zn mg (SD)	5.7 (2.9) [2,3,4]	6.5 (2.9) [1,3,4]	7.4 (3.3) [1,2,4]	9.5 (3.8) [1,2,3]	*<0.0001*
Vit C mg (SD)	56.1 (73.9) [2,3,4]	64.4 (78.5) [1,3,4]	76.7 (83.8) [1,2,4]	85.6 (95.0) [1,2,3]	*<0.0001*
Vit A	2257.0 (3323.2) [2,3,4]	2773.0 (3267.6) [1,3,4]	3522.7 (3536.8) [1,2,4]	4444.3 (4796.2) [1,2,3]	*<0.0001*
Folate	157.3 (88.7) [2,3,4]	190.6 (95.4) [1,3,4]	229.6 (106.9) [1,2,4]	308.3 (133.4) [1,2,3]	*<0.0001*
Vit B12	1.5 (1.5) [2,3,4]	1.7 (1.6) [1,3,4]	2.0 (1.8) [1,2,4]	2.6 (2.1) [1,2,3]	*<0.0001*

Q-quartile; median, SD- standard deviation; [1,2,3,4] indicate statistically significant results between quartiles (p-value <0.05).

Part II
Primary Analysis

Chapter 4

Depressive symptoms among Mexican adolescent girls in relation to iron status, anemia, body weight and pubertal status: results from a Latent Class Analysis

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Abstract

Objective: The study examined the association between depressive symptoms and iron status, anemia, body weight, and pubertal status among Mexican adolescent girls.

Design: In this cross-sectional study, depressive symptoms were assessed by the 6-item Kutcher Adolescent Depressive Scale (6-KADS), and latent class analysis (LCA) was used to identify and characterize groups of girls based on depressive symptoms. Iron status and inflammation were assessed using ferritin and soluble transferrin receptor, C-reactive protein and alpha-1-acid glycoprotein, respectively. Multiple logistic and linear regression were applied to model class membership as a function of iron status, anemia, body weight, and pubertal status.

Participants: We collected data from 408 girls aged 12-20 years.

Setting: Public schools in northern Mexico.

Results: LCA yielded three classes of depressive symptoms; 44.4% of the adolescents were "unlikely to be depressed", 41.5% were "likely to be depressed", and 14.1% were "highly likely to be depressed". Our analyses demonstrated that iron deficient girls had greater odds of being "likely depressed" (odds ratio, OR=2.01, 95% CI 1.01-3.00) or "highly likely depressed (OR=2.80, 95% CI 1.76-3.84). Linear regression analyses revealed that lower hemoglobin concentrations and higher body weight increased the probability of being "likely depressed". There was no evidence that depressive symptoms were associated to age at menarche and years since menstruation.

Conclusion: This study shows that iron deficient adolescent girls are more likely to suffer from depressive symptoms, and that lower concentrations of hemoglobin and higher body weight increased the probability of experiencing depressive symptoms.

Introduction

Previous observational studies have reported an association between iron deficiency and depressive symptoms in children and adolescents [1]. Adolescence is a period of intensive brain remodeling, and iron has a role in various neurological functions like myelin production, synaptogenesis, and production of neurotransmitters, i.e. serotonin, norepinephrine, and dopamine [2,3]. Disrupted myelination and lower concentration of these neurotransmitters are common in depressed persons [4,5]. Randomized controlled trials in women with postpartum depression have shown that iron supplementation improves depressive symptoms in iron deficient and non-iron deficient women [6]. In addition, low hemoglobin concentration has been associated with an increased odds of adult depression by 43% (95% CI 1.23 to 1.65) [4]. Anemia and depression share symptoms such as fatigue, which may be the result of altered cerebral oxygen transportation. However, little attention has been paid to the role of iron deficiency and anemia in depression among adolescents, especially girls who recently started their menses.

The low-degree chronic inflammation produced by adiposity might also represent a risk factor for depression. Longitudinal studies showed that body mass index (BMI) at an age of 14 years predicted BMI and inflammatory markers at age of 17 years, while inflammation was also associated with depressive symptoms [7]. In addition, depressive symptoms were associated with increased interleukin-6 responses among adolescents with higher BMI, but not among those with lower BMI [8]. The link between high BMI and depressive symptoms might not be explained exclusively through the biologically plausible pathways of adiposity and inflammation but also by psychosocial pathways of self-esteem, body dissatisfaction and social support [9–11]. Furthermore, we showed earlier that overweight and obesity increases the risk for iron deficiency [12].

After pubertal onset, prevalence of depression in girls is approximately twice that of boys [13–15]. In a survey among Mexican high school students, depressive symptoms were reported by 34% and 18% of female and male students, respectively [9]. Pubertal development may be the mechanism that underlies the gender difference in rates of depression. Clinical and epidemiological evidence suggests that fluctuations in hormonal concentrations, particularly in estrogens, may influence the regulation of the hypothalamic pituitary adrenal (HPA) axis and this may alter the neurotransmitter systems [10,16]. Anomalous HPA-axis function has been

associated with the onset of depression in adolescents, and this effect seems to be dependent on the pubertal stage [11]. In addition, the rise of androgens during puberty, most notably dihydrotestosterone and testosterone, are involved in the hippocampus development, and larger hippocampal volume is associated with risk of depression [17]. Thus, puberty is a critical period of development in which examining factors associated with depression is essential.

This study aimed to examine the association between iron status and depressive symptoms in Mexican adolescent girls. In addition, we also explored whether haemoglobin concentration, body weight and pubertal onset were associated with depressive symptoms.

Methods

Participants

We conducted a cross-sectional study in the cities of Santa Catarina and Monterrey, in Northern Mexico, from September 2018 to January 2019. Adolescent girls aged 12-20 years from public schools were recruited, and written informed consent was obtained from the adolescents and their parents. Exclusion criteria were: diagnosis of systemic disease that may affect iron status, history of major surgery in the last month, and regular use of medication (except contraceptives). After applying these criteria, a total of 408 adolescent girls were recruited. Five girls had incomplete questionnaires and therefore were excluded from this analysis.

Measurements

Depressive symptoms: In the remainder of this paper, "depressive symptoms" refers to symptoms experienced by adolescents, such as depressed mood, loss of interest, reduced energy leading to increased fatigability and diminished activity. To assess depressive symptoms, we used the 6-item Kutcher Adolescent Depression Scale (6-KADS) [18] (Appendix 1), a questionnaire that has been translated from English to Spanish and has been validated for use in Latino adolescents [19]. The questionnaire was applied individually and as privately as possible by trained staff members. Six questions briefly describe the characteristic symptomatology of depression, with a Likert scale of four points (0="almost never" to 3="all the time"). The overall score consisted of the sum of the score of all the items. Therefore, the total score ranges between 0-18. Traditionally, individuals are classified as not showing

depressive symptoms if the overall score is 0–6 and as showing depressive symptoms if the score is ≥6.

Iron status: A trained staff member drew venous blood samples for subsequent assessment of iron indicators (serum concentrations of ferritin and soluble transferrin receptor) and inflammatory markers (serum concentrations of C-reactive protein and alpha-1-acid glycoprotein). Blood samples were drawn at school facilities between 8:00 am to 12:00 pm approximately; not all the participants had fasted. Serum samples were stored at -80˚C. Iron and inflammation concentrations were measured using a sandwich enzyme-linked immunosorbent assay by the Vitmin Lab, Willstaett, Germany [20]. Depleted iron stores were defined as serum concentrations of ferritin <15µg/L [21]. The values for soluble transferrin receptor reported by the VitMin Lab are in the same range as the RAMCO assay. Therefore, iron-deficient erythropoiesis was defined as an soluble transferrin receptor concentration >8.3mg/L [20].

Anemia: Anemia was defined by capillary blood hemoglobin concentrations <120 g/L [22]. measured by a HemoCue 201+ portable photometer (Hemocue AB., Ängelholm, Sweden).

Anthropometric measurements: Body weight was measured on a calibrated platform scale with a bar (SECA 700), to the nearest 100g, and with participants wearing light clothes. Height was measured in centimeters with the subject barefoot using a stadiometer (SECA 213). Both body weight and height were measured in duplicate and using standardized techniques. A third measure was done if the two measures differ by more than 500g or 1cm, respectively. BMI-for-age (BAZ) was used to classify weight status according to the World Health Organization (WHO) growth standards [23]. BAZ was categorized as obese (> 2SD), overweight (> 1SD), normal (-2SD to 1SD), and thin (< -2SD).

Age at menarche*:* Self-reported age at menarche (first menstruation, reported in years) was used as a proxy indicator for onset of puberty.

Years since menarche: self-reported age at menarche was subtracted from the chronological age (reported in years).

Data analysis

The statistical program IBM SPSS 27.0 was used to calculate the descriptive statistics of general characteristics of the study population. Latent GOLD 5.1 (https://www.statisticalinnovations.com/latent-gold-5-1/) was used to undertake the Latent

Class Analysis (LCA). LCA is a statistical technique that aims to identify distinctive subgroups of people who share common characteristics so that people within the same subgroup have a similar scoring pattern on the measured variables [24]. To model the association between the different classes of depressive symptoms and the exposure variables (iron status, anemia, body weight and pubertal onset), three main steps were involved [25]: 1) estimating the model with the optimal number of latent classes; 2) classifying the adolescent girls into one of the classes based on the model selected in step 1; 3) examining the relationship between the classes and the exposure variables.

Responses to 6-KADS items from 403 Mexican adolescent girls were available and complete for LCA. First, explanatory LCA with 1-5 classes was conducted with the six 6-KADS items introduced as ordinal indicators. We selected the model with the lowest value for the Bayesian Information Criterion (BIC), which indicates the balance between model fit and model simplicity. It is a basic assumption from LCA that external variables are not correlated within the identified classes, which is known as conditional independence [24]. After identifying the optimal class solution, we examined the conditional independence between latent class indicators and the exposure variables by inspecting the bivariate residuals (BVR) after including each exposure variable one by one (**Supplemental Table S5.1**). BVR values higher than 3.0 indicate a residual association between variables [25]. We concluded that BMI-for-age was correlated with item-3 and item-6 of the 6-KADS questionnaire. We re-estimated the model, including BMI-for-age as an active covariate, thus correcting the encountered effect between BMI-for-age and items 3 and 6. This correction resulted in a reduction in the BIC value. Second, we used posteriori classification to assign each of the adolescent girls to one of the three latent classes from the corrected model, and this classification information was saved. Third, we used two types of exposure variables, nominal (for iron deficiency and iron-deficient erythropoiesis), and continuous (for hemoglobin, BMI z-scores, years since menstruation, and age at menarche), which we modelled using a multinomial logistic regression and linear regression, respectively. We conducted separate models for each exposure variable with class membership as the dependent variable. Maximum likelihood adjustment was used to correct for classification error bias.

Results

General characteristics of the study sample

Data of 403 Mexican adolescent girls (mean age 15.2 (SD 1.8) years) were analyzed, of whom 94 (23.3%) had a 6-KADS score equal or higher than 6, indicating evidence of depression. About a quarter of the participants suffered from anemia (21.8%), and 10.9% from iron deficiency. Overweight or obesity were present in almost half of the adolescents (42.7%). Detailed descriptive statistics are shown in **Table 4.1**.

Table 4.1. Summary statistics of the total sample and the three latent classes of depressive symptoms

Variable	Total sample	"Unlikely depressed"	"Likely depressed"	"Highly likely depressed"
N	403	187	159	57
Age (years)*	15.2 (1.8)	15.0 (1.7)	15.2 (1.9)	15.8 (1.8)
Age at menarche (years)*	11.8 (1.3)	11.8 (1.4)	11.7 (1.2)	12.0 (1.5)
Prevalence of depressive symptoms (6-KADS score >6)	94 (23.3%)	0 (0%)	37 (23.3%)	57 (100 %)
Prevalence of anemia	88 (21.8%)	33 (17.6%)	38 (23.9%)	17 (28.8%)
Prevalence of iron deficiency	44 (10.9%)	16 (8.6%)	19 (11.9%)	9 (15.8%)
Prevalence of iron deficiency erythropoiesis	14 (3.5%)	7 (3.7%)	6 (3.8%)	1 (1.8%)
Body mass index-for-age				
Normal (-2 SD to 1 SD)	228 (56.6%)	119 (63.6%)	78 (49.1%)	31 (54.4%)
Overweight (> 1 SD)	101 (25.1%)	42 (22.5%)	47 (29.6%)	12 (21.1%)
Obese (> 2 SD)	71 (17.6%)	25 (13.4%)	32 (20.1%)	14 (24.6%)
CRP (mg/L) †	0.3 (0.0, 1.1)	0.2 (0.0, 0.8)	0.3 (0.0, 1.9)	0.4 (0.0, 2.0)
AGP (g/L) †	0.6 (0.5, 0.8)	0.6 (0.5, 0.8)	0.7 (0.5, 0.8)	0.6 (0.5, 0.8)

*mean values(SD), †median values (Q_{25}, Q_{75}) or n(%); Depressive symptoms if 6-KADS score was ≥ 6; Anemia defined as hemoglobin levels <120 g/L; Iron deficiency was defined as concentrations of serum ferritin <15µg/L; Iron-deficient erythropoiesis was defined as an soluble transferrin receptor concentration >8.3mg/L. CRP, AGP: serum concentrations of C-reactive protein and α_1-acid glycoprotein

Heterogeneity of depressive symptoms

The LCA analysis indicated three distinctive classes for the degree of depressive symptoms. When BMI-for-age was entered in the model, the BIC value changed from 4332.5 to 4321.3, indicating the most optimal balance between model fit and model simplicity, and was therefore, the preferred solution for classification (**Table 4.2).** Based on this model, the percentages of individuals with self-reported scores of almost never in each of the six items of the 6-KADS questionnaire are shown in **Table 4.3**. Class 1 was the largest subgroup, constituting 44.4% of the adolescent girls, who were "unlikely depressed". Class 2 (41.5%) was labelled as "likely depressed" because the response pattern reflected moderate occurrence (sometimes) for four out of six of the 6-KADS items. Class 3 was composed of 14.1% of the adolescents and was described by a higher occurrence of depressive symptoms, with more adolescent girls reporting suicidal or self-harm ideation than the other two subgroups. Thus, the last class was labelled as "highly likely depressed."

