Relationships of
Diet Quality, Sedentary Behavior, and DNA Methylation Patterns
with Cardiometabolic Risk Factors
among Children and Adolescents

by

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Dedication

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Abstract

Among youth, there has been a parallel increase in the prevalence of obesity and cardiometabolic abnormalities, including central obesity, glucose intolerance, elevated blood pressure, and dyslipidemia. This cluster of cardiometabolic abnormalities is a risk factor for the incidence of cardiovascular disease (CVD), cardiovascular-related mortality, all-cause mortality, and other chronic conditions in adulthood. While CVD manifests in middle-late adulthood, the atherosclerotic process and impaired cardiometabolic regulation start in childhood and track into adulthood. Therefore, primary interventions aiming to control cardiometabolic abnormalities are worth implementing at a young age.

The recommended first line management protocol for cardiometabolic abnormalities in youth focuses on behavioral modifications of obesity. In fact, particular lifestyle behaviors (i.e., diet and sedentary and physical activity) are options for the prevention and management. Nevertheless, there is little evidence that addresses their effects during periods of rapid growth and maturation using a repeated measures, longitudinal study design. Such limitations may hinder our understanding of the precise preventive mechanisms of lifestyle behaviors, needed for tailoring cardiometabolic recommendations for youth. The current project will contribute much-needed evidence by longitudinally examine the dietary and sedentary patterns among Mexican adolescents in relation to cardiometabolic health.

The aforementioned lifestyle factors and genetic susceptibility are not enough to explain the increased prevalence in cardiometabolic abnormalities across populations. In fact, DNAm has been associated with the underlying pathology of CVD. DNA methylation (DNAm) refers to

the covalent link between the fifth carbon in a cytosine nucleotide and a methyl group (CH3). However, there is a scarcity of population-based studies among youth, highlighting the need for evidence regarding the associations between DNAm and cardiometabolic health during this sensitive life period. The current study will address these gaps in knowledge.

The three aims of this work used data from a pre-existing birth cohort called, **E**arly **L**ife **E**xposure in **M**exico to **EN**vironmental Toxicants (**ELEMENT**), where Mexican children and adolescents aged 8 – 21 years were included in the analysis.

Aim 1 investigated the repeated longitudinal associations between diet quality scores, measured using the Dietary Approaches to Stop Hypertension (DASH), Alternate Mediterranean Diet Score (aMedDiet), and Children's Dietary Inflammatory Index (C-DII^{TM}) scores and cardiometabolic risk factors. Higher DASH scores were associated with better insulin homeostasis. The aMedDiet and C-DII scores were associated with serum triglycerides. The positive association with C-DII and an inverse association with aMedDiet score confirmed a protective effect of these dietary patterns on serum triglycerides.

Aim 2 examined the repeated longitudinal associations between sedentary activity patterns and cardiometabolic risk factors. Results suggested screen time was positively associated with blood pressure, and other sedentary time (i.e., doing homework/reading, and commuting) was positively associated with serum glucose. Using isotemporal substitution paradigm, we observed substituting sedentary time with moderate-vigorous physical activity was inversely associated with waist circumference and serum triglycerides. Furthermore, substituting an uninterrupted five minutes of sedentary time, a sedentary bout, or even one minute of sedentary bout with light activity was inversely associated with serum insulin.

Aim 3 studied the associations between DNAm at Long Interspersed Nuclear Element–1 (LINE-1), *H19*, 11β-Hydroxysteroid Dehydrogenase type-2 (*11β-HSD-2*), and Peroxisome Proliferator-Activated Receptor alpha (*PPAR-α*) and cardiometabolic risk factors. DNAm was measured at multiple CpG sites per genomic region. DNAm at LINE-1 was inversely associated with repeated measures of serum glucose at site 1, and positively with high-density lipoprotein cholesterol at site 3. *11β-HSD-2* DNAm at CpG site 4 was positively associated with repeated measures of serum glucose.

This dissertation showed that higher diet quality, lower sedentary time, and replacing sedentary activity with higher intensities were associated with a better cardiometabolic profile among Mexican youth. In addition, we detected few associations between DNAm on the selected four genomic regions and cardiometabolic profile.

Chapter 1 Introduction

Cardiometabolic Health:

Cardiometabolic risk factors are central obesity, dyslipidemia (i.e., elevated serum triglycerides and/or reduced serum high-density lipoprotein cholesterol), glucose intolerance (i.e., elevated fasting blood glucose, oral glucose tolerance test, or hemoglobin A1C), and elevated blood pressure [1,2]. This cluster of abnormalities in turn constitutes a risk factor for the incidence of cardiovascular disease (CVD), cardiovascular-related mortality, all-cause mortality [3,4], and other chronic conditions [5,6] in adulthood. The manifestations of CVD events occur in middle and late adulthood; however, the atherosclerotic process starts in childhood [7-12] and tracks into adulthood [13-17].

The recommended first line management protocol for cardiometabolic abnormalities in children and adolescents lies on behavioral modifications [18,19]. In fact, the size of the protective effect is greater when the behavioral modifications entails modifying more than one lifestyle behavior compared to targeting only one behavior [19]. Intervening at an early stage is a necessity for effective primary interventions; however, this step should be preceded with identifying the behavioral determinants of cardiometabolic risk factors in youth for targeted intervention [15,20].

Identifying early determinants of impaired cardiometabolic health is of special interest to Mexican children and adolescents due to their disproportionate burden of metabolic disorders. Hispanic youth have higher prevalence of childhood obesity and impaired cardiometabolic health compared to their non-Hispanic white counterparts [21]. Furthermore, Mexican youth showed signs of insulin resistance even in the absence of overweight or obesity [22]. This tendency might be

explained in light of the documented difference in their body composition. Hispanic youth had higher body fat compared to non-Hispanic White peers [23,24]. Despite this disproportionate burden among populations of Hispanic origin, few longitudinal studies have examined the early determinants of impaired cardiometabolic health on Mexican youth.

Dietary Patterns/Quality:

Diet is a well-established risk factor for cardiometabolic abnormalities [25]. Diet patterns have been suggested over the traditional single-nutrient approach, to assess the relationship between diet and health outcomes [26]. A diet pattern indicator is a summary score used to evaluate a subject's diet and categorize their intake based on the degree of adherence to the eating recommendations used to construct the score [26,27]. There are three categories of the diet pattern indicators based on the components included; they are 1) food/food group based indicators, 2) nutrient-based indicators, and 3) food and nutrient indicators [27]. Multiple factors advocate for that shift in the analytical approach. To mention a few, collinearity between some nutrients may obscure the independent assessment of a given nutrient while keeping other highly correlated nutrients constant. Additionally, the effect size might be very small or undetectable for single nutrient or dietary factor relative to the effect of multiple factors [28]. Lastly, this multi-dimensional approach allows for detecting the collective impact of multiple nutrients and for delivering practical and holistic dietary messages [26], consistent with the public health recommendations.

Evidence relating diet patterns to cardiometabolic health highlights three dietary scores relevant to pediatric populations, which are Dietary Approaches to Stop Hypertension (DASH) and the alternate Mediterranean Diet (aMedDiet), and Children's Dietary Inflammatory Index (C-DII^{TM}). Both DASH and Mediterranean diet have been considered to provide the "most

evidence for CVD prevention"[29] relevant to other eating patterns. The DASH diet is an eating pattern for reducing blood pressure based on research findings sponsored by the U.S National Institutes of Health [30]. The plan is characterized by 1) reduced intake of cholesterol, saturated fat, total fat, lean red meat, sweets, added sugars, and sugar-containing beverages and 2) increased intake of fruits, vegetables, and fat-free or low-fat milk and milk products, whole grain products, fish, poultry, and nuts. Therefore, it is rich in potassium, magnesium, and calcium, as well as protein and fiber, and lower in sodium[30]. Secondly, the Mediterranean diet is an eating pattern traditionally consumed among or people living in the countries surrounded by the Mediterranean Sea [31]. This eating pattern is characterized by 1) the high consumption of fruits, vegetables, whole grains, nuts and seeds, and the use of olive oil as a predominant source of fat, 2) the low – moderate consumption of animal products (i.e., dairy products, red meats, fish and poultry), and 3) the moderate consumption of wine [31]. It has been shown that this dietary plan was inversely associated with obesity, cardiometabolic risk clustering [32], and cardiovascular health [33]. Although the aMedDiet and DASH scores were originally developed for use in adult populations [34,35], their scoring systems uses population-specific cut-offs for the food consumption, that allows for these scores to be used in pediatric populations [20,36]. The third approach of assessing diet quality was C-DII, which is a tool to assess the inflammatory potential of the diet and has been associated with multiple inflammatory markers in adolescents [37,38] and adults [39-43]. Use of the C-DII in cardiometabolic health is well justified in light of the established link between inflammation and cardiometabolic abnormalities [44-47].

Despite wide recognition of the role of diet in the development of cardiometabolic abnormalities, evidence is inconsistent about how adherence to each of these diet quality scores associates with cardiometabolic risk in pediatric populations [12,20,48-52]. This inconsistency

underscores the need for prospective cohort studies that investigate the relationship between adherence to diet patterns and the cardiometabolic cluster in youth [24,31,48,52,53]. Moreover, there is a dearth of knowledge for the association among children and adolescents using longitudinal dietary assessment to track the change in diet pattern over time. Most published longitudinal studies limited their analysis to baseline diet assessment in predicting the future occurrence of cardiometabolic risk factors [49,50]. This work planned to address this research gap assessing the longitudinal associations between repeated measures of diet quality scores, DASH, aMedDiet, and C-DII, with repeated measures of cardiometabolic risk factors, among healthy children and adolescents.

Physical Activity and Sedentary Behavior Patterns:

Both sedentary behavior and physical inactivity are considered modifiable risk factors for CVD [54], and promoting physical activity and reducing the sedentary behavior across all ages is a strategy for preventing CVD [54]. Sedentary behavior and physical inactivity are not identical concepts [55], and meeting the physical activity recommendations is not a guarantee of not being sedentary [56]. Sedentary behavior is defined as "any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents (METs), while in a sitting, reclining or lying posture" [57]. On the other hand, physical inactivity is defined as "insufficient physical activity level to meet present physical activity recommendations" [57].

A few considerations complicate the examination of physical activity in children and adolescents. "Activity is accumulated in bouts" [58]; a bout is defined as "[a] period of uninterrupted time" performing a specific activity [57]. A bout has three "quantitative dimensions," which are frequency, duration, and intensity; they collectively describe the activity patterns over a specific period [57-59]. Research has shown that bouts enrich our understanding of the activity

pattern beyond what total volume of activity may convey [60]. Children have distinct patterns in engaging and accumulating physical activity; their patterns are characterized by highly active and interrupted patterns [61]. Therefore, the assessment of total time spent in physical activity or sedentary behavior will not capture how sporadic patterns are associated with cardiometabolic health [58]. This fact underscores the need to examine activity patterns to refine current recommendations for combating diseases [58]. Although previous reports have examined the bouts in relation to cardiometabolic risk factors, a systematic review showed inconsistent evidence, and noted the lack of longitudinal studies among youth [58].

A longitudinal study design can also account for the documented decline in physical activity and increase in sedentary behavior during development and maturation. Dumith et al. quantified the reduction in physical activity among children and adolescents aged 10 -19 years from longitudinal studies and found that on average, the percentage of change in physical activity per year was -7.0 (95% CI: -8.8 to - 5.2) [62]. In addition to the decline in physical activity, the prevalence of sedentary behavior increases with age in children aged 6 – 11 years [63]. Using a longitudinal design, which acknowledges the change in activity patterns via repeated measures of activity level, is necessary to provide evidence about the effect of physical activity on health outcomes among youth.

Moreover, there is a need for ethnic-specific recommendations of physical activity to ameliorate cardiometabolic risk factors among youth. Previously, it was demonstrated that despite the similar total levels of physical activity and sedentary time among adolescents from different ethnic backgrounds, Hispanic Americans have fewer minutes of moderate and vigorous activity relative to European Americans [24]. The difference in the activity patterns might be a reason for the inconsistent associations between physical activity and cardiometabolic risk

factors across races/ethnicities. Bremer et al. showed that physical activity was favorably associated with insulin homeostasis, waist circumference, high density-lipoprotein cholesterol (HDL-C) and low density-lipoprotein cholesterol (LDL-C) among non-Hispanic White adolescents, but the number of favorable associations were less among Mexican American counterparts [64]. Another study conducted among multiethnic children aged 7 to 12 years, showed that total sedentary time was associated with higher serum glucose among Hispanic American children, but not among African Americans nor European Americans [24]. The importance of understating the contribution of activity patterns on cardiometabolic risk factors is of special interest to Hispanic children and adolescents, who showed signs of insulin resistance despite the lack of manifestations for either overweight or obesity [22] because of their higher body fat compared to non-Hispanic White peers [23,24].

The available evidence relating sedentary behavior, in particular TV watching, among children and adolescents with cardiometabolic risk is inconsistent, and this body of work has been described as providing "very low" evidence due to the "serious risk of bias and serious inconsistency" between studies [65]. Similarly, a summary of the prospective studies showed null evidence between sedentary time and cardiometabolic health [66], and other researchers claimed the available evidence as "unconvincing" [67]. The method of assessing sedentary behavior might be a reason for the inconsistent findings [68]. The accelerometer is an excellent tool to overcome the limitations of the self-reported questionnaires [68]. However, it has failed in distinguishing between posture settings (standing vs setting or lying down) [68], and in assessing the context of sedentary behavior (i.e., passive vs active screen time) [68]. Thus, this could be a possible justification for the conflicting findings between sedentary behavior assessed using self-reported questionnaires asking for the screen time and the objective measure of the sedentary behavior

using accelerometer data with regard to the CVD risk factors in children [68]. This leads to the endorsement of assessing the sedentary behavior using two methods whenever it is possible as they measure two dimensions of the same construct [68].

This work aimed to overcome the aforementioned limitations in examining the relationships between activity level and cardiometabolic health in youth. Using a repeated measure longitudinal design, the associations between repeated measures of sedentary behavior patterns and cardiometabolic risk factors were investigated among Mexican children and adolescents.

DNA Methylation:

Lifestyle factors – including diet, lack of physical activity, sedentary lifestyle – and genetic susceptibility are generally considered predisposing factors for cardiometabolic abnormalities [69]. However, they are not enough to explain the increase in prevalence of these abnormalities across populations [69]. Another plausible mechanism explaining the etiology of cardiometabolic abnormalities and CVD is thought to be through epigenetic modifications [70-76]. Epigenetics is defined as "the study of mitotically heritable regulators of gene expression that do not change the DNA sequence" [77]. Unlike genetics, epigenetic modifications are dynamic and reversible [70,78] and can respond to the environment. The major types of epigenetic modifications include DNA methylation (DNAm), histone modification, and non-coding RNA. DNAm is the most commonly studied approach in epidemiology, and it has been associated with health outcomes. DNAm refers to the presence of a covalent link between carbon number 5 in the cytosine nucleotide and a methyl group (CH_3)[79,80]. In vertebrate, the methylation typically occurs at CpG sites, meaning adjacent cytosine and guanine nucleotides linked by a phosphate bond [81]. The process of methylating DNA requires the presence of a methyl donor, S-Adenosyl

methionine (SAM), and an enzyme called DNA methyltransferase (DNMT), that transfers the methyl group from SAM to the cytosine nucleotide resulting in the formation of 5-Methylcytosine (5mC) [79]. In mammals, there are three different DNMTs, which are DNMT1, which is involved in maintenance DNAm during replication, and DMNT3a, and DNMT3b, whose roles are mainly in the *De novo* DNAm during embryogenesis [82,83].

One of the main functions for DNAm is its role in regulating gene expression. The impact of DNAm on gene expression is locus-specific. DNAm at promotor regions and gene bodies are typically associated with suppression and activation of gene expression, respectively [79]. Other critical functions of DNAm at CpG sites are 1) maintaining the genome stability by controlling the expression of the transposable elements, 2) involvement in genomic imprinting and X-linked activation [79].

Ample research has examined early life epigenetic programming, where DNAm was measured during early development, in relation to obesity and CVD risk later in life [84]. Epigenetic research often focuses on early development since DNA undergoes a broad demethylation wave followed by a re-methylation wave during early embryogenesis making this an important developmental time period for long term programming [81]. However, DNAm is a dynamic process as it involves in determining cell identity and the cell's response to an environmental stimuli [78], and the impact of lifestyle factors such as diet, smoking, physical activity, and others on DNAm have been widely recognized [85-87]. Previous work suggests that adolescence, in particular, is also a susceptible period when environmental stimuli can impact DNAm patterns [88,89], with implications for health outcomes [70,78]. Moreover, adolescence is associated with changes in body composition and hormonal milieu [90], considered the hallmark for cardiometabolic abnormalities [18]. Despite the rapid growth in research aimed at developing

epigenetic biomarkers for CVD's diagnosis, prognosis, and individualized treatment regimens [72,73,91], there are few studies of this relationship among children and adolescents.

DNAm quantification methods are classified based on the following 1) Global DNAm , 2) Gene/locus-specific methylation, and 3) Epigenome-wide methylation[78]. Each method has its pros and cons. A brief explanation focuses on the first two types, as they are the analytical methods used in the current project. The global DNAm method measures the percentage of total 5mC levels in the entire genomic sample [78] in relation to total cytosine contents [92]. The genomic sample could be either a specific sequence of the genome or multiple regions of the repetitive elements, such as long interspersed nuclear elements (LINE-1). A limitation of this method is the lack of information on methylation patterns at specific genes/loci [78]. The second method, gene/locus-specific methylation, involves quantifying the percentage of methylation at specific genes or at CpG sites within the genes [78,92]. Through this method, information about the association between lifestyle factors and epigenetic regulation of specific genes can be inferred. However, in gene/locus-specific methylation , the specificity DNAm is a concern for many epidemiological studies, because methylation is variable across cells and tissue [78].

For this dissertation work, DNAm was quantified at LINE-1 as a proxy measure for global DNAm [78], and at three genes previously associated with cardiometabolic-related outcomes (*H19* [93-96], 11β -hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*) [97-101], and peroxisome proliferator-activated receptor alpha (*PPAR-α*) [102-106]).

LINE-1 is the only autonomous, active non-LTR (long-terminal repeat) retrotransposon in humans [107], and it is commonly used as a proxy measure of global DNAm [78] since it makes up 15% - 17% of the human genome [107,108]. Active expression of LINE-1 (i.e., decreased LINE-1 methylation) is associated with genomic instability and CVD, independent from well-established

CVD risk factors in adults [109]. DNAm at the LINE-1 region has been associated with cardiometabolic risk factors in adults [106,110-112], but few studies have been conducted in children and adolescents [113,114]. Perng et al. found that quartiles of LINE-1 DNAm were inversely associated with a change in waist circumference z-score among Colombian boys aged 5 -12 years old after 2.5 years of follow-up [113]. Dunstan et al. reported null cross-sectional associations between salivary DNAm at LINE-1 and adiposity outcomes (body mass index (BMI) z score, waist circumference z score, and percent body fat in 431 adolescents, predominantly Caucasians, aged 10 - 15 years [114].

H19 is a gene for a long non-coding RNA—it does not code for protein and is an imprinted gene, where the maternally allele of the gene is expressed, while the paternally allele is imprinted or silenced. Genomic imprinting is an epigenetic phenomenon, and is defined as "monoallelic expression of a gene or chromosomal region depending on the parental origin of inheritance" [115]. *H19* has a role in regulating cell formation and proliferation, weight, adipogenesis, oxidative metabolism and brown adipose tissue thermogenesis [93,94]. A study of adult rats showed that subcutaneous and visceral adipose tissue *H19* expression were associated inversely with BMI, but positively with a marker for brown adipose tissue thermogenesis, Uncoupling Protein 1 (Ucp1) [94]. Few human studies examined the DNAm at *H19* in children in relation to adiposity [95,96]. A previous study in the **ELEMENT** population showed that DNAm at *H19* was positively associated with higher subcutaneous fat, but not with central obesity or BMI z score, among girls only [95]. Huang et al. reported a similar positive association with *H19* DNAm and subcutaneous fat in Australian adolescents [96]. There are no other studies, that we are aware of, examining DNAm at *H19* in relation to other cardiometabolic risk factors.

11β-HSD-2 converts cortisol to an inactive metabolite called cortisone, and abnormalities in this gene have been associated with hypertension [97]. The gene is located on chromosome 16 q22[116]. 11β-HSD2 enzyme protects the activation of mineralocorticoid receptors by intracellular cortisol [117]. Previous studies showed that DNAm at *11β-HSD-2* at the promotor region was associated with suppressing the gene expression [116,118], and impaired 11β-HSD-2 enzyme activity leads to elevation in the urinary cortisol: cortisone metabolites ratio [97]. DNAm at *11β-HSD-2* at the promoter region was positively associated with blood pressure in adults [97,98], and, lower 11β-HSD-2 enzyme activity was associated with higher blood pressure in children [99]. Drake et al. found a positive correlation between 11β-HSD2 methylation in blood and weight, waist circumference, and BMI, in Scottish adults [98]. In fact, previous research has shown the enzymatic activity of 11β-HSD-2 is associated with age [119], dietary intake, obesity [100], insulin sensitivity [100], type 2 diabetes [101], and physical activity [120], and is regulated by other epigenetic mechanisms such as miRNA[121].

Peroxisome Proliferator-Activated Receptor alpha (*PPAR-α*) is one isoform of the PPAR family that additionally encompasses PPAR *β/* PPAR *δ*, and PPAR *γ* [102,122], and it is located on chromosome 22 [103]. *PPAR-α* controls multiple lipid metabolism pathways, including fatty acid oxidation, triglycerides synthesis and breakdown, and bile acid metabolism and others [102,103]. As another function for *PPAR-α*, it regulates oxidative stress and inflammatory response [102,122]. Therefore, its contributions to dyslipidemia, diabetes, and obesity are biologically plausible [122]. The use of fibrates, *PPAR-α* agonist drugs, has been shown to significantly lower cardiovascular risk among high-risk adults [104]. Few studies have assessed the relationship between DNAm at *PPAR-α* and cardiometabolic risk factors. DNAm at *PPAR-α* from visceral adipose samples analyzed among adults showed a positive correlation between DNAm and serum triglycerides

[106]. Moreover, rats fed a high fructose diet for two weeks showed a significant increase in hepatic DNAm at one CpG site in the promoter region of *PPAR-α*, lowered mRNA expression, high serum triglycerides, total cholesterol, and higher hepatic lipid accumulation [105]. Furthermore, by comparing the *PPAR-α* expression in human cell culture studies from subjects diagnosed with obesity and normal weight controls, the obese cell culture had lower compensatory increase in *PPAR-α* expression after oversupply the cell culture with lipids [123].

We quantified global DNAm and the DNAm at three genes previously associated with cardiometabolic-related outcomes (*H19*, *11β-HSD-2*, and *PPAR-α*) to shed light on the role of DNAm in cardiometabolic risk in youth. The associations between DNAm at LINE-1, *H19*, and *11β-HSD-2* and repeated measures for cardiometabolic risk factors were investigated. For *PPAR-α*, we assessed the cross-sectional associations between DNAm and cardiometabolic health. We hope our findings will contribute to the body of evidence in relation to cardiometabolic health and support investigating the role of epigenetics during adolescence, as one environmentally sensitive period of growth and development.

The three aims of this project were conducted using the pre-existing data from the **E**arly **L**ife **E**xposure in **M**exico to **EN**vironmental **T**oxicants (**ELEMENT**) birth cohort in Mexico City. Briefly, mother/child dyads from low- to moderate-income populations visiting prenatal clinics [124-126] were recruited for the project between 1997 -2004. The research team conducted multiple follow-up visits for the offspring, and collected information on physical growth, maturation, diet, physical activity, epigenetics, and clinical biomarkers for cardiometabolic health. The outcomes for each aim were lipid profiles, glucose, blood pressure, and anthropometry. The use of a well-characterized cohort allowed for adjusting for multiple confounders measured at childbirth. Aim 1 investigated the repeated longitudinal associations

between diet quality scores, measured using the DASH, aMedDiet, and C-DII with cardiometabolic risk factors. Aim 2 examined the repeated longitudinal associations between patterns of sedentary activity and cardiometabolic risk factors. Lastly, Aim 3 studied the association between DNAm at LINE-1, *H19*, *11β-HSD-2*, and *PPAR-α* with cardiometabolic risk factors.

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Chapter 2 Diet Quality Scores and Cardiometabolic Risk Factors in Mexican Children and Adolescents: A Longitudinal Analysis

Abstract:

Background: There is limited evidence for the effect of diet on cardiometabolic health during the pubertal transition. We collected repeated measures of diet quality and cardiometabolic risk factors among Mexican youth.

Method: This analysis included 574 offspring of the **E**arly **L**ife **E**xposure in **M**exico to **En**vironmental **T**oxicants (**ELEMENT**) birth cohort followed-up to three time points. Dietary Approaches to Stop Hypertension (DASH), alternate Mediterranean Diet (aMedDiet), and Children Dietary Inflammatory Index (C-DII^{TM}) scores were constructed from food frequency questionnaires. Higher DASH and aMedDiet, and lower C-DII scores reflect a higher diet quality. Cardiometabolic risk factors were assessed including lipid profiles, glucose homeostasis, blood pressure, and waist circumference. Linear mixed models were used between quartiles of each diet score and cardiometabolic outcomes.

Results: The fourth DASH quartile was inversely associated with log insulin (μIU/mL) [β= -0.23, *p*= 0.0021] and log-Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) [β= -0.25, *p*= 0.0008] compared to the first quartile. Besides, DASH score was linearly associated with log-HOMA-IR [β= -0.02, *p*= 0.0045]. Serum log-triglycerides (mg/dL) was linearly associated with aMedDiet [β= -0.03, *p*= 0.0031] and C-DII scores [β= 0.03, *p*= 0.0027]. The highest C-DII quartile was associated with log-triglycerides (mg/dL) [β= 0.11, *p*=0.0037].

Conclusion: Higher diet quality was associated with a better cardiometabolic profile among Mexican youth.

Keywords: Cardiometabolic risk factors, diet quality, longitudinal analysis, population-based study, children and adolescent, Mexicans.

Introduction:

The prevalence of childhood obesity is increasing worldwide. In the Latin America region, the prevalence increased from 1.6%, and 1.8% in 1975 to 10.4%, and 13.4% in 2016 for girls and boys aged 5-19 years, respectively [1]. Childhood obesity is associated with increases in the risk and prevalence of cardiometabolic abnormalities [2-5]. The cluster of cardiometabolic abnormalities is a risk factor for the incidence of cardiovascular disease (CVD), cardiovascular-related mortality, all-cause mortality [6,7], and other chronic conditions in adulthood [8,9]. Targeting childhood obesity is crucial for effective primary interventions of "adulthood cardiometabolic sequela" [5], and understanding the determinants of cardiometabolic risk factors in youth can inform the design of risk reduction and prevention programs [4,10].

Diet is a well-established risk factor for cardiometabolic health [11]. Diet patterns have been suggested over the traditional single-nutrient approach, to assess the relationship between diet and health outcomes [12]. A diet pattern summary score can be used to evaluate a subject's overall diet and categorize their intake based on the degree of adherence to the eating recommendations used to construct the score [12,13]. This multi-dimensional approach allows for detecting collective impact of multiple nutrients and for delivering practical and holistic dietary messages [14,15], consisting with the public health recommendations.

Evidence relating diet patterns to cardiometabolic health highlights three dietary scores relevant to pediatric populations. The Dietary Approaches to Stop Hypertension (DASH) and the alternate Mediterranean Diet (aMedDiet) are considered to have "the most evidence for CVD prevention [16]" relevant to other eating patterns. The DASH is an eating pattern for reducing blood pressure based on research findings sponsored by the US National Institutes of Health [17]. Secondly, the aMedDiet is an eating pattern for people living in the countries surrounded by the

Mediterranean Sea [18] with favorable associations with obesity, cardiometabolic risk clustering [19], and cardiovascular health [20]. These two eating plans emphasize a higher consumption of fruits, vegetables, whole grain, and nuts [17,18]. Moreover, the Dietary Inflammatory Index (DII®) is a tool to assess the inflammatory potential of the diet, and it has been associated with multiple inflammatory markers in adolescents [21,22] and adults [23-27]. The use of the DII in cardiometabolic health is well justified in light of the established link between inflammation and cardiometabolic abnormalities [28-31]. Contrasting the associations of these scores with cardiometabolic risk factors is of interest, to build up evidence needed to formulate precise public health messages to prevent or manage cardiometabolic abnormalities in youth. Moreover, comparing the associations across the three scores, none of which was developed originally for the Mexican population, will help to shed light on the role of eating habits, traditions, and cultural values in facilitating the adoption of these scores across different populations [32,33].

Current evidence about adherence to each of these diet quality scores on cardiometabolic risk in pediatric populations is inconsistent across studies [10,34-39], underscoring the need for prospective cohort studies that investigate the relationship between diet quality and cardiometabolic risk factors [18,35,39-41]. Thus, the aim of the study was to investigate the relationship between diet quality scores, DASH, aMedDiet, and Children Dietary Inflammatory Index (C-DII™), and cardiometabolic risk factors using repeated-measures longitudinal study design, among healthy Mexican youth enrolled in the **E**arly **L**ife **E**xposure in **M**exico to **En**vironmental **T**oxicants (**ELEMENT**) birth cohorts. We hypothesized that a lower diet quality and more pro-inflammatory diets will be associated with impaired cardiometabolic profile, higher waist circumference, blood pressure, glucose homeostasis, and triglycerides (TG), but lower high-density lipoprotein cholesterol (HDL-C).

Methods:

Study population:

The analytic sample consists of children and adolescents from two of three cohorts comprising the **ELEMENT** project in Mexico City, Mexico [42-44]. A detailed description of the **ELEMENT** project has been published before [44]. In brief, during 1997 -2004, 1012 mother/child dyads were recruited from prenatal clinics, which serve low- to moderate-income populations [45]. At childbirth, mothers reported sociodemographic information. A sub-sample of mothers enrolled at Cohort 3 participated in a randomized controlled trial (RCT) of daily calcium supplementation (1200 mg) during their pregnancies up to one year postpartum [43,44]. Offspring were followed multiple time points in childhood to collect relevant data about growth, diet, and health outcomes.

The current analysis included 574 children and adolescents who attended at least one of three follow-up visits in late childhood and adolescence, had at least one of eight cardiometabolic risk factors (waist circumference, systolic and diastolic blood pressure, fasting glucose, TG, HDL-C, insulin, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)) and dietary information. At the 2011 follow-up visit, herein called Time 1, 250 children aged between 8 -14 years were included [44]. Time 2, a follow-up study conducted in 2015, re-recruited 554 children aged 10 -18 years [44]. In the 2018 visit, called Time 3, 518 adolescents aged 12 - 21 years completed the last follow-up visit. The sample sizes that were available for each diet quality score and the number of repeated measures for each score are presented in Figure 1. The National Institute of Public Health of Mexico and the University of Michigan institutional review boards approved the research protocol. The research team

collected written informed consent and assent from mothers upon their enrollment and from adolescents, respectively.

Cardiometabolic Risk Factors:

***Anthropometric measures*:** Trained research staff collected duplicate measurements for body weight (kilograms) to the nearest 0.1 kg and height (centimeters) to the nearest 0.5 cm using in Time 1 a digital scale (BAME Model 420; Catálogo Médico/Tanita Co. Tokyo, Japan with height rod (model WB-3000m [38]), and only for weight in Time 2 and 3 the body composition device Inbody (model 230, Gangnam-gu, Seoul 135-960 KOREA). For waist circumference (centimeters) duplicate measurements were also performed to the nearest 0.1 cm using a non-stretchable measuring tape (SECA (model 201, Hamburg, Germany [38])). The average of the two measurements was used for the analysis [46].

