Early Puberty: Adulthood Metabolic Consequences and Childhood Nutritional Determinants

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE UNIVERSITY OF MINNESOTA

BY

Noel Theodore Mueller

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Dr. Mark A. Pereira, Advisor

July 2013

Acknowledgements

The process of this research was supported by a T32 training grant in Cardiovascular Disease Epidemiology and Prevention from the National Heart Lung and Blood Institute administered by Aaron Folsom.

I want to express my deepest gratitude to my advisor, mentor, and comrade Mark Pereira, who, after widening my eyes to epidemiology, procured a healthy dose of scientific inspiration and skepticism throughout the years leading up to my epidemiologic coming-of-age. From the confines of Mark's office to the far-reaching jungles of Colombia, our mentoring relationship dynamically evolved, allowing for incredible academic and personal growth. Yet, most germane to this growth was Mark's steadfast advocacy for my success. For this I will remain eternally grateful. I want to thank my other dissertation committee members, David Jacobs, Ellen Demerath, Richard MacLehose, and Douglas Mashek for their wisdoms and helpful commentaries on my dissertation. I also want to thank Bruce Duncan and Maria Ines Schmidt, who were central to my involvement in The Brazilian Longitudinal Study of Adult Health, for the tremendous opportunity to live in Brazil working on this study, and for their invaluable insights on this research. Finally, I want to thank my family and friends for all the support they have provided throughout graduate school and this dissertation process.

ABSTRACT

Background: Accumulating evidence suggests puberty is occurring earlier today than ever before. Pubertal timing may be a harbinger for abnormal metabolic health. Thus, identifying its upstream determinants and downstream metabolic health consequences may provide an avenue to primordial prevention. The objective of this dissertation was to investigate pubertal timing in relation to type 2 diabetes (T2D), adipose depots, non-alcoholic fatty liver disease (NAFLD), and early-life nutrition.

Methods: This dissertation includes three separate but related research manuscripts. The first aimed to investigate pubertal timing and T2D in a Brazilian adult population (35-74y) who were born and came of age before the rise of overweight and obesity in Brazil. The second examined pubertal timing in relation NAFLD and fat depots in a biracial cohort of young adults (18-30 years at entry, 42-56 years at measurement of NAFLD) from the Coronary Artery Risk Development In Young Adults (CARDIA) study. Finally, the third paper determined the role of diet quality and risk of early pubertal timing in a biracial sample of girls (9-10 years) from the National Heart, Lung, and Blood Institute Growth and Health Study (NGHS). Multivariable linear regression was used for continuous outcomes (glucose homeostasis measures, adipose depots, liver attenuation, and age at menarche) and Poisson regression to generate prevalence ratios (PR) and relative risks (RR) for dichotomous outcomes (T2D, NAFLD, and early menarche). In the last project, a diet quality score, derived by summing standard deviation scores for the

densities of nutrients/chemicals indicative of prudent or Western dietary patterns, was analyzed in relation to incident early menarche.

Results: *In the first paper*: early menarche [<11 years vs. 13-14 years (reference)] was associated with 22% (95% confidence interval: 3-42%) higher prevalence of T2D in Brazilian adults, adjusted for confounders and BMI at age 20 years. In the same model, a 1-standard deviation higher relative leg length (RLL), a marker of early life growth and maturation, was associated with a 10% (5-15%) and 11% (7-15%) lower prevalence of T2D in women and men, respectively. The association between RLL and T2D was stronger among females with earlier menarche (*p* for interaction on multiplicative scale = 0.02), and among adults who were overweight or obese at age 20 years (*p* for interaction on multiplicative scale = 0.02) or 35-74 years (*p* for interaction on multiplicative scale = 0.03). *In the second paper*: in CARDIA, a 1-year increment in age at menarche was associated with 10% (2-18%) lower prevalence of NAFLD, and inversely associated with visceral, subcutaneous, and intra-abdominal muscle fat, after adjusting for confounders and BMI measured at 18-30 years. RLL was also associated with these measures, but was attenuated after control for BMI. Lastly, *in the third paper*: a better diet quality was associated with lower risk of early menarche (<11 years) after adjustment for confounders and % body fat from skinfolds (1-SD increment in diet quality score, RR=0.77; 95% CI: 0.66-0.90).

Conclusions: These results indicate that early menarche is associated with higher prevalence of diabetes in an adult Brazilian population that underwent nutritional

transition after coming of age, and non-alcoholic fatty liver disease in a biracial U.S. population, after adjusting for potential early-life confounders and early adulthood BMI. Relative leg length, while only loosely associate with age at menarche, was inversely associated with T2D but not NAFLD. Age at menarche and relative leg length did not appear to be preferentially related to specific adipose depots. These markers likely represent unique aspects of early-life growth and maturation, and depend on the context of the population under study. The results of the final research paper suggest that risk for early menarche may be reduced through dietary modification, independent of fatness. In light of the secular trend toward earlier age at puberty in the U.S. and abroad, it is important that future research continues in this arena under the realm of primordial prevention. Continued research is needed to determine the extent to which early-life modifiable lifestyle factors, such as dietary patterns and physical activity, can influence long-term health and disease through effects on pubertal timing.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

This proposal is organized into nine chapters. *Chapter 1* presents an overview of the gaps in the literature this dissertation will address. The second chapter provides an overview of puberty, its physiology, secular trends, determinants, and it summarizes the role of diet in the etiology of pubertal timing. The third chapter focuses on type 2 diabetes, its natural history, pathogenesis, and epidemiology, with a focus on Brazil. Risk factors are described with particular attention on the role of early-life markers of growth and development. *Chapter 4* focuses on non-alcoholic fatty liver disease, its natural history, pathogenesis, epidemiology, and risk factors, highlighting the role of markers of pubertal timing from etiologic perspectives. The fifth chapter describes the populations, design, and data collection for the three cohort studies included in this dissertation. *Chapter 6* provides background, methods, results, and discussion for manuscript 1 of the dissertation: the investigation of age at menarche and relative leg length, as markers of pubertal timing and early-life growth, respectively, in relation to type 2 diabetes in a cohort of Brazilian adults. *Chapter 7* provides background, methods, results, and discussion for manuscript 2: the investigation of age at menarche and relative leg length in relation to non-alcoholic fatty liver disease and fat depots in a biracial cohort of young adults from the U.S. *Chapter 8* provides background, methods, results, and discussion for manuscript 3: the prospective investigation of dietary quality in relation to early menarche in a biracial U.S. cohort of girls. The final chapter summarizes implications of

the findings in this dissertation, highlighting how these results should inform future research directions in this arena and be leveraged to guide primordial prevention in the U.S. and abroad.

CHAPTER 1: INTRODUCTION

Puberty represents a developmental window of rapid and profound change. The timing of puberty has physiologic variation between 4 and 5 years in human populations, and understanding the implications of this timing has been identified as a public health priority.[1] Beyond its importance to public health, the editors of *Science* recently labeled "What triggers puberty?" as one of the 100 most compelling questions facing science in the next century.[2] This topic has drawn scientific intrigue due to the hitherto unexplained etiology contributing to the recent downward secular trend in age at pubertal development, and the long-term health trajectories associated with early pubertal timing.

It is now widely accepted that girls reach pubertal milestones (e.g., breast budding and age at menarche) earlier today than ever before.[3] A recent study suggested boys are also reaching puberty earlier,[4] but the factors driving the trend in boys may be different than those for girls. In the Neolithic times, paleontological evidence suggests females reached puberty at an early age, and this was primarily driven by the evolutionary advantage of reaching reproductive capacity rapidly in a shortened lifespan.[5] However, as humans neared the Industrial Revolution, and hygiene deteriorated with increasing population density, it is believed that menarcheal age, the most distinguishable pubertal landmark in females, became delayed.[5] Yet, from the Industrial Revolution to the mid-1900s, the best available evidence suggests that menarcheal timing again embarked on a secular trend toward earlier puberty, likely due to improvements in environmental living conditions, reductions in infectious diseases, and better access to food.[5] Since the 1950s,

the downward secular trend in age at menarche appears to have continued, but less markedly and with distinct underlying drivers than previous shifts in pubertal timing.[3]

In 1970, Frisch et al. hypothesized that a critical body fat threshold had to be achieved before menarche could occur in girls.[6] Since then, several investigators have posited that recent shifts in pubertal timing are largely due to increases in childhood overweight and obesity.[7-9] Yet, mechanistic pathways underlying Frisch's 'critical weight' hypothesis are debated,[10] and, in many countries, including most European countries,[11-18] recent trends in pubertal age do not align with trends in childhood obesity. Therefore, other environmental or lifestyle factors, e.g., diet and physical activity, may play a role in timing of maturation independent of adiposity. The role of childhood nutrition in pubertal timing will be discussed in *Chapter 2*, and novel insights on diet quality will be examined in *Chapter 8* of this dissertation.

In many countries, secular trends in pubertal timing over the last several decades closely align with epidemics of type 2 diabetes and non-alcoholic fatty liver disease (NAFLD). These metabolic diseases share etiopathologic pathways, and their prevention holds potential for reducing nutrition-related morbidity and mortality. Several studies have investigated the role of pubertal timing, as measured by age at menarche in girls, in the development of type 2 diabetes in Western populations,[19-23] and prosperous Asian populations.[24,25] These studies offer conflicting evidence as to whether fatness explains the age at menarche–diabetes association, or whether alternative physiologic mechanisms are implicated. They also highlight the concept that the role of body fat in the association between timing of puberty and diabetes may vary based on the population being studied. More studies are needed from less prosperous populations in which the potential

confounding structure (i.e., underlying causes of pubertal timing and diabetes) for the association between pubertal timing and development of diabetes differs.

No studies to date have examined the association between markers of pubertal timing and NAFLD. Fraser et al. found that relative leg length, a marker for early-life growth and development, [26-30] was inversely associated with adult liver enzymes, implying that early life nutrition may play an important role in the development of liver disease.[31] These authors concluded with a call for further research on factors related to leg length that may influence adult liver function. According to the cephalocaudal gradient of growth, and empirical support,[26,27] earlier onset of puberty is associated with shorter legs relative to total stature. Estrogen, which peaks at puberty, is the dominant factor controlling cessation of long bone, i.e., leg, growth[28] through its effects on growth plate epiphysis.[29] Early puberty is associated with less linear growth before puberty, when the legs experience rapid gains, and more linear growth during and shortly after puberty, when torso elongation predominates; hence, short legs relative to stature.[30]

Recently, the Framingham Health Study investigated age at menarche and other reproductive factors in relation to adipose deposition. The main finding of their study was that age at menarche was associated with subcutaneous and visceral fat depots, but that these associations were no longer significant after adjusting for concurrently measured BMI.[32] The authors concluded that age at menarche is related to overall body fat and not preferential to one fat depot over another. Accumulation of fat in the liver, which can lead to NAFLD and more advanced states of hepatic fibrosis, has a different etiology and risk profile than visceral fat and deposition of fat in other regions.[33-35] Thus, while the findings from the Framingham study are intriguing, their conclusion that menarche age is

associated with overall, but not depot-specific, fat does not extend to hepatic fat accumulation. As fatty liver disease is an underlying risk factor for many cardiometabolic diseases (discussed in *Chapter 4*), many of which have been associated with early puberty, it is of biologic and public health interest to investigate whether markers of early pubertal timing (e.g., early age at menarche and, perhaps, shorter leg length relative to stature) are positively associated with NAFLD.

Many of the upstream nutritional determinants and downstream metabolic consequences of altered pubertal timing are poorly understood. Their discovery may facilitate primordial prevention of population health risks. Beyond its conjectural association with type 2 diabetes and NAFLD, early puberty is of public health poignancy for myriad reasons. Children who advance through puberty early have been shown to be at higher risk of adult obesity,[36] metabolic syndrome,[37] cardiovascular disease,[25,38-41] several cancers,[42,43] and incidence of adolescent risk behaviors (e.g., substance abuse, cigarette smoking, multiple sexual partners, conduct disorder).[44-46] In light of the purported downstream health consequences of pubertal timing, the triggers of puberty are a topical matter of scientific inquiry. Genetic factors[47] and some modifiable exposures, such as pre- and postnatal nutrition,[47] childhood diet,[48] childhood adiposity,[6] endocrine disruptors,[49] and stress,[50] may play roles. As these psychosocial, behavioral, and metabolic processes manifest around puberty, addressing them at this critical period may present opportunities for targeted public health interventions to prevent disease risk factors (i.e., primordial prevention).

THEORETICAL FRAMEWORK

Despite the well-accepted importance of early-life environment in the etiology of adult disease, relatively little is known about how pubertal timing influences long-term metabolic disease risk. The goal of this dissertation is to develop, explore, and address important gaps in the literature on modifiable nutritional determinants and metabolic consequences of early pubertal timing. Because there appears to be a sexual dimorphism in the etiology of puberty, and the reproductive strategy of the two sexes is quite distinct, the preponderance of this dissertation will focus on the female, in whom early puberty may have greater consequence on long-term health.

One literature gap in this field is how early pubertal development relates to type 2 diabetes in a Latin American population undergoing rapid nutritional transition. Another, yet unexplored, area of research related to this topic is the relation of early puberty and non-alcoholic fatty liver disease. The final literature gap to be investigated in this dissertation is the influence of diet quality on early pubertal timing. Before discussing how this dissertation will address these gaps, however, it is important to review several issues related to puberty (*Chapter 2*), type 2 diabetes (*Chapter 3*), and non-alcoholic fatty liver (*Chapter 4*) disease that provide contextual background for this work. **Figure 1.1** depicts a general theoretical framework through which early life environment might influence pubertal development, and early puberty may influence body composition, hepatic fat, and type 2 diabetes.

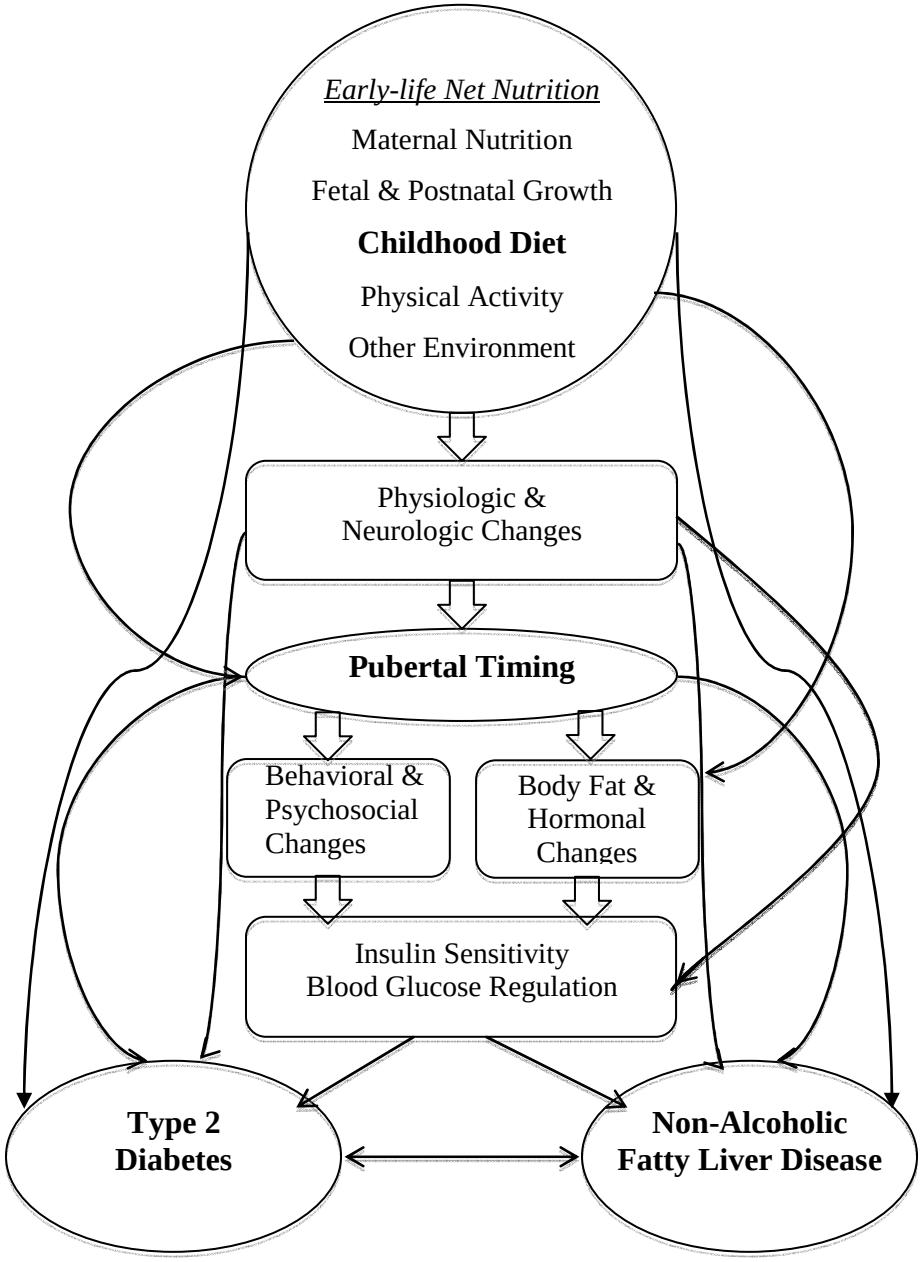

Figure 1.1: Theoretical framework on early life nutrition, pubertal timing, and development of non-alcoholic fatty liver disease and type 2 diabetes

CHAPTER 2: PUBERTY

OVERVIEW

Puberty is the developmental process by which reproductive capability is attained. This lifecourse milestone results from a complex series of molecular and physiological events, expressed in physical, behavioral, and hormonal changes. In girls, puberty begins with pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the GnRH neurons of the hypothalamus.[10] GnRH release stimulates primary gonadotrophs to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Once released, LH and FSH induce steroidogenesis and gametogenesis in the gonads, resulting in the dynamic physical changes characteristic of puberty. Nebulous still is the understanding of the factors underlying these pathways responsible for the variation in pubertal timing that is characteristic of human populations. Elucidating this etiology, particularly modifiable factors, such as nutrition, has biologic and public-health allure.

This chapter will briefly discuss *1*) physiology of the reproductive axis and the associated signals and pathways that affect puberty, *2*) recent trends in pubertal timing, and *3*) factors, with a focus on diet, that may influence physiologic variation in timing of puberty.

PHYSIOLOGY OF PUBERTAL TIMING

Physiologic changes involved in puberty are highly active during the pre- and perinatal (i.e., fetal), postnatal (i.e., infancy), and childhood periods of life (**Figure 2-1**).

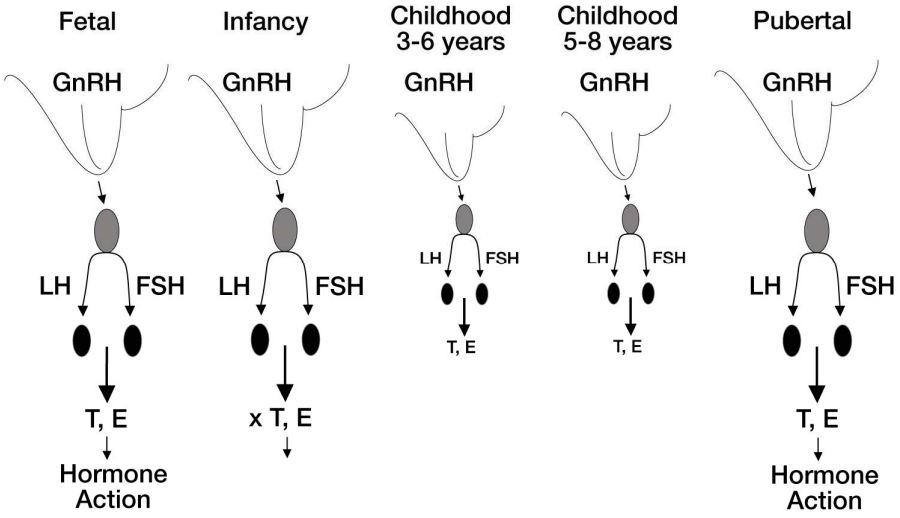

Figure 2-1: Hypothalamic-pituitary axis development until puberty *(modified from DiVall et al.*[10]*)*

Abbreviations—GnRH: Gonadotropin Releasing Hormone; LH: Luteinizing hormone; FSH: Follicle Stimulating Hormone; T: Testosterone; E: Estrogen

The first traces of GnRH neurons are found in the olfactory pit of the fetus around week six in gestation.[51] By week nine the neurons have already traveled *via* the forebrain to the hypothalamus. Here, in week 12, the pituitary begins to secrete LH and FSH into the fetal circulation.[52] Hypothalamic-pituitary axis activity increases rapidly thereafter, and in weeks 20-24 LH and FSH reach levels capable of contributing to ovarian development.[53] By late gestation, placental estrogen provides a negative feedback to the axis, causing LH and FSH levels to decrease.[54] As a result of this negative feedback, LH and FSH levels are low at birth. In the first months of life, as placental estrogens are removed, LH and FSH again begin to rise, peaking around one year of age. Yet, by two years of age LH and FSH again fall to nearly undetectable levels.

From around two years of age through mid-childhood the activity of the hypothalamic-pituitary axis remains relatively dormant. However, at this time in development the axis ramps up hormonal activity, as evidenced by outward physical changes. These patterns of hormonal and physical change follow a sexual dimorphism most easily discussed with reference to Tanner staging. For females, this is based on breast maturation and the appearance of pubic hair (**Figure 2-2**).[55] Tanner stage 1 represents pre-puberty. Rapid linear growth, i.e., age at take off, and development of breast tissue, usually appearing in Tanner stage 2, are the earliest outward signs of gonadarche in girls.[55] Menarche is achieved near the end of Tanner stage 4, after nearly a yearlong rise in estradiol output.[56] It is at this point in time that estradiol exhibits a negative feedback on the hypothalamic-pituitary axis to suppress its release, thereby leading to cyclic rise in estrogen levels and menstruation.[57]

In parallel to the rise in estradiol production by the gonad during puberty, the adrenal glands begin to secrete androgens. Adrenarche, the process by which the hypothalamic-pituitary-adrenal axis matures, gives rise to pubic hair growth, acne, body odor, and increases in growth velocity.[1] Pubic hair usually appears during Tanner stage 2. Although androgen production by the adrenal gland and estradiol production by the ovary coincide, they are independently regulated processes.

PUBERTAL ASSESSMENT

Pubertal timing can be assessed using a variety of contemporaneous (during a clinic visit) and retrospective (by the participant) modalities. Tanner staging, which uses a scale of 1-5 for breast development and pubic hair in girls (**Figure 2.2**) and testicular volume and

pubic hair for boys, is the most widely employed method for clinical assessment of puberty.[55] Tanner stage 2 indicates first pubertal development. In girls, pubertal timing can also be ascertained by age at menarche. This method is attractive for researchers because it is more objective than clinical evaluation of breast and pubic hair development, and self-recall of age at menarche is reliable on repeat questioning (intraclass correlation = 0.92).[58] In boys, puberty can be assessed contemporaneously in the clinic *via* orchidometry, the gold standard for measuring testicular volume, and retrospectively by recall of age at voice break and age at first nocturnal emission. However, these latter strategies may have less reliability.[4] Alternatively, for boys and girls, markers of growth and development can be used for assessment of pubertal timing. These include, age at take off, i.e., age at the onset of the adolescent growth spurt, age at peak height velocity, i.e., the timing of the maximum adolescent growth, and relative leg length, i.e., the ratio of leg length-to-sitting height or total stature. The heterogeneity in methods by which pubertal timing is assessed may be partially responsible for the discrepancies in reported trends and etiologic associations to be discussed later.

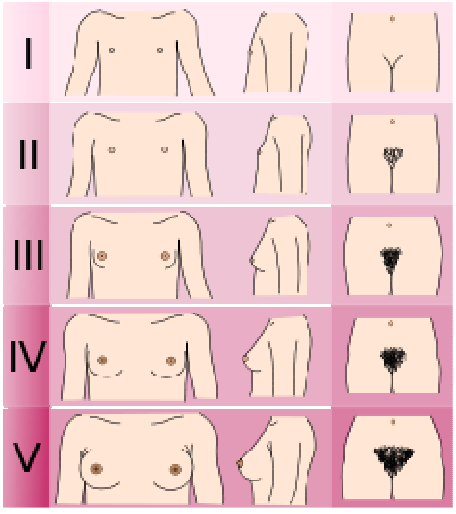

Figure 2-2: Female Tanner stages

SECULAR TREND IN PUBERTAL MARKERS

The onset of puberty in humans occurs over a wide age range. In the 1980s, James Tanner, through a series of longitudinal studies, found that the mean age at menarche, the most distinguishable pubertal event in girls, in the U.S. was 12.7 with a 1.3 year standard deviation; the mean age of breast budding (i.e., thelarche) was 10.7±1.0 years.[59] The age at onset of these and other pubertal landmarks continues to change over time.

The best available data suggest that there has been a downward secular trend in average age at menarche from the late 1800s to mid-1900s in the U.S.[3] Since the mid-1900s there has been disagreement among researchers in this field,[60-63] but an expert panel convened in 2003 concluded that the average age at onset of menarche and breast development, but not other pubertal markers, for girls in the U.S. decreased throughout the second half of the 20th century (**Figure 2.4**).[3] A key finding influencing this decision was from a study conducted by the American Academy for Pediatrics, which reported that girls were developing a year earlier than previously reported.[60] Other data from the U.S. indicate that the median age at menarche decreased modestly but significantly from 12.8 to 12.5 years in girls between the 1960s and early 1990s.[64,65] Age at onset of thelarche seems to have also declined, perhaps more drastically than menarche during the last two decades. Before the 1980s, the mean age at thelarche was ~11 years of age in the U.S.[3] Yet findings from NHANES III, conducted between 1988 and 1994, indicate a mean age at thelarche below 10 years.[66]

The trends toward earlier age at onset of thelarche and menarche in the US are driven by an entire shift in the age-at-pubertal-onset distribution, with African American girls having experienced greater decreases than Caucasians.[66,67] These findings have led

to revised guidelines, which propose that black and white girls showing breast development or pubic hair before age 6 and 7 years, respectively, should be evaluated for precocious puberty.[68] Secular trends in age at puberty in girls have been paralleled by trends in the incidence and prevalence of precocious puberty,[69] although this may be an artifact of higher referral rates rather than a true increase.

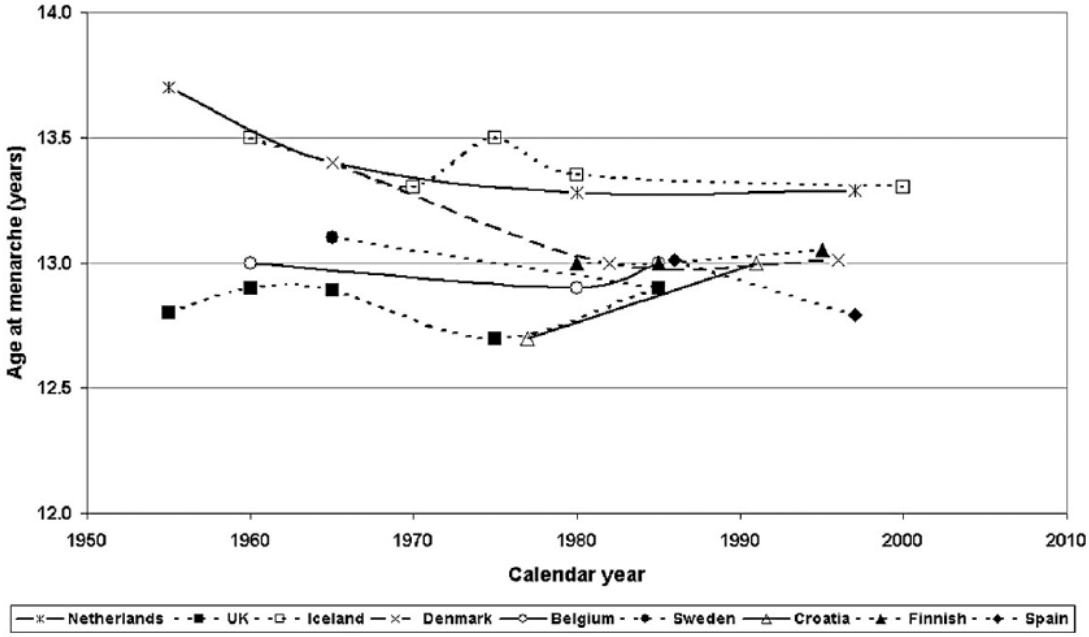

Figure 2-3: Secular changes in age at menarche in countries without consistent decline.

***Figure reproduced from Cheng et al.*[48]**

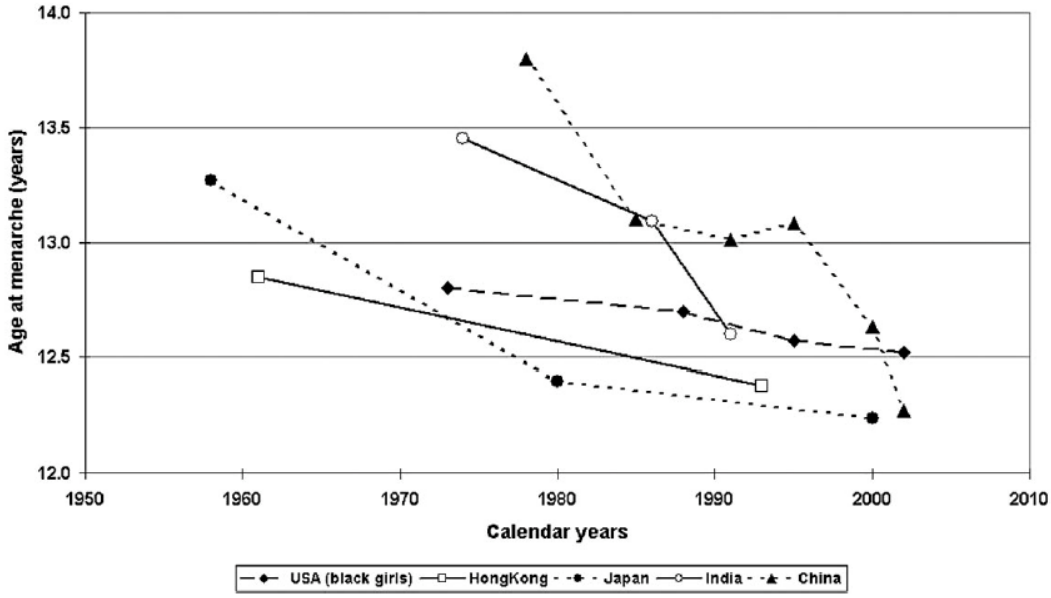

Figure 2-3: Secular changes in age at menarche in countries with consistent decline.

Figure reproduced from Cheng et al.[48]

In boys it has been harder to document secular changes due to the absence of an easy determined marker, such as menarche. Visual pubertal (Tanner) staging is also more difficult in boys than girls.[55] Orchidometry, i.e., measuring of testicular volume, is an intrusive procedure that is not a routine part of clinical exams. Nevertheless, a recent study in *Pediatrics*[4] reported that boys are reaching puberty, as assessed by testicular volume and achievement of Tanner stages 2-5, over a year earlier today than boys 40 years ago.[70] This study needs to be replicated before inference on trends in pubertal age in boys can be made.

Research on factors driving the trend toward earlier puberty is inconclusive. Many scientists in the field believe that earlier onset of puberty is a byproduct of the global rise in childhood obesity, citing studies that found pubertal development related to body fat accumulation.[6,71-74] But there are other possibilities, which are reviewed later in this

chapter, including direct effects of diet and physical activity, psychological and social stressors that might alter reproductive hormones, and the presence of environmental chemicals that can mimic the biological properties of estrogen. Many of these factors may be co-varying with trends in fatness over time. Therefore, the secular trend toward earlier age at menarche may have a multi-etiologic basis. Here, I review potential genetic and environmental factors and mechanisms underlying pubertal timing.

GENETIC AND PHYSIOLOGIC FACTORS IN PUBERTAL TIMING

Pubertal timing is believed to be largely heritable. Genome-wide association studies have identified single-nucleotide polymorphisms at 42 loci that influence age at menarche in European ancestry.[75] However, although the genetic composition of populations changes over time, due to migration and genetic drift, the observed secular trend in pubertal timing cannot be due to such small changes in genetic frequency. Rather, changes in pubertal timing are likely driven by non-genetic factors, or, in the broadest sense, the environment. In this light, the remaining review of factors related to pubertal timing focuses on *1*) physiologic processes that can be modified by the environment, and *2*) exposures that may be varying over time, including physical activity, diet, psychosocial stress, and environmental adulterants (e.g., endocrine disrupting agents).

Physiologic Processes

A full discussion of the molecular factors involved in induction of puberty in females is beyond the scope of this dissertation, but the major players are reviewed by DiVall and Radovick.[10] Here, the foci of discussion are physiologic factors that may comprise the common etiologic soil between altered pubertal timing and adult metabolic disease risk.

Growth Factors

The insulin-like growth factor 1 (IGF-1) axis has been implicated by several lines of evidence as a primary player in the physiology of pubertal timing. Reduced activity in this axis, due to rare human disorders, has been associated with delayed puberty.[76] IGF-1 gene polymorphisms have been linked to earlier menarche,[77] and a recent epidemiologic study in children found IGF-1 levels were an independent predictor of early menarche.[78] In experimental rodent models, intraventricular infusion of IGF-1 induced puberty,[79] while IGF-1 antibodies delayed puberty.[80] Adding to the picture, *in vitro* models have demonstrated that IGF-1 increases expression of GnRH.[81] These data suggest that the integration of growth, nutrition, and puberty may occur at the level of the GnRH neuron. Alternatively, as a function of its endocrine and autocrine actions, IGF-1 may stimulate the reproductive axis through effects on the hypothalamus, pituitary, and/or ovaries.[82]

Insulin

Hyperinsulinemia, resulting from insulin resistance, has also been proposed to play a role in the regulation of pubertal timing.[73] Starting in early puberty there is a transient increase in insulin concentration. Around mid-pubertal transition, insulin peaks then typically declines by late puberty in the absence of excess adiposity.[83-86] Insulin resistance may suppress IGF binding protein-1 and sex hormone binding globulin, leading to greater bioavailability of IGF-1 and sex steroids, respectively.[87,88] In addition to observational evidence, a randomized controlled trial found that metformin reduced insulin concentrations in addition to preventing early pubertal onset and menarche.[89,90]

Body Fat and Related Hormones

A large body of literature supports the conjecture that nutritional status at critical periods of growth and development plays an important role in the timing of puberty. Children with chronic malnutrition and infection have delayed pubertal onset,[91] and prolonged fasting in females has been linked to hypogonadism.[92] Moreover, a study in sheep found that GnRH pulse frequency was significantly reduced during food deprivation.[93] These findings suggest caloric restriction during critical periods of growth in childhood may inhibit pubertal onset *via* action in the hypothalamus.

In developed societies, overnutrition likely plays a larger role than undernutrition in determining the timing of puberty. In 1970, Frisch posited the 'critical weight' hypothesis, whereby a threshold of fatness must be attained before menarche can occur.[6] Others have called adiposity the 'gatekeeper of puberty'.[74] This notion makes evolutionary sense. For females, if energy and fat stores are limited, the body is not equipped to support a healthy fetus. Once a girl's body has enough fat for the energy demands of reproduction, hormonal signals clear the way for sexual reproduction. While salient in theory, the mechanistic pathways underlying this hypothesis have yet to be fully elucidated.

How body fat triggers pubertal development is a matter of ongoing investigation. Since the discovery of leptin, and its role as a signal of energy regulation to the hypothalamus,[94] several studies have investigated, with mixed results, the effect of leptin on GnRH neuronal activity and puberty. Leptin levels increase gradually through childhood and the pubertal progression,[95,96] and leptin has been shown to exert direct effects on gonadotropin secretion.[97] But, patients with lipoatrophic diabetes who

experience leptin deficiency experience normal timing of puberty.[98] In rodents, some studies found that leptin induced puberty,[99,100] whereas others have not found such an effect.[101,102] In primates, leptin concentrations were shown to have a null effect on pubertal onset.[103] Taken collectively, these findings suggest that leptin may not have a primary role in pubertal timing.

The epidemiologic literature, spanning ecologic, cross-sectional, and longitudinal data are mostly consistent with the notion that body fat plays a role in the nutritional regulation of puberty. For children 6-11 and 12-19 years of age living in the U.S. there has been a 3- to 6-fold increase in the prevalence of obesity since the 1970s.[104] Today, about 17% of U.S. children and adolescents are obese.[104] The timing of the childhood obesity epidemic roughly corresponds with recently observed drops in age of pubertal onset in the U.S. and other countries (**Figure 2.4**),[63] but not in most European countries (**Figure 2.3**).[11-18,48] The simultaneous trends for fatness and pubertal timing in some societies but not others implies that adiposity may have a permissive role, while other time-varying exposures such as diet, physical activity, stress, or environmental toxins (e.g., endocrine disruptors) that differ by geographic region have a primary role in determining the timing of puberty. And while several studies show strong associations,[71-73] and even a common genetic underpinning,[105] between childhood fatness and pubertal onset, others indicate that adiposity does not completely explain pubertal timing.[72,106] An important study on this issue showed associations between age at menarche and adult BMI remained significant after adjustment for BMI at age 5 years,[107] suggesting that menarcheal timing is related to adult body fat independent of prepubertal fatness.

Prenatal and Postnatal Growth

Early life factors might also influence pubertal timing. The predictive-adaptive response paradigm[108] posits that reduced fetal nutrition may result in earlier pubertal onset when constraints on postnatal growth, in an obesogenic environment, are minimal. Investigations into the association between birth size, as a measure of prenatal growth, and puberty have offered divergent results. Studies from prosperous[109-119] and less prosperous[120-122] populations have reported an association between lower birth weight and earlier menarche. Yet, some of these associations became null[113] or reversed [116] after controlling for postnatal growth. Other studies in more[123-125] and less economically developed[126-129] populations have reported no association between birth weight and pubertal timing, suggesting that the prenatal nutrition-pubertal timing association could be contextually specific rather than biologically based.

Rapid early weight gain has also been linked to earlier onset of puberty.[123,129-133] These associations may be mediated by insulin concentrations, sex steroid concentrations, IGF-1, adiposity, and leptin, as these factors have been associated with rapid growth and implicated in promoting GnRH activity.[130] To the best of our knowledge, no studies to date have examined whether the predictive-adaptive response paradigm extends to restricted growth during the first years of life coupled with an unrestricted obesogenic environment later in life. Epidemiologic investigation in a large cohort studies, that has markers of restricted early life growth and nutritional status later in life, would be a valuable first step in the exploration of this research question.

LIFESTYLE AND ENVIRONMENTAL FACTORS IN PUBERTAL TIMING

Diet

Beyond early life growth and body weight, dietary quality and quantity in the years preceding onset of puberty have been implicated in the timing of pubertal development. Germane to the aims of this dissertation, a number of prospective observational studies have investigated the association of mid-childhood diet and pubertal timing. These are summarized below, with focus on studies in females.

Energy intake

Based on the premise that excess body fat arises from long-term energy imbalance, several epidemiologic studies have tested the hypothesis that higher energy intake is associated with earlier pubertal timing. A study of Canadian girls aged 9-12 years girls[134] found that higher energy intake was associated with earlier age at menarche. However, a large number of other studies have reported conflicting findings,[135-140] suggesting energy intake is not related to timing of menarche or breast development. Methodological issues associated with self-reported diet assessment, such as social-desirability bias, are most pronounced for capturing total energy intake. Thus, one's ability to make causal inference regarding this literature is limited. Future research in this area should focus on objective measures of energy intake.

Dietary fiber

Two prospective cohort studies have reported an association between higher consumption of dietary fiber and later onset of menarche. In one study, Canadian girls whose fiber intake at 6-14 years of age was in the highest quartile (>25.5 g/day) had 46% reduced risk

of early menarche compared to those in the lowest quartile (≤18.2 g/day).[136] In a small study of Dutch girls, those with higher intake of grains at 10 years of age had later age at menarche and breast development.[141] In this same study, intake of grains was inversely associated with plasma concentrations of gonadotropins and estradiol. Fiber may influence pubertal timing through an insulin-mediated pathway or directly by reducing available estrogen.[141-143] Yet, Moisan et al. did not find an association between fiber intake (at 10-13 years of age) and age at menarche in Canadian girls.[134] Similarly, Cheng et al. did not observe a fiber-pubertal timing association in the DONALD study.[144] These findings may have been null due to measurement error or residual confounding of other whole-grain elements. More studies looking at the effects fiber, and fiber-rich dietary patterns, in relation to pubertal timing are warranted.