Table 4.2. Comparison of models with different number of classes derived from latent class analysis

Mexican adolescent girls (n=403)	BIC	AIC	Npar	L^2	Df	p-value
1-class model	4670.5	4598.5	18	999.3	385	<0.001
2-class model	4344.7	4244.7	25	631.5	378	<0.001
3-class model	4332.5	4204.5	32	577.3	371	0.31
4-class model	4356.5	4200.5	39	559.3	364	0.17
5-class model	4378.4	4194.5	46	539.3	357	0.61
3-class model *	**4321.3**	**4181.6**	**35**	**4111.6**	**365**	**0.39**

Bayesian Information Criterion (BIC), and Akaike Information Criterion (AIC) are both indicators of parsimony, i.e., the balance between model fit and model simplicity; the lower their value, the better this balance. Number of parameters (Npar); the lowest Npar indicates the model with the most parsimony. Degrees of freedom (df). Likelihood-ratio chi-squared goodness-of-fit statistic (L^2) indicates the amount of the association among the variables that remained unexplained after estimating the model; the lower the value, the better the fit of the model to the data. P-value from the bootstrap provides a more precise estimate by relaxing the assumption that the L^2 statistic follows a chi-square distribution; p-values < 0.05 indicate no improvement over the previous model. * Model with BMI-for-age as continuous covariate with direct effect on 6-KADS question 3 and 6. Bold values designates the selected model, with the best balance between model fit and model simplicity.

Table 4.3. Description of the selected 3-class latent class model with BMI-for-age as continuous covariate with direct effect on 6-KADS question 3 and 6.

Over the last week, how have you been "usually" regarding the following items:	Latent classes				
	Total sample	**"Unlikely depressed"**	**"Likely depressed"**	**"Highly likely depressed"**	**p-value**
Prevalence	**100%**	**44.4%**	**41.5%**	**14.1%**	
1. **Low mood, sadness, feeling blah or down, depressed, just can't be bothered.**					<0.0001
Almost never	0.315	0.615	0.101	0.000	
Sometimes	0.524	0.379	0.754	0.294	
Almost all the time	0.139	0.005	0.143	0.554	
All the time	0.022	0.000	0.003	0.151	
2. **Feelings of worthlessness, hopelessness, letting people down, not being a good person.**					0.010
Almost never	0.544	0.818	0.427	0.017	
Sometimes	0.355	0.178	0.527	0.407	
Almost all the time	0.069	0.004	0.046	0.347	
All the time	0.032	0.000	0.002	0.229	
3. **Feeling tired, feeling fatigued, low in energy, hard to get motivated, have to push to get things done, want to rest or lie down a lot.**					0.036
Almost never	0.223	0.322	0.185	0.022	
Sometimes	0.474	0.559	0.431	0.340	
Almost all the time	0.228	0.115	0.322	0.303	
All the time	0.074	0.004	0.062	0.335	
4. **Feeling that life is not very much fun, not feeling good when usually would feel good, not getting as much pleasure from fun things as usual.**					0.006
Almost never	0.697	0.960	0.603	0.132	
Sometimes	0.241	0.039	0.359	0.540	
Almost all the time	0.055	0.001	0.038	0.275	
All the time	0.007	0.000	0.000	0.053	

5. Feeling worried, nervous, panicky, tense, keyed up, anxious.					0.009
Almost never	0.385	0.548	0.326	0.029	
Sometimes	0.434	0.407	0.496	0.342	
Almost all the time	0.124	0.033	0.145	0.353	
All the time	0.057	0.011	0.033	0.276	
6. Thoughts, plans or actions about suicide or self-harm.					0.10
Almost never	0.888	0.988	0.882	0.587	
Sometimes	0.077	0.012	0.105	0.205	
Almost all the time	0.020	0.000	0.013	0.102	
All the time	0.015	0.000	0.000	0.106	

Values indicate conditional probabilities. For example, given membership of class 1, the probability of responding 'Almost never' to the first item is 0.615. Entries are given as response probabilities in each item of the 6-KADS questionnaire. Low p-values indicate that the questionnaire item was useful to differentiate between class membership.

Associations of class membership and iron status

We examined the association of depressive symptoms with iron deficiency and iron deficient erythropoiesis using multiple logistic regression models adjusted for other potential confounders, years since menstruation, BMI z-scores, and markers of inflammation (**Table 4.4**). Compared to their peers with normal ferritin concentrations, iron deficient girls (serum ferritin <15µg/L) had higher odds of being "likely depressed" and "highly likely depressed" (OR 2.01; 95%CI 1.01-3.00 and OR 2.80; 95% CI 1.76-3.84, respectively). In contrast, we found no evidence of an association between iron deficient erythropoiesis (soluble transferrin receptor concentration >8.3mg/L) and depressive symptoms.

Associations of class membership with hemoglobin, body weight, and puberty

The associations between depressive symptoms and hemoglobin concentration, body weight and puberty are reported in **Table 4.5**. Each unit-increment in hemoglobin concentration (1 g/L) increased the probability of membership in the class "unlikely depressed" by 16 percentage points and reduced the probability of membership in the class "likely depressed" by 17 percentage points (p=0.04). Each unit-increment in BMI-for-age (1 SD) increased the probability of being "likely depressed" by 22%, but reduced the probability of being "unlikely depressed" by 18% and "highly likely depressed" by 4%. Each increment in chronological age by 1 year increased the probability of being "highly likely depressed" by 20%. We assessed the association between puberty and depressive symptoms with two different indicators, years since menstruation and age at menarche, but we found no evidence of an association between puberty and depressive symptoms.

Table 4.4. Probability of latent class membership for exposure variables: results obtained by multinomial logistic regression analysis.

	Latent Classes			
Exposure variable	**"Unlikely depressed"**	**"Likely depressed"**	**"Highly likely depressed"**	**p-value**
	OR (95%CI)	OR (95%CI)	OR (95%CI)	
Iron deficiency				
Iron deficiency (serum ferritin concentration <15µg/L)				
Yes	1.0 [reference]	[reference]	[reference]	
No	1.0 [reference]	1.51 (0.59, 2.43)	2.34 (1.38, 3.30) [a]	0.23
Iron deficiency (serum ferritin concentration <15µg/L) [†]				
Yes	1.0 [reference]	[reference]	[reference]	
No	1.0 [reference]	2.01 (1.01, 3.00)	2.80 (1.76, 3.84)	0.11
Iron deficiency erythropoiesis				
Iron deficient erythropoiesis (sTfR concentration >8.3mg/L)				
No	1.0 [reference]	[reference]	[reference]	
Yes	1.0 [reference]	1.28 (-0.19, 2.75)	0.80 (-1.24, 2.83)	0.91
Iron deficient erythropoiesis (sTfR concentration >8.3mg/L) [†]				
No	1.0 [reference]	[reference]	[reference]	
Yes	1.0 [reference]	0.70 (-0.57, 1.97)	0.62 (-1.54, 2.78)	0.83

sTfR: soluble transferrin receptor

Values indicate odds ratios, obtained by multinominal logistic regression analyses with class 1 ("unlikely depressed") as the reference category. P-values are based on Wald statistics. sTfR, CRP, AGP: serum concentrations of soluble transferrin receptor, C-reactive protein and α1-acid glycoprotein. [a] Interpretation: girls with iron deficiency have 134.0 % higher odds of being "highly likely depressed" compared to girls without iron deficiency. [†]Iron deficiency model adjusted for years since menstruation, BMI z-scores, CRP and AGP, Iron deficient erythropoiesis model adjusted for years since menstruation, age at menarche, BMI z-scores, CRP and AGP.

Table 4.5. Probability of latent class membership for exposure variables: results obtained by linear regression analysis.

	Latent Classes			
Exposure variable	**"Unlikely depressed"**	**"Likely depressed"**	**"Highly likely depressed"**	**p-value**
	β (s.e)	β (s.e)	β (s.e)	
Hemoglobin				
Intercept	-1.65 (0.99)	2.50 (0.95)	-0.85 (0.98)	
Hemoglobin (g/L)	0.16 (0.08) [a]	-0.17 (0.07)	0.01 (0.07)	0.04
Body weight				
Intercept	0.55 (0.10)	0.14 (0.13)	-0.69 (0.13)	
BMI z-scores	-0.18 (0.07)	0.22 (0.09)	-0.04 (0.10)	0.004
Pubertal onset				
Age				
Intercept	2.63 (0.77)	1.37 (0.96)	-4.00 (1.18)	
Age (y)	-0.14 (0.05)	-0.06 (0.06)	0.20 (0.07)	0.005
Years since menstruation [†]				
Intercept	2.44 (1.01)	1.55 (<0.001)	-3.99 (<0.001)	
Years since menstruation (y)	0.52 (0.49)	-0.06 (0.06)	-0.46 (0.50)	0.38
Age at menarche				
Intercept	0.83 (0.76)	0.39 (0.93)	-1.22 (1.96)	
Age at menarche (y)	-0.04 (0.06)	-0.01 (0.08)	0.04 (0.10)	0.85

BMI: body mass index

Values indicate β estimates with standard errors, obtained by multinominal linear regression analyses. P-values are based on Wald statistics. [a] For example: Probability of being member of the class "unlikely depressed" = -1.65 + 0.16 (hemoglobin, g/L). Interpretation: each increment in hemoglobin, g/L by 1 unit increases the probability of membe rship in class "unlikely depressed" by 16 percentage points. [†]Model adjusted for age at menarche, and age

Discussion

Three distinct subtypes of depression were derived from the LCA, in which the severity of the symptoms was the source of heterogeneity. Our results indicate that depression was associated with iron deficiency, lower hemoglobin concentration, higher body weight, and age. There was no association between depression and puberty.

In the current study, iron deficient girls had a higher chance to be "likely depressed" (OR=2.01, 95% CI 1.01-3.00) or "highly likely depressed (OR=2.80, 95% CI 1.76-3.84) compared to non-iron deficient girls. These findings are in accordance with other cross-sectional studies indicating that depression scores were higher in women with iron deficiency (OR 2.84 95% CI:1.24–6.51) [26] and adolescents with iron deficiency anemia (OR = 2.34, 95% CI: 1.58 - 3.46) [27]. However, cross-sectional studies give no evidence of causality, and unfortunately, there is limited availability of longitudinal and intervention studies of iron status and depression among adolescents. A retrospective cohort study in Taiwan showed that 20-year-old women with iron deficiency anemia had a 49% increased risk of depression compared to women without iron deficiency anemia [28]. In addition, randomized controlled trials in women with postpartum depression demonstrated that iron supplementation improves the depressive symptoms in iron deficient and non-iron deficient women [6]. Previous studies have reported the effects of iron on different neurological activities, such as myelination and monoamine metabolism [29]. Therefore, it is plausible that iron deficiency contributes to depression.

Another important finding was that a higher body weight was associated with being in the "likely depressed" group and inversely associated with being "unlikely depressed". Similarly, results from the National Survey of Health and Nutrition in Mexico (ENSANUT-2012) showed that adult women with obesity had higher odds (OR 1.28 95% CI 1.07–1.53) of having depression in comparison with normal-weight women [30]. These findings are consistent with results from a meta-analysis that concluded that children and adolescents with obesity were more likely to be depressed (pooled OR 1.34; 95% CI 1.1, 1.64), but no association was observed for their peers with overweight [31]. Some longitudinal studies have shown that depressive symptoms at baseline were associated with obesity after one year follow-up in white, black and Hispanic American adolescents [32], in adults [33], and in women but not in men [34]. In contrast, baseline obesity did not predict depression at follow-up [32]. Whether

the association between body weight and depression is bidirectional is not entirely elucidated as it is complex and is mediated by multiple biological pathways and psychosocial factors.

Contrary to expectations, this study did not find evidence of an association between depression and pubertal development. A possible explanation for the inconsistency with other studies is the use of different indicators for pubertal stage [35–38]. Puberty comprises two distinct but overlapping processes, adrenarche (early stage) and gonadarche (later stage) [39]. A dramatic rise of steroid hormones marks adrenarche, and is associated with physical changes that include increased skin oil and acne, skeletal maturation, and pubic hair growth. Gonadarche instead is a gradual process that typically starts with breast development for girls and finalizes shortly after menarche [39]. Thus, pubertal timing and pubertal status likely play a different role in the development of depressive symptoms. Another possible explanation might be that the mean age at menarche was similar in the three subgroups of depression, indicating that most girls were in comparable pubertal stages. Future investigations should include participants at different pubertal stages and consider using various indicators of pubertal status and pubertal timing to elucidate the link between puberty and mental health.

The use of LCA as a statistical technique to identify subgroups of depression is a major strength of this study. The use of categorical diagnostic constructs can result in the loss of valuable information about the diagnosis, because those who score just below the diagnostic threshold are regarded as non-cases. LCA overcomes this pitfall and has additional benefits for understanding the biological pathways and treatment opportunities by discriminating subtypes of depression.

There were some limitations to this study which should be considered when interpreting our results. Primarily, because of the observational nature of our study, there may exist unmeasured or residual confounding in the associations of interest'. Furthermore, while our analysis adjusted for common demographic variables, other psychosocial indicators, which are likely to encompass the biological pathways that influence mental health, were not measured. Despite these limitations, our results are a starting point for future studies to investigate the role of nutritional status on mental health.

In conclusion, iron deficient adolescent girls were more likely to suffer from depressive symptoms, and low hemoglobin concentration and higher body weight increased the probability of depression. These findings suggest clinical trials to determine if nutritional status plays a role in depression and if improving nutritional status may alleviate some of its

symptoms. In addition, greater focus on screening and detecting depression in adolescent girls, especially among those with poor nutritional status is required.

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Supplemental Table S5.1. Bivariate residuals for the covariates in relation to the 3-class model of depressive symptoms among Mexican adolescent girls.

	Indicators					
Covariate	**Question 1**	**Question 2**	**Question 3**	**Question 4**	**Question 5**	**Question 6**
Age (y)	0.696	0.345	1.526	0.121	0.010	1.097
Hemoglobin (g/dL)	0.125	0.408	0.427	0.104	0.000	0.027
Ferritin (µg/L)	0.749	0.287	0.043	0.003	0.026	0.287
sTfR (mg/L)	0.158	1.860	0.015	0.190	1.957	0.344
Age at menarche (y)	0.902	0.229	0.022	0.041	0.028	2.536
BMI-for-age (SD)	0.002	0.000	**3.716**	0.518	1.203	**2.859**
CRP (mg/L)	0.502	0.914	0.018	0.372	0.027	0.011
AGP (g/L)	0.888	0.340	0.133	0.030	0.825	0.828

BVR obtained by including the covariates one by one in step-one 3-class model. Using a cut-off point of 3.0 we can conclude that BMI-for-age has direct effect on items 3 and 6. This suggests that the probability of having a pattern of responses for these items is conditional on BMI. Therefore, we need to include BMI-for-age in the model as covariate, and the two encountered direct effects. sTfR, CRP, AGP: serum concentrations of soluble transferrin receptor, C-reactive protein and α1-acid glycoprotein

Chapter 5

Gut Microbiota Composition and Depressive Symptoms in Mexican Adolescent Girls.