Cardiometabolic biomarkers: Duplicate readings for systolic and diastolic blood pressure were recorded in seated position using a mercury sphygmomanometer (TXJ - 10 MD 3000 model, Homecare, China), and the average of the two measurements was used for the analysis. Fasting blood samples were used to analyze serum glucose via automated chemiluminescence immunoassay (Immulite 1000; Siemens Medical Solutions) [46], and TG and HDL-C using a biochemical analyzer (Cobas Mira Plus; Roche Diagnostics) [46]. Levels of insulin were quantified via enzyme-linked immunosorbent assay chemiluminescence method with IMMULITE® 1000, Erlangen, Germany equipment [38]. A Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as [fasting plasma glucose (mmol/l)*fasting serum insulin (mU/l))/ 22.5] [47]; higher values represents low insulin sensitivity/insulin resistance [47].

Diet quality scores:

Dietary intake was assessed using a semi-quantitative food frequency questionnaire (FFQ) adapted from the nationally representative 2006 Mexican National Health and Nutrition Survey [48]. The FFQ contains 101 food items in 14 food groups: 1) Dairy Products; 2) Fruits; 3) Vegetables; 4) Homemade Fast Food; 5) Meat, Sausages, and Eggs; 6) Fish and Seafood; 7) Legumes; 8) Cereal and Starchy Vegetables; 9) Corn Products; 10) Beverages; 11) Snack, Sweet and Desserts; 12) Soups, Creams, and Pasta; 13) Miscellaneous, and 14) Tortillas. The FFQ queries usual intake over the previous week [38,48]. The frequency of consumption fell into 8 categories, ranging from never to 6 times a day [38]. Mothers of children younger than 11 years of age attended the study visit and helped in the FFQ session to improve the accuracy and validity of children's answers. FFQs were analyzed using a food composition software developed by the National Institute of Public Health, Mexico [49,50]. The average daily intake was calculated by multiplying the nutrient content for each food item by its frequency of the reported consumption. Then, all intake values of all nutrients were summed to compute the daily consumption for each nutrient.

The construction of the DASH and aMedDiet scores was completed after grouping FFQ food items according to their nutritional properties. DASH and aMedDiet scores were calculated similarly to the methods proposed by Fung, et al. (2008) [51] and Fung, et al. (2005) [52], respectively (Table 1 (Supplementary) and Table 2 (Supplementary)). The possible range of values is 8 – 40 for the DASH score and 0 – 8 for aMedDiet – down from 9 due to the exclusion of the alcohol group; higher values indicate higher adherence to the diet pattern (i.e., individuals consumed more food/groups that characterized the dietary pattern). To account for the age and sex effects on dietary intake, we grouped our sample into 20 strata based on two-year increments by sex using a previously published approach [10,53].

Collected FFQ data at each Time point, was used to calculated the validated C-DII that included 25 components [54] (Table 3 (Supplementary)). An inflammatory effect score was given to each C-DII sub-component according to their relationship with various inflammatory markers, which was based on past literature [23]. To calculate the z-score for each component of the C-DII score, each child's dietary information was mapped with a population-based food consumption database composed of means and standard deviations from children in approximately 14 nations, which are referred to as global means and standard deviations [54]. The z-scores were calculated by subtracting the participants' intake from the global means and dividing by the global standard deviation. The z-scores were standardized per 1000 calories to adjust for between-person variability in energy intake [55]. The scores were converted into centered percentiles by doubling the value and then subtracting 1 to minimize the right-skewed distributions. The resulting percentiles were multiplied by their corresponding inflammatory effect score to obtain a component-specific C-DII value. Lastly, each child's C-DII score was the sum of its component-specific C-DII scores. The range of values for the C-DII in the current study was -4, +4, where positive values indicate, a pro-inflammatory diet and negative values represent an anti-inflammatory diet [38].

Covariates:

Based on prior knowledge, potential confounders assessed for this research study were: 1) baseline characteristics assessed at delivery, including sex, gestational age**,** mode of delivery, birth weight, duration of breastfeeding, and mothers' age, marital status, parity, years of education, and enrollment in the calcium supplementation RCT during pregnancy, and 2) Follow-up characteristics for the children that were measured at each of the three time points.

The follow-up characteristics were child's age, body mass index (BMI), total daily caloric intake, physical activity measured as metabolic equivalents, and puberty status.

Mothers reported household and demographic information, including their age, marital status (married, or others – includes free union, single, separated, and divorced), parity status (0, 1, ≥ 2), and years of education (<12 yrs., 12 yrs., or >12 yrs.), gestational age estimated by a registered nurse, mode of delivery (vaginal, or C-section childbirth), and enrollment in the calcium supplementation RCT (not enrolled or, enrolled). The newborns were followed until 5 years of age, and information about self-reported breastfeeding duration was quantified across the visits [56].

A physical activity questionnaire based on the Youth Activity Questionnaire (YAQ), and it was validated relative to 24 hours physical activity recall among Mexican school-children aged 10 to 14 years in Mexico City [57]. The questionnaire was used to calculate total metabolic equivalents. For each self-reported physical activity, the corresponding metabolic equivalent [58] was multiplied by the activity intensity. The total metabolic equivalents per week were calculated by summing the metabolic equivalents for all activities. Tanner stages for sexual maturation were assessed by a trained pediatrician including female breast development, male genitalia and female and male pubic hair [59] with values ranging from 1 for pre-pubertal status till 5 for fully mature status [60,61]. In this study, pubertal onset was indicated by a value greater than 1 for one or more Tanner stages [62].

Statistical Analysis:

The outcomes were 1) waist circumference (cm), 2) systolic and 3) diastolic blood pressure (mm Hg), 4) fasting glucose (mg/dL), 5) TG (mg/dL), 6) HDL-C (mg/dL), 7) insulin (μIU/mL), and 8) HOMA-IR. The residuals for TG, insulin, and HOMA-IR indicated skewness;

thus, log transformation was done. Demographic characteristics of the study participants were presented as mean (SD) for continuous variables and frequency (proportions) for categorical variables. We ran partial Spearman's correlations between diet quality scores across each study visit adjusting for age, sex, and total caloric intake. Linear mixed effects models with a compound symmetry error structure were used to examine the repeatedly assessed relationship between diet quality scores and cardiometabolic risk factors. Residuals of the final models were checked for assessing the mixed effects assumptions. Dietary quality scores were calculated by categorizing the continuous exposures into quartiles to assess dose-response relationships and detect any non-linear associations. We assigned the median value for each quartile, and examined the linearity of trends across quartiles by modeling the quartiles as continuous exposure. Findings are presented as β (SE), and p-value.

The crude model included quartiles of each diet score, and fully adjusted models included covariates that were considered potential confounders. Potential confounders were selected based on prior knowledge of the cardiometabolic health literature and their association with the quartiles of each diet quality score. We had repeated measures for the following covariates: age, total daily caloric intake, physical activity measured via metabolic equivalents, and puberty onset. All models were adjusted for total caloric intake and age, and sex when models included boys and girls together. Also, we additionally adjusted waist circumference models for BMI to account for body size [63]. We present our results from the overall sample and sex-stratified. To account for the multiple testing according to Bonferroni method, a p-value of < 0.00625 (0.05/8 [number of outcomes]) was considered for a significant finding. SAS statistical software package, version 9.4, was used for analyses (SAS Corp, NC, USA).

Results:

Figure 1 summarizes the study design, sample sizes that were available for each diet quality score and the number of repeated measures for each score. Table 4 shows the demographic characteristics of the children stratified by the study visit. The mean (SD) age of the sample was 10.32 (1.67), 14.50 (2.12), and 16.44 (2.14) years at Time1, 2, and 3, respectively. There was variability in the mean values of the cardiometabolic risk factors and diet quality scores across the follow-up visits. Time 1 had the highest values for the diet quality scores (i.e. higher DASH, and aMedDiet scores, and lower C-DII score (anti-inflammatory diet)); while Time 3 had the lowest diet quality scores (Table 4). The Spearman's correlation coefficients [r_s] between DASH and aMedDiet scores ranges from 0.39 – 0.45, for DASH and C-DII scores ranges from $r_{s=}$ -0.53 – -0.57, and for C-DII and aMedDiet ranges from r_s = -0.43 – -0.47 across the three follow-up study visits; all correlations were significant (p-value <0.0001) (Table 5 (Supplementary)).

Association between DASH diet scores and cardiometabolic risk factors:

The distributions of potential confounding factors were examined across quartiles of the DASH diet score. DASH scores had medians of 19, 23, 26 and 29 in each quartile. The DASH quartiles were associated with several factors, including mothers' characteristics (such as enrollment in the calcium intervention study, parity status, and years of education) and youths' characteristics (such as pubertal onset and metabolic equivalents) (Table 6 (Supplementary)). In adjusted models, girls in the second DASH quartile had higher waist circumference (cm) [β= 1.12, p= 0.0036] compared to those in the lowest DASH quartile. An inverse association was detected with log serum insulin among participants in the highest DASH quartile compared to the lowest DASH quartile [β= -0.23, p= 0.0021], corresponding to 23% reduction in serum insulin. Although the DASH score was linearly associated with HOMA-IR [β= -0.02, p=

0.0045], corresponding to 2.0% reduction for every unit increase in DASH score, the difference in HOMA-IR between the DASH quartiles was significant only between the highest vs. lowest quartile with 22.0% reduction [β= -0.25, p= 0.0008]. No association was found with other cardiometabolic risk factors in the overall sample or the sex-stratified analysis (Table 7).

Association between aMedDiet score and cardiometabolic risk factors:

The aMedDiet scores had medians of 2, 3, 5 and 6 in each quartile. The aMedDiet quartiles were associated with following confounding factors, including mothers' characteristics (such as enrollment in the calcium intervention study, and mode of childbirth) and youths' characteristics (such as pubertal onset and metabolic equivalents) (Table 8 (Supplementary)). In adjusted models, an inverse linear trend association was detected for log-serum TG [β= -0.03, p= 0.0031]. This change represented a reduction by 3.0% in serum TG for every unit increase in aMedDiet score. No association was found with other cardiometabolic risk factors either in the overall sample or the sex-stratified analysis (Table 9).

Association between C-DII score and cardiometabolic risk factors:

The C-DII scores had medians of -1.809, -0.630, 0.367, and 1.627 in each quartile. The C-DII quartiles were associated with several confounding factors, including mothers' characteristics (such as enrollment in the calcium intervention study, parity status, and years of education) and youth-related factors (such as pubertal onset and metabolic equivalents) (Table 10 (Supplementary)). In adjusted models, the C-DII scores were positively associated with serum TG. These associations were captured in the linear trend of the log-serum TG scale [β= 0.03, *p*= 0.0027], with an increase by 3.0% in serum TG for every unit increase in C-DII score. Moreover, a positive association was detected with log-serum TG among participants in the highest quartile [β= 0.11, *p*= 0.0037] compared to the lowest quartile, with a change by 11.0%. In sex stratified

analysis, the positive association for log-serum TG was detected with a linear trend [β= 0.04, *p*= 0.0051] among boys, and the highest quartile [β= 0.16, *p*= 0.0028] differed from the lowest quartile among boys. No association was found with other cardiometabolic risk factors either in the overall sample or the sex-stratified analysis (Table 11).

Discussion:

In this longitudinal study, we examined the relationships between three diet quality scores and cardiometabolic risk factors among Mexican children and adolescents aged 8 – 21 years across the three study visits. Our study showed that TG, out of the cardiometabolic risk factors, was associated with two diet quality scores; aMedDiet scores were negatively associated, while C-DII scores were positively associated. Both findings indicate higher dietary quality was associated with lower serum TG. Insulin and HOMA-IR were inversely associated with the DASH scores. As far as we know, our study is one of the few prospective studies with repeated measures of multiple dietary quality scores and cardiometabolic risk factors conducted among Mexican youth.

Our findings showed that serum TG was linearly associated with the aMedDiet and the C-DII scores. The increase from a quartile to the next was associated with small effect sizes (i.e., 2.75% for aMedDiet scores, and 3.20% for C-DII scores), and it might not be of clinical significance. However, a four-unit increase in diet quality, captured by the change from the first to the highest quartiles, was associated with 11% reduction, and 13% increase in serum TG for aMedDiet and C-DII scores, respectively. Our results indicating higher diet quality is associated with favorable control of serum TG was consistent with the established role of diet in managing hypertriglyceridemia [64-66]. Beside, diet impacts serum TG via the consumed dietary fatty acid, and synthesized fatty acids from excess glucose via the *de novo* lipogenesis pathway [64]. This

evidence collectively endorses controlling for serum TG as a potential primary intervention among youth to mitigate future dire cardiometabolic consequences given the evidence supporting the role of TG as an established risk factor for CVD among adults [67-70].

We found an inverse association between DASH score and HOMA-IR and serum insulin. Our results are consistent with findings from a meta-analysis of RCTs among adults [71], as well as a randomized cross-over clinical trial of 6 weeks of DASH intervention conducted among adolescent girls [72]. The nutrients rich in DASH diet, which are potassium, magnesium, calcium, [17] and folic acid [73], have potential roles in insulin and glucose homeostasis [74-76]. The inverse associations with insulin sensitivity were of special interest for the Hispanic youth because insulin resistance can be identified in Mexican children without evidence of overweight or obesity [77]. Insulin sensitivity is a driver for adipose tissue partitioning [78] and abnormal fat deposition may be a potential risk for the pathology of obesity [79]. Regarding the association between DASH score and blood pressure, our null results are consistent with null associations reported in other studies [37,80].

We identified few longitudinal studies conducted among Mexican youth with which to compare our results [38,81]. In a sub-sample of young adults in the **ELEMENT** cohort (N=100, and mean age= 21.5 years), Betanzos-Robledo et al. examined the association between DII scores, as a cumulative exposure from the first year of life until 21 years of age, with few cardiometabolic risk factors, and only blood pressure was positively associated with DII scores [38]. Moreover, Barragán-Vázquez et al. investigated the longitudinal association between C-DII scores and adiposity, assessed at 5, 7, and 11 years among Mexican children [81]. They found no association with waist circumference, which was consistent with our conclusions. However, they showed that a one unit increase in the C-DII score was associated with a change of 0.41% in waist

circumference among girls [81]. Future longitudinal studies are worth conducting to solidify the evidence by examining the role of diet and cardiometabolic health in youth from different analytical perspectives. For example, by assessing repeated longitudinal associations, the role of cumulative effect of diet across childhood might reveal additional information about the long-term exposure [38], and the heterogeneity in diet quality among the study population can be investigated as well [81].

We found a positive association between higher DASH score and waist circumference among girls. Waist circumference is an effective non-invasive tool for assessing truncal fat among children and adolescents [82]. However, repeated measures of waist circumference in childhood have to interpret with caution. Waist circumference captures information about subcutaneous fat, muscle, intramuscular fat, visceral fat, and bone [83]. The documented increase in waist circumference that parallels growth in children and adolescents [81,84,85] may not necessarily reflect a high-fat mass [84]. Additionally, it should also be noted that waist circumference is also affected by genetic and environmental factors [85], which may highlight the possibility of residual confounding in our analysis. Lastly, our dietary assessment may not capture habitual intake, as the used FFQ assessed the consumption over the last week [86], and that could increase the measurement error in our analysis.

Our sample had relatively lower diet quality and variability assessed by the three scores, which were consistent with other studies conducted on youth [87,88]. A plausible explanation might be because neither DASH or aMedDiet scores was originally developed accommodating Mexican traditions and eating habits. Eating habits are influenced by culture [32], which is captured via methods of preparing foods, social norms about food consumption, the availability of certain foods, and other factors [33]. This emphasizes the need to assess the cultural context when applying

diet quality scores across different populations [33]. Previously, it was shown that identifying empirically-driven dietary patterns were not necessary capturing the overall dietary pattern; rather these patterns reflected the meal patterns within households among adolescents enrolled in the **ELEMENT** cohort (N=550) [89]. In addition, the study found no evidence that suggest a distinction between "westernized" or "traditional" patterns, as they were simultaneously incorporated into eating patterns among adolescents [89]. Consistent with this observation, a Brazilian study showed higher consumption of both protective and unhealthy DASH score components among adolescents in the highest tertiles of the DASH score [87]. This evidence showed the importance of considering the cultural context when assessing diet quality among youth.

Differences in the associations between each diet score and cardiometabolic risk factors can be justified with several reasons. We found moderate associations; others also have reported both moderate [90,91] and higher associations [92,93] among diet quality scores. Secondly, the differences in the analytical methods deriving each scores could be a reason for the moderate associations [92,94]. Moreover, each score captures slightly distinct characteristics of the diet as each is composed of different foods and food groups and represents different dietary recommendations. We found that DASH score was associated with lower fat intake from all types. In contrast, aMedDiet and C-DII scores were positively associated with all types of fat, except for an inverse association for saturated fat and polyunsaturated fat, for aMedDiet and C-DII scores, respectively (Data not shown). DASH eating plan is characterized by reducing the intake of fat, and red meat [17]. On the other hand, aMedDiet and C-DII scores emphasize on fat quality, either by promoting food sources rich in the intake of nuts and seeds, and olive oil use, and the low-moderate consumption of animal products [18], or considering monounsaturated fat,

and polyunsaturated fat, for their anti-inflammatory potential, and cholesterol, saturated fat, and total fat for pro-inflammatory properties [95], respectively.

It should be kept in mind the few protective associations detected in the current study might have been with larger effect sizes if we had long duration of follow-up and large variabilities in diet quality and cardiometabolic risk factors. Children and adolescents are generally metabolically healthy [96,97] and dietary exposures might require long duration to manifest their impacts on clinical biomarkers of cardiometabolic health. Winpenny et al. assessed the role of three diet quality scores and cardiometabolic health in a sample from the UK aged 11 – 60 years, and noted the role of age in modifying the association. In the overall sample, they found inverse associations between diet quality scores and standardized metabolic syndrome, and component z scores, except for positive association with HDL-C z score. However, stratified analysis showed null association among subjects aged 11- 18 years – except for a protective association between a diet quality score and HDL-C z score, but stronger associations among the group aged 36 – 60 years compared to the other age strata [98]. Further studies with longer follow-up duration are worth conducting to examine the cardiometabolic abnormalities among youth as these associations may be pronounced in middle age.

The current study has several strengths. The **ELEMENT** birth cohort is a well-characterized cohort, and permits adjustment for multiple confounders at baseline. We examined the overall associations in addition to sex-stratified associations due to the plausible differences among boys and girls in their eating patterns and their cardiometabolic profile during pubertal transition. Moreover, most of the longitudinal studies conducted, limited their analysis to baseline diet assessment in predicting the future occurrence of cardiometabolic risk factors [36,37]. Repeated assessment of dietary intake enhances our understanding of the short and long-term

effects of adhering to certain dietary patterns because children change their eating patterns during development.

However, the current study has several limitations that should be considered when interpreting the findings. The aMedDiet and the DASH scores use "population-specific" cut-offs for the food consumption, and that allowed for these scores to be used in pediatric populations [10,53] despite their original application in adults [51,52]. Nevertheless, this may inflate type 2 error because of the reduction in diet variability in homogenous populations [99,100]. Another concern is that our sample might have different scores if other cut-off values were used [33,94,101]. To illustrate, the ratio between monounsaturated to saturated fatty acid component of aMedDiet was 1.6, and 0.93 in studies conducted in Greece [102], and the UK [103], respectively. To circumvent the inherent limitation of population-based cut-offs, we used C-DII scores as a third approach to assess diet quality. The C-DII scores use a population based food consumption database from multiple countries as a reference to calculate z-scores [54,104]. The standardization of reference values in C-DII score enhances cross-studies comparability, and reduces the inherited bias that might occur if using the study population as a reference.

Moreover, dietary assessment in children and adolescents is subject to reporting errors due to limited skills in retrieving the information, estimating the portion size and other factors [86,105]. Diet quality patterns might not be a precise measure for overall healthy habits among adolescents [87,89] because they are not a comprehensive dietary assessment [106,107]. Also, the FFQs used in this study queried the intake in the previous week, which may not capture the habitual intake [86]. However, we assessed diet at multiple time points to capture the change in consumption. Another limitation is that the FFQ used has not been formally validated, but has been used in the National Nutrition Survey of Mexico, which offers advantages of a culturally

relevant food list and comparable diet assessment [48]. Lastly, the possibility of residual confounding could not be ruled out.

In conclusion, in this study, we found a protective association for higher diet quality on selected cardiometabolic risk factors, e.g., TG and HOMA-IR among apparently healthy Mexican adolescents. Further studies are needed to validate the use of diet quality scores among children and adolescents and examine their reflection of the overall diet. Researchers have highlighted the importance of complementing diet assessment with measures that consider the culture of eating (such as watching media while eating, unhealthy snacks between meals, and others) [108]. Further studies are warranted to expand on this approach and validate the Composition and Culture of Eating questionnaire [108] in other populations. Lastly, it has shown that healthy diet patterns could have null or modest effect on cardiometabolic health outcomes compared to larger effect sizes for unhealthy eating patterns [109]. Thus, we endorse supplementing the diet quality assessment with indices for unhealthy eating behaviors, i.e., the consumption of processed foods. This eating behavior is of a great interest not only because it has been associated with impaired metabolic health [110], but also because Mexico had the highest annual retail sales per capita of ultra-processed food and drink products across Latin America [111,112], and the fourth rank worldwide [111].

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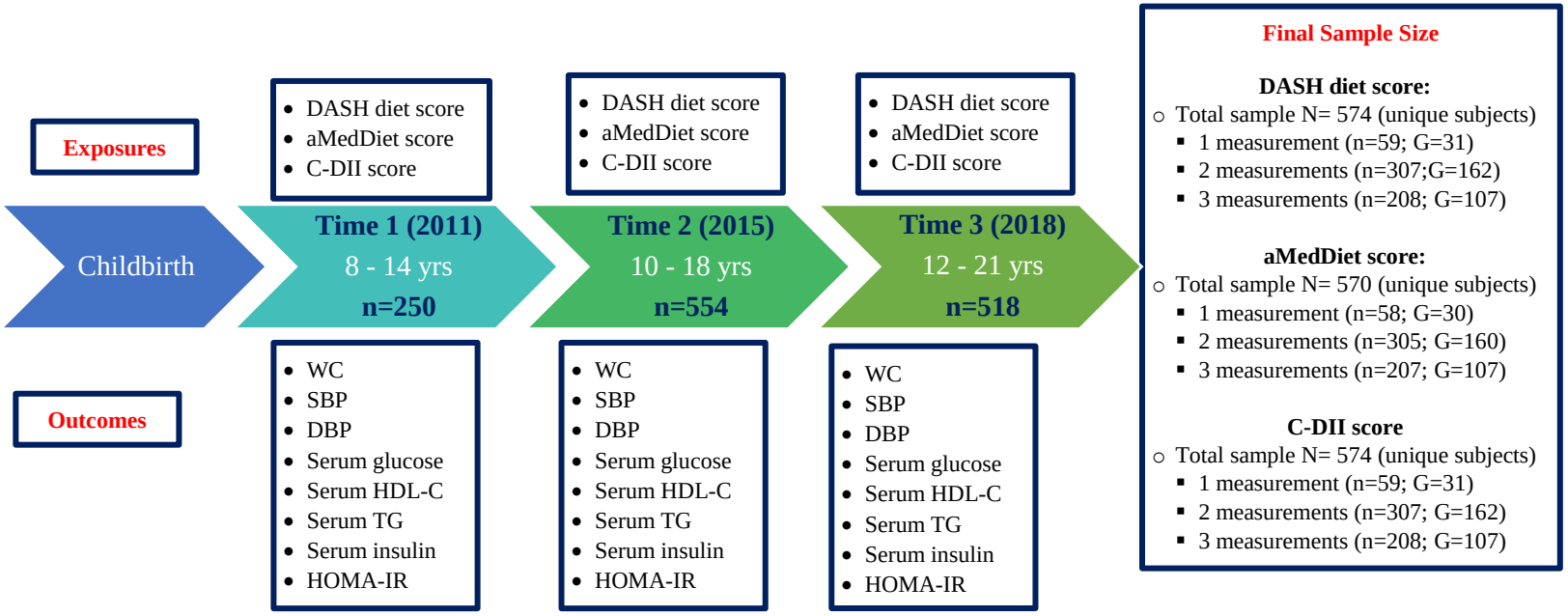
Figure 2.1: Flowchart Summary of Analytical Samples of Early Life Exposures in Mexico to ENvironmental Toxicants (**ELEMENT**) Cohort:



Abbreviations: DASH= Dietary Approach to Stope Hypertension; aMedDiet= Alternate Mediterranean Diet; C-DII= Children Dietary Inflammatory Index; WC= waist circumference; SBP= systolic blood pressure; DBP= diastolic blood pressure; HOMA-IR= Homeostatic Model Assessment for Insulin Resistance; TG= triglycerides; HDL-C =high density lipoprotein cholesterol; G=Girls

Table 2.1 (Supplementary): Scoring Criteria for Dietary Approaches to Stop Hypertension (DASH) Score:

No	Food groups (gm/day)	Foods included	Scoring criteria [1]
1	Fruits	All fruits and fruit juices	Q1 = 1 point; Q2 = 2 points; Q3 = 3 points; Q4 = 4 points; Q5 = 1 points
2	Vegetables	All vegetables except potatoes and legumes	
3	Nuts and legumes	Nuts and peanut butter, dried beans, peas, tofu	
4	Whole grains	Brown rice, whole wheat bread, cooked cereal, whole-grain cereal, other grains, popcorn, wheat germ, bran	
5	Low-fat dairy [2]	Skim milk, yogurt, cottage cheese	
6	Sodium [3]	Sum of sodium content of all foods in FFQ	Q1 = 5 points; Q2 = 4 points; Q3 = 3 points; Q4 = 4 points; Q5 = 1 point
7	Red and processed food	Beef, pork, lamb, deli meats, organ meats, hot dogs, bacon	
8	Sweetened beverages	Carbonated and noncarbonated sweetened beverages	

1: Sex- and age-specific quintiles.
2: The FFQ did not distinguish between low fat and high fat dairy products, so we included all dairy products in one group
3: Sodium intake was ranked without age or sex stratification.

Table 2.2 (Supplementary): Scoring Criteria for Alternate Mediterranean Diet (aMedDiet) Score:

No	Food groups (gm/day)	Foods included	Criteria for 1 point
1	Fruits	All fruit and juices	Greater than sex- and age-specific median intake
2	Vegetables	All vegetables except potatoes	
3	Legumes	Tofu, string beans, peas, beans	
4	Nuts	Nuts, peanut butter	
5	Whole grains	Whole-grain ready-to-eat cereals, cooked cereals, crackers, dark breads, brown rice, other grains, wheat germ, bran, popcorn	
6	Fish	Fish and shrimp, breaded fish	
7	Ratio of monounsaturated to saturated fat		
8	Red and processed food	Hot dogs, deli meat, bacon, hamburger, beef	Less than or equal to sex- and age-specific median intake

Table 2.3 (Supplementary): Availability of Children's Dietary Inflammatory Index (C-DII) Sub-components used in the Current Study:

No	DII Sub-components	Availability
1	Alcohol (g)	✓
2	Vitamin B12 (mcg)	✓
3	Vitamin B6 (mg)	✓
4	Beta Carotene (mcg)	✓
5	Carbohydrate (g)	✓
6	Cholesterol (mg)	✓
7	Energy (kcal)	✓
8	Fat (g)	✓
9	Fiber (g)	✓
10	Folic Acid (mcg)	✓
11	Iron (mg)	✓
12	Magnesium (mg)	✓
13	Monounsaturated fatty acids (g)	✓
14	Niacin (mg)	✓
15	Protein (g)	✓
16	Polyunsaturated fatty acids (g)	✓
17	Riboflavin (mg)	✓
18	Saturated Fat (g)	✓
19	Selenium (mcg)	✓
20	Thiamin (mg)	✓
21	Vitamin A (RE)	✓
22	Vitamin C (mg)	✓
23	Vitamin D (mcg)	X
24	Vitamin E (mg)	✓
25	Zinc (mg)	✓

Table 2.4: Descriptive Statistics of Mother and Child Characteristics of the Early Life Exposures in Mexico to ENvironmental Toxicants (**ELEMENT**) Analytical Sample:

	Time 1 N= 250	**Time 2** N= 554	**Time 3** N= 518
Maternal Characteristics (at time of child's birth)			
Years of education, %			
< 12 years	123 (49.20)[1]	284 (51.26)[2]	265 (51.16)[2]
12 years	91 (36.40)[1]	187 (33.75)[2]	171 (33.01)[2]
> 12 years	35 (14.00)[1]	78 (14.08)[2]	77 (14.86)[2]
Age at childbirth, (years)	26.80 (5.63)[1]	26.36 (5.40)[3]	26.38 (5.44)[3]
Parity, %			
0	1 (0.40)[1]	4 (0.72)[2]	4 (0.77)[2]
1	92 (36.80)[1]	205 (37.00)[2]	190 (36.68)[2]
≥ 2	156 (62.40)[1]	340 (61.37)[2]	319 (61.58)[2]
Marital Status, %			
Married	178 (71.20)[1]	390 (70.40)[4]	363 (70.08)[4]
Others (includes free union, single, separated, or divorced)	71 (28.40)[1]	157 (28.34)[4]	148 (28.57)[4]
Enrollment in calcium supplementation study, %			
Not enrolled	154 (61.60)[1]	399 (72.02)[2]	375 (72.39)[2]
Enrolled during pregnancy	95 (38.00)[1]	150 (27.08)[2]	138 (26.64)[2]
Child Characteristics (at birth)			
Girls, %	132 (52.80)	286 (51.62)	273 (52.70)
Gestational age, (weeks)	38.85 (1.49)[5]	38.76 (1.61)[6]	38.75 (1.60)[6]
Mode of delivery, %			
Vaginal delivery	144 (57.60)[7]	352 (63.54)[8]	329 (63.51)[8]
C Section	103 (41.20)[7]	194 (35.02)[8]	181 (34.94)[8]
Birth weight, (kg)	3.15 (0.45)[9]	3.15 (0.49)[4]	3.15 (0.48)[4]
Breastfeeding duration, (months)	8.10 (5.88)[1]	8.05 (6.07)[2]	8.00 (5.98)[2]
Child Characteristics (at follow-up visit)			
Age, (years)	10.32 (1.67)	14.50 (2.12)	16.43 (2.14)
Body mass index, (kg/m^2)	19.38 (3.60)	21.62 (4.15)	22.81 (4.46)
Body mass Z score for age	0.84 (1.24)	0.50 (1.25)[8]	0.50 (1.25)[10]
Pubertal onset, %	175 (70.00)	545 (98.38)	515 (99.42)[11]
Metabolic equivalents, (METs/week)	31.39 (19.82)	57.23 (39.01)	44.95 (35.18)[1]
Cardiometabolic risk factors			
Waist circumference, (cm)	70.75 (10.67)	79.56 (11.38)	85.53 (11.80)[1]
Systolic blood pressure, (mm Hg)	102.68 (10.20)	98.66 (9.92)	101.53 (9.83)[1]
Diastolic blood pressure, (mm Hg)	65.52 (7.32)	63.03 (6.86)	64.14 (7.20)[1]
Fasting glucose, (mg/dL)	87.02 (9.36)	77.81 (7.27)[12]	90.22 (8.41)[13]
HDL-C, (mg/dL)	58.68 (11.94)	43.06 (8.60)[12]	44.70 (9.03)[13]
TG, (mg/dL)	87.54(44.41)	103.97 (55.85)[12]	105.52 (50.09)[13]
Insulin, (μIU/mL)	6.26 (11.03)[14]	19.06 (11.84)[12]	19.21 (12.62)[15]
HOMA-IR	1.59 (3.51)[14]	3.69 (2.31)[12]	4.32 (2.94)[15]
Diet quality scores			
DASH diet score	24.84 (4.06)	24.23 (3.99)	24.00 (4.00)

aMedDiet score	4.26 (1.83)	3.81 (1.67)	3.77 (1.69)
C-DII score	-0.16 (1.35)	-0.11 (1.43)	-0.10 (1.46)

Means (SD) or count (percentages) are presented for continuous or categorical variables, respectively
Number of missing values 1.n=1; 2. n=5; 3.n=6; 4.n=7; 5.n=4; 6.n=9; 7.n=3; 8.n=9; 9.n=2; 10.n=65; 11.n=11; 12.n=154; 13.n= 142; 14.n=174; 15.n=143
Abbreviations: HDL-C =high density lipoprotein cholesterol; TG= triglycerides; HOMA-IR= Homeostatic Model Assessment of Insulin Resistance; DASH= Dietary Approach to Stope Hypertension; aMedDiet= Alternate Mediterranean Diet;C-DII= Children's Dietary Inflammatory Index

Table 2.5 (Supplementary): Spearman Correlations Coefficients between Diet Quality Scores:

		aMedDiet score	DII score
1 N=250	**DASH diet sore**	0.44966	-0.57187
	aMedDiet score	-	-0.44865
2 N=554	**DASH diet score**	0.38868	-0.52886
	aMedDiet score	-	-0.42776
3 N=518	**DASH diet score**	0.44159	-0.56334
	aMedDiet score	-	-0.47128

All p-value <0.0001

Table 2.6 (Supplementary): Overall Associations between Potential Confounders and Dietary Approaches to Stop Hypertension (DASH) Score:

	DASH Diet Score			
	Quartile 1 Median= 19 n=332	**Quartile 2** Median= 23 n=355	**Quartile 3** Median=26 n=351	**Quartile 4** Median= 29 n=284
Maternal Characteristics (at time of child's birth)				
Years of education, %				
< 12 years	54.24	47.71	53.43	49.47
12 years	31.82	34.00	34.57	37.01
> 12 years	13.94	18.29	12.00	13.52
Age at childbirth, (years)	26.40	26.68	26.34	26.38
Parity, %				
0	0.61	0.86	0.57	0.71
1	30.30	41.14	38.57	38.43
≥ 2	69.09	58.00	60.86	60.85
Marital Status, %				
Married	72.73	70.20	68.88	73.67
Others (includes free union, single, separated, or divorced)	27.27	29.80	31.12	26.33
Enrollment in calcium supplementation study, %				
Not enrolled	69.39	70.86	73.71	68.68
Enrolled during pregnancy	30.61	29.14	26.29	31.32
Child Characteristics (at birth)				
Girls, %	49.40	48.73	53.28	58.80
Gestational age, (weeks)	38.78	38.84	38.72	38.74
Mode of delivery, %				
Vaginal delivery	62.20	62.75	61.85	67.14
C Section	37.80	37.25	38.15	32.86
Birth weight, (kg)	3.17	3.12	3.12	3.19
Breastfeeding duration, (months)	7.82	7.84	8.06	8.53
Child Characteristics (at follow-up visit)				
Age, (years)	14.37	14.44	14.61	14.43
Body mass index, (kg/m^2)	21.77	21.40	21.74	21.77
Body mass Z score for age	0.64	0.51	0.54	0.60
Pubertal onset, %	85.71	86.53	87.07	84.75
Metabolic equivalents, (METs/week)	44.41	47.55	48.19	50.37
Total caloric intake, (kcal/day)	2365.55	2219.02	2308.09	2280.74

Means or percentages are presented for continuous or categorical variables, respectively.
Red color indicts the covariates included in the fully adjusted models for DASH diet score.