Dietary isoflavones

Only one study to date has explored the influence of isoflavones on the timing of puberty. Investigators in the DONALD study reported that, after adjustment for prepubertal BMI and fiber intake, girls whose dietary isoflavone intake was in the highest tertile experienced peak height velocity approximately seven months later and Tanner stage 2 for breast development approximately eight months later than girls whose intake was in the lowest tertile.[144] Dietary isoflavones were not associated with pubertal timing markers in boys. The lack of other observational research published on this topic suggests that negative publication bias may be an issue.

Nevertheless, there is biologic rationale behind the isoflavone-pubertal development association. Beyond having structural similarity to estradiol, which may result in biologic interaction with estrogen receptors,[145] isoflavones have been shown to

inhibit the activity of aromatase, the rate-limiting step in the conversion of androstenedione to testosterone and estrone to estradiol.[146] Soy phytoestrogens may also inhibit 17β-hydroxysteroid dehydrogenase, which catalyzes the conversion of 17β-keto steroids to active 17β-hydroxyl sex steroids.[147] Nevertheless, despite biologic plausibility and provocative findings from one longitudinal study, more observational and experimental research is needed before a causal association can be speculated.

Dietary fat

Several observational studies have investigated the association between consumption of dietary fat in relation to age at menarche. Many studies on this topic found no evidence for an association between overall dietary fat and pubertal markers in girls.[139,141,148,149] Other studies reported that polyunsaturated fat intake, during various prepubertal periods, was associated with earlier onset of puberty in girls.[135,137,138,140] Conversely, higher intake of saturated fat has been linked to later onset of menarche in some studies.[136,137] The incongruent findings on this topic, and lack of clear mechanistic rationale, imply that reported associations may be due to residual confounding, or publication bias influenced by the surge in nutritional epidemiologic investigation on dietary fats that took place in the 1990s. Thus, based on the best available data, dietary fat does not appear to be directly related to the timing of puberty.

Dietary protein

The observation that animal protein stimulates IGF-1 secretion[150] led to the hypothesis that high animal protein consumption during childhood is related to earlier pubertal onset. In the DONALD study, Gunther et al. found that boys and girls in the highest animal-protein-intake tertile had age at menarche, voice break, take off, and peak height velocity

approximately seven months earlier than those in the lowest tertile.[151] Berkey et al. observed that, in three to five year old girls, eight g/day (one standard deviation) higher animal protein intake was associated with seven month earlier onset of age at menarche.[135] In British girls, a 1 standard deviation increment in animal protein (1.1 g/day) was associated with a 17% higher risk earlier menarche occurrence.[140] Remer et al. also observed that higher animal protein intake was associated with earlier age at take off, peak height velocity, menarche, and voice break.[152]

A few studies have investigated the association between animal protein food groups and pubertal timing in females. Gunther et al. found that children five to six years of age in the highest tertile of cow milk and dairy intake experienced age at take off approximately five months earlier than those in the lowest tertile.[151] Mechanistically topical to this finding, intake of animal protein from dairy sources has been implicated in regulation of the IGF-1 axis.[153] Rogers and colleagues[140] reported that higher meat intake in childhood was strongly associated with earlier age at menarche. This aligns with a study by Kissinger and Sanchez, which found U.S. girls in the highest quartile for animal protein reached menarche 6 months earlier than girls in the lowest quartile.[148]

The role of plant protein in pubertal development has also been investigated. Berkey et al. found that in girls aged 3-5 years, a 1 standard deviation higher intake of vegetable protein was associated with 10 month later onset of menarche.[135] Consistent with this study, de Ridder et al. reported an association of higher vegetable protein intake with later onset of breast development.[141] Most recently, Gunther et al. observed that children three to four years of age whose vegetable protein was in the highest tertile reached their age at takeoff, age at peak height velocity, and menarche/voice break

approximately six months later than those whose intake was in the lowest tertile.[151] As a whole, this literature suggests that animal protein may advance pubertal development while plant-based protein may delay its onset.

Dietary quality

The majority of studies on this topic have examined pubertal development in relation to single nutrients or foods. Although these investigations are valuable, the single nutrient or single food approach to nutritional epidemiology may be too reductionist to account for the complicated nature of nutrient and food interactions that take place within the dietary patterns in which they are consumed.[154] People eat meals comprised of correlated nutrient and non-nutrient structures. Thus, to better understand the role of diet in pubertal timing, it is important to combine foods or nutrients in patterns with which they are consumed.

To this end, one study has investigated overall dietary quality in relation to pubertal timing. The DONALD study was conducted in 222 children (119 girls 103 boys), and found that a higher dietary quality score was associated with later age at take off in boys and girls, independent of prepubertal body composition.[155] Overall dietary quality in this study was defined by an *a priori* dietary index that was informed by the literature. A higher dietary quality in this study was one with lower intake of total fat and higher intake of carbohydrates, fiber, and micronutrients. However, one key limitation to this study was the absence of analysis in relation to age at menarche, the pubertal marker most robustly associated with chronic diseases. Further prospective studies examining dietary quality, using nutrient- and food-based dietary indices, e.g., prudent vs. Western, in relation to age at menarche and other landmarks of pubertal timing is needed to

determine if modification of dietary patterns has the potential to prevent early age at menarche, and thereby reduce incidence of menarche age-related chronic diseases.

Physical Activity

There are limited, if any, data on how timing of puberty is influenced by high levels of sedentary activity, which is an increasing public health problem in Western societies. However, several lines of evidence support the hypothesis that intensive exercise during mid-childhood delays pubertal development.[156] Delayed pubertal onset has been observed in highly trained runners[157,158] and elite gymnasts.[159] A recent cross-sectional study in Colombia found that menarche was delayed in women who practiced physical activity ≥2 hours/day.[160] Physical activity increases energy expenditure and can improve body composition. As such, the effects of physical activity may be mediated by adiposity and related hormones (e.g., leptin).[159] It has also been demonstrated that elite childhood athletes usually have increased glucocorticoid levels induced by chronic activation of the hypothalamic-pituitary-adrenal axis.[157,158] Glucocorticoids in excess can inhibit growth hormone secretion,[161] skeletal maturation,[162] and suppress hypothalamic-pituitary GnRH pulsatile exertion.[156] Thus, while literature on sedentary activity (e.g., screen time) is lacking, there is evidence, and biologic mechanisms, to suggest moderate and vigorous activity during childhood is related to later onset of puberty.

Other Environmental Factors

Socioeconomic factors, such as urban/rural residence, family size, family income, and level of parental education may also influence the onset of puberty. On average, girls from families with a high socioeconomic status experience menarche at an earlier age

than girls from families with lower socioeconomic status.[163,164] Higher parental education has been associated with earlier timing of puberty.[160,165] Absence of a biological father is associated with earlier menarche,[166] whereas the presence of older sisters in the household while growing up is associated with later menarche.[167] It is also well documented that girls living in a rural environment have later menarche than those living in an urban setting.[168]

Girls adopted into a developed country from a developing country experience earlier menarche than their native-born counterparts.[169] Cumulative level of childhood social hardships has also been associated with later age at menarche.[170] Moreover, psychosocial stressors,[166,167,171,172] acute/chronic illness,[173] and war conditions[174] have all been linked to delayed onset of puberty. These stress-related exposures may influence pubertal timing through suppression the hypothalamic-pituitary gonadal axis.[173] Stress-related exposures may also influence pubertal timing indirectly through effects on lifestyle choices, such as level of physical activity, sleep, and diet.

An emerging body of literature (reviewed by Ozen et al.[175]) suggests that endocrine-disrupting chemicals play a role in altered pubertal timing. Endocrine disrupting agents are used in plastics (e.g., bisphenol A), plasticizers (e.g., phthalates), industrial solvents/lubricants (polychlorinated biphenyls, and dioxins), pesticides (e.g., DDT), fungicides (e.g., vinclozolin), and pharmaceutical agents (e.g., diethylstilbestrol).[176] Several endocrine disruptors have structural similarity to estrogen and thus likely influence pubertal timing through action on estrogen receptors. Others, such as fungicides, likely interact with the androgen receptor.[175] Endocrine disruptors have also been shown to inhibit steroidogenesis, aromatase activity, and modulate

controls in the central nervous system.[175] In sum, available evidence indicates that endocrine-disrupting agents play a role in pubertal timing. Yet, the extent to which these exposures are responsible for the declining trend in age at menarche, independent of childhood body fatness, has yet to be determined.

SUMMARY

The best available evidence indicates that the average timing and progression of puberty in girls, and perhaps boys, has probably advanced in many populations over the last half-century. The influences driving this trend are not firmly established, although it is likely that changes in non-genetic factors, such as body weight, diet, physical activity, psychosocial stress, and other environmental adulterants play significant roles in modulating the physiologic triggers of puberty.

Identifying modifiable factors involved in pubertal development provides a window of opportunity for ameliorating the adult health consequences of altered pubertal timing. As dietary habits are formed in adolescence, prior to and during puberty, identifying beneficial and pernicious dietary components and patterns during this time has value for long-term improvement of public health. The literature on diet and pubertal timing and progression is still relatively small. Some evidence suggests that a diet rich in fiber, a proxy for a prudent diet vegetable based diet, is related to later onset of puberty. Other nutrient markers of a prudent diet that are related to obesity and metabolic risk, such as Mg, K, vitamin C, and vitamin E have not been thoroughly investigated. Furthermore, no studies have investigated the role of nutrient proxies for a Western diet, or calorically sweetened beverages, which may influence pubertal timing through insulin-

mediated pathways, in advanced pubertal development. Only one study, using a dietary quality score, has investigated overall diet in relation to pubertal timing, however, this study was limited in sample size and did not examine diet quality in association with early age at menarche. As such, in *Chapter 8* of this dissertation, I will examine the role of diet quality, as indicated by the density of nutrients and food elements characteristic of a prudent vs. Western diet, in relation to incident early age at menarche.

Before this, however, I will provide an overview of type 2 diabetes (*Chapter 3*) and non-alcoholic fatty liver disease (*Chapter 4*)—diseases that may constitute an important part of the long-term morbidity and mortality associated with early puberty. In doing so, I will establish clear rationale for investigating pubertal timing in relation to these metabolic disease endpoints in *Chapter 6* and *Chapter 7* of this dissertation.

CHAPTER 3: TYPE 2 DIABETES

OVERVIEW

Type 2 diabetes is a major cause of mortality and morbidity worldwide,[177,178] and its prevalence has risen to epidemic proportions in most parts of the world in the last two decades.[179] Disability and complications associated with diabetes put a major burden on health systems in both the developed and developing worlds.[179-181] As such, prevention of type 2 diabetes is a major priority for health systems worldwide.

NATURAL HISTORY OF TYPE 2 DIABETES

The natural history of diabetes has been described in many populations and eloquently reviewed by DeFronzo.[182] Type 2 diabetes etiology comprises a complex interplay of genes and environment. Glucose homeostasis is achieved through a coordinated effort of tissues and hormones that regulate glucose uptake and release. The homeostatic controls of glucose metabolism commensurate with the obligate metabolic needs of the human body in the fasting and postprandial glycemic states.

Individuals with type 2 diabetes are more likely than their non-diabetic counterparts to have inherited a set of genes that make their tissues susceptible to insulin resistance.[183] Ultimately, however, it is the environment that triggers the defect in insulin action that underlies insulin resistance. Over time, generalized insulin resistance perpetuates a state of hyperinsulinemia, impaired glucose tolerance, and, consequentially,

overt type 2 diabetes, in which production of insulin from the pancreatic β-cell is insufficient to overcome insulin insensitivity in peripheral tissues.

Insulin Resistance, β-cell Function, and Type 2 Diabetes

In individuals with type 2 diabetes, insulin sensitivity in the liver and muscle is severely reduced or nonexistent.[182] To understand this pathogenesis, it is important to differentiate between insulin resistance in the fasting vs. postprandial state. In muscle, insulin resistance manifests by impaired glucose uptake following ingestion of a carbohydrate-rich meal and results in postprandial hyperglycemia.[182,184] Hepatic insulin resistance, on the other hand, is characterized by overproduction of glucose during the fasting state despite elevated concentrations of fasting insulin,[185] and impaired suppression of hepatic glucose production in response to insulin,[186] as normally occurs following a meal.[184]

The brain has an obligate need for glucose; it has been shown that it is responsible for nearly 50% of glycemic utilization under fasting conditions.[182] Glucose demand, in the fasting state, is met primarily by glucose production in the liver (~85%) and to a smaller extent the kidneys (~15%).[187] In type 2 diabetics, the rate of hepatic glucose production continues to rise in light of increasing levels of fasting plasma glucose. This overproduction of glucose in the liver occurs in the presence of fasting plasma insulin concentrations that can be increased nearly threefold, indicating significant resistance to the suppressive effect of insulin on hepatic glucose production.[188] As such, measures of fasting insulin are most reflective of hepatic insulin resistance.

Although the origins of insulin resistance can be traced to genetics,[183] the epidemic of diabetes in economically prosperous societies is driven by an increasingly

obesogenic environment.[189,190] This environment engenders an insulin-resistant state that places major stress on pancreatic β-cells to augment secretion of insulin to offset defects in insulin action.[182] Normal glucose homeostasis is maintained so long as β-cells are able to boost their insulin secretion to compensate for reduced insulin sensitivity.[182] Yet, over time, more rapidly in an environment of nutritional excess, β-cells fatigue, exhaust, or dedifferentiate,[191] thereby leading to a rise in postprandial, followed by fasting, glucose levels.[182] The resulting hyperglycemic state and poor metabolic control may cause further decline in insulin sensitivity, but degeneration of β-cells ultimately determines the rate of disease progression.[182]

The plasma insulin response to the development of insulin resistance is generally increased during the natural history of diabetes.[182] This does not imply, however, that the β-cell is functioning normally.[188] An increase in fasting and postprandial plasma insulin concentrations can be interpreted as an adaptive response of the β-cells to counter lack of insulin sensitivity in the tissues and preserve glucose homeostasis.[188] As severity of insulin resistance progresses, β-cell mass and function becomes compromised, and insulin secretion is no longer sufficient to overcome a generalized state of insulin insensitivity. Around this time, hepatic glucose production, the main source of glucose in fasting conditions, begins to rise abnormally.[188] The increase in glucose production from the liver is explained almost entirely by an increase in hepatic gluconeogenesis.[188]

In addition to insulin resistance in the liver, other factors contribute to the accelerated rate of hepatic glucose production. These include an increase in circulating glucagon levels, enhanced hepatic sensitivity to glucagon, lipotoxicity leading to increased expression of phosphoenolpyruvate carboxylase and pyruvate carboxylase, and

glucotoxicity, which may result in increased expression of glucose-6-phosphatase, the rate-limiting enzyme for hepatic glucose production.[188] This increased release of glucose from the liver exacerbates the hyperglycemic state, further limiting the ability of the pancreatic β-cell to respond. If this state is not remediated, through environment or therapy, blood glucose will increase beyond a normal range.

Insulin concentrations remain elevated in the progression from normal to abnormal glucose homeostasis. Between normoglycemia and manifestation of overt diabetes lies a state of impaired fasting glucose (IFG), characterized by a fasting blood glucose concentration of 100 to 125 mg/dL.[192] The intermediate state of postprandial glucose metabolism is known as impaired glucose tolerance (IGT), and diagnosed by blood glucose concentrations between 140 and 199 mg/dL measured 2 hours after a 75-gram bolus of glucose.[192] As an individual progresses from IFG to IGT their β-cell loses ability to maintain glucose homeostasis in response to hyperglycemia. Insulin secretion declines due to decreased β-cell function, dysfunction, and/or apoptosis. If β-cell function continues to degenerate, an individual progresses to frank diabetes, as indicated by fasting glucose ≥ 126 mg/dL, 2-hour postprandial glucose ≥ 200 mg/dL, or glycated hemoglobin (A1C) ≥ 6.5%.[192]

DIAGNOSIS OF TYPE 2 DIABETES

Diabetes mellitus is diagnosed through clinical practice, which relies on measurement of blood glucose levels (whole blood or plasma). Diabetes is a heterogeneous metabolic disease, thus, diagnosis may be made with or without the presence of hyperglycemic

symptoms. The early stages of diabetes often have a symptom-free insidious onset. Thus one third or more of cases, depending on the population, may be undiagnosed.[193-195]

The gold standard criterion for diagnosis of diabetes is postprandial plasma glucose concentration of ≥ 200 mg/dL (11.1 mmol/L), which is measured 2-hours after ingestion of a 75-gram glucose solution [i.e., oral glucose tolerance test (OGTT)].[192] This cut point was chosen for clinical diagnosis because it predicted risk of diabetic retinopathy better than other cut offs.[196] This cut point is also thought to be the inflection point at which insulin secretion starts to decline.[197]

A fasting plasma glucose value of ≥ 126 mg/dL (7.0 mmol/L) is also used to diagnose diabetes.[192] The fasting glucose test has lower reliability than the 2-hour plasma glucose from OGTT. As such, it is recommended that a positive finding be repeated on a different day unless there is unequivocal hyperglycemia with corroborating evidence from other metabolic symptoms.[192]

Most recently, the World Health Organization (WHO) and the American Diabetes Association (ADA) have recommended the use of glycated hemoglobin (A1C) ≥ 6.5% for the diagnosis of diabetes.[192,198] While A1C tests may be less sensitive than the 2-hour plasma glucose from OGTT and fasting plasma glucose, the relative ease of taking A1C (e.g., patient does not have to be fasted) makes this an attractive, if not the preferred, diagnostic measure for clinicians. Fasting plasma glucose, 2-hour plasma glucose, and A1C may not always be in accordance, and have been shown to vary with age, sex, race, and ethnicity.[199-208] As such, the choice of diagnostic criteria for diabetes may have important implications for overall- and sub-population prevalence of diabetes and, consequentially, the devotion of research and health care resources.

EPIDEMIOLOGY OF TYPE 2 DIABETES

In the late 20th and early 21st centuries it has been suggested, based on limited surveillance data, that an epidemic of type 2 diabetes began taking hold in many parts of the world.[209] Today, the global burden of diabetes appears to be rising as developing populations' age, urbanize, and adopt lifestyles characterized by increased sedentary activity and consumption of calorie dense, nutrient poor diets. The International Diabetes Federation (IDF) estimated that in the year 2011, 366 million adults had diabetes. This prevalence is expected to swell to 552 million by 2030.[209] At this growth rate, the epidemic of type 2 diabetes promises to challenge health-care systems across the globe.

Diabetes prevalence rates vary markedly by geographic region. These differences are likely due to a confluence of factors, including genetics, lifestyle, urbanization, and age structure. By region, the Middle East and North Africa have the highest rates of diabetes in the world, with an adjusted prevalence of 12.5% in 2011, and the rest of Africa has the lowest, at 5% in 2011.[209] The most recent estimates from the U.S. suggest that approximately 9.3% of adults 20-79 years of age have diabetes,[193] ranking the U.S. third for the number of people with diabetes in 2011.[209] Diabetes prevalence increases with age, reaching 21.6% for those ≥ 65 years in the US.[193] While *diagnosed* diabetes rates appear to be similar by sex, more men than women have undiagnosed diabetes.[193] There are also differences by race/ethnicity. Compared to non-Hispanic whites, diagnosed diabetes prevalence was twice as high in non-Hispanic blacks and Mexican Americans; the prevalence of undiagnosed diabetes in the U.S. was similar by race/ethnicity.[193]

Type 2 Diabetes in Brazil

Brazil has one of the most rapidly growing populations in the world, and its inhabitants have high prevalence of type 2 diabetes at low body mass index.[195,210] Thus, studies identifying markers of growth and development—other than body mass index—that predict future development of diabetes have potential for facilitating primordial prevention in this population. In Brazil, the IDF estimated 10.2% of adults had diabetes in 2011 and 12.3% will have diabetes in 2030.[209] However, these prevalence estimates are based on self-reported diabetes from two studies: *1*) a nation-wide survey in 2006[210] that did not take into account undiagnosed diabetes, and *2*) a study of urban adults in 1986 to 1988 that did not reflect epidemiologic and nutritional changes that have occurred in Brazil over the last two and a half decades.[195] As such, current estimates of diabetes prevalence in Brazil[209] that are based on these studies may be grossly understated. In addition to examining markers of early-life growth and development in relation to type 2 diabetes in Brazilian adults, *Chapter 6* of this dissertation provides contemporary estimates of diagnosed and undiagnosed diabetes prevalence in Brazil among an occupational cohort of adults. These estimates will help demonstrate the burden of diabetes in Brazil and inform public health capacity building for diabetes prevention and management.

LIFESTYLE AND TYPE 2 DIABETES

The purported increase in type 2 diabetes over the last two to three decades implies that environment, not genetics, is primarily responsible for the epidemic. Galvanizing this notion are findings from several randomized controlled studies that examined the effect

of physical activity, smoking cessation, and healthy diet on the incidence of type 2 diabetes in high-risk participants. Collectively, these trials reported about a 50% reduction in the incidence of type 2 diabetes after on average three years intensive lifestyle modification.[211-219] Much about the role of lifestyle in diabetes etiology can also be gleaned from epidemiologic studies. Danaei et al. used the parametric g-formula to estimate 24-year risk of diabetes in healthy middle-aged women under hypothetical lifestyle interventions, including: smoking cessation, losing weight by 5% every 2 years if overweight/obese, exercising at least 30 min/day, eating less than 3 servings/week of red meat, eating at least 2 servings/day of whole grain, drinking 2 or more cups of coffee/day, drinking 5 or more grams of alcohol/day, and drinking less than 1 serving/week of soda.[220] These hypothetical interventions were informed by observational[221-225] and experimental[226,227] data. The 24-year risk when all interventions were imposed was 55% lower than baseline. The most effective interventions were weight loss (24% lower risk), physical activity (19%), and moderate alcohol use (19%). These findings largely align with the epidemiologic literature base on relative importance of lifestyle risk factors for diabetes prevention.

Therefore, through a most optimistic lens that assumes perfect long-term participant adherence, approximately half of diabetes cases could be prevented through lifestyle intervention. However, as evidenced in numerous trials, efforts to lose weight and reduce risk for diabetes and its complications through lifestyle changes later in life pose tremendous challenges.[228,229] As such, *primordial* prevention of obesity and insulin resistance, through identification of their early-life antecedents, should be a global priority.

PLASTICITY AND EARLY LIFE ANTECEDENTS

Plasticity implies that the development of an organism's phenotype reflects variations in the quality and quantity of environmental exposures required for life.[230] Here, we use this to mean that during periods of critical growth and development tissues can grow to various sizes, and maturation can occur at varying timing and tempo. Plasticity of human growth is at an all time high in the prenatal period, early infancy, and childhood through puberty. After puberty, the preponderance of human growth and development has occurred. From the perspective of developmental plasticity, birth weight/size/body composition, growth in recumbent length and weight during the first two years, relative leg length, and age at puberty/menarche are indicators for prenatal, postnatal, childhood, and pubertal growth and maturation, respectively.

During times of plasticity, growth is especially sensitive to shortage/surplus of nutrition, infection, parasites, psychosocial stressors, and other adverse environmental conditions.[231] Together these factors can be thought of as *net-nutrition*.[231] In the simplest case, tissues and organs compete for scarce nutrients, and the organs, i.e., brain, with obligate nutrient needs, get priority over other tissues, i.e., long bones, and organs, i.e., liver and pancreas. In this context of developmental plasticity lay more complex theories, including the 'thrifty phenotype' hypothesis,[232] 'fetal programming' hypothesis,[233] and 'predictive adaptive response' hypothesis.[234] In general, each of these hypotheses predicts that the vital organs of the head, thorax, and abdomen are protected from adversity at the expense of less vital tissues, e.g., limbs, and the cellular machinery for hormonal and metabolic regulation. Here, I review several early-life markers of net nutrition during these times of developmental plasticity, and their relation to type 2 diabetes.

Fetal Origins

Hales et al. pioneered the notion that fetal growth was related to development of type 2 diabetes through the observation that, among 64-year-old men born in the UK, those born with the lowest birth weight were six times more likely to have type 2 diabetes than men with higher birth weights.[235] These findings led to the thrifty phenotype hypothesis, i.e., poor intrauterine nutrition causes metabolic adaptations in the developing fetus that would support its survival in conditions of continued postnatal deprivation, but would be disadvantaged metabolically in conditions of postnatal and later life nutritional excess.[232] This hypothesis is one possible explanation for why rapidly developing countries, such as those in Latin America, Asia, and the Middle East bear greater burden of diabetes at lower body weight than Europids. In Brazil, rural to urban migration, and rapid Westernization, has set the stage for metabolic perturbations that can result from early-life undernutrition followed by overnutrition later in life.

Famine studies have provided direct evidence for the role of intrauterine nutrient deprivation in the development of type 2 diabetes. Individuals exposed to the Dutch famine *in utero* had higher postprandial glucose and insulin concentrations at age 50 compared to those born the year before the famine.[236] Similar results emerged from a study of adults prenatally exposed to the Chinese famine (1950-1960), in which *in utero* famine exposure was associated with increased risk of adult obesity and hyperglycemia.[237] Yet, in some populations, individuals with higher birth weight had increased risk of type 2 diabetes; this association may be explained by maternal fatness and gestational diabetes.[238-240]

Sibling-pair studies in the Pima Indian population provide evidence for a direct association of gestational diabetes on risk of type 2 diabetes in offspring. Participants born to diabetic mothers had increased risk of diabetes compared to their siblings born when their mothers had normal glycemia.[241] While some have suggested that the maternal-offspring association may be explained by shared familial (environment and genetic) risk factors rather than by intrauterine environment,[242] in the Pima Indian population, increased risk of developing diabetes in offspring was specific to the maternal diabetes (no association for paternal diabetes).[238,239] These findings suggest a causal role for intrauterine exposure to gestational diabetes and risk of offspring type 2 diabetes.

Intrauterine exposure to maternal obesity, independent of gestational diabetes status, has also been found to be associated with insulin resistance and diabetes in offspring. Using maternal umbilical cord blood, one study found that fetuses of obese mothers had greater insulin resistance than fetuses born to non-obese mothers.[243] Furthermore, at age 11, children with obese mothers who were born large for gestational age were at nearly twice the risk of developing metabolic syndrome than children who were born to non-obese mothers.[244]

Postnatal Origins

A rapidly emerging literature base has identified the early postnatal period as another important window during which glucose-insulin metabolism can be programmed in a manner predisposing or protecting individuals from development of type 2 diabetes and other metabolic complications. Specifically, it has been hypothesized that the

metabolically pernicious effects of poor intrauterine nutrition are exacerbated if followed by a period of accelerated postnatal weight gain, i.e., catch-up growth.

Several epidemiologic studies support the catch-up growth hypothesis. At one year of age, small for gestational age children that underwent catch-up weight gain had higher insulin concentrations than those who experienced normal weight gain.[245] However, those children who were small-for-gestational age and had accelerated gains in height did not have increased insulin concentrations.[245] A meta-analysis on this topic, which included 57 studies of birth weight and postnatal growth, concluded that low birth weight followed by catch-up growth is indeed associated with insulin resistance.[246] Moreover, in the Helinski birth cohort, children who had low birth weight and high BMI at age 11 had the greatest odds of developing type 2 diabetes later in life.[247]

Breastfeeding has been linked to slower postnatal weight gain and more favorable body composition during childhood and adolescence, even after control for maternal obesity.[248] Yet, it is still debated whether breastfeeding is associated with long-term improved insulin sensitivity and lower risk of diabetes. Previous studies have suggested inverse associations of breastfeeding with insulin resistance in childhood[249] and adulthood,[250] as well as with risk of diabetes.[251] These associations have not been observed in other populations.[252] A meta-analysis of observational studies found that compared to formula feeding, breastfeeding was associated with ~40% lower risk of adult type 2 diabetes.[253] However, only few studies were eligible for inclusion in the meta-analysis, and residual confounding by socioeconomic determinants of breastfeeding is a validity threat to the estimates obtained in this literature.

As a whole, the above evidence suggests that pre- and postnatal periods are important for programming of glucose-insulin metabolism, and environmental conditions of malnutrition during these periods predisposes one to risk of adult diabetes.

Childhood Origins

While a large body of literature has investigated the role of birth weight and early catch-up growth in relation to metabolic disorders, there are relatively few studies on markers of prepubertal nutrition and pubertal timing in relation to development of type 2 diabetes.

Onset of puberty represents an important event during human growth and it is influenced by myriad factors including genetics, ethnicity, environmental conditions, and nutrition.[111] Early age at menarche is a marker for childhood growth, adiposity, and related hormonal and metabolic changes.[254] Relative leg length may reflect prepubertal net nutrition, i.e., the combination of nutritional input and the demands of disease, stress, and physical activity.[231] Leg growth is most sensitive to environmental conditions between 0 and 2 years, but because its growth slows and eventually discontinues during and after puberty relative leg length has been proposed as a proxy for pubertal timing (*discussed in next section*). Shorter relative leg length has been found to be associated with earlier pubertal onset,[255,256] peak height velocity,[257] and age at menarche.[255,258] Unlike leg length, sitting height continues elongating through puberty, and thus has not been found to be related to pubertal timing.[30] Here, I review the literature on leg length, as marker of prepubertal growth and development, and age at menarche, as indicator of pubertal timing, in relation to development of type 2 diabetes.

Relative leg length

Leitch was the first to propose that leg length relative to stature could be a good indicator of early-life nutrition.[259] In reviewing the literature (pre-1950), Leitch found that improved nutrition during infancy and childhood resulted in greater increase in leg length than in total height, suggesting leg length as a marker of better constitution. These observations aligned with the 'reserve capacity' hypothesis—an individual with greater leg length likely exceeded their minimal requirements for sustaining life, with reserve capacity that can result in greater growth, better health, and slower rates of senescence.[260]

Several authors have suggested that relative leg length (i.e., leg length relative to sitting height or total stature) is a crude marker of pubertal timing and peak height velocity.[256,257] According to the cephalocaudal principle, prior to the pubertal growth spurt, growth is more rapid in the legs, whereas during and after the pubertal growth spurt, growth is more rapid in the trunk.[30] As such, relative leg length increases until around the age of peak height velocity in both males and females and then begins to decline.[30] An estrogen surge in the early stages of pubertal development is the dominant factor controlling peak height velocity and fusion of the epiphysis growth plate,[29] This latter phenomenon results in cessation of long bone, but not torso, growth. Thus, one would expect earlier puberty or peak height velocity to result in lower relative leg length in fully-grown adults.

Due to the growing recognition of the importance of early-life conditions in long-term health, several epidemiologic studies have examined relative leg length, as proxy of the plasticity of the human body to early-life environment and pubertal timing, in relation to a number of chronic disease outcomes.

A few studies have investigated the association between relative leg length and type 2 diabetes. In the National Health and Nutrition Examination Survey (NHANES) III, leg length and leg length-to-stature were inversely associated with prevalence of type 2 diabetes. This association persisted after adjustment for potential confounders, such as parental history of diabetes, socioeconomic status, and adult BMI.[261] In the Atherosclerosis Risk In Communities study, Weitzman et al. found that in white men and women, both leg length and leg length-to-height ratio were inversely associated with risk of type 2 diabetes after adjustment for potential confounders and BMI at age 25. The risk for diabetes per standard deviation (5-cm) increment in leg length was 15% lower in white men and 21% lower in white women. Similar associations were observed for leg length-to-stature ratio. However, associations were not significant in African American adults.[262] In a cross-sectional study carried out on 4286 English women of age 60-79 years, a standard deviation (4.3 cm) increment in leg length was associated with 13% lower odds of diabetes after full adjustment for confounders and adult measures of adiposity, blood pressure, and lipids. Similar associations were observed for leg length-to-sitting height ratio.[263]

From non-Western populations there has been only one study on the association between relative leg length and diabetes. In the Shanghai Health Study, a one standard deviation increment in leg length-to-sitting height ratio was associated with a 9% decreased risk of diabetes in women and a 12% decreased risk of diabetes in men. These associations were attenuated after adjustment for adult BMI.[264] However, adjustment for BMI in late adulthood as a potential confounder is not appropriate, as it constitutes an intermediate variable on the causal pathway from pubertal timing to diabetes.

Thus, gleaning from studies in Western and non-Western populations, it appears that relative leg length, as proxy of early childhood nutrition and development, is associated with type 2 diabetes risk later in life. But, whether this association is driven by the quality of early life nutrition or the timing of puberty is still unclear. Moreover, it is still unknown whether the association is independent of early-life indicators of adiposity, or modified by measures of nutritional status later in life (e.g., BMI), as one might hypothesize under the predictive adaptive response paradigm.[232,265] More research is warranted in these areas.

Age at menarche

The first cohort studies to investigate age at menarche and type 2 diabetes did not find a statistically significant association.[22,266] However, these studies were potentially underpowered and focused on older postmenopausal women. More recently, larger cohort studies of pre- and postmenopausal women have addressed the question of whether earlier age at menarche is associated with higher risk for type 2 diabetes.

In an investigation of the Nurses Health Study I, women with menarche ≤11 years (vs. 13 years) had an 18% increased risk of diabetes after adjustment for multiple confounders.[19] In the Nurses Health Study II, a younger cohort, risk for diabetes among women with menarche ≤11 years was 98% higher than those who had menarche at 13 years.[19] After adjustment for adult BMI, measured at baseline, risk for diabetes was attenuated to 40%. In the EPIC-Norfolk study, the odds for diabetes diagnosed at baseline or within 8-years of follow up were 10% lower for each 1 year later age at menarche.[21] This association was no longer significant after adjustment for BMI measured at baseline

between 40 and 75 years of age. In a cross-sectional study of 1,503 German women, aged 32-81 years, a 1-year lower age at menarche was associated with a 11% reduced prevalence of pre-diabetes or diabetes, after adjustment for potential confounding variables including BMI (measured at 32-81 years).[22]

In the light of these findings from predominantly white cohorts studies, Dreyfus et al. examined the association between age at menarche and type 2 diabetes in a biracial sample of women from the Atherosclerosis Risk in Community (ARIC) study who were followed prospectively (on average 9 years) for diabetes.[267] In this study, the authors observed increased odds of diabetes at baseline, and increased risk of diabetes over follow-up among white women who reported menarche between 8-11 years, before adjusting for adult BMI. There was no association between age at menarche and diabetes among African American women.

In perhaps the most informative study on this topic, Pierce and Hardy examined the association between age at menarche and type 2 diabetes using data from a British birth cohort study of women born in 1946, with contemporaneous recording of age at menarche, BMI at age 2, 7, 15 and 20-53 years, and diabetes status to 53 years.[23] This study design allowed for control of body weight both before and after menarche, a limitation of previous studies on this topic. The authors found a significant association between later age at menarche and type 2 diabetes risk (27% lower risk per 1-year increment in age at menarche; p=0.02). This association was attenuated by adjustment for adult BMI (15% lower risk per year older age at menarche; p=0.2). However, the association was not markedly attenuated by adjustment for BMI at age 2, or at age 7. This study provides evidence that the age at menarche-diabetes association is *not* confounded

by prepubertal BMI, implying that age at menarche may be a clinically useful tool for identifying women at risk for type 2 diabetes early in life.

Collectively, studies from Western populations suggest that age at menarche is associated with type 2 diabetes, independent of many potential confounders, including prepubertal and early-life BMI. However, these associations may vary by population, due to contextually unique influences on pubertal timing and diabetes.

To date, there have been only two studies that have examined the association between age at menarche and diabetes in non-Western populations, both of which were from Asia. In the Singapore Chinese Health Study, after adjustment for potential confounders and adult BMI (measured between 45-74 years of age), we observed 18% increased prevalence of diabetes among women with menarche ≤12 years (vs. 13-14 years). In addition, women who reported menarche between 15-16 years and ≥17 years (vs. 13-14 years) had 17% lower prevalence of diabetes.[268] In an analysis of the Shanghai Women's Health Study, Conway et al. reported 25% reduced risk of diabetes in women who were in the highest (vs. lowest) quintile of age at menarche, after adjustment for birth cohort, education, and income.[264] This association was attenuated to 12%, with confidence intervals including the null, after adjusting for BMI at age 20 and baseline. Yet, as noted above, adjusting for BMI in late adulthood as a confounder may be over adjustment as it is on the causal pathway between age at menarche and diabetes. Thus, the findings from Asian populations are largely consistent with those from Western populations in suggesting that earlier age at menarche is associated with higher risk of type 2 diabetes. More research on this topic is needed from less prosperous populations,

where potential confounding structures to the age at menarche-type 2 diabetes association may differ.

SUMMARY

Type 2 diabetes continues to increase in prevalence worldwide. Current lifestyle recommendations, including weight loss, improved diet, and increased physical activity remain first in line for prevention of type 2 diabetes. However, successes in sustained adherence and compliance to long-term lifestyle changes are few and far between,[228] and, alone, will not suffice in averting a diabetes pandemic.

Primordial prevention, loosely defined as preventing the risk factors before they develop, is an ideal approach for reducing diabetes incidence across diverse populations, especially those from low- and middle-income countries who lack resources and capacity to deal with the burden of type 2 diabetes. Brazil is one such country where the prevalence of diabetes is believed to be increasing in response to an aging and increasingly urbanized population. Few studies in Brazil have investigated markers of early-life growth and development in relation to type 2 diabetes. Such studies are paramount for identifying potential periods of intervention, when it's not too late to promote healthful lifestyle habits and alter health trajectories. In Manuscript 1 of this dissertation (*Chapter 6*) we will address these research gaps by investigating age at menarche and relative leg length in relation to development of type 2 diabetes.

CHAPTER 4: NON-ALCOHOLIC FATTY LIVER DISEASE

OVERVIEW

Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver disease from hepatic steatosis to steatohepatitis (**Figure 4.1**).[269] Simple hepatic steatosis is defined as the presence of cytoplasm triglyceride droplets in more than 5% of hepatocytes and can be self-limiting but often progresses to non-alcoholic steatohepatitis (NASH).[270] NASH is distinguished from simple steatosis by the presence of hepatocyte injury (e.g., hepatocyte ballooning and cell death), inflammatory infiltrate, and/or collagen deposition (fibrosis).[271] In patients with NAFLD, approximately 30% with isolated steatosis will progress to NASH.[272] Of those individuals with NASH, roughly 20% develop cirrhosis, and of those with cirrhosis, 30-40% decompensate and succumb to liver related death within 10 years.[272]

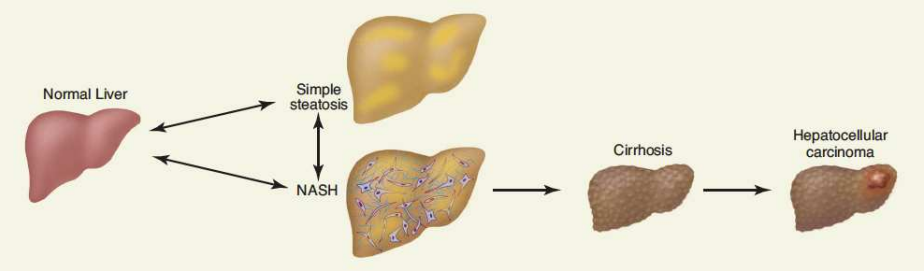

Figure 4-1: Progression from normal liver function to hepatocellular carcinoma.

NAFLD has risen to epidemic proportions in across many parts of the world. In the U.S. an estimated 30% are afflicted with the disease.[273] As well as being a leading cause of chronic liver disease in North America,[274] NAFLD is associated with significant

long-term *non*-liver related morbidity and mortality.[275] The characterization of NAFLD as a disease is relatively recent, and the gold standard used for clinical diagnosis, biopsy, is invasive and not suitable for population-based studies. Therefore, most studies have used elevated levels of serum aminotransferases, i.e., liver enzymes, as a proxy for fatty liver disease. A review of using alanine aminotransferase (ALT) and aspartate aminotransferase (AST) screening for chronic liver diseases indicated this type of screening has moderate specificity and sensitivity for detecting liver diseases.[276] The spectrum of NAFLD seems to encompass a wider range of subjects than those identified by elevated serum liver chemistries, and it is possible to have NAFLD without elevated ALT or AST.