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Abstract

Depression is a common problem among adolescents. Given the growing evidence of gut microbiota being involved in psychiatric disorders including depression, we aimed to identify differences in gut microbiota composition between subgroups of Mexican adolescent girls with depressive symptoms. Fecal samples were collected from 139 girls (N= 71 unlikely depressed, 48 likely depressed, and 20 highly likely depressed). The relative quantification of bacterial taxa was done using 16S ribosomal RNA gene amplicon sequencing. Beta-diversity revealed no significant differences in bacterial composition between participants within the different subgroups of depressive symptoms. Phyla and genera showed no nominal differences ($p < 0.05$) between subgroups. However, several other variables (BAZ, ferritin, CRP, and AGP) that may be associated with depression seemed to explain some of the variation in beta diversity, and suggest a potential link between iron metabolism, inflammation and depression.

Introduction

Depressive disorders are one of the leading causes of disability worldwide, affecting an estimated of 280 million people [1]. In a survey among Mexican high school students in 2007, depressive symptoms were reported by 34% and 18% of female and male students, respectively [2]. Although depression in adolescents is a major public health concern in Mexico, the available data on contributing factors for depression at the national level is scarce. Characterized by low mood or loss of interest and often accompanied by feelings of guilt, hopelessness and changes in appetite and sleep, major depressive disorder (MDD) significantly impairs daily functioning [3]. The clinical and diagnostic features of depression in adolescents and adults are similar, except for irritability, a diagnostic symptom in adolescents [4]. Psychiatric illnesses frequently first manifest during the teenage years (10-19 years). After pubertal onset, prevalence of depression in girls is approximately twice that of boys. Pubertal development may be the mechanism that underlies the sex difference in rates of depression [5,6]. Clinical and epidemiological evidence suggests that fluctuations in hormonal concentrations, particularly in estrogens, may influence the regulation of the hypothalamic pituitary adrenal (HPA) axis, which in turn alters the neurotransmitter systems [7]. All these physiological changes mirrored inside the intestine [8].

Depressive disorders are caused by multiple factors and are the result of a complex interaction between an individual's genetics and the environment [9]. Epidemiological data highlight the association between nutrition and mental health but do not provide information about causality or underlying mechanism [10–12]. The composition, structure and function of the brain are dependent on the availability of appropriate nutrients, including iron [12]. In a previous study we found that iron deficient adolescent girls are more likely to suffer from depressive symptoms, and that lower concentrations of hemoglobin increased the probability of experiencing depressive symptoms [13].

There is a growing appreciation of the gut microbiota's role in all aspects of health and disease, including mental health, especially in psychiatric and neurological conditions such as MDD. Results from a systematic review show higher relative abundance of the genera *Anaerostipes, Blautia, Clostridium, Klebsiella, Lachnospiraceae incertae sedis, Parabacteroides, Parasutterella, Phascolarctobacterium, and Streptococcus,* and lower relative abundance of *Bifidobacterium, Dialister*, *Escherichia/Shigella, Faecalibacterium,* and *Ruminococcus* in participants with depression compare to controls [14]. The same literature review found

differences in beta-diversity between participants with depression and controls, but in terms of alpha diversity, results were divergent [14].

The aim of this paper is to investigate the relationship between the gut microbiota composition and the presence of depressive symptoms in relation to iron deficiency in Mexican adolescent girls.

Methods

Study participants

To evaluate differences in gut microbiota composition between participants with different patterns of depressive symptoms, fecal samples from 139 adolescent girls enrolled in the Ten2Twenty project in Mexico, who voluntarily donated a fecal sample. The Ten2Twenty-Mexico project is a cross-sectional study with a total of 408 participants aged 12-20 years old, which aims to investigate the interrelationships between nutritional exposure and health outcomes among adolescent girls in Mexico, with specific focus on iron deficiency and depression. Exclusion criteria were: diagnosis of systemic disease that may affect iron status, history of major surgery in the last month, and regular use of antibiotics and other medication (except contraceptives). We obtained written informed consent from the adolescents and their legal guardians [13]. The study was approved by the Research, Biosafety and Bioethics Committees of the School of Medicine of the Universidad de Monterrey, Mexico (Reference 04072018-CEI).

To assess depressive symptoms, we used the 6-item Kutcher Adolescent Depression Scale (6-KADS) [15]. The 6-KADS briefly describe the characteristic symptomatology of depression in 6 questions, with a Likert scale of four points (0="almost never" to 3="all the time"). The overall score consisted of the sum of the score of all the items. Therefore, the total score ranges between 0-18. Traditionally, individuals are classified as not showing depressive symptoms if the overall score is 0‑6 and as showing depressive symptoms if the score is ≥6. Using Latent Class Analysis, participants were classified into three groups with unlikely, likely and highly likely occurrence of depressive symptoms [13]. Additionally, sociodemographic and anthropometric measurements were obtained, and blood samples were collected to measure biomarkers of iron and inflammation status [13].

Microbiota methods and measures

Sample collection, preparation and sequencing

Study participants were provided with a stool collection container and instructed on how to collect and store (at 4 ◦C) a stool sample at home 24 hours prior to the research visit. During the research visit, stool samples were collected and then transported to the laboratory of Microbiology at University of Monterrey to be aliquoted and frozen at -80◦C. The samples were exported to the Netherlands on dry ice and stored at -80 ◦C at the laboratory of Human Nutrition and Health at Wageningen University and Research, until being processed.

Bacterial DNA was extracted by using the Maxwell 16 Total RNA system (Promega, Wisconsin, USA) with fecal sample (0.01 to 0.13g) and Stool Transport and Recovery Buffer (STAR; Roche Diagnostics Corporation, Indianapolis, IN) based on previous literature [16]. The V4 region of 16S ribosomal RNA (rRNA) gene was amplified in duplicate, generating amplicons with a length of around 290bp. Each PCR reaction comprised of 10µl of 5xPhusion Green HF Buffer (Thermo Scientific, US), 1µl of 10µM barcoded primers 515F-n(5'-GTGYCAGCMGCCGCGGTAA-3') and 806R-n(5'- GGACTACNVGGGTWTCTAAT-3') [17,18], 1µl of 10mM dNTPs mix (Promega Corporation, US), 0.5µl of 2U/µl Phusion Green Hot Start II High-Fidelity DNA polymerase (Thermo Scientific, US), 36.5µl of Nuclease-free water and 1µl of 20ng/µl DNA template. PCR was carried out as described earlier with modification: initial denaturation (98°C, 30s), 25 cycles of denaturation (98°C, 10s), annealing (50°C, 10s), extension (72°C, 10s) and elongation (72°C, 7min). The presence and length of PCR products was then verified by gel electrophoresis. PCR products were purified by the HighPrep® PCR kit (MagBio Genomics, Alphen aan den Rijn, Netherlands), according to the instructions of the kit. DNA concentration of purified samples was measured using a fluorometer (DS-11; DeNovix) with Qubit® dsDNA BR Assay Kit (Life Technologies, Leusden, Netherlands). 200ng of barcoded samples was pooled in libraries comprising 69 uniquely tagged samples, two of which were artificial control communities representative of human gut microbiota. The mixture was purified again by HighPrep® PCR kit to a final volume of 40µl. Purified amplicons were then used for 16S rRNA sequencing (Illumina platform) at the Eurofins Genomics Laboratory in Germany. The data of the sequencing was processed in the pipeline NG-*Tax* 2.0 [19] of which taxonomic assignment of amplicon sequence variants (ASVs) was conducted with SILVA_132_SSU 16S rRNA gene reference database[20], a pipeline for analysis of 16S rRNA amplicons from complex biomes using an Illumina Hiseq2000 instrument [21].

Within sample diversity: Three alpha-diversity metrics were applied at the ASV level: (1) chao2, counting the presence or absence of unique ASV in each sample; (2) Shannon diversity index [22], which takes into consideration not only the number of observed unique ASV but also their abundance; and (3) the phylogenetic richness estimator, which estimates microbial diversity across a phylogenetic tree (Faiths' phylogenetic diversity) [23]. The alpha-diversity metrics were calculated using the R packages *Microbiome* [24] and *Picante* [25], and then compared between participants depression groups (i.e., unlikely-, likely-, and highly likely-depressed) using Kruskal-Wallis test.

Between-Sample Diversity Metrics: To assesses beta-diversity, we used the weighted UniFrac distance metric, a phylogenetic-based assessment of the difference in overall bacterial community composition. To analyze the beta-diversity, multivariate statistics were conducted using ADONIS and betadisper functions in the R package *vegan* (version 2.5–7) [26]. Through ADONIS, we determined if the tested variables (i.e., depressive score or group of depressive symptoms) influenced beta-diversity. Betadisper measures the variability in ASV composition among depression groups [27]. To determine and visualize the differences in microbial composition between depression groups, we performed Principal Coordinates Analysis (PCoA) by using the R function *phyloseq::ordinate*.

Taxonomic Composition Analysis and Associations with Depressive Symptoms: Taxonomic composition of the gut microbiota was investigated at the phylum and genus levels after transforming the sequencing read counts into microbial relative abundance (normalization step). Any unknown taxonomic level (e.g., unknown genus) was assigned to the next highest known taxonomic rank (e.g., family). The composition analysis was calculated using the function *tax_glom* from *phyloseq* package 1.36.0 [28]. We used Linear discriminant analysis Effect Size (LEfSe; https://huttenhower.sph. harvard.edu/galaxy) for statistical analysis and visualization of the results. LEfSe uses non-parametric statistics (which is less sensitive to the extreme values), in our case the Kruskal–Wallis sum-rank test, to identify (nominal) statistical differences in the relative abundance of gut microbiota between participants in different groups of depressive symptoms.

Redundancy Analysis: To determine how much variation in microbial composition was explained by external variables such as depressive symptoms score and the selected confounders (i.e., biomarkers of inflammation (C-reactive protein (CRP), and alpha-1-acid

glycoprotein (AGP)), ferritin and hemoglobin concentrations, BMI-for-age z-scores (BAZ), and age) and to investigate if the abundance of species is related to depressive symptoms and the selected confounders, we conducted a multivariate canonical ordination analysis method (i.e., redundancy analysis: RDA) at the genus level [26]. Samples with missing values in confounder variables were omitted. Forward and reverse automatic stepwise model selection for constrained ordination was performed to build a model with variables that significantly explain variation in the data.

Results

Participants' characteristics

The general characteristics of the studied subsample are presented in **Table 5.1**. Mean age was 15.0 years. Median values for hemoglobin, ferritin and CRP concentrations were similar among the three groups of depressive symptoms. BAZ and AGP values were significantly lower in the group unlikely depressed compared to the likely depressed. group

Microbiota measures

Within-and between-sample diversity metrics: None of the three alpha-diversity (within-sample diversity) measures showed significant differences between the three groups of depressive symptoms (**Figure S5.1**). Beta-diversity (between-sample diversity), assessed by betadisper, showed that the unlikely depressed group had a smaller variation in the gut microbiota composition, which means a higher taxonomic similarity (within the group) compared to the likely depressed group (P = 0.047; **Figure 5.1** and **Figure S5.2**). ADONIS revealed no significant influence of depressive symptoms in the beta-diversity. The variation in the data was not explained by depression score, neither by group of depressive symptoms. The univariate variation in the data was to some extent explained by concentrations of ferritin (N = 139; variance explained = 2.4%; P = 0.020), BAZ (N = 139; variance explained = 2.0%; P = 0.018), CRP (N=139, variance explained = 3.5%; P = 0.004), and AGP (N = 139; variance explained 1.6%; P = 0.069) (**Table 5.2**). Additionally, PCoA based on weighted UniFrac distance did not show a clear discrimination of microbial composition between three depression groups (Figure S5.2).

Table 5.1. Demographic and clinical characteristics of the participants

Characteristics	Total subsample	Unlikely depressed	Likely depressed	Highly-likely depressed	*P*-value [a]
	n=139	n=71	n=48	n=20	
Depression score	3.0 (2.0, 5,0)	2.0 (1.0, 2.5)	4.0 (4.0, 5.0)	9.0 (8.0, 11.0)	
Age (years)	15.0 (14.0, 16.0)	15.0 (13.5, 16.0)	15.5 (14.0, 16.0)	16.0 (15, 16)	0.277
BAZ (SD)	0.7 (-0.1, 1.7)	0.5 (-0.3, 1.0)	1.1 (0.2, 1.9)	0.6 (-0.2, 1.7)	0.022[b]
Hemoglobin (g/L)	13.2 (12.4, 14.0)	13.4 (12.8, 14.2)	12.9 (12.0, 14.8)	13.1 (12.4, 13,6)	0.343
Ferritin (µg/L)	41.2 (24.1, 71.4)	43.0 (22.1, 68.1)	47.0 (30.9, 79.2)	32.0 (20.9, 53.2)	0.460
CRP (mg/L)	0.3 (0.0, 0.9)	0.2 (0.0, 0.7)	0.3 (0.0, 2.6)	0.2 (0.1, 0.7)	0.086
AGP (g/L)	0.59 (0.51, 0.72)	0.55 (0.49, 0.72)	0.64 (0.57, 0.76)	0.57 (0.50, 0.68)	0.026[b]

Values are presented as median (interquartile range); [a] Kruskal-Wallis test was applied to compare medians for unlikely, likely and highly likely depressed; [b] Pairwise comparisons indicate the median value in the unlikely depressed group differs from the likely depressed group. BMI-for-age z-score (BAZ)

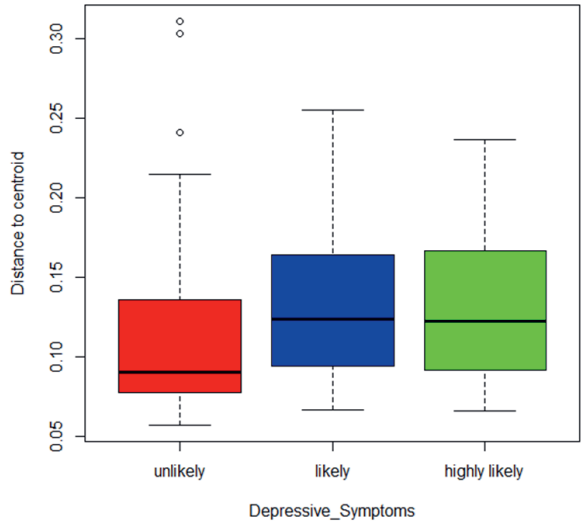

Figure 5.1 Boxplot of multivariate homogeneity of groups' dispersions (betadisper) of participants with unlikely, likely, highly likely occurrence of depressive symptoms. Box plots represent median with whiskers on ± 1.5 IQR. *Pseudo-F = 1.3214, P = 0.231.