Table 2.7: Linear Mixed Regression Models for the Relationship between Quartile of Dietary Approaches to Stop Hypertension (DASH diet) Score with Cardiometabolic Risk Factors:

DASH score [1]		Waist circumference (cm)			Systolic blood pressure (mm Hg)			Diastolic blood pressure (mm Hg)			Log glucose (mg/dL)			Log TG (mg/dL)			log HDL-C (mg/dL)			Log insulin (µIU/mL)			Log HOMA-IR		
		All N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300
Crude model [2]																									
Quartile 1 Median= 19		(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
Quartile 2 Median= 23	β SE	0.3290 0.7379	-1.7641 0.9053	2.6736 1.1428	-0.2851 0.7010	-1.1086 1.0205	0.5242 0.9425	0.1422 0.5197	-0.3087 0.7595	0.6257 0.7036	-0.01721 0.01014	-0.03203 0.01391	-0.00364 0.01452	-0.01155 0.03466	-0.00835 0.04758	-0.00631 0.04972	0.009501 0.01885	0.05156 0.02721	-0.03006 0.02563	-0.1001 0.07586	-0.1137 0.1062	-0.08177 0.1075	-0.1217 0.07668	-0.1546 0.1071	-0.08626 0.1091
	P-value	0.6557	0.0520	0.0197	0.6843	0.2778	0.5783	0.7844	0.6846	0.3741	0.0899	0.0218	0.8021	0.7389	0.8607	0.8990	0.6143	0.0587	0.2415	0.1875	0.2850	0.4474	0.1129	0.1496	0.4295
Quartile 3 Median= 26	β SE	0.09201 0.7617	-2.7801 0.9663	2.8635 1.1434	-0.6746 0.7160	-2.1720 1.0700	0.9136 0.9372	0.1368 0.5285	-0.8724 0.7908	1.2081 0.6986	-0.00466 0.01005	-0.01281 0.01413	0.002958 0.01403	-0.03880 0.03537	-0.08675 0.04988	0.005701 0.04932	0.02000 0.01913	0.05558 0.02814	-0.01575 0.02551	-0.02026 0.07616	-0.04442 0.1087	-0.00571 0.1060	-0.02314 0.07702	-0.06669 0.1096	0.01215 0.1076
	P-value	0.9039	**0.0042**	0.0125	0.3463	0.0428	0.3300	0.7958	0.2704	0.0842	0.6429	0.3650	0.8330	0.2730	0.0827	0.9080	0.2963	0.0488	0.5374	0.7903	0.6830	0.9571	0.7639	0.5432	0.9102
Quartile 4 Median= 29	β SE	-0.9829 0.8514	-2.5003 1.1382	0.5892 1.2309	-0.4748 0.7877	-0.3090 1.2243	-0.05971 0.9996	0.003925 0.5772	-0.6580 0.8948	0.8226 0.7432	-0.02157 0.01058	-0.02724 0.01530	-0.01239 0.01442	-0.09454 0.03906	-0.09441 0.05781	-0.09647 0.05247	0.02863 0.02098	0.07668 0.03173	-0.01502 0.02721	-0.1323 0.08199	-0.1339 0.1223	-0.1484 0.1104	-0.1602 0.08306	-0.1634 0.1234	-0.1683 0.1125
	P-value	0.2485	0.0285	0.6323	0.5468	0.8008	0.9524	0.9946	0.4624	0.2687	0.0418	0.0758	0.3907	0.0157	0.1031	0.0666	0.1727	0.0160	0.5811	0.1071	0.2743	0.1798	0.0541	0.1865	0.1353
Linear	β SE	-0.08280 0.08159	-0.2866 0.1078	0.07905 0.1193	-0.05908 0.07507	-0.1037 0.1157	0.01289 0.09584	0.002651 0.05492	-0.08367 0.08410	0.09677 0.07117	-0.00155 0.001002	-0.00197 0.001446	-0.00086 0.001370	-0.00898 0.003738	-0.01096 0.005494	-0.00792 0.005047	0.002905 0.002001	0.007333 0.003001	-0.00108 0.002616	-0.00913 0.007728	-0.00990 0.01134	-0.01073 0.01051	-0.01100 0.007835	-0.01242 0.01146	-0.01164 0.01071
	P-value	0.3104	0.0081	0.5077	0.4314	0.3705	0.8930	0.9615	0.3202	0.1744	0.1220	0.1735	0.5314	0.0165	0.0466	0.1172	0.1470	0.0149	0.6786	0.2378	0.3833	0.3077	0.1608	0.2794	0.2777
Adjusted model [3,4,5]																									
Quartile 1 Median= 19		(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
Quartile 2 Median= 23	β SE	0.5597 0.2437	0.1304 0.2955	1.1217 0.3838	-0.3081 0.6901	-0.8217 1.0078	0.4695 0.9375	0.1619 0.5150	-0.08272 0.7466	0.6631 0.7011	-0.01725 0.01002	-0.02903 0.01387	-0.00373 0.01445	-0.00602 0.03400	0.005145 0.04634	0.006695 0.04905	0.009699 0.01523	0.03435 0.02164	-0.02150 0.02095	-0.1371 0.06644	-0.1550 0.09863	-0.09573 0.08908	-0.1519 0.06687	-0.1838 0.09814	-0.1006 0.09108
	P-value	0.0219	0.6593	**0.0036**	0.6554	0.4153	0.6167	0.7532	0.9118	0.3446	0.0855	0.0369	0.7965	0.8594	0.9116	0.8915	0.5244	0.1131	0.3054	0.0393	0.1169	0.2832	0.0234	0.0618	0.2702
Quartile 3 Median= 26	β SE	-0.03826 0.2508	-0.3449 0.3157	0.3845 0.3829	-0.5970 0.7021	-1.7154 1.0591	0.9465 0.9297	0.1527 0.5214	-0.4362 0.7783	1.2120 0.6943	-0.00373 0.009942	-0.01195 0.01424	0.004203 0.01410	-0.03738 0.03464	-0.06697 0.04875	0.01268 0.04865	0.02193 0.01558	0.03916 0.02273	-0.00074 0.02095	-0.08174 0.06692	-0.06098 0.1013	-0.09701 0.08859	-0.08305 0.06746	-0.07986 0.1009	-0.08291 0.09067
	P-value	0.8788	0.2752	0.3156	0.3953	0.1059	0.3090	0.7697	0.5754	0.0814	0.7075	0.4017	0.7657	0.2809	0.1703	0.7945	0.1595	0.0855	0.9718	0.2223	0.5474	0.2741	0.2187	0.4293	0.3611
Quartile 4 Median= 29	β SE	-0.01285 0.2810	-0.2079 0.3714	0.2549 0.4103	-0.1041 0.7723	0.1039 1.2063	0.1270 0.9890	0.1898 0.5691	-0.3482 0.8719	0.9246 0.7363	-0.01938 0.01055	-0.02584 0.01543	-0.01159 0.01445	-0.08453 0.03829	-0.07182 0.05620	-0.08762 0.05156	0.02736 0.01731	0.06742 0.02609	-0.00929 0.02240	-0.2266 0.07349	-0.2590 0.1158	-0.2044 0.09330	-0.2513 0.07439	-0.2957 0.1162	-0.2209 0.09579
	P-value	0.9635	0.5759	0.5347	0.8928	0.9314	0.8979	0.7388	0.6898	0.2097	0.0666	0.0947	0.4231	0.0275	0.2020	0.0899	0.1143	0.0101	0.6784	**0.0021**	0.0259	0.0290	**0.0008**	0.0113	0.0216
Linear	β SE	-0.01514 0.02697	-0.03377 0.03521	0.00505 9 0.03970	-0.02551 0.07354	-0.05500 0.1141	0.03115 0.09481	0.01784 0.05409	-0.04429 0.08207	0.1043 0.07051	-0.00133 0.001001	-0.00190 0.001464	-0.00076 0.001378	-0.00824 0.003663	-0.00860 0.005352	-0.00727 0.004964	0.002874 0.001656	0.006240 0.002479	-0.00027 0.002159	-0.01836 0.006949	-0.01913 0.01078	-0.01846 0.008907	-0.02004 0.007044	-0.02221 0.01083	-0.01923 0.009156
	P-value	0.5746	0.3380	0.8987	0.7288	0.6301	0.7426	0.7415	0.5896	0.1395	0.1837	0.1942	0.5839	0.0247	0.1087	0.1438	0.0830	0.0122	0.8992	0.0084	0.0768	0.0388	**0.0045**	0.0410	0.0362

1: median values of DASH score at each quartile
2: model includes DASH score quartiles as fixed effects and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
3: models additionally adjusted for the following fixed effects mother's enrollment in the calcium intervention study, parity status, years of education at childbirth, child age, pubertal onset, metabolic equivalents, and calories
4: sex is an additional fixed effect in the adjusted models for the overall sample
5: BMI is an additional fixed effect in the waist circumference model

Table 2.8 (Supplementary): Overall Associations between Potential Confounders and Alternate Mediterranean Diet (aMedDiet) Score:

	aMedDiet score			
	Quartile 1 Median= 2 n=298	**Quartile 2** Median= 3 n=269	**Quartile 3** Median=5 n=522	**Quartile 4** Median= 6 n=233
Maternal Characteristics (at time of child's birth)				
Years of education, %				
< 12 years	48.82	52.63	50.00	55.65
12 years	36.36	32.33	35.14	31.74
> 12 years	14.81	15.04	14.86	12.61
Age at childbirth, (years)	26.13	26.85	26.48	26.34
Parity, %				
0	0.00	1.50	0.39	1.30
1	43.10	33.83	36.10	35.65
≥ 2	56.90	64.66	63.51	63.04
Marital Status, %				
Married	70.95	70.19	71.57	72.05
Others (includes free union, single, separated, or divorced)	29.05	29.81	28.43	27.95
Enrollment in calcium supplementation study, %				
Not enrolled	70.37	71.43	72.97	65.65
Enrolled during pregnancy	29.63	28.57	27.03	34.35
Child Characteristics (at birth)				
Girls, %	52.68	52.79	50.38	55.36
Gestational age, (weeks)	38.83	38.91	38.68	38.74
Mode of delivery, %				
Vaginal delivery	56.23	65.41	64.01	68.58
C Section	43.77	34.59	35.99	31.42
Birth weight, (kg)	3.15	3.21	3.12	3.14
Breastfeeding duration, (months)	7.61	8.17	7.80	9.00
Child Characteristics (at follow-up visit)				
Age, (years)	14.48	14.58	14.61	13.99
Body mass index, (kg/m^2)	22.00	21.67	21.71	21.11
Body mass Z score for age	0.65	0.58	0.54	0.51
Pubertal onset, %	87.03	87.12	88.05	79.31
Metabolic equivalents, (METs/week)	44.69	47.02	49.05	48.37
Total caloric intake, (kcal/day)	1834.11	2049.71	2400.28	2918.90

Means or percentages are presented for continuous or categorical variables, respectively.
Red color indicts the covariates included in the fully adjusted models for aMedDiet score.

Table 2.9: Linear Mixed Regression Models for the Relationship between Quartile of Alternate Mediterranean Diet (aMedDiet) Score with Cardiometabolic Risk Factors:

aMedDiet score [1]		Waist circumference (cm)			Systolic blood pressure (mm Hg)			Diastolic blood pressure (mm Hg)			Log glucose (mg/dL)			Log TG (mg/dL)			log HDL-C (mg/dL)			Log insulin (µIU/mL)			Log HOMA-IR		
		All N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297	**All** N= 570	**Boys** N= 273	**Girls** N= 297
Crude model [2]																									
Quartile 1 Median = 2		(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
Quartile 2 Median = 3	β SE	-0.00397 0.7844	0.7594 0.9800	-0.9840 1.2059	0.4164 0.7559	1.3783 1.1285	-0.5512 0.9909	-0.08385 0.5620	-0.05524 0.8456	-0.07809 0.7386	-0.00518 0.01138	-0.00354 0.01614	-0.00745 0.01572	0.004121 0.03755	0.008136 0.05186	-0.00694 0.05352	-0.01521 0.02024	0.003428 0.03015	-0.03848 0.02689	-0.00645 0.08416	0.1204 0.1207	-0.1224 0.1160	-0.01854 0.08514	0.1047 0.1217	-0.1314 0.1181
	P-value	0.9960	0.4388	0.4149	0.5818	0.2225	0.5783	0.8814	0.9479	0.9158	0.6492	0.8266	0.6357	0.9126	0.8754	0.8969	0.4527	0.9095	0.1531	0.9389	0.3189	0.2919	0.8277	0.3900	0.2668
Quartile 3 Median = 5	β SE	-0.2745 0.7210	0.02010 0.9054	-0.6808 1.1045	0.2544 0.6813	2.1387 1.0115	-1.7062 0.8957	-0.2516 0.5025	0.4121 0.7494	-0.9031 0.6661	-0.01286 0.009678	-0.01415 0.01374	-0.01357 0.01335	-0.05454 0.03380	-0.04724 0.04667	-0.06745 0.04807	-0.01042 0.01806	0.01679 0.02651	-0.03923 0.02436	-0.01953 0.07260	0.07325 0.1042	-0.1016 0.09973	-0.03485 0.07356	0.05275 0.1052	-0.1128 0.1018
	P-value	0.7035	0.9823	0.5379	0.7089	0.0349	0.0572	0.6167	0.5826	0.1756	0.1843	0.3034	0.3099	0.1070	0.3121	0.1612	0.5642	0.5269	0.1079	0.7879	0.4827	0.3091	0.6357	0.6165	0.2685
Quartile 4 Median = 6	β SE	-2.2631 0.9080	-1.6717 1.1817	-3.0287 1.3487	0.1265 0.8487	0.4668 1.2956	-0.2324 1.0866	0.1132 0.6229	-0.5120 0.9521	0.7676 0.8072	-0.00407 0.01145	-0.01868 0.01659	0.006951 0.01551	-0.1098 0.04148	-0.1004 0.05936	-0.1269 0.05713	0.07574 0.02206	0.08852 0.03312	0.05865 0.02903	-0.2324 0.08983	-0.03947 0.1333	-0.4040 0.1199	-0.2375 0.09109	-0.07271 0.1346	-0.3854 0.1226
	P-value	0.0128	0.1578	0.0251	0.8815	0.7187	0.8307	0.8558	0.5910	0.3420	0.7223	0.2610	0.6543	0.0082	0.0915	0.0268	**0.0006**	0.0078	0.0439	0.0099	0.7673	**0.0008**	0.0093	0.5895	**0.0018**
Linear	β SE	-0.3730 0.1952	-0.2823 0.2557	-0.4632 0.2881	0.02046 0.1813	0.3241 0.2771	-0.2624 0.2326	-0.01863 0.1329	0.008106 0.2023	-0.02874 0.1732	-0.00214 0.002451	-0.00481 0.003520	-0.00024 0.003348	-0.02610 0.008975	-0.02317 0.01287	-0.03016 0.01233	0.01139 0.004809	0.01536 0.007151	0.007683 0.006382	-0.03660 0.01891	-0.00433 0.02759	-0.06626 0.02565	-0.03876 0.01917	-0.01101 0.02788	-0.06427 0.02619
	P-value	0.0563	0.2700	0.1085	0.9101	0.2426	0.2597	0.8885	0.9681	0.8682	0.3836	0.1723	0.9417	**0.0037**	0.0724	0.0148	0.0180	0.0322	0.2292	0.0532	0.8754	0.0102	0.0436	0.6932	0.0145
Adjusted model [3,4,5]																									
Quartile 1 Median = 2		(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
Quartile 2 Median = 3	β SE	-0.1420 0.2601	0.03933 0.3208	-0.3250 0.4021	0.5688 0.7468	1.4686 1.1171	-0.3590 0.9875	-0.1003 0.5602	0.02544 0.8347	-0.06161 0.7391	-0.00288 0.01119	-0.00199 0.01599	-0.00305 0.01555	-0.00303 0.03688	-0.00109 0.05089	-0.02578 0.05269	-0.00733 0.01643	0.02287 0.02385	-0.03676 0.02212	0.01505 0.07354	0.1104 0.1112	-0.08190 0.09746	0.002362 0.07401	0.08538 0.1102	-0.08758 0.09979
	P-value	0.5853	0.9025	0.4194	0.4464	0.1892	0.7163	0.8580	0.9757	0.9336	0.7972	0.9009	0.8446	0.9346	0.9830	0.6249	0.6556	0.3382	0.0973	0.8379	0.3214	0.4012	0.9745	0.4391	0.3806
Quartile 3 Median = 5	β SE	-0.3911 0.2458	-0.4860 0.3045	-0.3533 0.3778	0.4032 0.6911	2.3156 1.0287	-1.4274 0.9170	-0.3545 0.5140	0.4406 0.7576	-0.8536 0.6845	-0.00804 0.009857	-0.00869 0.01416	-0.00641 0.01358	-0.06943 0.03411	-0.06048 0.04709	-0.08546 0.04831	-0.00428 0.01529	0.03426 0.02200	-0.04444 0.02067	-0.00806 0.06635	0.02150 0.1003	-0.03184 0.08694	-0.01460 0.06712	0.001387 0.1002	-0.03219 0.08932
	P-value	0.1118	0.1111	0.3501	0.5598	0.0248	0.1201	0.4905	0.5610	0.2128	0.4152	0.5396	0.6373	0.0421	0.1998	0.0775	0.7794	0.1201	0.0321	0.9034	0.8303	0.7143	0.8278	0.9890	0.7187
Quartile 4 Median = 6	β SE	0.1843 0.3214	-0.2824 0.4054	0.4886 0.4887	0.4832 0.8972	1.1195 1.3507	0.04273 1.1819	0.01543 0.6632	-0.07551 0.9867	0.7504 0.8815	0.005378 0.01236	-0.01142 0.01784	0.02016 0.01707	-0.1112 0.04372	-0.08739 0.06131	-0.1442 0.06138	0.03456 0.01965	0.06864 0.02857	-0.00140 0.02636	-0.1271 0.08661	-0.08917 0.1332	-0.1504 0.1124	-0.1148 0.08776	-0.1210 0.1334	-0.1066 0.1156
	P-value	0.5665	0.4864	0.3179	0.5903	0.4075	0.9712	0.9814	0.9390	0.3949	0.6636	0.5224	0.2382	0.0111	0.1548	0.0192	0.0790	0.0167	0.9577	0.1427	0.5035	0.1817	0.1913	0.3649	0.3570
Linear	β SE	-0.03617 0.06905	-0.1337 0.08779	0.03707 0.1043	0.08198 0.1912	0.4483 0.2894	-0.2245 0.2515	-0.05365 0.1413	0.07090 0.2097	-0.05280 0.1879	-0.00034 0.002645	-0.00297 0.003810	0.002002 0.003657	-0.02788 0.009408	-0.02286 0.01329	-0.03310 0.01312	0.004778 0.004243	0.01342 0.006196	-0.00406 0.005685	-0.01990 0.01830	-0.01840 0.02795	-0.01786 0.02386	-0.01819 0.01855	-0.02371 0.02807	-0.01133 0.02452
	P-value	0.6006	0.1283	0.7225	0.6681	0.1219	0.3725	0.7042	0.7354	0.7788	0.8972	0.4361	0.5844	**0.0031**	0.0861	0.0120	0.2605	0.0309	0.4757	0.2773	0.5107	0.4546	0.3272	0.3988	0.6443

1: median values of aMedDiet score at each quartile
2: model includes aMedDiet score quartiles as fixed effects and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
3: models additionally adjusted for the following fixed effects mother's enrollment in the calcium intervention study, parity status, mode of childbirth, child age, pubertal onset, metabolic equivalents, and calories
4: sex is an additional fixed effect in the adjusted models for the overall sample
5: BMI is an additional fixed effect in the waist circumference models

Table 2.10 (Supplementary): Overall Associations between Potential Confounders and Children's Dietary Inflammatory Index (C-DII) Score:

	C-DII score			
	Quartile 1 Median= -1.809 n=330	**Quartile 2** Median= -0.630 n=331	**Quartile 3** Median= 0.367 n=331	**Quartile 4** Median= 1.627 n=330
Maternal Characteristics (at time of child's birth)				
Years of education, %				
< 12 years	47.06	47.11	55.45	55.32
12 years	35.29	36.17	31.21	34.35
> 12 years	17.65	16.72	13.33	10.33
Age at childbirth, (years)	26.74	26.64	26.09	26.35
Parity, %				
0	0.93	0.30	0.61	0.91
1	38.70	38.91	39.09	31.91
≥ 2	60.37	60.79	60.30	67.17
Marital Status, %				
Married	72.05	71.87	69.91	71.12
Others (includes free union, single, separated, or divorced)	27.95	28.13	30.09	28.88
Enrollment in calcium supplementation study, %				
Not enrolled	71.83	65.35	71.21	74.77
Enrolled during pregnancy	28.17	34.65	28.79	25.23
Child Characteristics (at birth)				
Girls, %	57.88	51.36	49.55	50.30
Gestational age, (weeks)	38.76	38.79	38.75	38.80
Mode of delivery, %				
Vaginal delivery	65.20	61.35	64.13	62.61
C Section	34.80	38.65	35.87	37.39
Birth weight, (kg)	3.14	3.14	3.12	3.19
Breastfeeding duration, (months)	7.92	7.48	8.59	8.17
Child Characteristics (at follow-up visit)				
Age, (years)	14.54	14.05	14.44	14.84
Body mass index, (kg/m^2)	22.25	21.17	21.63	21.60
Body mass Z score for age	0.73	0.50	0.54	0.51
Pubertal onset, %	84.80	85.67	85.37	88.54
Metabolic equivalents, (METs/week)	51.61	48.21	45.84	44.49
Total caloric intake, (kcal/day)	2162.51	2315.62	2384.56	2307.86

Means or percentages are presented for continuous or categorical variables, respectively.
Red color indicts the covariates included in the fully adjusted models forC-DII score.

Table 2.11: Linear Mixed Regression Models for the Relationship between Quartile of Children's Dietary Inflammatory Index (C-DII) with Cardiometabolic Risk Factors:

C-DII score [1]		Waist circumference (cm)			Systolic blood pressure (mm Hg)			Diastolic blood pressure (mm Hg)			Log glucose (mg/dL)			Log TG (mg/dL)			log HDL-C (mg/dL)			Log insulin (µIU/mL)			Log HOMA-IR		
		All N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300	**All** N= 574	**Boys** N= 274	**Girls** N= 300
Crude model [2]																									
Quartile 1 Median = -1.809		(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
Quartile 2 Median = -0.630	β SE	-1.7767 0.7480	-1.5876 0.9488	-1.6627 1.1287	-0.8237 0.7132	-0.8569 1.0841	-1.0464 0.9262	-0.8375 0.5289	-1.2088 0.8073	-0.5412 0.6915	0.008648 0.01016	0.01467 0.01453	0.003467 0.01392	0.04094 0.03449	0.08152 0.04795	0.01564 0.04866	0.02078 0.01881	0.01736 0.02810	0.02682 0.02502	-0.04829 0.07708	-0.03451 0.1137	-0.05160 0.1041	-0.04128 0.07801	-0.02215 0.1146	-0.05077 0.1060
	P-value	0.0177	0.0950	0.1413	0.2484	0.4296	0.2590	0.1136	0.1349	0.4341	0.3949	0.3132	0.8035	0.2356	0.0899	0.7480	0.2695	0.5370	0.2843	0.5312	0.7616	0.6202	0.5969	0.8469	0.6321
Quartile 3 Median = 0.367	β SE	-0.7154 0.7746	0.9719 0.9874	-2.3282 1.1678	-0.01267 0.7302	0.8179 1.1069	-1.0997 0.9509	-0.3229 0.5389	0.006068 0.8185	-0.7512 0.7087	0.01869 0.01022	0.03326 0.01447	0.002780 0.01418	0.07652 0.03565	0.09498 0.04894	0.07509 0.05097	0.003954 0.01935	-0.00060 0.02839	0.01082 0.02632	0.02633 0.07764	0.05897 0.1117	0.01183 0.1077	0.04014 0.07861	0.08463 0.1127	0.009923 0.1097
	P-value	0.3559	0.3255	0.0467	0.9862	0.4603	0.2479	0.5492	0.9941	0.2895	0.0678	0.0219	0.8446	0.0321	0.0899	0.1414	0.8381	0.9832	0.6813	0.7346	0.5979	0.9126	0.6097	0.4532	0.9280
Quartile 3 Median = 1.627	β SE	-0.4730 0.8139	0.5592 1.0580	-1.3017 1.2065	0.3410 0.7557	1.0353 1.1543	-0.5672 0.9737	-0.1045 0.5543	0.06080 0.8452	-0.3924 0.7246	0.008168 0.01039	0.01963 0.01500	-0.00252 0.01413	0.1172 0.03781	0.1706 0.05359	0.07392 0.05228	0.005732 0.02038	0.009810 0.03038	0.007625 0.02709	-0.06520 0.07842	-0.1412 0.1145	0.02099 0.1068	-0.05938 0.07950	-0.1222 0.1157	0.01296 0.1090
	P-value	0.5613	0.5974	0.2811	0.6519	0.3701	0.5604	0.8505	0.9427	0.5883	0.4322	0.1912	0.8585	**0.0020**	**0.0016**	0.1580	0.7786	0.7469	0.7784	0.4060	0.2183	0.8443	0.4553	0.2916	0.9054
Linear	β SE	-0.02710 0.2294	0.3636 0.3003	-0.4014 0.3386	0.1637 0.2122	0.4279 0.3244	-0.1571 0.2728	0.01930 0.1556	0.1336 0.2373	-0.1228 0.2030	0.003014 0.002901	0.006732 0.004178	-0.00070 0.003949	0.03424 0.01062	0.04648 0.01506	0.02450 0.01469	0.000128 0.005722	0.000998 0.008499	0.000844 0.007622	-0.01163 0.02195	-0.03093 0.03203	0.01011 0.02992	-0.00955 0.02226	-0.02485 0.03237	0.007784 0.03055
	P-value	0.9060	0.2266	0.2363	0.4406	0.1876	0.5650	0.9013	0.5736	0.5452	0.2991	0.1079	0.8594	**0.0013**	**0.0022**	0.0959	0.9822	0.9066	0.9119	0.5964	0.3348	0.7356	0.6681	0.4431	0.7990
Adjusted model [3,4,5]																									
Quartile 1 Median = -1.809		(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)	(Ref)
Quartile 2 Median = -0.630	β SE	-0.1622 0.2481	-0.4641 0.3073	0.1158 0.3783	-0.9515 0.7032	-0.9685 1.0724	-0.9390 0.9205	-0.8455 0.5245	-1.2116 0.7953	-0.4926 0.6885	0.009419 0.01003	0.01684 0.01441	0.006022 0.01382	0.04627 0.03392	0.09234 0.04686	0.002851 0.04794	0.01068 0.01518	-0.00189 0.02216	0.02140 0.02040	-0.05002 0.06788	-0.00704 0.1058	-0.09846 0.08748	-0.04387 0.06849	0.00781 5 0.1052	-0.09504 0.08982
	P-value	0.5133	0.1317	0.7597	0.1763	0.3669	0.3081	0.1072	0.1282	0.4746	0.3481	0.2431	0.6633	0.1729	0.0495	0.9526	0.4819	0.9321	0.2948	0.4614	0.9470	0.2610	0.5220	0.9408	0.2906
Quartile 3 Median = 0.367	β SE	-0.05138 0.2572	0.1094 0.3210	-0.2653 0.3921	-0.4100 0.7205	0.3755 1.0970	-1.2512 0.9455	-0.5400 0.5348	-0.2713 0.8052	-0.8539 0.7058	0.01666 0.01014	0.03328 0.01437	0.002557 0.01416	0.07787 0.03508	0.08906 0.04783	0.06151 0.05028	-0.00699 0.01576	-0.00828 0.02259	-0.00006 0.02169	0.02622 0.06887	0.07926 0.1042	-0.04227 0.09117	0.03146 0.06957	0.1008 0.1038	-0.04884 0.09372
	P-value	0.8417	0.7333	0.4990	0.5694	0.7322	0.1862	0.3128	0.7363	0.2268	0.1008	0.0210	0.8568	0.0267	0.0633	0.2217	0.6573	0.7142	0.9978	0.7035	0.4474	0.6432	0.6512	0.3319	0.6025
Quartile 3 Median = 1.627	β SE	-0.06379 0.2710	0.2387 0.3430	-0.3789 0.4077	-0.1990 0.7460	0.4404 1.1387	-0.9106 0.9770	-0.4396 0.5500	-0.3937 0.8245	-0.6105 0.7282	0.005773 0.01036	0.01965 0.01495	-0.00551 0.01437	0.1083 0.03724	0.1568 0.05225	0.05402 0.05202	0.008566 0.01682	0.007604 0.02460	0.01520 0.02257	-0.06494 0.07076	-0.09318 0.1084	-0.04209 0.09288	-0.06121 0.07177	-0.06606 0.1087	-0.05399 0.09568
	P-value	0.8139	0.4868	0.3531	0.7897	0.6990	0.3517	0.4243	0.6332	0.4021	0.5777	0.1894	0.7015	**0.0037**	**0.0028**	0.2995	0.6107	0.7574	0.5011	0.3590	0.3905	0.6507	0.3939	0.5436	0.5729
Linear	β SE	-0.00700 0.07627	0.1167 0.0974 7	-0.1327 0.1143	-0.00199 0.2097	0.2405 0.3203	-0.2696 0.2738	-0.08756 0.1545	- 0.01362 0.2313	-0.1931 0.2041	0.002124 0.002898	0.006508 0.004173	-0.00170 0.004026	0.03150 0.01046	0.04137 0.01471	0.01913 0.01462	0.000836 0.004735	0.001550 0.006911	0.002360 0.006364	-0.01141 0.01987	-0.01867 0.03034	-0.00738 0.02610	-0.01048 0.02016	-0.01090 0.03045	-0.01127 0.02688
	P-value	0.9269	0.2319	0.2461	0.9924	0.4531	0.3253	0.5711	0.9531	0.3444	0.4638	0.1196	0.6732	**0.0027**	**0.0051**	0.1912	0.8600	0.8227	0.7110	0.5659	0.5387	0.7774	0.6033	0.7204	0.6751

1: median values of C-DII score at each quartile
2: model includes C-DII score quartiles as fixed effects and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
3: models additionally adjusted for the following fixed effects mother's enrollment in the calcium intervention study, mother's years of education at childbirth, child age, pubertal onset, metabolic equivalents, and calories
4: sex is an additional fixed effect in the adjusted models for the overall sample
5: BMI is an additional fixed effect in the waist circumference models

Chapter 3 Sedentary Patterns and Cardiometabolic Risk Factors in Mexican Children and Adolescents: A Longitudinal Analysis

Abstract:

Background: Sedentary behavior is a modifiable risk factor for cardiometabolic health; however, the assessment of total sedentary time may not capture youth's highly active and interrupted activity patterns. Because of limited knowledge among Hispanic youth, who have a disproportionate burden of metabolic diseases, this study examined the longitudinal association between sedentary activity patterns and cardiometabolic risk factors among Mexican youth.