Non-invasive radiological modalities, including magnetic resonance imaging and ultrasonography have also been used, but nut for diagnosis of NAFLD or grading of steatosis.[277] Liver attenuation, assessed by unenhanced computed tomography, has been shown to be an effective predictor of hepatic fat content [suggested cut point for NAFLD $\leq$ 48 HU],[278,279] and the best measure of liver steatosis in large epidemiologic studies. While work on establishing an effective non-invasive yet widely available tool to diagnose steatosis severity continues, there have been efforts to coalesce existing estimates of NAFLD prevalence to describe its epidemiology.

EPIDEMIOLOGY OF NAFLD

The majority of studies on NAFLD from U.S., which are based on cryptogenic abnormal liver function test results, autopsy samples, ultrasound, and magnetic resonance spectroscopy have reported a prevalence range of 10-35%.[273] A population-based

estimate from National Health and Nutrition Examination Study-III reported 19% of U.S. adults had NAFLD, as diagnosed by ultrasound.[280] NAFLD is not unique to the U.S.; estimates from diverse populations in other parts of the world indicate a range of 6-35% and a median of 20%.[273] Estimates of NAFLD prevalence still lack precision due to the heterogeneous modalities used to measure NAFLD, as well as marked differences that have been observed across diverse populations.

A growing number of studies suggest NAFLD rates differ by race and ethnicity.[273,281] In a large cohort study from the U.S. that used magnetic resonance spectroscopy, one-third of the population was found to have hepatic steatosis. This prevalence varied markedly by race and ethnicity; 45% of Hispanics, 33% of Caucasians and 24% of African Americans were affected.[282] The notion that NAFLD rates vary by ethnicity is supported by another study that found higher prevalence of NALFD in Hispanics compared to non-Hispanics, after controlling for measures of fatness.[283]

The prevalence of NAFLD and NAFLD-related fibrosis has been reported to increase with age.[273] Furthermore, in a study of biopsy-proven NAFLD adults, Frith et al. showed that degree of fibrosis increased with age.[284] Other studies have found older NAFLD patients have higher probability of disease progression and mortality than their younger counterparts.[285,286] Yet, in an analysis of NHANES by Dunn et al., it was found that *younger* adults (aged 45-54 years) who had suspected NAFLD, as determined by liver enzymes, were at increased risk of all-cause and cardiovascular disease morality, but older adults (aged 55-85 years) were not.[287]

Pediatric NAFLD also exists. Although the pathology is still poorly understood, it is believed to be distinct from the corresponding adult condition. A review by Patton et

al. highlights important pathologic differences between pediatric NAFLD and adult NAFLD, and notes that there are no data to support the extrapolation of pediatric studies to the adult population in this field.[288] The scope of our investigation in this dissertation (*Manuscript 2; Chapter 7*) is limited to development of NAFLD in adults.

The preponderance of literature suggests that adult NAFLD is more common among males than females. Compared to females, biopsy-proven NAFLD males were found to have higher aminotransferase levels, prevalence of histological NASH, hepatic fibrosis, and overall mortality.[281,285] Differences by sex has led some to suggest that the threshold for normal alanine aminotransferase in women should be reduced from an upper normal limit of ≤ 30 U/L to ≤ 19 U/L.[289] However, prevalence of 'lean NAFLD' phenotype appears to be higher in females,[290] and risk of liver steatosis has been shown to increase markedly after menopause.[291]

Beyond predisposing demographical factors, it has been shown that NAFLD is more prevalent in persons with cardiometabolic conditions. Excess adiposity is the strongest risk factor for NAFLD. The prevalence of NAFLD was as high as 57% in overweight and 98% in non-diabetic obese adults attending outpatient clinics.[273] Insulin resistance is also centrally involved in NAFLD etiology. In one study, ~70% of type 2 diabetes patients were found to have NAFLD, as determined by ultrasound.[292] NAFLD is also associated with hypertension and hypertriglyceridemia.[293] The association between NAFLD and this constellation of metabolic risk factors has led some to name it the hepatic manifestation of the metabolic syndrome.[293]

PATHOGENESIS OF NAFLD

Much headway has been made in elucidating the core mechanisms underlying the progression from steatosis to more advanced liver inflammation, fibrosis, and steatohepatitis. The current pathogenic understanding of NAFLD is discussed below, with the anticipation that such knowledge will inform a conceptual model relating pubertal timing to development of liver steatosis.

A 'two-hit' mechanism was originally proposed to describe the pathogenesis of NAFLD.[294] The primary hit, known as steatosis, occurs when fat is deposited. This is attributed to *1*) elevated *de novo* lipogenesis and *2*) accumulation and re-esterification of nonesterified fatty acids. Steatosis itself is sufficient to trigger inflammatory responses and induce hepatic insulin resistance.[295] Yet, a more pernicious result of fat sequestration in the liver is increased hepatocyte vulnerability to secondary hits from circulating inflammatory cytokines, mitochondrial dysfunction, and oxidative stress.[295] Together, these events are believed to exacerbate hepatic inflammatory responses in NAFLD and thereby advance steatosis to steatohepatitis. It has also been posited that hepatic fat deposition, inflammatory response, and insulin resistance are involved in a latent cycle that leads to development of steatohepatitis.[295,296]

The two-hit model of NAFLD pathogenesis was recently revised in the recognition that free fatty acids (FFAs) play a central role in promoting liver injury.[297] FFAs arise from *1*) lipolysis (the hydrolysis of FFA and glycerol from triglyceride) within adipose tissue, *2*) dietary sources, and *3*) *de novo* lipogenesis.[288] Once in the liver, FFAs may *1*) be used *via* β-oxidation; *2*) be packaged and exported out of the liver as very low-density lipoprotein; or *3*) undergo glycerol esterification to form triglycerides

that are stored as lipid droplets (i.e., steatosis). As such, steatosis can occur as a result of decreased fat oxidation, decreased fat export, increased fat synthesis, and/or increased fat delivery.[288] Furthermore, FFAs may directly cause hepatic toxicity by increasing oxidative stress and activating pathways of inflammation.[298] Triglyceride formation may actually be a mechanism to prevent the toxic effects of nonesterified FFAs.[299]

Inadequate hepatocyte proliferation has been proposed as a potential 'third-hit' in NAFLD pathogenesis.[300] In a healthy state of the liver, hepatic apoptosis stimulates replication of mature hepatocytes, which results in an increased number of hepatic progenitor cells.[300] Progenitor cells can differentiate into hepatocyte-like cells. It has been observed that progenitor cells and hepatocyte-like cells are strongly correlated with fibrosis stage, suggesting that hepatocyte loss promotes accumulation and differentiation of progenitor cells into hepatocytes.[300] When individuals are in states of chronic fibrosis or cirrhosis they depend on efficient hepatocyte regeneration. Thus, impaired proliferation of hepatocyte progenitor cells may underlie the third hit of NAFLD pathogenesis.[301]

Insulin Resistance

Insulin sensitivity plays an instrumental role in the pathology of NAFLD. In insulin-sensitive individuals, the process by which insulin binds to its receptors leads to phosphorylation of several substrates, including receptor substrates (IRS)-1, -2, -3, and -4, which propagate the insulin signal.[288,302] The stimulation of IRS-1 and IRS-2 by insulin activates intracellular phosphoinositide 3-kinase and protein kinase B (PKB) pathways, which are centrally involved in mediating metabolic effects of insulin.[288] Activation of

the PKB pathway leads to translocation of vesicles containing glucose transporter type 4 to the plasma membrane.[288] In addition to facilitating glucose uptake, this cascade of events leads to increased expression of key lipogenic genes with a concomitant decrease in glycogenic gene expression *via* regulation of the fork head transcription factor activity.[288]

Circulating insulin is a potent suppressor of adipose tissue lipolysis. During a state of insulin resistance this insulin-dependent suppression is impaired, leading to increased efflux of FFA from adipocytes.[303] The hyperinsulinemia that results from insulin resistance can *1*) upregulate of the transcription factor sterol regulatory element binding protein 1-c, which regulates genes involved in *de novo* lipogenesis,[304] and *2*) inhibit β-oxidation of FFA, thereby promoting accumulation of fat in hepatocytes.[288]

Many metabolic abnormalities associated with NAFLD contribute to insulin resistance by interfering with the insulin-signaling cascade. NAFLD is associated with FFAs, tumor necrosis factor-alpha (TNF-α), and nuclear factor kappa B (NF-κB), among other metabolic factors involved in insulin-signaling.[305] Increased production of lipid metabolites, such as diacylglycerol, has also been shown to interfere with insulin signaling *via* inhibition of insulin receptor activity and modulation of IRS-2 phosphorylation.[288] Similar interference of the insulin-signaling cascades in skeletal muscles may also occur, leading to a more ubiquitous state of insulin resistance.

GENETICS AND NAFLD

The genetic study of NAFLD was motivated by the observation that some individuals with healthy metabolic profiles develop NAFLD, whereas others with many metabolic

risk factors do not.[306,307] More specific motivation for this line of research came from a case series showing familial clustering of NAFLD[308] and the racial and ethnic differences in the prevalence of NAFLD.[282,309] Moreover, among patients of comparable weight and insulin resistance, fatty liver disease was more prevalent in Latin American children descent than in African American children.[306]

An important step in determining whether a disease or trait may have genetic origins is examining the phenotypic heritability, i.e., the fraction of the total phenotypic variation from one person to the next that is due to underlying genetic or familial factors. Until recently, estimates of the heritability of NAFLD were lacking because of the methodological challenge of accurately determining NALFD phenotype.

In 2009, to test the hypothesis that NAFLD is a heritable condition, Schwimmer et al. conducted a family study using MRI to assess hepatic steatosis in 33 children with NAFLD and 11 overweight controls without NAFLD.[310] Depending on whether hepatic steatosis was modeled continuously or dichotomously (using a cut off of >5% lipid concentration of lipid droplets in the hepatocytes), the heritability estimates ranged from 40% to 100%, after adjusting for age, gender, race, and BMI. This study confirmed what was observed in previous studies: NAFLD runs in families and is more common in certain ethnic groups. Yet, it left open the question of whether familial and ethnic clustering is due to genetics, environment (which families and ethnic groups share), or both.

PNPLA3 variants

Accumulating evidence now links individual genetic variants to NAFLD. In 2008, Romeo and colleagues with the Dallas-Heart Study performed a genome wide association

study (GWAS) to identify single nucleotide polymorphisms (SNPs) associated with NAFLD. The authors found rs738409 G allele of adiponutrin [patatin-like phospholipase domain containing 3 (PNPLA3)] was significantly associated with NAFLD.[311] The G allele variation of PNPLA3 was significantly associated with liver fat content in all ethnicities in the study independent of BMI, diabetes, alcohol consumption, and ancestry. Hepatic fat accumulation was two-fold greater for G allele homozygotes than for non-carriers of this allele. Furthermore, consistent with ethnic differences in prevalent NAFLD, the SNP rs738409 was most frequent in Latinos, followed by Caucasians, then African Americans. GWAS from several ethnic cohorts have since corroborated the PNPLA3 variant association with hepatic fat, independent of age, gender, BMI, and insulin resistance.[312-314] These findings provide compelling evidence for a NAFLD genotype.

APOC3 variants

While genetic association studies linking PNPLA3 variant (SNP rs738409) with hepatic fat suggest development of NAFLD is independent of insulin resistance, other studies show a strong relationship among NAFLD and hepatic insulin resistance.[315-317]

To better understand the genetic basis of the insulin resistance-NAFLD pathology, Peterson et al. studied a cohort of 95 healthy Asian Indian men.[317] They sequenced two SNPs (rs2854116 and rs2854117) in the apolipoprotein C3 (APOC3) allele known to be associated with hypertriglyceridemia. They also sequenced the SNP rs738409 within PNPLA3. The APOC3 variants were associated with 30% higher fasting apolipoprotein C3 concentration, 60% higher fasting plasma triglyceride concentration, and a two-fold higher postprandial plasma triglyceride and retinyl fatty acid ester

concentrations. Moreover, 38% of the Asian Indian men with APOC3 variants had NAFLD compared to 0% in the APOC3 wild-type homozygote group. Men with APOC3 variants and NAFLD had significantly higher levels of insulin resistance compared to their wild-type homozygote counterparts. The PNPLA3 allele was also associated with NAFLD, but it was not reported whether subjects with the PNPLA3 variant and NAFLD were more insulin resistant than those without the variant. These findings in Asian Indian men were confirmed in non-Asian Indian men; the prevalence of APOC3 variants was similar among Asian and non-Asian Indian men.[317]

In summary, the heritability of NAFLD makes it a strong candidate for genetic linkage and association studies. Genetic studies of NAFLD have already led to new etiologic insights. More studies are warranted to discover new SNPs associated with NAFLD, better use the current SNPs to elucidate the pathology of NAFLD, and determine how genetic variation might account for racial and ethnic differences in the propensity to accumulate hepatic fat.

LIFESTYLE AND NAFLD

While the genetic studies are provocative, the contemporary global increase in NAFLD prevalence suggests that environmental, not genetic, factors play the central role in NAFLD etiology. The prevailing lifestyle recommendations for NAFLD patients, i.e., secondary prevention, are to lose weight and increase physical activity. However, primary prevention of NAFLD through lifestyle modification is also important. Here, we review lifestyle risk factors for NAFLD, emphasizing studies of nutrition and physical activity.

Diet

As this dissertation focuses on early growth and development and pubertal timing in relation to NAFLD, a comprehensive synthesis of the literature relating diet and NAFLD is beyond the scope of this review. In brief, the limited evidence on this topic stems from small case-control and cross-sectional studies of limited sample size and poor methodological rigor. These studies have reported a positive association between NAFLD/NASH and consumption of fructose and sugar-sweetened beverages,[318-320] meat,[319] saturated fat and cholesterol.[321,322] Conversely, higher consumption of fiber,[323] fish or omega-3 fatty acids,[319,323] PUFA,[322] some vitamins,[321] coffee,[324] and moderate consumption of alcohol [324] have been associated with lower rates of NAFLD. One study investigated dietary patterns and NAFLD in adolescents, finding that a Western dietary pattern was associated with greater odds of NAFLD, and a 'healthy' dietary pattern was associated with lower odds of NAFLD.[325] Future research on the role of diet in NAFLD, including prospective cohort studies and experimental human trials, is warranted.

Physical Activity

The literature on physical activity and sedentary behaviors in association with measures of liver fat is sparse and limited to small cross-sectional studies. In one study, patients with NAFLD reported engaging in less physical activity, of any sort, than their non-NALFD counterparts, after adjustment for insulin resistance, adiponectin, and diet.[326] Another study showed an inverse association between physical fitness and NAFLD, independent of BMI.[327] These findings have been corroborated by a biopsy study of 37 NAFLD patients, in which there was a lower cardiorespiratory fitness among NAFLD

patients with a higher NAFLD activity score.[328] Thus, while increased physical activity and decreased sedentary activity are important for chronic disease prevention in general, there is limited evidence to infer a causal role for these factors in the prevention or therapy of NAFLD.

In summary, the majority of findings on diet and physical activity in relation to fatty liver disease are from small cross-sectional or case-control studies that may be prone to temporal or selection bias, respectively. However, the limited evidence provided by these studies does indeed suggest nutrition and physical activity may play important roles in the prevention and amelioration of NAFLD. To provide stronger evidence for causal inference on this topic, large prospective studies and clinical trials are need to determine whether these modifiable risk factors can prevent or reduce the occurrence of NAFLD.

Even if weight change, dietary restriction/modification, and physical activity are causally related to development of NAFLD, long-term changes to these lifestyle factors, once they are established, are rarely maintained in the long term.[229] Moreover, there is limited evidence for the efficacy of long-term lifestyle modifications in the prevention or remediation of NAFLD.[329] Therefore, primordial prevention of NAFLD should be a public health priority. The first step in primordial prevention is identifying antecedents of disease that provide early-life windows of opportunity for establishing healthful lifestyle behaviors before disease risk factor onset.

EARLY LIFE ANTECEDENTS OF NAFLD

Understanding the antecedents of adult NAFLD is a first step toward primordial prevention of liver and non-liver related morbidity and mortality. In addition to progressing to severe steatohepatitis, cirrhosis, and hepatocellular carcinoma, NAFLD independently increases risk for developing type 2 diabetes and cardiovascular disease in adults. The literature base on the early-life origins of liver function and NAFLD is still in its infancy. A review on the topic has concluded that more studies are needed to shed light on early-life antecedents of hepatic fat accumulation and overt NAFLD and NASH.[330] Here, we summarize the literature on the developmental origins of NAFLD.

Fetal and Postnatal Origins

According to the 'fetal programming' hypothesis, prenatal undernutrition in mid- and late-gestation results in brain maintenance at the expense of the growth of other organs, including the liver.[233,331] Such allocation of scarce resources may result in abnormal liver function later in life.[332]

Support for this hypothesis comes from both human and animal studies. In rats, a low-protein diet during gestation resulted in reduced liver size of the offspring, but no alteration in growth of the brain or the heart.[333] In addition to reduced liver size, maternal low-protein diets in rats have been shown to permanently alter hepatic structure and function in offspring.[334] Magee et al. showed that intrauterine growth restricted rats displayed down regulation of peroxisome proliferator-activated receptor (PPAR) α and γ and up regulation of sterol regulatory element-binding protein-1 and fatty acid

synthase.[335] These proteins are involved in the pathogenesis of NAFLD *via* regulation of lipid metabolism and lipid-related inflammatory response.[336]

Human studies offer further support for the fetal programming hypothesis. In a hospital-based case-control study, Nobili et al. found that the prevalence of small-for-gestational-age was 4 times higher in 90 Italian children admitted with biopsy-proven NAFLD compared to a control group selected from the general pediatric population in the hospital.[246] In another study of 50 human infants matched on gestational age, those born small-for-gestational-age had reduced liver and kidney dimensions, but not spleen dimensions.[337,338] Direct evidence for the hypothesis comes from a large (*n*=10,7533) Danish prospective linkage study, in which the strongest association between measures of birth weight/length and cause-specific mortality was observed for deaths attributed to liver cirrhosis [Hazard ratio for a 500g increase in birth weight was 0.73 (95% CI: 0.58, 0.91) and for a 5 cm increase in length was 0.55 (95% CI: 0.36, 0.85)].[339] Perhaps the strongest evidence implicating intrauterine growth in the etiology of adult liver disease comes from the British Women's Heart and Health Study. In this study of 2,101 British women aged 60-79, increasing birth weight was associated with decreases in ALT and gamma glutamyl transferase (GGT).[340] The birth size-liver enzyme associations were attenuated by adjustment for components of the metabolic syndrome. This suggests that the associations with elevated liver enzymes were more likely due to NAFLD—i.e., the hepatic manifestation of the metabolic syndrome—than other liver disease.

Childhood Origins

Childhood growth and maturational development have been linked to adult obesity, metabolic syndrome, and type 2 diabetes (reviewed in *Chapter 3*). Despite the interrelated nature of hepatic steatosis and steatohepatitis with these cardiometabolic diseases, no epidemiologic studies have investigated prepubertal nutrition and pubertal timing in relation to NAFLD or NASH. An incipient step in exploring early-life conditions and long-term health and disease is the use of markers of childhood nutritional status and developmental timing that can be ascertained in adults. Two markers shown to serve well in this regard are relative leg length and age at menarche.

Relative leg length

Relative leg length has been shown to be a proxy for prepubertal living conditions, including quality of childhood nutrition,[231] and timing to pubertal onset.[255,257,258,341] In addition to a crude proxy for pubertal timing, short legs relative to height may indicate adverse nutrition during the first years of life.[231] Poor nutrition during in the first years of life alters the metabolic programming of vital organs, such as the pancreas and liver, in ways that may increase metabolic disease risk later in life.[265] In this light, Fraser et al. investigated leg length in relation to liver enzymes in British women, and found that leg length was inversely associated with levels of ALT, GGT, and ALP, after adjusting for childhood and adulthood social class, physical activity, smoking, alcohol consumption, waist-to-hip ratio, and trunk height.[31] These findings suggest that quality of childhood nutrition and/or pubertal timing may play a role in the early origins of liver dysfunction.

Age at menarche

Hyperinsulinemia, resulting from insulin resistance at the hepatic and systemic level, is thought be a driver of early menarche.[342] Higher insulin concentrations have been shown to increase genetic expression of lipogenic enzymes and decrease expression of fatty acid oxidation, thereby engendering hepatic steatosis.[295,296] Despite this mechanistic support, no studies have examined the association between age at menarche and NAFLD.

Tangentially, a few studies have investigated age at menarche in relation to hepatocellular cancer. A case-control study found that a 1-year increment in age at menarche was associated with 21% lower odds of hepatocellular carcinoma, although this association did not reach statistical significance (p=0.10). Cases of liver cancer in this study also had higher levels of testosterone and sex hormone binding protein and reduced levels of IGF-1 and its binding protein.[343] In a case-control study in Taiwan, age at menarche was inversely associated with odds of hepatocellular carcinoma (odds ratio for age at menarche ≤12 vs. ≥16 years was 2.65 (95% CI: 1.27, 5.52) after multivariable adjustment].[344] These studies provide evidence that age at menarche, and the steroid hormones involved in its regulation, may be involved in the pathogenesis of liver disease.

More direct evidence for the association between early menarche and hepatic steatosis comes from human trials. Ibanez and colleagues conducted a series of highly informative randomized trials in girls with low birth weight (birth weight <-1.5 standard deviation score for gestational age) and either early puberty (stage 2 breast development at age 8-9 years) or precocious puberty (pubic hair <8 years). In one trial, 38 low-birth-weight, precocious-puberty girls, 8 years of age, were randomized to either metformin or no treatment for 4 years, and followed until 2 years past menarche. After the trial, the age

at menarche was 11.4 (standard deviation 0.1) years in untreated girls and 12.5 (standard deviation 0.2) years in metformin treated girls.[342] In measurements taken after onset of menarche, the metformin-treated girls were taller with less visceral and hepatic fat, and had more favorable levels of circulating insulin, androgens, and lipids than the untreated girls. In another trial from this same group of high-risk girls, early (8-12 years) metformin treatment was superior to late (13-14 years) metformin treatment for preventing or delaying the onset of hirutism, androgen excess, oligomehorrhea, and PCOS.[262] In a separate trial of low birth weight girls with early puberty (i.e., stage 2 breast development at age 8-9 years), metformin therapy for 36 months delayed onset of menarche, augmented attained height, and was associated with lower levels of insulin, IGF-1, and leptin and higher levels of SHBG and IGF-1 binding protein.[90] Collectively, these findings demonstrate that metformin, an insulin-sensitizing drug, taken pre-menarche can delay menarcheal onset, increase stature, and reduce hepatic fat deposition. Moreover, they provide evidence for biologic interplay between menarcheal onset, insulin resistance, and hepatic steatosis.

SUMMARY

NAFLD is increasing in prevalence worldwide and now stands as the leading cause of chronic liver disease in the U.S.[274] Its association with fatness, insulin resistance, hypertension, and hypertriglyceridemia have led some to call NAFLD the hepatic manifestation of the metabolic syndrome.[293] Perhaps most poignant is the significant long-term liver and non-liver related morbidity and mortality associated with NAFLD.[275]

In addition to 5-10% weight loss, modification of lifestyle, including increased physical activity and a prudent diet, has been recommended for remediation of NAFLD.[264] Yet, maintaining successful long-term change in these programmed lifestyle habits once metabolic conditions manifest is a behavioral-health challenge.[228] Even if achieved, such therapy will not stem the growth of the NAFLD epidemic at the societal level. Detecting the etiologic antecedents of NAFLD can circumvent such futile secondary-prevention lifestyle change efforts, and thereby provide a path to primordial prevention of NAFLD and its liver- and non-liver related sequelae.

Identifying markers of early-life growth and development that predict development of NAFLD should thus be a public health priority. In addition to being associated with NAFLD-related metabolic conditions, age at menarche has been linked to the development of hepatocellular carcinoma.[343,344] Relative leg length, a marker of early-life net nutrition and pubertal timing, has also been linked to liver function in adulthood.[31] Furthermore, experimental data indicates that administration of metformin delays age at menarche, increases stature, and reduces liver inflammation.[89,90,262,342] As a whole, these studies provide theoretical support for the hypothesis that earlier age at menarche and shorter legs relative to stature are associated with increased risk of NAFLD. Large cohort studies with measures of liver fat are now needed to test whether direct associations between age at menarche, relative leg length, and NAFLD indeed exist.

CHAPTER 5: STUDY DESIGNS AND DATA COLLECTION

OVERVIEW

This dissertation relies on data from three distinct cohort studies: Manuscript 1, Estudo Longitudinal de Saúde do Adulto (ELSA)-Brasil (i.e., The Brazilian Longitudinal Study of Adult Health); Manuscript 2, Coronary Artery Risk Development in Young Adults (CARDIA) study; and Manuscript 3, National Heart, Lung, And Blood Institute (NHLBI) Growth and Health Study (NGHS). Here, I describe these studies' populations, design, aims, and data collection methods.

ESTUDO LONGITUDINAL DE SAÚDE DO ADULTO (ELSA)-BRASIL

Study Design and Population

ELSA-Brasil is a cohort study of 15,105 civil servants from 5 universities and 1 research institute located in different regions of Brazil: the federal universities of Bahia, Espirito Santo, Minas Gerais, and Rio Grande do Sul; the University of São Paulo; and the Oswaldo Cruz Foundation.[345] The main study objectives of ELSA-Brasil were to investigate the incidence and progression of diabetes and cardiovascular diseases and their biologic, behavioral, environmental, occupational, psychological, and social factors.

Active or retired employees, aged 35–74 years, of the 6 institutions were eligible for the study. Exclusion criteria were current or recent (< 4 months prior to the first interview) pregnancy, intention to quit working at the institution in the near future, severe cognitive or communication impairment, and, if retired, residence outside of a study center's corresponding metropolitan area.

There were two phases to participant recruitment. In the first recruitment phase, lasting approximately one hour, informed consent was obtained and an initial interview was conducted at the participant's job site. The second phase, consisting of additional interviews and exams, lasted approximately six hours and was conducted at a study clinic. To be considered a participant, an individual must have completed the initial interview, an electrocardiogram, fasting blood collection, and blood pressure measurement.

The final ELSA sample included volunteers (76% of the final sample) and actively recruited participants (24%), the latter being recruited from listings of civil servants. The research protocol was approved by the ethics committee of each institution and also by Brazil's National Research Ethics Committee. In addition to the usual informed consent, participants were also asked to consent to the storage of biologic samples.

Data for the current analysis comes from the first (baseline) exam, carried out from 2008 through 2010. At baseline, detailed information about health status was collected, including clinical exams. The cohort is currently being followed annually to verify general health status. At three-year intervals participants will return to the ELSA Investigation Centers to complete detailed evaluations, including clinical exams.

Data Collection

Independent variables for this analysis include age at menarche and relative leg length (i.e., leg length-to-sitting height ratio). Women were asked at baseline exam, "At what age did you have your first menses?" This information was used to derive age at

menarche. Adult retrospective reports of menarcheal age are highly correlated (r = 0.79) with original adolescent reports.[346] Leg length is the difference between stature and sitting height. In ELSA-Brasil, sitting height was measured from the vertex of the head to the seated buttocks (*See* **Figure 5.1** adapted from NHANES anthropometric manual). Anatomically, leg length is defined as the sum of the lengths of the femur and tibia. It assumes that in the seated position the proximal landmark is the hip joint, which can be difficult to locate. In ELSA-Brasil, as in other epidemiological studies in which ease of measurement was considered, leg length comprises the sum of lengths of the femur, tibia, and foot (tibia-talus to the ground). Relative leg length is calculated as height – sitting height / height, and constitutes the proportion of total stature composed by the legs. Depending on the setting, relative leg length has been shown to reflect *1*) net nutritional status during prepubertal physical growth (in more impoverished populations), and *2*) time until peak height velocity or pubertal timing (in more affluent populations).[231]

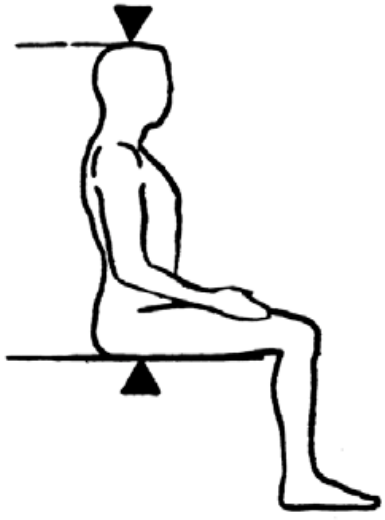

Figure 5-2: Sitting height measurement

Laboratory measures

A 2-hour 75-g oral glucose tolerance test (OGTT) was administered to all participants *without* known diabetes. Glucose was measured by the hexokinase/glucose-6-phosphate dehydrogenase method (Roche Diagnostics). Glycated hemoglobin (A1C) was measured using a Tinaquant II immunoassay method (Roche Diagnostics, Basel, Switzerland).

Assessment of Diabetes

A participant was considered to have diagnosed diabetes if they responded in the affirmative to either *1*) "Have you been previously told by a physician that you had diabetes (sugar in the blood)?" or *2*) "Have you used medication for diabetes in the past 2 weeks?" Individuals without diagnosed diabetes were then classified as having undiagnosed diabetes if they exceeded the thresholds for fasting plasma glucose ≥126 mg/dL or 2-hour plasma glucose ≥200 or A1C ≥6.5%. Total diabetes was calculated as a sum of diagnosed and undiagnosed diabetes.

CORONARY ARTERY RISK DEVELOPMENT IN YOUNG ADULTS (CARDIA) STUDY

Study Design and Population

CARDIA is an ongoing multi-center, longitudinal, community-based study funded by the National Heart, Lung, and Blood Institute. CARDIA was undertaken to study the evolution of coronary heart disease risk factors from young adulthood onward. Young adults were recruited mostly by phone from Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. Geographically based recruitment stratification was used (a list of Kaiser-Permanente health plan members was used in Oakland) to obtain nearly equal numbers of African Americans and Caucasians, younger (18-24 years) and older (25-30 years) individuals, and those with more (≤12 years) and less (>12 years) education.[347] A decision to select only one person per household was made to ensure each participant was statistically independent.

In total, there were 5,115 black and white men and women, aged 18-30 examined in 1985-1986. Participants have now been followed for 25 years, including 8 clinic exams conducted in 1985/6 (baseline, or exam year 0), 1987/8 (year 2), 1990/1 (year 5), 1992/3 (year 7), 1995/6 (year 10), 2000/1 (year 15), 2005/6 (year 20), and 2010/11 (year 25). Informed consent was obtained from all participants at each exam, and the institutional review board at each of the clinical centers approved all protocols.

Data Collection

Information on several potential risk factors for NAFLD was collected in CARDIA. Starting at baseline, the following variables relevant to our analyses were measured: age,

sex, race, height, weight, educational level (years of schooling), past physical activity (before high school, during high school), current physical activity (The CARDIA Physical Activity History Questionnaire[348]) graded exercise test, cigarette smoking history (current, former, or never), alcohol consumption, and dietary history (focused on past month and based on modified Burke method[341]), and medical history. Time-dependent variables were collected at each exam. Age at menarche was ascertained from the open-ended question at year 0 and repeated in year 2, "How old where you when you began menstruating?" Sitting height was directly measured at the year 2 exam.

Anthropometric assessment was made after participants changed into light clothing and removed shoes. Trained and certified technicians made all measurements, and regular quality control checks were made throughout the exam cycle. Standing height was measured with the participant standing erect on the floor with his or her back against a vertical-mounted centimeter ruler, looking straight ahead with his or her head in the Frankfort horizontal plane (the plane that includes the lower margin of the bony orbit and the upper margin of the external auditory meatus). A plastic triangle was placed on the top of the head, and the participant's height was measured to the nearest 0.5 cm. Sitting height was measured with the participant seated in an erect position on a stool with a horizontal surface, and the participant's back in contact with the wall-mounted metal ruler at both the scapula and buttocks and the head held in the Frankfort horizontal plane. Measurement was then made to the nearest 0.5 cm using a plastic triangle placed snugly on the top of the participant's head. Leg length was calculated as standing height minus sitting height. Body mass index was calculated as weight in kilograms divided by the square of the height in meters.

Clinical and laboratory measures

Clinic measurements were standardized for all field centers at the year 25 visit and have been consistent across examinations. Blood was drawn by venipuncture after a 12 hour fast. Glucose was measured as hexokinase coupled to glucose-6-phosphate dehydrogenase (Millipore, Inc, Bellerica, MA), and insulin was quantified using an Elecsys sandwich immunoassay (Roche Diagnostics Corporation, Indianapolis, IN).

Liver Attenuation and Fat Depots

A unique feature of CARDIA is the computed tomography (CT) taken at year 25 exam. Women who were pregnant or potential pregnancy were excluded from the CT exam. Individuals weighing greater than the CT couch limit (400 lbs) or unable to fit within the CT gantry were excluded. CT images acquired at the field centers were electronically transmitted to the central CT reading center located at Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Liver attenuation

Liver attenuation was measured on non-contrast CT images of the upper abdomen acquired in an axial scan mode. A total of 9 regions of interest were measured on three slices spaced at 5 mm a part along the z-axis of the patient (head-to-foot). The 9 regions were averaged to provide mean hepatic attenuation in Hounsfield Units (HU). Analysts performing the measurements were trained to avoid placing regions of interest such that they included hepatic vasculature or common hepatic lesions (cysts and hemangiomas).

Adipose tissue depots

CT scans of the abdomen were reconstructed into 5 mm slices with the maximum 50 cm field-of-view to include the whole abdomen for body composition. Total and adipose

tissues were measured volumetrically from two 5 mm contiguous slices located at the level of the lumbar disk between the 4^{th} and 5^{th} vertebra. Tissues with attenuation between -190 to -30 Hounsfield units were defined as adipose tissue. Experienced analysts used the Medical Image Processing, Analysis, and Visualization (MIPAV--http://mipav.cit.nih.gov/index.php) application to segment the images based on anatomic boundaries (skin, subcutaneous fat-muscle interface and peritoneum) into the entire abdomen, abdominal wall and intra-abdominal compartments. In each compartment, total abdominal volume, total abdominal adipose tissue, subcutaneous adipose tissue and visceral adipose tissue contained within the 10 mm slice located at L4-5 were quantified.

THE NATIONAL HEART, LUNG, AND BLOOD INSTITUTE GROWTH AND HEALTH STUDY (NGHS)

Study Design and Population

The NGHS cohort was formed in 1987-1988 to investigate racial differences in diet, physical activity, and familial and psychosocial factors in relation to obesity. The study enrolled 2,379 African American and Caucasian girls aged 9 and 10 from three clinical centers: University of California at Berkeley, University of Cincinnati/Cincinnati Children's Hospital Medical Center, and Westat, Inc./Group Health Association in Rockville, Maryland. The Maryland Medical Research Institute in Baltimore is the Coordinating Center, and The Johns Hopkins University Department of Lipid Research is the Central Lipid Laboratory.

Girls were eligible for enrollment in NGHS if: *1*) they declared themselves as being either black or white; *2*) they were within 2 weeks of age 9 or 10 at the time of the first clinical visit; *3*) they had parents or guardians who identified themselves as the same race as the child; *4*) their parents or guardians completed a household demographic information form and gave consent.

Data Collection

Participants were seen annually for 10 visits, including data from age 9 to 19. Trained study staff using standardized assessment protocols at each examination measured height and weight. Almost 90% of the girls originally enrolled in the cohort participated through year 10. The institutional review boards of each participating institution approved the study protocol, and an independent monitoring board provided study oversight.

Demographic information was collected at study entry from girls and their parents (or guardians). Race (black or white) was defined by self-report, by using US Census categories. Participants' age was recorded as their age at last birthday. BMI (weight in kilograms divided by height in meters squared) was calculated annually on the basis of the research staff's measures of girls' height and weight.

Diet Assessment

During each of 8 exam years dietary data were collected using 3-day diet records, which comprised 2 weekdays and 1 weekend day. Study nutritionists trained participants to record detailed dietary information using standard household measuring instruments for the estimation of portion sizes. The research dietitian excluded diet records considered unreliable.

Dietary data were entered into the Nutrition Data System of the University of Minnesota, Minneapolis, to estimate the intake of total calories, macronutrients, and micronutrients.[349] The Nutrition Data System also outputs food codes for each food and each ingredient from composite foods. The Nutrition Data System food code data were combined with the US Department of Agriculture's survey food code database, the Food and Nutrient Database for Dietary Studies, version 2.0. By matching these food codes, the child's average daily intake was derived in each of the 5 major food groups and in all the subgroups as defined by Nutrition and Your Health: Dietary Guidelines for Americans by the US Department of Agriculture (Nutrition and Your Health: Dietary Guidelines for Americans. 5th ed. Washington, DC US Government Printing Office. 2000).

Anthropometric assessment

Anthropometry in this study included height, weight, hip and waist circumferences, and triceps, subscapular, and suprailiac skin folds. All measurements were taken twice, with a third measurement if the first two differed markedly. For skin folds this difference criterion was 1.0 mm, and for circumference measurements, 1.0 cm. When three measurements were taken, the closest two were averaged. To ensure anthropometric procedure consistency across study sites, a master anthropometric trainer was designated to train local trainers, who then provided training to field staff in each study site, with recertification annually. The NGHS anthropometric techniques have been described in detail elsewhere.[350]

Pubertal Assessment

Participants were queried annually regarding onset of menstruation to determine the date of menarche. At each annual visit, trained female examiners assessed breast development and pubic hair stage (Tanner stages 1-5), using criteria established by Marshall and Tanner.[55] Participants were examined in standard measurement gowns.

SUMMARY OF STUDY DESIGNS AND DATA COLLECTION

This dissertation uses three distinct cohort studies to answer unique questions related to childhood nutrition, pubertal timing, and metabolic disease risk. The aim of first of these studies is to investigate how relative leg length and age at menarche, markers of early-life nutrition and pubertal timing, associate with the development of diabetes in an adult Brazilian population undergoing rapid epidemiologic and nutritional transitions. While these markers have been shown to predict diabetes in Western and prosperous Asian

populations, there have been no investigations on this topic in Latin American populations. The rich data from ELSA-Brasil afford the opportunity to study age at menarche and relative leg length in relation to type 2 diabetes after accounting for potential confounding by factors, e.g., paternal socioeconomic status, paternal diabetes, birth weight, and body mass index at 20 years of age, as a proxy for childhood BMI. Moreover, the large sample size allows for evaluation of effect modification on these associations by measures of nutritional status throughout the lifecourse. Discovery of early-life antecedents of diabetes in this population represents an important step toward primordial prevention in Brazil.

Manuscript 2 (*Chapter 7*) of this dissertation represents the first study to explore the association between markers of pubertal timing and NAFLD. This paper will also shed light on the association between pubertal timing and specific fat depots in adulthood. The CARDIA Study is uniquely apt to answer the question of whether earlier age at menarche and shorter relative leg length are associated with higher prevalence of non-alcoholic fatty liver. In addition to having a vast array of demographic, lifestyle, psychosocial, and physiologic variables that can be assessed as potential confounding, mediating, or modifying factors, the CARDIA study has a large sample of subjects with CT-derived intra-abdominal fat measures and liver attenuation. This is rare in the field of epidemiology. Assessing liver steatosis in a large number of subjects requires a significant research investment, which is why there are few large studies with data on hepatic steatosis and NAFLD. As such, the CARDIA data set can be viewed as an invaluable resource for determining for the first time whether pubertal timing is associated with specific fat deposition, and the development of NAFLD.

The objective of the third manuscript of this dissertation (*Chapter 8*) is to explore the prospective association between diet quality and early menarche in a biracial cohort of girls from the U.S. To achieve this aim, we use the NGHS, a longitudinal dataset that includes detailed assessment of dietary behaviors at baseline and 7 other time points throughout the 10-year study follow-up period. In addition to having excellent measures of diet and pubertal assessment throughout the study, the NGHS dataset provides a comprehensive array of demographic, lifestyle, psychosocial, and physiologic variables to control for potential confounding. Further, the NGHS has collected skin-fold measurements that allow for careful control of fatness in these analyses. In sum, the NGHS dataset affords an exceptional opportunity to identify the role of diet quality in the timing of puberty.