Table 5.2: Beta diversity analysis

Variable N=139	**R^2**	**Pseudo-F**	**P-value**
Depressive symptoms (groups)	0.019	1.32	0.231
Age (years)	0.007	0.97	0.396
Ferritin	0.024	3.41	**0.020**
Hemoglobin	0.013	1.87	0.108
BAZ	0.020	2.85	**0.018**
CRP	0.035	5.06	**0.004**
AGP	0.016	2.19	0.069

Results of ADONIS on weighted UniFrac dissimilarity matrix. Tests were conducted separately for these factors: depressive symptoms, age, ferritin, hemoglobin, BMI-for-age z-scores (BAZ), C-reactive protein (CRP), and alpha-glycoprotein 1(AGP) variables; R^2 = variance explained, a measure of effect size; Pseudo-F= indicator of the number of clusters, the larger the Pseudo-F value the greater between group variation than the within variation. In bold, significant p-values <0.05.

Redundancy Analysis

Redundancy analysis showed that the total accumulative variation in microbiota composition explained by depressive symptoms score and confounding variables was 6.6 %. Depressive symptoms score was not significantly associated with the variation in microbiota composition. However, ferritin (variance explained = 7.1%; *P*=0.011), CRP (variance explained = 4.0 %; *P*=0.058), hemoglobin (variance explained = 4.2%; *P*=0.072) were identified as significant drivers of differences in the gut microbiota composition of Mexican adolescent girls. Higher concentrations of hemoglobin were associated with higher relative abundances of *Prevotella*, while CRP and ferritin concentrations were inversely correlated with the relative abundances of uncultured bacteria within *Clostridia_DTU014* and *Puniceicoccaceae* family, and *Eubacterium_fissicatena* group, and positively correlated with depressive symptoms score.

Taxonomic composition analysis and associations with depressive symptoms

Compositional analysis of our samples revealed that *Bacteroidetes, Firmicutes*, and *Actinobacteria* were the most predominant phyla in our data (Table S5.2). Based on LEfSe, there were no significant differences in relative abundances of any of these phyla between depression groups. At the genus level, differences in the gut microbiota composition of participants in the different groups of depressive symptoms were not significant.

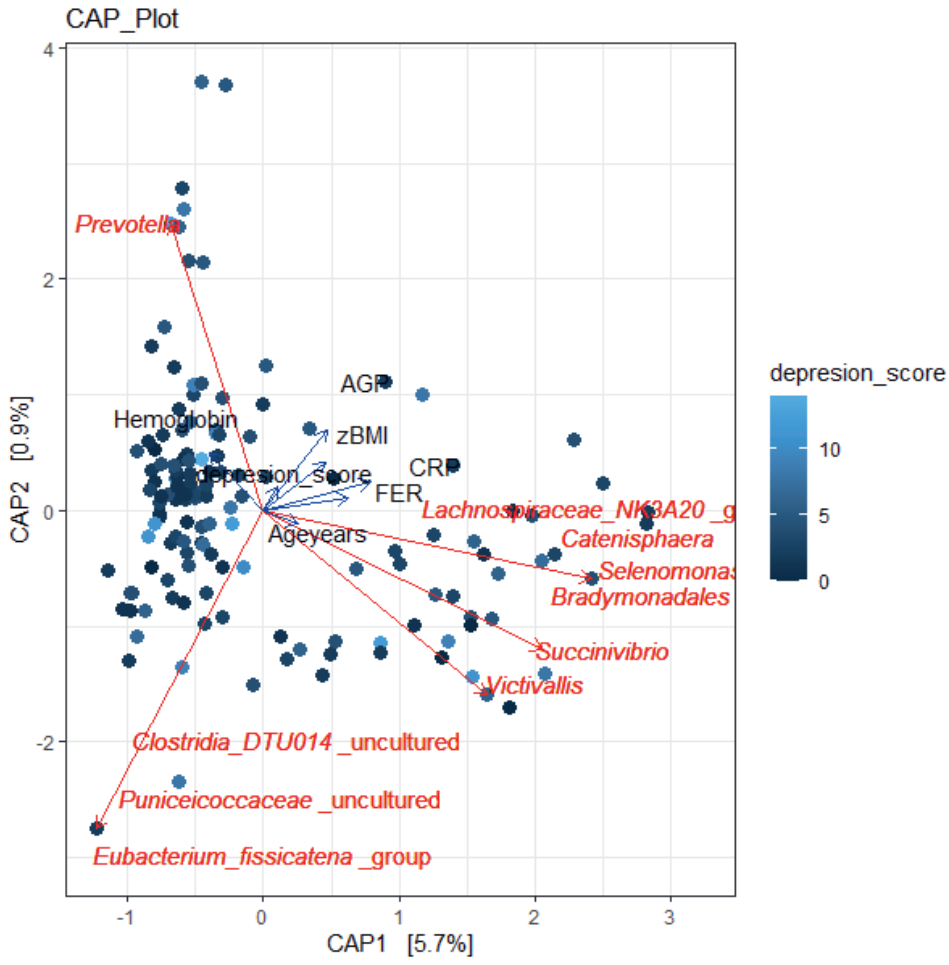

Figure 5.2. Redundancy analysis on microbiota composition of Mexican adolescent girls at the genus level. Explanatory variables shown by blue arrows explain variation in the gut microbiota composition. Colored points indicate participants depressive symptoms score.

Discussion

In this study, we aimed to determine the differences in gut microbiota composition between adolescent girls within the different subgroups of depressive symptoms, and the association between depressive symptoms and the relative abundance of selected genera. Our results did neither show general differences in microbiota composition (beta-diversity) between the groups of depressive symptoms, nor at the genus level, where the relative abundance of the main genera was not associated with the depressive symptoms. However, other variables of nutritional status (BAZ), iron status (ferritin), and inflammation status (CRP and AGP) slightly contributed to the variation between samples in our study sample. The redundancy analysis confirmed that depressive symptoms had no influence in the variation in microbiota composition. Moreover, higher concentrations of hemoglobin were associated with higher relative abundance of *Prevotella*, and higher concentrations of ferritin and CRP were inversely associated with uncultured bacteria within *Clostridia_DTU014* and *Puniceicoccaceae* family, and *Eubacterium_fissicatena* group, and positively correlated with depressive symptoms score.

Despite current theories that propose a role of gut microbiota in the pathophysiology of depression, the available studies show inconsistent results [29]. For instance, a case-control study conducted in China found higher alpha-diversity in adults with MDD compared to the healthy controls, and *Proteobacteria* and *Actinobacteria* were strongly increased, whereas *Firmicutes* were significantly reduced in the MDD group when compared to the healthy controls [30]. Jiang et al., also found increased alpha diversity in adults with MDD, and increased abundance of *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*, whereas the abundance of *Firmicutes* was significantly reduced in participants with MDD compared to healthy controls [31]. In our study, the gut microbiota composition of participants in the likely depressed and highly-likely depressed group did not differ significantly from participants in the unlikely depressed group. Consistent with our findings, Thapa et al. did not observe significant group differences in alpha and beta diversity, nor differential abundance of bacterial taxa in adolescents with MDD, and controls [32]. The reason for the lack of replication between studies could be due to methodological differences. These include differences in the method of DNA extraction [33], 16S rRNA gene region [34], bioinformatic pipeline, data processing and analysis [35], sample size, tool employed to assess depressive symptoms, and study design. Moreover, the age range (adolescents vs. adults) and other differences between studies, such

as locality, diet and non-shared environmental influences can lead to differences in microbiota composition and etiology of depression [36].

This study should be viewed in the context of several strengths and limitations. Our strengths include the assessment of indicators of nutritional status (BAZ), and biomarkers of inflammation and iron status, which are potential confounders in the association between gut microbiota composition and depressive symptoms. The limitations of our study are; fecal samples were collected at only one time point, the study was based on a convenience sampling method uncertainty if the sample size was large enough to capture sufficient heterogeneity in microbiota composition, we only look at depressive symptoms and not at clinically diagnosed depression, the fact that we were not able to collect information on lifestyle (alcohol and tobacco consumption), dietary patterns, and other variables that might alter the gut microbiota composition.

In conclusion, we did not find a significant association between gut microbiota composition and the presence of depressive symptoms in Mexican adolescent girls. However, several other variables (BAZ, ferritin, CRP, and AGP) that may be associated with depression seem to explain some of the variation between subjects, and suggest a potential link between iron metabolism, inflammation and depression. Further studies should identify potential gut-brain mechanisms, and further explore the role of biomarkers of the iron status and inflammation in the etiology of depression.

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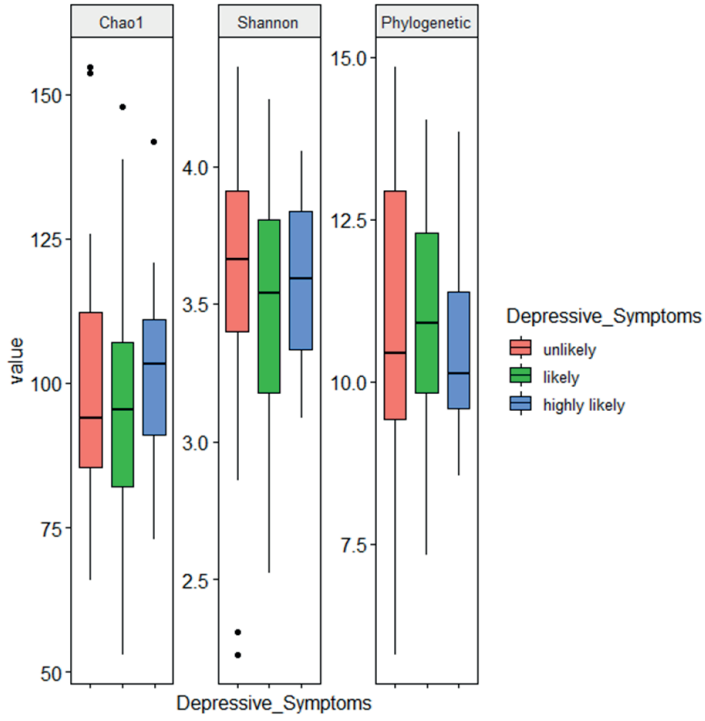

Figure S5.1. Alpha diversity comparison of the gut microbiota between participants with unlikely, likely and highly likely occurrence of depressive symptoms. Alpha diversity was indicated by Chao1 (P=0.388), Shannon index (P=0.307), and Phylogenetic diversity (P=0.841).

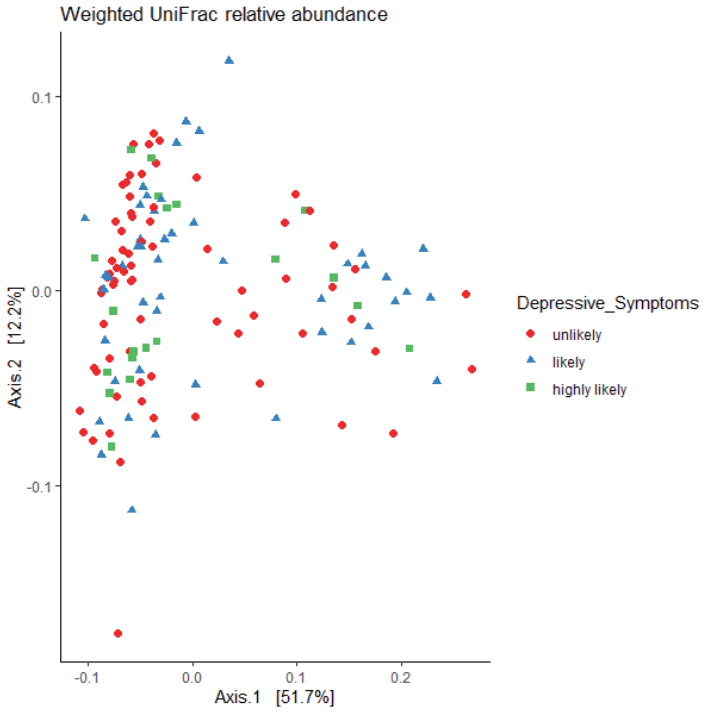

Figure S5.2. Principal coordinates analysis (PCoA) of weighted UniFrac distances representing the microbial composition of participants with unlikely, likely and highly likely occurrence of depressive symptoms. The first two components are plotted with the percentage of the variance explained by each principal component. Each point represents an individual sample.

Table S5.2. Relative abundance of bacterial phyla of all participants in our study

	Unlikely depressed	**Likely depressed**	**Highly likely depressed**	**p-value**
	Median (IQR)	Median (IQR)	Median (IQR)	
Bacteroidetes	0.19 (0.14, 0.28)	0.25 (0.14, 0.40)	0.20 (0.12, 0.28)	**0.157**
Firmicutes	0.68 (0.57, 0.76)	0.70 (0.61, 0.97)	0.66 (0.53, 0.73)	**0.077**
Actinobacteria	0.07 (0.03, 0.12)	0.06 (0.03, 0.11)	0.04 (0.03, 0.10)	**0.677**

N=139; IQR = interquartile range.

6

Chapter 6

Adjusting biomarkers of iron status for inflammation with alternative transformation methods for CRP and AGP

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Abstract

The assessment of iron deficiency is a challenge when inflammation is present. The use of regression coefficients to correct iron indicators for inflammation has substantially changed the estimates of iron deficiency prevalence. However, a key problem of using regression coefficients is rooted in the log-transformation of inflammatory markers. We propose alternative approaches to transform the inflammatory markers when adjusting ferritin (SF) and soluble transferrin receptor (sTfR) for inflammation. For that purpose, inflammatory marker concentrations C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were transformed in several ways (via logarithmic, fractional polynomial, cubic root, and inverse hyperbolic sine transformation) to achieve homoscedasticity, to improve model fit, and to normalize the distribution. The balance between model fit and model simplicity on SF and sTfR was tested with Akaike and Bayesian Information Criteria (AIC/BIC) and visual inspection of the residual plots. The best model for SF, with the lowest AIC and BIC values (806.58 and 814.46), included CRP transformed by inverse hyperbolic sine but did not include AGP, whereas sTfR did not benefit from any adjustment. The alternative approaches to transform CRP led to an increase by up to 5.0 percentual points in the estimation of iron deficiency (SF<15 µg/L). On the other hand, transforming CRP and AGP did not modify the estimation of iron-deficient erythropoiesis (sTfR >8.3 mg/L). In conclusion, the estimation of iron deficiency can be statistically improved by using alternative methods to transform the markers of inflammation. More research is needed to determine the reproducibility of these alternative approaches to adjust iron status indicators in settings with varying degrees of inflammation.