Design: 570 subjects in the **E**arly **L**ife **E**xposure in **M**exico to **EN**vironmental **T**oxicants (**ELEMENT**) birth cohort, who were followed up to three time points during adolescence, were included. Bout duration, and frequency and percentages of waking time spent in specific-intensities of activity, were quantified using ActiGraph GT3X+ wrist accelerometers. Self-reported questionnaires were used to query usual duration of different sedentary behaviors. Outcomes were lipid profile, markers for glucose homeostasis, anthropometry, and blood pressure. Linear mixed models and an isotemporal substitution approach were used, adjusting for potential confounders.

Results: Each hour of self-reported screen-based time was positively associated with diastolic blood pressure (mm Hg) [$\beta = 0.30$, $p < 0.01$], and an hour of other sedentary time was associated with log serum glucose (mg/dL) [$\beta = 0.01$, $p < 0.01$]. Replacing 1% of sedentary time with moderate to vigorous physical activity (MVPA) was associated with lower waist circumference

(cm) [β= -0.27, p <0.01] and log serum triglycerides (mg/dL) [β=-0.02, p<0.01]. Substituting one uninterrupted sedentary bout with light activity was associated with lower insulin (μIU/mL) [β= -0.06, p <0.01].

Conclusion: Sedentary time was associated with cardiometabolic risk factors in a context-specific manner and replacing sedentary time with higher intensities improved some cardiometabolic markers.

Key words: physical activity; sedentary behavior; screen time, bouts, cardiovascular health; metabolic health; adolescent health.

Introduction:

Sedentary behavior and physical inactivity, which is "an insufficient physical activity level to meet present physical activity recommendations" [1], are independent modifiable factors for CVD risk factors [2]. Thus, promoting physical activity and reducing sedentary behavior across the lifespan are strategies for preventing CVD [2]. That is consistent with the cardiometabolic abnormalities management protocol among children and adolescents [3]. Children have distinct patterns in engaging and accumulating physical activity; their patterns are characterized for being highly active and interrupted [4]. Therefore, the assessment of total time spent in physical activity or sedentary behavior will not capture how sporadic patterns are associated with cardiometabolic health [5]. Therefore, there is a need to examine the activity patterns to refine current recommendations for combating diseases [5].

One way to approach this need is to examine the forms of activity accumulation via the assessment of activity bouts [5], which is defined as uninterrupted time performing an intensity-specific activity. Bout assessment enriches our understanding of the activity pattern beyond what total minutes of activity convey [6]. Previous studies have examined the associations between bouts of activity and cardiometabolic health in children and adolescents. Nevertheless, inconsistent evidence was reported among youth, due to limited studies holistically assessing the entire spectrum of intensity levels, and cardiometabolic risk factors other than adiposity. In addition, there is a need for longitudinal studies to examine causal relationships [5]. Longitudinal designs can also address the age effect on the activity patterns, due to a decline in physical activity level and an increase in sedentary behavior during development and maturation [7,8].

Previously, it was demonstrated that despite similar total levels of physical activity and sedentary time among adolescents from different ethnic backgrounds, Hispanic Americans have

fewer minutes of moderate and vigorous activity relative to European Americans [9]. The difference in the activity patterns might be a reason for the inconsistent associations between physical activity and cardiometabolic risk factors across races/ethnicities. Bremer et al. showed physical activity was favorably associated with insulin homeostasis, waist circumference, triglycerides (TG), high density-lipoprotein cholesterol (HDL-C) and low density-lipoprotein cholesterol (LDL-C) among non-Hispanic White adolescents, but only with insulin homeostasis, TG, and HDL-C among Mexican American counterparts [10]. Additionally, among Hispanic American children, total sedentary time was associated with higher serum glucose, but not among African Americans nor European Americans [9]. The importance of understating the contribution of activity patterns on cardiometabolic risk factors is of special interest to Hispanic children and adolescents; they showed sign of insulin resistance despite the presence of overweight or obesity [11] because of their higher body fat compared to non-Hispanic White peers [9,12].

Therefore, the aim of the study was to assess the longitudinal associations between repeated measures of sedentary activity patterns with cardiometabolic risk factors, among children and adolescents in a Mexico City birth cohort study.

Methods:

Study population:

The study population was composed of children and adolescents enrolled at the **ELEMENT** project in Mexico City, Mexico [13,14]. A description of the **ELEMENT** birth cohorts has been published elsewhere [15]. Briefly, 1012 mother/child dyads from low- to moderate-income populations visiting prenatal clinics [16] were recruited for the project between 1997 -2004. At childbirth, mothers completed self-reported sociodemographic questionnaires. A subset of

670 mothers participated in a randomized controlled trial (RCT) of daily calcium supplementation (1200 mg) during their pregnancies until 1-year postpartum [14,15]. The research team conducted multiple follow-up visits for the offspring, and collected information on physical growth, maturation, diet, physical activity, and clinical biomarkers of cardiometabolic health.

The 2011 follow-up visit, herein called Time 1 was composed of 250 children aged between 8 -14 years [15]. Time 2 is a 2015 follow-up visit, and 554 children in the middle of pubertal transition-aged 10 -18 years were recruited [15]. Five hundred and eighteen adolescents aged 12 - 21 years completed the last follow-up visit, which was conducted in 2018, herein called Time 3. During Time 1 until Time 3, self-reported physical activity questionnaire assessment was collected while the objective physical activity assessment, using accelerometer, was completed at Time 2, and 3 only. The current sample size was 570 children and adolescents who attended at least one of the follow-up three visits and have information of sedentary behavior patterns and any cardiometabolic risk factors (waist circumference, systolic and diastolic blood pressure, fasting glucose, TG, HDL-C, insulin, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)). Figure 1 illustrates the number of repeated measures and the final sample size for each form of sedentary behavior assessment. The National Institute of Public Health of Mexico and the University of Michigan institutional review boards approved the research protocols followed in the **ELEMENT**'s project. Upon the subjects' enrollments in the project, the research team collected written informed consent and assent from mothers and adolescents, respectively.

Cardiometabolic Risk Factors:

***Anthropometric measures*:** Trained research staff collected duplicate measurements for body weight (kilograms) to the nearest 0.1 kg and height (centimeters) to the nearest 0.5 cm

using a digital scale (BAME Model 420; Catálogo Médico/ Tanita Co. Tokyo, Japan with height rod (model WB-3000m) [17], and waist circumference (centimeters) to the nearest 0.1 cm using a non-stretchable measuring tape (SECA (model 201, Hamburg, Germany)) [17]. The average of the two measurements was used for the analysis [18].

Cardiometabolic biomarkers: Duplicated readings for systolic and diastolic blood pressure were recorded in seated position using a mercury sphygmomanometer (TXJ - 10 MD 3000 model, Homecare, China), and the average of the two measurements was used for the analysis. Blood samples after fasting for ≥8 hours were used to analyze serum glucose via automated chemiluminescence immunoassay (Immulite 1000; Siemens Medical Solutions) [18], and TG and HDL-C using a biochemical analyzer (Cobas Mira Plus; Roche Diagnostics) [18]. Levels of insulin were quantified via enzyme-linked immunosorbent assay chemiluminescence method with IMMULITE® 1000, Erlangen, Germany equipment [17]. A Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as [fasting plasma glucose (mmol/L)*fasting serum insulin (mIU/mL))/ 22.5] [19]; higher values represent lower insulin sensitivity/insulin resistance [19].

Physical activity assessment:

At each of the three follow-up visits, questionnaires modified from the Youth Activity Questionnaire (YAQ) and validated relative to 24 hours physical activity recall among Mexican school-children aged 10 to 14 years in Mexico City [20], were administered by research staff. The questionnaire queried usual frequency of sedentary and select moderate-to-vigorous activities in the previous month. The questionnaires included four types of sedentary activities: 1) hours spent watching TV, 2) hours spent watching movies or videos on video cassette recorder (VCR) or digital versatile disc (DVD), 3) hours spent doing homework or reading, and 4) hours spent in

commuting (i.e., riding a bus or car). The answers for each type of sedentary activity were categorical responses. Total metabolic equivalents per week were calculated by summing the metabolic equivalents (METs) for all physical activities in the questionnaire. METs for each activity were calculated by multiplying the corresponding METs based on Ainsworth's et al., compendium [21] by activity intensity.

The self-reported sedentary activity exposures used in this analysis were (1) total sedentary time per day derived by adding up the number of hours spent in all four types of sedentary activities, (2) screen-based sedentary time per day calculated by combining the number of hours spent watching TV or movies, and (3) other sedentary time per day was calculated by adding up the number of hours spent doing homework or reading and commuting.

During the last two follow-up visits, an objective physical activity assessment was obtained using the ActiGraph GT3X+ (ActiGraph LLC, Pensacola, FL). The water-resistant device [22] was worn on the non-dominant wrist throughout the day for seven consecutive days (entire 24-hour period), and a wristband was used to secure the ActiGraph snugly on the wrist. Children and adolescents who had at least three weekdays and one weekend day [23,24] were included in the analysis. A day with less than 10 hours of accelerometer data was counted as an invalid day and removed from the analysis [16]. The collected data were processed with ActiLife program (ActiGraph LLC. 2009, Version 6.13.3). Pruned dynamic programming separated the waking time from the sleeping time [25]. Actigraphy data were summarized into 5-second epochs, and Chandler's Vector Magnitude cutoffs were used to classify the daily waking time into the following three categories of physical activity intensities: (1) sedentary, (2) light, (3) moderate and vigorous physical activity (MVPA) [26]. Out of all available days per subject, the average total minutes per day of physical activities were calculated and then used to calculate the objective

physical activity exposures. A bout was defined as 5 minutes of uninterrupted time performing a specific activity. Within a bout, we allowed for up to 30-second of change in the physical activity intensity before terminating the bout.

The objective physical activity exposures were:

- The percentage of sedentary activity per day (100*total minutes of sedentary activity per day / total minutes of waking time spent).
- The percentage of light activity per day (100*total minutes of light activity per day / total minutes of waking time spent).
- The percentage of MVPA per day (100*total minutes of MVPA per day / total minutes of waking time spent).
- Bouts frequency (bouts/day) as the sum of all bouts that occurred per day for each of the physical activity intensity
- Bouts duration (minutes/day) as the sum of bouts minutes occurred performing bouts throughout the day for each of the physical activity intensity

Potential confounders:

Based on prior knowledge, potential confounders included: 1) maternal and childbirth characteristics measured at baseline, e.g., sex, gestational age**,** mode of delivery, parity, mother's age, marital status, years of education, and enrollment in the calcium supplementation study, as well as duration of breastfeeding, and 2) follow-up characteristics for the children, measured at each of the three visits, e.g., child's age, body mass index (BMI), total daily caloric intake, and pubertal onset.

After childbirth, mothers reported household and demographic information, including their ages, marital status (married, or others – includes free union, single, separated, and

divorced), parity status (≤ 1, ≥ 2), and years of education (continuous), gestational age (continuous) estimated by a registered nurse, and mode of delivery (vaginal, or C-section childbirth), enrollment at the RCT for calcium supplementation (not enrolled or, enrolled). The newborns were followed until 5 years of age, and information about self-reported breastfeeding duration (continuous) was quantified across the visits [27].

Total caloric intake was calculated from a semi-quantitative food frequency questionnaire (FFQ) administered at each study visit [28-30]. Sexual maturation was coded based on trained pediatrician assessment for the breast, pubic hair, and girls genitalia [31] to assess Tanner stage (i.e., the range of values were 1 for pre-pubertal status up to 5 for fully mature status) [32,33]. In our analysis, we coded the pubertal onset as follow a value greater than 1 for the Tanner Stage for pubic hair or genital development for boys and pubic hair or breast development girls, respectively [34].

Statistical Analysis:

Exposure variables included daily total sedentary time, screen-based sedentary time, and other sedentary time. Objective assessments included the percentage of waking time spent in specific-intensities of physical activity, bout duration, and bout frequency of specific-intensities of physical activity. Outcome measures were 1) waist circumference (cm), 2) systolic and 3) diastolic blood pressure (mm Hg), 4) fasting glucose (mg/dL), 5) TG (mg/dL), 6) HDL-C (mg/dL), 7) insulin (μIU/mL), and 8) HOMA-IR. The HDL-C, TG, insulin, and HOMA-IR variables were log-transformed to minimize skewedness of their distributions. Mean (standard deviation) and frequency (proportions) were presented for the study population's continuous and categorical demographic characteristics, respectively.

We examined the relationship between sedentary time and physical activity patterns with the study outcome using linear mixed models with compound symmetry error structure for repeatedly assessed data within each participant. Residuals of the final models were checked for assessing the mixed effects assumptions. Findings are presented as β (standard errors [SE]) and p-value.

For each exposure, the crude model included only a continuous variable of the exposure. Some of the pre-specified set of confounders that were based on prior knowledge were included in the fully adjusted models if they were confounders to our study population based on their association with the outcomes. Age, sex, pubertal status, and METs for subjective exposures total time of physical activity for objective exposures were included in the adjusted models even if they did not associate with the exposures. Also, we additionally adjusted waist circumference models for BMI to account for body size [35].

We used substitution models for objective sedentary behavior exposures [36,37]. We included the percentage of waking time spent in physical activity (total) and the corresponding variables for light physical activity and MVPA. The beta coefficient of percentage of light activity in these models would be interpreted as the change in outcome for substituting a percentage of sedentary activity with a percentage of light activity while keeping the activity level constant. The same interpretation is applied for substituting a percentage of MVPA for a percentage of sedentary activity. For bout analysis, we included the continuous variable of sedentary activity with the corresponding continuous value for either light or MVPA in the same model. Beta coefficients of replacing the sedentary activity with light activity were calculated by taking the difference in the point estimates of light and sedentary activities. We followed the same approach for MVPA bout models. A corrected p-value of < 0.00625 (0.05/8 [number of

outcomes]) was considered a statistically significant association to account for the multiple testing according to the Bonferroni method. SAS statistical software package, version 9.4, was used for analyses (SAS Corp, NC, USA).

Results:

Figure 1 illustrates the study design and sample sizes used for subjective and objective assessments. The final sample size for self-reported sedentary time was 570 subjects, with three repeated measurements at the maximum per subject. For objective sedentary and physical activity assessment, 533 subjects were included with two repeated measurements per subject at the maximum (Figure 1). Table 1 shows the demographic characteristics of the study population by the time point. The mean (SD) age of the sample was 10.32 (1.67) years, 14.50 (2.12) years and, 16.43 (2.14) years at Time 1, 2, and 3, respectively. Among cardiometabolic risk factors, the mean values for waist circumference, triglycerides, insulin, and HOMA-IR rose across the three visits. Self-reported sedentary time was relatively stable across the three visits, while the objective assessment using accelerometer showed increases in sedentary activity and decreases in MVPA activity in Time 2 relevant to values reported at Time 1 (Table 1).

Association between self-reported sedentary time and cardiometabolic risk factors:

The distributions of potential confounders were examined across quartiles of self-reported total sedentary time (i.e., daily hours) (Table 2 (Supplementary)). Mothers' enrollment in the calcium intervention study, parity, and mode of childbirth showed notable differences across the quartiles, and thus they were included in the fully adjusted models. In adjusted models, 1 hour of screen-based sedentary time was positively associated with diastolic blood pressure [β= 0.3044 (mm Hg), p= 0.0038], and one hour spent in other sedentary activities (i.e.,

doing homework or reading and commuting) was associated with log-serum glucose [β= 0.01049, *p*= 0.0015] (i.e., 1% of change) (Table 3).

Associations for substituting percentages of sedentary time with physical activity on cardiometabolic risk factors:

Quartiles of the percentage of MVPA showed slightly different distributions for mothers' enrollment in the calcium intervention study, parity, mode of childbirth, sex, and pubertal status (Table 4 (Supplementary)). In adjusted models, substituting 1% of individual's daily sedentary time (median = 6 minutes) with an equal percentage of MVPA (median = 0.7 minutes) was associated with a reduction in waist circumference by 0.2707 cm ($p < 0.0001$), and log-serum TG [β= -0.02106, p= 0.0036] (i.e., 2% of change). Replacing 10% of individual sedentary time (median = 61 minutes) with same percentage of MVPA (median= 7 minutes) resulted in a proportionally greater effect size for waist circumference (cm) [β =-2.7066], and log-serum TG [β= -0.2106] (i.e., 19% of change) (Table 5).

Associations for substituting bouts of sedentary time with physical activity on cardiometabolic risk factors:

Among the covariates, tertiles of MVPA bouts frequency (Table 6 (Supplementary)) and bout duration (Table 7 (Supplementary)) were associated with mothers' enrollment in the calcium intervention study, parity, sex and pubertal status. Replacing one sedentary bout – a bout was defined as 5 minutes of uninterrupted time performing a specific intensity of activity– with an equal quantity of light activity was inversely associated with log-serum insulin [β= -0.05847, p= 0.0055] (i.e., 6% of change). Moreover, substituting 1 minute of spent in sedentary bouts with an equal quantity of light activity was inversely associated with log-serum insulin [β= -0.00868, p= 0.0041] (i.e., 0.9% of change) (Table 8).

Discussion:

The current study is one of the few prospective studies with repeated measures of both objective and self-reported sedentary patterns conducted among Mexican youth aged 8 – 21 years. Although we found null associations between total self-reported sedentary time and cardiometabolic risk factors, partitioning sedentary time by its context revealed that screen-time was positively associated with diastolic blood pressure, and other sedentary time (i.e., doing homework or reading and commuting) was positively associated with serum glucose. For an objective assessment of sedentary time, substituting the percentage of sedentary time with MVPA was associated with decrease in waist circumference and serum TG. Replacing sedentary bouts by light activity was associated with a reduction in serum insulin.

Our null association between total sedentary time and cardiometabolic risk factors were consistent with previous studies [6,38-40], but contradicted other studies that found positive associations [9,38,41]. It is worth noting that multiple systematic reviews and meta-analysis of observational studies, including prospective and cross-sectional study designs, have found limited or lack of evidence of an association between sedentary time and cardiometabolic health among youth [42-45]. Furthermore, evidence from a randomized cross-over study conducted among healthy youth supported the lack of any detrimental effects on cardiometabolic health after eight hours of uninterrupted sedentary activity [46]. Children and adolescents are metabolically healthy [47,48] and a short exposure might not show noticeable impact compared to cumulative exposure over decades among middle-aged adult populations [48]. Despite the limited evidence for sedentary time among youth, several national public health authorities have incorporated the reduction of sedentary time in their physical activity guidelines [49,50] as sedentary behavior is a modifiable risk factor for cardiovascular health across the lifespan [2].

We found a positive association between diastolic blood pressure and screen time. In fact, our effect size was similar to one reported among adolescents aged 11–13 years in the US in a predominantly Hispanic population [39] . Other studies have detected detrimental associations between screen time and other cardiometabolic risk factors such as waist circumference, lipid profile, fat mass, and BMI [39,51,52] . Our positive association with blood pressure is justified in light of the prior evidence showing TV watching is associated with higher caloric consumption [53-55], impaired diet quality [53,56], and shorter sleep duration [57], each of which are plausible contributor for impaired cardiometabolic health . Nevertheless, three reviews concluded that there was little evidence from observational studies regarding the association between screen time and cardiometabolic health, including blood pressure, in youth [58-60] and flagged heterogeneity concerns across studies [58,59].

A positive association between other sedentary time (i.e., doing homework or reading and commuting) and serum glucose was detected in our study. Previous experimental studies showed an increase in the mean ad libitum energy intake after cognitive-based sedentary tasks (i.e., reading and writing or computerized test-battery) relative to the control group (i.e., sitting in a comfortable chair) [61,62]. Similarly, an adult study revealed an increase in caloric consumption before major work deadlines [63]. Moreover, higher mean cortisol and larger variability in serum glucose and insulin while performing cognitive-based sedentary tasks have been reported [62]. This evidence suggests that cognitive-based sedentary time might contribute to positive energy balance and weight gain in the long-term [55,61,62]; future studies are warranted to expand the assessment of the sedentary behavior beyond the screen-time among youth.

Our substitution models showed inverse associations on waist circumference (β=- 0.27 cm) and serum TG (β=-0.81 mg/dL) when replacing sedentary time with MVPA. Similarly, other

studies have shown favorable associations for replacing sedentary time with MVPA on cardiometabolic health among youth [44,64]. However, our effect sizes were smaller than other studies [44,64]. The duration of replacement should be considered when comparing studies. We assessed replacing 1% of sedentary time (median 6 minutes) compared to 10 minutes [64] and 60 minutes [44]. We showed that a higher effect size resulted from higher percentages of replacement; however, it is noteworthy that even a small increase in MVPA resulted in a favorable impact. In short, our results were consistent with the recommendations to replace sedentary time with activity at higher intensities to improve cardiometabolic health related outcomes among youth [42,60,65].

We found that replacing a sedentary bout with light activity was associated with a reduction in serum insulin. Studies have found inconsistent results [6,38,47,66-68], with limited evidence from several reviews and meta-analysis [5,42,45,60]. Some methodological related factors in defining bouts could be a source for the heterogeneity as there is no consensus on defining the duration of a bout [6,42,57,67], and standardizing the exposure assessment would increase the robustness of the extracted evidence from studies [5,42,45,64]. Moreover, the differences in total minutes of activity and the partitioning into different intensities is important to consider when comparing studies. For example, our sample had higher minutes of sedentary activity [44,47,64], lower minutes of light activity [44,64], but higher minutes of MVPA [41,44,47,64]. Lastly, other studies showed that overweight and obesity have been associated with larger effect sizes for the association between sedentary and physical activity on cardiometabolic health [39,66], and body fat percentages partially explained the association [69]. Thus, future studies are needed to examine if body composition modulates the association between activity and health outcomes.

Our study has several strengths. The use of a well-characterized cohort allowed for adjusting for multiple confounders at childbirth. Additionally, multiple limitations of the previous works were addressed through our longitudinal design with repeated measures. Using the repeated measures of activity acknowledges the change in activity patterns during growth and maturation [7,8]. Different analytic perspectives were used; we examined the association of self-reported sedentary time, and substituting sedentary behavior pattern with higher intensity in relation to cardiometabolic risk factors. We also examined the 24-hours of activity for 7 consecutive days, as subjects wore the accelerometer continuously, as facilitated by the use of a water resistant device [25].

The study has several limitations, however. The sedentary time calculated from self-reported activity questionnaires has not been validated against an objective measure. We tried to address the change in activity pattern across the weekend and weekday for school-age youth [70], by including subjects who had at least four valid days out of the 7 days, one of which had to be a weekend day. However, some researchers have claimed that four days may not fully represent variability in movement behaviors in youth[70], and could be a source of random error [71]. Moreover, to address the youth's highly active and interrupted activity patterns [4], we summarized Actigraphy data into 5 second epochs [26] to reduce the measurement error and the miss-classification associated with using higher epoch length. However, there is no consensus about the epoch length used to summarize the accelerometer data; and that is a concerning point as previous research showed the association between bouts of activity and metabolic health was influenced by the epoch length [6].

Despite the common use of accelerometers as a feasible objective assessment tool for activity in epidemiological studies [72-74], it is not a gold standard for assessing sedentary behavior

[47]. Accelerometers don't distinguish between posture settings [4,67,74,75], which could misclassify light activity (i.e., static standing) as sedentary time [67], and they fail to capture the context of sedentary behavior because they provide a crude summary of total time of activity over the day [39,74,76]. Not all sedentary-context are equal in their impacts on health due to their differences in caloric and food consumption [53-56,61,77], energy expenditure and biological homeostasis [62,77] and other differences [57,78,79]. Lastly, we could not rule out the possibility of residual confounding in our analysis due to the use of crude assessment of some covariates or unknown confounding, such as family history of chronic diseases.

In conclusion, we reported detrimental impacts of screen-time and other sedentary time (i.e., doing homework or reading and commuting), and protective associations of replacing sedentary time by higher intensities on a few cardiometabolic risk factors among Mexican youth. Further studies are needed to consolidate the evidence around assessing sedentary and physical activity patterns using accelerometers. Currently, there is no consensus about the best approach to summarize accelerometer data, epoch length, and defining bouts, and that is needed to enhance the comparability of research findings across studies, and reduce measurement errors, and activity's misclassifications (i.e., misclassifying a duration of activity at different intensities) [6,39,42,57,67]. For the sedentary time assessment, validation studies are needed to examine the use of objective assessment tools, that can capture the context of the sedentary behavior. Furthermore, future studies are warranted to examine the context of sedentary behavior in relation to health outcomes to facilitate the incorporation of context-specific sedentary behavior recommendations among youth.