CHAPTER 6: MANUSCRIPT 1—AGE AT MENARCHE, RELATIVE LEG LENGTH, AND TYPE 2 DIABETES IN BRAZILIAN ADULTS

Background: Early puberty has been linked to higher rates of type 2 diabetes in prosperous societies, yet whether markers of pubertal timing are associated with diabetes in developing countries, where the etiology of puberty and diabetes may differ, is unknown. In a population-based cross-sectional sample of Brazilian adults (34-75y) we tested the hypothesis that age at menarche and relative leg length, as markers of pubertal timing and growth and development, are associated with presence of type 2 diabetes, independent of demographic and lifestyle factors, and indicators of early-life fatness.
Methods: Participants (8032 women; 6736 men) from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) with complete information on age at menarche, height components, and variables used to diagnosis type 2 diabetes (i.e., self-reported diagnosis, medication use, and laboratory values) were included. Poisson regression with robust variance was used to generate prevalence ratios (PR) and 95% confidence intervals (CI).
Results: Early menarche [8-11 years vs. 13-14 years (reference)] was associated with higher prevalence of type 2 diabetes (PR=1.22; 95% CI: 1.03-1.45) after control for potential early-life demographic and lifestyle confounders, and BMI at age 20 years. In the same model, greater relative leg length was associated with lower prevalence of type 2 diabetes in women (PR for 1 SD increment in relative leg length=0.90; 95% CI: 0.85-0.95). The association was of similar magnitude in men (PR for 1 SD increment=0.89;

95% CI: 0.85-0.93). The association between relative leg length and diabetes was stronger among females with earlier menarche (*p* multiplicative interaction=0.02), and among adults overweight or obese at age 20 (*p* interaction=0.02) or at baseline (*p* interaction=0.03).

Conclusion: These findings support the hypothesis that age at menarche and relative leg length are independently associated with presence of type 2 diabetes in Brazilian adults.

BACKGROUND

Type 2 diabetes has emerged over the past two decades as a major priority in the health agenda of the underdeveloped world.[177,179] Developing countries, while still suffering nutritional burdens of scarcity and infection, are faced with the challenge of managing type 2 diabetes and micro- and macro-vascular diseases resulting from hyperglycemia.[177,178] In most low- and middle-income countries, type 2 diabetes is now a leading cause of mortality and morbidity, due to its long duration and devastating consequences with respect to quality of life and economic burden. In Brazil, this phenomenon has been documented by routine surveillance and periodic surveys on chronic diseases and their risk factors collected by the Ministry of Health.[351]

The rise of type 2 diabetes in Brazil is multi-factorial in origin. In addition to the aging Brazilian population, there has been a rapid rate of migration from rural to urban environments.[352] Social, behavioral, nutritional, and environmental changes accompanying this transition are believed to underlie increases in fatness, insulin resistance, and ultimately type 2 diabetes.[353] Once obesity and diabetes manifest, their remediation through behavior change poses tremendous challenges.[228,229] And even if weight-loss through intensive lifestyle intervention is achieved in diabetics, it may be too late to reduce incidence of cardiovascular events.[354] This emphasizes the need for prevention before type 2 diabetes and its risk factors develop (i.e., primordial prevention).

Identifying valid markers of pubertal timing could pave the lifecourse avenue to primordial prevention of type 2 diabetes and its risk factors at the preclinical stage. Pubertal timing is influenced by genetic and environmental factors, including childhood nutrition and obesity.[163-165,167,168,256] In females, menarche is the most distinguishable

pubertal marker. There is evidence for a trend towards earlier menarche in Brazil over the last several decades,[355] and this is not explained by childhood obesity.[356] Relative leg length (i.e., leg length-to-sitting height ratio) has been proposed as a crude but objective marker of pubertal timing that can be used in both sexes.[24] It is sensitive to prepubertal growth rate and duration,[164,256] and greater relative leg length has been associated with better early-life nutrition,[255] earlier menarche,[258] and earlier peak height velocity.[256,357]

Studies from more prosperous Western and Asian populations have reported that earlier age at menarche[19,21-23,264,267,268] and lower relative leg length[261-263264] are associated with increased type 2 diabetes risk. No studies have examined these associations in a economically and nutritionally transitioning Latin American population where contextual influences on age at menarche and relative leg length, in relation to body fat and type 2 diabetes, may differ in important ways. Further, no studies have examined whether these associations are modified by measures of nutritional status later in life. According to the thrifty phenotype and predictive-adaptive response hypotheses, mismatch between early- and late-life nutrition may interact synergistically to increase diabetes risk.[232,265]

Considering the current gaps in knowledge, the aim of this manuscript was to examine age at menarche and relative leg length in relation to type 2 diabetes in Brazilian adults. Specifically, we will test the hypotheses that *1*) earlier age at menarche is associated with higher prevalence of diabetes, and *2*) greater relative leg length is associated with lower prevalence of diabetes. We will further evaluate whether markers of nutritional status throughout the lifecourse (e.g., age at menarche or relative leg length, BMI in early adulthood, and BMI in later adulthood) modify these associations.

METHODS

Study participants

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a prospective cohort study designed to identify risk factors for diabetes and cardiovascular disease. The details of the study, including design, eligibility criteria, sources and methods of recruitment, have been described in detail in *Chapter 5* of this dissertation and elsewhere.[345] Briefly, the cohort comprises 15,105 civil servants (8218 females), aged 35 to 74 years at baseline (2008-2010), who were sampled from 5 universities and 1 research institute located in different regions of Brazil: the University of São Paulo (*n*=5061); the federal universities of Minas Gerais (*n*=3115), Rio Grande do Sul (*n*=2069), Bahia (*n*=2029), and Espirito Santo (*n*=1055); and the Oswaldo Cruz Foundation (*n*=1784). All data for the current analyses were collected during initial interviews (~1 hour) and the baseline clinic visit (~5 hours). The ELSA-Brasil sample included volunteers (76% of the final sample) and actively recruited participants (24%); the latter being recruited from listings of civil servants. The ethics committee of each institution approved the research protocol. In addition to the usual informed consent, participants were also asked to consent to the storage of biologic samples and to follow up interviews and hospital chart review.

For the current analyses, participants were excluded if they were missing lab information on all three measures of glycemia (fasting glucose, A1C, and 2-hour glucose) used to ascertain diabetes status in participants without a history of diabetes at baseline (*n*=5). We additionally excluded women with missing age at menarche (*n*=37), and all participants with missing height (*n*=6), or sitting height (*n*=3). We also excluded females

who reported age at menarche <8 years (*n*=7) or >18 years (*n*=11) as we were interested in studying women within a normal range of menarcheal age. To reduce influence from extreme values that were likely data entry errors, we excluded participants with sitting height (*n*=37) or height (*n*=39) greater than three standard deviations (SDs) from the sex-specific mean (i.e., male height > 193.5 cm or < 150.6 cm, female height >178.4 cm or < 139.6 cm; male sitting height >102.6 cm or <79.0 cm, female sitting height > 95.9 cm or < 73.7 cm). We further excluded participants who were missing race/color (*n*=172). We created a dummy variable for participants missing birth weight (~14%). Study exclusions/inclusions did not materially alter results. Our final analytic sample comprised 8032 women and 6736 men.

Data collection

Exposure assessment

All variables used in the current cross-sectional study were collected at baseline examination that was carried out from 2008 through 2010. Age at menarche was assessed by the open-ended question, "At what age did you have your first menses?" Adult retrospective reports of menarcheal age have a moderately high (r = 0.79) correlation with original adolescent reports.[346] Height and sitting height (vertex of the head to the seated buttocks) were measured according to standard equipment and techniques. Leg length was determined by taking total height minus sitting height. Relative leg length (i.e., leg length-to-sitting height ratio) was calculated by taking leg length divided by sitting height. Sex- and race-specific quintiles of relative leg length were calculated.

Outcome assessment

Diabetes status was assessed at the baseline using blood glucose measurements and self-reported information about diabetes. A participant was considered to have previously diagnosed diabetes if they answered, “yes” to either “Have you been previously told by a physician that you had diabetes (sugar in the blood)?” or “Have you used medication for diabetes in the past 2 weeks?” We excluded participants whose self-reported age of diabetes diagnosis was earlier than 30 years to decrease the chance of including type 1 diabetes cases.

If individuals answered “no” to both aforementioned diabetes questions they were evaluated for undiagnosed diabetes based on their laboratory values for glucose. A 12-hour fasting blood sample was drawn by venipuncture soon after the patient arrived at the baseline clinic visit. A 2-hour 75-g oral glucose tolerance test (OGTT) was administered only to participants without known diabetes. Glucose was measured by the hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois). Glycated hemoglobin (A1C) was measured using a high pressure liquid chromatography (Bio-Rad Laboratories, Hercules, California), and insulin using an immunoenzymatic assay (ELISA) (Siemens). Participants without previously diagnosed diabetes were considered to have diabetes if they exceeded the thresholds for fasting plasma glucose ≥126 mg/dL, 2-hour plasma glucose ≥200 mg/dL, or A1C ≥6.5%. Total type 2 diabetes was the sum of previously diagnosed and newly classified diabetes.

Covariate assessment

A comprehensive set of questionnaires, tests, and measurements was carried out to control for co-varying parameters. Age at baseline visit (years), race/skin color,

educational achievement of the participant and their mother, parental history of diabetes, birth weight, polycystic ovary syndrome, menopause, parity, oral contraceptive use, hormone therapy, smoking status, alcohol use, physical activity, and diet were ascertained by questionnaire. Body mass index (BMI) was calculated as weight divided by height squared. Participants were asked to recall their weight at age 20 years to determine their young adult BMI. Hip circumference was measured at the maximal protrusion of the hips and waist circumference was measured at the midpoint between the iliac crest and lower costal border along the mid-auxiliary line with the participant standing erect.

Statistical analysis

All analyses were performed using SAS 9.2 (SAS institute, Cary, NC). We summarized the characteristics of the cohort using unadjusted means and standard deviations for continuous variables and percentages for categorical variables according to menarcheal age categories (<11, 11-12, 13-14, 15-16, and 17-18 y) and relative leg length quintiles.

To determine whether age at menarche was uniquely related to specific height dimensions, we used multivariable linear regression to estimate adjusted means for components of height (total, sitting, leg) in relation to a 1-year increment in menarcheal age before and after adjustment for age at enrollment, race/ethnicity, education, and birth weight. We then stratified these analyses by birth weight and educational level, to evaluate whether the associations occur at different levels of intrauterine growth and socioeconomic status indicators. Evidence for departure from linearity was determined by examining the means of height components across age at menarche categories.

To address the main hypotheses of our study, we used multivariable Poisson regression with robust variance.[358] This allowed us to estimate prevalence ratios (PRs) and 95% confidence intervals (CIs) for prevalent diabetes at study enrollment according to categories of menarcheal age and quintiles relative leg length. Multivariable models were contrasted in a series beginning with an age-at-interview (years) adjusted model (Model 1), followed by adjustment for potential confounders. Model 2 included further adjustment for study center, race/skin color, parental history of diabetes (yes/no), maternal education (no formal education, less than eighth grade, completed eighth grade but not high school, completed high school but no college, some college), and birth weight (<2500 grams, 2500-4000 grams, >4000 grams, missing). As we were interested in potential confounding by BMI, we further adjusted Model 2 for BMI at age 20 years (the earliest measure of this variable) (Model 3). We consider this a proxy for BMI prior to puberty, as BMI has been shown to track from childhood to young adulthood.[359] Lastly, in models that included only females, we added relative leg length (age at menarche analyses) or age at menarche (relative leg length analyses) to determine if these two markers of growth and development were independent of each other (Model 4). Further inclusion of participant education level (less than high school, completed high school but not college, completed college but no graduate school, graduate school plus), smoking status (never, former, current), alcohol use (yes/no), leisure time physical activity (light, moderate, hard), polycystic ovary syndrome (yes/no; menarche analyses only), postmenopausal (yes/no; menarche analyses only), and parity (0, 1-2, 3-4, 5+; menarche analyses only) did not materially alter the results. As these factors are likely on

the exposure-outcome causal pathway (i.e., mediators), they were not retained in the final model.

We evaluated effect measure modification in the final model by including cross-product terms between our exposures and age at interview (median split; <55 vs. ≥55 years), race/skin color (black vs. white), maternal education (<high school vs. ≥high school), birth weight (< 2500 vs. ≥ 2500 grams), age at menarche (≤12 vs. >12), BMI at 20 years (25kg/m^2 vs. ≥25 kg/m^2), and BMI at baseline visit (25kg/m^2 vs. ≥25 kg/m^2).

In supplemental analyses, we used multivariable linear regression to assess age at menarche categories and relative leg length quintiles in relation to continuous outcome measures for glucose and insulin. For these analyses we excluded participants with known diabetes at study enrollment so as to reduce bias from diabetes diagnosis-related medication use or changes in lifestyle. All statistical tests were two-sided and significance was defined at $p < 0.05$.

RESULTS

Study cohort

The mean (SD) age at menarche for the 8,032 women eligible for this analysis was 12.7 (1.7) years of age. Participant characteristics according to categories of age at menarche are presented in **Table 1**. Earlier menarche was associated with younger age at enrollment into the study, lower grandmultiparity (>4 births), higher maternal and participant educational attainment, and higher paternal diabetes. In addition, earlier menarche was associated with shorter leg length (but not sitting height), higher BMI at

age 20 and baseline visit, and higher hip and waist circumference measured at baseline (**Table 1**).

The mean (SD) leg length-to-sitting height ratio (i.e., relative leg length) in men and women (*n*=14,768) was 0.88 (0.05). In both men and women, greater relative leg length was associated with older age at enrollment into study, lower educational achievement, and lower prevalence of paternal diabetes. Relative leg length was also inversely associated with BMI at age 20 and BMI measured at baseline exam, and hip and waist circumference measured at baseline exam (**Table 2**).

Age at menarche and height components

After adjusting for age, race, education, and birth weight, later age at menarche was associated with greater leg length, but was not associated with sitting height. These associations persisted across categories of participant educational achievement and birth weight (**Table 3**). There was modest statistical evidence that the association between age at menarche and leg length was stronger in those with higher educational achievement (*p* for multiplicative interaction = 0.05).

Age at menarche and type 2 diabetes

Approximately 19.5% of Brazilian adults aged 35–74 years at interview in 2008 through 2010 had diabetes (10.0% were diagnosed before baseline and 9.5% were newly classified at baseline). The association between age at menarche and total diabetes after multiple levels of covariate adjustment is shown in **Table 4**. Age at menarche <11 years [vs. 13-14 years (referent)] was associated with prevalent diabetes after adjusting for potential early-life confounders (Model 2, PR=1.30; 95% CI: 1.10, 1.53). This association was modestly attenuated after further adjustment for BMI at age 20 years

(Model 3, PR= 1.22; 95% CI: 1.03, 1.45), and was not explained by relative leg length (Model 4, PR = 1.20; 95% CI: 1.01, 1.41). There was no association between later categories of age at menarche and prevalent diabetes (**Table 4**). In additional multivariable-adjusted analyses among those without diagnosed diabetes at baseline (*n*=7265), age at menarche was associated with A1C, but not 2-hour glucose, fasting glucose, or insulin concentration (**Table 6**).

Relative leg length and type 2 diabetes

In both men and women, relative leg length was inversely associated with prevalence of type 2 diabetes. After adjustment for potential early-life confounders and BMI at age 20 years (Model 2), a 1-SD (0.05) increment in relative leg length was associated with 10% (95% CI: 5-15%) and 11% (7-15%) lower risk of diabetes in women and men, respectively (**Table 5**). In women, the strength of the inverse association in the upper quintiles was modestly weakened by further adjustment for age at menarche (Model 4). In additional multivariable analyses among those without diagnosed diabetes at baseline (*n*=7265 for women; *n*=5863 for men), relative leg length was inversely associated with all glucose homeostasis and fasting insulin measures in both women and men (**Table 7**). Further adjustment for hip or waist circumference measured at study enrollment did not explain the observed associations (not shown).

In the evaluation of effect measure modification on the multiplicative scale, we observed evidence that the association between relative leg length and type 2 diabetes was modified by menarche age (≤12 years vs. >12 years; *p* for interaction = 0.02), BMI at 20 years of age (<25 kg/m^2 vs. ≥25 kg/m^2; *p* for interaction = 0.02), and BMI measured at baseline exam (<25 kg/m^2 vs. ≥25 kg/m^2; *p* for interaction = 0.03; **Figure 1**). The

association was stronger among women with earlier menarche, and men and women who were overweight or obese at age 20 years and at study enrollment (35 to 74 years). There was no evidence of effect measure modification by birth weight, age at enrollment, education, or smoking on the relative leg length and diabetes association, nor was there evidence of effect measure modification by these measures on the age at menarche and diabetes association.

DISCUSSION

In this large cohort of adult Brazilian men and women, who were born and came-of-age before the economic and epidemiologic transition in Brazil, early menarche (<11 years) was associated with higher prevalence of diabetes. This association remained after adjustment for potential confounders, including race, maternal education, parental diabetes, birth weight, and BMI at age 20 years. Relative leg length, which had a positive but weak association with age at menarche, was inversely associated with diabetes in men and women after full adjustment for potential confounders. The association between relative leg length and diabetes was stronger in adults who were overweight or obese at 20 years of age and at baseline, and in females who reported earlier menarche onset.

Our findings on age at menarche and diabetes are consistent with the literature on this topic from populations that, similar to ours, underwent rapid epidemiologic and nutritional transition after coming of age. Conway et al. found that in 69,385 middle-aged adult Chinese women from the Shanghai Women's Health Study, later age at menarche was associated with lower risk of diabetes, but this association was attenuated upon adjustment for BMI measured at age 40 to 70 years.[24] In the Singapore Chinese Health

Study we found that earlier menarche was associated with increased prevalence of diabetes even after adjustment for BMI, which was self-reported at baseline when the participants were aged 45-74 years.[25]

Studies from countries with longer-standing economic prosperity have also reported an association between earlier menarche and increased risk of diabetes.[20,2319,21,22] Yet, similar to more recently transitioning populations, evidence from developed societies on whether this association is attenuated after adjustment for BMI is discordant. Findings from our study suggest that the association between early age at menarche and diabetes is not explained by BMI measured at 20 years of age (the earliest measure of BMI in our cohort). These results are consistent with those from the ARIC study[20] and a British birth cohort[23] which found that BMI measured at age 25 and pre-menarche, respectively, did not explain the age at menarche-diabetes association. Moreover, our study was the first to examine and find that age at menarche was associated with diabetes independent of relative leg length, implying that these two markers represent unique aspects of early-life growth and development.

Our findings on relative leg length and diabetes largely agree with previous studies on this topic. In middle-aged adults (women aged 40-70 and men aged 40-74 years at baseline) from the Shanghai Health Study, relative leg length was inversely associated with diabetes risk after adjustment for birth cohort, education, and income, but this association was attenuated after adjustment for BMI at baseline.[24] The authors did not provide a parameter estimate from a parsimonious model, restricted to potential confounders and early-life BMI, so we cannot determine whether BMI measured around the time of puberty explained this association. Yet, other studies from Western

populations have reported an association between relative leg length and diabetes that is independent of BMI measured at 25 years of age,[360] as well as BMI[261] and weight and waist-to-hip ratio[263] measured later in adulthood at the time of diabetes ascertainment. Studies that adjust for adulthood BMI and other measures of adiposity, which are on the causal pathway from puberty to diabetes, do so under the presupposition that all potential confounders of the exposure-outcome and mediator-outcome are controlled for. They must also assume no effect modification of the exposure-outcome by the mediator—an assumption that does not hold true in our study. Violation of these assumptions, which is common in the literature on this topic, may lead to biased estimates and a misunderstanding of the mechanisms driving such associations.

The observation in our study that age at menarche in females was related to leg length, but not sitting height, aligns with observations from other populations.[164,256,258] The biologic mechanism most likely governing this association is the cephalocaudal gradient of growth, which posits that growth of the legs occurs more rapidly than the torso early in life up until pubertal onset at which time estrogen surges to cause the cessation of long bone (leg), but not torso.[231] This provides one explanation why early puberty correlates with shorter leg length but not sitting (torso) height. However, in our investigation, the association between menarche timing and leg length was small—a 3 mm change in leg length per 1-year increment in age at menarche—and age at menarche only explained a small percent of variation in relative leg length (~2%). Thus, in ELSA participants, differences in relative leg length appear to be largely driven by factors other than pubertal timing.

Beyond the influence of pubertal timing on prepubertal *growth duration*, relative leg length is sensitive to environmental conditions that affect growth rate during the first years of life when the leg growth is most sensitive to nutritional status.[231] Whether *1*) early-life *growth rate* or *2*) prepubertal *growth duration* plays the largest role in determining relative leg length seems to be determined by the societal context. It has been suggested that as a population transitions from undernutrition to overnutrition, the effects of nutrition on prepubertal growth rate surpass those of prepubertal growth duration.[258] This is supported in our study by the finding that pubertal timing and leg length were more strongly associated among more educated participants. In Brazil when ELSA participants were born (1934-1975) or 5 years old (1939-1980), undernutrition was more common than overnutrition. In population-based surveys from 1974/5, stunting among 5-9 year old girls in Brazil was 26.7%, compared to 12.6% in 1989, and 6.3% in 2008/9. Meanwhile, overweight among 5-9 year old girls increased from 8.6% to 11.9% to 32.0% in 1974/5, 1989, and 2008/9, respectively.[361] Thus, in our study population, which underwent economic and epidemiologic transition between the time participants came of age and were assessed for diabetes status, low relative leg length may reflect inadequate early-life growth rate resulting from undernutrition, more so than advanced pubertal onset (i.e., shorted prepubertal growth duration) due to childhood overnutrition.

If one assumes relative leg length is a marker for early-life net nutrition in this population, our finding of an inverse association between relative leg length and diabetes prevalence suggest that environmental conditions contributing to inadequate nutrition early in life independently increase diabetes risk in adulthood. Accumulating evidence now links early nutritional deprivation to long-term metabolic alterations, including

higher risk for adult obesity[362] and diabetes.[363] For example, it is now widely accepted that intrauterine growth restriction increases risk for insulin resistance and diabetes later in life.[363] Inadequate nutritional status in the first years of life may also cause long-term metabolic perturbations, as physiologic and metabolic mechanisms are not fully matured at birth and continue maturing in the immediate postnatal period.[364]

In support of this notion, several studies have shown that inadequate infant weight gain was associated with coronary heart disease independent of birth weight.[365,366] Another study reported that postnatal undernutrition during the first year of life was associated with higher 2-hour glucose and insulin levels after an OGTT.[367] This may be due to a decrease in β-cell function rather than insulin sensitivity.[368] In addition, studies in adolescents and adult women from Brazil have found that short stature or stunting, a common result of infection or malnutrition,[369] was associated with higher levels of glucose,[370] insulin,[370] and insulin resistance,[370,371] as well as related metabolic drivers, including abdominal obesity,[370-372] reduced lipid oxidation,[373] and reduced total energy expenditure.[374] These findings lend support to the hypothesis that restricted postnatal growth and development, which relative leg length may represent in our study, alters regulation of physiologic mechanisms responsible for energy conservation, resulting in excess adiposity and increased risk of metabolic diseases such as diabetes.

Findings from our study hint at a new wrinkle to this hypothesis. To our knowledge, we are the first to examine and find that early age at menarche and BMI in early and late adulthood modified the association between relative leg length and type 2 diabetes. There are several biologic mechanisms that may explain these statistical interactions. Age at menarche, similar to BMI at age 20 years, may represent increased

early-life adiposity in our study. In this context, effect modification by menarcheal age and early-life BMI on the relative leg length-diabetes association may reflect a mechanistic interaction between height components and body fat stores at different periods of the lifespan. Compared to persons with long legs relative to torso, those with relatively longer torso would seemingly have greater propensity store adipose tissue in more metabolically pernicious abdominal depots. Research is needed to test this hypothesis.

Another potential explanation for the observed statistical interactions lies in developmental plasticity theory. The thrifty phenotype hypothesis posits that adulthood metabolic diseases result from mismatch between early- and late-life nutrition.[232] More recently, the predictive-adaptive response hypothesis proposed that poor early-life environmental conditions induce metabolic changes that maximize health and fitness in similarly poor later-life conditions, but reduce fitness if later-life conditions improve.[265] Thus, if we presuppose that relative leg length is a proxy for early-life net nutrition, our findings suggest that inadequate nutrition early in life and nutritional excess later in life (e.g., early menarche and fatness) may interact synergistically (i.e., beyond their individual influence) to increase diabetes risk. Other cohort studies positioned in societies that have recently undergone nutritional and epidemiologic transition are needed to replicate these findings. If consistent in other populations, alternative study designs, using more direct measures of postnatal, early-life, and adult nutritional adequacy are needed to shed light on the potential causes and mechanisms governing this interaction.

Strengths and limitations

This research took advantage of a rich database in a unique population to study the role of early-life growth and development factors in relation to development of type 2 diabetes. An important limitation to consider when interpreting the results of this analysis was the self-recall of age at menarche many years after the event; thus, misclassification was inevitable. However, adulthood retrospective reports of menarcheal age are highly correlated (r=0.79) with original adolescent reports.[346] One might also consider the cross-sectional nature of this study a limitation. Yet, in our study, age at menarche occurred before diabetes onset, and leg length is fully determined by teenage years. Thus temporality between these exposures and type 2 diabetes is not in question. We cannot rule out the potential for residual confounding by unmeasured socioeconomic or lifestyle factors related to both age at menarche and type 2 diabetes. ELSA-Brasil does not have childhood anthropometry (e.g., BMI) or physiologic measures. Childhood fatness and related hormones may cause early puberty and diabetes. However, based on evidence[359] that BMI tracks well through life, we believe that adjustment for BMI at age 20 years is a good proxy for pre-pubertal BMI adjustment. Finally, ELSA-Brasil comprises employees of universities in Brazilian capitals, thus results may not generalize to all Brazilian adults.

Novelty and public health relevance

Our study provides estimates of average age at menarche in a sample of Brazilian women participating in a large, free-living occupational cohort, born between 1934-1975 and raised during a period when the average gross domestic product of Brazil increased by 81%.[375] Earlier age at menarche was associated with younger age at enrollment into the

study, likely due to the secular trend toward earlier age at menarche in Brazil.[356] While this trend may have been influenced by increases in childhood fatness, our study, corroborating evidence from others,[20,23] suggests that the association between pubertal timing and type 2 diabetes is not accounted for by BMI measured early in life. Our study adds an important piece of evidence to this literature base, showing early menarche was associated with diabetes after full multivariable adjustment, in a context where the confounding structure differs from the previously studied, more prosperous, populations.

We also examined relative leg length, finding it was inversely associated with type 2 diabetes, independent of early-life confounding factors, and BMI. Age at a menarche and relative leg length were independently associated with diabetes, implying these markers reflect unique early life circumstances. The finding that age at menarche was only weakly associated with leg length in our population also supports this notion. In less-prosperous societal contexts, like Brazil during the time ELSA participants were coming of age, low relative leg length may reflect effects of slow growth due to undernutrition, rather than shortened prepubertal growth span (i.e., early puberty) due to overnutrition. These findings highlight the importance of understanding the environmental context of the population under study when using relative leg length (and age at menarche) as a marker of early life growth and development.

Perhaps most the most novel finding of this study was the observation that timing of menarche, and BMI in early and late adulthood, modified the inverse association between relative leg length and diabetes. If we assume that relative leg length is a marker for net nutritional status in the first years of life, this finding suggests that inadequate nutrition early in life may interact with overnutrition later in life to synergistically

increase risk for diabetes. Research in other settings is needed to replicate these findings and elucidate the biologic mechanisms that may underlie these statistical interactions.

Understanding how factors along the lifecourse impact type 2 diabetes, and how such factors can be cost-effectively and sustainably modified, are among the transcendent public health challenges of our time. These challenges are especially critical for population health in low- and middle-income countries, including those in South America, in which changing demographics, in addition to behavioral and environmental risks, are producing rapid increases in diabetes, and where the alternative to risk factor prevention—the widespread use of costly drug- and device-related interventions—is neither practical nor cost-effective. As such, beyond its potential for extending quality years of life, investigation into the primordial prevention of type 2 diabetes and other cardiometabolic diseases is critically important to the future economic viability of health care globally.

Conclusions

We have carefully examined age at menarche and relative leg length, separately, finding that these measures are independently associated with type 2 diabetes. This research provides a platform for continued exploration of the myriad behavioral and environmental factors that alter markers of growth and development and thereby increase risk of type 2 diabetes in Brazil. Moreover, these findings add additional evidence to support the possibility of attenuating metabolic diseases in low- and middle-income countries through identification of, and eventual intervention on, primordial markers of disease etiology.

Table 6.1. Mean and standard deviation (unless otherwise noted) of baseline characteristics by age at menarche in females aged 35-74 years from The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

	8-11 years	11-12 years	13-14 years	15-16 years	17-18 years
N	653	3104	3161	911	203
Age at enrollment	51.4±8.4	51.3±8.9	52.3±8.9	53.5±8.7	53.9±8.3
Race/skin color (% Black)	19.7	18.2	16.8	18.1	22.1
Maternal education (% ≥ HS)	26.0	24.9	22.1	16.9	12.1
Participant education (% ≥ HS)	90.9	92.1	90.5	86.2	86.8
Maternal diabetes (%)	22.3	20.0	19.4	19.2	21.6
Paternal diabetes (%)	19.7	14.9	12.1	10.9	10.8
Premature birth (%)	7.6	5.5	5.1	5.9	6.5
Low birth weight (%)	9.9	8.7	7.5	12.0	10.8
PCOS (%)	14.8	11.1	10.7	9.3	11.8
Post-menopause (%)	60.5	56.7	59.6	64.4	64.7
Grandmultiparous (%)	9.9	9.6	9.4	11.4	11.3
Current smoker (%)	15.9	12.3	10.4	13.5	11.8
Physically active (%)	18.2	20.7	20.0	20.7	16.7
Alcohol (% none)	16.2	13.6	16.7	18.3	24.5
Leg height (cm)	73.0±4.2	73.9±4.0	74.5±4.1	74.7±4.3	75.7±4.2
Sitting height (cm)	84.7±3.7	84.9±3.5	84.8±3.5	84.6±3.5	84.4±3.4
BMI (kg/m^2) at age 20	21.8±3.6	21.1±3.0	20.6±2.8	20.4±3.2	20.0±3.0
BMI (kg/m^2) at baseline	29.0±5.6	27.5±5.2	26.5±4.8	26.6±4.9	26.5±4.8
Hip circumference (cm)	105.7±11.0	103.8±11.0	102.1±.8	102.2±10.7	102.1±9.4
Waist circumference (cm)	91.4±14.0	88.3±12.6	86.6±12.1	87.4±12.5	87.6±12.2

Table 6.2. Mean and standard deviation (unless otherwise noted) of baseline characteristics by quintiles of leg length-to-sitting height in adults aged 35-74 years from The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

	Q1 (0.68-0.86)	Q2 (0.83-0.88)	Q3 (0.86-0.91)	Q4 (0.88-0.94)	Q5 (0.91-1.13)
Women (n)	1606	1606	1607	1608	1605
Age at enrollment	50.8±8.1	51.1±8.6	51.6±8.8	52.2±9.1	54.2±9.3
Race/skin color (% Black)	17.9	17.9	17.9	17.9	17.9
Maternal education (% ≥ HS)	22.1	22.6	23.9	23.8	20.8
Participant education (% ≥ HS)	92.2	91.8	91.8	90.1	86.9
Maternal diabetes (%)	18.7	21.8	19.6	20.2	19.2
Paternal diabetes (%)	14.8	13.8	14.1	12.9	12.5
Premature birth (%)	6.6	4.7	5.6	5.2	5.8
Low birth weight (%)	10.2	8.1	9.6	7.7	7.9
Menarche age (years)	12.4±1.7	12.6±1.7	12.7±1.7	12.8±1.7	13.0±1.8
Current smoker (%)	10.8	12.5	12.1	12.0	12.4
Physically active (%)	20.5	20.1	20.4	20.2	19.4
Alcohol (% none)	15.2	14.6	15.5	16.0	18.0
Leg height (cm)	70.0±2.9	72.6±2.8	74.2±2.9	75.8±3.1	78.4±3.5
Sitting height (cm)	86.2±3.3	85.4±3.2	84.9±3.3	84.3±3.3	83.2±3.6
BMI (kg/m^2) at age 20	21.1±3.0	21.0±3.1	20.8±2.9	20.7±3.1	20.6±3.0
BMI (kg/m^2) at baseline	28.3±5.5	27.6±5.1	26.9±4.9	26.5±5.0	26.2±4.6
Hip circumference (cm)	104.4±11.9	103.5±9.8	102.8±10.5	102.3±10.5	102.2±9.5
Waist circumference (cm)	88.4±13.2	88.1±12.5	87.2±12.3	87.1±12.8	88.1±12.0

Table 6.2. Mean and standard deviation (unless otherwise noted) of baseline characteristics by quintiles of leg length-to-sitting height in adults aged 35-74 years from The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

	Q1 (0.75-0.87)	Q2 (0.85-0.91)	Q3 (0.88-0.93)	Q4 (0.90-0.96)	Q5 (0.93-1.22)
Men (n)	1347	1348	1346	1350	1345
Age at enrollment	50.7±8.6	51.4±9.1	52.3±9.3	52.5±9.5	53.7±9.8
Race/skin color (% Black)	13.8	13.8	13.8	13.8	13.8
Maternal education (% ≥ HS)	24.5	26.4	27.3	24.2	23.2
Participant education (% ≥ HS)	84.5	85.3	85.0	83.2	79.1
Maternal diabetes (%)	19.3	19.4	19.2	19.4	17.2
Paternal diabetes (%)	13.8	11.6	11.4	10.6	11.1
Premature birth (%)	4.6	5.3	4.2	4.9	4.9
Low birth weight (%)	9.3	7.4	6.1	8.3	8.8
Current smoker (%)	13.9	14.3	13.4	13.2	16.3
Physically active (%)	24.1	28.1	28.5	26.3	25.6
Alcohol (% none)	5.5	4.1	5.2	4.4	3.6
Leg height (cm)	76.7±3.3	79.6±3.1	81.2±3.4	82.9±3.4	85.6±3.8
Sitting height (cm)	92.4±3.6	91.5±3.5	91.0±3.7	90.3±3.6	88.9±3.8
BMI (kg/m^2) at age 20	22.1±3.1	21.8±2.9	21.7±3.0	21.6±3.0	21.4±3.0
BMI (kg/m^2) at baseline	28.4±4.7	27.4±4.2	26.7±4.2	26.4±3.9	26.0±4.0
Hip circumference (cm)	101.7±9.0	100.5±7.7	99.7±7.8	99.7±8.0	99.1±7.9
Waist circumference (cm)	96.8±12.7	95.7±11.9	94.7±12.2	94.8±11.0	94.8±11.7

Table 6.3. Adjusted means (and 95% confidence intervals) from linear associations of 1-year increment in menarche with height and height components adjusted and stratified by education and birth weight categories in female adults aged 35-74 years from ELSA-Brasil.

	Multivariable adjusted models		Multivariable models stratified by levels of education			Multivariable models stratified by levels of birth weight		
	Age at enrollment and race adjusted*	Age at enrollment, race, education, and birth weight adjusted	<High school	High School	>High school	Birth weight <2.5 kg	Birth weight 2.5-4.0 kg	Birth weight >4.0 kg
n	8032	6905	756	2330	4946	603	5911	391
Stature (mm)	3.0 (2.2-3.7)	3.7 (3.0-4.5)	3.4 (1.2-5.7)	2.8 (1.5-4.2)	4.1 (3.1-5.1)	2.0 (-0.4-4.5)	3.9 (3.0-4.8)	4.5 (1.3-7.7)
Leg length (mm)	3.0 (2.4-3.5)	3.4 (2.9-4.0)	2.2 (0.4-4.0)	3.0 (2.1-4.0)	3.8 (3.0-4.5)	2.6 (1.0-4.3)	3.5 (2.9-4.1)	3.8 (1.6-6.0)
Sitting height (mm)	0.0 (-0.4-0.4)	0.3 (-0.2-0.7)	1.0 (-0.2-2.2)	0.1 (-0.6-0.8)	0.4 (-0.1-0.9)	-0.7 (-1.9-0.7)	0.4 (-0.1-0.8)	0.7 (-0.9-2.3)

*There is suggestion of effect modification ($p = 0.05$) by participant education on the association between relative leg length and age at menarche, but no such evidence of effect modification by birth weight ($p = 0.46$).

Table 6.4. Prevalence ratios (and 95% confidence intervals) for type 2 diabetes by menarcheal age in females aged 35-74 years from ELSA-Brasil.

	8-11 years	11-12 years	13-14 years	15-16 years	17-18 years
cases/*n*	139/653	481/3104	510/3161	158/911	33/203
Model 1	1.41 (1.19, 1.66)	1.02 (0.91, 1.14)	1.0 (Referent)	1.01 (0.86, 1.18)	1.02 (0.75, 1.38)
Model 2	1.30 (1.10, 1.53)	1.01 (0.91, 1.13)	1.0 (Referent)	0.95 (0.81, 1.11)	1.03 (0.71, 1.29)
Model 3	1.22 (1.03, 1.45)	0.99 (0.88, 1.10)	1.0 (Referent)	0.96 (0.82, 1.13)	1.03 (0.77, 1.38)
Model 4	1.20 (1.01, 1.41)	0.98 (0.88, 1.09)	1.0 (Referent)	0.98 (0.84, 1.15)	1.05 (0.78, 1.42)

Model 1: adjusted for age at enrollment
Model 2: adjusted for Model 1 + study center, race/color, maternal education, maternal diabetes, paternal diabetes, and birth weight
Model 3: adjusted for Model 2 + BMI at age 20
Model 4: adjusted for Model 3 + relative leg length

Table 6.5. Prevalence ratios (and 95% confidence intervals) for type 2 diabetes by quintiles of leg length-to-sitting height ratio in adults aged 35-74 years from ELSA-Brasil.

	Q1	Q2	Q3	Q4	Q5	Continuous*
Women, cases/*n*	303/1606	254/1606	243/1607	253/1608	272/1605	
Model 1	1.0 (Referent)	0.81 (0.70, 0.94)	0.75 (0.64, 0.87)	0.74 (0.64, 0.86)	0.72 (0.62, 0.84)	0.96 (0.91, 1.01)
Model 2	1.0 (Referent)	0.79 (0.68, 0.92)	0.73 (0.63, 0.85)	0.72 (0.62, 0.84)	0.69 (0.60, 0.80)	0.89 (0.84, 0.94)
Model 3	1.0 (Referent)	0.85 (0.77, 0.94)	0.75 (0.68, 0.83)	0.79 (0.72, 0.88)	0.71 (0.64, 0.79)	0.90 (0.85, 0.95)
Model 4	1.0 (Referent)	0.80 (0.67, 0.97)	0.77 (0.61, 0.97)	0.77 (0.58, 1.04)	0.76 (0.50, 1.15)	0.90 (0.85, 0.95)
Men, cases/*n*	344/1347	324/1348	275/1346	317/1350	291/1345	
Model 1	1.0 (Referent)	0.91 (0.80, 1.03)	0.74 (0.65, 0.85)	0.84 (0.74, 0.95)	0.73 (0.64, 0.84)	0.92 (0.88, 0.97)
Model 2	1.0 (Referent)	0.91 (0.81, 1.04)	0.75 (0.65, 0.86)	0.84 (0.73, 0.95)	0.71 (0.62, 0.81)	0.89 (0.85, 0.93)
Model 3	1.0 (Referent)	0.90 (0.80, 1.03)	0.75 (0.66, 0.86)	0.83 (0.73, 0.95)	0.71 (0.62, 0.81)	0.89 (0.85, 0.93)

*Expressed as per standard deviation change.
Model 1: adjusted for age at enrollment
Model 2: adjusted for Model 1 + study center, race/color, and maternal education, maternal diabetes, paternal diabetes, and birth weight
Model 3: adjusted for Model 2 + BMI at age 20
Model 4: adjusted for Model 3 + age at menarche

Table 6.6. Adjusted means and standard errors of fasting glucose, fasting insulin, 2-hour glucose, and A1C by age at menarche in females aged 35-74 years in ELSA-Brasil without previously diagnosed diabetes.

	8-11 years	11-12 years	13-14 years	15-16 years	17-18 years	F test *p* value
N	564	2828	2869	819	185	
Fasting glucose	103.8±0.6	103.9±0.3	103.2±0.3	103.3±0.5	104.7±1.0	0.22
Fasting insulin	8.2±0.7	8.1±0.3	6.9±0.3	7.2±0.6	7.2±1.2	0.04
A1C	5.37±0.03	5.32±0.01	5.28±0.01[a]	5.30±0.02[a]	5.32±0.04	0.02
2-hour glucose	130.3±1.7	128.0±0.7	127.2±0.7	128.9±1.4	128.6±2.9	0.47

Adjusted for age at enrollment, study center, race/color, maternal education, maternal diabetes, paternal diabetes, birth weight, and BMI at age 20

Table 6.7. Adjusted means (±standard error) of fasting glucose, fasting insulin, 2-hour glucose, and A1C by quintiles of leg length-to-sitting height in adults aged 35-74 years in ELSA-Brasil without previously diagnosed diabetes.