Introduction

Accurate assessment of iron status represents a challenge in settings of low- and mild- degree of inflammation. Although different methods have been proposed to adjust for inflammation when assessing iron status, no international agreement exists on which approach is more accurate. For this study, we used data from the Ten2Twenty-Mexico project, which consist of adolescent girls living in a setting with mild- degree of inflammation. We compared the current methods to adjust iron biomarkers for inflammation with alternative approaches.

Serum ferritin (SF) and soluble transferrin receptor (sTfR) are the most common biomarkers to evaluate iron status at the population level [1]. Ferritin is the major iron storage protein and is also considered an acute-phase protein that increases with an inflammatory response. sTfR reflects erythropoietic activity and cellular iron status [1, 2]. Although sTfR is less influenced by inflammation than SF, it may be affected by inflammation as a result of the redistribution of iron over body tissues [3]. Therefore, it is recommended to assess iron status with the concurrent measurement of inflammation biomarkers, such as C-reactive protein (CRP) and α1-acid glycoprotein (AGP) [3–7].

Different approaches have been proposed about how to use CRP and AGP to account for inflammation and infections to increase the sensitivity of detecting depleted iron stores at the population level. These approaches include, raising the cut-off value for ferritin to define iron deficiency, excluding individuals with elevated CRP or AGP from prevalence calculations, applying arithmetic coefficients to correct SF and sTfR, and using regression coefficients to correct markers of iron status [4,6,8].

The most recent approach to assess iron status in settings of inflammation was proposed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. The BRINDA project is a multiagency and multi-country partnership designed to improve the interpretation of nutrient biomarkers in settings of inflammation [6]. BRINDA has proposed the use of regression coefficients to adjust SF and sTfR for inflammation. This approach shows statistical improvements compared to the approaches proposed earlier [9,10]. However, recent publications bring attention to specific downsides that could confound the use of the BRINDA method when adjusting for inflammation. First, the assumption of linearity may be incorrect, leading to a miss-specified model in which a relevant variable may not be included for having a non-linear relationship with the outcome. Second, the limits of detection

and the lower limits of quantification for the inflammation biomarkers assays may be unknown or even variable [11,12].

These shortcomings suggest that the current methods can be improved to deal with the flaws in statistical efficiency, validity and interpretation. Therefore, this paper aims to compare the commonly used and the alternative methods, and to identify the best approach to determine iron status in Mexican adolescent girls within a setting of mild-burden of inflammation.

Methods

Participants

This cross-sectional study was conducted in Santa Catarina and Monterrey, in Northern Mexico, from September 2018 to January 2019. Adolescent girls aged 12-20 years from public schools were recruited, and written consent was obtained from the participants and their parents. Exclusion criteria were: diagnosis of systemic disease that may affect iron status, history of major surgery in the last month, and regular use of medication (except contraceptives). After applying these criteria, a total of 408 adolescent girls were recruited for assessment. Twenty-six girls had no serum samples and therefore were excluded from this analysis (**Figure 6.1**).

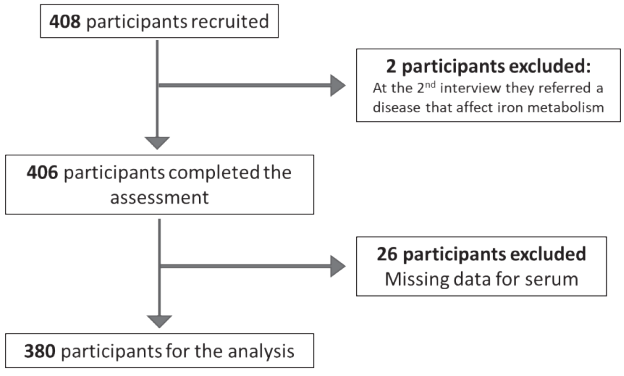

Figure 6.1. Selection process of the study sample.408 participants were recruited, but only those with serum samples available were included in this analysis.

Iron and inflammation status assessment

A trained staff member drew venous blood samples for subsequent assessment of iron indicators (SF and sTfR) and inflammatory markers (CRP and AGP). Blood samples were drawn between 8:00 am to 12:00 pm approximately; not all the participants had fasted. Serum samples were stored at -80˚C. Iron and inflammation concentrations were measured using a sandwich enzyme-linked immunosorbent assay by the Vitmin Lab [13].

Statistical analysis

Depleted iron stores were defined as serum concentrations of SF <15μg/L [8]. Iron-deficient erythropoiesis was defined as an sTfR >8.3mg/L [13].

Descriptive statistics and bivariate analysis were performed with R version 3.6.1. Fractional Polynomial models were conducted with Stata (StataCorp, version 16). Normal distribution of the variables was inspected with Kolmogorov-Smirnov and Levine's normality test. The iron and inflammation biomarkers were not normally distributed even after transformation. Therefore, we show the median and the 25th and 75th percentiles. The prevalence of iron deficiency and iron deficiency erythropoiesis was estimated with and without adjustment for inflammation. Various approaches were explored to adjust SF and sTfR for inflammation. CRP contained zero values; thus, to transform the variable by natural logarithm (ln) we imputed the lowest value (0.005). Based on a linear regression model, we visually inspected the expected ln-SF and ln-sTfR concentrations with ln-(CRP + 0.005) and ln-AGP as independent predictors.

Traditional approaches to account for inflammation when assessing iron status

We applied three methods that are commonly used to estimate iron deficiency in the presence of inflammation. The first method consists of raising the cut-off value for ferritin to define iron deficiency, or excluding individuals with elevated CRP or AGP from prevalence calculations as advised by the WHO [8]. The second method was proposed by Thurnham et al. and uses arithmetic coefficients to correct SF and sTfR [4]. Finally, we also applied the regression coefficient method proposed by BRINDA. This method is widely used to adjust SF and sTfR by CRP and AGP concentrations on a continuous scale [3,5]. In addition, we re-ran the regression coefficient approach in a subset of the study sample after excluding 104 participants with CRP values equal to zero.

Alternative approaches for ln-transformation of biomarkers of inflammation

An obstacle in the regression coefficient method is how to deal with non-linearity in the relationship between an outcome and a continuous predictor. The traditional assumption of linearity may be incorrect, leading to a mis-specified model in which a relevant variable may not be included because its true relationship with the outcome is non-monotonic, or in which the assumed functional form differs from the unknown true form [14]. Royston and Altman have alternatively suggested to keep the variable continuous and to allow some form of non-linearity [15]. Following that logic, we allowed non-linearity of the value distribution of the inflammatory markers by alternative transformations (i.e. fractional polynomials, cubic root, and inverse hyperbolic sine transformation (IHS)). These approaches have the advantages of being weaker at low values and having a defined value at a base of zero.

Untransformed markers of inflammation: with inflammatory markers left untransformed, the prediction model for the ln-SF and ln-sTfR outcomes were linear functions:

$$\ln[SF_{adj}] = \ln[SF_{unadj}] - \beta_1(CRP_{adj} - CRP_{ref}) - \beta_2\,(AGP_{adj} - AGP_{ref})$$

$$\ln[sTfR_{adj}] = \ln[sTfR_{unadj}] - \beta_1\,(CRP_{adj} - CRP_{ref}) - \beta_2\,(AGP_{adj} - AGP_{ref})$$

Fractional polynomials: polynomial functions are conventionally used for curve fitting. They comprise a family of linear functions that include one or more terms with non-negative integer powers of x (linear, quadratic, cubic, quartic, etc.). By contrast, fractional polynomials also include terms with powers that can be negative integers or fractions. A second-degree fractional polynomial for a single independent variable x has been defined as a function with the form:

$$y = \beta_0 + \beta_1 x^p + \beta_2 x^q$$

where x>0. In practice, powers p and q are usually selected from a restricted set S = {–2, –1, –0.5, 0, 0.5, 1, 2, 3}, where *x^0* denotes $log_e(x)$. The set includes no transformation (p = 1) and the reciprocal, logarithmic, square root, and square transformations. If nonpositive values of x can occur, then a preliminary transformation of x to ensure positivity is needed. Common cases are where x is a count, when log(x+1) is traditionally used; where x is a positive random

variable such as a physical quantity for which recorded values can be zero, due to imprecise measurement and/or rounding of observations; or where x is a difference or log ratio between two quantities [15]. The best model is found by fitting models with every possible combination of powers p and q in the set and selecting the pair of p and q that is associated with the maximum likelihood or, equivalently, with the lowest value for the deviance D or one or more information criteria. Elimination of the term $\beta_2 x^{\mathrm{q}}$ *results in* a one-degree polynomial that includes the logarithmic function (p=0), which is the method for adjustment as advocated by the BRINDA group [16], as well as the linear function (p=1). One-degree polynomials with positive p values, or two-degree polynomials with positive values for p and q, are of particular interest because they are defined at zero. Models can be easily extended by adding terms for other continuous variables or categorical variables.

The use of fractional polynomials to transform CRP and AGP to correct iron status biomarkers leads to the following formulas

$$In(SFadj) = \ln\left[SF_{unadj}\right] - \beta_1 CRP^p - \beta_2 AGP^q$$

$$In(sTfRadj) = \ln\left[sTfR_{unadj}\right] - \beta_1 CRP^p - \beta_2 AGP^q$$

Cube root transformation: as applied to independent variables in regression models, the cube root transformation ($\sqrt[3]{x}$) is a particular case of a fractional polynomial that is not included in the set of power functions described in the preceding section:

$$\ln\left[SF_{adj}\right] = \ln\left[SF_{unadj}\right] - \beta_1\left(\sqrt[3]{CRP_{unadj}} - \sqrt[3]{CRP_{ref}}\right) - \beta_2\left(\sqrt[3]{AGP_{unadj}} - \sqrt[3]{AGP_{ref}}\right)$$

$$\ln\left[sTfR_{adj}\right] = \ln\left[sTfR_{unadj}\right] - \beta_1\left(\sqrt[3]{CRP_{unadj}} - \sqrt[3]{CRP_{ref}}\right) - \beta_2\left(\sqrt[3]{AGP_{unadj}} - \sqrt[3]{AGP_{ref}}\right)$$

Inverse hyperbolic sine transformation: the inverse hyperbolic sine transformation of x is defined as:

$$\ln(x+\sqrt{(x^2+1)})$$

At x=0, the formula reduces to ln(1)=0 (it goes through the origin). Except for small values of x, the inverse sine is approximately equal to ln(2x) or "ln" (2) + "ln" (x), so it mimics a log-transformed variable such as employed in the BRINDA method in most of the range of x≥0. Analogous to this method, the following formulas were derived.

$$ln(SF_{adj}) = \ln[SF_{unadj}] - In\left(CRP\sqrt{CRP^2+1}\right) + In\left(AGP\sqrt{AGP^2+1}\right)$$

$$ln(sTfR_{adj}) = \ln[sTfR_{unadj}] - In\left(CRP\sqrt{CRP^2+1}\right) + In\left(AGP\sqrt{AGP^2+1}\right)$$

Model selection

To assess possible violations of homoscedasticity assumption, we inspected bivariate residuals versus fit plots. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to assess the trade-off between overall model fit and model simplicity. The models with the lowest AIC and BIC were preferred. For the selected models, we computed estimates of iron status and compared these results with those obtained using the traditional approaches.

Results

The general characteristics of the study sample are shown in **Table 6.1**. Complete data were available for 380 adolescent girls. Inflammation was present in 14.7% of the participants, whether assessed by CRP or AGP. The prevalence of iron deficiency was 11.6% and iron deficient erythropoiesis 3.7%.

Table 6.1. Summary of iron and inflammatory markers of Mexican adolescent girls (n=380)

Characteristics	Median (Q_{25}, Q_{75})	Iron deficiency (%)
Ferritin (µg/L)	39.5 (23.9, 62.5)	11.6
sTfR (mg/L)	5.1 (4.4, 6.0)	3.7
CRP (mg/L)	0.3 (0.0, 1.1)	-
AGP (g/L)	0.6 (0.5, 0.8)	-

Iron deficiency was defined as concentrations of serum ferritin <15µg/L; Iron-deficient erythropoiesis was defined as an soluble transferrin receptor concentration >8.3mg/L. sTfR, CRP, AGP: serum concentrations of soluble transferrin receptor, C-reactive protein and α1-acid glycoprotein.

ln-SF was positively associated with ln-CRP and ln-AGP (**Figure 6.2**). These plots suggest that ln-SF models would benefit from CRP and AGP adjustments while ln-sTfR models would only benefit from AGP adjustments because there was no evidence of a linear association between ln-sTfR and CRP. Therefore, only AGP was considered in models with ln-sTfR in further analyses.

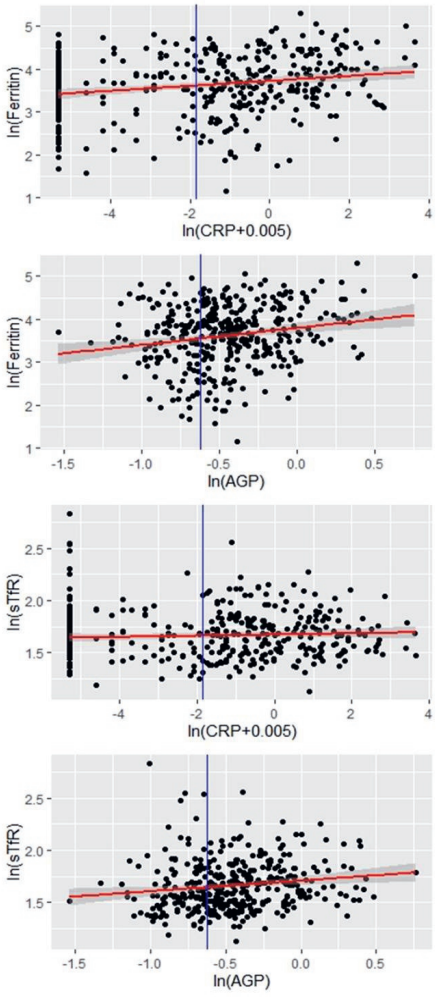

Figure 6.2. Association of markers of iron status and markers of inflammation. The red line indicates the expected ln-ferritin and ln-sTfR concentrations given ln-(CRP+0.005) and ln-AGP based on a linear regression model. The blue line indicates the reference value suggested by BRINDA (CRP: 0.16 mg/L and AGP: 0.53g/L); when using the BRINDA approach, ferritin and sTfR concentration are adjusted only if the participants have CRP and AGP values above the reference.