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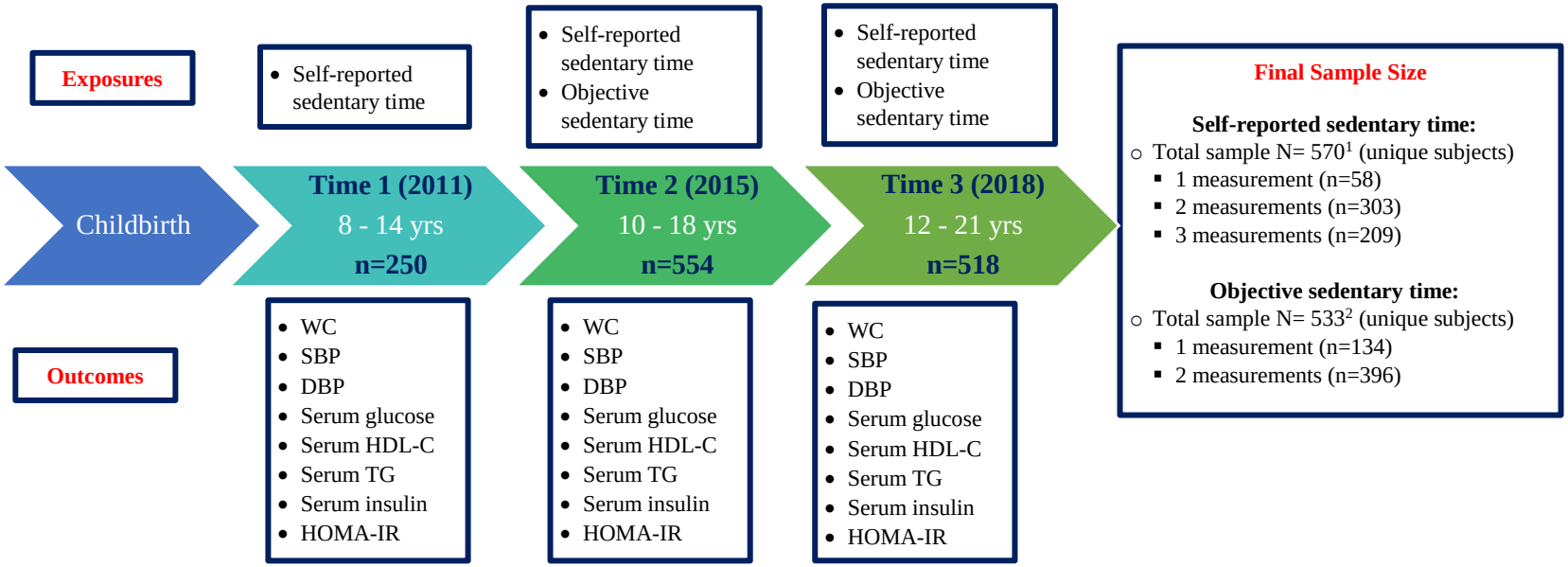
Figure 3.1: Flowchart Summary of Analytical Samples of Early Life Exposures in Mexico to ENvironmental Toxicants (**ELEMENT**) Cohort:



Abbreviations: PA= physical activity; WC= waist circumference; SBP= systolic blood pressure; DBP= diastolic blood pressure; HDL-C =high density lipoprotein cholesterol; TG=TG; HOMA-IR= homeostatic model assessment for insulin resistance

[1] the sample size for serum glucose, HDL-C, and TG is 432, and for serum insulin and HOMA-IR is 407

[2] the sample size for serum glucose, HDL-C, TG, insulin and HOMA-IR is 390

Table 3.1: Descriptive Statistics of Mother and Child Characteristics of the Early Life Exposures in Mexico to ENvironmental Toxicants (**ELEMENT**) Analytical Sample:

	Time 1 N= 250	**Time 2** N= 554	**Time 3** N= 518
Maternal Characteristics (at time of child's birth)			
Years of education, (years)	11.00 (2.79)	10.93 (2.91) [2]	10.98 (2.91) [2]
Age at childbirth, (years)	26.80 (5.63) [1]	26.36 (5.40)[3]	26.38 (5.44)[3]
Parity (≥ 2), %	156 (62.40) [1]	340 (61.37) [2]	319 (61.58) [2]
Marital Status (married), %	178 (71.20) [1]	390 (70.40) [4]	363 (70.08) [4]
Enrolled in calcium supplement study, %	95 (38.00) [1]	150 (27.08) [2]	138 (26.64) [2]
Child Characteristics (at birth)			
Girls, %	132 (52.80)	286 (51.62)	273 (52.70)
Gestational age, (weeks)	38.85 (1.49)[5]	38.76 (1.61)[6]	38.75 (1.60)[6]
Mode of delivery (vaginal delivery), %	144 (57.60)[7]	352 (63.54)[8]	329 (63.51)[8]
Breastfeeding duration, (months)	8.10 (5.88) [1]	8.05 (6.07) [2]	8.00 (5.98)[2]
Child Characteristics (at follow-up visit)			
Age, (years)	10.32 (1.67)	14.50 (2.12)	16.43 (2.14)
Body mass index, (kg/m^2)	19.38 (3.60)	21.62 (4.15)	22.81 (4.46)
Body mass Z score for age	0.84 (1.24)	0.50 (1.25)[8]	0.50 (1.25)
Pubertal onset, %	104 (41.60)	509 (91.88)	518 (100.00)
Total caloric intake, (kcal/day)	2627.32 (837.77)	2299.06 (922.41)	2124.47 (835.68)
Child Cardiometabolic risk factors			
Waist circumference, (cm)	70.75 (10.67)	79.56 (11.38)	85.53 (11.80) [1]
Systolic blood pressure, (mm Hg)	102.68 (10.20)	98.66 (9.92)	101.53 (9.83) [1]
Diastolic blood pressure, (mm Hg)	65.52 (7.32)	63.03 (6.86)	64.14 (7.20) [1]
Fasting glucose, (mg/dL)	87.02 (9.36)	77.81 (7.27) [9]	90.22 (8.41)[10]
HDL-C, (mg/dL)	58.68 (11.94)	43.06 (8.60)[9]	44.70 (9.03)[10]
TG, (mg/dL)	87.54(44.41)	103.97 (55.85)[9]	105.52 (50.09)[10]
Insulin, (μIU/mL)	6.26 (11.03)[11]	19.06 (11.84)[9]	19.21 (12.62)[12]
HOMA-IR	1.59 (3.51)[11]	3.69 (2.31)[9]	4.32 (2.94)[12]
Self-reported sedentary time and physical activity			
Total sedentary time, (hours/day)	5.48 (1.91)	5.88 (2.26)	5.37 (2.11)[1]

Metabolic equivalents, (METs/week)	31.39 (19.82)	57.23 (39.01)	44.95 (35.18) [1]
Objective assessment for sedentary and physical activity patterns			
Total time of physical activity, (minutes/day)	N/A	915.15 (56.74) [13]	924.06 (67.57)[14]
Time of minutes of sedentary activity, (minutes/day)	N/A	599.91 (73.42) [13]	630.78 (630.78)[14]
% of sedentary activity	N/A	65.54 (6.74) [13]	68.24 (6.71)[14]
Number of sedentary bouts, (bout/day)	N/A	36.72 (9.83)[13]	40.48 (9.44)[14]
Duration of sedentary bouts, (minutes/day)	N/A	322.70 (104.69)[13]	374.85 (109.80)[14]
Time of minutes of light activity, (minutes/day)	N/A	236.76 (41.37) [13]	226.19 (47.26)[14]
% of light activity	N/A	25.87 (4.33)[13]	24.47 (4.65)[14]
Number of light bouts, (bout/day)	N/A	0.62 (0.83)[13]	0.85 (1.01)[14]
Duration of light bouts, (minutes/day)	N/A	3.89 (5.53)[13]	5.53 (7.03)[14]
Time of minutes of MVPA activity, (minutes/day)	N/A	78.48 (28.50) [13]	67.08 (25.23)[14]
% of MVPA activity	N/A	8.59 (3.13)[13]	7.29 (2.71)[14]
Number of MVPA bouts, (bout/day)	N/A	0.19 (0.53)[13]	0.13 (0.31)[14]
Duration of MVPA bouts (minutes/day)	N/A	1.26 (3.60)[13]	0.88 (2.30)[14]

Means (SD) or count (percentages) are presented for continuous or categorical variables, respectively.
Number of missing values 1.n=1, 2. n=5, 3. n=6, 4.n=7, 5.n=4, 6.n=9, 7.n=3, 8.n=9, 9.n=154, 10= 142, 11. n=174, 12. n=143, 13.n=36, 14.n=83
Abbreviations: High density lipoprotein cholesterol: HDL-C, TG: TG, Homeostatic Model Assessment of Insulin Resistance: HOMA-IR, METs: metabolic equivalents, MVPA: moderate and vigorous physical activity

Table 3.2 (Supplementary): Overall Associations between Potential Confounders and Total Sedentary Time:

	Total Sedentary Time			
	Quartile 1 Median= 3.25 n=328	**Quartile 2** Median= 4.63 n=331	**Quartile 3** Median=6.00 n=331	**Quartile 4** Median= 8.00 n=331
Maternal Characteristics (at time of child's birth)				
Years of education, (years)	10.73	10.95	11.06	11.13
Age at childbirth, (years)	26.76	26.63	26.20	26.25
Parity (≥ 2), (%)	65.55	64.35	60.42	56.19
Marital Status (married), (%)	69.21	73.11	68.58	71.00
Enrolled in calcium supplement study, (%)	28.96	32.63	28.40	25.98
Child Characteristics (at birth)				
Girls, (%)	49.70	49.55	54.08	55.59
Gestational age, (weeks)	38.92	38.78	38.75	38.66
Mode of delivery (vaginal delivery), (%)	64.63	65.26	60.73	58.91
Breastfeeding duration, (months)	7.63	8.32	8.15	8.07
Child Characteristics (at follow-up visit)				
Age, (years)	14.50	14.37	14.25	14.74
Body mass index, (kg/m^2)	21.58	21.66	21.49	21.90
Pubertal onset, (%)	85.98	83.69	84.89	87.61
Total caloric intake, (kcal/day)	2194.93	2272.28	2322.66	2377.76
Metabolic equivalents, (METs/week)	45.92	46.23	47.33	50.65

Means or percentages are presented for continuous or categorical variables, respectively
Red color indicts the covariates included in the fully adjusted models for total sedentary time, screen-based sedentary time, and other sedentary time.

Table 3.3: Linear Mixed Regression Models for the Relationship between Sedentary Time and Cardiometabolic Risk Factors:

	Waist circumference (cm) N= 570, # obs= 1291			**Systolic blood pressure (mm Hg)** N= 570, # obs= 1290			**Diastolic blood pressure (mm Hg)** N= 570, # obs= 1290			**Log glucose (mg/dL)** N= 432, # obs = 1008		
	All sedentary time	Screen-based sedentary time	Other sedentary time	All sedentary time	Screen-based sedentary time	Other sedentary time	All sedentary time	Screen-based sedentary time	Other sedentary time	All sedentary time	Screen-based sedentary time	Other sedentary time
Crude models [1]												
B (SE)	-0.1372 (0.1283)	-0.8739 (0.1495)	1.5799 (0.2283)	0.03736 (0.1207)	0.1320 (0.1429)	-0.1905 (0.2212)	0.1534 (0.08891)	0.2848 (0.1050)	-0.1669 (0.1634)	-0.00059 (0.001751)	-0.00440 (0.002084)	0.008415 (0.003210)
P-value	0.2852	**<0.0001**	**<0.0001**	0.7570	0.3560	0.3894	0.0848	0.0068	0.3071	0.7377	0.0352	0.0089
Adjusted models [2,3]												
β (SE)	-0.05715 (0.04269)	-0.1031 (0.05157)	0.04846 (0.08128)	0.08744 (0.1189)	0.1725 (0.1421)	-0.1219 (0.2273)	0.1638 (0.08821)	0.3044 (0.1048)	-0.1855 (0.1688)	-0.00021 (0.001729)	-0.00444 (0.002070)	0.01049 (0.003293)
P-value	0.1810	0.0457	0.5511	0.4624	0.2252	0.5918	0.0636	**0.0038**	0.2719	0.9015	0.0323	**0.0015**

	Log TG (mg/dL) N= 432, # obs = 1008			**log HDL-C (mg/dL)** N= 432, # obs = 1008			**Log insulin (µIU/mL)** N= 407 , # obs= 837			**Log HOMA-IR** N= 407 , # obs= 837		
	All sedentary time	Screen-based sedentary time	Other sedentary time	All sedentary time	Screen-based sedentary time	Other sedentary time	All sedentary time	Screen-based sedentary time	Other sedentary time	All sedentary time	Screen-based sedentary time	Other sedentary time
Crude models [1]												
B (SE)	-0.00190 (0.006112)	-0.00375 (0.007302)	0.002368 (0.01100)	-0.00309 (0.003296)	0.01026 (0.003919)	-0.03357 (0.005837)	0.01062 (0.01306)	-0.01815 (0.01566)	0.07654 (0.02359)	0.009777 (0.01323)	-0.02378 (0.01586)	0.08649 (0.02385)
P-value	0.7564	0.6078	0.8296	0.3484	0.0090	**<0.0001**	0.4167	0.2469	**0.0012**	0.4602	0.1342	**0.0003**
Adjusted models [2]												
β (SE)	-0.00191 (0.006001)	0.004518 (0.007304)	-0.01776 (0.01135)	-0.00179 (0.002683)	-0.00045 (0.003277)	-0.00535 (0.005076)	0.007004 (0.01157)	0.006264 (0.01402)	0.009590 (0.02149)	0.007336 (0.01168)	0.002668 (0.01418)	0.01917 (0.02169)
P-value	0.7502	0.5364	0.1182	0.5038	0.8912	0.2918	0.5450	0.6552	0.6555	0.5301	0.8508	0.3771

[1] Models includes either all sedentary time, screen-based sedentary time, or other sedentary time as a fixed effect and compound symmetry error matrix structure
[2] Models additionally adjusted for the following fixed effects: mother's enrollment in the calcium intervention study, parity status, mode of childbirth at childbirth, child age, sex, metabolic equivalents, and pubertal onset.
[3] Waist circumference models were additionally adjusted for body mass index

Table 3.4 (Supplementary): Overall Associations between Potential Confounders and Percentage of Moderate to Vigorous Physical Activity (MVPA):

	% of MVPA			
	Quartile 1 Median= 4.77 n=238	**Quartile 2** Median= 6.81 n=238	**Quartile 3** Median= 8.62 n=238	**Quartile 4** Median= 11.52 n=238
Maternal Characteristics (at time of child's birth)				
Years of education, (years)	11.18	11.05	10.96	10.71
Age at childbirth, (years)	26.76	26.47	26.72	25.62
Parity (≥ 2), (%)	64.71	57.98	59.41	61.34
Marital Status (married), (%)	73.95	72.69	66.95	67.65
Enrolled in calcium supplement study, (%)	22.69	27.73	29.29	31.09
Child Characteristics (at birth)				
Girls, (%)	44.12	48.74	59.83	57.98
Gestational age, (weeks)	38.72	38.76	38.76	38.83
Mode of delivery (vaginal delivery), (%)	66.39	64.29	59.83	58.82
Breastfeeding duration, (months)	8.76	8.20	8.17	7.23
Child Characteristics (at follow-up visit)				
Age, (years)	16.22	15.47	15.18	14.37
Body mass index, (kg/m^2)	22.36	21.91	22.42	21.92
Pubertal onset, (%)	97.48	97.90	97.90	91.18
Total caloric intake, (kcal/day)	2181.56	2217.18	2164.57	2345.59
Total minutes of activity, (minutes/day)	925.28	917.24	922.96	911.38

Means or percentages are presented for continuous or categorical variables, respectively
Red color indicts the covariates included in the fully adjusted substituting models for percentage of sedentary activity.

Table 3.5: Linear Mixed Regression Models for the Relationship between Percentages of Physical Activities and Cardiometabolic Risk Factors:

			WC (cm) N= 530, # Obs = 926	**SBP (mm Hg)** N= 530, # Obs = 925	**DBP (mm Hg)** N= 530, # Obs = 925	**Log glucose (mg/dL)** N= 388, # Obs = 679	**Log TG (mg/dL)** N= 388, # Obs = 679	**log HDL-C (mg/dL)** N= 388, # Obs = 679	**Log insulin (µIU/mL)** N= 388, # Obs = 679	**Log HOMA-IR** N= 388, # Obs = 679
Crude models [1]										
1 % of daily sedentary time	Sedentary Median (interquartile range [IQR]) minutes= 6 (1)	Ref.								
	Light Median (IQR) minutes=2 (1)	β (SE) P-value	0.2526 (0.08578) **0.0033**	0.08478 (0.08846) 0.3381	-0.1441 (0.06489) 0.0266	-0.00004 (0.001301) 0.9753	0.000881 (0.004764) 0.8533	-0.00448 (0.001944) 0.0216	0.007166 (0.005429) 0.1873	0.004759 (0.005832) 0.4148
	MVPA Median (IQR) minutes =0.70 (0.37)	β (SE) P-value	-1.0842 (0.1323) **<0.0001**	-0.4941 (0.1363) **0.0003**	-0.09894 (0.09992) 0.3224	-0.00412 (0.001892) 0.0300	-0.02057 (0.006950) **0.0032**	0.002022 (0.002839) 0.4766	-0.01530 (0.007923) 0.0540	-0.02337 (0.008507) **0.0062**
Adjusted models [2,3]										
1 % of daily sedentary time	Sedentary Median (IQR) minutes = 6 (1)	Ref.								
	Light Median (IQR) minutes =2 (1)	β (SE) P-value	0.05021 (0.03550) 0.1676	0.1359 (0.08456) 0.1085	-0.1085 (0.06275) 0.0841	0.000644 (0.001333) 0.6295	0.000117 (0.004835) 0.9808	-0.00392 (0.001959) 0.0458	0.003950 (0.005454) 0.4692	0.003774 (0.005870) 0.5205
	MVPA Median (IQR) minutes =0.70 (0.37)	β (SE) P-value	-0.2707 (0.05718) **<0.0001**	-0.1729 (0.1353) 0.2016	0.08365 (0.09984) 0.4023	-0.00167 (0.001952) 0.3937	-0.02106 (0.007214) **0.0036**	0.003905 (0.002947) 0.1856	-0.01746 (0.008151) 0.0325	-0.01836 (0.008761) 0.0364
5 % of daily sedentary time	Sedentary Median (IQR) minutes = 31 (5)	Ref.								
	Light Median (IQR) minutes =12 (3)	β (SE)	0.2511 (0.1775)	0.6793 (0.4228)	-0.5425 (0.3137)	0.003218 (0.006667)	0.000583 (0.02417)	-0.01960 (0.009796)	0.01975 (0.02727)	0.01887 (0.02935)
	MVPA Median (IQR) minutes =3.51 (1.85)	β (SE)	-1.3533 (0.2859)	-0.8647 (0.6767)	0.4183 (0.4992)	-0.00833 (0.009760)	-0.1053 (0.03607)	0.01952 (0.01473)	-0.08730 (0.04075)	-0.09182 (0.04380)
10 % of daily sedentary time	Sedentary Median (IQR) minutes = 61 (10)	Ref.								
	Light Median (IQR) minutes = 23 (6)	β (SE)	0.5021 (0.3550)	1.3587 (0.8456)	-1.0850 (0.6275)	0.006436 (0.01333)	0.001165 (0.04835)	-0.03920 (0.01959)	0.03950 (0.05454)	0.03774 (0.05870)
	MVPA Median (IQR) minutes =7.01 (3.71)	β (SE)	-2.7066 (0.5718)	-1.7294 (1.3534)	0.8365 (0.9984)	-0.01666 (0.01952)	-0.2106 (0.07214)	0.03905 (0.02947)	-0.1746 (0.08151)	-0.1836 (0.08761)

[1] Models includes percentage of light and percentage of MVPA as fixed effects and compound symmetry error matrix structure.
[2] Models additionally adjusted for the following fixed effects: mother's enrollment in the calcium intervention study, mode of childbirth, parity status, child's age, sex, total time of physical activity, and pubertal onset
[3] Waist circumference models were additionally adjusted for body mass index

Table 3.6 (Supplementary): Overall Associations between Potential Confounders and Bout Frequency for Moderate to Vigorous Physical Activity (MVPA):

	Bout frequency of MVPA		
	Tertile 1 Median= 0 n=663	**Tertile 2** Median= 0.17 n=178	**Tertile 3** Median= 0.71 n=112
Maternal Characteristics (at time of child's birth)			
Years of education, (years)	10.91	10.92	11.48
Age at childbirth, (years)	26.49	25.94	26.55
Parity (≥ 2), (%)	61.54	61.80	55.36
Marital Status (married), (%)	70.44	69.10	71.43
Enrolled in calcium supplement study, (%)	28.21	23.60	31.25
Child Characteristics (at birth)			
Girls, (%)	54.75	53.37	39.29
Gestational age, (weeks)	38.76	38.63	39.01
Mode of delivery (vaginal delivery), (%)	62.44	60.67	64.29
Breastfeeding duration, (months)	8.35	7.19	7.97
Age, (years)	15.43	15.20	14.74
Child Characteristics (at follow-up visit)			
Body mass index, (kg/m^2)	22.37	22.19	20.83
Pubertal onset, (%)	96.53	93.82	92.86
Total caloric intake, (kcal/day)	2201.69	2306.27	2252.19
Total minutes of activity, (minutes/day)	918.42	917.56	926.56

Means or percentages are presented for continuous or categorical variables, respectively
Red color indicts the covariates included in the fully adjusted substituting models for sedentary bout frequency.

Table 3.7 (Supplementary): Overall Associations between Potential Confounders and Bout Duration for Moderate to Vigorous Physical Activity (MVPA):

	Bout duration of MVPA		
	Tertile 1 Median= 0 n=663	**Tertile 2** Median= 1.00 n=149	**Tertile 3** Median= 4.03 n=141
Maternal Characteristics (at time of child's birth)			
Years of education, (years)	10.91	10.93	11.36
Age at childbirth, (years)	26.49	25.99	26.38
Parity (≥ 2), (%)	61.54	64.43	53.90
Marital Status (married), (%)	70.44	68.46	71.63
Enrolled in calcium supplement study, (%)	28.21	23.49	29.79
Child Characteristics (at birth)			
Girls, (%)	54.75	56.38	39.01
Gestational age, (weeks)	38.76	38.62	38.94
Mode of delivery (vaginal delivery), (%)	62.44	59.73	64.54
Breastfeeding duration, (months)	8.35	7.05	7.98
Age, (years)	15.43	15.23	14.80
Child Characteristics (at follow-up visit)			
Body mass index, (kg/m^2)	22.37	22.36	20.93
Pubertal onset, (%)	96.53	94.63	92.20
Total caloric intake, (kcal/day)	2201.69	2255.38	2317.09
Total minutes of activity, (minutes/day)	918.42	918.44	923.78

Means or percentages are presented for continuous or categorical variables, respectively

Red color indicts the covariates included in the fully adjusted substituting models for sedentary bout duration.

Table 3.6: Linear Mixed Regression Models for the Relationship between Bout Durations and Frequencies with Cardiometabolic Risk Factors:

			WC (cm) N= 533 # Obs = 932	**SBP (mm Hg)** N= 533 # Obs = 931	**DBP (mm Hg)** N= 533 # Obs = 931	**Log glucose (mg/dL)** N= 390 # Obs = 683	**Log TG (mg/dL)** N= 390 # Obs = 683	**log HDL-C (mg/dL)** N= 390 # Obs = 683	**Log insulin (µIU/mL)** N= 390 # Obs = 683	**Log HOMA-IR** N= 390 # Obs = 683
Bout duration of light activity	Crude model[1]	β (SE)	0.06778 (0.04667)	0.1674 (0.04827)	0.02252 (0.03587)	0.002086 (0.000730)	-0.00473 (0.002608)	0.000769 (0.001041)	-0.01078 (0.002911)	-0.00780 (0.003174)
		P-value	0.1469	**0.0005**	0.5303	**0.0044**	0.0702	0.4604	**0.0002**	0.0142
	Adjusted model[2]	β (SE)	-0.01385 (0.01992)	0.04997 (0.04856)	-0.04407 (0.03653)	0.001188 (0.000772)	-0.00347 (0.002717)	0.001316 (0.001075)	-0.00868 (0.003016)	-0.00748 (0.003266)
		P-value	0.4870	0.3038	0.2281	0.1245	0.2025	0.2214	**0.0041**	0.0224
Bout duration of MVPA activity	Crude model[3]	β (SE)	-0.2943 (0.08881)	-0.1378 (0.09502)	-0.08750 (0.07070)	-0.00061 (0.001355)	-0.00966 (0.004681)	0.001272 (0.001874)	-0.01070 (0.005290)	-0.01254 (0.005711)
		P-value	**0.0010**	0.1473	0.2162	0.6548	0.0394	0.4977	0.0436	0.0285
	Adjusted model[4]	β (SE)	-0.07251 (0.03712)	-0.1246 (0.09133)	-0.07165 (0.06911)	-0.00046 (0.001343)	-0.00787 (0.004695)	0.002188 (0.001869)	-0.00846 (0.005263)	-0.00891 (0.005677)
		P-value	0.0512	0.1728	0.3001	0.7337	0.0941	0.2420	0.1083	0.1168
Bout frequency of light activity	Crude model[5]	β (SE)	0.4983 (0.3249)	1.1808 (0.3331)	0.1677 (0.2475)	0.01453 (0.005091)	-0.03476 (0.01810)	0.006116 (0.007286)	-0.07497 (0.02023)	-0.05413 (0.02206)
		P-value	0.1256	**0.0004**	0.4983	**0.0045**	0.0553	0.4016	**0.0002**	0.0144
	Adjusted model[6]	β (SE)	-0.1096 (0.1370)	0.3549 (0.3339)	-0.3162 (0.2511)	0.007432 (0.005377)	-0.02484 (0.01884)	0.008066 (0.007511)	-0.05847 (0.02096)	-0.05147 (0.02270)
		P-value	0.4240	0.2881	0.2083	0.1673	0.1879	0.2834	**0.0055**	0.0237
Bout frequency of MVPA activity	Crude model[7]	β (SE)	-2.1226 (0.6238)	-0.9965 (0.6658)	-0.5794 (0.4955)	-0.00310 (0.009371)	-0.07169 (0.03266)	0.01262 0.01325	-0.06038 0.03705	-0.07362 (0.03995)
		P-value	**0.0007**	0.1348	0.2426	0.7405	0.0285	0.3412	0.1037	0.0658
	Adjusted model[8]	β (SE)	-0.5410 (0.2585)	-0.8765 (0.6383)	-0.4441 (0.4838)	-0.00340 (0.009315)	-0.05840 (0.03278)	0.02026 0.01318	-0.04285 0.03686	-0.04669 (0.03975)
		P-value	0.0368	0.1701	0.3589	0.7149	0.0753	0.1248	0.2455	0.2406

[1] Model one includes bout duration spent in sedentary and light physical activity as fixed effect and compound symmetry error matrix structure
[2] Model one additionally adjusted for the following fixed effects: mother's enrollment in the calcium intervention study, parity status, child's age, sex, total time of physical activity, and pubertal onset
[3] Model two includes bout duration spent in sedentary and MVPA physical activity as fixed effect and compound symmetry error matrix structure
[4] Model two additionally adjusted for the following fixed effects: mother's enrollment in the calcium intervention study, parity status, child's age, sex, total time of physical activity, and pubertal onset
[5] Model three includes bout frequency spent in sedentary and light physical activity as fixed effect and compound symmetry error matrix structure
[6] Model three additionally adjusted for the following fixed effects: mother's enrollment in the calcium intervention study, parity status, child's age, sex, total time of physical activity, and pubertal onset
[7] Model four includes bout frequency spent in sedentary and MVPA physical activity as fixed effect and compound symmetry error matrix structure
[8] Model four additionally adjusted for the following fixed effects: mother's enrollment in the calcium intervention study, parity status, child's age, sex, total time of physical activity, and pubertal onset
Waist circumference models were additionally adjusted for body mass index

Chapter 4 DNA Methylation and Cardiometabolic Risk Factors in Mexican Children and Adolescents

Abstract:

Background: DNA methylation (DNAm) has been associated with cardiometabolic abnormalities. However, this relationship has rarely been assessed in youth.

Methods: This analysis included 402 healthy children of the Early Life Exposure in Mexico to Environmental Toxicants (**ELEMENT**) birth cohort who participated in follow-up visits in late childhood/adolescence (referred to as Time 1 and Time 2). DNAm was quantified in blood leukocytes at long interspersed nuclear elements (LINE-1), *H19,* and 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*). At Time 2, DNAm at peroxisome proliferator-activated receptor alpha (*PPAR-α*) was measured. At each time point, cardiometabolic risk factors were assessed including lipid profiles, glucose, blood pressure, and anthropometry.

Results: Time 1 DNAm at two LINE-1 loci was associated with repeat measures of log glucose (β=-0.029, p-value=0.0006 and β=0.027, p-value=0.0160, respectively), but with log high-density lipoprotein cholesterol at another LINE-1 CpG site (β=0.063, p-value =0.0072). *11β-HSD-2* DNAm at site 1 associated with systolic (β= -1.753, p=0.0360) and diastolic blood pressure (β= -1.147, p=0.0336), and at site 4 with log glucose (β= -0.017, p =0.0033). DNAm at *PPAR-α* site 2 was cross-sectionally associated with waist circumference (β= -1.681, p=0.0097).

Conclusion: Blood leukocyte DNAm was associated with cardiometabolic risk factors among youth in a locus-specific manner.

Key words: epigenetics; cardiovascular health; metabolic health; population-based study; adolescent health.

Introduction:

Metabolic syndrome (MetS) is a cluster of physiological conditions, which are central obesity, dyslipidemia, glucose intolerance, and elevated blood pressure [1,2]. MetS is considered a risk factor for cardiovascular disease incidence (CVD), cardiovascular-related mortality, all-cause mortality [3,4], and other chronic diseases [5,6], and rising prevalence of MetS may be a driver of the CVD and type-2 diabetes epidemics [7]. Even though CVD outcomes are manifested in middle and late adulthood, cardiometabolic risk factors may become evident during childhood [8-13], and track into adulthood [14-16]. Obesity has been associated with increases in the risk and prevalence of cardiometabolic abnormalities among youth [16-18]. In fact, obesity is rising worldwide among children aged 5-19 years. In Latin America and the Caribbean region, prevalence rose over a 40-year period from 1.6%, and 1.8% in 1975 to 10.4%, and 13.4% in 2016 for girls and boys, respectively [19]. As intervening at an early stage is a necessity for effective primary interventions, identifying the determinants of cardiometabolic risk factors in youth is a prior fundamental step for risk reduction and prevention [16,20].

Lifestyle factors – including diet, lack of physical activity, sedentary lifestyle – and genetic susceptibility are considered predisposing factors for cardiometabolic abnormalities. However, they are not enough to explain the increase in MetS prevalence across populations. Another plausible risk factor for and a possible mechanism explaining the etiology of cardiometabolic abnormalities and CVD is thought to be through epigenetic modifications [21-27], defined as mitotically heritable regulators of gene expression that do not change the DNA sequence [28]. Unlike genetics, epigenetic modifications are dynamic and reversible [21,29] and can respond to the environment. DNA methylation (DNAm) is a commonly studied epigenetic modification, and it refers to the covalent link between the fifth carbon in a cytosine nucleotide

and a methyl group (CH_3) [30,31]. The impact of DNAm on gene expression is locus-specific; DNAm at promotor regions and gene bodies are typically associated with suppression and activation of gene expression, respectively [30].

DNAm has been associated with the underlying pathology of CVD [21-27], which led to a rapid growth in research testing epigenetics as a potential biomarker for CVD's diagnosis, prognosis, and individualized treatment regimens [23,24,32]. However, there is a scarcity of population-based studies among children and adolescents focusing on epigenetics and cardiometabolic outcomes, highlighting the need to study this important life course period. The current study will address the gap in knowledge by examining the association of DNAm with repeated measures of cardiometabolic risk factors among Mexican children and adolescents aged 8 – 18 years. Specifically, we quantified CpG site-specific DNAm at repetitive elements (long interspersed nuclear element-1, (LINE-1)), and three genes previously associated with cardiometabolic-related outcomes (*H19* [33-36], 11β -hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*) [37-41], and peroxisome proliferator-activated receptor alpha (*PPAR-α*) [42-46] in blood leukocyte samples. In a sub-sample, we quantified gene expression of *PPAR-α* and assessed the correlation between expression and DNAm. Based on functions of the genes and results from other related studies, we hypothesized that hyper-methylation at *11β-HSD-2* and *PPAR-α,* and hypo-methylation at LINE-1 and *H19* would associate with impaired cardiometabolic risk factors.

Methods:

Study population:

The analytical sample consisted of subjects who participated in two of three sequentially-enrolled birth cohorts comprising the Early Life Exposure in Mexico to Environmental Toxicants

(**ELEMENT**) project in Mexico City, Mexico. A comprehensive description for the **ELEMENT** project, and the eligibility and exclusion criteria is available elsewhere [47]. Briefly, the **ELEMENT** project included mother-child dyads recruited from maternity hospitals representing women from low- to moderate-income populations from 1997 to 2005 [48]. Mothers recruited for one of the birth cohorts, were enrolled in randomized control trial (RCT) that examined the role of daily calcium supplementation during pregnancy (1200 mg/day) in mitigating the effect of lead exposure on the offsprings' neurobehavioral and physical developmental outcomes [47]. Offspring were followed at multiple time points in childhood and through adolescence as well. We utilized data from two follow-up visits in late childhood/adolescence. The first follow-up visit, herein called Time 1, included children aged between 8 -15 years [47]. A second follow up visit, Time 2, recruited children in the middle of the pubertal transition (ages 10 -18 years). Most children completed both Time 1 and 2 visits [47]. The National Institute of Public Health of Mexico and the University of Michigan institutional review boards approved the research protocols. Written informed consents were collected from mothers upon their enrollments and assent from adolescents, respectively.

Laboratory measurements and outcomes:

DNA methylation analysis:

The current study limits its focus to four genomic regions, which previously have been associated with cardiometabolic risk factors. Long Interspersed Nuclear Element-1 (LINE-1) repetitive elements comprises 15% - 17% of the human genome [49,50], and DNAm of these elements is used commonly as a proxy measure for global DNAm [29]. DNAm at LINE-1 was found to be associated with CVD independent from well-established CVD risk factors in adults [51]. The other three regions selected were *H19, 11β-HSD-2,* and *PPAR-α* genes. *H19* is an

imprinted gene located on chromosome 11 [36], and it has been associated with weight regulation and adipogenesis [34]. *11β-HSD-2* coverts cortisol to an inactive metabolite called cortisone, and abnormalities in this gene have been associated with hypertension [37]. Lastly, *PPAR-α* was considered because it controls multiple lipid metabolism pathways, including fatty acid oxidation, triglyceride synthesis and breakdown, and bile acid metabolism [43]; therefore, its contributions in dyslipidemia, diabetes, and obesity are biologically plausible [52].