	Q1	Q2	Q3	Q4	Q5	F test *p* value
Women, *n*	1458	1459	1469	1461	1418	
Fasting glucose	105.0±0.4	104.1±0.4	103.3±0.4[a]	103.3±0.4[a]	102.1±0.4[a]	<.0001
Fasting Insulin	8.7±0.4 [a]	7.4±0.4[a]	7.4±0.4[a]	7.3±0.4[a]	6.7±0.4[a]	0.02
A1C	5.36±0.02	5.30±0.02[a]	5.29±0.02[a]	5.29±0.02[a]	5.29±0.02[a]	0.007
2-hour glucose	132.1±1.0	129.6±1.0[a]	126.5±1.0[a]	126.9±1.0[a]	124.8±1.0[a]	<.0001
Men, *n*	1171	1166	1194	1161	1171	
Fasting glucose	111.9±0.6	110.2±0.6	109.6±0.6[a]	110.0±0.6[a]	107.5±0.6[a]	<.0001
Fasting Insulin	9.9±0.2	9.1±0.2	8.1±0.2[a]	8.4±0.2[a]	8.1±0.2[a]	<.0001
A1C	5.34±0.02	5.34±0.02	5.29±0.02[a]	5.34±0.02	5.28±0.02[a]	0.04
2-hour glucose	143.8±1.5	138.0±1.4[a]	134.4±1.4[a]	136.1±1.5[a]	128.9±1.5[a]	<.0001

Adjusted for age at enrollment, study center, race/color, maternal education, maternal diabetes, paternal diabetes, birth weight, and BMI at age 20

Table 6.8. Prevalence ratios (and 95% confidence intervals) for diabetes according to a 1-standard deviation increment in leg length-to-sitting height ratio in adults from ELSA-Brasil, stratified by menarcheal age (females), BMI at 20 years, and BMI at 35-74 years.

	1-SD increment*	*p* for interaction on multiplicative scale
Menarche ≤ 12 years,	0.83 (0.77, 0.90)	0.02
Menarche > 12 years,	0.94 (0.88, 1.01)	
BMI < 25 kg/m^2 at 20 years	0.93 (0.90, 0.97)	0.02
BMI ≥ 25 kg/m^2 at 20 years	0.88 (0.81, 0.96)	
BMI < 25 kg/m^2 at baseline	1.01 (0.93, 1.09)	0.03
BMI ≥25 kg/m^2 at baseline	0.95 (0.91, 0.98)	

*Expressed as per standard deviation (0.05) change.
Adjusted for age at enrollment, study center, race/color, maternal education, maternal diabetes, paternal diabetes, birth weight, and BMI at age 20

CHAPTER 7: MANUSCRIPT 2—AGE AT MENARCHE AND RELATIVE LEG LENGTH IN RELATION TO FAT DEPOTS AND NON-ALCOHOLIC FATTY LIVER DISEASE: CARDIA STUDY

Background: Early pubertal timing is related to cardiometabolic diseases later in life, but whether markers of pubertal timing are associated with nonalcoholic fatty liver disease (NAFLD), or specific abdominal fat depots, is unclear. In a community-based cross-sectional sample of middle-aged adults free from liver disease, we tested the hypothesis that age at menarche and relative leg length (leg length-to-sitting height ratio), as markers of pubertal timing, are related to presence of NAFLD, and specific fat depots, after control for potentially confounding demographic and lifestyle factors, as well as generalized fatness.

Methods: Participants from the Coronary Artery Risk Development in Young Adults study with complete measures of age at menarche (year 0 and year 2; females), height components (year 2), and computed tomographic (CT) quantification of liver fat, visceral fat (VAT), subcutaneous fat (SAT), and intramuscular abdominal fat (IAAT) (year 25). NAFLD was defined as liver attenuation ≤ 48 Hounsfield Units after exclusion of other causes of liver fat. We used multivariable linear regression for continuous outcome measures and Poisson regression for binary outcomes.

Results: The mean (±standard deviation) age at menarche for the 1214 women eligible for this analysis was 12.6±1.5 years of age. Of the 2148 eligible men and women the mean leg length-to-sitting height ratio was 0.92±0.06. Age at menarche was associated with presence of NAFLD (1-year increment PR=0.86; 95% CI: 0.78, 0.98), liver attenuation (1-year increment =0.8±0.2 HU), VAT (1-year increment =-6.7±1.2 cc), SAT (1-year increment =-19.7±3.3 cc), and IAAT (1-year increment =-7.4±1.4 cc) after adjustment for early-life demographic and lifestyle factors (all *p* for trend <0.01). These associations remained after adjustment for BMI measured early in adulthood (age 18-30 y), but were attenuated (all *p* for trend >0.05) after adjustment for BMI later in adulthood (age 43-55 y) at the time of CT. Relative leg length, while related to age at menarche, was not associated with presence of NAFLD in men and women combined. There was an association between relative leg length and adipose depots (all *p* for trend <0.01), but these were attenuated (all *p* for trend >0.05) by adjustment for early adulthood BMI.

Conclusion: These findings suggest that age at menarche is associated with presence of NAFLD later in life, and that this association is independent of BMI measured in early adulthood, shortly after age at menarche. Relative leg length, which was loosely associated with menarcheal timing, was not associated with NAFLD. Measurement of age at menarche may add to the prediction of NAFLD above and beyond measurement of early adulthood BMI.

BACKGROUND

Identifying early-life antecedents of visceral adiposity and non-alcoholic fatty liver disease (NAFLD) may facilitate opportunities for primordial prevention of cardiometabolic diseases. Puberty represents an important event during human growth in which patterns of adult health are established. Its timing is influenced by myriad factors, including genetics, ethnicity, environmental conditions, and nutrition.[163-165,167,168,256]

Menarche occurs during the later pubertal stages in females, around the time of peak height velocity.[355] Relative leg length (e.g., leg length-to-sitting height ratio) is a marker of prepubertal growth rate and duration,[164,256] with greater relative leg length being associated with better early-life nutrition,[255] earlier age at menarche,[258] and earlier peak height velocity.[256,357] In separate literatures, studies have found early age at menarche and lower relative leg length to be associated with increased risk of obesity,[7] metabolic syndrome,[37,39] elevated liver enzymes,[31] type 2 diabetes[19,20,262,264,268] and cardiovascular disease.[268] However, no studies to date have examined whether these developmental markers are related to NAFLD, and only one study[32] has investigated whether age at menarche is differentially related to specific fat depots, e.g., visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and intramuscular abdominal adipose tissue (IAAT).

In light of these literature gaps, the objective of this study is to examine whether age at menarche and relative leg length are associated with liver attenuation, NAFLD (as

determined by computed tomography (CT) liver attenuation ≤ 48 HU), and specific adiposity depots (VAT, SAT, and IAAT). Based on prior knowledge, we hypothesize that: *1*) earlier age at menarche is associated with higher prevalence of NALFD and elevated VAT; *2*) lower relative leg length is associated with lower prevalence of NAFLD) and elevated VAT; and finally *3*) these associations are attenuated by adjustment for body mass index (BMI) measured early in adulthood.

METHODS

Study population

CARDIA is an ongoing multi-center, longitudinal, community-based study funded by the National Heart, Lung, and Blood Institute. CARDIA was undertaken to study the evolution of cardiovascular disease risk factors in 5,115 young adults initially aged 18-30 years at baseline examination in 1985-1986. Young black and white adults were recruited from the population in Birmingham, AL; Chicago, IL; Minneapolis, MN; and from a health maintenance organization in Oakland, CA. Participants were nearly balanced on race (black vs. white), age (18-24 vs. 25-30 years), and education (≤12 vs. >12 years schooling).[347] Participants have been followed for 25 years, including 8 clinic exams conducted in 1985/6 (baseline, or exam year 0), 1987/8 (year 2), 1990/1 (year 5), 1992/3 (year 7), 1995/6 (year 10), 2000/1 (year 15), 2005/6 (year 20), and 2010/11 (year 25). The retention rates among survivors at year 2 and 25 exams were 91 and 72%, respectively. Informed consent was obtained from all participants at each exam, and the

institutional review board at each of the clinical centers approved all protocols. The present study comprises a subset of participants who underwent CT of the abdomen and thorax as part of the year 25 exam.

There were 3,499 participants who attended the CARDIA year 25 exam. Women who were pregnant and individuals weighing greater than the CT couch limit (400 lbs) or otherwise unable to fit within the CT gantry were excluded (*n*=318). Participants were excluded from analysis if they were missing measurements of liver attenuation, VAT, SAT, or IAAT (*n*=30). Of the remaining 3,152 participants with all 4 measures complete, we excluded participants with history of liver disease (*n*=92), or secondary cause of fatty liver, which included alcohol consumption ≥20 g/day in women and ≥30 g/day in men (*n*=459), self-reported HIV (*n*=21), a history of intravenous drug use (*n*=82) and participants taking hormone therapy (*n*=115).

For age at menarche analyses, we additionally excluded women missing age at menarche at both year 0 and year 2 (*n*=7), women whose reported menarche age at year 0 and year 2 were more than 2 years apart (*n*=142), and women who's reported menarche age between year 0 and year 2 was >18 years (*n*=1), as we were interested in studying women within the normal range of menarcheal age. For the relative leg length analyses we further excluded individuals missing height or sitting height (*n*=66), and, to reduce influence from extreme values that may have been data entry or measurement errors, we excluded participants with height or sitting height (*n*=18) greater than 3 standard

deviations from the sex-specific mean (height > 198.2 cm or <156.5 cm for men and > 184.2 cm or < 144.2 cm for women; sitting height > 106.4 cm or < 76.6 cm for men and > 101.3 cm and < 70.7 cm for women). The results observed after exclusions were not materially different than those before exclusions. After these exclusions we were left with 1214 women for the age at menarche analyses, and 2148 men and women for the relative leg length analyses.

Measurements

Clinical outcomes and data from exam years 0, 2, 7, 20, and 25 (CT measures) were used. Standardized protocols for data collection were used across study centers.[347] At year 0 the following variables relevant to our analyses were measured: age, sex, race, height, weight, BMI, waist circumference, educational level (years of schooling), fasting insulin, childhood physical activity (before high school), cigarette smoking status (current, former, or never), alcohol consumption, and dietary history (focused on past month and based on modified Burke method[341]), and medical history. If participants were missing year 0 BMI (*n*=7) or year 0 smoking status (*n*=10), values from year 2 were used. For those who were missing dietary data at year 0 (*n*=30), information from subsequent visits (year 7 and year 20) was used.

We derived our main exposure variable, age at menarche, based on self-report at year 0 (baseline) and again at year 2 exam. At both exams females were asked the open-ended question, "How old where you when you began menstruating?" The intraclass

correlation between year 0 and year 2 self-report of menarche was 0.89. As noted above, if there was a discrepancy of >2 years, females were excluded from the analysis. If both reports of age at menarche were available, the average of the two was taken.

Anthropometric assessment was made after participants changed into light clothing and removed shoes. Trained and certified technicians took all measurements. Quality control checks were made at regular intervals throughout the exam cycle. Standing height was measured with the participant standing erect on the floor with his or her back against a vertical-mounted centimeter ruler. The participant was instructed to look straight ahead with his or her head in the Frankfort horizontal plane, i.e., the plane that includes the lower margin of the bony orbit and the upper margin of the external auditory meatus. A plastic triangle was placed on the top of the head, and the participant's height was measured to the nearest 0.5 cm. Sitting height was measured with the participant seated in an erect position on a stool with a horizontal surface, with his or her back in contact with the wall-mounted metal ruler at both the scapula and buttocks and the head held in the Frankfort horizontal plane. Measurement was then made to the nearest 0.5 cm using a plastic triangle placed snugly on the top of the participant's head. Leg length was calculated as standing height minus sitting height. Relative leg length was derived from the calculation of leg length divided by sitting height. Body weight was measured to the nearest 0.2 kg with a calibrated balance-beam scale. BMI was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference

was measured with a tape measure in duplicate to the nearest 0.5 cm around the minimal abdominal girth identified laterally midway between the iliac crest and the lowest portion of the rib cage and midway between the xiphoid process and the umbilicus.

Standard questionnaires were used to assess demographic characteristics (age, sex, race, participant education, parental education, and parental diabetes). Education was represented as years of schooling. Level of pre-high school physical activity (low, medium, high) was determined by self-report at year 0. Total daily alcohol consumption was calculated from an interviewer-administered questionnaire. Medication use was self-reported and participants were asked to bring their medications for verification. Birth weight was ascertained through birth records in a subsample of participants. Number of pregnancies, i.e., parity, was determined by self-report at each examination.

Laboratory measures

Clinic measurements were standardized for all field centers at the year 25 visit and have been consistent across examinations. Before each exam participants were asked to fast for at least 12 hours and to avoid smoking or engaging in heavy physical activity for at least 2 hours. Blood was drawn from participants in the seated position, separated, and then (plasma) frozen at -70C prior to analysis in a central laboratory.[347,376] From this fasting blood sample insulin was quantified using an Elecsys sandwich immunoassay (Roche Diagnostics Corporation, Indianapolis, IN).

Computed tomography (CT) scan protocol

CT images were acquired at each CARDIA field center and then electronically transmitted to the central CT reading center located at Wake Forest University School of Medicine, Winston-Salem, NC. The protocol entailed a non-contrast CT scan of the abdomen and was performed using GE [GE 750HD and GE LightSpeed VCT Birmingham and Oakland Centers, respectively; GE Healthcare, Waukesha, WI] or Siemens [Sensation, both Chicago and Minneapolis Centers; Siemens Medical Solutions, Erlangen, Germany] multidetector CT scanners. Image analysis and quality control were performed at the central reading center (Wake Forest University Health Sciences, Winston-Salem, NC). The protocol included scout images through the lower abdomen.

Assessment of hepatic steatosis

The CT diagnosis of fat infiltration in the liver can be made by measuring CT attenuation in Hounsfield Units (HU) which has been shown to be inversely correlated with fatty filtration of the liver as seen on liver biopsy.[278,377] Liver attenuation was measured on non-contrast CT images of the upper abdomen acquired in an axial scan mode. Specifically, measurement of liver attenuation was performed in the right lobe of the liver using CT slices at the T12-L1 intervertebral space. Liver attenuation was based on the mean HU of 3 circular regions of interest, measuring 100 mm^2 in the parenchyma of the right lobe of the liver across 3 separate CT slices. Analysts performing the measurements were trained to avoid placing regions of interest that included large hepatic vessels or

common hepatic lesions (cysts and hemangiomas).[377,378] The correlation coefficient between 2 different readers on a randomly selected sample of 156 participants was 0.98 for liver attenuation, indicating high reproducibility. As the amount of liver fat increases, the measured liver attenuation decreases based on the HU scale in which fat has negative values.[377] Our study used a sensitive[279] hepatic attenuation of ≤ 48 HU to indicate moderate to severe macrovesicular steatosis. As there is some disagreement on a clinically meaningful cut point, we performed sensitivity analyses using a cut point of ≤ 40 HU. This cut off has also been supported by literature.[278,379,380] These cutoffs were used to define NAFLD after exclusion criteria had been met.

Assessment of adipose tissue depots

CT scans of the abdomen were reconstructed into 5 mm slices with the maximum 50 cm field-of-view to include the whole abdomen for body composition. The abdominal muscular wall was first manually traced and the adipose tissue in different compartments was measured by a semiautomatic segmentation technique. Adipose tissue depots were measured volumetrically from 2 contiguous 5 mm slices located at the level of the lumbar disk between the 4th and 5th (L4-L5) vertebra. Volume Analysis software (Advantage Windows, GE Healthcare, Waukesha, WI) was used to segment and characterize each individual voxel as a tissue attenuation of fat. Tissues with attenuation between -190 to -30 HU were defined as adipose tissue. Analysts used the Medical Image Processing, Analysis, and Visualization (http://mipav.cit.nih.gov/index.php) application to segment

the images based on anatomic boundaries (skin, subcutaneous fat-muscle interface and peritoneum) into the entire abdomen, abdominal wall and intra-abdominal compartments. In each compartment, total abdominal volume, total abdominal adipose tissue, SAT, VAT, and IAAT were quantified. VAT volumes were the sum of 24 slices located within the intra-abdominal cavity.[378,381] The interclass correlation coefficient for inter-reader comparisons was 0.99 for VAT, and intra- and inter-reader error were 2.4% and 6.7%, respectively, in 156 scans that were blinded and reevaluated.

Statistical analysis

All analyses were performed using SAS 9.2 (SAS institute, Cary, NC). We summarized the characteristics of the cohort with unadjusted means and standard deviations for continuous variables and percentages for categorical data across age at menarche categories (8-11.9, 12-13.9, 14-18 years) and quintiles of relative leg length (i.e., leg length-to-sitting height ratio). We used multivariable linear regression to estimate adjusted means for components of height (total, sitting, leg) in relation to a 1-year increment in menarcheal age after adjustment for age at year 25 (in lieu of birth year), race/ethnicity, and education. We also performed a sensitivity analysis that included additional control for birth weight in the subsample of participants who had that variable (n=309). We then stratified these analyses by educational level, to evaluate whether the associations occur at different levels of socioeconomic status.

We used multivariable linear regression to assess the association between continuous measures of liver attenuation (HU), VAT, SAT, and IAAT and age at menarche and relative leg length categories. We chose covariates *a priori* based on their association with the dependent variable, age at menarche or relative leg length, and the independent variable, adipose tissue depots or NAFLD. Model 1, a potential confounder model, included age at exam year 25, sex, race, study center, parental educational attainment, maternal diabetes, paternal diabetes, and pre-high school physical activity. Following this model we additionally adjusted Model 2 for year 0 BMI as a proxy for pre-puberty BMI. In a further model we adjusted for year 25 BMI to determine if this indicator of general adiposity measured at the same time as CT explained observed associations. Year 25 BMI is on the causal pathway between markers of pubertal timing and NAFLD. Thus, obtaining unbiased estimates from this analysis relies on the presupposition of no unmeasured confounding between the exposure-outcome and mediator-outcome association, no effect modification, and that the association is linear. Since these assumptions may not be met in this analysis, the parameter estimate from this final model should be interpreted cautiously. Further model inclusion of participant education level (less than high school, completed high school but not college, completed college but no graduate school, graduate school plus), year 0 diet score, smoking status (never, former, current), alcohol use (yes/no), postmenopausal (yes/no; age at menarche analyses only), parity (0, 1-2, 3-4, 5+; age at menarche analyses only), and fasting insulin

(year 0 exam) did not materially alter the results. As these factors are likely on the exposure-outcome causal pathway they were not included.

We used multivariable Poisson regression with robust error variance[358] to estimate prevalence ratios (PRs) and 95% confidence intervals (CIs) for our primary endpoint, presence of NAFLD, according to categories of menarcheal age and relative leg length. We present a multivariable adjusted model that includes variables from multivariable linear regression Model 1 and Model 2. We further adjusted for year 25 BMI to determine if the association was explained by a general measure of fatness measured at the time of CT. We evaluated effect measure modification by including cross-product terms in the models for our exposures and age at year 25 exam (<50 vs. ≥50 years), race (black vs. white), smoking (ever vs. never), education (<high school vs. ≥high school), age at menarche (≤12 vs. >12; in relative leg length models), and BMI at year 0 exam (25kg/m^2 vs. ≥25 kg/m^2). All statistical tests were two-sided and significance was defined at $p < 0.05$.

RESULTS

Study cohort

The median age at menarche for the 1214 women who were eligible for the age at menarche analyses was 12.6 (SD=1.5) years of age. Baseline participant characteristics according to categories of age at menarche are presented in **Table 1**. Participants were 43 to 55 years of age at the year 25 exam. Participants who self-identified as black were

more likely to have menarche < 12 years of age. Earlier menarche was associated with lower maternal and paternal education, higher rates of paternal diabetes, shorter legs, greater BMI, waist circumference, and fasting insulin. There were no marked differences in birth weight across age at menarche categories.

The mean relative leg length (i.e., leg length-to-sitting height ratio) in men and women included in the leg length analyses (*n*=2,418) was 0.92 (SD=0.06). Baseline participant characteristics according to quintiles of relative leg length are presented by sex in **Table 1**. In men and women, greater relative leg length was associated with lower BMI, waist circumference, fasting insulin, and, in females, later age at menarche.

Age at menarche and height components

After adjusting for age, race, and educational achievement, later age at menarche was associated with 0.33 cm longer leg length, and a 0.15 cm shorter sitting height. These associations persisted after adjustment for birth weight, and were consistent across categories of participant educational achievement (**Table 3**).

Age at menarche in relation to liver attenuation and fat depots

In multivariable linear regression analysis with continuous CT outcomes (**Table 4**), we observed a positive association between menarcheal age and liver attenuation and an inverse association between menarcheal age and VAT, IAAT, and SAT (all $p < 0.001$). These associations were slightly attenuated (all p for trend <0.05) by adjustment for year

0 BMI (Model 2). These were largely explained by adjustment for BMI measured at the year 25 exam (all *p* for trend > 0.05).

Age at menarche and NAFLD

Of the 1214 women eligible for the age at menarche analyses, 179 (14.7%) had CT-diagnosed NAFLD. The association between age at menarche and NAFLD is shown in **Table 5**. After adjusting for multiple potential confounders, but before adjustment for BMI, a later age at menarche associated with lower prevalence of NAFLD (Model 1, PR for 1-year increment in menarche=0.86; 95% CI: 0.78, 0.94). This association was modestly attenuated after adjustment for BMI at year 0 exam (Model 2, PR for 1-year increment in menarche=0.90; 95% CI: 0.82, 0.98). This association was attenuated further after additional adjustment for BMI measured at year 25 exam (Model 3, PR for 1-year increment in menarche=0.93; 95% CI: 0.85, 1.02).

Relative leg length in relation to liver attenuation and fat depots

In multivariable linear regression analysis examining the association between relative leg length and body composition (**Table 6**), we did not observe a positive association between relative leg length and liver attenuation. We did observe an inverse association between relative leg length and VAT, IAAT, and SAT (Model 1, all $p < 0.001$), but these were attenuated after adjusting for year 0 BMI (Model 2, all *p* for trend > 0.05).

Relative leg length and NAFLD

Of the 2148 men and women eligible for relative leg length analyses, 401 (18.7%) had CT-diagnosed NAFLD. Similar to the findings on relative leg length and liver attenuation (as a continuous measure), there was little evidence of an association between relative leg length and NAFLD in this sample (**Table 7**).

Evaluation of effect measure modification

We found no statistical evidence of effect measure modification on the multiplicative scale by age at year 25 exam, race, BMI at year 0, or education (p for interaction > 0.05) on the age at menarche-NAFLD or relative leg length-NAFLD associations. We thus pooled results across these strata for presentation.

DISCUSSION

Our findings suggest that later menarche is associated with higher prevalence rates NAFLD after adjusting for potential demographic and lifestyle confounding factors. This association remained after adjusting for BMI measured between 18 and 30 years (our earliest measure). Relative leg length, while loosely positively related to menarcheal age, was not associated with NAFLD. These findings were consistent by race and sex.

To the best of our knowledge, this is the first study to examine a marker of pubertal timing in relation to NAFLD. Earlier age at menarche has been found to be associated with several cardiometabolic outcomes related to NAFLD, including adult obesity,[7] metabolic syndrome,[37,39] type 2 diabetes,[19,20,262,264,268] and cardiovascular

disease.[268] Menarcheal age has also been linked to development of advanced liver disease and carcinoma.[344] The hypothesized mechanism linking earlier menarche to metabolic disease outcomes relates to pathways of adiposity and insulin resistance. Accumulation of body fat and insulin resistance are key etiologic drivers for NAFLD, thus, it is not surprising age at menarche and NAFLD are associated with each other.

When we controlled for BMI, measured when the participants in our study were 18-30 years of age (year 0), we found that the association between age at menarche and liver attenuation persisted. It has been shown that BMI tracks from childhood to young adulthood.[359] A stronger BMI-NAFLD association would be expected for BMI measured closer to the measurement of hepatic steatosis. Thus, the observed findings are not likely to be confounded by a general fatness measured early in life. However, when we further adjusted our model for BMI measured at year 25 exam, the same exam at which CT liver attenuation was performed, the associations were further attenuated to include the null in the confidence bounds. This measure of association should be interpreted cautiously as the aforementioned assumptions for mediation analysis were likely not met.

We are also unaware of any other study to examine the association of relative leg length and NAFLD. Relative leg length has been linked to NAFLD-related endpoints, including adult obesity[261], insulin resistance,[261,263] type 2 diabetes,[360] and elevated liver enzymes.[31] Yet, we did not find evidence of an association between relative leg length and liver attenuation or relative leg length and CT-diagnosed NAFLD. We did find an

association between relative leg length and fat depots, but these associations were largely explained by adjustment for BMI measured when participants were between 18 and 30 years of age. These results suggest relative leg length, which has been proposed as a marker for early life growth and development in some populations, is associated with generalized, rather than specific, adiposity in adulthood. They do not support an independent association between relative leg length and hepatic steatosis. In this study, relative leg length and age at menarche, which were loosely associated with each other, likely represent distinct landmarks of growth and maturation.

One previous study has examined the association between age at menarche and visceral and subcutaneous adipose tissue accumulation. In an analysis of women from the Framingham Study, Trikudanathan et al. found that earlier age at menarche is associated with greater BMI, WC, VAT, and SAT.[32] These associations were attenuated close to null after further adjustment for BMI measured concurrently with CT. A model adjusting for early-life BMI was not presented. The authors of this study concluded that age at menarche is associated with generalized adiposity, but that it is not differentially related to one fat depot over another. Our findings largely support their conclusions. We observed that age at menarche was associated with each adiposity depot, independent of demographic and lifestyle confounding factors. These associations remained after adjusting for BMI measured early in adulthood. However, similar to the Framingham Study, we found that these associations were largely explained by adjustment for BMI

measured at year 25 exam, when specific fat depots were also measured. Similar to our liver attenuation findings, these results suggest that generalized body early in life may not confound so much as mediate the association between age at menarche and pernicious deposition of fat. Thus, if BMI and age at menarche are both measured early in life, menarcheal age might add to the prediction of visceral adiposity and liver steatosis. Further research to determine whether age at menarche improves NAFLD risk prediction above and beyond traditional CVD risk factors is warranted.

The strengths of the current study include the large, well-characterized community-based sample of whites and blacks, the highly sensitive measurement of body composition and liver attenuation, and a wide array of demographic, lifestyle, and physiologic measures to assess cofounding and effect modification. Another strength was our definition of NAFLD, which we obtained by CT liver attenuation after excluding participants with known chronic liver disease or those with secondary causes of liver dysfunction, such as alcohol use and medication. Nevertheless, this was an epidemiologic definition that should be validated in future studies by using alternative measures of NAFLD and follow-up for hard clinical endpoints.

There were also limitations to the current study. The findings were from a cross-sectional assessment of body fat depots and liver attenuation, limiting our ability to establish temporality in the observed associations. Specific body fat stores may have been present prior to puberty. Another limitation was the self-recall of age at menarche many

years after the event; thus, misclassification was inevitable. Yet, CARDIA participants recalled age at menarche in young adulthood, between 18-30 years of age and again at 20-32 years of age. Original adolescent reports of menarcheal age have been shown to correlate well (r=0.79) with adulthood retrospective reports.[346] We also cannot rule out the potential for residual confounding by unmeasured early-life socioeconomic, lifestyle, or physiologic measures, such as pre-pubertal diet and BMI. However, based on the evidence[359] that BMI tracks well through life, we believe our measure of BMI between 18 and 30 years is a good proxy for childhood BMI. Some might also consider our definition of NAFLD a limitation because CT is less sensitive than liver biopsy.[275,278] However, liver biopsy is an invasive procedure, not practical in a population-based study. We accounted for the lack of data on liver function or viral hepatitis serologies in the present study by excluding participants with a history of HIV, intravenous drug use, or use of medications or excessive alcohol known to contribute to steatosis. Many individuals identified with NAFLD by our definition will not progress to other disease beyond what would occur with any fat deposition. More work is needed to understand whether epidemiologic NAFLD definitions using CT liver attenuation, such as ours, are indeed clinical NAFLD. This work may include using adjunct measures, including alternative measures of liver dysfunction and inflammation.

CONCLUSIONS

These findings provide evidence that age at menarche is associated with the presence of NAFLD 25 plus years later after controlling for demographic and lifestyle confounding factors. This association is modestly attenuated after adjustment for early-adulthood BMI. Under the presupposition that BMI measured between 18-30 years of life is a proxy for childhood BMI, this finding suggests that general indicators of fatness do not confound this association. Associations between age at menarche and fat depots measured at year 25 also persisted after adjusting for early adulthood BMI measured at year 0. Thus, measurement of age at menarche may add to the prediction of NAFLD and pernicious storage of adiposity above and beyond BMI measured in adulthood, shortly after puberty. Early puberty may be an early-life harbinger for risk of NAFLD. Intervening on environmental factors related to pubertal timing and/or targeting early maturing females for lifestyle intervention may hold potential for the primordial prevention of NAFLD and related cardiometabolic diseases. Future studies on this topic should use alternative markers of pubertal timing and progression, include early-life measures of fatness and diet, and be prospective in nature.

Table 7.1. Characteristics of females by age at menarche: CARDIA study.

	8-12 years	12-13.9 years	14-18 years
Women (*N*)	293	692	229
Age at year 0 exam	24.7 (3.7)	25.1 (3.6)	24.8 (3.8)
Black race (%)	62.1	50.4	52.4
Education > high school (%)	77.8	79.8	76.9
Maternal education > high school (%)	35.3	42.7	43.8
Paternal education > high school (%)	37.2	44.4	48.6
Maternal diabetes (%)	13.0	10.7	9.6
Paternal diabetes (%)	13.3	11.6	8.3
Birth weight (g) (*n*=38/93/33)	3031.9 (708.3)	3072.7 (548.0)	3078.9 (678.1)
Parity at year 25 exam	2.6 (2.0)	2.7 (2.0)	2.5 (1.9)
Postmenopausal at year 25 exam (%)	56.3	56.6	59.8
Early-life lifestyle variables			
Highest level of pre-high school PA (%)	28.3	30.2	40.2
Diet score at year 0 exam	62.6 (12.4)	63.9 (13.1)	63.2 (13.6)
Ever smoker at year 0 exam (%)	23.5	19.9	25.8
Body proportions			
Height (cm)	163.8 (7.2)	164.1 (6.5)	164.3 (7.4)
Leg length (cm)	77.8 (5.2)	78.3 (4.4)	78.7 (8.5)
Sitting height (cm)	86.1 (3.9)	85.8 (3.8)	85.6 (8.2)
Year 0 anthropometric fat indicators			
BMI at year 0 exam (kg/m^2)	26.3 (5.8)	24.6 (5.5)	23.2 (4.9)
Obese at year 0 exam (%)	23.2	14.2	10.5
Waist girth at year 0 exam (cm)	77.2 (11.9)	74.2 (11.3)	72.3 (10.2)
Abdominal obesity at year 0 exam (%)	17.5	10.9	7.4
Year 25 anthropometric fat indicators			

Table 7.1. Characteristics of females by age at menarche: CARDIA study.

	8-12 years	12-13.9 years	14-18 years
BMI at year 25 exam (kg/m^2)	33.6 (8.1)	31.3 (8.1)	28.7 (7.5)
Obese at year 25 exam (%)	63.5	51.0	39.3
Waist girth at year 25 exam (cm)	95.9 (15.8)	92.2 (16.6)	87.3 (16.4)
Abdominal obesity at year 25 exam (%)	68.3	55.8	41.7
Blood measures			
Year 0 Fasting insulin (uU/dL) (*n*=286/685/227)	12.3 (7.2)	11.1 (8.0)	11.2 (8.4)
Year 25 Fasting insulin (uU/dL) (*n*=289/684/226)	13.2 (17.1)	11.3 (8.4)	10.6 (8.9)

Values presented are unadjusted means (and standard deviations) unless otherwise indicated.

Table 7.2. Characteristics of participants by quintiles (Q) of leg length-to-sitting height: CARDIA study

	Q1 (0.66-0.89)	Q2 (0.85-0.92)	Q3 (0.88-0.95)	Q4 (0.90-0.99)	Q5 (0.92-1.24)
Women (*N*)	239	240	239	237	240
Age at year 0 exam	25.4 (3.8)	25.0 (3.6)	25.0 (3.4)	24.7 (3.7)	24.6 (3.7)
Black race (%)	53.1	53.7	53.1	54.0	53.7
Education > high school (%)	76.2	77.5	78.2	79.7	83.7
Maternal education > high school (%)	40.4	37.4	41.0	40.8	47.0
Paternal education > high school (%)	41.6	45.5	45.2	41.1	44.7
Maternal diabetes (%)	12.6	14.6	8.8	10.6	8.4
Paternal diabetes (%)	16.7	12.9	7.1	9.3	10.5
Age at menarche (years)	12.1 (1.3)	12.4 (1.5)	12.6 (1.5)	12.6 (1.4)	12.9 (1.5)
Birth weight (g)	3028.4 (587.9)	2908.3 (622.9)	3238.1 (554.5)	3140.3 (636.3)	3016.4 (536.2)
Early-life lifestyle variables					
Highest level pre-high school PA (%)	31.0	28.3	33.1	25.7	33.1
Diet score at year 0 exam	64.1 (11.8)	63.7 (13.6)	64.0 (13.9)	62.7 (12.4)	62.6 (13.7)
Ever smoker at year 0 exam (%)	21.8	23.8	19.2	21.1	23.0
Body proportions					
Height (cm)	161.1 (6.0)	163.4 (5.8)	164.5 (6.1)	164.8 (6.6)	166.9 (6.9)
Leg length (cm)	73.5 (3.4)	76.8 (2.9)	78.5 (2.9)	79.9 (3.2)	83.1 (4.2)
Sitting height (cm)	87.5 (3.5)	86.6 (3.3)	86.0 (3.6)	84.9 (3.9)	83.9 (3.8)
Year 0 anthropometric fat indicators					
Waist girth at year 0 exam (cm)	77.5 (13.1)	74.8 (10.5)	73.3 (10.7)	73.9 (10.4)	73.3 (11.6)
Abdominal obesity at year 0 exam (%)	18.5	13.8	9.7	9.7	4.2
BMI at year 0 exam (kg/m^2)	27.2 (6.8)	25.2 (5.2)	24.2 (5.0)	23.9 (4.6)	23.1 (5.1)
Obese at year 0 exam (%)	27.2	17.9	12.6	9.7	9.6
Year 25 anthropometric fat indicators					
Waist girth at year 25 exam (cm)	94.6 (17.3)	92.8 (15.3)	90.7 (16.6)	91.9 (16.8)	88.7 (16.5)

Table 7.2. Characteristics of participants by quintiles (Q) of leg length-to-sitting height: CARDIA study

	Q1 (0.66-0.89)	Q2 (0.85-0.92)	Q3 (0.88-0.95)	Q4 (0.90-0.99)	Q5 (0.92-1.24)
Abdominal obesity at year 25 exam (%)	64.3	59.7	50.1	54.4	51.9
BMI at year 25 exam (kg/m^2)	33.7 (8.7)	31.9 (7.6)	30.9 (8.1)	30.8 (7.9)	29.6 (7.5)
Obese at year 25 exam (%)	64.9	55.0	48.1	46.8	43.9
	Q1 (0.78-0.92)	Q2 (0.86-0.96)	Q3 (0.89-0.99)	Q4 (0.92-1.03)	Q5 (0.95-1.20)
Men (*N*)	190	191	189	191	189
Age at year 0 exam	25.4 (3.4)	25.1 (3.7)	24.8 (3.5)	24.9 (3.6)	25.0 (3.7)
Black race (%)	43.2	43.4	43.4	42.9	42.9
Education > high school (%)	79.5	77.5	78.3	78.0	69.8
Maternal education > high school (%)	46.6	43.2	47.4	43.9	40.1
Paternal education > high school (%)	53.5	46.3	50.0	42.7	39.7
Maternal diabetes (%)	7.9	7.9	9.0	6.8	12.2
Paternal diabetes (%)	7.4	7.9	10.1	8.9	7.4
Birth weight	3230.1 (516.6)	3188.9 (572.4)	3279.8 (822.4)	3145.9 (630.0)	3118.4 (523.4)
Early-life lifestyle variables					
Highest level pre-high school PA (%)	43.2	42.9	39.7	44.5	42.9
Diet score at year 0 exam	61.9 (11.9)	60.6 (12.5)	62.2 (12.1)	59.9 (11.9)	60.0 (13.1)
Ever smoker at year 0 exam (%)	20.5	21.5	18.0	26.7	25.4
Body proportions					
Height (cm)	175.2 (6.2)	175.6 (5.9)	177.6 (6.3)	179.5 (5.7)	179.8 (6.9)
Leg length (cm)	81.3 (3.7)	83.4 (3.2)	85.8 (3.2)	88.0 (3.2)	90.6 (4.2)
Sitting height (cm)	93.9 (3.4)	92.1 (3.5)	91.8 (3.9)	91.5 (3.5)	89.3 (4.2)
Year 0 anthropometric fat indicators					
Waist girth at year 0 exam (cm)	84.6 (10.6)	82.3 (9.3)	82.3 (9.6)	81.5 (9.2)	80.8 (9.5)
Abdominal obesity at year 0 exam (%)	8.5	4.2	5.3	2.1	1.1
BMI at year 0 exam (kg/m^2)	26.2 (4.5)	24.9 (3.6)	24.7 (3.9)	23.9 (3.6)	23.4 (3.5)
Obese at year 0 exam (%)	14.7	9.4	7.9	5.2	3.7
Year 25 anthropometric fat indicators					

Table 7.2. Characteristics of participants by quintiles (Q) of leg length-to-sitting height: CARDIA study

	Q1 (0.66-0.89)	Q2 (0.85-0.92)	Q3 (0.88-0.95)	Q4 (0.90-0.99)	Q5 (0.92-1.24)
Waist girth at year 25 exam (cm)	103.0 (15.3)	99.7 (14.7)	98.1 (14.1)	98.0 (13.8)	98.6 (13.0)
Abdominal obesity at year 25 exam (%)	49.7	37.4	32.8	34.0	32.3
BMI at year 25 exam (kg/m^2)	32.0 (6.6)	30.4 (6.2)	29.5 (5.6)	29.0 (5.5)	28.6 (5.1)
Obese at year 25 exam (%)	57.4	45.5	39.7	36.1	29.6

Values presented are unadjusted means (and standard deviations) unless otherwise indicated.

Table 7.3. Adjusted means (and 95% confidence intervals) from linear associations of 1-year increment in menarche with height and height components in females from CARDIA.

	Age-race-adjusted	Age-race-education-adjusted	Age-race-education-birth weight-adjusted	≤High school	>High school
N	2314	2314	309	922	1392
Stature (cm)	0.18 (0.00, 0.35)	0.18 (0.00, 0.35)	0.15 (-0.33, 0.63)	0.12 (-0.15, 0.40)	0.21 (-0.02, 0.44)
Leg length (cm)	0.33 (0.21, 0.45)	0.33 (0.21, 0.45)	0.37 (0.05, 0.69)	0.28 (-0.09, 0.48)	0.36 (0.21, 0.52)
Sitting height (cm)	-0.15 (-0.25,-0.06)	-0.15 (-0.25,-0.06)	-0.22 (-0.48, 0.05)	-0.16 (-0.32, 0.00)	-0.15 (-0.27,-0.03)

Table 7.4. Adjusted means and standard errors for liver attenuation (HU) and adipose tissue depots (cc) by age at menarche in women from CARDIA.