Bivariate analysis

Raw and smoothed residuals for ln-SF and ln-sTfR were derived from bivariate linear regression models with inflammatory markers entered as single independent variables (**Figure 6.3**). The models for SF with transformed inflammatory markers had more stable variances compared to the model with untransformed inflammatory markers. Stable variances were more evident in models with CRP than in those with AGP as a covariate. The residual plots suggest that the association between ln-SF and the markers of inflammation might not be linear. In addition, balance between model fit and model simplicity improved with the transformation of CRP values, as indicated by the comparison of information criteria values in **Table 6.2**. The AIC and BIC values reduced from 817.22 and 829.04 with untransformed CRP to 807.33 and 819.15 with an inverse hyperbolic sine transformation and to 806.58 and 814.46 with fractional polynomial transformation. For sTfR, the transformation of AGP did not improve the balance between model fit and model simplicity regardless of the type of transformation used (**Table 6.3**).

Model comparison

For SF, the lowest AIC and BIC values, 806.58 and 814.46, were achieved with a fractional polynomial model that included CRP (power -0.5) transformed with pre-transformation scaling. Nevertheless, the model with CRP inversely hyperbolic sine transformed was preferred because it did not require pre-transformation scaling of independent variables with zero values and performed similarly to fractional polynomial model. The inclusion of AGP in the models did not improve the balance between model simplicity and model fit.

The lowest information criteria values for sTfR included ln-transformed AGP (AIC -7.48 and BIC 4.34). However, the model with untransformed AGP was preferred because the balance between model fit and model simplicity hardly improved after transformation of AGP. Furthermore, fractional polynomial results indicated insufficient evidence of an association between sTfR and the biomarkers of inflammation.

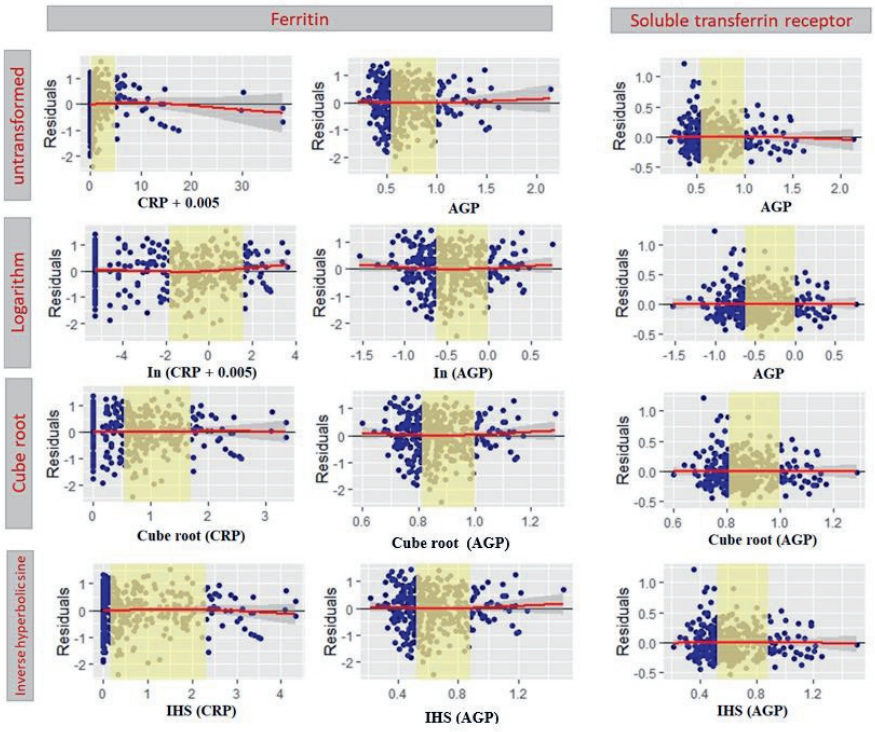

Figure 6.3. Model fit with various transformations of inflammatory markers

Ferritin and sTfR concentrations were ln-transformed. Dots indicate raw residuals obtained in linear models with CRP or AGP entered as single independent variables, either untransformed, ln-transformed, or cube-root or inverse hyperbolic sine transformation (from the top row to bottom row). Locally linear smoothed residuals are shown as red lines with 95% pointwise CI. Horizontal lines at y=0 indicate the expected value of the residuals if the model is correct. The horizontal limits of the yellow areas indicate reference concentration values used by the BRINDA group (C-reactive protein: 0.16 mg/L; α1-acid glycoprotein: 0.53 g/L) and Thurnham et al. (2010) (C-reactive protein: 5 mg/L; α1-acid glycoprotein: 1 g/L. The graphs for soluble transferrin receptor and CRP are not shown because there was no evidence of an association between these variables.

Table 6.2. Comparison of models for predicting ln-ferritin concentrations with various transformation approaches for inflammation markers.

Transformation approach for inflammatory markers	CRP		AGP		CRP and AGP	
	AIC	BIC	AIC	BIC	AIC	BIC
Untransformed (linear)	817.22	829.04	813.83	825.65	812.26	828.02
Logarithmic	813.82	825.64	817.56	829.38	814.02	829.78
Fractional polynomials [1]	FP1(-0.5)		FP1(1)		FP1(-0.5) and FP1(out)	
	806.58	814.46	812.52	820.40	806.58	814.46
Cube root	809.43	821.25	814.92	826.74	810.23	825.99
Inverse hyperbolic sine	807.33	819.15	819.04	830.88	807.92	823.68

AIC, BIC: Akaike information criterion, Bayesian Information Criterion. AIC/BIC values indicate the trade-off between overall model fit and model simplicity, with lower values indicating better trade-off. When comparing AIC/BIC values within columns, the number of parameters are identical, so that comparison is by overall model fit. [1] FP1: one-degree polynomial; the number between brackets indicate power p for CRP and AGP, respectively.

Table 6.3. Comparison of models for predicting ln-soluble transferrin receptor concentrations with various transformation approaches for inflammation markers.

Transformation method for Inflammatory markers	AGP		CRP and AGP	
	AIC	BIC	AIC	BIC
Untransformed (linear)	-6.95	4.87	-8.34	7.42
Logarithmic	-7.48	4.34	-6.13	9.63
Fractional polynomials [1]	FP1(out)		FP1(out) and FP1(out)	
	-4.93	-0.99	-4.93	-0.99
Cube root transformation	-7.47	4.35	-7.39	8.37
Inverse hyperbolic sine transformation	-7.42	4.40	-8.14	7.62

AIC, BIC: Akaike information criterion, Bayesian Information Criterion. AIC/BIC values indicate the trade-off between overall model fit and model simplicity, with lower values indicating better trade-off. When comparing AIC/BIC values within columns, the number of parameters are identical, so that comparison is by overall model fit. [1] FP1: one-degree polynomial; the number between brackets indicate power p for CRP and AGP, respectively.

Estimated parameters for the selected models

The prediction model for SF included CRP with inverse hyperbolic sine transformation (IHS-CRP). This prediction model indicated that each unit increment in IHS-CRP (mg/L) increased the geometric mean of serum ferritin concentration by 20.8% (**Table 6.4).** In contrast, the prediction of sTfR concentrations was not affected by the biomarkers of inflammation.

Table 6.4. Selected model for serum concentrations of ferritin

	ln Ferritin β (95% CI)		**Ferritin β (95% CI)**		**P**
Inverse hyperbolic sine transformed CRP, mg/L	0.189 [1]	(0.114, 0.263)	20.8	(12.1, 30.1 %)	<0.005
Constant	3.487[2]	(2.620, 4.353)	32.7	(13.7, 77.7µg/L)	<0.005

CRP, IHS : C-reactive protein, inverse hyperbolic syne transformed. [1] For example: In-Ferritin=3.487 + 0.189 (IHS-CRP, mg/L). Interpretation: each increment in IHS-CRP, mg/L by 1 unit increases geometric mean serum ferritin concentration by 20.8%, [2] In adolescents with CRP=0 mg/L, the estimated geometric mean for serum ferritin concentration is 32.7µg/L.

Comparison of the estimated parameters of iron deficiency based on diverse methods of adjustment for inflammation

Table 6.5 summarizes the median of SF and sTfR concentrations along with the estimated prevalence of iron deficiency using the commonly used and the alternative adjustment methods. For the regression coefficient method (using logarithmic transformation), the estimated median for SF was 5.86 µg/L lower when calculated with imputed values for CRP as compared when calculated after the exclusion of censored CRP values. The prevalence of iron deficiency (SF <15µg/L) increased by 2.6 – 6.8 percentual points in models adjusted for CRP. . The prevalence of iron deficiency (SF <15µg/L) increased by 2.6 – 6.8 percentual points in models adjusted for CRP. The prevalence of iron-deficient erythropoiesis (sTfR > 8.3 mg/L) remained almost the same after adjusting for AGP, regardless of the transformation method.

Table 6.5. Comparison of estimates of iron status based on the commonly used and alternative methods of adjustment for inflammation

Correction method	Median SF concentration	Prevalence of iron deficiency	Median sTfR concentration	Prevalence of iron deficiency erythropoiesis
	µg/L (95% CI)	% (95% CI)[1]	mg/L (95% CI)	% (95% CI)[1]
Untransformed (linear)	39.50 (23.9, 62.5)	11.6% (8.7, 15.2)	5.08 (4.4, 6.0)	3.7% (2.2, 6.1)
Higher cut-off point	-	20.5% (16.8, 24.9)	-	-
Exclusion criteria (n=324)[a]	36.76 (22.7, 56.1)	13.3% (10.0, 17.4)	5.00 (4.4, 6.0)	3.7% (2.1, 6.4)
Coefficient Factor	36.44 (22.7, 56.12)	12.6% (11.1, 18.6)	5.02 (4.4, 5.9)	3.4% (2.1, 6.0)
Logarithmic [2]	30.39 (18.3, 48.5)	18.4% (14.8, 22.6)	4.79 (4.2, 5.6)	2.6% (1.4, 4.8)
Logarithmic [2] (exclusion CRP=0, n=276)[b]	36.25 (18.2, 49.8)	17.9% (13.8, 22.9)	-	-
Fractional polynomials [3]	CRP: FP1(-0.5)		-	-
	35.68 (20.1, 58.7)	16.6% (13.2, 20.6)	-	-
Cube root transformation	32.75 (20.4, 53.4)	16.1% (12.7, 20.1)	4.90 (4.3, 5.8)	2.6% (1.4, 4.8)
IHS transformation	34.30 (21.6, 54.0)	14.2% (11.1, 18.1)	4.82(4.2, 5.6)	2.6% (1.4, 4.8)

SF, sTfR, CI, CRP, IHS: serum ferritin, soluble transferrin receptor, confidence intervals, inverse hyperbolic syne, [1] 95% CI calculated using Wilson's method. [2] Values in geometric mean for ln-transformed outcomes. [3] For ferritin, FP1 includes only CRP with power -0.5. For sTfR, there was no sufficient evidence of an association with CRP and AGP, [a] 56 cases excluded for having higher values of CRP and AGP, [b] 104 cases excluded for having CRP values equal to zero.

Discussion

We tested diverse models to predict adjusted ferritin and soluble transferrin receptor by applying alternative methods to transform CRP and AGP. Ferritin prediction obtained the best trade-off between model fit and model simplicity by the fractional polynomial and by the inverse hyperbolic syne-transformation of CRP, whereas the prediction of soluble transferrin receptor did not benefit from any adjustment. These findings suggest that compared to logarithmic transformation, the alternative approaches to transform CRP and AGP can statistically improve the assessment of iron status.

The alternative transformation approaches of CRP decreased the estimate median of ferritin concentration by 3 to 9 µg/L and consequently increased the estimated prevalence of iron deficiency by up to 5 percentual points. The estimated prevalence of iron deficiency was 11.6% when ignoring the inflammation markers. Using the regression coefficient method resulted in an estimated iron deficiency prevalence of 18.4 % and 17.9% when adjusting SF with CRP with imputed values and adjusting SF excluding censored CRP values, respectively. It can therefore be assumed that ignoring inflammation likely results in underestimating iron deficiency prevalence, but the logarithmic transformation of CRP represent statistical flaws that can result in overestimation of iron deficiency.

In contrast, the prediction of soluble transferrin receptor did not benefit from adjustments for inflammation regardless of the approach used. These results are in line with previous studies that demonstrate that soluble transferrin receptor concentrations are less susceptible to inflammation than ferritin [7,17,18]. Nonetheless, sTfR lacks assay standardization, which hinders the comparison of sTfR data between studies unless the same assay has been used [1]. Given the different roles of each of the iron indicators as well as the potential confounding effects of inflammation and infection, the use of both indicators is recommended to assess iron deficiency, particularly in areas where inflammation is prevalent.

The use of regression coefficients, the method proposed by the BRINDA project, shows statistical improvements compared to previous approaches to assess iron deficiency in the presence of inflammation [9,10]. However, recent publications bring attention to two specific downsides of the use of regression coefficients that could confound its use when adjusting for inflammation [11,12]. First, the assumption of linearity may be incorrect, leading to a mis-specified model in which a relevant variable may not be included if it has a non-linear

relationship with the outcome. Second, the limits of detection and the lower limits of quantification for the inflammation biomarkers assays may be unknown or even variable CRP values under the limit of detection are often censored, as shown in our dataset and previous studies. Thus, CRP does not benefit from logarithmic transformation [11]. We offer a potential solution to these flaws by using alternative transformation methods that allow both the non-linearity of the CRP and AGP values distribution, and transforming values close or equal to zero.

Although the proposed approaches improved the estimation of iron status indicators, the markers used in this study do not reflect the true iron status, but they are the most commonly used indicators of iron deficiency. The absence of stainable iron in the bone marrow is considered the gold standard marker for iron deficiency. In practice, this method is not widely used because it is a costly and invasive technique, and consequently, the assessment of iron status relies on proxy indicators, such as ferritin and soluble transferrin receptor [2]. The proxy indicators of iron status adjusted for inflammation remain to be validated with the "gold standard."

In summary, the proposed approaches to transform the biomarkers of inflammation complement the BRINDA method. Further research on these alternative approaches is recommended in settings of a low- to mild- degree of inflammation to assess replicability and elucidate the effects of acute and chronic inflammation on iron deficiency estimation.