Whole blood samples were collected via venipuncture into tubes containing ethylenediaminetetraacetic acid (EDTA) preservative (Paxgene and BD Vacutainer) by trained staff following standard protocols. High-molecular-weight DNA was extracted from blood leukocytes with the PAXgene Blood DNA kit (PreAnalytix, Switzerland) or the Flexigene kit (Qiagen). The extracted DNA samples were treated with sodium bisulfite using Epitect (Qiagen, Valencia, CA) or EZ DNA Methylation kits (Zymo Research, Irvine, CA) following the standard methods previously published [53]. The purpose of bisulfite treatment was to convert the unmethylated cytosines to uracil and preserve the methylated cytosines. The bisulfite-treated DNA samples were amplified using HotStarTaq Master Mix (Qiagen) and primers designed to amplify each region of interest. Pyrosequencing was performed using either PyroMark Q96 MD (Qiagen) or PyroMark Q96 ID (Qiagen). Pyro Q-CpG Software calculated the percent methylation and performed internal quality control checks. At Time 1, DNAm was quantified for *H19 (*4 CpG sites *in the imprinting control region),* for LINE-1 (4 CpG sites in a conserved region), and for *11β-HSD-2* (5 CpG sites in the promoter region), and at Time 2 for *PPAR-α* (2 CpG sites in the promoter region) following the protocols published previously [54-56]. Information of these genomic regions and the primer sequences is presented in Table 1 (Supplementary) [48]. More than 10% of all samples and controls of human DNA with known percentages of DNAm

(0%, 25%, 50%, 75%, and 100%) were run in duplicate and included in each pyrosequencing batch (96-well plate). The average of duplicate samples was used when applicable [57]. DNAm data from LINE-1, *11β-HSD-2*, and *H19* suggested a batch effect, and the methylation percentages were standardized to adjust for the batch effects as described previously [57]. We then standardized DNAm values for each region to have mean 0 standard deviation 1 based on the sample's mean and standard deviation values, and these z-scores used in statistical analysis.

A small sample of the participants enrolled at Time 2 (n=65) provided blood leukocyte samples for RNA isolation, and those with the best quality and quantity of RNA were selected for next-generation sequencing of RNA ('RNA-Seq'). *PPAR-α* gene expression data from the RNA-seq was used to assess the relationship between DNAm and gene expression for *PPAR*-α. The protocol followed to quantify the gene expression via RNA-seq was described previously [58].

Cardiometabolic risk factors

***Anthropometric measures*:** Duplicate measurements were collected by trained research staff in body weight to nearest 0.1 kilogram using a digital scale (BAME Model 420; Catálogo Médico), and InBody 230 (InBody Co.), height to the nearest 0.5 centimeter, and waist circumference to the nearest 0.1 centimeters using a non-stretchable measuring tape (SECA 201; SECA). The average of the two measurements was used for the analysis [59].

Blood pressure: Duplicate readings of systolic and diastolic blood pressure were recorded in seated position using a mercury sphygmomanometer (TXJ - 10 MD 3000 model, Homecare, China), and the average of the two measurements was used for the analysis.

Metabolic biomarkers: Fasting blood samples were used to analyze serum glucose via automated chemiluminescence immunoassay (Immulite 1000; Siemens Medical Solutions) [59],

and triglycerides and high density lipoprotein cholesterol using a biochemical analyzer (Cobas Mira Plus; Roche Diagnostics) [59].

Potential confounders:

Based on prior knowledge on cardiovascular and metabolic health, potential confounders assessed for this research were classified as 1) maternal and child characteristics around the time of birth (sex, birth weight, gestational age, mode of delivery, duration of breastfeeding, and mothers' age, marital status, parity, years of education, and enrollment in the calcium supplementation study during pregnancy) and 2) follow-up characteristics for the children, that were measured at each time point, e.g., child's age, total caloric intake, physical activity measured as metabolic equivalent, and pubertal onset.

After childbirth, mothers reported household and demographic information, including their ages, marital status (married compared to any other status), parity status (0, 1, ≥ 2), and years of education (<12 yrs, 12 yrs, or >12 yrs), gestational age estimated by a registered nurse, and mode of delivery (vaginal, or C-section childbirth). The newborns were followed until 5 years of age, and information about self-reported breastfeeding duration was quantified [60]. Since cohort 3 was a RCT for daily calcium supplementation (1200 mg/day) during the first trimester of pregnancy until 1-year postpartum and cohort 2 participants were not part of a trial, we created a binary indicator for mothers who received the calcium treatment (yes/no) with all mothers from cohort 2 falling into the 'no' category [47,61].

During each of two follow-up visits, total caloric intake was quantified using semi-quantitative food frequency questionnaire (FFQ), that captured the intake over the previous week [62]. The FFQ was adapted from the Mexican National Health and Nutrition Survey [62], and FFQs were analyzed using a food composition software developed by the National Institute of Public

Health, Mexico [63,64]. A physical activity questionnaire was developed based on the Youth Activity Questionnaire (YAQ) and validated relative to 24 hours physical activity recall among Mexican school-children aged 10 to 14 years in Mexico City [65]. For each self-reported physical activity, the corresponding metabolic equivalent was multiplied by the activity intensity [66]. The total metabolic equivalent per week was calculated by summing the metabolic equivalent for all activities. Puberty was assessed through Tanner staging for breast, pubic hair, and girls genitalia by trained physicians [67]. Tanner stages range from 1 (pre-puberty) till 5 (full maturation) [68,69]. Pubertal onset was classified as follows: a value greater than 1 for the Tanner Stage for pubic hair or genital development for boys and pubic hair or breast development girls, respectively [70].

Statistical analysis:

Outcomes were cardiometabolic risk factors: systolic blood pressure, diastolic blood pressure, waist circumference, high-density lipoprotein cholesterol, and triglycerides. Demographic characteristics of the study participants were presented as mean (SD) and counts (proportions) for continuous and categorical variables, respectively. To assess potential confounders, associations were examined between quartile of averaged DNAm across all sites in the region and potential confounders at childbirth and follow-up characteristics using either analysis of variance or Kruskal-Wallis H tests for continuous variables that were normally and non-normally distributed, respectively, and a chi-squared test for categorical variables. We aimed to have parsimonious models; thus, covariates that were statistically associated with the DNAm ($p < 0.05$), were included in the final models. All final models included age and sex. In LINE-1 models, we also adjusted for breastfeeding duration. In *11β-HSD-2* models, we included metabolic equivalent per week, total caloric intake, and maternal enrollment in a calcium

supplementation trial during pregnancy. For *H19* and *PPAR-α,* only age and sex were adjusted for.

DNAm data was quantified at multiple loci (CpG sites) located within the same genomic region. For each gene, all sites were included together as repeated measures of the same predictor in models of each outcome [71,72]. Additionally, we ran models where DNAm was averaged across sites within a genomic region; however, the site-specific models are of main interest in this work because our crude analysis showed variability in the direction and magnitude of the association between the sites and outcomes. To examine the relationship between DNAm at Time 1 at *11β-HSD-2, H19,* and LINE-1 and each longitudinally assessed cardiovascular risk factor outcome, separate linear mixed models with compound symmetry covariance structure were used with each outcome to model the covariance structure of the repeated measurements at Time 1 and 2. We used linear regression to assess the cross-sectional association between DNAm at *PPAR-α* and the outcomes because this gene was only measured at Time 2. Collinearity was assessed in the linear regression models using variance inflation factors. We conducted sensitivity analyses. We additionally adjusted for the pubertal onset at Time 1 for *11β-HSD-2, H19* and LINE-1 and at Time 2 for *PPAR-α* when it crudely associated with DNAm; given that puberty has been associated with DNAm at some genes [73]. We also ran the analysis after one excluding outlier value in DNAm percentage for *H19.* SAS statistical software package, version 9.4, was used for analyses (SAS Corp, NC, USA), and a p-value < 0.05, was considered a statistically significant association. We also discuss results in terms of significance following correction for multiple testing of six outcomes ($p<0.008$ or 0.05/6).

Results:

The final sample sizes for LINE-1, *H19*, and *11β-HSD-2* were 242, 245, and 228 subjects, respectively, with DNAm at Time 1 and outcomes at Time 1 and/or 2. For *PPAR-α,* 345 subjects had DNAm and outcomes at Time 2 (Figure 1). Table 2 shows the demographic characteristics of the 402 children by the time point. At Time 1, the mean (SD) age of the sample was 10.34 (1.67) years and 53.25% were girls. At Time 2, the mean age was 14.08 (2.03) years and 51.32 % were girls. Among cardiometabolic risk factors, only waist circumference and serum triglycerides values were higher at Time 2 than Time 1.

Associations between DNAm at LINE-1 and repeated measures of cardiometabolic risk factors:

Quartiles of DNAm percentages were associated crudely with age, sex, pubertal onset, and breastfeeding duration (Table 3 (Supplementary)). DNAm was higher in boys compared to girls at LINE-1. In adjusted models, LINE-1 methylation levels were associated with log serum fasting glucose inversely at site 1 [β= -0.029, p=0.0006], and positively at site 2 [β= 0.027, p =0.0160]. In addition, a positive association was detected between DNAm at site 3 and log serum high-density lipoprotein cholesterol [β= 0.063, p=0.0072] (Table 4). The inverse associations between DNAm at site 1 with fasting glucose, and the positive association between DNAm at site 3 with fasting high-density lipoprotein cholesterol are the only statistically significant associations after adjusting for multiple testing (p-value <0.008). Sensitivity analyses (i.e., additionally adjusting for pubertal onset) did not attenuate the detected associations (Table 5 (Supplementary)).

Associations between DNAm at *11β-HSD-2* and repeated measures of cardiometabolic risk factors:

Quartiles of DNAm were associated crudely with age, metabolic equivalents, pubertal onset, total caloric intake, and mothers' enrollment at the calcium intervention during pregnancy (Table 6 (Supplementary)). *11β-HSD-2* methylation prospectively associated inversely with systolic (mmHg) [β= -1.753, *p* =0.0360] and diastolic blood pressure (mmHg [β= -1.147, *p* =0.0336]. Moreover, DNAm at site 4 showed an inverse association with log serum fasting glucose [β= -0.017, *p* =0.0033] (Table 7). The inverse association between DNAm at site 4 and fasting glucose was the only statistically significant relationship after correcting for multiple testing (p-value <0.008). In sensitivity analysis (i.e., additionally adjusting for pubertal onset), all associations maintained similar magnitude and significance (Table 8 (Supplementary)).

Associations between DNAm at *H19* and repeated measures of cardiometabolic risk factors:

Among the covariates, quartiles of DNAm were associated crudely with pubertal onset only (Table 9 (Supplementary)).In adjusted models, none of the CpG sites were associated with any of the cardiometabolic risk factors (Table 10 (Supplementary)).Additionally, adjusting for pubertal onset as a sensitivity analysis (Table 11 (Supplementary)) or removing an outlier value (Table 12 (Supplementary)) did not change the conclusion.

Association between DNAm at *PPAR-α* and cardiometabolic risk factors:

At Time 2, tertiles of DNAm were crudely associated with age and pubertal onset (Table 13 (Supplementary)). In a cross-sectional analysis, DNAm at *PPAR-α* at site 2 was inversely associated with waist circumference (cm) [β= -1.681, *p* =0.0097] (Table 14). The sensitivity analysis showed the same result (cm) [β= -1.697, *p* =0.0091] (Table 15 (Supplementary)). This association was not statistically significant at the adjusted p-value (p-value <0.008).

Regarding the correlation between DNAm and gene expression for *PPAR-α,* RNA-seq data was available for 65 subjects at the same time point. Weak non-significant positive correlations were identified between mRNA and DNAm (site 1: Spearman's correlation [r_s] = 0.14, (*p*-value = 0.26); site 2: r_s =0.10, (*p*-value = 0.42); Average of the two sites r_s =0.12, (*p*-value = 0.33)).

Discussion:

In this study, the relationships between DNAm at LINE-1, *H19, 11β-HSD-2,* and *PPAR-α* with cardiometabolic risk factors were investigated among Mexican children enrolled in a well-characterized birth cohort from Mexico City. Out of the measured cardiometabolic components, blood pressure, fasting glucose, high-density lipoprotein cholesterol, and waist circumference, were associated with DNAm of at least one gene region each.

Epigenetic modification is a plausible underlying mechanism in the etiology of obesity, cardiometabolic abnormalities, and CVD [21-27]. However, there is limited study of this relationship among children and adolescents, where the identification and assessment of potential causative factors for adverse cardiometabolic health outcomes is critical in order to design evidence based prevention and intervention strategies [74]. Ample research has examined early life epigenetic programming, where DNAm was measured during early development, in relation to obesity and CVD risk later in life [75]; DNA undergoes waves of demethylation and re-methylation during early embryogenesis making this an important developmental time period for long term programming [76]. While not as drastic, adolescence is also a susceptible period when environmental stimuli can impact DNAm patterns [73,77], with implications for health outcomes [21,29]. Moreover, adolescence is characterized by changes in body composition and hormonal milieu [78], which is considered the hallmark for the cardiometabolic abnormalities [79].

Our findings indicated an inverse prospective relationship between DNAm at LINE-1 and serum glucose. Each one standard deviation increase in the LINE-1 methylation at sites 1 and 3, was associated with a 3.0% decrease of fasting glucose, and a 6.0% increase of high-density lipoprotein cholesterol, respectively. It is worth nothing that the detected changes might not be of a clinical significance because of the small effect size. Having said that, our results are in agreement with findings from adult studies [46,80-82]. Using peripheral blood samples, Martin-Nunez et al. showed that baseline DNAm at LINE-1 was inversely associated with carbohydrate metabolism after one year of follow-up among middle-aged adults [80]. An analysis using target tissue (i.e., visceral adipose tissue) for DNAm assessment also reported an inverse association between DNAm at LINE-1 and fasting glucose [46,81]. Moreover, our finding is in line with other studies that have shown LINE-1 hypomethylation is associated with genomic instability and CVD [51,82-84]. To the best of our knowledge, there were few studies conducted on children assessing the relationship between LINE-1 DNAm and adiposity outcomes [85,86]. Perng et al. found that quartiles of LINE-1 DNAm was inversely associated with a change in waist circumference Z-score among Colombian boys aged 5 -12 years old after 2.5 years of follow-up [85]. Dunstan et al. reported null cross-sectional associations between salivary LINE-1 DNAm and adiposity outcomes (BMI z score, waist circumference z score, and percent body fat in 431 adolescents, predominantly Caucasians, aged 10 - 15 years) [86]. However, comparing our findings with the previous youth studies should be done with caution due to the miss-match in the study endpoints, age of the sample, source of DNA, and other factors.

We found that *11β-HSD-2* methylation was inversely associated with blood pressure, and fasting glucose. These associations had small effect sizes as a one-SD unit of DNAm change was associated with a 2 mmHg change in blood pressure, and a decrease of 2% in fasting glucose.

However, our findings fit in with the available body of evidence. 11β-HSD-2 enzyme converts cortisol to an inactive metabolite called cortisone [87,88]. Previous studies showed that DNAm at *11β-HSD-2* at the promotor region was associated with suppressing the gene expression [89,90], and impaired 11β-HSD-2 enzyme activity, evident elevation in the urinary cortisol: cortisone metabolites ratio [37]. DNAm at *11β-HSD-2* at the promoter region was positively associated with blood pressure in adults [37,38]. Additionally, lower 11β-HSD-2 enzyme activity was associated with higher blood pressure in children [39]. However, our inverse association between DNAm at the promotor region of *11β-HSD-2* and blood pressure was reported earlier by Drake et al. [38]. The regulation of gene expression is complex and can vary by region of the gene and by type of regulator (i.e. DNAm versus other mechanisms). Previous research has shown the enzymatic activity of 11β-HSD-2 is associated with age [91] , dietary intake, and physical activity [92], and is regulated by other epigenetic mechanisms including miRNA[93]. Future studies examining the role of DNAm at *11β-HSD-2* should be supplemented with analysis of mRNA expression and measurement of its enzymatic activity.

Adult studies found that *11β-HSD-2* activity was associated positively with obesity [40] and inversely with insulin sensitivity [40]. A previous study found higher *11β-HSD-2* activity among adults diagnosed with type 2 diabetes diagnosis than controls, despite the lack of difference in mRNA expression [41]. Future studies examining the role of DNAm at *11β-HSD-2* with other cardiometabolic risk factors – in addition to what is already known about blood pressure, are worth conducting to build upon past research in adults and our findings in adolescence.

In the present study using repeated measures of outcomes, DNAm at *H19* was not associated with any of the cardiometabolic risk factors for the site-specific models. *H19* is a long non-coding RNA and is a maternally expressed imprinted gene. *H19* has a role in regulating

cell formation and proliferation, weight, adipogenesis, oxidative metabolism and brown adipose tissue thermogenesis [33,34]. *H19* transgenic mice which had overexpression of *H19*, were less susceptible to the adverse cardiometabolic effects of high fat diet such as insulin insensitivity[34]. We previously reported an association between DNAm at *H19* with higher subcutaneous fat, but not with central obesity or BMI z score among girls, but not boys, from the **ELEMENT** cohort [35]. Huang et al. reported a similar positive association with *H19* DNAm and subcutaneous fat in Australian adolescents [36]. Age and sex may be important modifiers in the relationship between *H19* DNAm and these outcomes.

We found that higher *PPAR-α* methylation at CpG site 2 in the promotor region, was inversely associated with waist circumference. Each one standard deviation increase in the *PPAR-α* methylation was associated with approximately 2 cm smaller waist circumference, a magnitude of potential clinical significance. In general, DNAm at gene promoter regions is associated with gene repression [30]; however, our analysis showed weak positive correlations between DNAm at the CpG sites assessed and gene expression. Thus, the reported inverse association is in agreement with the biological function known for *PPAR-α*. *PPAR-α* enhances the oxidation of fatty acid, breakdown of triglyceride-rich particles, and high density lipoprotein cholesterol synthesis, removing excess cholesterol from the liver, and reducing the accumulation of triglycerides [42,43]. The use of fibrates, a *PPAR-α* agonist drug, has shown to significantly lower cardiovascular risk among high-risk subjects [44]. *PPAR-α* also regulates oxidative stress and inflammatory response [42]. Few studies assessed the relationship between DNAm at *PPAR-α* and cardiometabolic risk factors. DNAm at *PPAR-α* from visceral adipose samples analyzed among adults showed a positive correlation between DNAm and serum triglycerides [46]. Moreover, rats fed a high fructose diet for two weeks showed a significant increase in hepatic

DNAm at one CpG site in the promoter region of *PPAR-α*, lowered mRNA expression, high serum triglycerides, total cholesterol, and higher hepatic lipid accumulation [45]. Other possible physiological conditions could regulate *PPAR-α* expression such as stress, insulin, leptin, adiponectin, growth hormones [43], which could confound our findings.

The present study has multiple strengths, including the prospective assessment of the association between DNAm at three of the genes and two repeat measures of cardiometabolic risk factors during a sensitive period of growth, development, and maturation. We used a robust statistical model to account for the longitudinal data structure, and conducted a site-specific analysis for examining the association between the epigenetic modifications and cardiometabolic risk factors. As noted earlier, the association between DNAm and outcome might be site-specific[81]. Averaging all sites within a region, a common analytical practice in epigenetic studies, might hinder the ability to detect site-specific associations between DNAm and outcomes. Our data come from a well-characterized birth cohort, **ELEMENT**, which allowed for assessing multiple confounders, including mother's sociodemographic and reproductive characteristics. In regard to the study limitations, we measured DNAm in blood, which is not the target tissue for cardiometabolic related outcomes. However, it has been shown in epigenome-wide studies that DNAm in blood correlates with DNAm in adipose tissue [94-96], and skeletal muscle [97] in many genes. Additionally, this work aims to inform the development of potential biomarkers for cardiometabolic risk among children and adolescents; thus an accessible tissue such as blood is necessary to use for this purpose [98]. Lastly, the use of bisulfite treatment to measure DNAm does not distinguish between cytosine methylation (5mC) and cytosine hydroxymethylation (5hmC) [99], and 5hmC has its own distinct impact on gene regulation which is not captured by our method.

In conclusion, we observed site-specific associations between DNAm at LINE-1, *11β-HSD-2*, and *PPAR-α* with cardiometabolic risk factors in a sample of Mexican children. Future studies are needed to replicate and expand on the association between DNAm and cardiometabolic health in adolescents. Such findings if validated and replicated in other cohorts could open the door for the use of blood DNAm biomarkers to predict risk and develop targeted interventions among youth. Moreover, since our study only focused on 4 genomic regions, we recommend future studies employ epigenome-wide approaches and assess gene expression to identify all important genes for these outcomes in children.

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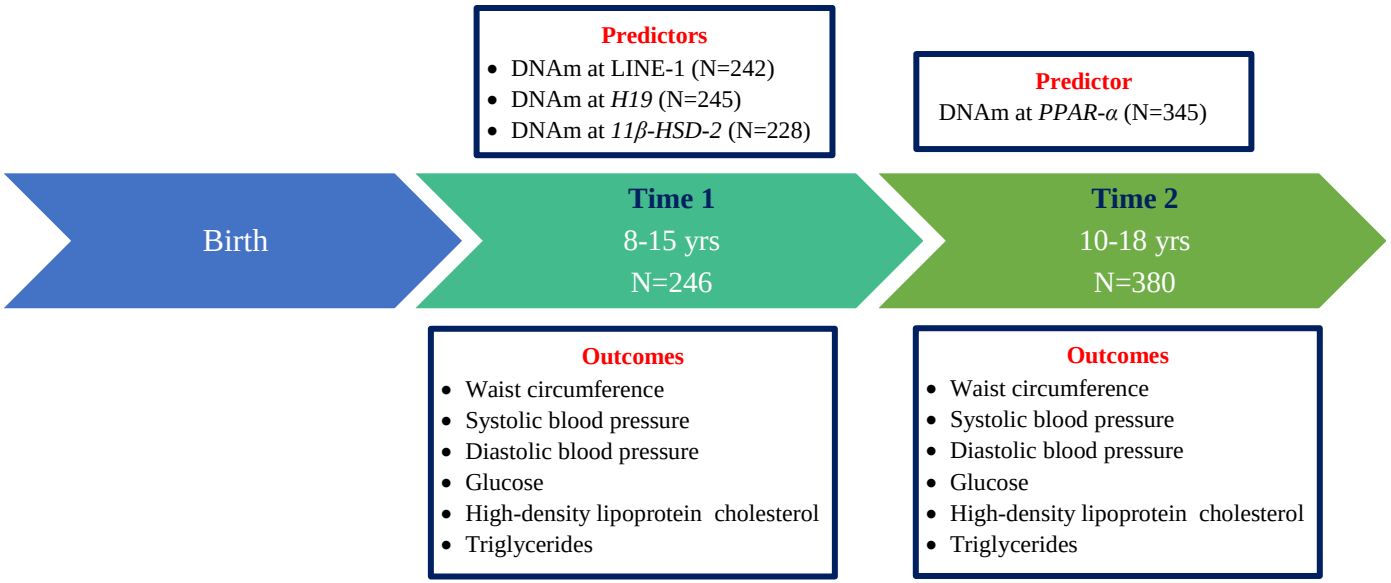
Table 4.1 (Supplementary): Primer Sequences and Details of CpG Sites Assessed:

Gene or Element Name	# of CpG Sites Assessed	Loci of CpG Sites [a]	Primer Sequences			Locus of Amplified Region
			Forward	Reverse [b]	Sequencing	
LINE-1	4	Various [c]	TTGAGTTAGGTGTGG GATATAGTT	CAAAAAATCAAAAAAT TCCCTTTCC	AGGTGTGGAT ATAGT	Various[3]
H19	4	chr11: 2003031, 2003029, 2003027, and 2003024	TTTGTTGATTTTATTA AGGGAG	CTATAAATAAACCCCA ACCAAAC	GTGTGGAATT AGAAGT	chr11: 2002966-2003111
PPAR-α	2	chr22: 46149160 and 46149179	GGAGGTTTTTATGAG GATGTAGTT	ACACATATTAACCAAC AATAACTATCAT	GGATGTGGTT GTTTG	chr22: 46149046-46149244
11β-HSD-2	5	chr16: 67430541, 67430543, 67430562, 67430564, and 67430580	TTAAGTTTTGGAAGG AAAGGGAAAGA	ACATCCCCATACCCTT TACTAATC	AGTTTTTGTTT TAGGTAGG	chr16: 67430512-67430745

Notes:
a. Loci are based off genome build GRCh38/hg38
b. All reverse primers for pyrosequencing are 5'biotinylated.
c. A consensus sequence found in all LINE-1s (located throughout the genome) is amplified and sequenced here. The specific sequence is as follows: 5'-CTCGTGGTGCGCCGTTTCTTAAGCCG
Long interspersed nuclear elements (LINE-1); 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*); Peroxisome proliferator-activated receptor alpha (*PPAR-α*).

Figure 4.1: Summary of Main Predictors and Outcomes for this Study and Number of Participants with the Data from the Early Life Exposures in Mexico to ENvironmental Toxicants (**ELEMENT**) Cohort:



The associations between DNAm at LINE-1, *H19*, and *11β-HSD-2*, and repeated measures of cardiometabolic risk factors were examined. For *PPAR-α*, the cross-sectional associations were investigated.
The sample size for LINE-1 (N=242); 43 observations had information at Time 1 only, and 199 observations had information at the two time points.
The sample size for *H19* (N=245); 44 observations had information at Time 1 only, and 201 had information at the two time points.
The sample size for *11β-HSD-2* (N=228), 43 observations had information at Time 1 only, and 185 had information at the two time points.
The sample size for *PPAR-α* (N=345), they were all at Time 2 only.
Long interspersed nuclear elements (LINE-1); 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*); Peroxisome proliferator-activated receptor alpha (*PPAR-α*).

Table 4.2: Descriptive Statistics of Mother and Child Characteristics of the Early Life Exposures in Mexico to ENvironmental Toxicants (**ELEMENT**) Analytical Sample:

	Time 1 N= 246	**Time 2** N= 380
Maternal Characteristics (at time of child's birth)		
Years of education, %		
< 12 years	121 (49.19)[1]	196 (51.58)[2]
12 years	90 (36.59) [1]	131 (34.47)[2]
> 12 years	34 (13.82)[1]	52 (13.68)[2]
Age at childbirth, (years)	26.86 (5.64)[1]	26.47 (5.46) [2]
Parity, %		
0	1 (0.41)[1]	4 (1.05)[2]
1	89 (36.18)[1]	140 (36.84)[2]
≥ 2	155 (63.01)[1]	235 (61.84)[2]
Marital Status, %		
Married	175 (71.14)[1]	274 (72.11) [2]
Others (includes free union, single, separated, or divorced)	70 (28.46) [1]	105 (27.63) [2]
Enrollment in calcium supplementation study, %		
Not enrolled	152 (61.79)[1]	257 (67.63)[2]
Enrolled during pregnancy	93 (37.80)[1]	122 (32.11)[2]
Child Characteristics (at birth)		
Girls, %	131 (53.25)	195 (51.32)
Gestational age, (weeks)	38.85 (1.49)[3]	38.79 (1.61)[4]
Mode of delivery, %		
Vaginal delivery	140 (56.91)[5]	220 (57.89) [6]
C Section	103 (41.87)[5]	158 (41.58) [6]
Birth weight, (kg)	3.15 (0.45)[7]	3.15 (0.48)[6]
Breastfeeding duration, (weeks)	8.15 (5.91)[1]	8.09 (6.07)[2]
Child Characteristics (at follow-up visit)		
Age, (years)	10.34 (1.67)	14.08 (2.03)
Body mass index Z score for age	0.85 (1.24)	0.53 (1.26)[6]
Metabolic equivalent (minutes/week)	31.38 (19.97)	60.63 (38.76)
Total caloric intake, (kcal/day)	2636.32 (839.83)	2371.62 (936.82)
Pubertal onset, %		
No	143 (58.13)	25 (6.58)[8]
Yes	103 (41.87)	348 (91.58)[8]
Outcomes (cardiometabolic risk factors)		
Waist circumference, (cm)	70.81 (10.71)	79.14 (11.42)
Systolic blood pressure, (mm Hg)	102.74 (10.24)	97.23 (9.62)
Diastolic blood pressure, (mm Hg)	65.58 (7.31)	62.24 (6.71)
Fasting glucose, (mg/dL)	86.98 (9.38)	77.48 (7.05) [9]
High density lipoprotein cholesterol, (mg/dL)	58.76 (11.92)	42.95 (8.87) [9]
Triglycerides, (mg/dL)	87.89 (44.40)	105.81 (57.47) [9]
Predictors (DNAm values)		
LINE-1 DNAm, % (averaged across 4 CpG sites)	78.49 (2.31)[5]	N/A
***H19* DNAm, %** (averaged across 4 CpG sites)	58.31(5.16)[1]	N/A
***11β-HSD-2* DNAm, %** (averaged across 5 CpG sites)[a]	-0.85 (1.34)	N/A

***PPAR- α* DNAm, %** (averaged across 2 CpG sites)	N/A	10.62 (2.09)[10]

1.n=245 , 2. n= 379, 3. n= 242, 4.n=377, 5.243, 6.n=378, 7.n=244, 8.n=373, 9. n= 342, 10.n=358.
DNAm=DNA methylation
Means (SD) or count (percentages) are presented for continuous or categorical variables, respectively.
a. Negative values appear for *11β-HSD-2* because values are standardized to controls included on each plate to reduce the impact of pyrosequencing batch effects (for Time 1).
Long interspersed nuclear elements (LINE-1); 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*); Peroxisome proliferator-activated receptor alpha (*PPAR-α*).