		8-12 years	12-13.9 years	14-15.9 years	16-18 years	1-year increment
	N	293	692	189	40	
LA	Model 1	55.0±0.6	57.1±0.4[a]	58.6±0.8[a]	61.5±1.7[a]	0.8±0.2*
	Model 2	55.8±0.6	57.1±0.4	57.7±0.8[a]	60.7±1.6[a]	0.5±0.2*
	Model 3	56.1±0.6	57.2±0.4	57.1±0.7	59.4±1.5[a]	0.2±0.2
VAT	Model 1	124.6±3.6	116.3±2.4	100.5±4.5[a]	86.8±9.8[a]	-6.7±1.2*
	Model 2	118.6±3.4	116.7±2.2	107.2±4.2[a]	92.3±9.2[a]	-4.0±1.3*
	Model 3	114.6±2.7	115.7±1.7	113.9±3.4[a]	104.5±7.3[a]	-1.4±0.9
IAAT	Model 1	139.4±4.1	131.4±2.7	113.3±5.1[a]	97.3±11.2[a]	-7.3±1.4*
	Model 2	132.3±3.8	131.9±2.5	121.1±4.8[a]	103.8±10.3[a]	-4.1±1.3*
	Model 3	128.0±3.0	130.8±1.9	128.4±3.8[a]	117.7±8.1[a]	-1.3±1.0*
SAT	Model 1	427.5±9.9	397.4±6.4[a]	337.7±12.3[a]	337.8±26.7[a]	-19.6±3.3*
	Model 2	402.9±8.2	399.0±5.3	365.1±10.2[a]	360.7±22.1	-8.4±2.8*
	Model 3	392.9±4.7	395.2±3.1	382.9±5.9[a]	400.1±12.8	-1.3±1.6

Model 1: Adjusted for age at year 25, race, study center, parental education, maternal diabetes, paternal diabetes, pre-high school physical activity.
Model 2: Model 1 + year 0 BMI
Model 3: Model 2 + year 25 BMI

Table 7.5. Prevalence ratios (95% confidence intervals) for NAFLD by categories of menarche age in women from CARDIA.

		8-12 years	12-13.9 years	14-18 years	1-year increment
	cases/*n*	54/293	104/692	21/229	
NAFLD (Liver attenuation ≤ 48 HU)	Model 1	1 (referent)	0.82 (0.61, 1.11)	0.51 (0.32, 0.82)	0.86 (0.78, 0.94)
	Model 2	1 (referent)	0.89 (0.66, 1.19)	0.63 (0.39, 0.99)	0.90 (0.82, 0.98)
	Model 3	1 (referent)	0.94 (0.70, 1.26)	0.78 (0.49, 1.23)	0.93 (0.85, 1.02)

Model 1: Adjusted for age at year 25 exam, race, study center, parental education, maternal diabetes, paternal diabetes, pre-high school physical activity.
Model 2: additional adjustment for BMI at year 0 exam.
Model 3: additional adjustment for BMI at year 25 exam.

Table 7.6. Adjusted means and standard errors for liver attenuation (HU) and adipose depots (cc) by quintiles (Q) of leg length-to-sitting height ratio in adults from CARDIA.

		Q1	Q2	Q3	Q4	Q5	Continuous
	N	429	431	428	428	428	
LA	Model 1	54.5±0.6	55.0±0.5	56.3±0.5	56.5±0.5	54.7±0.6	0.4±0.3
	Model 2	56.0±0.5	55.4±0.5	56.2±0.5	56.2±0.5	55.9±0.5	-0.8±0.3
VAT	Model 1	145.3±3.4	136.0±3.4	125.4±3.4	126.3±3.4	128.9±3.4	-6.7±1.8
	Model 2	133.7±3.2	133.5±3.1	126.5±3.1	131.0±3.1	137.5±3.2	-2.1 ±1.8
IAAT	Model 1	159.4±3.8	151.0±3.8	139.0±3.8	141.3±3.8	142.5±3.8	-6.8±2.1
	Model 2	145.7±3.6	148.0±3.5	140.3±3.5	146.7±3.5	152.5±3.6	3.4±2.0
SAT	Model 1	374.6±7.6	352.7±7.5	336.1±7.5	331.0±7.6	320.4±7.7	-23.6±4.1
	Model 2	334.9±6.4	344.1±6.2	339.9±6.2	346.7±6.2	349.3±6.4	5.8±3.5

Model 1: Adjusted for age at year 25 exam, race, study center, parental education, maternal diabetes, paternal diabetes, pre-high school physical activity.
Model 2: additional adjustment for BMI at year 0 exam.

Table 7.7. Prevalence ratios (and 95% confidence intervals) of NAFLD by quintiles (Q) of leg length-to-sitting height ratio in adults from CARDIA.

		Q1	Q2	Q3	Q4	Q5	Continuous
	cases/*n*	105/431	79/430	69/430	64/428	84/429	
NAFLD (LA ≤48 HU)	Model 1	1 (referent)	0.82 (0.63, 1.07)	0.90 (0.70, 1.15)	0.78 (0.59, 1.04)	0.99 (0.76, 1.28)	0.94 (0.85, 1.05)

Model 1: Adjusted for age at year 25 exam, sex, race, study center, parental education, maternal diabetes, paternal diabetes, pre-high school physical activity.

Supplemental chapter 7 table. Prevalence ratios (and 95% confidence intervals) for alternative NAFLD definition by categories of menarche age in women from CARDIA.

		<12 years	12-13.9 years	≥14 years	1-Year Increment
	cases/*n*	29/293	53/692	13/229	
NAFLD (Liver attenuation ≤ 40 HU)	Model 1	1 (referent)	0.76 (0.49, 1.16)	0.57 (0.31, 1.07)	0.89 (0.78, 1.02)
	Model 2	1 (referent)	0.81 (0.53, 1.24)	0.69 (0.37, 1.28)	0.92 (0.81, 1.06)
	Model 3	1 (referent)	0.88 (0.58, 1.34)	0.90 (0.49, 1.66)	0.97 (0.85, 1.12)

Model 1: Adjusted for age at year 25 exam, race, study center, parental education, maternal diabetes, paternal diabetes, pre-high school physical activity.
Model 2: additional adjustment for BMI at year 0 exam.
Model 3: additional adjustment for BMI at year 25 exam.

CHAPTER 8: MANUSCRIPT 3—DIET QUALITY IN CHILDHOOD AND INCIDENT EARLY MENARCHE: NATIONAL HEART, LUNG, AND BLOOD INSTITUTE GROWTH AND HEALTH STUDY

Background: Early pubertal maturation has been linked to increased risk of several adult chronic diseases, yet prospective research on diet and early puberty is sparse.

Methods: The National Heart, Lung, and Blood Institute Growth and Health Study recruited girls aged 9-10 years from Richmond, California, Cincinnati, Ohio, and Washington, DC. There were 2,379 (1213 African-American, 1166 Caucasian) girls enrolled in the study. We excluded those who were menarcheal at baseline, did not report age at menarche during follow-up, had baseline diabetes, or were missing key exposure variables, yielding a final analytic sample of 1988 girls. Exposure variables, including diet, physical activity and anthropometry were collected at baseline, prior to menarche. Girls were seen annually for 10 visits to ascertain incident age at menarche.

Results: Mean age at menarche in our sample was 12.4±1.1 years. A better diet quality, as indicated by higher nutrient-element based diet quality score, was associated with lower risk of early menarche (<11 years). Of the individual components included in the diet quality score, early menarche was positively associated with caffeine and aspartame, and negatively associated with α-tocopherol. These associations remained after control for potential confounders and % body fat measured at baseline.

Conclusion: A diet rich in nutrients typically found in vegetables, fruits, and nuts, and poor in nutrients found in red meat and chemical elements found in soft drinks is associated with lower risk of early menarche, independent of fatness. These results suggest that diet, independent of adiposity, contributes to the timing of puberty.

BACKGROUND

A growing body of literature, including our own (in *Chapters 6 & 7* of this dissertation) suggests that early puberty is on the causal pathway to a number of non-communicable diseases in adulthood, including adult obesity,[36] metabolic syndrome,[37] type 2 diabetes,[19,21-23,261-264,267,268] non-alcoholic fatty liver disease (*Chapter 7*), cardiovascular disease,[25,38-41] hormone-related cancers,[42,43] and the incidence of adolescent risk behaviors (e.g., substance abuse, cigarette smoking, multiple sexual partners, conduct disorder).[44-46] As the average age at menarche continues to decline,[63] and the evidence for a link between maturational timing and health and disease strengthens, it is increasingly important to identify and understand possible modifiable factors underlying the timing of puberty.

Due to the coinciding secular increases in childhood obesity and declines in timing of menarche in many countries,[63] the preponderance of research on factors related to pubertal timing has focused on the role of childhood adiposity and related hormonal changes.[71-73] Yet, in some populations, the secular trends in pubertal timing are not explained by trends in childhood obesity (*Chapter 2*, **Figure 2.3**),[48] suggesting that beyond adiposity, environmental factors such as pre-pubertal nutrition may play an influential role in the pace of pubertal development.

A small but growing body of literature has examined the role of mid-childhood nutrition and pubertal timing.[134-144,151,152] The majority of studies on this topic have

focused on specific components of foods, often single nutrients. Several but not all (reviewed by Cheng et al),[48] have reported significant associations. Although these studies provide insight into the role of dietary components in pubertal timing, restricting analyses to single nutrients fails to consider the complexity of the diet, which includes multiple correlations between nutrients and foods that may have both interactive and synergistic properties with respect to human health and disease. Dietary patterns complement the reductionist isolated nutrient or food approach by addressing diet complexity through a holistic approach.[154]

Generally, dietary patterns are derived based on evidence or expert knowledge of foods or nutrients. A drawback of the food-based diet patterns is that many indices (e.g., Mediterranean diet) cannot be directly applied to diverse populations. Another drawback is the lack of nutritional evidence linking individual foods to less-frequently studied outcomes (e.g., age at menarche). As such, rather paradoxically, evidence from nutrient-disease studies is often used to construct *a priori* food-based diet indices.[382] Estimated nutrients are structurally weighted sums of foods and, when appropriately combined, can serve as a proxy for food-based dietary patterns. Moreover, nutrient-based diet quality indices are robust and adaptable to diverse populations.

To date, one study has examined a nutrient-based dietary quality index in relation to markers of pubertal timing. In 222 children (119 girls and 103 boys) from the DONALD study, a higher nutritional quality score, rich in carbohydrates, dietary fiber,

vitamin C, folate, vitamin E, sodium, iron, and calcium, and low in total and saturated fat was associated with later age at take off, i.e., age at onset of the adolescent growth spurt, independent of pre-pubertal body fat.[155] Yet, this study was limited in sample size and did not determine the association of the score with age at menarche, the pubertal timing marker most robustly associated with long-term chronic diseases.

Considering this gap in the literature, the purpose of the current investigation was to examine the prospective association between indices of a prudent diet and onset of menarche in a biracial sample of girls from the US. We hypothesize that a diet rich in nutrients characteristic of a prudent diet and relatively low in nutrients and other chemicals typically found in a Western diet is associated with lower risk for early menarche.

METHODS

Study population

The NGHS cohort was formed in 1987-1988 to investigate racial differences in diet, physical activity, and familial and psychosocial factors in relation to obesity. The details of the study cohort, including design, eligibility criteria, sources and methods of recruitment and data collection, and means of assessing pubertal status have been described in detail in *Chapter 5* of this dissertation and elsewhere.[383] The study enrolled 2,379 (1213 African-American, 1166 Caucasian) girls aged 9 and 10 from 3 centers: University of California, Berkeley, University of Cincinnati/Cincinnati Children's

Medical Center, and Westat, Rockville, Maryland. The choice of these clinical centers was informed by census track information to ensure a wide distribution of household income and parental education within each race. Girls were eligible for enrollment in NGHS if they self-identified as non-Hispanic African American or Caucasian, had racially concordant parents or guardians, and were within 2 weeks of age 9 or 10 at the time of the first clinical visit. Girls in the study gave assent and their parents or guardians gave consent. The institutional review boards of each participating institution approved the study protocol, and an independent monitoring board provided study oversight.

NGHS participants were followed annually for 10 visits, between 1986 and 1997. Data collection occurred at clinic centers and home visits using a standard protocol. Annual follow-up rates through year 10 varied from 74% to 95%; almost 90% of the girls originally enrolled in the cohort participated through year 10. Girls attended an average of 8.8 visits (8.6 for white girls and 9.0 for black girls).

Dietary assessment

Dietary assessment for this study included a 3-day food record that provided high reporting accuracy, as documented in the initial validation study.[384] We used baseline dietary assessment to allow for temporality between diet and incident menarche. Dietitians were trained at the University of Minnesota Nutrition Coordinating Center (NCC) and retrained in later years by staff using age-appropriate materials to instruct girls to record all food and drink for 3 consecutive days (2 weekdays and 1 weekend

day). Dietitians reviewed the completed food records individually with the girls using standardized probes to clarify incomplete responses. Default values from the NCC were used for missing information on food amounts or preparations. Study staff had a notebook of labels and pictures to help girls describe the foods and thereby minimize the amount of defaults. Food records were coded and analyzed for nutrients and other food chemicals using the Food Table version 19 of the NCC nutrient database;[349] updated versions were used as the study progressed. The research dietitian excluded diet records that were considered unreliable.

Our diet quality score (DQS) was derived to determine the extent to which a child consumed various nutrients and other food chemicals specific to a prudent or Western dietary pattern. DQS indicates diet quality expressed as nutrient or food chemical density. Food elements with a skewed distribution (vitamin C, caffeine, and aspartame) were normalized by log-transformation. To derive the DQS, we took the sum of individual nutrient standard deviation scores (SDS) [(participant intake of nutrient element – sample mean intake of nutrient)/standard deviation of nutrient]. A positive SDS was given to diet elements indicative of prudent dietary habits (dietary fiber, folate, magnesium (Mg), potassium (K), α-tocopherol (active form of vitamin E), vitamin C, and polyunsaturated-to-saturated fat ratio) and a negative SDS was given to diet elements indicative of a Western dietary pattern (dietary cholesterol, caffeine, and aspartame). We tried not to include nutrients ubiquitously found in prudent and Western foods, such as total sugar,

which is found in fruit but also sugar-sweetened beverages, or nutrients for which the literature suggests no consistent association with obesity or chronic disease risks. Our DQS had a mean of 0 and scores range from -17 to 25; higher scores indicate better diet quality. We also calculated Keys score [1.35 × (2 × % energy from saturated fat − % energy from polyunsaturated fat) + 1.5 × √(mg cholesterol /1000 kcal)] to characterize quality of dietary fat intake.[385]

Pubertal assessment

Participants were queried annually regarding onset of menstruation to determine their date of menarche to the closest month. At each annual visit participants were examined in standard measurement gowns by trained female examiners. Pubic hair stage (Tanner stages 1-5) was determined by using criteria established by Marshall and Tanner, and areolar stage by using Garn-Falkner areolar stages (1-5).[55]

Covariate assessment

Demographic information was collected at baseline from girls and their parents (or guardians). Race (black or white) was defined by self-report using U.S. Census categories. Participants' age was recorded as their age at last birthday. Parental educational achievement ranged from 0 years of education to graduate school. Girls completed a habitual physical activities questionnaire that assessed frequencies of several activities described previously.[386] Information on activity intensity, duration, and

frequency was used to derive the physical activity score. The last question on the activity questionnaire was used to estimate the total number of hours per week the girls usually watched videos or television. This total indicates sedentary behavior and was not included in the physical activity score.

A stadiometer and a calibrated scale were used to measure height and weight while girls were wearing socks and light indoor clothing. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured at the narrowest part of the torso. Other anthropometry included triceps, subscapular, and supra-iliac skinfolds were also measured at baseline. All anthropometrics were taken twice, with a third measurement if the first 2 differed markedly. For skinfolds this difference criterion was 1.0 mm, and for circumference measurements, 1.0 cm. When 3 measurements were taken, the closest 2 were averaged. Measurement techniques for skinfolds and circumferences have been described in detail.[350] To ensure anthropometric procedure consistency across study sites, a master anthropometric trainer was designated to train local trainers, who then provided training to field staff in each study site, with recertification annually. Percent body fat was derived from the triceps and subscapular skinfolds according to the Slaughter et al. formula.[387]

Of the 2379 girls enrolled in NGHS at baseline, we excluded those who had already reached menarche at baseline exam (n=96), as we were interested in exposures prior to onset of menarche. We also excluded those who never reported menarche during

the 10-year follow-up (*n*=22). We further excluded girls with diabetes at baseline (*n*=5), and those who were missing information on diet (*n*=210), body mass index (*n*=10), percent body fat from skinfolds (*n*=7), physical activity (*n*=40), and parent education (*n*=1), yielding a final sample of 1988 for the current analyses.

Statistical analysis

All analyses were performed using SAS 9.2 (SAS institute, Cary, NC). We summarized the characteristics of the cohort with adjusted and unadjusted means and standard deviations for continuous variables and percentages for categorical data across categories (8-11, 11-11.9, 12-12.9, 13-13.9, 14-18) of incident age at menarche.

We categorized height, BMI, percent body fat, physical activity levels, nutrients, DQS, and Keys score into tertiles. Other variables were categorized using logical cut points that allowed for sufficient cases at each level. We used menarche at < 11 years as our primary outcome because we were interested in risk factors for early menarche as opposed to menarcheal timing. We used Poisson models with robust error variance to estimate relative risk and 95% confidence intervals for early age at menarche.[358] We began with a crude model (Model 1), and then added variables if the literature and our data suggested they were associated with diet quality and age at menarche, and not on the causal pathway of this association. In Model 2 we adjusted for age at baseline (years), study center, race, parental education (<college, college, graduate school), total calories (continuous), and physical activity score (continuous) (Model 2). In Model 3, we further

adjusted for BMI at baseline. We also evaluated parental smoking reported at baseline exam as a covariate but did not include it in the final model as it was not associated with our main exposure (DQS) and there were many missing values (n=164).

We evaluated effect modification by including cross-product terms in the models for our exposures and race (black vs. white) and BMI at baseline visit (median split; 17.5 kg/m^2). All statistical tests were two-sided and significance was defined at $p < 0.05$.

RESULTS

Girls included in the present analyses (n=1988) were 9-10 y old at baseline and at the end of follow-up had a mean (±SD) age at menarche of 12.4±1.1 years. One hundred sixty five (8.3%) of the girls had their first menses by age 11 years. These girls were, on average, taller and fatter than the rest of the cohort at the start of the study. Compared to their later maturing counterparts, early menarche girls were also more likely to be black, younger at enrollment, and from parents of lower educational status who were more likely to smoke (**Table 1**).

Girls with a lower quality diet according to the DQS had greater BMI and percent fat, and were from less educated parents. These girls had a higher Keys score and consumed more energy overall. A greater percent of their calories came from dietary fat, specifically saturated fat, and cholesterol, whereas they consumed lower quantities of polyunsaturated fats, protein, and carbohydrates. They also had lower intakes of most

vitamins and minerals, and higher intakes of caffeine and aspartame, compared to their healthier eating counterparts (**Table 2**).

Relative risks of early menarche (<11 years) for non-diet variables after multivariable adjustment are shown in **Table 3**. Black girls had 2.42 (1.77, 3.32) times the risk of early menarche compared to their white counterparts. Girls in the highest tertile of height, BMI, and percent body fat had 4.78 (3.23, 6.07), 2.46 (1.70, 3.58), and 1.59 (1.11, 2.23) times greater risk of early menarche, respectively, as girls in the lowest tertile. The height-early menarche association persisted after further adjustment for percent body fat (RR for 3rd vs. 1st tertile = 4.46; 95% CI: 2.96, 6.70). After multivariable adjustment there was little evidence that early menarche was associated with parental education, parental smoking, or physical activity score.

Associations for dietary components included in the DQS and early menarche are presented in **Table 4**. Higher intakes of caffeine (RR for 3rd vs. 1st tertile = 1.72; 95% CI: 1.04, 2.85), and aspartame (RR for 3rd vs. 1st tertile = 2.43; 95% CI: 1.51, 3.90) were positively associated with early menarche after adjustment for study center, baseline age, race (black/white), parental education, physical activity score, and percent body fat measured from skinfolds at baseline. Higher intake of α-tocopherol was associated with lower risk of early menarche after full multivariable adjustment (RR for 3rd vs. 1st tertile = 0.70; 95% CI: 0.49, 0.99). The association between intakes of other nutrients and early menarche were not as strong, with confidence intervals that included the null (**Table 4**),

but most were consistently in the direction supporting the literature or our *a priori* hypothesis. Early menarche was not associated with total energy intake, or energy-adjusted intakes of protein, carbohydrate, or dietary fat (not shown).

Before and after adjustment for potential confounders and percent body fat, girls consuming a higher DQS were at lower risk of early menarche (Multivariable RR for 1 SD change in DQS = 0.80; 95% CI: 0.66, 0.90). A higher Keys score was not associated with risk of early menarche (RR for 1 SD change in Keys score = 1.05; 95% CI: 0.92, 1.21) (**Table 5**). There was no evidence that these associations differed across strata of race (black versus white; *p* for multiplicative interaction=0.62) or BMI (<17.5 versus ≥17.5 kg/m^2; *p* for multiplicative interaction=0.92).

DISCUSSION

Our study suggests that pre-menarcheal girls consuming a relatively high quality diet, as indicated here by a nutrient and food chemical density-based index, have a lower risk for early age at menarche. This association remained after control for confounding variables and pre-menarcheal percent body fat, suggesting diet quality is associated with early menarche independent of fatness. These associations were consistent across race strata.

Consistent with a large body of literature,[64,118,388-391] black girls in our study had earlier menarche than white girls after adjustment for multiple potential confounders, including pre-menarcheal BMI. With respect to anthropometry, our study corroborated previous findings from NGHS that pre-menarcheal height, BMI, and skinfold-thickness

indices are higher in early maturing females.[391] These investigators found that BMI and sum of three skinfolds remained higher throughout follow-up in early maturing females. Yet, *pre*-menarcheal height was positively associated with earlier menarche, whereas *post*-menarcheal height was inversely associated with early menarche. This observation aligns with the notion that early-maturing females reach peak height velocity at a younger age than late-maturing females.[357391] In our analyses, the association between pre-menarcheal height and age at menarche remained after adjusting for percent body fat. This implies that mechanisms driving early peak height velocity in early-developers are important to pubertal-timing etiology and may be independent of pre-menarcheal accretion of body fat.

Many of the dietary elements included in our DQS, e.g., fiber, Mg, K, vitamin C, and PUFA-SFA ratio were only suggestively associated with menarcheal timing. Yet, other DQS components showed a strong signal. Higher intake of caffeine and aspartame was associated with risk of early menarche after adjustment for potential confounders and percent body fat measured at baseline. In this sample of 9 and 10 year old girls, caffeine and aspartame likely represent intake of regular and diet soft drinks, respectively, but we do not have information on beverage consumption to test this conjecture. To the best of our knowledge, no previous studies have examined the association of soft drinks, diet or regular, in relation to puberty. Considering that hyperinsulinemia is a major driver of early maturation,[90] and sugar-sweetened consumption has been associated with greater

insulin resistance,[392] further study of sugar-sweetened soft drinks and pubertal timing association is warranted. Diet beverages have been linked to higher cardiometabolic risk in some studies but not others (reviewed by Pereira et al.[393]). de Konig and colleagues[394] found that the association between artificially sweetened beverages and diabetes is attenuated by adjustment for previous weight change and dieting behaviors, factors that we were unable to control for in the current study. Other mechanisms linking aspartame or their main vehicle, diet beverages, to early puberty may also exist.

Higher intake of (non-supplemental) α-tocopherol, the active form of vitamin E, was found to be inversely associated with risk of early menarche in our study. Most sources of α-tocopherol are high in plant-based fat, including wheat germ oil, dried nuts and seeds, and fats extracted from nuts and seeds.[395] Among fruits, good sources include avocados and mangos. For vegetables, good sources include soybeans, dark leafy greens, broccoli, and asparagus. Epidemiologic studies have found that suboptimal intake of dietary α-tocopherol and other antioxidants increases risk of several menarche-associated diseases, including diabetes,[396] heart disease,[397] and breast cancer.[398] Moreover, the PIVENS trial found that natural vitamin E improved liver steatosis.[399] Based on these results, one might speculate that dietary sources of α-tocopherol may alter pubertal timing and impart health benefits through shared mechanisms. However, our findings, one small piece of evidence for this conjecture, should be viewed only as hypothesis generating.

Replication, with more expanded analyses and preferably including food groups, of these findings in other studies is needed.

The nutrients chosen for inclusion into our score have been shown to indicate dietary quality in the NGHS and in other populations. In a previous report from the NGHS, Moore et al[400] showed that, compared to girls who consumed high red meat and low whole grain diets, girls who consumed low red meat/high whole grain diets had higher intakes of vitamin C and Mg (vitamin E, folate, and K were not compared). Moreover, those who consumed high non-meat protein/high whole grain and high non-meat protein/high fruit and vegetable diets had higher vitamin C and Mg compared to their low non-meat protein, whole-grain, and fruit-and-vegetable consuming counterparts. Other studies have also shown "healthy" dietary patterns were positively associated with the nutrients in our diet quality score. In a cohort of UK adolescents, a "Traditional/health conscious" dietary pattern had the strongest positive correlations ($r > .5$) with fiber, K, Mg, vitamin C, and folate.[401] In the Jackson Health Study, a cohort of black and white adults, a 'Fast Food' dietary pattern had significantly lower serum concentrations of α-tocopherol.[402] This corroborated a previously found positive association between healthy diet and serum α-tocopherol in a study of U.S. Hispanic and non-Hispanic adults.[403]

We did not observe an association between Keys score and risk of early menarche in our study. Keys score takes into account polyunsaturated fat, saturated fat, and cholesterol, reflecting the atherogenic potential of the diet.[385] Previous studies on dietary

fat and age at menarche have suggested a positive association between intake of polyunsaturated fat and early menarche,[135,137,140] and a negative association between saturated fat and early menarche.[136,137] In contrast to these findings, we found a suggestive inverse association between polyunsaturated-to-saturated fat ratio and risk of early age at menarche. We also found a weak, non-monotonic positive association between cholesterol and risk of early menarche. A higher polyunsaturated-to-saturated fat ratio and higher cholesterol may indicate higher meat intake, which has been shown to be associated with early age at menarche.[135,140,151] Collectively, these findings suggest dietary fat intake is not strongly implicated in the timing of menarche. Rather, the pattern with which fatty acids are consumed, animal-based diet versus plant-based diet, may have greater relevance to maturational timing and health-relevant outcomes. Future studies of animal- versus vegetable-based foods and food-based dietary patterns would be an informative next research step to address this hypothesis.

Our findings have more direct public health implications than past studies that have focused on single nutrients. Analyzing single nutrients may not accurately represent the correlated nutrient milieu embedded in dietary patterns. Combining nutrients, such as those commonly found in a prudent diet can provide insight into how nutrient and non-nutrient components of food interact synergistically to alter health and disease. Because it is not yet known which dietary factors may influence the timing of menarche, we decided to first examine a nutrient-density based index in this regard. A welcomed next step in

this area of research would be to examine a dietary patterns using principal component or cluster analysis of food groups. Examining nutrients and food groups in concert will help shed light on dietary patterns, and the dietary factors they comprise, in relation to age at menarche.

The higher risk of early menarche observed in girls with a lower diet quality may have long-term consequences for risk of menarche-associated chronic diseases, including adult obesity,[36] metabolic syndrome,[37] type 2 diabetes,[19,21-23,261-264,267,268] non-alcoholic fatty liver disease (*Chapter 7*), cardiovascular disease,[25,38-41] certain cancers,[42,43] and mortality.[404,405] More research is needed to determine if menarcheal timing is indeed on the causal pathway between pre-menarcheal diet quality and risk of menarche-linked adulthood chronic diseases, and, if it is, the extent to which modifying diet can alter these outcomes.

There are several strengths to our study. First, the 3-day recall has been previously validated against, and performed better than, a 24-hour recall and 5-day food frequency questionnaire in this sample of 9 and 10 year olds.[384] Our approach of combining nutrients representative of prudent and non-prudent aspects of diet considers the whole diet and the potentially synergistic effects of nutrients, rather than attempting to isolate the effects of individual nutrients, which may be too small to detect.[406] Other strengths include annual prospective measurement of age at menarche to the exact month, a high retention rate over the study's 10-year duration, and 3 clinical sites allowing for

socioeconomic diversity.

Our study is not without limitations. As with all assessments of food intake in nutritional epidemiology, food records may misclassify diet, contributing to measurement error in the point estimates that may potentially result in biased results. However, on average, such misclassification would be non-differential, biasing our results toward the null. Another limitation to our study is that our earliest dietary information was at age 9-10 years. Different effects on menarcheal timing have occurred from dietary intake of nutrients earlier in childhood. This study defined maturation by timing of menarche, rather than onset of puberty. We were unable to capture onset of breast budding or first appearance of pubic hair in a substantial number of participants because 48% of black and 22% of white 9 year-old girls had reached Tanner stage 2, as had 78% of black and 45% of white 10 year-old girls. The correlation between age at menarche and pubertal onset ranges from 0.39 to 0.86.[407-409] These differences may reflect accuracy of pubertal ascertainment in each study. Onset of menarche, breast budding, and age at appearance of pubic hair may not represent parallel pubertal events, thus, results of this study should not be generalized to other measures of pubertal onset or tempo. Nevertheless, age at menarche remains the most relevant measure of maturational timing because of the reliability with which it is reported and the robustness of its association with long-term chronic disease risk. Finally, we did not have direct measures of intrauterine or postnatal growth, which have been shown to be associated with menarcheal timing.[123] These

factors have not been shown to be strongly associated with diet quality in early childhood, thus we do not believe they would confound the observed results. We cannot, however, rule out unmeasured or residual confounding by other early-life factors.

CONCLUSIONS

Consistent with some but contrary to other studies (reviewed by Cheng et al.[48]), our findings suggest that better dietary quality, as indicated by higher intake of nutrients found in a prudent vegetable-based diet, and lower intake of nutrients and other dietary elements commonly found in a Western diet was associated with reduced risk of early menarche, independent of fatness. Improving childhood diet quality may thereby herald long-term reductions in risks for adult chronic diseases associated with earlier menarche.

Table 8.1. Characteristics by age at menarche at the end of follow-up in a biracial cohort of females from the U.S. aged 9-10 years at baseline.

	Age at menarche categories (years)				
	9-11y	11-12y	12-13y	13-14y	14-18y
N	165	547	705	410	161
Age at entry into study	9.7±0.04	10.0±0.02	10.1±0.02	10.0±0.03	10.1±0.04
Race (% black)	67.3	58.1	46.2	35.8	23.6
Height (cm)	143.8±0.6	143.0±0.3	140.8±0.3	138.4±0.3	138.1±0.6
% fat from skinfolds	23.0±0.6	22.3±0.4	21.4±0.3	19.7±0.4	18.0±0.7
BMI* (kg/m^2)	19.7±0.3	19.2±0.2	18.6±0.1	17.6±0.2	17.0±0.3
Parent education* (% college plus)	70.1	75.0	77.6	75.9	86.2
Parent smoked* (%)	57.6	47.3	53.0	49.1	35.5
TV per week* (hours)	31.2±1.3	30.2±0.7	31.2±0.6	30.7±0.8	29.5±1.3
Activity score* (mets)	30.2±1.5	32.0±0.8	32.9±0.7	32.2±1.0	32.7±1.5
Diet variables†					
Nutrition quality score	-1.1±0.4	-0.3±0.2	0.2±0.2	0.1±0.3	1.2±0.4
Calories (kcal)	1863±42	1829±23	1827±19	1802±26	1833±41
Fat (% kcal)	35.6±0.4	36.3±0.2	35.9±0.2	35.5±0.3	36.0±0.4
Keys score	45.4±0.7	45.0±0.4	44.4±0.3	43.9±0.5	44.7±0.7
PUFA-SFA ratio	0.45±0.01	0.48±0.01	0.48±0.01	0.48±0.01	0.49±0.01
Diet cholesterol (mg/1000 kcal)	132.9±4.4	125.7±2.4	124.9±2.1	120.6±2.8	134.6±4.4
Protein (% kcal)	14.3±0.2	14.4±0.1	14.2±0.1	14.1±0.1	14.3±0.2
CHO (% kcal)	51.2±0.5	50.4±0.3	51.1±0.3	51.6±0.3	51.0±0.5
Fiber (g/1000kcal)	6.2±0.2	6.2±0.1	6.4±0.1	6.3±0.1	6.7±0.2
K (mg/1000kcal)	1128.1±18.7	1133.8±10.3	1138.1±9.0	1126.8±11.8	1175.3±18.9
Mg (mg/1000kcal)	118.4±2.0	118.0±1.1	119.2±1.0	119.1±1.3	123.2±2.1
Vitamin A (mg/1000kcal)	522.7±31.2	446.4±17.1	470.1±15.0	460.4±19.7	494.4±31.6
Vitamin C (mg/1000kcal)	47.2±2.6	48.6±1.4	51.4±1.3	49.3±1.7	55.9±2.6
Vitamin D (mg/1000kcal)	3.1±0.1	3.1±0.1	3.1±0.1	3.1±0.1	3.2±0.1
Ca (mg/1000kcal)	457.4±10.5	446.0±5.8	448.8±5.0	451.4±6.7	452.8±10.7
Caffeine (mg/1000kcal)	10.6±0.8	9.1±0.5	8.7±0.4	8.8±0.5	6.7±0.8
Aspartame (mg/1000kcal)	8.4±1.5	5.9±0.8	5.3±0.7	3.2±0.9	1.9±1.5
Sugar (g/1000kcal)	64.5±1.3	62.2±0.7	64.5±0.6	64.5±0.8	64.7±1.3

*Adjusted for race; †Adjusted for race and total calories

Data are presented as mean ± standard error unless otherwise indicated.

Table 8.2. Characteristics by tertiles (T) of nutrition quality index (DQS) in a biracial cohort of females from the U.S. aged 9-10 years at baseline.

	T1	T2	T3
N	662	663	663
Age at entry into study	10.0±0.02	10.0±0.02	10.0±0.02
Race (% black)	45.0	48.3	48.6
Height (cm)*	141.3±0.3	140.8±0.3	140.8±0.3
% fat from skinfolds*	22.5±0.3	20.8±0.3	20.2±0.3
BMI* (kg/m^2)	19.1±0.1	18.3±0.1	18.1±0.1
Parent education* (%college +)	72.2	75.9	82.0
Parent smoked* (% ever)	50.7	50.7	47.3
TV per week* (hours)	31.7±0.7	30.2±0.7	30.1±0.7
Activity score* (mets)	32.3±0.7	31.5±0.7	33.2±0.7
Diet variables†			
Keys score	49.1±0.3	45.0±0.3	39.6±0.3
Calories (kcal)	1866.4±20.4	1837.4±20.4	1784.1±20.4
Fat (% kcal)	37.2±0.2	36.2±0.2	34.1±0.2
SFA (% kcal)	14.6±0.1	13.7±0.1	12.3±0.1
PUFA (% kcal)	5.7±0.1	6.1±0.1	6.9±0.1
Diet cholesterol (mg/1000kcal)	141.3±2.1	125.9±2.1	109.0±2.1
Protein (% kcal)	13.9±0.1	14.4±0.1	14.4±0.1
CHO (% kcal)	49.6±0.3	50.4±0.3	53.1±0.3
Fiber (g/1000kcal)	4.9±0.1	6.2±0.1	8.0±0.1
K (mg/1000kcal)	977.2±7.8	1128.7±7.8	1304.1±7.8
Mg (mg/1000kcal)	101.3±0.8	116.9±0.8	139.1±0.8
Vitamin A (mg/1000kcal)	349.0±14.9	445.6±14.9	609.0±14.9
Vitamin C (mg/1000kcal)	33.4±1.2	47.7±1.2	69.5±1.2
Vitamin D (mg/1000kcal)	2.8±0.1	3.0±0.1	3.5±0.1
Ca (mg/1000kcal)	435.0±5.5	449.6±5.5	464.1±5.5
Caffeine (mg/1000kcal)	13.5±0.4	7.5±0.4	5.5±0.4
Aspartame (mg/1000kcal)	9.1±0.7	3.5±0.7	2.4±0.7
Sugar (g/1000kcal)	64.5±0.6	62.2±0.6	65.0±0.6

DQS sums the *z* scores of dietary intake of (+) fiber, (+) folate, (+) Mg, (+) K, (+) α-tocopherol, vitamin C, polyunsaturated-to-saturated fat ratio, (-) cholesterol, (-) caffeine, and (-) aspartame

Keys score is calculated by: $1.35 \times (2 \times \%$ energy from saturated fat $- \%$ energy from polyunsaturated fat$) + 1.5 \times \sqrt{}$(mg cholesterol /1000 kcal)

*Adjusted total calories

Table 8.3. Relative risk (RR) of menarche before 11 years of age, according selected variables at the start of a biracial cohort of females from the U.S. aged 9-10 years at baseline.

Variable (units)	Category level (range)	Menarche <11 y, *n* (%)	RR of menarche <11 y (95% CI)
Race*	White	54 (5.2)	1.0 (reference)
	Black	111 (11.8)	2.42 (1.77, 3.32)
Parent education†	HS or less	51 (11.0)	1.0 (reference)
	Some college	60 (7.8)	0.70 (0.49, 1.01)
	College graduate	54 (7.2)	0.85 (0.57, 1.26)
Parent smoking‡	Never	66 (7.2)	1.0 (reference)
	Ever	86 (9.5)	1.19 (0.88, 1.62)
Physical activity score‡	T1	63 (9.5)	1.0 (reference)
	T2	55 (8.4)	0.94 (0.67, 1.32)
	T3	47 (7.1)	0.84 (0.59, 1.20)
Height (cm)‡	T1 (117.2-137.8)	30 (4.4)	1.0 (reference)
	T2 (137.8-143.9)	51 (7.9)	2.14 (1.40, 3.28)
	T3 (144.0-169.0)	84 (12.7)	4.78 (3.23, 7.07)
BMI (kg/m^2) ‡	T1 (11.2-16.3)	30 (4.5)	1.0 (reference)
	T2 (16.3-19.1)	60 (9.1)	2.14 (1.47, 3.12)
	T3 (19.1-34.5)	75 (11.3)	2.46 (1.70, 3.58)
% fat from skinfolds‡	T1 (7.3-15.9)	46 (6.9)	1.0 (reference)
	T2 (16.4-23.3)	54 (8.1)	1.35 (0.93, 1.95)
	T3 (23.6-54.5)	65 (10.0)	1.59 (1.11, 2.23)

Table 8.4. Relative (RR) of menarche before11 years of age, according to intake of nutrients included in nutrition quality index (DQS) at start of study in a biracial cohort of females from the U.S. enrolled at 9-10 years.