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Chapter 7

General Discussion

As described in **Chapter 1**, the main aim of this thesis is to investigate the interrelationships between nutritional exposure and health outcomes among adolescent girls in Mexico, with specific focus on iron deficiency and depression. We also explored the relationship of these two health concerns with pubertal onset, body weight, and gut microbiota composition. This last chapter includes for each objective a summary of the main findings, an overview of the methodological challenges, and a discussion on the possible implications on adolescents' social, economic, and nutritional trajectories in Mexico and globally. The findings of this thesis are summarized in Table 7.1.

Age at menarche and the effect of pubertal onset on health

In Mexico, AAM decreased with 0.99 years between the 1920s (13.6 years) and the 1980s (12.6 years) (**Chapter 2**). This is similar to high-income countries where AAM decreased with 1.2 years from 13.5 years for births in the 1930s to 12.3 years for births between 1970 and 1984 [1]. Data that we collected in the North of Mexico (**Chapter 4-6**) shows a mean AAM of 11.8 years in girls born between 1999 and 2006. AAM in this group of girls occurred approximately 0.5 to 3.3 years earlier than in healthy girls from LMICs (mean AAM 12.3 years) and in girls living in LMICs where nutrition has improved to a lesser extent (for example, 15.1 years in the rural Gambia)[1]. Decreasing AAM has been observed globally, but the timing and pace have varied among populations. Environmental disruptors (nutrition, health, socioeconomic status, and psychosocial stress) may explain the difference in AAM trends [2].

In chapter 2, we observed an association between AAM and adult health. AAM in Mexican women was positively associated with height and inversely associated with body weight. In addition, Mexican women with later AAM had a lower risk of diabetes type 2 and hypercholesterolemia. **In chapter 4,** we were not able to observe associations between AAM and depression. This might be because almost all (96.1%) of the participants attained menarche, which means there was little variability in terms of pubertal status.

Implications of an earlier AAM in the health, social, and economic trajectories

An early AAM predicts obesity, some non-communicable diseases [1], and depressive symptoms [3] in later life. A cohort study showed that Mexican women with early menarche (< 11.0 years) had a higher risk of mortality for diabetes type 2, breast cancer and other types of cancer compared to women with later mean AAM at 13 years [4]. This evidence suggests

that AAM, which might be affected by environmental factors during childhood, could be an important marker of adulthood risk of morbidity and mortality.

Menstruation is still a taboo in some societies, and as a result many young girls lack information regarding menstrual hygiene and reproductive health [5]. In terms of reproductive health, earlier AAM indicates earlier sexual maturation and increased risk of teenage pregnancy. The fertility rate in 2011 for women between 12 and 19 years old was 37 births per 1,000, higher than that of 2005, which was 30. The increment in births at this age was 23.3% between 2005 and 2011 [6]. Adolescent pregnancy compromises the nutritional status of the girl and fetus, and compounds disadvantages for girls by dropping out from school, limiting employment opportunities, and perpetuating the poverty cycle [1]. Several reports suggest that incidence of pregnancy during adolescence predicts a range of negative outcomes like labor market challenges and increasing the number of working days lost [7].

Dietary patterns and the double burden of malnutrition in Mexican adolescents

In Mexico, overweight and obesity coexist with micronutrient deficiencies, anemia, and stunting. From **chapter 3**, we observed that about 30% of the adolescents were overweight or obese at national level. The prevalence of anemia was higher in adolescent girls (10%) than in boys (6.3 and 3.6, respectively for young and older adolescent boys). Approximately 3.0-5.0% of the adolescents in Mexico were stunted or anemic and simultaneously suffered from overweight or obesity.

Diet is an important contributor to adolescents' nutrition. **In chapter 3**, we described the dietary patterns of Mexican adolescents and their association with nutritional status. We identified four dietary patterns: non-traditional and breakfast-type, Western, plant-based, and protein-rich. The prevalence of overweight and obesity was higher in adolescents who scored high in the Western and plant-based patterns. Both patterns were also inversely associated with stunting. On the other hand, high scores on the non-traditional and breakfast-type pattern were inversely associated with anemia, while the probability of anemia was higher in adolescents who scored high in the Western pattern. In the past 40 years, the Mexican diet has shifted from mainly fresh and unprocessed foods to ultra-processed products high in sugar, salt, and fat [8]. Repeated cross-sectional data from 1999 to 2011 indicates that adolescents' diets are relatively diverse but high in ultra-processed foods, and sugar-sweetened beverages [9]. From 1984 to 2016, the total daily energy purchased that comes from ultra-processed foods increased from

10.5% to 23.1% kcal [10]. Rapid changes in the diets and food systems of LMICs, such as Mexico, have led to change in energy balance and weight gain, which increases the risk for the double burden of malnutrition [8,11]. The combined prevalence of overweight and obesity in female adolescence increased from 11.1% in 1999 to 35.8% in 2012, showing the highest and marked increase between 1988 and 1999 (17.2±0.9 pp) [12].

Implications of malnutrition on the social, economic and nutritional trajectories

Overweight, obesity and poor metabolic profile in adolescence are associated with chronic disease later in life [13,14], and with iron deficiency during adolescence [15]. Stunting in adolescence leads to short height attained at adulthood [13] Stunting is also associated with higher body fat and cardiometabolic risk later in life [16,17]. While the prevalence of overweight and obesity have increased in Mexico, the prevalence of stunting and anemia among adolescents also persist.

Throughout adolescence, nutritional status is interrelated with social and economic trajectories. Stunting among adolescents is associated with impaired cognitive development and school achievement, reduced economic productivity, and poor reproductive health outcomes for females [14,18]. Anemia and iron deficiency anemia in adolescents are also associated with lower school grade attained and with poorer cognitive skills [18]. The economic effect of overweight and obesity is usually measured by the cost of illness and by reduced productivity. It is estimated that in Mexico, undernutrition costs 1.7% of the GDP, and overweight and obesity 0.6% [19]. In addition, foods highly dense in energy and poorly dense in essential nutrients density predominate in Mexican dietary patterns across all age groups, contributing importantly to the attributable burden of disease, accounting for more than 10% of DALYs in 2013 [20].

Depressive symptoms in relation to iron deficiency, anemia, body weight and gut microbiota

We identified three subgroups of depressive symptoms by using Latent Class Analysis (**Chapter 4**): 44.4% of the adolescents were “unlikely”, 41.5% were “likely”, and 14.1% were “highly likely” to present depressive symptoms. Similarly, 35.8% of adolescents living in Chiapas, Mexico, reported suffering from depressive symptoms [21]. To our knowledge, data on depression among Mexican adolescents at the national level is inexistent, and comparing

results with similar studies is challenging due to the different tools used to assess depressive symptoms. In alignment with the available literature, we found that iron deficiency [22], lower hemoglobin concentration [23,24], and higher body weight [25,26] were related with the severity of depressive symptoms, whereas pubertal status was not associated.

Despite significant advances in our understanding of the etiology of depression, the existing knowledge is incomplete. Recent research suggests a potential role of the gut microbiota in the etiology of depression [27]. **In Chapter 5**, we explored the relationship between the gut microbiota composition and the presence of depressive symptoms in Mexican adolescent girls. Our results did neither show general differences in microbiota composition (beta-diversity) between the groups of depressive symptoms, nor at the genus level, where the relative abundance of the main genera was not associated with the depressive symptoms. Significant differences have been found in the gut microbiota composition between depressed and healthy subjects, with a higher abundance of the *Lachnospiraceae* and *Bifidobacteriaceae* families, and of the *Roseburia, Blautia, Streptococcus, Oscillibacter, Parabacteroides, Alistipes* and *Eggerthella* genus in presence of depression. [27–31]. However, alpha and beta diversity results were inconsistent across the literature [28–31]. While the link between gut microbiota and depression is to some extent supported byscientific evidence, the major research gap still is the causality in the connection between the two. Depression, as a mental disorder, may alter the intestinal physiology and the microbiota through the gut-brain axis and, on the other hand, alterations in the gut microbiota may contribute to the occurrence of depression.

Implications of depression on the social, economic and nutritional trajectories

Depression during adolescence may increase the risk of clinically diagnosed depression, anxiety, substance abuse, and suicide in adulthood [32,33]. In LMICs, adolescents with depressive symptoms were more likely to engage in risky sexual behavior and substance use compared with nondepressed adolescents [34]. A negative perception of oneself as well as one's appearance are associated with onset and maintenance of eating disorders and depression [35,36]. In a recent study on 15- to 25-year-old females, those with lifetime major depressive disorder or anxiety disorder were four times more likely to have a lifetime eating disorder [37]. In addition, a meta-analysis showed that depressed adolescents had a 70% increased risk of being obese [38]. There are several possible mechanisms linking depression and eating disorders or obesity, including behavioral and lifestyle factors (changes in appetite and dietary

patterns, sedentary activity) as well as biological (inflammation, impaired neuroendocrine mechanisms, dysregulation of the HPA-axis) and genetic factors [38].

Depression in adolescence also increases the probability of dropping out of school by 76%, of unemployment by 66%, and of becoming adolescent parents by 38%, as compared to adolescents without depression [39]. Depressive disorders accounted for nearly 10% of YLDs in 2013 and became a leading cause of disability in Mexican women [20]. The availability of mental health care remains insufficient throughout Mexico. Particularly, large treatment gaps are observed in 18–29 year-olds diagnosed with depression, which might be associated with the increased risk of suicide in this population [20].

It is estimated that cost-of-illness in adolescents with depression is higher (Ratio of means: 2.70; 1.69-4.79) than in non-depressed adolescents (Ratio of means : 2.70; 1.69-4.79), but this calculation was based on two studies only, which is why no generalizable conclusions on the cost-of-illness of depression can be drawn [40].

Assessing iron deficiency in the context of the double burden of malnutrition

Low-grade inflammation due to excessive adiposity may negatively affect iron status [41]. In addition, low iron status in overweight individuals may result from a combination of nutritional and functional iron deficiency [41,42]. Thus, assessing iron status in populations with a high prevalence of overweight and obesity should consider inflammation and use biomarkers of inflammatory to correct iron status, especially in LMICs, where ID is still prevalent and overweight and obesity are on the rise.

In chapter 6, we proposed alternative methods to transform the markers of inflammation in order to improve the estimation of iron deficiency prevalence. The best model for correcting ferritin, in our study population, included CRP transformed by inverse hyperbolic sine but did not include AGP, whereas sTfR did not benefit from any adjustment. The alternative approaches to transform CRP led to an increase by up to 5.0 percentual points in the prevalence of iron deficiency. Contrary to the findings of the BRINDA project, adjusting sTfR concentrations with AGP did not modify the estimation of iron-deficient erythropoiesis. The BRINDA group found a positive but weak association between sTfR concentrations and AGP concentrations in their multi-country study. When applying the logarithmic regression approach, the prevalence of iron-deficient erythropoiesis decreased by an extent of 1.9 (in

Laos) to 13.8 (in particular Cameroon) percentual points for adjusted sTfR compared to unadjusted prevalence. [43]. The inconsistencies in the adjusted and unadjusted prevalence of iron deficiency and iron deficiency-erythropoiesis [43,44] suggest that datasets must be carefully inspected to determine the association between iron status biomarkers and inflammation biomarkers and then explore the different approaches to transform the markers of inflammation.

Overall methodological considerations

To obtain more insight into Mexican adolescents' current health and nutrition situation, we performed a combination of secondary and primary cross-sectional analyses. Methodological considerations were addressed in each chapter; however, this section will elaborate on issues to consider when interpreting the findings or applying the methods used in this thesis in a different setting.

Study design

Chapters 2 and 3 were based on secondary cross-sectional data from nationally representative surveys (ENSA-2000 and ENSANUT-2006). A key strength of ENSA and ENSANUT is the probabilistic sampling design. The sample size was determined to make national inferences on the urban and rural areas in the four geographic regions (north, center, Mexico City, and south) [45]. In addition, some of the results in **chapters 2 and 3** may be comparable with those of later ENSANUT surveys (if the variables of interest were measured in previous or most recent versions of the survey), which will allow to identify potential changes in the health and nutritional status of the Mexican population over time. **Chapters 4-6** were based on primary cross-sectional data collected in the North of Mexico. Data were collected by convenience in public schools located in Northern Mexico, which prevents us from drawing inferences at national level, but provides us with a good insight of the health and nutritional status of adolescent girls attending to public schools in that region. Nevertheless, inferences of any possible causalities are speculative, and our findings are limited to the description of observed associations.

Selection of the participants

Initially, we planned to conduct an intervention study among 162 iron-deficient participants. Since the prevalence of iron deficiency in Mexican adolescent girls was 36% in urban areas

[46], we determined that we would need to screen 450 adolescent girls to find a large enough number of iron-deficient participants for our intervention study. Hence, this dictated the sample size of our survey.

An important methodological consideration is the selection of the study participants. Selection bias may occur in the voluntarily enrolment of schools and participants. For **chapters 4-6**, we recruited girls attending public schools located in low socioeconomic neighborhoods in industrialized cities. Whether our results can be translated to adolescent boys, or to girls who have dropped out of school, and adolescent girls with different sociodemographic characteristics remains to be demonstrated. Selection bias in a study affects the generalizability of the results but does not compromise the internal validity.

Measurement error

It is worth to mention that in **chapter 3**, the use of 7-day food frequency questionnaire to asses habitual dietary intake, may be subject to information bias, including social desirable answers and recall bias. Further, standard data collection and interviewer training ensure reliability. Likewise, the FFQ (**chapter 3**) and the 6-KADS (**chapters 4 and 5**) are validated tools, which were also pre-tested and adapted, minimizing any possible information bias, influencing our findings.

In **chapters 4 and 5**, the analysis of factors associated to depressive symptoms were exploratory. We modelled several potential exploratory variables available in the data. Nonetheless, we could not account for all possible explanatory variables in the etiology of depression. Notably, dietary intake and other social and psychological variables were not available and thus unaccounted for in the statistical models. Accordingly, we cannot rule out the effect of residual confounding in our statistical models.

Indicators used in the assessment of exposure variables and outcomes.

First, the self-reported information on AAM may introduce potential misclassification bias. However, this is likely to be a non-differential bias, which will bias the findings to null. Second, age at menarche is only one variable that reflects the timing (onset) of puberty. Future studies can benefit from including more variables on the timing (Tanner Stage, Pubertal Developmental Scale, age at menarche, hormonal levels) and tempo (can be extracted from growth models, requires more than one point data) of pubertal development, nutritional status prior to pubertal onset, maternal nutritional status, and expanding the age range of participants

by including younger girls. Finally, 96.1% of the participants from the primary analysis (**chapter 4-6**) had attained menarche. The homogeneity in pubertal status have prevented us from finding associations of pubertal status with nutritional status and depressive symptoms, as we hypothesized based on the literature.