Table 4.3 (Supplementary): Average DNAm at LINE-1 and Confounders Selection:

	Average DNA methylation z score at LINE-1				
	Q 1 N= 143	**Q 2** N= 143	**Q 3** N=144	**Q 4** N=143	**P-value**
Maternal Characteristics (at time of child's birth)					
Years of education, %					
< 12 years	53.15	52.45	45.83	51.05	0.3035
12 years	27.66	34.97	38.89	37.76	
> 12 years	18.44	12.59	15.28	11.19	
Age at childbirth, (years)	26.79	27.01	26.28	26.26	0.5697
Parity, %					
0	0.00	0.70	2.08	0.70	0.4233
1	33.33	35.66	37.50	41.26	
≥ 2	66.67	63.64	60.42	58.04	
Marital Status, %					
Married	68.79	69.93	72.92	74.83	0.6601
Others (includes free union, single, separated, or divorced)	31.21	30.07	27.08	25.17	
Enrollment in calcium supplementation study, %					
Not enrolled	60.99	64.34	69.44	64.34	0.5154
Enrolled during pregnancy	39.01	35.66	30.56	35.66	
Child Characteristics (at birth)					
Girls, %	58.04	56.64	50.69	38.46	0.0034*
Gestational age, (weeks)	38.72	38.64	38.87	38.93	0.2243
Mode of delivery, %					
Vaginal delivery	53.90	56.64	53.85	65.25	0.1696
C Section	46.10	43.36	46.15	34.75	
Birth weight, (kg)	3.14	3.14	3.17	3.16	0.8982
Breastfeeding duration, (weeks)	6.82	7.78	9.38	7.99	0.0018*
Child Characteristics (at follow-up visit)					
Age, (years)	12.14	12.83	12.21	12.54	0.1317
Body mass Z score for age	0.64	0.79	0.67	0.51	0.3063
Metabolic equivalents, (METs/ week)	49.49	49.92	47.33	49.06	0.8888
Pubertal onset, %	62.41	80.99	72.03	69.72	0.0068*
Total caloric intake, (kcal/day)	2452.21	2441.99	2545.91	2509.13	0.7121

Means or percentages are presented for continuous or categorical variables, respectively.
Long interspersed nuclear elements (LINE-1)
*P-value < 0.05

Table 4.4: Associations between DNAm at LINE-1 and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models (N=242):

	LINE-1 site 1		**LINE-1 site 2**		**LINE-1 site 3**		**LINE-1 site 4**		**Average of All sites**		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =441)											
Model 1	-0.5960 (1.0435)	0.5684	1.1418 (1.4217)	0.4227	-0.4783 (1.1510)	0.6781	0.2997 (0.9013)	0.7398	**Model 3**	0.3366 (0.6796)	0.6208
Model 2	0.5615 (1.0072)	0.5777	0.9837 (1.3686)	0.4730	-1.7757 (1.1106)	0.1111	0.3214 (0.8710)	0.7124	**Model 4**	0.07382 (0.6786)	0.9135
Systolic blood pressure (mm Hg) (number of observation used =441)											
Model 1	-0.4560 (0.8541)	0.5939	-0.1855 (1.1698)	0.8741	0.1632 (0.9435)	0.8628	0.9703 (0.7361)	0.1887	**Model 3**	0.2637 (0.5575)	0.6366
Model 2	-0.9634 (0.8928)	0.2817	-0.00023 (1.2181)	0.9999	0.4640 (0.9898)	0.6397	0.8922 (0.7676)	0.2464	**Model 4**	0.1597 (0.6019)	0.7911
Diastolic blood pressure (mm Hg) (number of observation used =441)											
Model 1	-0.5185 (0.5769)	0.3697	0.1316 (0.7927)	0.8682	0.2271 (0.6379)	0.7221	0.3619 (0.4966)	0.4669	**Model 3**	-0.1096 (0.3767)	0.7713
Model 2	-0.6759 (0.5947)	0.2570	-0.04549 (0.8136)	0.9555	0.3404 (0.6613)	0.6072	0.3674 (0.5094)	0.4716	**Model 4**	-0.09573 (0.4009)	0.8115
Log transformed fasting glucose (mg/dL) (number of observation used = 438)											
Model 1	-0.01570 (0.007838)	0.0463*	0.02427 (0.01086)	0.0263*	-0.00357 (0.008708)	0.6825	-0.00361 (0.006726)	0.5917	**Model 3**	0.003541 (0.005172)	0.4943
Model 2	-0.02864 (0.008211)	0.0006*	0.02729 (0.01124)	0.0160*	0.01135 (0.009149)	0.2162	-0.00142 (0.007028)	0.8402	**Model 4**	0.009024 (0.005689)	0.1141
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used = 438)											
Model 1	0.02078 (0.01893)	0.2733	-0.02664 (0.02610)	0.3083	0.01023 (0.02099)	0.6265	-0.01677 (0.01627)	0.3039	**Model 3**	-0.00961 (0.01244)	0.4404
Model 2	-0.01466 (0.02111)	0.4881	-0.02801 (0.02873)	0.3306	0.06331 (0.02334)	0.0072*†	-0.00571 (0.01822)	0.7543	**Model 4**	0.01239 (0.01431)	0.3878
Log transformed triglycerides (mg/dL) (number of observation used = 438)											
Model 1	-0.05170 (0.04055)	0.2035	-0.03424 (0.05541)	0.5372	0.05445 (0.04481)	0.2255	-0.00392 (0.03498)	0.9109	**Model 3**	-0.03059 (0.02656)	0.2506
Model 2	-0.02698 (0.03945)	0.4947	-0.04343 (0.05383)	0.4205	0.05072 (0.04378)	0.2477	0.009633 (0.03392	0.7766	**Model 4**	-0.01358 (0.02666)	0.6109

- Long interspersed nuclear elements (LINE-1)
- Model 1: includes LINE-1 z scores at site 1, 2, 3, and 4 as fixed effects, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additionally adjusted for the following fixed effects: age, sex, and duration of breastfeeding
- Model 3: includes average LINE-1 z score at site 1, 2, 3, and 4 as fixed effect, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 4: model 3 additionally adjusted for the following fixed effects: age, sex, and duration of breastfeeding

*P-value < 0.05; † P-value < 0.008

Table 4.5 (Supplementary): Associations between DNAm at LINE- 1 and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models Adjusting for Pubertal Onset (N=242):

	LINE-1 site 1		LINE-1 site 2		LINE-1 site 3		LINE-1 site 4		Average of All sites		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =441)											
Model 1	-0.5960 (1.0435)	0.5684	1.1418 (1.4217)	0.4227	-0.4783 (1.1510)	0.6781	0.2997 (0.9013)	0.7398	**Model 3**	0.3366 (0.6796)	0.6208
Model 2	0.6325 (1.0098)	0.5317	1.0269 (1.3678)	0.4535	-1.9266 (1.1252)	0.0881	0.3154 (0.8699)	0.7172	**Model 4**	0.05462 (0.6795)	0.9360
Systolic blood pressure (mm Hg) (number of observation used =441)											
Model 1	-0.4560 (0.8541)	0.5939	-0.1855 (1.1698)	0.8741	0.1632 (0.9435)	0.8628	0.9703 (0.7361)	0.1887	**Model 3**	0.2637 (0.5575)	0.6366
Model 2	-0.6264 (0.8602)	0.4672	0.2907 (1.1720)	0.8043	-0.3020 (0.9613)	0.7537	0.8606 (0.7375)	0.2444	**Model 4**	0.03881 (0.5785)	0.9466
Diastolic blood pressure (mm Hg) (number of observation used =441)											
Model 1	-0.5185 (0.5769)	0.3697	0.1316 (0.7927)	0.8682	0.2271 (0.6379)	0.7221	0.3619 (0.4966)	0.4669	**Model 3**	-0.1096 (0.3767)	0.7713
Model 2	-0.4927 (0.5769)	0.3940	0.1524 (0.7887)	0.8469	-0.09831 (0.6458)	0.8791	0.3551 (0.4932)	0.4723	**Model 4**	-0.1387 (0.3881)	0.7212
Log transformed fasting glucose (mg/dL) (number of observation used = 438)											
Model 1	-0.01570 (0.007838)	0.0463*	0.02427 (0.01086)	0.0263*	-0.00357 (0.008708)	0.6825	-0.00361 (0.006726)	0.5917	**Model 3**	0.003541 (0.005172)	0.4943
Model 2	-0.02747 (0.008217)	0.0010*†	0.02862 (0.01124)	0.0115*	0.008495 (0.009213)	0.3575	-0.00145 (0.007023)	0.8362	**Model 4**	0.008756 (0.005687)	0.1250
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used = 438)											
Model 1	0.02078 (0.01893)	0.2733	-0.02664 (0.02610)	0.3083	0.01023 (0.02099)	0.6265	-0.01677 (0.01627)	0.3039	**Model 3**	-0.00961 (0.01244)	0.4404
Model 2	-0.00710 (0.02059)	0.7305	-0.02295 (0.02796)	0.4127	0.04707 (0.02299)	0.0417*	-0.00636 (0.01771)	0.7197	**Model 4**	0.008877 (0.01388)	0.5230
Log transformed triglycerides (mg/dL) (number of observation used = 438)											
Model 1	-0.05170 (0.04055)	0.2035	-0.03424 (0.05541)	0.5372	0.05445 (0.04481)	0.2255	-0.00392 (0.03498)	0.9109	**Model 3**	-0.03059 (0.02656)	0.2506
Model 2	-0.02828 (0.03955)	0.4752	-0.04454 (0.05388)	0.4093	0.05366 (0.04424)	0.2263	0.009718 (0.03392)	0.7747	**Model 4**	-0.01339 (0.02668)	0.6161

- Long interspersed nuclear elements (LINE-1)
- Model 1: includes LINE-1 z score at site 1, 2, 3, and 4 as fixed effects, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additionally adjusted for the following fixed effects: age, sex, duration of breastfeeding, and pubertal onset
- Model 3: includes average LINE-1 z score at site 1, 2, 3, and 4 as fixed effect, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 3 additionally adjusted for the following fixed effects: age, sex, duration of breastfeeding, and pubertal onset

*P-value < 0.05; † P-value < 0.008

Table 4.6 (Supplementary): Average DNAm at *11β-HSD-2* and Confounders Selection:

	Average DNA methylation z score at *11β-HSD-2*				
	Q 1 N= 134	**Q 2** N= 135	**Q 3** N=134	**Q 4** N=134	**P-value**
Maternal Characteristics (at time of child's birth)					
Years of education, %					
< 12 years	53.73	54.07	49.25	49.25	0.2002
12 years	32.09	37.04	38.06	34.33	
> 12 years	14.18	8.89	11.19	16.42	
Age at childbirth, (years)	26.72	26.54	26.52	26.86	0.9538
Parity, %					
0	0.00	2.22	0.00	1.49	0.2014
1	33.58	35.56	37.31	39.55	
≥ 2	66.42	62.22	61.19	58.96	
Marital Status, %					
Married	66.42	73.33	69.40	78.36	0.1313
Others (includes free union, single, separated, or divorced)	33.58	26.67	29.10	21.64	
Enrollment in calcium supplementation study, %					
Not enrolled	56.72	77.78	62.69	64.18	0.0064*
Enrolled during pregnancy	43.28	22.22	35.82	35.82	
Child Characteristics (at birth)					
Girls, %	55.97	51.11	52.99	48.51	0.8091
Gestational age, (weeks)	38.80	38.73	38.92	38.74	0.7167
Mode of delivery, %					
Vaginal delivery	56.72	54.81	62.69	55.22	0.4120
C Section	42.54	45.19	35.07	44.03	
Birth weight, (kg)	3.10	3.11	3.18	3.22	0.1277
Breastfeeding duration, (weeks)	8.20	7.48	8.55	8.31	0.3030
Child Characteristics (at follow-up visit)					
Age, (years)	11.38	13.69	12.89	11.64	<0.0001*
Body mass Z score for age	0.69	0.61	0.59	0.69	0.7855
Metabolic equivalents, (METs/week)	42.02	54.03	54.61	38.45	0.0005*
Pubertal onset, %	56.72	85.93	78.36	54.48	<0.0001*
Total caloric intake, (kcal/day)	2634.96	2440.82	2396.67	2522.42	0.0265*

Means or percentages are presented for continuous or categorical variables, respectively.
11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*)
*P-value < 0.05

Table 4.7: Associations between DNAm at *11β-HSD-2* and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models (N=228):

	***11β-HSD-2* site 1**		***11β-HSD-2* site 2**		***11β-HSD-2* site 3**		***11β-HSD-2* site 4**		***11β-HSD-2* site 5**		**Average of all sites**		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =413)													
Model 1	-0.3085 (1.0403)	0.7671	-0.1795 (0.7979)	0.8223	0.1743 (0.9688)	0.8574	0.4602 (0.7678)	0.5496	0.2848 (0.7244)	0.6946	**Model 3**	0.1115 (0.6867)	0.8712
Model 2	-1.1409 (0.9965)	0.2535	0.1966 (0.7703)	0.7988	0.6050 (0.9244)	0.5135	0.4888 (0.7320)	0.5050	-0.1429 (0.6916)	0.8365	**Model 4**	-0.3122 (0.6598)	0.6366
Systolic blood pressure (mm Hg) (number of observation used =413)													
Model 1	-1.5839 (0.8347)	0.0590	-0.7899 (0.6400)	0.2184	1.2452 (0.7780)	0.1109	0.3529 (0.6179)	0.5685	-0.4642 (0.5801)	0.4245	**Model 3**	-0.6991 (0.5581)	0.2116
Model 2	-1.7532 (0.8309)	0.0360*	-0.4561 (0.6411)	0.4776	1.2370 (0.7702)	0.1098	0.5984 (0.6121)	0.3294	-0.1707 (0.5751)	0.7669	**Model 4**	-0.4597 (0.5573)	0.4105
Diastolic blood pressure (mm Hg) (number of observation used =413)													
Model 1	-0.9272 (0.5538)	0.0955	-0.8573 (0.4245)	0.0446*	0.3562 (0.5165)	0.4911	0.4559 (0.4108)	0.2683	-0.01583 (0.3845)	0.9672	**Model 3**	-0.5909 (0.3725)	0.1142
Model 2	-1.1472 (0.5364)	0.0336*	-0.6452 (0.4133)	0.1200	0.4374 (0.4972)	0.3800	0.6087 (0.3960)	0.1257	0.08813 (0.3705)	0.8122	**Model 4**	-0.5068 (0.3631)	0.1644
Log transformed fasting glucose (mg/dL) (number of observation used =410)													
Model 1	-0.00084 (0.007539)	0.9118	0.002076 (0.005797)	0.7206	0.006432 (0.007029)	0.3612	-0.01860 (0.005607)	0.0011*†	0.002604 (0.005242)	0.6198	**Model 3**	0.006014 (0.005094)	0.2392
Model 2	0.008404 (0.007906)	0.2891	-0.00140 (0.006109)	0.8192	0.000587 (0.007321)	0.9362	-0.01733 (0.005830)	0.0033*†	0.008617 (0.005479)	0.1173	**Model 4**	0.01106 (0.005340)	0.0396*
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used =410)													
Model 1	0.004448 (0.01863)	0.8115	-0.00790 (0.01432)	0.5820	-0.01054 (0.01736)	0.5445	-0.01287 (0.01383)	0.3528	0.007295 (0.01297)	0.5743	**Model 3**	-0.00234 (0.01222)	0.8485
Model 2	0.02602 (0.01970)	0.1879	-0.02181 (0.01523)	0.1535	-0.02503 (0.01825)	0.1717	-0.01469 (0.01448)	0.3115	0.02421 (0.01367)	0.0781	**Model 4**	0.009358 (0.01317)	0.4782
Log transformed triglycerides (mg/dL) (number of observation used =410)													
Model 1	0.02273 (0.04134)	0.5829	0.03775 (0.03175)	0.2358	0.006459 (0.03849)	0.8669	0.01930 (0.03054)	0.5281	-0.01121 (0.02881)	0.6975	**Model 3**	0.02331 (0.02737)	0.3954
Model 2	0.009899 (0.04004)	0.8050	0.03468 (0.03095)	0.2637	0.01336 (0.03709)	0.7191	0.02312 (0.02947)	0.4336	-0.01934 (0.02777)	0.4869	**Model 4**	0.01315 (0.02639)	0.6189

- 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*)
- Model 1: the model includes *11β-HSD-2* z scores for sites 1, 2, 3, 4, and 5 as fixed effects and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additionally adjusted for the following fixed effects: age, sex, metabolic equivalents, total caloric intake, and maternal enrollment in calcium supplementation study
- Model 3: the model includes average *11β-HSD-2* z score for sites 1, 2, 3, 4, and 5 as fixed effect and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 4: model 3 additionally adjusted for the following fixed effects: age, sex, metabolic equivalents, total caloric intake, and maternal enrollment in calcium supplementation study

*P-value < 0.05; † P-value < 0.008

Table 4.8 (Supplementary): Associations between DNAm at *11β-HSD-2* and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models Adjusting for Pubertal Onset (N=228):

	***11β-HSD-2* site 1**		***11β-HSD-2* site 2**		***11β-HSD-2* site 3**		***11β-HSD-2* site 4**		***11β-HSD-2* site 5**		**Average of all sites**		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =413)													
Model 1	-0.3085 (1.0403)	0.7671	-0.1795 (0.7979)	0.8223	0.1743 (0.9688)	0.8574	0.4602 (0.7678)	0.5496	0.2848 (0.7244)	0.6946	**Model 3**	0.1115 (0.6867)	0.8712
Model 2	-1.1569 (0.9972)	0.2472	0.2025 (0.7702)	0.7929	0.6391 (0.9291)	0.4923	0.4385 (0.7454)	0.5569	-0.1585 (0.6928)	0.8192	**Model 4**	-0.3101 (0.6594)	0.6386
Systolic blood pressure (mm Hg) (number of observation used =413)													
Model 1	-1.5839 (0.8347)	0.0590	-0.7899 (0.6400)	0.2184	1.2452 (0.7780)	0.1109	0.3529 (0.6179)	0.5685	-0.4642 (0.5801)	0.4245	**Model 3**	-0.6991 (0.5581)	0.2116
Model 2	-1.8682 (0.8201)	0.0237*	-0.4356 (0.6324)	0.4916	1.5518 (0.7646)	0.0436*	0.1210 (0.6181)	0.8450	-0.3009 (0.5684)	0.5971	**Model 4**	-0.4251 (0.5470)	0.4380
Diastolic blood pressure (mm Hg) (number of observation used =413)													
Model 1	-0.9272 (0.5538)	0.0955	-0.8573 (0.4245)	0.0446*	0.3562 (0.5165)	0.4911	0.4559 (0.4108)	0.2683	-0.01583 (0.3845)	0.9672	**Model 3**	-0.5909 (0.3725)	0.1142
Model 2	-1.2107 (0.5318)	0.0238*	-0.6472 (0.4095)	0.1154	0.6383 (0.4964)	0.1998	0.2989 (0.4033)	0.4594	0.01191 (0.3679)	0.9742	**Model 4**	-0.4740 (0.3569)	0.1856
Log transformed fasting glucose (mg/dL) (number of observation used =410)													
Model 1	-0.00084 (0.007539)	0.9118	0.002076 (0.005797)	0.7206	0.006432 (0.007029)	0.3612	-0.01860 (0.005607)	0.0011*†	0.002604 (0.005242)	0.6198	**Model 3**	0.006014 (0.005094)	0.2392
Model 2	0.007630 (0.007860)	0.3328	-0.00149 (0.006069)	0.8063	0.003090 (0.007327)	0.6736	-0.02129 (0.005954)	0.0004*†	0.007724 (0.005453)	0.1581	**Model 4**	0.01135 (0.005349)	0.0351*
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used =410)													
Model 1	0.004448 (0.01863)	0.8115	-0.00790 (0.01432)	0.5820	-0.01054 (0.01736)	0.5445	-0.01287 (0.01383)	0.3528	0.007295 (0.01297)	0.5743	**Model 3**	-0.00234 (0.01222)	0.8485
Model 2	0.02284 (0.01931)	0.2381	-0.02102 (0.01492)	0.1602	-0.01731 (0.01798)	0.3368	-0.02643 (0.01448)	0.0694	0.02091 (0.01341)	0.1205	**Model 4**	0.009940 (0.01293)	0.4430
Log transformed triglycerides (mg/dL) (number of observation used =410)													
Model 1	0.02273 (0.04134)	0.5829	0.03775 (0.03175)	0.2358	0.006459 (0.03849)	0.8669	0.01930 (0.03054)	0.5281	-0.01121 (0.02881)	0.6975	**Model 3**	0.02331 (0.02737)	0.3954
Model 2	0.01078 (0.04006)	0.7881	0.03456 (0.03094)	0.2652	0.01098 (0.03732)	0.7689	0.02680 (0.03016)	0.3752	-0.01839 (0.02782)	0.5093	**Model 4**	0.01289 (0.02639)	0.6257

- 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD-2*)
- Model 1: the model includes *11β-HSD-2* z scores for sites 1, 2, 3, 4, and 5 as fixed effects and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additionally adjusted for the following fixed effects: age, sex, metabolic equivalents, total caloric intake, mother enrollment in calcium supplementation study, and pubertal onset
- Model 3: the model includes average *11β-HSD-2* z score for sites 1, 2, 3, 4, and 5 as fixed effect and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 4: model 3 additionally adjusted for the following fixed effects: age, sex, metabolic equivalents, total caloric intake, mother enrollment in calcium supplementation study, and pubertal onset

*P-value < 0.05; † P-value < 0.008

Table 4.9 (Supplementary): Average DNAm at *H19* and Confounders Selection:

	Average DNA methylation z score at *H19*				
	Q 1 N= 147	**Q 2** N= 148	**Q 3** N=148	**Q 4** N=148	**P-value**
Maternal Characteristics (at time of child's birth)					
Years of education, %					
< 12 years	46.26	49.32	54.73	48.65	0.2168
12 years	35.37	34.46	37.84	37.84	
> 12 years	17.69	15.54	7.43	13.51	
Age at childbirth, (years)	26.63	26.57	26.76	26.38	0.9462
Parity, %					
0	0.68	1.35	0.00	1.35	0.4834
1	44.22	35.81	30.41	35.81	
≥ 2	54.42	62.16	69.59	62.84	
Marital Status, %					
Married	74.15	75.00	71.62	66.89	0.6709
Others (includes free union, single, separated, or divorced)	25.17	24.32	28.38	33.11	
Enrollment in calcium supplementation study, %					
Not enrolled	65.99	63.51	66.89	65.54	0.9517
Enrolled during pregnancy	33.33	35.81	33.11	34.46	
Child Characteristics (at birth)					
Girls, %	54.42	53.38	48.65	51.35	0.8746
Gestational age, (weeks)	38.79	38.78	38.73	38.93	0.6611
Mode of delivery, %					
Vaginal delivery	53.06	58.11	60.14	56.76	0.7641
C Section	45.58	40.54	39.86	42.57	
Birth weight, (kg)	3.16	3.18	3.11	3.18	0.5626
Breastfeeding duration, (weeks)	8.25	8.16	7.83	8.42	0.9021
Child Characteristics (at follow-up visit)					
Age, (years)	12.52	12.30	12.96	12.40	0.1925
Body mass Z score for age	0.83	0.61	0.48	0.60	0.0865
Metabolic equivalent, (METs/week)	50.38	44.31	49.84	48.10	0.5308
Pubertal onset , %	70.07	70.95	79.05	64.19	0.0021*
Total caloric intake, (kcal/day)	2468.28	2420.31	2423.70	2666.31	0.0506

Means or percentages are presented for continuous or categorical variables, respectively
*P-value < 0.05

Table 4.10 (Supplementary): Associations between DNAm at *H19* DNAm and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models (N=245):

	***H19* site 1**		***H19* site 2**		***H19* site 3**		***H19* site 4**		**Average of all sites**		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =446)											
Model 1	-2.0199 (2.3712)	0.3951	0.4201 (0.9290)	0.6515	-0.1023 (1.0516)	0.9226	1.7246 (2.3175)	0.4575	**Model 3**	-0.1567 (0.6716)	0.8157
Model 2	-0.4958 (2.2859)	0.8285	0.07485 (0.9050)	0.9342	-0.01468 (1.0179)	0.9885	1.0578 (2.2350)	0.6364	**Model 4**	0.5336 (0.6513)	0.4135
Systolic blood pressure (mm Hg) (number of observation used =446)											
Model 1	2.9254 (1.9347)	0.1318	0.5769 (0.7460)	0.4402	-0.3888 (0.8519)	0.6485	-2.0551 (1.8928)	0.2786	**Model 3**	0.8583 (0.5457)	0.1171
Model 2	2.1289 (2.0092)	0.2905	0.6308 (0.7785)	0.4188	-0.3848 (0.8852)	0.6643	-1.4683 (1.9667)	0.4561	**Model 4**	0.6896 (0.5713)	0.2287
Diastolic blood pressure (mm Hg) (number of observation used =446)											
Model 1	1.9218 (1.3080)	0.1430	0.07059 (0.5004)	0.8880	-0.1770 (0.5740)	0.7580	-1.1655 (1.2803)	0.3635	**Model 3**	0.6313 (0.3673)	0.0870
Model 2	1.7656 (1.3295)	0.1855	0.07927 (0.5086)	0.8763	-0.1730 (0.5822)	0.7667	-1.0460 (1.3026)	0.4228	**Model 4**	0.5994 (0.3773)	0.1136
Log transformed fasting glucose (mg/dL) (number of observation used =443)											
Model 1	-0.00046 (0.01808)	0.9796	0.002770 (0.006771)	0.6829	0.004283 (0.007883)	0.5875	-0.00728 (0.01773)	0.6816	**Model 3**	-0.00270 (0.005061)	0.5948
Model 2	-0.01426 (0.01900)	0.4536	0.006626 (0.007262)	0.3627	0.001784 (0.008333)	0.8307	-0.00365 (0.01861)	0.8448	**Model 4**	-0.01179 (0.005433)	0.0311*
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used =443)											
Model 1	0.08297 (0.04325)	0.0562	-0.00522 (0.01640)	0.7507	-0.00854 (0.01895)	0.6527	-0.09188 (0.04237)	0.0311*	**Model 3**	-0.01575 (0.01221)	0.1984
Model 2	0.04381 (0.04758)	0.3582	0.009569 (0.01869)	0.6091	-0.01912 (0.02112)	0.3663	-0.08270 (0.04654)	0.0769	**Model 4**	-0.04359 (0.01364)	0.0016*†
Log transformed triglycerides (mg/dL) (number of observation used =443)											
Model 1	-0.09145 (0.09240)	0.3233	0.03856 (0.03589)	0.2838	-0.02407 (0.04087)	0.5566	0.1266 (0.09037)	0.1626	**Model 3**	0.03357 (0.02621)	0.2015
Model 2	-0.04746 (0.08976)	0.5975	0.03792 (0.03481)	0.2772	-0.02790 (0.03962)	0.4820	0.09088 (0.08786)	0.3020	**Model 4**	0.03886 (0.02560)	0.1303

- Model 1: the model includes *H19* z scores for site 1, 2, 3, and 4 as fixed effects, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additionally adjusted for the following fixed effects: age and sex
- Model 3: the model includes average *H19* z score for site 1, 2, 3, and 4 as fixed effects, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 4: model 3 additionally adjusted for the following fixed effects: age and sex

*P-value < 0.05; † P-value < 0.008

Table 4.11 (Supplementary): Associations between DNAm at *H19* DNAm and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models Adjusting for Pubertal Onset (N=245):

	***H19* site 1**		***H19* site 2**		***H19* site 3**		***H19* site 4**		**Average of all sites**		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =446)											
Model 1	-2.0199 (2.3712)	0.3951	0.4201 (0.9290)	0.6515	-0.1023 (1.0516)	0.9226	1.7246 (2.3175)	0.4575	**Model 3**	-0.1567 (0.6716)	0.8157
Model 2	-0.4601 (2.2842)	0.8405	0.1017 (0.9050)	0.9106	0.02333 (1.0184)	0.9817	1.0305 (2.2331)	0.6449	**Model 4**	0.5850 (0.6548)	0.3726
Systolic blood pressure (mm Hg) (number of observation used =446)											
Model 1	2.9254 (1.9347)	0.1318	0.5769 (0.7460)	0.4402	-0.3888 (0.8519)	0.6485	-2.0551 (1.8928)	0.2786	**Model 3**	0.8583 (0.5457)	0.1171
Model 2	2.2231 (1.9256)	0.2495	0.8875 (0.7469)	0.2360	-0.1066 (0.8494)	0.9003	-1.6144 (1.8851)	0.3926	**Model 4**	0.9724 (0.5503)	0.0785
Diastolic blood pressure (mm Hg) (number of observation used =446)											
Model 1	1.9218 (1.3080)	0.1430	0.07059 (0.5004)	0.8880	-0.1770 (0.5740)	0.7580	-1.1655 (1.2803)	0.3635	**Model 3**	0.6313 (0.3673)	0.0870
Model 2	1.7623 (1.2916)	0.1737	0.2580 (0.4953)	0.6030	-0.01024 (0.5665)	0.9856	-1.1167 (1.2655)	0.3784	**Model 4**	0.7436 (0.3670)	0.0439*
Log transformed fasting glucose (mg/dL) (number of observation used =443)											
Model 1	-0.00046 (0.01808)	0.9796	0.002770 (0.006771)	0.6829	0.004283 (0.007883)	0.5875	-0.00728 (0.01773)	0.6816	**Model 3**	-0.00270 (0.005061)	0.5948
Model 2	-0.01454 (0.01894)	0.4434	0.008114 (0.007262)	0.2652	0.003016 (0.008321)	0.7174	-0.00410 (0.01855)	0.8254	**Model 4**	-0.01093 (0.005447)	0.0460*
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used =443)											
Model 1	0.08297 (0.04325)	0.0562	-0.00522 (0.01640)	0.7507	-0.00854 (0.01895)	0.6527	-0.09188 (0.04237)	0.0311*	**Model 3**	-0.01575 (0.01221)	0.1984
Model 2	0.04758 (0.04618)	0.3039	0.01380 (0.01815)	0.4478	-0.01387 (0.02052)	0.4996	-0.08614 (0.04517)	0.0577	**Model 4**	-0.03746 (0.01334)	0.0054*†
Log transformed triglycerides (mg/dL) (number of observation used =443)											
Model 1	-0.09145 (0.09240)	0.3233	0.03856 (0.03589)	0.2838	-0.02407 (0.04087)	0.5566	0.1266 (0.09037)	0.1626	**Model 3**	0.03357 (0.02621)	0.2015
Model 2	-0.04758 (0.08978)	0.5966	0.03750 (0.03488)	0.2834	-0.02833 (0.03968)	0.4760	0.09109 (0.08788)	0.3010	**Model 4**	0.03848 (0.02570)	0.1356

- Model 1: the model includes *H19* z scores for site 1, 2, 3, and 4 as fixed effects, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additional adjusted for the following fixed effects: age, sex, and pubertal onset
- Model 3: the model includes average *H19* z score for site 1, 2, 3, and 4 as fixed effect, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 4: model 3 additional adjusted for the following fixed effects: age, sex, and pubertal onset

*P-value < 0.05; † P-value < 0.008

Table 4.12 (Supplementary): Associations between DNAm at *H19* DNAm and Repeated Measures of Cardiometabolic Risk Factors using Mixed Models after Removing Outlier DNAm Value (N=244):

	***H19* site 1**		***H19* site 2**		***H19* site 3**		***H19* site 4**		**Average of all sites**		
	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (number of observation used =444)											
Model 1	-1.9711 (2.3738)	0.4072	1.7287 (1.9031)	0.3646	-0.9859 (1.5380)	0.5221	1.8050 (2.3215)	0.4376	**Model 3**	-0.1645 (0.6804)	0.8091
Model 2	-0.4707 (2.2913)	0.8374	0.5902 (1.8478)	0.7497	-0.3633 (1.4905)	0.8076	1.0906 (2.2422)	0.6271	**Model 4**	0.5617 (0.6605)	0.3959
Systolic blood pressure (mm Hg) (number of observation used =444)											
Model 1	2.9096 (1.9392)	0.1348	0.1319 (1.5394)	0.9318	-0.08892 (1.2463)	0.9432	-2.0820 (1.8985)	0.2739	**Model 3**	0.8114 (0.5527)	0.1434
Model 2	2.1309 2.0126	0.2909	0.5685 (1.6014)	0.7230	-0.3428 (1.2944)	0.7914	-1.4729 (1.9721)	0.4559	**Model 4**	0.6441 (0.5793)	0.2675
Diastolic blood pressure (mm Hg) (number of observation used =444)											
Model 1	1.9019 (1.3105)	0.1480	-0.5299 (1.0351)	0.6092	0.2280 (0.8388)	0.7860	-1.2025 (1.2836)	0.3498	**Model 3**	0.6256 (0.3723)	0.0942
Model 2	1.7543 (1.3311)	0.1889	-0.4459 (1.0506)	0.6717	0.1809 (0.8503)	0.8317	-1.0820 (1.3054)	0.4081	**Model 4**	0.5953 (0.3831)	0.1217
Log transformed fasting glucose (mg/dL) (number of observation used =441)											
Model 1	-0.00042 (0.01812)	0.9813	0.004047 (0.01413)	0.7749	0.003419 (0.01151)	0.7667	-0.00720 (0.01778)	0.6859	**Model 3**	-0.00312 (0.005130)	0.5435
Model 2	-0.01405 (0.01903)	0.4610	0.01456 (0.01502)	0.3333	-0.00357 (0.01217)	0.7696	-0.00310 (0.01866)	0.8684	**Model 4**	-0.01271 (0.005509)	0.0220 *
Log transformed high density lipoprotein cholesterol (mg/dL) (number of observation used =441)											
Model 1	0.08260 (0.04335)	0.0579	-0.01677 (0.03406)	0.6229	-0.00074 (0.02769)	0.9788	-0.09260 (0.04249)	0.0303*	**Model 3**	-0.01587 (0.01238)	0.2010
Model 2	0.04405 (0.04770)	0.3568	0.01789 (0.03828)	0.6407	-0.02474 (0.03092)	0.4245	-0.08213 (0.04670)	0.0799	**Model 4**	-0.04540 (0.01382)	0.0012 *†
Log transformed triglycerides (mg/dL) (number of observation used =441)											
Model 1	-0.08894 (0.09237)	0.3365	0.1086 (0.07369)	0.1420	-0.07136 (0.05967)	0.2329	0.1309 (0.09040)	0.1488	**Model 3**	0.03282 (0.02656)	0.2177
Model 2	-0.04632 (0.08982)	0.6066	0.08574 (0.07154)	0.2319	-0.06016 (0.05788)	0.2997	0.09421 (0.08801)	0.2854	**Model 4**	0.03746 (0.02597)	0.1505