Variable	Category level (range)	Menarche <11y, *n* (%)	Unadjusted RR of menarche <11y	Multivariable model, RR of menarche <11y	Multivariable model +BMI, RR of menarche < 11 y
Fiber (mg/1000kcal)	T1 (1.9-5.3)	64 (9.7)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (5.3-6.9)	54 (8.1)	0.84 (0.60, 1.19)	0.83 (0.59, 1.16)	0.85 (0.61, 1.20)
	T3 (6.9-16.2)	47 (7.1)	0.73 (0.51, 1.05)	0.76 (0.53, 1.09)	0.78 (0.55, 1.12)
Mg (mg/1000 kcal)	T1 (50.4-106.2)	66 (10.0)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (106.2-126.7)	54 (8.1)	0.82 (0.58, 1.15)	0.84 (0.60, 1.17)	0.87 (0.62, 1.21)
	T3 (126.8-308.5)	45 (6.8)	0.68 (0.47, 0.98)	0.74 (0.51, 1.06)	0.76 (0.53, 1.09)
Folate (mg/1000 kcal)	T1 (26.8, 100.1)	58 (8.8)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (100.1, 141.2)	55 (8.3)	0.95 (0.67, 1.35)	0.87 (0.62, 1.22)	0.90 (0.64, 1.27)
	T3 (141.2, 530.4)	52 (7.8)	0.90 (0.63, 1.28)	0.91 (0.64, 1.31)	0.95 (0.66, 1.36)
K (mg/1000 kcal)	T1 (464.4, 1021.4)	62 (9.4)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (1022.9, 1224.4)	53 (8.0)	0.85 (0.60, 1.21)	0.88 (0.63, 1.24)	0.87 (0.62, 1.22)
	T3 (1224.5, 2343.5)	50 (7.5)	0.81 (0.56, 1.15)	0.88 (0.62, 1.25)	0.88 (0.62, 1.26)
Vitamin C (mg/1000 kcal)	T1 (3.8, 32.9)	60 (9.1)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (32.9, 56.4)	52 (7.8)	0.87 (0.61, 1.23)	0.86 (0.60, 1.21)	0.88 (0.62, 1.24)
	T3 (56.5, 487.5)	53 (8.0)	0.88 (0.62, 1.26)	0.90 (0.63, 1.28)	0.92 (0.65, 1.32)
α tocopherol (mg/1000 kcal)	T1 (1.1, 3.2)	66 (10.0)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (3.2, 4.3)	50 (7.5)	0.76 (0.53, 1.07)	0.77 (0.55, 1.10)	0.79 (0.56, 1.11)
	T3 (4.3, 33.5)	49 (7.4)	0.74 (0.52, 1.06)	0.68 (0.48, 0.97)	0.70 (0.49, 0.99)
PUFA-SFA ratio	T1 (0.1-0.4)	58 (8.8)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	T2 (0.4-0.5)	57 (8.6)	0.98 (0.69, 1.39)	0.87 (0.62, 1.22)	0.84 (0.60, 1.19)
	T3 (0.5-1.5)	50 (7.5)	0.86 (0.60, 1.24)	0.75 (0.53, 1.06)	0.74 (0.52, 1.05)
Diet cholesterol (mg/1000)	T1 (24.6, 94.8)	43 (6.5)	1.0 (reference)	1.0 (reference)	1.0 (reference)

	T2 (94.9, 134.4)	61 (9.2)	1.42 (0.97, 2.06)	1.37 (0.94, 1.98)	1.37 (0.94, 1.98)
	T3 (134.5, 538.3)	61 (9.2)	1.42 (0.97, 2.06)	1.30 (0.90, 1.89)	1.26 (0.86, 1.83)
Caffeine (mg/day)	0-25	124 (7.9)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	25-50	27 (16.4)	1.14 (0.77, 1.70)	1.45 (0.97, 2.18)	1.37 (0.92, 2.05)
	≥50	14 (12.4)	1.57 (0.94, 2.64)	1.95 (1.19, 3.19)	1.72 (1.04, 2.85)
Aspartame (mg/day)	0	133 (7.8)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	<50	14 (9.1)	1.17 (0.69, 1.97)	1.45 (0.87, 2.42)	1.41 (0.84, 2.35)
	≥50	18 (14.1)	1.80 (1.14, 2.85)	2.67 (1.68, 4.24)	2.43 (1.51, 3.90)

*Adjusted for study center, baseline age, race (black/white), parental education, total calories, and physical activity score

†Adjusted for study center, baseline age, race (black/white), parental education, total calories, physical activity score, and baseline BMI

Table 8.5. Relative risk (RR) of menarche before 11 years of age, according to indices of dietary quality at start of study in a biracial cohort of females from the U.S. enrolled at age 9-10 years.

	Category level (range)	Menarche <11y, *n* (%)	Unadjusted RR of menarche <11y	Multivariable model, RR of menarche <11y	Multivariable model +BMI, RR of menarche < 11 y
DQS*	T1	72 (10.9)	1 (reference)	1 (reference)	1 (reference)
	T2	50 (7.5)	0.69 (0.49, 0.98)	0.64 (0.46, 0.89)	0.67 (0.48, 0.94)
	T3	43 (6.5)	0.60 (0.42, 0.86)	0.56 (0.39, 0.80)	0.59 (0.41, 0.86)
	1 SD (4.9) Δ	-	0.79 (0.67, 0.91)	0.75 (0.64, 0.88)	0.77 (0.66, 0.90)
Keys score†	T1	54 (8.2)	1 (reference)	1 (reference)	1 (reference)
	T2	51 (7.7)	0.94 (0.65, 1.36)	0.95 (0.67, 1.37)	0.95 (0.66, 1.36)
	T3	60 (9.1)	1.11 (0.78, 1.57)	1.11 (0.79, 1.56)	1.06 (0.75, 1.49)
	1 SD (9.3) Δ	-	1.06 (0.93, 1.21)	1.07 (0.94, 1.23)	1.05 (0.92, 1.21)

*DQS sums the *z* scores of dietary intake of (+) fiber, (+) folate, (+) Mg, (+) K, (+) α-tocopherol, vitamin C, polyunsaturated-to-saturated fat ratio, (-) cholesterol, (-) caffeine, and (-) aspartame

†Keys score is calculated by: $1.35 \times (2 \times \%$ energy from saturated fat $-\%$ energy from polyunsaturated fat$) + 1.5 \times \sqrt{}$ (mg cholesterol /1000 kcal)

CHAPTER 9: SUMMARY

Early life exposures during times of rapid growth and development are increasingly recognized for their contribution to health and disease throughout the lifecourse. Puberty, the attainment of reproductive competence, is central to the life history of humans. The timing of puberty is influenced by myriad factors including genetics, ethnicity, environmental conditions, and nutrition. Accumulating evidence suggests that approximately two centuries ago puberty, as defined by age at menarche in girls, embarked on a secular trend toward younger age at onset that continues today in many parts of the world (*see Chapter 2*). Literature on modifiable determinants and metabolic consequences of this trend toward earlier pubertal timing is young but expanding, with future research in this arena holding potential for discovery of novel insights into the pathology and primordial prevention of human disease.

Germane to this dissertation is the investigation of pubertal timing as a harbinger for development of metabolic diseases. The association between pubertal timing and type 2 diabetes has been explored in developed populations, but research on this topic is lacking in low- and middle-income countries (*see Chapter 3*). In Latin America this research is topical due to the high rates of type 2 diabetes resulting from continued regional shifts in epidemiology and nutrition.[351] In *Chapter 6* of this dissertation we addressed this literature gap by examining age at menarche and relative leg length, as markers of pubertal timing and early-life growth and development, in relation to type 2 diabetes. Compared with the median age at menarche (13-14 years), early menarche (8-11 years) in Brazilian females was associated with a 30% increased prevalence of type 2

diabetes after adjustment for potential demographic and lifestyle confounding factors. This was modestly attenuated by control for BMI at age 20 years. In this same study, relative leg length was inversely associated with type 2 diabetes, in a monotonic fashion, after full adjustment for potential confounders and early-adulthood BMI. In females, age at menarche and relative leg length were loosely related, and associated with diabetes independently of each other, suggesting that they represent unique elements of growth, development, and pubertal maturation in this nutritionally transitioning Brazilian population.

Interestingly, the association between relative leg length and diabetes was stronger among early maturing females, and adults who were overweight or obese at age 20 years and at 35-74 years. If relative leg length indicates early-life net nutrition in this population, these findings suggest that inadequate nutrition early in life coupled with nutritional excess later in life may interact synergistically to increase risk for development of diabetes. If true, this finding provides evidence that, under the predictive-adaptive response paradigm, metabolic programing may extend to development during the first years of life. Longitudinal birth cohort studies in developing populations, using more sensitive measures of early-life growth and development, are needed to replicate this association.

Accretion of fat in the liver and visceral depots may be on the pathway to type 2 diabetes. Whether pubertal timing is uniquely related to NAFLD or specific deposition of adipose had not been previously examined (*reviewed in Chapter 4*). In *Chapter 7* of this dissertation, age at menarche was found to be inversely associated with prevalence of NAFLD after adjustment for potential confounders and early adulthood BMI. Relative

leg length, on the other hand, was not associated with NAFLD. Age at menarche and relative leg length were associated with all adipose depots, but the associations between relative leg length and adipose depots were explained by early-adulthood BMI. Neither age at menarche nor relative leg length appeared to be preferentially associated with one adipose depot over another. These findings suggest that, in a biracial U.S. cohort, age at menarche, but not relative leg length, may add to prediction of NAFLD above and beyond traditional indices of fatness measured early in adulthood.

As the secular trend toward earlier pubertal timing in the U.S. and abroad continues, and research linking earlier puberty to long-term health and disease grows, it is increasingly important to identify early-life modifiable risk factors for early age at puberty. This was the aim of paper three (*Chapter 8*) of this dissertation. In a biracial cohort of pre-menarcheal females, we found that girls who consumed a diet rich in nutrients typically found in vegetables, fruits, and nuts, and poor in nutrients found in red meat and chemical elements found in soft drinks, had lower risk of early menarche, independent of fatness. These results suggest that diet, independent of adiposity, contributes to the timing of puberty.

The mechanisms underlying the associations between early-life nutrition, pubertal timing, and development of type 2 diabetes and fatty liver disease are likely myriad and interrelated. One extensively studied pathway is the triggering of puberty by adipocytes and related hormones. Accretion of fat (and therefore leptin) has been shown to promote hypothalamic GnRH pulse generator activity,[97,130] thereby attenuating gonadal feedback suppression of LH secretion, [410] and augmenting aromatase activity in the ovarian

granulosa cells.[411] However, our findings, which were largely independent of fatness, provide evidence that alternative mechanisms are at play.

A growing body of literature suggests insulin-related mechanisms may underlie associations between nutrition, puberty, and metabolism. Perhaps the most enlightening evidence comes from a series of experimental trials in low-birth weight precocious-puberty females, in which administration of metformin, an insulin-sensitizing medication commonly used to treat type 2 diabetes, was shown to delay age at menarche, increase total stature, and reduce post-menarcheal insulin resistance and fatty liver.[89,90,262,342] Therefore, insulin-resistance and hyperinsulinemia that manifests early in life may be the primary pathologic perturbation driving the associations observed in this dissertation. Serum SHBG and IGF binding protein-1, which rise concomitantly with insulin concentrations, may alter unbound estrogen and IGF-1 that act upon the endometrium and epiphyseal growth plate. Higher insulin concentrations and IGF-1 may also increase aromatase activity and estrogen synthesis in the ovary.[412] Further research from cell, animal, and human studies is needed to shed etiologic light on the interplay of insulin resistance, SHBG, estrogen, IGF axis, body fat, and leptin, among other biologic intermediaries.

In our study diet quality was associated with early menarche independent of pre-menarcheal adiposity. Diet, like metformin, can improve insulin sensitivity[211-219] and thereby may modulate pubertal timing, and reduce risk of fatty liver disease and type 2 diabetes. From the holistic perspective of public health, dietary intervention is preferable to administration of drugs in children for disease proto-prophylaxis. However, further research is needed to determine whether insulin-sensitizing treatments, such as dietary

modulation, can reduce risk for metabolic conditions (e.g., diabetes and liver steatosis) by normalizing the endocrine-metabolic state, pubertal growth, and menarcheal timing.

In conclusion, earlier menarche was associated with higher prevalence of type 2 diabetes and non-alcoholic fatty liver disease after adjustment for potential confounding factors and indicators of fatness measured in early adulthood. Moreover, the risk of early menarche was reduced through improved childhood diet quality, independent of pre-menarcheal adiposity. These findings suggest that measurement of pubertal timing affords a unique early-life glimpse into an individual's long-term metabolic trajectory, and that targeted interventions on diet in childhood holds promise for reducing risk of early puberty, independent of fatness. Future studies examining food-based dietary patterns and pubertal landmarks other than menarche are needed, as is research on the extent to which diet, and other modifiable lifestyle factors in childhood, such as physical activity, can influence long-term health and disease through their effects on pubertal timing. Continued investigation in this realm will continue to inform our understanding of the early origins of adult health and disease and, in doing so, highlight potential public health initiatives to facilitate primordial prevention.

REFERENCES

1. Golub MS, Collman GW, Foster PM, et al. Public health implications of altered puberty timing. *Pediatrics.* Feb 2008;121 Suppl 3:S218-230.

2. So Much More to Know. *Science.* 1 July 2005 2005;309(5731):25.

3. Euling SY, Herman-Giddens ME, Lee PA, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics.* Feb 2008;121 Suppl 3:S172-191.

4. Herman-Giddens ME, Steffes J, Harris D, et al. Secondary sexual characteristics in boys: data from the Pediatric Research in Office Settings Network. *Pediatrics.* Nov 2012;130(5):e1058-1068.

5. Gluckman PD, Bergstrom CT. Evolutionary biology within medicine: a perspective of growing value. *BMJ.* 2011;343:d7671.

6. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science.* Jul 24 1970;169(3943):397-399.

7. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. The relation of menarcheal age to obesity in childhood and adulthood: the Bogalusa heart study. *BMC Pediatr.* Apr 30 2003;3:3.

8. Biro FM, Khoury P, Morrison JA. Influence of obesity on timing of puberty. *Int J Androl.* Feb 2006;29(1):272-277; discussion 286-290.

9. Walvoord EC. The timing of puberty: is it changing? Does it matter? *J Adolesc Health.* Nov 2010;47(5):433-439.

10. DiVall SA, Radovick S. Pubertal development and menarche. *Ann N Y Acad Sci.* 2008;1135:19-28.

11. Helm P, Helm S. Decrease in menarcheal age from 1966 to 1983 in Denmark. *Acta obstetricia et gynecologica Scandinavica.* 1984;63(7):633-635.

12. Helm P, Grolund L. A halt in the secular trend towards earlier menarche in Denmark. *Acta obstetricia et gynecologica Scandinavica.* Feb 1998;77(2):198-200.

13. Tryggvadottir L, Tulinius H, Larusdottir M. A decline and a halt in mean age at menarche in Iceland. *Ann Hum Biol.* Mar-Apr 1994;21(2):179-186.

14. Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM. Pubertal development in The Netherlands 1965-1997. *Pediatr Res.* Oct 2001;50(4):479-486.

15. Dann TC, Roberts DF. Menarcheal age in University of Warwick young women. *J Biosoc Sci.* Oct 1993;25(4):531-538.

16. Hauspie RC, Vercauteren M, Susanne C. Secular changes in growth and maturation: an update. *Acta Paediatr Suppl.* Nov 1997;423:20-27.

17. Vercauteren M, Susanne C. The secular trend of height and menarche in Belgium: are there any signs of a future stop? *Eur J Pediatr.* Nov 1985;144(4):306-309.

18. Lindgren GW, Degerfors IL, Fredriksson A, et al. Menarche 1990 in Stockholm schoolgirls. *Acta Paediatr Scand.* Oct 1991;80(10):953-955.

19. He C, Zhang C, Hunter DJ, et al. Age at menarche and risk of type 2 diabetes: results from 2 large prospective cohort studies. *American journal of epidemiology.* Feb 1 2010;171(3):334-344.

20. Dreyfus JG, Lutsey PL, Huxley R, et al. Age at menarche and risk of type 2 diabetes among African-American and white women in the Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia.* Sep 2012;55(9):2371-2380.
21. Lakshman R, Forouhi N, Luben R, et al. Association between age at menarche and risk of diabetes in adults: results from the EPIC-Norfolk cohort study. *Diabetologia.* May 2008;51(5):781-786.
22. Stockl D, Doring A, Peters A, et al. Age at menarche is associated with prediabetes and diabetes in women (aged 32-81 years) from the general population: the KORA F4 Study. *Diabetologia.* Mar 2012;55(3):681-688.
23. Pierce MB, Kuh D, Hardy R. The role of BMI across the life course in the relationship between age at menarche and diabetes, in a British Birth Cohort. *Diabetic medicine : a journal of the British Diabetic Association.* May 2012;29(5):600-603.
24. Conway BN, Shu XO, Zhang X, et al. Age at menarche, the leg length to sitting height ratio, and risk of diabetes in middle-aged and elderly Chinese men and women. *PloS one.* 2012;7(3):e30625.
25. Mueller NT, Odegaard AO, Gross MD, Koh WP, Yuan JM, Pereira MA. Age at menarche and cardiovascular disease mortality in Singaporean Chinese women: the Singapore Chinese Health Study. *Annals of epidemiology.* Oct 2012;22(10):717-722.
26. Onland-Moret NC, Peeters PH, van Gils CH, et al. Age at menarche in relation to adult height: the EPIC study. *American journal of epidemiology.* Oct 1 2005;162(7):623-632.
27. Schooling CM, Jiang CQ, Heys M, et al. Is leg length a biomarker of childhood conditions in older Chinese women? The Guangzhou Biobank Cohort Study. *Journal of epidemiology and community health.* Feb 2008;62(2):160-166.
28. Juul A. The effects of oestrogens on linear bone growth. *Hum Reprod Update.* May-Jun 2001;7(3):303-313.
29. Perry RJ, Farquharson C, Ahmed SF. The role of sex steroids in controlling pubertal growth. *Clin Endocrinol (Oxf).* Jan 2008;68(1):4-15.
30. Vizmanos B, Marti-Henneberg C, Cliville R, Moreno A, Fernandez-Ballart J. Age of pubertal onset affects the intensity and duration of pubertal growth peak but not final height. *Am J Hum Biol.* May-Jun 2001;13(3):409-416.
31. Fraser A, Ebrahim S, Davey Smith G, Lawlor DA. The associations between height components (leg and trunk length) and adult levels of liver enzymes. *Journal of epidemiology and community health.* Jan 2008;62(1):48-53.
32. Trikudanathan S, Pedley A, Massaro JM, et al. Association of female reproductive factors with body composition: the framingham heart study. *The Journal of clinical endocrinology and metabolism.* Jan 2013;98(1):236-244.
33. Fabbrini E, Magkos F, Mohammed BS, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci U S A.* Sep 8 2009;106(36):15430-15435.
34. Nguyen-Duy TB, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab.* Jun 2003;284(6):E1065-1071.

35. Kim LJ, Nalls MA, Eiriksdottir G, et al. Associations of visceral and liver fat with the metabolic syndrome across the spectrum of obesity: the AGES-Reykjavik study. *Obesity (Silver Spring).* Jun 2011;19(6):1265-1271.
36. Pierce MB, Leon DA. Age at menarche and adult BMI in the Aberdeen children of the 1950s cohort study. *The American journal of clinical nutrition.* Oct 2005;82(4):733-739.
37. Stockl D, Meisinger C, Peters A, et al. Age at menarche and its association with the metabolic syndrome and its components: results from the KORA F4 study. *PloS one.* 2011;6(10):e26076.
38. Kivimaki M, Lawlor DA, Smith GD, et al. Association of age at menarche with cardiovascular risk factors, vascular structure, and function in adulthood: the Cardiovascular Risk in Young Finns study. *The American journal of clinical nutrition.* Jun 2008;87(6):1876-1882.
39. Feng Y, Hong X, Wilker E, et al. Effects of age at menarche, reproductive years, and menopause on metabolic risk factors for cardiovascular diseases. *Atherosclerosis.* Feb 2008;196(2):590-597.
40. Cui R, Iso H, Toyoshima H, et al. Relationships of age at menarche and menopause, and reproductive year with mortality from cardiovascular disease in Japanese postmenopausal women: the JACC study. *Journal of epidemiology / Japan Epidemiological Association.* Sep 2006;16(5):177-184.
41. Remsberg KE, Demerath EW, Schubert CM, Chumlea WC, Sun SS, Siervogel RM. Early menarche and the development of cardiovascular disease risk factors in adolescent girls: the Fels Longitudinal Study. *The Journal of clinical endocrinology and metabolism.* May 2005;90(5):2718-2724.
42. Gong TT, Wu QJ, Vogtmann E, Lin B, Wang YL. Age at menarche and risk of ovarian cancer: A meta-analysis of epidemiological studies. *Int J Cancer.* Nov 23 2012.
43. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* Oct 2004;13(10):1558-1568.
44. Kaltiala-Heino R, Kosunen E, Rimpela M. Pubertal timing, sexual behaviour and self-reported depression in middle adolescence. *J Adolesc.* Oct 2003;26(5):531-545.
45. Kaltiala-Heino R, Rimpela M, Rissanen A, Rantanen P. Early puberty and early sexual activity are associated with bulimic-type eating pathology in middle adolescence. *J Adolesc Health.* Apr 2001;28(4):346-352.
46. Kaltiala-Heino R, Marttunen M, Rantanen P, Rimpela M. Early puberty is associated with mental health problems in middle adolescence. *Soc Sci Med.* Sep 2003;57(6):1055-1064.
47. Silventoinen K, Haukka J, Dunkel L, Tynelius P, Rasmussen F. Genetics of pubertal timing and its associations with relative weight in childhood and adult height: the Swedish Young Male Twins Study. *Pediatrics.* Apr 2008;121(4):e885-891.

48. Cheng G, Buyken AE, Shi L, et al. Beyond overweight: nutrition as an important lifestyle factor influencing timing of puberty. *Nutrition reviews.* Mar 2012;70(3):133-152.
49. Mouritsen A, Aksglaede L, Sorensen K, et al. Hypothesis: exposure to endocrine-disrupting chemicals may interfere with timing of puberty. *Int J Androl.* Apr 2010;33(2):346-359.
50. Semiz S, Kurt F, Kurt DT, Zencir M, Sevinc O. Factors affecting onset of puberty in Denizli province in Turkey. *Turk J Pediatr.* Jan-Feb 2009;51(1):49-55.
51. Schwanzel-Fukuda M, Crossin KL, Pfaff DW, Bouloux PM, Hardelin JP, Petit C. Migration of luteinizing hormone-releasing hormone (LHRH) neurons in early human embryos. *J Comp Neurol.* Mar 11 1996;366(3):547-557.
52. Kaplan SL, Grumbach MM. Pituitary and placental gonadotrophins and sex steroids in the human and sub-human primate fetus. *Clin Endocrinol Metab.* Nov 1978;7(3):487-511.
53. Gulyas BJ, Hodgen GD, Tullner WW, Ross GT. Effects of fetal or maternal hypophysectomy on endocrine organs and body weight in infant rhesus monkeys (Macaca mulatta): with particular emphasis on oogenesis. *Biol Reprod.* Mar 1977;16(2):216-227.
54. Kaplan SL, Grumbach MM, Aubert ML. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of central nervous system regulation of anterior pituitary function. *Recent Prog Horm Res.* 1976;32:161-243.
55. Tanner JM. The assessment of growth and development in children. *Archives of disease in childhood.* Feb 1952;27(131):10-33.
56. Legro RS, Lin HM, Demers LM, Lloyd T. Rapid maturation of the reproductive axis during perimenarche independent of body composition. *The Journal of clinical endocrinology and metabolism.* Mar 2000;85(3):1021-1025.
57. Hansen JW, Hoffman HJ, Ross GT. Monthly gonadotropin cycles in premenarcheal girls. *Science.* Oct 10 1975;190(4210):161-163.
58. Heys M, Schooling CM, Jiang C, et al. Age of menarche and the metabolic syndrome in China. *Epidemiology.* Nov 2007;18(6):740-746.
59. Tanner JM, Davies PS. Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr.* Sep 1985;107(3):317-329.
60. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics.* Apr 1997;99(4):505-512.
61. Finlay F, Jones R. Precocious puberty. *Pediatrics.* Jul 2000;106(1 Pt 1):162-163.
62. Rosenfield RL, Bachrach LK, Chernausek SD, et al. Current age of onset of puberty. *Pediatrics.* Sep 2000;106(3):622-623.
63. Himes JH. Examining the evidence for recent secular changes in the timing of puberty in US children in light of increases in the prevalence of obesity. *Molecular and cellular endocrinology.* Jul 25 2006;254-255:13-21.

64. Anderson SE, Dallal GE, Must A. Relative weight and race influence average age at menarche: results from two nationally representative surveys of US girls studied 25 years apart. *Pediatrics.* Apr 2003;111(4 Pt 1):844-850.
65. Chumlea WC, Schubert CM, Roche AF, et al. Age at menarche and racial comparisons in US girls. *Pediatrics.* Jan 2003;111(1):110-113.
66. Sun SS, Schubert CM, Chumlea WC, et al. National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics.* Nov 2002;110(5):911-919.
67. Wu T, Mendola P, Buck GM. Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Pediatrics.* Oct 2002;110(4):752-757.
68. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. *Pediatrics.* Oct 1999;104(4 Pt 1):936-941.
69. Mogensen SS, Aksglaede L, Mouritsen A, et al. Diagnostic work-up of 449 consecutive girls who were referred to be evaluated for precocious puberty. *The Journal of clinical endocrinology and metabolism.* May 2011;96(5):1393-1401.
70. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Archives of disease in childhood.* Feb 1970;45(239):13-23.
71. Rosenfield RL, Lipton RB, Drum ML. Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. *Pediatrics.* Jan 2009;123(1):84-88.
72. Aksglaede L, Juul A, Olsen LW, Sorensen TI. Age at puberty and the emerging obesity epidemic. *PloS one.* 2009;4(12):e8450.
73. Ahmed ML, Ong KK, Dunger DB. Childhood obesity and the timing of puberty. *Trends Endocrinol Metab.* Jul 2009;20(5):237-242.
74. Burt Solorzano CM, McCartney CR. Obesity and the pubertal transition in girls and boys. *Reproduction.* Sep 2010;140(3):399-410.
75. Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet.* Dec 2010;42(12):1077-1085.
76. Veldhuis JD, Roemmich JN, Richmond EJ, Bowers CY. Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. *Endocrine reviews.* Apr 2006;27(2):101-140.
77. Zhao J, Xiong DH, Guo Y, Yang TL, Recker RR, Deng HW. Polymorphism in the insulin-like growth factor 1 gene is associated with age at menarche in caucasian females. *Hum Reprod.* Jun 2007;22(6):1789-1794.
78. Thankamony A, Ong KK, Ahmed ML, Ness AR, Holly JM, Dunger DB. Higher levels of IGF-I and adrenal androgens at age 8 years are associated with earlier age at menarche in girls. *The Journal of clinical endocrinology and metabolism.* May 2012;97(5):E786-790.
79. Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL. Insulin-like growth factor I of peripheral origin acts centrally to accelerate the initiation of female puberty. *Endocrinology.* Sep 1996;137(9):3717-3728.

80. Pazos F, Sanchez-Franco F, Balsa J, Lopez-Fernandez J, Escalada J, Cacicedo L. Regulation of gonadal and somatotropic axis by chronic intraventricular infusion of insulin-like growth factor 1 antibody at the initiation of puberty in male rats. *Neuroendocrinology.* Jun 1999;69(6):408-416.

81. Zhen S, Zakaria M, Wolfe A, Radovick S. Regulation of gonadotropin-releasing hormone (GnRH) gene expression by insulin-like growth factor I in a cultured GnRH-expressing neuronal cell line. *Mol Endocrinol.* Jul 1997;11(8):1145-1155.

82. Daftary SS, Gore AC. IGF-1 in the brain as a regulator of reproductive neuroendocrine function. *Exp Biol Med (Maywood).* May 2005;230(5):292-306.

83. Moran A, Jacobs DR, Jr., Steinberger J, et al. Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes.* Oct 1999;48(10):2039-2044.

84. Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. *Diabetes.* Nov 2001;50(11):2444-2450.

85. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. *Pediatr Res.* Dec 2006;60(6):759-763.

86. Moran A, Jacobs DR, Jr., Steinberger J, et al. Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. *The Journal of clinical endocrinology and metabolism.* Oct 2002;87(10):4817-4820.

87. Holly JM, Smith CP, Dunger DB, et al. Levels of the small insulin-like growth factor-binding protein are strongly related to those of insulin in prepubertal and pubertal children but only weakly so after puberty. *J Endocrinol.* May 1989;121(2):383-387.

88. Caprio S. Insulin: the other anabolic hormone of puberty. *Acta Paediatr Suppl.* Dec 1999;88(433):84-87.

89. Ibanez L, Ong K, Valls C, Marcos MV, Dunger DB, de Zegher F. Metformin treatment to prevent early puberty in girls with precocious pubarche. *The Journal of clinical endocrinology and metabolism.* Aug 2006;91(8):2888-2891.

90. Ibanez L, Valls C, Ong K, Dunger DB, de Zegher F. Metformin therapy during puberty delays menarche, prolongs pubertal growth, and augments adult height: a randomized study in low-birth-weight girls with early-normal onset of puberty. *The Journal of clinical endocrinology and metabolism.* Jun 2006;91(6):2068-2073.

91. Simon D. Puberty in chronically diseased patients. *Horm Res.* 2002;57 Suppl 2:53-56.

92. Vigersky RA, Andersen AE, Thompson RH, Loriaux DL. Hypothalamic dysfunction in secondary amenorrhea associated with simple weight loss. *The New England journal of medicine.* Nov 24 1977;297(21):1141-1145.

93. I'Anson H, Manning JM, Herbosa CG, et al. Central inhibition of gonadotropin-releasing hormone secretion in the growth-restricted hypogonadotropic female sheep. *Endocrinology.* Feb 2000;141(2):520-527.

94. Rosenbaum M, Leibel RL. The role of leptin in human physiology. *The New England journal of medicine.* Sep 16 1999;341(12):913-915.

95. Clayton PE, Gill MS, Hall CM, Tillmann V, Whatmore AJ, Price DA. Serum leptin through childhood and adolescence. *Clin Endocrinol (Oxf).* Jun 1997;46(6):727-733.
96. Blum WF, Englaro P, Hanitsch S, et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *The Journal of clinical endocrinology and metabolism.* Sep 1997;82(9):2904-2910.
97. Kaplowitz PB. Link between body fat and the timing of puberty. *Pediatrics.* Feb 2008;121 Suppl 3:S208-217.
98. Andreelli F, Hanaire-Broutin H, Laville M, Tauber JP, Riou JP, Thivolet C. Normal reproductive function in leptin-deficient patients with lipoatropic diabetes. *The Journal of clinical endocrinology and metabolism.* Feb 2000;85(2):715-719.
99. Chehab FF, Mounzih K, Lu R, Lim ME. Early onset of reproductive function in normal female mice treated with leptin. *Science.* Jan 3 1997;275(5296):88-90.
100. Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS. Leptin accelerates the onset of puberty in normal female mice. *The Journal of clinical investigation.* Feb 1 1997;99(3):391-395.
101. Bronson FH. Puberty in female mice is not associated with increases in either body fat or leptin. *Endocrinology.* Nov 2001;142(11):4758-4761.
102. Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA. Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology.* Feb 1997;138(2):855-858.
103. Plant TM, Durrant AR. Circulating leptin does not appear to provide a signal for triggering the initiation of puberty in the male rhesus monkey (Macaca mulatta). *Endocrinology.* Oct 1997;138(10):4505-4508.
104. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. *JAMA : the journal of the American Medical Association.* Feb 1 2012;307(5):483-490.
105. Johnson W, Choh AC, Curran JE, et al. Genetic risk for earlier menarche also influences peripubertal body mass index. *Am J Phys Anthropol.* Jan 2013;150(1):10-20.
106. Demerath EW, Towne B, Chumlea WC, et al. Recent decline in age at menarche: the Fels Longitudinal Study. *Am J Hum Biol.* Jul-Aug 2004;16(4):453-457.
107. Mamun AA, Hayatbakhsh MR, O'Callaghan M, Williams G, Najman J. Early overweight and pubertal maturation--pathways of association with young adults' overweight: a longitudinal study. *Int J Obes (Lond).* Jan 2009;33(1):14-20.
108. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science.* Sep 17 2004;305(5691):1733-1736.
109. Cooper C, Kuh D, Egger P, Wadsworth M, Barker D. Childhood growth and age at menarche. *Br J Obstet Gynaecol.* Aug 1996;103(8):814-817.
110. Blell M, Pollard TM, Pearce MS. Predictors of age at menarche in the newcastle thousand families study. *J Biosoc Sci.* Jul 2008;40(4):563-575.
111. Delemarre-van de Waal HA, van Coeverden SC, Engelbregt MT. Factors affecting onset of puberty. *Horm Res.* 2002;57 Suppl 2:15-18.

112. Luo ZC, Cheung YB, He Q, Albertsson-Wikland K, Karlberg J. Growth in early life and its relation to pubertal growth. *Epidemiology.* Jan 2003;14(1):65-73.
113. Persson I, Ahlsson F, Ewald U, et al. Influence of perinatal factors on the onset of puberty in boys and girls: implications for interpretation of link with risk of long term diseases. *American journal of epidemiology.* Oct 1 1999;150(7):747-755.
114. Romundstad PR, Vatten LJ, Nilsen TI, et al. Birth size in relation to age at menarche and adolescent body size: implications for breast cancer risk. *Int J Cancer.* Jun 20 2003;105(3):400-403.
115. Tam CS, de Zegher F, Garnett SP, Baur LA, Cowell CT. Opposing influences of prenatal and postnatal growth on the timing of menarche. *The Journal of clinical endocrinology and metabolism.* Nov 2006;91(11):4369-4373.
116. dos Santos Silva I, De Stavola BL, Mann V, Kuh D, Hardy R, Wadsworth ME. Prenatal factors, childhood growth trajectories and age at menarche. *International journal of epidemiology.* Apr 2002;31(2):405-412.
117. Ibanez L, Ferrer A, Marcos MV, Hierro FR, de Zegher F. Early puberty: rapid progression and reduced final height in girls with low birth weight. *Pediatrics.* Nov 2000;106(5):E72.
118. Morris DH, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Determinants of age at menarche in the UK: analyses from the Breakthrough Generations Study. *Br J Cancer.* Nov 23 2010;103(11):1760-1764.
119. Opdahl S, Nilsen TI, Romundstad PR, Vanky E, Carlsen SM, Vatten LJ. Association of size at birth with adolescent hormone levels, body size and age at menarche: relevance for breast cancer risk. *Br J Cancer.* Jul 8 2008;99(1):201-206.
120. Bhargava SK, Ramji S, Srivastava U, et al. Growth and sexual maturation of low birth weight children: a 14 year follow up. *Indian Pediatr.* Sep 1995;32(9):963-970.
121. Lazar L, Pollak U, Kalter-Leibovici O, Pertzelan A, Phillip M. Pubertal course of persistently short children born small for gestational age (SGA) compared with idiopathic short children born appropriate for gestational age (AGA). *Eur J Endocrinol.* Nov 2003;149(5):425-432.
122. Koziel S, Jankowska EA. Effect of low versus normal birthweight on menarche in 14-year-old Polish girls. *J Paediatr Child Health.* Jun 2002;38(3):268-271.
123. Terry MB, Ferris JS, Tehranifar P, Wei Y, Flom JD. Birth weight, postnatal growth, and age at menarche. *American journal of epidemiology.* Jul 1 2009;170(1):72-79.
124. Epplein M, Novotny R, Daida Y, Vijayadeva V, Onaka AT, Le Marchand L. Association of maternal and intrauterine characteristics with age at menarche in a multiethnic population in Hawaii. *Cancer Causes Control.* Feb 2010;21(2):259-268.
125. Maisonet M, Christensen KY, Rubin C, et al. Role of prenatal characteristics and early growth on pubertal attainment of British girls. *Pediatrics.* Sep 2010;126(3):e591-600.
126. Semiz S, Kurt F, Kurt DT, Zencir M, Sevinc O. Pubertal development of Turkish children. *J Pediatr Endocrinol Metab.* Oct 2008;21(10):951-961.

127. Chaudhari S, Otiv M, Hoge M, Pandit A, Mote A. Growth and sexual maturation of low birth weight infants at early adolescence. *Indian Pediatr.* Mar 2008;45(3):191-198.
128. Boyne MS, Thame M, Osmond C, et al. Growth, body composition, and the onset of puberty: longitudinal observations in Afro-Caribbean children. *The Journal of clinical endocrinology and metabolism.* Jul 2010;95(7):3194-3200.
129. Adair LS. Size at birth predicts age at menarche. *Pediatrics.* Apr 2001;107(4):E59.
130. Dunger DB, Ahmed ML, Ong KK. Early and late weight gain and the timing of puberty. *Molecular and cellular endocrinology.* Jul 25 2006;254-255:140-145.
131. Sloboda DM, Hart R, Doherty DA, Pennell CE, Hickey M. Age at menarche: Influences of prenatal and postnatal growth. *The Journal of clinical endocrinology and metabolism.* Jan 2007;92(1):46-50.
132. Hui LL, Leung GM, Wong MY, Lam TH, Schooling CM. Small for gestational age and age at puberty: evidence from Hong Kong's "Children of 1997" birth cohort. *American journal of epidemiology.* Nov 1 2012;176(9):785-793.
133. Hui LL, Wong MY, Lam TH, Leung GM, Schooling CM. Infant growth and onset of puberty: prospective observations from Hong Kong's "Children of 1997" birth cohort. *Annals of epidemiology.* Jan 2012;22(1):43-50.
134. Moisan J, Meyer F, Gingras S. Diet and age at menarche. *Cancer Causes Control.* Sep 1990;1(2):149-154.
135. Berkey CS, Gardner JD, Frazier AL, Colditz GA. Relation of childhood diet and body size to menarche and adolescent growth in girls. *American journal of epidemiology.* Sep 1 2000;152(5):446-452.
136. Koo MM, Rohan TE, Jain M, McLaughlin JR, Corey PN. A cohort study of dietary fibre intake and menarche. *Public Health Nutr.* Apr 2002;5(2):353-360.
137. Maclure M, Travis LB, Willett W, MacMahon B. A prospective cohort study of nutrient intake and age at menarche. *The American journal of clinical nutrition.* Oct 1991;54(4):649-656.
138. Merzenich H, Boeing H, Wahrendorf J. Dietary fat and sports activity as determinants for age at menarche. *American journal of epidemiology.* Aug 15 1993;138(4):217-224.
139. Meyer F, Moisan J, Marcoux D, Bouchard C. Dietary and physical determinants of menarche. *Epidemiology.* Sep 1990;1(5):377-381.
140. Rogers IS, Northstone K, Dunger DB, Cooper AR, Ness AR, Emmett PM. Diet throughout childhood and age at menarche in a contemporary cohort of British girls. *Public Health Nutr.* Dec 2010;13(12):2052-2063.
141. de Ridder CM, Thijssen JH, Van 't Veer P, et al. Dietary habits, sexual maturation, and plasma hormones in pubertal girls: a longitudinal study. *The American journal of clinical nutrition.* Nov 1991;54(5):805-813.
142. Goldin BR, Woods MN, Spiegelman DL, et al. The effect of dietary fat and fiber on serum estrogen concentrations in premenopausal women under controlled dietary conditions. *Cancer.* Aug 1 1994;74(3 Suppl):1125-1131.

143. Arts CJ, Govers CA, van den Berg H, Wolters MG, van Leeuwen P, Thijssen JH. In vitro binding of estrogens by dietary fiber and the in vivo apparent digestibility tested in pigs. *J Steroid Biochem Mol Biol.* May 1991;38(5):621-628.
144. Cheng G, Remer T, Prinz-Langenohl R, Blaszkewicz M, Degen GH, Buyken AE. Relation of isoflavones and fiber intake in childhood to the timing of puberty. *The American journal of clinical nutrition.* Sep 2010;92(3):556-564.
145. Knight DC, Eden JA. Phytoestrogens--a short review. *Maturitas.* Nov 1995;22(3):167-175.
146. Adlercreutz H, Bannwart C, Wahala K, et al. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol.* Feb 1993;44(2):147-153.
147. Gunnarsson C, Ahnstrom M, Kirschner K, et al. Amplification of HSD17B1 and ERBB2 in primary breast cancer. *Oncogene.* Jan 9 2003;22(1):34-40.
148. Sanchez A, Kissinger DG, Phillips RI. A hypothesis on the etiological role of diet on age of menarche. *Medical hypotheses.* Nov 1981;7(11):1339-1345.
149. Petridou E, Syrigou E, Toupadaki N, Zavitsanos X, Willett W, Trichopoulos D. Determinants of age at menarche as early life predictors of breast cancer risk. *Int J Cancer.* Oct 9 1996;68(2):193-198.
150. Budek AZ, Hoppe C, Michaelsen KF, Bugel S, Molgaard C. Associations of total, dairy, and meat protein with markers for bone turnover in healthy, prepubertal boys. *The Journal of nutrition.* Apr 2007;137(4):930-934.
151. Gunther AL, Karaolis-Danckert N, Kroke A, Remer T, Buyken AE. Dietary protein intake throughout childhood is associated with the timing of puberty. *The Journal of nutrition.* Mar 2010;140(3):565-571.
152. Remer T, Shi L, Buyken AE, Maser-Gluth C, Hartmann MF, Wudy SA. Prepubertal adrenarchal androgens and animal protein intake independently and differentially influence pubertal timing. *The Journal of clinical endocrinology and metabolism.* Jun 2010;95(6):3002-3009.
153. Hoppe C, Udam TR, Lauritzen L, Molgaard C, Juul A, Michaelsen KF. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. *The American journal of clinical nutrition.* Aug 2004;80(2):447-452.
154. Jacobs DR, Jr., Gross MD, Tapsell LC. Food synergy: an operational concept for understanding nutrition. *The American journal of clinical nutrition.* May 2009;89(5):1543S-1548S.
155. Cheng G, Gerlach S, Libuda L, et al. Diet quality in childhood is prospectively associated with the timing of puberty but not with body composition at puberty onset. *The Journal of nutrition.* Jan 2010;140(1):95-102.
156. Malina RM. Menarche in athletes: a synthesis and hypothesis. *Ann Hum Biol.* Jan-Feb 1983;10(1):1-24.
157. Luger A, Deuster PA, Kyle SB, et al. Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *The New England journal of medicine.* May 21 1987;316(21):1309-1315.