Stainable iron in the bone marrow is considered the gold standard biomarker to assess iron deficiency; however, it is invasive and costly [47]. Ferritin and soluble transferrin receptor are commonly used as proxy indicators of iron status. Nonetheless, these biomarkers are sometimes independently influenced by inflammation. The regression approach to correct biomarkers of iron status for inflammation provides an improvement in estimating iron deficiency in settings where inflammation is prevalent [48]. However, there is still room to improve this method statistically, as we explored with some alternative methods in **chapter 6.**

We used the Kutcher Adolescent Depression Scale (6-KADS) to assess the severity of depressive symptoms in **chapter 4**. While standardized diagnostic interviews using established criteria are the gold standard for assessing depression, less time-consuming measures of depressive symptoms, such as 6-KADS, are commonly used for practical reasons. However, the use of categorical diagnostic constructs can result in misclassification bias. To overcome this pitfall, we used latent class analysis to identify subgroups of depressive symptoms. Latent class analysis aims to identify subgroups of people who share common characteristics in such a way that people within the subgroups have a similar scoring pattern on the measured variables, while the difference in scoring patterns between the subgroups are as distinctly different as possible. Such a technique has additional benefits for understanding the biological pathways of subtypes of depression.

General Conclusions and implications for future research

The research in this thesis illustrates a decrease in AAM in Mexican women, consistent with the trends in HICs. Furthermore, in our secondary and primary data analysis, we show that the double burden of malnutrition is present in Mexican adolescents and that iron deficiency, anemia, and overweight and obesity are associated with the severity of depressive symptoms in our study sample. However, the timing of pubertal onset and the gut microbiota composition are not. Based on our findings and reflections we recommend the following important areas for further research:

1. Providing more evidence for the adverse health consequences of the interaction between nutritional status and mental health can contribute to a better understanding of the problem and will help to create awareness and potential solutions.
2. Future nutritional interventions in Mexico that target adolescents should consider malnutrition in all its forms, as well as mental health.
3. The challenges of assessing iron deficiency in settings of acute inflammation have been widely recognized, yet understanding the mechanism in which low-degree inflammation related to overweight and obesity affects iron status remains to be explored more deeply, and existing methods for iron status assessment in these populations can be further improved.

Table 7.1. Summary of the results

Exposure or outcome of interest	Aim	Study design and population	Results	Chapter
Pubertal onset (AAM) and risk of disease	To assess if AAM is associated with disease risk in Mexican women	Cross-sectional study (ENSA-2000), 30628 women >20y	• AAM decreased in the 20th century from 13.6 y in 1920s to 12.6 y in 1980s. • AAM was inversely associated with BMI, and positively with height. • Women with later AAM had lower risk of diabetes and hypercholesterolemia.	2
	To examine the association between pubertal onset and depressive symptoms.	Cross-sectional study (Ten2Twenty-Mexico) 408 adolescent girls aged 12-20y	• There was no evidence that depressive symptoms were associated to age at menarche and years since menstruation.	4
Dietary patterns	To describe the dietary patterns of Mexican adolescents.	Cross-sectional study (ENSANUT-2006), 7380 adolescent boys and girls aged 12-19y	• Four dietary patterns were identified: non-traditional and breakfast-type, Western, plant-based and protein-rich.	3
	To study the association between dietary patterns and nutritional status.	Cross-sectional study (ENSANUT-2006), 7380 adolescent boys and girls aged 12-19y	• The Western and plant-based pattern were simultaneously associated with overweight-obesity and at least one indicator of undernutrition.	3
Depressive symptoms	To identify and characterize groups of girls based on depressive symptoms	Cross-sectional study (Ten2Twenty-Mexico) 408 adolescent girls aged 12-20y	• 44.4% of the adolescents were “unlikely to be depressed”, 41.5% were “likely to be depressed”, and 14.1% were “highly likely to be depressed”.	4
	To examine the association between iron status, hemoglobin concentration, body weight, and pubertal status	Cross-sectional study (Ten2Twenty-Mexico) 408 adolescent girls aged 12-20y	• Iron-deficient adolescent girls are more likely to suffer from depressive symptoms, and lower concentrations of hemoglobin and higher body weight increased the	4

	with depressive symptoms in Mexican adolescent girls		probability of experiencing depressive symptoms.	
	To investigate the relationship between gut microbiota composition and the presence of depressive symptoms in Mexican adolescent girls.	Cross-sectional study (Ten2Twenty-Mexico) 139 adolescent girls aged 12-20y	• The gut microbiota diversity of participants is not explained by depressive symptoms. • At the genus and phyla level, differences in the gut microbiota composition of participants in the different groups of depressive symptoms were not significant.	5
Iron deficiency	To compare commonly used and alternative methods to correct iron biomarkers for inflammation.	Cross-sectional study (Ten2Twenty-Mexico) 380 adolescent girls aged 12-20y	• The best correction model for ferritin included CRP transformed by inverse hyperbolic sine but did not include AGP, whereas sTfR did not benefit from any adjustment.	6
	To identify the best approach to determine iron status in Mexican adolescent girls within a setting of mild-burden of inflammation.	Cross-sectional study (Ten2Twenty-Mexico) 380 adolescent girls aged 12-20y	• The alternative approach with inverse hyperbolic sine transformation of CRP led to an increase of 5 percent points in the estimation of iron deficiency prevalence. On the other hand, transforming CRP and AGP did not modify the prevalence estimation of iron-deficient erythropoiesis.	6

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Summary

Previous studies have also reported an association between iron deficiency and depressive symptoms in children and adolescents. Nonetheless, there is limited information on the prevalence of iron deficiency and depression among adolescent girls in Mexico, and the potential link between iron deficiency and depression is not completely elucidated. Therefore, this thesis aimed to investigate the interrelationships between nutritional exposures and health outcomes among adolescent girls in Mexico, focusing on iron deficiency and depression.

In **chapter 2**, we investigated the time trends in age at menarche (AAM) during the 20th century in Mexico, and we tested the association between age at menarche and the risk of non-communicable diseases in adult life, using data from adult women (n = 30,628) from the Mexican National Health Survey (ENSA-2000). Linear and log-binomial regression were used for nutritional and disease outcomes, while Welch-ANOVA was used to test for a time trend. AAM decreased from 13.6 y in 1920s to 12.6 y in 1980s. AAM was negatively associated with weight (β = -1.01 kg; 95% CI -1.006, -1.004) and body mass index (BMI) (β = -1.01 kg/m²; -1.007, -1.006), and positively with height (β = 0.18 cm; 0.112, 0.231). AAM was associated with diabetes (RR = 0.95; 0.93, 0.98) and hypercholesterolemia (RR = 0.93; 0.90, 0.95), but not with hypertension, breast cancer or arthritis. We concluded that AAM decreased significantly during the 20th century in Mexico, and earlier AAM was positively associated with chronic disease in adulthood.

Chapter 3 explored the existing dietary patterns among Mexican adolescents and examined their association with nutritional status. This chapter used data from adolescents aged 12–19 years (n = 7380) from the National Survey of Health and Nutrition (ENSANUT-2006). Principal component analysis was used to derive the dietary patterns (DPs). Associations between DP and nutritional status were determined by prevalence ratios. We identified four DPs: nontraditional and breakfast-type, Western, plant-based, and protein-rich. The prevalence of overweight and obesity was higher in adolescents who scored high on the Western pattern (PR: 1.15, 95% CI: 1.08–1.21) or the plant-based pattern (PR: 1.09, 95% CI:1.03–1.17). The Western pattern was also positively associated with anemia in girls (PR: 1.18, 95% CI:1.03–1.35), while the nontraditional and breakfast-type pattern was inversely associated with anemia in younger adolescents (aged 12–15 years; PR: 0.87, 95% CI: 0.76–0.99) and in girls (PR: 0.84, 95% CI: 0.75–0.97), but not in older adolescents and boys. The Western pattern was simultaneously associated with overweight–obesity, and anemia. In the context of the double burden of malnutrition, dietary advice must consider malnutrition in all its forms.

Chapters 4-6, result from a cross-sectional study conducted in Santa Catarina and Monterrey, Northern Mexico, from September 2018 to January 2019. We collected data from 408 girls aged 12-20 years from public schools. Exclusion criteria were: diagnosis of systemic disease that may affect iron status, history of major surgery in the last month, and regular use of medication (except contraceptives). To assess depressive symptoms, we used the 6-item Kutcher Adolescent Depression Scale (6-KADS). Blood samples were collected for subsequent assessment of iron indicators (ferritin and soluble transferrin receptor) and inflammatory markers (C-reactive protein and alpha-1-acid glycoprotein), while fecal samples were collected to evaluate gut microbiota composition. In addition, anthropometrics and other sociodemographic variables were also collected.

Chapter 4 studies the subtypes of depressive symptoms and their relation with iron status, body weight, and pubertal onset. Latent class analysis (LCA) was used to identify and characterize groups of girls based on depressive symptoms. LCA yielded three classes of depressive symptoms; 44.4% of the adolescents were "unlikely to be depressed," 41.5% were "likely to be depressed," and 14.1% were "highly likely to be depressed." Our analyses demonstrated that iron-deficient girls had greater odds of being "likely depressed" (odds ratio, OR=2.01, 95% CI 1.01-3.00) or "highly likely depressed (OR=2.80, 95% CI 1.76-3.84). Linear regression analyses revealed that lower hemoglobin concentrations and higher body weight increased the probability of being "likely depressed." This study shows that iron-deficient adolescent girls are more likely to suffer from depressive symptoms and that lower concentrations of hemoglobin and higher body weight increase the probability of experiencing depressive symptoms.

Chapter 5 investigated if gut microbiota composition in girls with depressive symptoms differs from that of girls without depressive symptoms. Fecal samples were collected from 139 adolescent girls. The relative quantification of bacterial taxa was done using 16S ribosomal RNA gene amplicon sequencing. Beta-diversity revealed no significant differences in bacterial composition between participants within the different subgroups of depressive symptoms. Phyla and genera showed no nominal statistical differences between subgroups. However, several other variables (BAZ, ferritin, CRP, and AGP) that may be associated with depression seem to explain slightly the variation in the microbiota composition and suggest a potential link between iron metabolism, inflammation, and depression

Chapter 6 examines the existing methods to adjust biomarkers of iron status for inflammation and proposes an alternative method to account for inflammation when assessing iron status. For that purpose, inflammatory marker concentrations CRP and AGP were transformed in several ways (via logarithmic, fractional polynomial, cubic root, and inverse hyperbolic sine transformation) to achieve homoscedasticity, to improve model fit, and to normalize the distribution. The balance between model fit and model simplicity on SF and sTfR was tested with Akaike and Bayesian Information Criteria (AIC/BIC) and visual inspection of the residual plots. The best model for SF in our dataset, included CRP transformed by inverse hyperbolic sine but did not include AGP, whereas sTfR did not benefit from any adjustment. The alternative approaches to transform CRP led to an increase by up to 5.0 percentual points in the estimation of iron deficiency (SF<15 μg/L). On the other hand, transforming CRP and AGP did not modify the estimation of iron-deficient erythropoiesis (sTfR >8.3 mg/L). In conclusion, the estimation of iron deficiency can be statistically improved by using alternative methods to transform the markers of inflammation. More research is needed to determine the reproducibility of these alternative approaches to adjust iron status indicators in settings with varying degrees of inflammation.

Finally, **Chapter 7** summarizes the main findings and integrates them to fill in some of the research gaps mentioned in this introductory chapter. Overall, the research in this thesis illustrates a decrease in AAM in Mexican women. Furthermore, in our secondary and primary data analysis, we show that the double burden of malnutrition persists in Mexican adolescents and that iron deficiency, anemia, and overweight and obesity are associated with the severity of depressive symptom.

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About the author

Arli Zarate-Ortiz was born in Mexico. She completed her Bachelor in Nutrition and Wellness at Tecnologico de Monterrey, Mexico City. As part of her Bachelor studies, Arli had one-year academic internship at Universidad de Chile and she volunteered in the project "Study of Growth and Obesity (ECO)" at the Institute for Nutrition and Food Technology (INTA), Chile. After completing her Bachelor, Arli became research assistant at The National Institute for Public Health (INSP) in Mexico. Three years later she decided to pursued a master degree in Human Nutrition with specialization in Public Health and Epidemiology at Wageningen University. She did her MSc thesis entitled "A glance to physical inactivity and policies to promote physical activity in the European Union" at the National Institute for Public Health and the Environment (RIVM), The Netherlands. Additionally, she completed a minor thesis called "Infant and young children feeding practices in Mexico" at the department of Human Nutrition in Wageningen. In 2016, Arli started her PhD program as part Ten2Twenty project. Among other activities, she conducted a cross-sectional study in the North of Mexico and supported with educational activities.

Overview of completed training activities

Discipline-Specific activities	Organizing Institute	Year
Exposure Assessment in Nutrition Research	VLAG	2016
Pregnancy and programming and later risk of obesity and related disease	Parker Institute	2016
Gender, food safety and nutrition for Latin America and The Caribbean	FAO	2016
NutriScience	VLAG	2017
Food Composition	Human Nutrition & Health	2017
International Symposium on Understanding the Double Burden of Malnutrition for Effective Interventions"	IAEA	2018
LatinAmerican Society of Nutrition (SLAN) congress	SLAN	2018
Intestinal Microbiome of humans and animals	VLAG	2019

General courses	Organizing Institute	Year
VLAG PhD week	VLAG	2016
Competence Assessment	WGS	2016
Data Management Planning	WGS	2016
Information Literacy including EndNote introduction	WGS	2016
Chemometrics	VLAG	2017
Estimación de distribuciones de ingestión dietética habitual en poblaciones utilizando Recordatorio de 24 horas	Institute for Public Health and Nutrition (INSP), Mexico	2017
Essentials of Scientific Writing and Presenting	WGS	2018
Effective Behaviour	WGS	2018
Scientific Writing	WGS	2021
Intensive writing week	WGS	2021
Career perspectives	WGS	2021
Latent Class course	Statistical Innovation	2021

Other activities	Organizing Institute	Year
Preparation of research proposal	Human Nutrition & Health	2016
Weekly group meetings	Human Nutrition & Health	2017-2021
Advanced Analytical Epidemiology (HNH-33403)	Human Nutrition & Health	2018
PhD study tour to Canada + organizing committee	Human Nutrition & Health	2019

Colophon

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