- Model 1: the model includes *H19* z scores for site 1, 2, 3, and 4 as fixed effects, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 2: model 1 additionally adjusted for the following fixed effects: age and sex
- Model 3: the model includes average *H19* z score for site 1, 2, 3, and 4 as fixed effect, and compound symmetry matrix structure to model the covariance structure of the repeated measurements for each outcome
- Model 4: model 3 additionally adjusted for the following fixed effects: age and sex

*P-value < 0.05; † P-value < 0.008

Table 4.13 (Supplementary): Average DNAm at *PPAR- α* and Confounders Selection:

	Average DNA methylation z score at *PPAR-α*			
	Q 1 N= 119	**Q 2** N= 120	**Q 3** N=119	**P-value**
Maternal Characteristics (at time of child's birth)				
Years of education, %				
< 12 years	43.70	56.67	53.78	0.5221
12 years	40.34	33.33	31.09	
> 12 years	15.97	9.17	15.13	
Age at childbirth, (years)	27.14	26.26	25.98	0.2051
Parity, %				
0	3.36	0.00	0.00	0.0758
1	40.34	35.83	36.97	
≥ 2	56.30	63.33	63.03	
Marital Status, %				
Married	80.67	70.83	66.39	0.2163
Others (includes free union, single, separated, or divorced)	19.33	28.33	33.61	
Enrollment in calcium supplementation study, %				
Not enrolled	73.95	68.33	63.03	0.2068
Enrolled during pregnancy	26.05	30.83	36.97	
Child Characteristics (at birth)				
Girls, %	45.38	52.50	57.14	0.3336
Gestational age, (weeks)	38.85	38.85	38.66	0.4964
Mode of delivery, %				
Vaginal delivery	54.62	54.17	67.23	0.3513
C Section	44.54	45.00	32.77	
Birth weight, (kg)	3.15	3.13	3.17	0.8723
Breastfeeding duration, (weeks)	8.50	8.39	7.47	0.1503
Child Characteristics (at follow-up visit)				
Age, (years)	14.55	14.10	13.69	0.0084*
Body mass Z score for age	0.57	0.57	0.45	0.6289
Metabolic equivalents, (METs/ week)	60.67	61.66	59.47	0.7728
Pubertal onset , %	92.44	93.33	90.76	<0.0001*
Total caloric intake, (kcal/day)	2401.42	2412.75	2266.42	0.7909

Means or percentages are presented for continuous or categorical variables, respectively.
Peroxisome proliferator-activated receptor alpha (*PPAR-α*)
*P-value < 0.05

Table 4.14: Cross-sectional Associations between DNAm at *PPAR- α* and Cardiometabolic Risk Factors using Linear Regression (N=345):

	***PPAR- α* site 1**		***PPAR- α* site 2**		**Average *PPAR- α***		
	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (N= 345)							
Model 1	0.71915 (0.71474)	0.3150	-1.70941 (0.65445)	0.0094*	**Model 3**	-1.25724 (0.72517)	0.0839
Model 2	0.99917 (0.70529)	0.1575	-1.68127 (0.64618)	0.0097*	**Model 4**	-1.00482 (0.71708)	0.1620
Systolic blood pressure (mm Hg) (N= 345)							
Model 1	0.58582 (0.60305)	0.3320	-1.02922 (0.55218)	0.0632	**Model 3**	-0.63619 (0.61069)	0.2983
Model 2	0.49623 (0.57982)	0.3927	-0.66490 (0.53123)	0.2116	**Model 4**	-0.31745 (0.58645)	0.5886
Diastolic blood pressure (mm Hg) (N= 345)							
Model 1	0.58530 (0.42242)	0.1668	-0.57466 (0.38679)	0.1383	**Model 3**	-0.15115 (0.42804)	0.7242
Model 2	0.58072 (0.40724)	0.1548	-0.34026 (0.37311)	0.3624	**Model 4**	0.09596 (0.41231)	0.8161
Log transformed fasting glucose (mg/dL) (N=310)							
Model 1	0.00598 (0.00614)	0.3305	0.00016627 (0.00600)	0.9779	**Model 3**	0.00519 (0.00653)	0.4274
Model 2	0.00282 (0.00609)	0.6443	0.00159 (0.00596)	0.7900	**Model 4**	0.00401 (0.00646)	0.5355
Log transformed high density lipoprotein cholesterol (mg/dL) (N= 310)							
Model 1	-0.00813 (0.01303)	0.5329	0.01206 (0.01273)	0.3445	**Model 3**	0.00566 (0.01386)	0.6830
Model 2	-0.00419 (0.01309)	0.7490	0.00857 (0.01280)	0.5035	**Model 4**	0.00535 (0.01388)	0.7001
Log transformed triglycerides (mg/dL) (N= 310)							
Model 1	0.01232 (0.03058)	0.6873	0.00118 (0.02989)	0.9684	**Model 3**	0.01155 (0.03249)	0.7225
Model 2	0.02086 (0.03057)	0.4956	-0.01116 (0.02989)	0.7092	**Model 4**	0.00596 (0.03242)	0.8543

- Peroxisome proliferator-activated receptor alpha (*PPAR-α*)
- Model 1: the model includes *PPAR- α* z scores for site 1 and 2
- Model 2: model 1 additionally adjusted for age, and sex
- Model 3: the model includes average *PPAR- α* z score for site 1 and 2
- Model 4: model 3 additionally adjusted for age and sex

*P-value < 0.05; † P-value < 0.008

Table 4.15 (Supplementary): Cross-sectional Associations between DNAm at *PPAR- α* and Cardiometabolic Risk Factors using Linear Regression Adjusting for Pubertal Onset (N=345):

	***PPAR- α* site 1**		***PPAR- α* site 2**		**Average *PPAR- α***		
	Estimate (SE)	P-value	Estimate (SE)	P-value		Estimate (SE)	P-value
Waist circumference (cm) (N= 345)							
Model 1	0.71915 (0.71474)	0.3150	-1.70941 (0.65445)	0.0094*	**Model 3**	-1.25724 (0.72517)	0.0839
Model 2	1.01860 (0.70558)	0.1498	-1.69669 (0.64638)	0.0091*	**Model 4**	-1.00584 (0.71725)	0.1617
Systolic blood pressure (mm Hg) (N= 345)							
Model 1	0.58582 (0.60305)	0.3320	-1.02922 (0.55218)	0.0632	**Model 3**	-0.63619 (0.61069)	0.2983
Model 2	0.51018 (0.58026)	0.3799	-0.67598 (0.53158)	0.2044	**Model 4**	-0.31821 (0.58672)	0.5879
Diastolic blood pressure (mm Hg) (N= 345)							
Model 1	0.58530 (0.42242)	0.1668	-0.57466 (0.38679)	0.1383	**Model 3**	-0.15115 (0.42804)	0.7242
Model 2	0.59773 (0.40664)	0.1425	-0.35377 (0.37253)	0.3430	**Model 4**	0.09503 (0.41164)	0.8176
Log transformed fasting glucose (mg/dL) (N=310)							
Model 1	0.00598 (0.00614)	0.3305	0.00016627 (0.00600)	0.9779	**Model 3**	0.00519 (0.00653)	0.4274
Model 2	0.00302 (0.00609)	0.6204	0.00131 (0.00595)	0.8258	**Model 4**	0.00389 (0.00645)	0.5469
Log transformed high density lipoprotein cholesterol (mg/dL) (N= 310)							
Model 1	-0.00813 (0.01303)	0.5329	0.01206 (0.01273)	0.3445	**Model 3**	0.00566 (0.01386)	0.6830
Model 2	-0.00391 (0.01310)	0.7655	0.00819 (0.01281)	0.5234	**Model 4**	0.00518 (0.01388)	0.7092
Log transformed triglycerides (mg/dL) (N= 310)							
Model 1	0.01232 (0.03058)	0.6873	0.00118 (0.02989)	0.9684	**Model 3**	0.01155 (0.03249)	0.7225
Model 2	0.02104 (0.03063)	0.4927	-0.01140 (0.02996)	0.7038	**Model 4**	0.00586 (0.03247)	0.8568

- Peroxisome proliferator-activated receptor alpha (*PPAR-α*).
- Model 1: the model includes *PPAR- α* z scores for site 1 and 2 as fixed effects
- Model 2: additional adjusted for the following fixed effects: age, sex, and pubertal onset.
- Model 3: the model includes average *PPAR- α* z score for site 1 and 2
- Model 4: model 3 additionally adjusted for age, sex, and pubertal onset

*P-value < 0.05; † P-value < 0.008

Chapter 5 Conclusion

Summary of Main findings:

The overall aim for this dissertation work was to examine the determinants of cardiometabolic risk factors among free-living Mexican children and adolescents during their pubertal transition. More specifically, the associations between cardiometabolic risk factors and diet quality, which was measured with the Dietary Approaches to Stop Hypertension (DASH), Alternate Mediterranean Diet (aMedDiet), and Children's Dietary Inflammatory Index (C-DII^{TM}) scores, were investigated using a repeated measures-longitudinal study design with up to three measurements per subject. Moreover, we analyzed the relationship between sedentary patterns – including sedentary time, and replacing sedentary time with different intensities of physical activity – on cardiometabolic risk factors using a repeated measures-longitudinal study design. Total sedentary time, and the context of sedentary time, were assessed with a subjective tool (i.e., physical activity questionnaires), and sedentary time and bout, which reflects a pattern of activity accumulation, were assessed using an objective tool (i.e., ActiGraph GT3X+ wrist accelerometers). Lastly, we examined the association between cardiometabolic heath and DNA methylation (DNAm), quantified in blood leukocytes, at long interspersed nuclear elements (LINE-1), *H19,* 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD*-2), and peroxisome proliferator-activated receptor alpha (*PPAR-α*). DNAm at LINE-1, *H19,* and *11β-HSD*-2 were analyzed prospectively using a repeated measures longitudinal design of the cardiometabolic risk factors, and DNAm at *PPAR-α* was appraised using a cross-sectional study design. The three

aims of this project were executed using the pre-existing data of children and adolescents who participated in the **E**arly **L**ife **E**xposure in **M**exico to **EN**vironmental **T**oxicants (**ELEMENT**) birth cohort Mexico City, Mexico [1-3].

In chapter 2, associations between diet quality and cardiometabolic risk factors were identified among healthy youth aged between 8 – 21 years. Participants in the fourth quartile of DASH score had lower insulin, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) compared to the lowest quartile (i.e., reference group). An additional linear trend association was detected between HOMA-IR and DASH score. Serum triglycerides was associated with aMedDiet and C-DII scores. An inverse linear trend association was detected with aMedDiet score, and a positive linear trend association was found with C-DII scores. In addition, participants in the fourth quartile of the C-DII scores had higher serum triglycerides relative to the reference group.

Our inverse associations between insulin homeostasis and DASH score are consistent with findings from meta-analysis of randomized control trials (RCTs) among adults [4] and a randomized cross-over clinical trial DASH intervention conducted among adolescent girls [5]. The DASH diet is characterized by: 1) reducing the intake of cholesterol, saturated fat, total fat, lean red meat, sweets, added sugars, and sugar-containing beverages; and 2) increasing the intake of fruits, vegetables, and fat-free or low-fat milk and milk products, whole grains, fish, poultry, and nuts [6]. Therefore, DASH diet is rich in protein, fiber, potassium, magnesium, calcium [6], and folic acid [7] and low in sodium [6] and fat. Some of these nutrients have potential roles in insulin and glucose homeostasis [8-10]. The identified inverse associations are important for Mexican youth because insulin resistance can be identified in Mexican children without evidence of overweight or obesity [11]. Moreover, insulin sensitivity has been considered the driver for the adipose tissue

partitioning [12] . This is very crucial as abnormal fat deposition may be a potential risk for the pathology of obesity [13]. Tailoring nutritional recommendations to tackle insulin resistance would be a feasible strategy to endorse even for healthy youth.

The detected associations between aMedDiet and C-DII scores with serum triglycerides are biologically plausible as higher aMedDiet scores and lower C-DII scores reflect higher diet quality. Our findings support the established role of diet as a cornerstone of hypertriglyceridemia management [14-16]. Furthermore, serum triglycerides are influenced by diet in two ways. The first one is the direct source from the consumed dietary fatty acids, and the second one is the indirect pool from the synthesized fatty acids from glucose via the *de novo* lipogenesis pathway [14]. Regarding the connection between serum triglycerides and cardiovascular disease (CVD), the available evidence from adults' studies showed that serum triglycerides is an established risk factor [17-20]. Therefore, controlling serum triglycerides via dietary strategies might be an effective first line approach in youth to prevent consequences of impaired cardiometabolic health.

It is worth noting that the differences in associations detected between each diet score and cardiometabolic risk factors are plausible given the moderate associations between them. A potential explaining factor for moderate associations lies in the analytical differences followed to construct each score [21,22]. In addition, the differences between scores stems from the fact that each score captures slightly distinct dietary recommendations, and is composed of different foods, food groups, and nutrients. We found that DASH score is associated with lower intake from all types of fat. In contrast, aMedDiet and C-DII scores were positively associated with all types of fat, except for an inverse association with saturated fat and polyunsaturated fat, for aMedDiet and C-DII scores, respectively. The DASH eating plan restricts the intake from fat, and red meat [6]. On the other hand, aMedDiet and C-DII scores emphasize fat quality. The

aMedDiet recommendations promote the consumption of nuts and seeds, and olive oil use, and lower the intake from animal products [23]. For C-DII, the monounsaturated fat and polyunsaturated fat have anti-inflammatory potential, but cholesterol, saturated fat, and total fat are considered pro-inflammatory nutrients [24]

In chapter 3, we detected adverse associations of sedentary time in a context-specific manner, and protective associations of replacing sedentary time with light or moderate to vigorous physical activity on cardiometabolic risk factors. Additional one hour of screen-based sedentary time was associated with higher diastolic blood pressure, and of other sedentary time (i.e., doing homework or reading and commuting) was associated with higher serum glucose. Moreover, replacing 1% of sedentary time with moderate to vigorous physical activity was inversely associated with waist circumference and serum triglycerides. Substituting an uninterrupted five minutes of sedentary bout or one minute of a sedentary bout with light activity was inversely associated with serum insulin levels.

The detected effect size for the positive association between diastolic blood pressure and screen time was similar to one reported previously among adolescents aged 11–13 years [25]. Unfavorable associations between screen time and other cardiometabolic risk factors such as waist circumference, lipid profile, fat mass, and BMI also have been reported [25-27] . It is worth noting that these unfavorable associations reported earlier are consistent with the augmented evidence of association between TV watching and higher caloric consumption [28-30], lower diet quality [28,31], and disturbed sleep duration [32], each of which are a plausible contributor to impaired cardiometabolic health. Despite this biological plausibility, evidence from observational studies examining the association between screen time and cardiometabolic health in youth, did not support the adverse association [33-35] and flagged concerns about heterogeneity [33,34].

We found a positive association between other sedentary time (i.e., doing homework or reading and commuting) and serum glucose. The association between cognitive or knowledge based sedentary behaviors and metabolic health has been examined before among adults [36-38]. Two experimental studies showed that higher mean ad libitum energy intake after cognitive-related tasks (i.e., reading and writing or computer-based automated test-battery) relative to the control sedentary condition (i.e., sitting in a comfortable chair) [36,37]. Another study also reported an increase in caloric consumption while working on major work deadlines [38]. Regarding metabolic homeostasis, performing sedentary cognitive tasks have been associated with higher mean cortisol level and larger variability in serum glucose and insulin concentrations compared to a relaxed sedentary setting [37]. Therefore, it is plausible to consider cognitive-based sedentary time as a potential driver for positive energy balance and weight gain in the long-term [30,36,37]. Future studies are warranted to expand the assessment of the sedentary behavior beyond that screen-based sedentary time among youth.

Our substitution models showed inverse associations between replacing sedentary time with moderate to vigorous physical activity on waist circumference and serum triglycerides, and these associations were consistent with the previous studies [39,40]. We assessed replacing 1% of sedentary time (median of 6 minutes) compared to 10 minutes [40] and 60 minutes [39], which justified our lower effect sizes compared to the other studies [39,40]. In fact, we showed that higher effect sizes resulted when higher percentages of sedentary time were replaced (i.e., 5% of change [median of 31 minutes] and 10% of change [median of 61 minutes). Despite that, our study showed that a small and feasible increase in moderate to vigorous physical activity to replace sedentary time resulted in a favorable cardiometabolic impact. Our conclusion was consistent with the recommendations for replacing sedentary time with higher intensities of physical

activity to promote cardiometabolic benefits among youth [35,41], and the conclusion drawn from a meta-analysis of observational studies used isotemporal substitution to examine replacing sedentary behavior with higher intensities of physical activity [42].

We assessed the activity pattern via bouts of accumulating activity. We found that replacing a sedentary bout with light activity was associated with a reduction in serum insulin. In the current research on activity bouts among youth, studies have found inconsistent results for investigating the relationship with cardiometabolic risk factors [43-48]. Moreover, there is limited evidence distilled from several reviews and meta-analysis reviews [35,41,49,50]. Some methodological related factors in defining bouts could be a source of heterogeneity as there is no consensus on defining the duration of a bout [32,41,46,48]. Standardizing the exposure assessment would increase the robustness of pooled evidence extracted from multiple studies [40], and reduce the heterogeneity concerns raised in the reviews which assessed bouts of activity [41,49,50].

In chapter 4, we found evidence supporting the gene-specific and site-specific associations between DNAm and cardiometabolic risk factors. We showed that DNAm at LINE-1 loci was inversely associated with repeated measures of serum glucose at site 1, but positively related to serum glucose and high-density lipoprotein cholesterol at site 2 and 3, respectively. DNAm at *11β-HSD-2* was inversely associated with repeated measures of systolic and diastolic blood pressure at site 1 and with serum glucose at site 4. An inverse cross-sectional association was detected between DNAm at *PPAR-α* at site 2 with waist circumference.

Findings from Chapter 4 emphasize the importance of DNAm as a potential mechanism of the cardiometabolic health among youth. Firstly, our inverse prospective relationship between DNAm at LINE-1 and glucose are in agreement with findings from adult studies [51-54]. Additionally, other studies have shown LINE-1 hypomethylation was associated with genomic

instability and CVD [54-57]. Few epidemiological studies have been conducted on children to investigate the relationship between global DNAm, measured by DNAm at LINE-1, and adiposity outcomes [58,59]. An inverse linear association between quartiles of DNAm and a change in waist circumference z-score was found among Colombian boys aged 5 -12 years old after 2.5 years of follow-up [58]. A null cross-sectional finding was reported between salivary DNAm at LINE-1 and adiposity outcomes – BMI z-score, waist circumference z-score, and percent body fat – in 431 adolescents aged 10 - 15 years [59]. `

We also found that higher DNAm at *11β-HSD-2* was inversely associated with blood pressure and fasting glucose. The biological function for 11β-HSD-2 enzyme is to convert cortisol to an inactive metabolite called cortisone [60,61]. Our findings disagree with other studies that showed DNAm at the promotor region for *11β-HSD-2* was associated with lower gene expression [62,63], impaired 11β-HSD-2 enzyme activity [64], and higher blood pressure in adults [64,65], and that impaired enzymatic activity was positively associated with blood pressure in children [66]. However, others have shown the possibility for identifying inverse association between DNAm at the promotor region of *1β-HSD-2* and blood pressure [65]. Reflecting on the association identified with glucose, research conducted on adults has shown that *11β-HSD-2* activity was positively associated with obesity [67] and inversely with insulin sensitivity [67]. Moreover, a previous study found that higher *11β-HSD-2* activity among adults diagnosed with type 2 diabetes diagnosis than controls, despite the lack of difference in mRNA expression [68]. This inconsistency in findings across studies shed light on the complexity of the regulation of genes, and how the control can vary by region of the gene, type of regulator (i.e. DNAm versus miRNA[69]), and other physiological and behavioral factors such as age [70] , dietary intake, and physical activity [71].

We found that each one standard deviation increase in the DNAm at the promotor region of *PPAR-α* at site 2 was associated with approximately 2 cm smaller waist circumference. Previous evidence showed that DNAm at promoter regions of genes is associated with gene repression [72]. However, our *PPAR-α* expression data (N=65), showed weak non-significant positive correlations between DNAm at the two CpG sites located in the promotor regions and *PPAR-α* expression**.** The inverse association with waist circumference was in agreement with *PPAR-α* biological functions, which are enhancing fatty acid oxidation, breaking down triglyceride-rich particles, removing excess cholesterol from the liver, and regulating oxidative stress and inflammatory response [73,74]. We identified two studies investigating the DNAm at *PPAR-α* and cardiometabolic risk factors. The first one showed that DNAm at *PPAR-α* – quantified from visceral adipose samples – was positively correlated with serum triglycerides among adults [52]. The second experiment revealed that feeding rats a high fructose diet for two weeks resulted in significant increase in hepatic DNAm at the promoter region of *PPAR-α,* decrease in mRNA expression of *PPAR-α*, and increase in serum triglycerides, total cholesterol, and hepatic lipid accumulation [75]. Other possible physiological conditions could regulate *PPAR-α* expression such as stress, insulin, leptin, adiponectin, growth hormones [74], and we acknowledge that these factors could confound our findings.

Strengths and limitations:

Several strengths are worth highlighting in this dissertation. First, our data came from a well-characterized birth cohort, **ELEMENT**, which allowed us adjusting for multiple confounders at childbirth. Another unique strength was that we assessed the cardiometabolic risk factors at multiple time points during pubertal transition. We acknowledged that the majority of evidence for cardiometabolic health and lifestyle factors comes from studies on Mexican

American and Hispanic American youth. Findings from previous studies can not necessarily be generalized to the Mexican youth due to the regional and cultural context and available resources and assets. Therefore, our reliance on youth who were residents in Mexico City made our study unique, and our conclusions would be generalizable to other Mexican youth from urban areas.

In chapter 2 and 3, we had repeated measures for exposures, which were diet quality scores, and sedentary and physical activity patterns, respectively. This unique strength of the longitudinal study design allowed us to capture changes in lifestyle behaviors during a critical period of development and growth. In chapter 2, we assessed the diet quality using three different measures, each of which represents a specific set of dietary recommendations. The simultaneous multiple appraisal of the same construct, diet quality, allowed us to derive a precise conclusion regarding the relationship between dietary recommendations and cardiometabolic risk factors. For chapter 3, we examined not only the independent associations between daily total and context-specific sedentary time with cardiometabolic risk factors, but also we investigated the impact of replacing sedentary time and sedentary bouts, a pattern for accumulating activity, with cardiometabolic health. Furthermore, we also examined the 24-hours of activity for 7 consecutive days, as subjects wore the accelerometer continuously, as facilitated by the use of a water resistant device [76].

One of the main strengths in chapter 4 was the prospective assessment of the association between DNAm at LINE-1, *H19,* and *11β-HSD*-2 and repeated measures of cardiometabolic risk factors during a sensitive period of growth, development, and maturation. We conducted a site-specific analysis for examining the association between the epigenetic modifications and cardiometabolic risk factors because it was noted earlier, the association between DNAm and outcome might be site-specific [53].

Nevertheless, this dissertation has several limitations that should be discussed. Primarily, the original aim for the **ELEMENT** project was understand the impact of environmental toxicants, more precisely lead, on health outcomes. Despite the later expansion of the project aim to include cardiometabolic health, some important information related to cardiometabolic health was missing. Examples of the missing covariates were family history of metabolic and cardiovascular diseases, and a thorough assessment of smoking status. Therefore, we acknowledge that residual confounding due to unmeasured or crudely measured covariates could still be present in our findings.

In chapter 2, dietary assessment in children and adolescents was subject to potential reporting errors due to youth limited skills in retrieving information, estimating the portion size and other related factors [77,78]. Furthermore, diet quality patterns might not be a precise measure for overall healthy habits among children and adolescents [79,80] as they are not a comprehensive assessment of all aspects of diet [81,82]. In addition, our food frequency questionnaire (FFQ) did not capture habitual intake as it queried the intake in the previous week [78] , and has not been validated despite its use in the National Nutrition Survey of Mexico, a national representative survey [83]. Lastly, the aMedDiet and the DASH scores use "population-specific" cut-offs for food consumption, and that may inflate type 2 error, reduce the variability in the intake among homogenous populations [84,85], and hinder the cross-study comparability. However, we also used C-DII scores, where population based food consumption database from multiple countries was used as a reference [86,87] , and that would enhance cross-studies comparability, and reduce the inherent bias that might occur for using the study population as a reference.

In chapter 3, the sedentary time calculated from self-reported activity questionnaires has not been validated against objective methods. Despite the common use of accelerometer as a

feasible objective assessment tool [88-90], it is not a gold standard for assessing sedentary behavior [44] because of its failure in distinguishing between posture settings [46,90-92] and capturing the context of sedentary behavior [25,90,93]. This is crucial information as different sedentary behaviors might not have an equal impact on health due to the differences in caloric and food consumption [28-31,36,94], energy expenditure and biological homeostasis [37,94] and other differences [32,95,96] associated with distinct forms of sedentary time. We addressed the change in activity between weekends and weekdays for school-age youth [97] by including subjects who had at least four valid days – one of which had to be a weekend day. Despite this attempt, some researchers claim that four days might not be a good representation of the variable movement behaviors among youth [97]. Thus, we acknowledge that imperfect representation could be a source of random error [98]. We summarized accelerometer data into 5 second epoch length [99] to reduce the measurement error and the misclassification concern associated with using higher epoch length for assessing highly variable children's movement behaviors [91]. Nevertheless, there have been no consensus about the epoch length used to summarize the accelerometer data, and that is a concerning point as previous research showed the association between activity bout and metabolic health was influenced by the epoch length [48].

In chapter 4 we measured DNAm in blood, which is not a target tissue for cardiometabolic related outcomes. Nonetheless, epigenome-wide studies have showed that DNAm in blood for multiple genes were correlated with DNAm in adipose tissue [100-102], and skeletal muscle [103], which are target tissues. Our work aimed to inform the development of potential biomarkers for cardiometabolic risk among children and adolescents, therefore, an accessible tissue such as blood is necessary to use for this purpose [104]. Lastly, the use of bisulfite treatment to measure DNAm does not distinguish between cytosine methylation (5mC) and

cytosine hydroxymethylation (5hmC) [105], and 5hmC has its own distinct impact on gene regulation which is not captured by our method. Therefore, our DNAm values might be confounded by the hydroxymethylation because both 5hmC and 5mC were qualified as 5mC, DNAm, after the bisulfite treatment.

Implications, recommendations and scope of future research:

The implications of the current dissertation are numerous. First and the most important, we shed light on determinants of cardiometabolic health (i.e., diet quality, sedentary and activity patterns, and DNAm) using a repeated measures longitudinal study design. Moreover, the use of a sample comprised of youth allowed us to provide insights on potential primary prevention strategies for cardiometabolic health. Besides, identifying the early determinants of cardiometabolic health is of special interest to Mexican youth due to their disproportionate burden of obesity and its related metabolic disorders. Hispanic youth have higher prevalence of childhood obesity and impaired cardiometabolic markers compared to their non-Hispanic White counterparts [106]. To add more, healthy Mexican youth showed signs of insulin resistance [11], which could be explained by their body higher body fat compared to non-Hispanic White peers [107,108]. Despite this disproportionate burden among Mexican youth, few longitudinal studies have been conducted aiming to mitigate the dire consequences of impaired cardiometabolic health at young age. Moreover, our findings could provide insights to guide the design and evaluation of novel interventions to improve diet quality and reduce sedentary behavior patterns. These studies could help in solidifying the evidence regarding the role of lifestyle interventions on mitigate the burden of cardiometabolic abnormalities among Mexican youth.

We suggest several recommendations for future studies on this topic. In this dissertation work, we used at the maximum three follow-up visits per subject whenever data was available.

However, there was age variability among participants at each visit. The age range was 8 – 14 years, 10 – 18 years, and 12 – 21 years for study visit 1, 2, and 3, respectively. We encourage future studies to pay additional attention for the youth's age by recruiting subjects at the same age to the extent this is feasible, and standardizing time between follow-up visits for all subjects to reduce influence of age on cardiometabolic health. Examples of these influences are the change during pubertal transition on lifestyle behaviors (i.e., smoking, alcohol composition, sedentary and activity level, and diet quality), and biological systems (i.e., body composition, and hormonal milieu). Besides, future studies should aim to conduct powered sex-stratified analysis as it might reveal critical information about sexual dimorphism in the early development of cardiometabolic diseases. Further studies with longer follow-up duration are worth conducting to examine the long-term association between the lifestyle determinants of cardiometabolic risk factors; the associations may be pronounced in middle adulthood to reflect the chronic and cumulative exposures.

The scope for future research regarding chapter 2 is to validate the use of diet quality scores among children and adolescents and examine the extent to which they represent the overall diet. We endorse the importance of complementing diet assessment with culture of eating measures – such as watching media while eating, and unhealthy snacks between meals, and others [109] – and the social-cultural context of the food consumption. Furthermore, because Mexico has the highest annual retail sales per capita of ultra-processed food and drink products across Latin America [110,111], ranked fourth worldwide [110], we encourage futures studies to use a diet quality score that captures the consumption of processed foods. Lastly, to apprise the detected unexpected positive association between higher diet quality (i.e., DASH score) and waist circumference among girls, we recommend longitudinal studies supplement the waist

circumference measurement with a valid tool to quantify the abdominal fat such as Dual-energy X-ray absorptiometry (DEXA). By doing so, the longitudinal changes in abdominal fat mass will not be confounded with the parallel growth and maturation in the abdominal compartment – muscle mass and bone mass.

Future directions for chapter 3 are validating the physical activity questionnaires to capture the duration and context of various sedentary behaviors among youth. Similarly, additional studies are needed to validate the use of accelerometers in assessing sedentary time and enhance its feature to capture body posture. In light of the claim questioning the representation of four assessment days of physical activity [97], we recommend evaluating the required number of days to get representative habitual physical activity patterns. Additional work has to be done to reach consensus about the proper epoch length for summarizing accelerometer data, cut-off points to define activity intensity, define bouts, and non-wear time to enhance the comparability of findings across studies, and reduce measurement errors [25,32,41,46,48].

For chapter 4, we recommend future studies to validate the use of blood DNAm as a proxy for DNAm in the target tissue for cardiometabolic health, adipose tissue and skeletal mulches, among children and adolescents. The blood DNAm validation is a necessary step toward developing potential epigenetic biomarkers for cardiometabolic risk using blood samples [104]. Moreover, due to the wide recognition for cytosine hydroxymethylation (5hmC) as a distinct epigenetic modification than cytosine methylation (5mC) or DNAm, future studies should apply laboratory techniques that allow for distinguishing between 5hmC and 5mC in order to get pure assessment of DNAm. Besides, we recommend future studies to employ epigenome-wide approaches to identify all-important genes for cardiometabolic outcomes in youth. DNAm is not the only approach to modulate gene expression; in fact, gene expression could be influenced by

multiple factors – including other epigenetic modifications, physiological conditions, and others. Thus, we recommend future studies to supplement the assessment of DNAm with gene expression instead of gauging the gene expression using only DNAm data.

Conclusions:

This dissertation project showed evidence supporting the associations between higher diet quality, lower sedentary time, and replacing sedentary time with higher activity intensities and better cardiometabolic profile among healthy youth. In addition, we detected few associations between DNAm on the selected 4 genomic regions and cardiometabolic profiles. Despite the inclusion of healthy Mexican youth, we have identified associations with small effect sizes supporting the role of lifestyle modifications on metabolic health among the high-risk population for metabolic disorders. Therefore, advocating for a healthy lifestyle at an early age could result in a protective impact on cardiometabolic health, which might mitigate the dire CVD consequences that would manifest in adulthood.

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