158. MacConnie SE, Barkan A, Lampman RM, Schork MA, Beitins IZ. Decreased hypothalamic gonadotropin-releasing hormone secretion in male marathon runners. *The New England journal of medicine.* Aug 14 1986;315(7):411-417.
159. Weimann E, Blum WF, Witzel C, Schwidergall S, Bohles HJ. Hypoleptinemia in female and male elite gymnasts. *Eur J Clin Invest.* Oct 1999;29(10):853-860.
160. Chavarro J, Villamor E, Narvaez J, Hoyos A. Socio-demographic predictors of age at menarche in a group of Colombian university women. *Ann Hum Biol.* Mar-Apr 2004;31(2):245-257.
161. Burguera B, Muruais C, Penalva A, Dieguez C, Casanueva FF. Dual and selective actions of glucocorticoids upon basal and stimulated growth hormone release in man. *Neuroendocrinology.* Jan 1990;51(1):51-58.
162. Daughaday WH, Herington AC, Phillips LS. The regulation of growth by endocrines. *Annu Rev Physiol.* 1975;37:211-244.
163. Wronka I, Pawlinska-Chmara R. Menarcheal age and socio-economic factors in Poland. *Ann Hum Biol.* Sep-Oct 2005;32(5):630-638.
164. Mishra GD, Cooper R, Tom SE, Kuh D. Early life circumstances and their impact on menarche and menopause. *Womens Health (Lond Engl).* Mar 2009;5(2):175-190.
165. Junqueira Do Lago M, Faerstein E, De Souza Lopes C, Werneck GL. Family socio-economic background modified secular trends in age at menarche: evidence from the Pro-Saude Study (Rio de Janeiro, Brazil). *Ann Hum Biol.* May-Jun 2003;30(3):347-352.
166. Tither JM, Ellis BJ. Impact of fathers on daughters' age at menarche: a genetically and environmentally controlled sibling study. *Dev Psychol.* Sep 2008;44(5):1409-1420.
167. Ellis BJ, Garber J. Psychosocial antecedents of variation in girls' pubertal timing: maternal depression, stepfather presence, and marital and family stress. *Child Dev.* Mar-Apr 2000;71(2):485-501.
168. Padez C. Social background and age at menarche in Portuguese university students: a note on the secular changes in Portugal. *Am J Hum Biol.* May-Jun 2003;15(3):415-427.
169. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden. I. Menarcheal age. *Acta Paediatr Scand.* Aug-Sep 1991;80(8-9):852-858.
170. Boynton-Jarrett R, Harville EW. A prospective study of childhood social hardships and age at menarche. *Annals of epidemiology.* Oct 2012;22(10):731-737.
171. Ellis BJ. Timing of pubertal maturation in girls: an integrated life history approach. *Psychol Bull.* Nov 2004;130(6):920-958.
172. Moffitt TE, Caspi A, Belsky J, Silva PA. Childhood experience and the onset of menarche: a test of a sociobiological model. *Child Dev.* Feb 1992;63(1):47-58.
173. Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. *The Journal of clinical endocrinology and metabolism.* Jun 1998;83(6):1827-1834.

174. Prebeg Z, Bralic I. Changes in menarcheal age in girls exposed to war conditions. *Am J Hum Biol.* Jul 2000;12(4):503-508.
175. Ozen S, Darcan S. Effects of environmental endocrine disruptors on pubertal development. *J Clin Res Pediatr Endocrinol.* 2011;3(1):1-6.
176. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine reviews.* Jun 2009;30(4):293-342.
177. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet.* May 27 2006;367(9524):1747-1757.
178. Danaei G, Lawes CM, Vander Hoorn S, Murray CJ, Ezzati M. Global and regional mortality from ischaemic heart disease and stroke attributable to higher-than-optimum blood glucose concentration: comparative risk assessment. *Lancet.* Nov 11 2006;368(9548):1651-1659.
179. Danaei G, Finucane MM, Lu Y, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet.* Jul 2 2011;378(9785):31-40.
180. Bjork S, Kapur A, King H, Nair J, Ramachandran A. Global policy: aspects of diabetes in India. *Health Policy.* Oct 2003;66(1):61-72.
181. MacLeod KM, Tooke JE. Direct and indirect costs of cardiovascular and cerebrovascular complications of type II diabetes. *Pharmacoeconomics.* 1995;8 Suppl 1:46-51.
182. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. *Med Clin North Am.* Jul 2004;88(4):787-835, ix.
183. Groop L, Lyssenko V. Genes and type 2 diabetes mellitus. *Curr Diab Rep.* Jun 2008;8(3):192-197.
184. Ferrannini E, Simonson DC, Katz LD, et al. The disposal of an oral glucose load in patients with non-insulin-dependent diabetes. *Metabolism: clinical and experimental.* Jan 1988;37(1):79-85.
185. DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism: clinical and experimental.* Apr 1989;38(4):387-395.
186. Groop LC, Bonadonna RC, DelPrato S, et al. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *The Journal of clinical investigation.* Jul 1989;84(1):205-213.
187. Grill V. A comparison of brain glucose metabolism in diabetes as measured by positron emission tomography or by arteriovenous techniques. *Ann Med.* Jun 1990;22(3):171-176.
188. Defronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes.* Apr 2009;58(4):773-795.

189. Raynor LA, Pankow JS, Duncan BB, et al. Novel Risk Factors and the Prediction of Type 2 Diabetes in the Atherosclerosis Risk in Communities (ARIC) Study. *Diabetes care.* Jan 2013;36(1):70-76.
190. Odegaard AO, Koh WP, Vazquez G, et al. BMI and diabetes risk in Singaporean Chinese. *Diabetes care.* Jun 2009;32(6):1104-1106.
191. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell.* Sep 14 2012;150(6):1223-1234.
192. Diagnosis and classification of diabetes mellitus. *Diabetes care.* Jan 2012;35 Suppl 1:S64-71.
193. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. *Diabetes care.* Jun 2006;29(6):1263-1268.
194. Yang SH, Dou KF, Song WJ. Prevalence of diabetes among men and women in China. *The New England journal of medicine.* Jun 24 2010;362(25):2425-2426; author reply 2426.
195. Malerbi DA, Franco LJ. Multicenter study of the prevalence of diabetes mellitus and impaired glucose tolerance in the urban Brazilian population aged 30-69 yr. The Brazilian Cooperative Group on the Study of Diabetes Prevalence. *Diabetes care.* Nov 1992;15(11):1509-1516.
196. Jarrett RJ, Keen H. Hyperglycaemia and diabetes mellitus. *Lancet.* Nov 6 1976;2(7993):1009-1012.
197. Sayegh HA, Jarrett RJ. Oral glucose-tolerance tests and the diagnosis of diabetes: results of a prospective study based on the Whitehall survey. *Lancet.* Sep 1 1979;2(8140):431-433.
198. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes: response to the International Expert Committee. *Diabetes care.* Dec 2009;32(12):e159; author reply e160.
199. James C, Bullard KM, Rolka DB, et al. Implications of alternative definitions of prediabetes for prevalence in U.S. adults. *Diabetes care.* Feb 2011;34(2):387-391.
200. Kim CH, Kim HK, Bae SJ, Park JY, Lee KU. Discordance between fasting glucose-based and hemoglobin A1c-based diagnosis of diabetes mellitus in Koreans. *Diabetes research and clinical practice.* Jan 2011;91(1):e8-e10.
201. Christensen DL, Witte DR, Kaduka L, et al. Moving to an A1C-based diagnosis of diabetes has a different impact on prevalence in different ethnic groups. *Diabetes care.* Mar 2010;33(3):580-582.
202. Unwin N, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabetic medicine : a journal of the British Diabetic Association.* Sep 2002;19(9):708-723.
203. Olson DE, Rhee MK, Herrick K, Ziemer DC, Twombly JG, Phillips LS. Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria. *Diabetes care.* Oct 2010;33(10):2184-2189.

204. Mostafa SA, Khunti K, Srinivasan BT, Webb D, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, to detect people with impaired glucose regulation in a UK multi-ethnic cohort. *Diabetes research and clinical practice.* Oct 2010;90(1):100-108.

205. Ziemer DC, Kolm P, Weintraub WS, et al. Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. *Annals of internal medicine.* Jun 15 2010;152(12):770-777.

206. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. Is haemoglobin A1c a step forward for diagnosing diabetes? *BMJ.* 2009;339:b4432.

207. Borch-Johnsen K, Colagiuri S. Diagnosing diabetes--time for a change? *Diabetologia.* Nov 2009;52(11):2247-2250.

208. Herman WH, Ma Y, Uwaifo G, et al. Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. *Diabetes care.* Oct 2007;30(10):2453-2457.

209. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice.* Dec 2011;94(3):311-321.

210. Schmidt MI, Duncan BB, Hoffmann JF, Moura L, Malta DC, Carvalho RM. Prevalence of diabetes and hypertension based on self-reported morbidity survey, Brazil, 2006. *Rev Saude Publica.* Nov 2009;43 Suppl 2:74-82.

211. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England journal of medicine.* Feb 7 2002;346(6):393-403.

212. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England journal of medicine.* May 3 2001;344(18):1343-1350.

213. Pan XR, Li GW, Hu YH, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes care.* Apr 1997;20(4):537-544.

214. Dyson PA, Hammersley MS, Morris RJ, Holman RR, Turner RC. The Fasting Hyperglycaemia Study: II. Randomized controlled trial of reinforced healthy-living advice in subjects with increased but not diabetic fasting plasma glucose. *Metabolism: clinical and experimental.* Dec 1997;46(12 Suppl 1):50-55.

215. Kosaka K, Noda M, Kuzuya T. Prevention of type 2 diabetes by lifestyle intervention: a Japanese trial in IGT males. *Diabetes research and clinical practice.* Feb 2005;67(2):152-162.

216. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). *Diabetologia.* Feb 2006;49(2):289-297.

217. Bo S, Ciccone G, Baldi C, et al. Effectiveness of a lifestyle intervention on metabolic syndrome. A randomized controlled trial. *J Gen Intern Med.* Dec 2007;22(12):1695-1703.

218. Roumen C, Corpeleijn E, Feskens EJ, Mensink M, Saris WH, Blaak EE. Impact of 3-year lifestyle intervention on postprandial glucose metabolism: the SLIM

study. *Diabetic medicine : a journal of the British Diabetic Association.* May 2008;25(5):597-605.

219. Lindahl B, Nilsson TK, Borch-Johnsen K, et al. A randomized lifestyle intervention with 5-year follow-up in subjects with impaired glucose tolerance: pronounced short-term impact but long-term adherence problems. *Scand J Public Health.* Jun 2009;37(4):434-442.

220. Danaei G, Pan A, Hu FB, Hernan MA. Hypothetical midlife interventions in women and risk of type 2 diabetes. *Epidemiology.* Jan 2013;24(1):122-128.

221. Hu FB, Manson JE, Stampfer MJ, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *The New England journal of medicine.* Sep 13 2001;345(11):790-797.

222. Mozaffarian D, Kamineni A, Carnethon M, Djousse L, Mukamal KJ, Siscovick D. Lifestyle risk factors and new-onset diabetes mellitus in older adults: the cardiovascular health study. *Archives of internal medicine.* Apr 27 2009;169(8):798-807.

223. Malik VS, Popkin BM, Bray GA, Despres JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes care.* Nov 2010;33(11):2477-2483.

224. Huxley R, Lee CM, Barzi F, et al. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: a systematic review with meta-analysis. *Archives of internal medicine.* Dec 14 2009;169(22):2053-2063.

225. Freeman SR, Drake AL, Heilig LF, et al. Statins, fibrates, and melanoma risk: a systematic review and meta-analysis. *J Natl Cancer Inst.* Nov 1 2006;98(21):1538-1546.

226. Knowler WC, Fowler SE, Hamman RF, et al. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet.* Nov 14 2009;374(9702):1677-1686.

227. Lindstrom J, Louheranta A, Mannelin M, et al. The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. *Diabetes care.* Dec 2003;26(12):3230-3236.

228. Fabricatore AN. Behavior therapy and cognitive-behavioral therapy of obesity: is there a difference? *J Am Diet Assoc.* Jan 2007;107(1):92-99.

229. Wadden TA, Webb VL, Moran CH, Bailer BA. Lifestyle modification for obesity: new developments in diet, physical activity, and behavior therapy. *Circulation.* Mar 6 2012;125(9):1157-1170.

230. Lasker GW. Human biological adaptability. The ecological approach in physical anthropology. *Science.* Dec 19 1969;166(3912):1480-1486.

231. Bogin B, Varela-Silva MI. Leg length, body proportion, and health: a review with a note on beauty. *Int J Environ Res Public Health.* Mar 2010;7(3):1047-1075.

232. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia.* Jul 1992;35(7):595-601.

233. Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. *International journal of epidemiology.* Dec 2002;31(6):1235-1239.

234. Gluckman PD, Hanson MA, Beedle AS. Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am J Hum Biol.* Jan-Feb 2007;19(1):1-19.
235. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ.* Oct 26 1991;303(6809):1019-1022.
236. Ravelli AC, van der Meulen JH, Michels RP, et al. Glucose tolerance in adults after prenatal exposure to famine. *Lancet.* Jan 17 1998;351(9097):173-177.
237. Li Y, He Y, Qi L, et al. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. *Diabetes.* Oct 2010;59(10):2400-2406.
238. Petitt DJ, Bennett PH, Knowler WC, Baird HR, Aleck KA. Gestational diabetes mellitus and impaired glucose tolerance during pregnancy. Long-term effects on obesity and glucose tolerance in the offspring. *Diabetes.* Jun 1985;34 Suppl 2:119-122.
239. Pettitt DJ, Aleck KA, Baird HR, Carraher MJ, Bennett PH, Knowler WC. Congenital susceptibility to NIDDM. Role of intrauterine environment. *Diabetes.* May 1988;37(5):622-628.
240. Pettitt DJ, Knowler WC. Long-term effects of the intrauterine environment, birth weight, and breast-feeding in Pima Indians. *Diabetes care.* Aug 1998;21 Suppl 2:B138-141.
241. Dabelea D, Hanson RL, Lindsay RS, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes.* Dec 2000;49(12):2208-2211.
242. Fleten C, Nystad W, Stigum H, et al. Parent-offspring body mass index associations in the Norwegian Mother and Child Cohort Study: a family-based approach to studying the role of the intrauterine environment in childhood adiposity. *American journal of epidemiology.* Jul 15 2012;176(2):83-92.
243. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes care.* Jun 2009;32(6):1076-1080.
244. Mingrone G, Manco M, Mora ME, et al. Influence of maternal obesity on insulin sensitivity and secretion in offspring. *Diabetes care.* Sep 2008;31(9):1872-1876.
245. Soto N, Bazaes RA, Pena V, et al. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. *The Journal of clinical endocrinology and metabolism.* Aug 2003;88(8):3645-3650.
246. Nobili V, Alisi A, Panera N, Agostoni C. Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. *Pediatr Endocrinol Rev.* Dec 2008;6(2):241-247.
247. Barker DJ, Osmond C, Kajantie E, Eriksson JG. Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol.* Sep-Oct 2009;36(5):445-458.
248. Gunderson EP. Breast-feeding and diabetes: long-term impact on mothers and their infants. *Curr Diab Rep.* Aug 2008;8(4):279-286.

249. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet.* Mar 29 2003;361(9363):1089-1097.
250. Ravelli AC, van der Meulen JH, Osmond C, Barker DJ, Bleker OP. Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. *Archives of disease in childhood.* Mar 2000;82(3):248-252.
251. Mayer-Davis EJ, Dabelea D, Lamichhane AP, et al. Breast-feeding and type 2 diabetes in the youth of three ethnic groups: the SEARCh for diabetes in youth case-control study. *Diabetes care.* Mar 2008;31(3):470-475.
252. Lawlor DA, Riddoch CJ, Page AS, et al. Infant feeding and components of the metabolic syndrome: findings from the European Youth Heart Study. *Archives of disease in childhood.* Jun 2005;90(6):582-588.
253. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *The American journal of clinical nutrition.* Nov 2006;84(5):1043-1054.
254. Karapanou O, Papadimitriou A. Determinants of menarche. *Reprod Biol Endocrinol.* 2010;8:115.
255. Schooling CM, Jiang CQ, Lam TH, et al. Leg length and age of puberty among men and women from a developing population: the Guangzhou Biobank Cohort study. *Am J Hum Biol.* Sep-Oct 2010;22(5):683-687.
256. Lorentzon M, Norjavaara E, Kindblom JM. Pubertal timing predicts leg length and childhood body mass index predicts sitting height in young adult men. *J Pediatr.* Mar 2011;158(3):452-457.
257. Sandhu J, Ben-Shlomo Y, Cole TJ, Holly J, Davey Smith G. The impact of childhood body mass index on timing of puberty, adult stature and obesity: a follow-up study based on adolescent anthropometry recorded at Christ's Hospital (1936-1964). *Int J Obes (Lond).* Jan 2006;30(1):14-22.
258. McIntyre MH. Adult stature, body proportions and age at menarche in the United States National Health and Nutrition Survey (NHANES) III. *Ann Hum Biol.* Nov 2011;38(6):716-720.
259. Leitch I. Growth and health. *The British journal of nutrition.* 1951;5(1):142-151.
260. Bogin B. Childhood, adolescence, and longevity: A multilevel model of the evolution of reserve capacity in human life history. *Am J Hum Biol.* Jul-Aug 2009;21(4):567-577.
261. Asao K, Kao WH, Baptiste-Roberts K, Bandeen-Roche K, Erlinger TP, Brancati FL. Short stature and the risk of adiposity, insulin resistance, and type 2 diabetes in middle age: the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. *Diabetes care.* Jul 2006;29(7):1632-1637.
262. Ibanez L, Lopez-Bermejo A, Diaz M, Marcos MV, de Zegher F. Early metformin therapy (age 8-12 years) in girls with precocious pubarche to reduce hirsutism, androgen excess, and oligomenorrhea in adolescence. *The Journal of clinical endocrinology and metabolism.* Aug 2011;96(8):E1262-1267.
263. Lawlor DA, Ebrahim S, Davey Smith G. The association between components of adult height and Type II diabetes and insulin resistance: British Women's Heart and Health Study. *Diabetologia.* Aug 2002;45(8):1097-1106.

264. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *Journal of hepatology.* Aug 2010;53(2):372-384.
265. Gluckman PD, Hanson MA, Spencer HG. Predictive adaptive responses and human evolution. *Trends Ecol Evol.* Oct 2005;20(10):527-533.
266. Cooper GS, Ephross SA, Sandler DP. Menstrual patterns and risk of adult-onset diabetes mellitus. *Journal of clinical epidemiology.* Nov 2000;53(11):1170-1173.
267. Dreyfus JG, Lutsey PL, Huxley R, et al. Age at menarche and risk of type 2 diabetes among African-American and white women in the Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia.* Jul 4 2012.
268. Mueller NT, Odegaard AO, Gross MD, Koh WP, Yuan JM, Pereira MA. Age at menarche and cardiovascular disease mortality in Singaporean Chinese women: the Singapore Chinese Health Study. *Annals of epidemiology.* Aug 30 2012.
269. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology.* Jun 2012;55(6):2005-2023.
270. Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *Journal of hepatology.* Jan 2005;42(1):132-138.
271. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc.* Jul 1980;55(7):434-438.
272. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis.* Aug 2004;8(3):521-533, viii.
273. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary pharmacology & therapeutics.* Aug 2011;34(3):274-285.
274. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol.* Jun 2011;9(6):524-530 e521; quiz e560.
275. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med.* Dec 2011;43(8):617-649.
276. Wedemeyer H, Hofmann WP, Lueth S, et al. [ALT screening for chronic liver diseases: scrutinizing the evidence]. *Z Gastroenterol.* Jan 2010;48(1):46-55.
277. Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *Journal of hepatology.* Sep 2009;51(3):433-445.
278. Kodama Y, Ng CS, Wu TT, et al. Comparison of CT methods for determining the fat content of the liver. *AJR Am J Roentgenol.* May 2007;188(5):1307-1312.

279. Pickhardt PJ, Park SH, Hahn L, Lee SG, Bae KT, Yu ES. Specificity of unenhanced CT for non-invasive diagnosis of hepatic steatosis: implications for the investigation of the natural history of incidental steatosis. *Eur Radiol.* May 2012;22(5):1075-1082.

280. Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic Fatty liver disease in the United States: the third national health and nutrition examination survey, 1988-1994. *American journal of epidemiology.* Jul 1 2013;178(1):38-45.

281. Kallwitz ER, Kumar M, Aggarwal R, et al. Ethnicity and nonalcoholic fatty liver disease in an obesity clinic: the impact of triglycerides. *Digestive diseases and sciences.* May 2008;53(5):1358-1363.

282. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology.* Dec 2004;40(6):1387-1395.

283. Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology.* Jan 2003;124(1):71-79.

284. Frith J, Day CP, Henderson E, Burt AD, Newton JL. Non-alcoholic fatty liver disease in older people. *Gerontology.* 2009;55(6):607-613.

285. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *Journal of hepatology.* Oct 2008;49(4):608-612.

286. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology.* Jul 2005;129(1):113-121.

287. Dunn W, Xu R, Wingard DL, et al. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. *Am J Gastroenterol.* Sep 2008;103(9):2263-2271.

288. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *The Journal of clinical investigation.* Mar 2008;118(3):829-838.

289. Kariv R, Leshno M, Beth-Or A, et al. Re-evaluation of serum alanine aminotransferase upper normal limit and its modulating factors in a large-scale population study. *Liver Int.* May 2006;26(4):445-450.

290. Younossi ZM, Stepanova M, Negro F, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine (Baltimore).* Nov 2012;91(6):319-327.

291. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol.* Mar 2006;40 Suppl 1:S5-10.

292. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int.* Jan 2009;29(1):113-119.

293. Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Jarvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? *Dig Liver Dis.* May 2010;42(5):320-330.

294. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology.* Apr 1998;114(4):842-845.
295. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology.* Nov 2010;52(5):1836-1846.
296. Sanyal AJ. Mechanisms of Disease: pathogenesis of nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol.* Jan 2005;2(1):46-53.
297. Day CP. From fat to inflammation. *Gastroenterology.* Jan 2006;130(1):207-210.
298. Feldstein AE, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology.* Jul 2004;40(1):185-194.
299. Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology.* Jun 2007;45(6):1366-1374.
300. Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. *Semin Liver Dis.* Nov 2008;28(4):370-379.
301. Roskams T, Yang SQ, Koteish A, et al. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. *Am J Pathol.* Oct 2003;163(4):1301-1311.
302. Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol.* Aug 2000;1(2):113-118.
303. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocrine reviews.* Apr 2002;23(2):201-229.
304. Stefan N, Kantartzis K, Haring HU. Causes and metabolic consequences of Fatty liver. *Endocrine reviews.* Dec 2008;29(7):939-960.
305. McCullough AJ. Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol.* Mar 2006;40 Suppl 1:S17-29.
306. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics.* Oct 2006;118(4):1388-1393.
307. Kim HJ, Kim HJ, Lee KE, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Archives of internal medicine.* Oct 25 2004;164(19):2169-2175.
308. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol.* Oct 2001;96(10):2957-2961.
309. Weston SR, Leyden W, Murphy R, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology.* Feb 2005;41(2):372-379.
310. Schwimmer JB, Celedon MA, Lavine JE, et al. Heritability of nonalcoholic fatty liver disease. *Gastroenterology.* May 2009;136(5):1585-1592.

311. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* Dec 2008;40(12):1461-1465.
312. Kotronen A, Johansson LE, Johansson LM, et al. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. *Diabetologia.* Jun 2009;52(6):1056-1060.
313. Sookoian S, Castano GO, Burgueno AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res.* Oct 2009;50(10):2111-2116.
314. Valenti L, Nobili V, Al-Serri A, et al. The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. *Journal of hepatology.* Dec 2011;55(6):1409-1414.
315. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *The Journal of clinical endocrinology and metabolism.* Jul 2002;87(7):3023-3028.
316. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes.* Mar 2005;54(3):603-608.
317. Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *The New England journal of medicine.* Mar 25 2010;362(12):1082-1089.
318. Assy N, Nasser G, Kamayse I, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol.* Oct 2008;22(10):811-816.
319. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, et al. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *Journal of hepatology.* Nov 2007;47(5):711-717.
320. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *Journal of hepatology.* Jun 2008;48(6):993-999.
321. Musso G, Gambino R, De Michieli F, et al. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology.* Apr 2003;37(4):909-916.
322. Yasutake K, Nakamuta M, Shima Y, et al. Nutritional investigation of non-obese patients with non-alcoholic fatty liver disease: the significance of dietary cholesterol. *Scand J Gastroenterol.* 2009;44(4):471-477.
323. Cortez-Pinto H, Jesus L, Barros H, Lopes C, Moura MC, Camilo ME. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clin Nutr.* Oct 2006;25(5):816-823.
324. Catalano D, Martines GF, Tonzuso A, Pirri C, Trovato FM, Trovato GM. Protective role of coffee in non-alcoholic fatty liver disease (NAFLD). *Digestive diseases and sciences.* Nov 2010;55(11):3200-3206.

325. Oddy WH, Herbison CE, Jacoby P, et al. The Western dietary pattern is prospectively associated with nonalcoholic fatty liver disease in adolescence. *Am J Gastroenterol.* May 2013;108(5):778-785.
326. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, et al. Role of leisure-time physical activity in nonalcoholic fatty liver disease: a population-based study. *Hepatology.* Dec 2008;48(6):1791-1798.
327. Church TS, Kuk JL, Ross R, Priest EL, Biltoft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology.* Jun 2006;130(7):2023-2030.
328. Krasnoff JB, Painter PL, Wallace JP, Bass NM, Merriman RB. Health-related fitness and physical activity in patients with nonalcoholic fatty liver disease. *Hepatology.* Apr 2008;47(4):1158-1166.
329. Centis E, Marzocchi R, Suppini A, et al. The Role of Lifestyle Change in the Prevention and Treatment Of NAFLD. *Curr Pharm Des.* Feb 4 2013.
330. Alisi A, Panera N, Agostoni C, Nobili V. Intrauterine growth retardation and nonalcoholic Fatty liver disease in children. *Int J Endocrinol.* 2011;2011:269853.
331. Gruenwald P. Chronic Fetal Distress and Placental Insufficiency. *Biol Neonat.* 1963;5:215-265.
332. Barker DJ. Fetal programming of coronary heart disease. *Trends Endocrinol Metab.* Nov 2002;13(9):364-368.
333. Desai M, Crowther NJ, Lucas A, Hales CN. Organ-selective growth in the offspring of protein-restricted mothers. *The British journal of nutrition.* Oct 1996;76(4):591-603.
334. Burns SP, Desai M, Cohen RD, et al. Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. *The Journal of clinical investigation.* Oct 1 1997;100(7):1768-1774.
335. Magee TR, Han G, Cherian B, Khorram O, Ross MG, Desai M. Down-regulation of transcription factor peroxisome proliferator-activated receptor in programmed hepatic lipid dysregulation and inflammation in intrauterine growth-restricted offspring. *Am J Obstet Gynecol.* Sep 2008;199(3):271 e271-275.
336. Tilg H, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. *Trends Endocrinol Metab.* Dec 2008;19(10):371-379.
337. Fagerberg B, Bondjers L, Nilsson P. Low birth weight in combination with catch-up growth predicts the occurrence of the metabolic syndrome in men at late middle age: the Atherosclerosis and Insulin Resistance study. *Journal of internal medicine.* Sep 2004;256(3):254-259.
338. Latini G, De Mitri B, Del Vecchio A, Chitano G, De Felice C, Zetterstrom R. Foetal growth of kidneys, liver and spleen in intrauterine growth restriction: "programming" causing "metabolic syndrome" in adult age. *Acta Paediatr.* Dec 2004;93(12):1635-1639.
339. Andersen AM, Osler M. Birth dimensions, parental mortality, and mortality in early adult age: a cohort study of Danish men born in 1953. *International journal of epidemiology.* Feb 2004;33(1):92-99.

340. Fraser A, Ebrahim S, Smith GD, Lawlor DA. The associations between birthweight and adult markers of liver damage and function. *Paediatr Perinat Epidemiol.* Jan 2008;22(1):12-21.
341. McDonald A, Van Horn L, Slattery M, et al. The CARDIA dietary history: development, implementation, and evaluation. *J Am Diet Assoc.* Sep 1991;91(9):1104-1112.
342. Ibanez L, Lopez-Bermejo A, Diaz M, Marcos MV, de Zegher F. Early metformin therapy to delay menarche and augment height in girls with precocious pubarche. *Fertility and sterility.* Feb 2011;95(2):727-730.
343. Mucci LA, Kuper HE, Tamimi R, Lagiou P, Spanos E, Trichopoulos D. Age at menarche and age at menopause in relation to hepatocellular carcinoma in women. *BJOG : an international journal of obstetrics and gynaecology.* Mar 2001;108(3):291-294.
344. Yu MW, Chang HC, Chang SC, et al. Role of reproductive factors in hepatocellular carcinoma: Impact on hepatitis B- and C-related risk. *Hepatology.* Dec 2003;38(6):1393-1400.
345. Aquino EM, Barreto SM, Bensenor IM, et al. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): objectives and design. *American journal of epidemiology.* Feb 15 2012;175(4):315-324.
346. Must A, Phillips SM, Naumova EN, et al. Recall of early menstrual history and menarcheal body size: after 30 years, how well do women remember? *American journal of epidemiology.* Apr 1 2002;155(7):672-679.
347. Friedman GD, Cutter GR, Donahue RP, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *Journal of clinical epidemiology.* 1988;41(11):1105-1116.
348. Sidney S, Jacobs DR, Jr., Haskell WL, et al. Comparison of two methods of assessing physical activity in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *American journal of epidemiology.* Jun 15 1991;133(12):1231-1245.
349. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc.* Oct 1988;88(10):1268-1271.
350. Campaigne BN, Morrison JA, Schumann BC, et al. Indexes of obesity and comparisons with previous national survey data in 9- and 10-year-old black and white girls: the National Heart, Lung, and Blood Institute Growth and Health Study. *The Journal of pediatrics.* May 1994;124(5 Pt 1):675-680.
351. Schmidt MI, Duncan BB, Azevedo e Silva G, et al. Chronic non-communicable diseases in Brazil: burden and current challenges. *Lancet.* Jun 4 2011;377(9781):1949-1961.
352. United Nations. Dept. of International Economic and Social Affairs., United Nations. Dept. for Economic and Social Information and Policy Analysis., United Nations. Dept. of Economic and Social Affairs. Population Division. World urbanization prospects. New York: United Nations:v.
353. Monteiro CA, Conde WL, Popkin BM. The burden of disease from undernutrition and overnutrition in countries undergoing rapid nutrition transition: a view from Brazil. *Am J Public Health.* Mar 2004;94(3):433-434.

354. Wing RR, Bolin P, Brancati FL, et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *The New England journal of medicine.* Jul 11 2013;369(2):145-154.
355. Pantsiotou S, Papadimitriou A, Douros K, Priftis K, Nicolaidou P, Fretzayas A. Maturational tempo differences in relation to the timing of the onset of puberty in girls. *Acta Paediatr.* Feb 2008;97(2):217-220.
356. Kac G, Auxiliadora de Santa Cruz C, Velasquez-Melendez G. Secular trend in age at menarche for women born between 1920 and 1979 in Rio de Janeiro, Brazil. *Ann Hum Biol.* Jul-Aug 2000;27(4):423-428.
357. Buyken AE, Karaolis-Danckert N, Remer T. Association of prepubertal body composition in healthy girls and boys with the timing of early and late pubertal markers. *The American journal of clinical nutrition.* Jan 2009;89(1):221-230.
358. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. *American journal of epidemiology.* Aug 1 2005;162(3):199-200.
359. Herman KM, Craig CL, Gauvin L, Katzmarzyk PT. Tracking of obesity and physical activity from childhood to adulthood: the Physical Activity Longitudinal Study. *Int J Pediatr Obes.* 2009;4(4):281-288.
360. Weitzman S, Wang CH, Pankow JS, Schmidt MI, Brancati FL. Are measures of height and leg length related to incident diabetes mellitus? The ARIC (Atherosclerosis Risk in Communities) study. *Acta Diabetol.* Sep 2010;47(3):237-242.
361. Estatistica IBdGe. *Pesquisa de Orc□amentos Familiares 2008-2009*2010.
362. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *The American journal of clinical nutrition.* Nov 1999;70(5):811-816.
363. de Rooij SR, Painter RC, Roseboom TJ, et al. Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. *Diabetologia.* Apr 2006;49(4):637-643.
364. Waterlow JC. Protein-energy malnutrition: the nature and extent of the problem. *Clin Nutr.* Mar 1997;16 Suppl 1:3-9.
365. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* Sep 9 1989;2(8663):577-580.
366. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early growth and coronary heart disease in later life: longitudinal study. *BMJ.* Apr 21 2001;322(7292):949-953.
367. Gonzalez-Barranco J, Rios-Torres JM, Castillo-Martinez L, et al. Effect of malnutrition during the first year of life on adult plasma insulin and glucose tolerance. *Metabolism: clinical and experimental.* Aug 2003;52(8):1005-1011.
368. Martins PA, Sawaya AL. Evidence for impaired insulin production and higher sensitivity in stunted children living in slums. *The British journal of nutrition.* May 2006;95(5):996-1001.
369. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organization technical report series.* 1995;854:1-452.

370. da Luz Santos CD, Clemente AP, Martins VJ, Albuquerque MP, Sawaya AL. Adolescents with mild stunting show alterations in glucose and insulin metabolism. *Journal of nutrition and metabolism.* 2010;2010:943070.
371. Florencio TT, Ferreira HS, Cavalcante JC, Stux GR, Sawaya AL. Short stature, abdominal obesity, insulin resistance and alterations in lipid profile in very low-income women living in Maceio, north-eastern Brazil. *Eur J Cardiovasc Prev Rehabil.* Apr 2007;14(2):346-348.
372. Sawaya AL, Grillo LP, Verreschi I, da Silva AC, Roberts SB. Mild stunting is associated with higher susceptibility to the effects of high fat diets: studies in a shantytown population in Sao Paulo, Brazil. *The Journal of nutrition.* Feb 1998;128(2 Suppl):415S-420S.
373. Hoffman DJ, Sawaya AL, Verreschi I, Tucker KL, Roberts SB. Why are nutritionally stunted children at increased risk of obesity? Studies of metabolic rate and fat oxidation in shantytown children from Sao Paulo, Brazil. *The American journal of clinical nutrition.* Sep 2000;72(3):702-707.
374. Hoffman DJ, Sawaya AL, Coward WA, et al. Energy expenditure of stunted and nonstunted boys and girls living in the shantytowns of Sao Paulo, Brazil. *The American journal of clinical nutrition.* Oct 2000;72(4):1025-1031.
375. Monteiro CA, Benicio MH, Gouveia Nda C. Secular growth trends in Brazil over three decades. *Ann Hum Biol.* Jul-Aug 1994;21(4):381-390.
376. Folsom AR, Jacobs DR, Wagenknecht LE, et al. Increase in fasting insulin and glucose over seven years with increasing weight and inactivity of young adults. The CARDIA Study. Coronary Artery Risk Development in Young Adults. *Am. J. Epidemiol.* 1996;144(3):235-246.
377. Davidson LE, Kuk JL, Church TS, Ross R. Protocol for measurement of liver fat by computed tomography. *J Appl Physiol.* Mar 2006;100(3):864-868.
378. Liu J, Musani SK, Bidulescu A, et al. Fatty liver, abdominal adipose tissue and atherosclerotic calcification in African Americans: the Jackson Heart Study. *Atherosclerosis.* Oct 2012;224(2):521-525.
379. Park SH, Kim PN, Kim KW, et al. Macrovesicular hepatic steatosis in living liver donors: use of CT for quantitative and qualitative assessment. *Radiology.* Apr 2006;239(1):105-112.
380. Boyce CJ, Pickhardt PJ, Kim DH, et al. Hepatic steatosis (fatty liver disease) in asymptomatic adults identified by unenhanced low-dose CT. *AJR Am J Roentgenol.* Mar 2010;194(3):623-628.
381. Liu J, Fox CS, Hickson D, Bidulescu A, Carr JJ, Taylor HA. Fatty liver, abdominal visceral fat, and cardiometabolic risk factors: the Jackson Heart Study. *Arterioscler Thromb Vasc Biol.* Nov 2011;31(11):2715-2722.
382. Wirt A, Collins CE. Diet quality--what is it and does it matter? *Public Health Nutr.* Dec 2009;12(12):2473-2492.
383. Obesity and cardiovascular disease risk factors in black and white girls: the NHLBI Growth and Health Study. *Am J Public Health.* Dec 1992;82(12):1613-1620.

384. Crawford PB, Obarzanek E, Morrison J, Sabry ZI. Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9- and 10-year-old girls. *J Am Diet Assoc.* Jun 1994;94(6):626-630.
385. Keys A, Anderson JT, Grande F. Serum cholesterol in man: diet fat and intrinsic responsiveness. *Circulation.* Feb 1959;19(2):201-214.
386. Obarzanek E, Schreiber GB, Crawford PB, et al. Energy intake and physical activity in relation to indexes of body fat: the National Heart, Lung, and Blood Institute Growth and Health Study. *The American journal of clinical nutrition.* Jul 1994;60(1):15-22.
387. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol.* Oct 1988;60(5):709-723.
388. Koprowski C, Ross RK, Mack WJ, Henderson BE, Bernstein L. Diet, body size and menarche in a multiethnic cohort. *Br J Cancer.* Apr 1999;79(11-12):1907-1911.
389. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. Relation of age at menarche to race, time period, and anthropometric dimensions: the Bogalusa Heart Study. *Pediatrics.* Oct 2002;110(4):e43.
390. Reagan PB, Salsberry PJ, Fang MZ, Gardner WP, Pajer K. African-American/white differences in the age of menarche: accounting for the difference. *Soc Sci Med.* Oct 2012;75(7):1263-1270.
391. Biro FM, McMahon RP, Striegel-Moore R, et al. Impact of timing of pubertal maturation on growth in black and white female adolescents: The National Heart, Lung, and Blood Institute Growth and Health Study. *J Pediatr.* May 2001;138(5):636-643.
392. Bremer AA, Auinger P, Byrd RS. Relationship between insulin resistance-associated metabolic parameters and anthropometric measurements with sugar-sweetened beverage intake and physical activity levels in US adolescents: findings from the 1999-2004 National Health and Nutrition Examination Survey. *Arch Pediatr Adolesc Med.* Apr 2009;163(4):328-335.
393. Pereira MA. Diet beverages and the risk of obesity, diabetes, and cardiovascular disease: a review of the evidence. *Nutrition reviews.* Jul 2013;71(7):433-440.
394. de Koning L, Malik VS, Rimm EB, Willett WC, Hu FB. Sugar-sweetened and artificially sweetened beverage consumption and risk of type 2 diabetes in men. *The American journal of clinical nutrition.* Jun 2011;93(6):1321-1327.
395. Maras JE, Bermudez OI, Qiao N, Bakun PJ, Boody-Alter EL, Tucker KL. Intake of alpha-tocopherol is limited among US adults. *J Am Diet Assoc.* Apr 2004;104(4):567-575.
396. Mayer-Davis EJ, Costacou T, King I, Zaccaro DJ, Bell RA. Plasma and dietary vitamin E in relation to incidence of type 2 diabetes: The Insulin Resistance and Atherosclerosis Study (IRAS). *Diabetes care.* Dec 2002;25(12):2172-2177.
397. McCullough ML, Feskanich D, Stampfer MJ, et al. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. *The American journal of clinical nutrition.* Dec 2002;76(6):1261-1271.
398. Frazier AL, Rosenberg SM. Preadolescent and adolescent risk factors for benign breast disease. *J Adolesc Health.* May 2013;52(5 Suppl):S36-40.

399. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *The New England journal of medicine.* May 6 2010;362(18):1675-1685.
400. Moore LL, Singer MR, Qureshi MM, Bradlee ML, Daniels SR. Food group intake and micronutrient adequacy in adolescent girls. *Nutrients.* Nov 2012;4(11):1692-1708.
401. Northstone K, Smith AD, Cribb VL, Emmett PM. Dietary patterns in UK adolescents obtained from a dual-source FFQ and their associations with socio-economic position, nutrient intake and modes of eating. *Public Health Nutr.* Jun 20 2013:1-10.
402. Talegawkar SA, Johnson EJ, Carithers TC, Taylor HA, Jr., Bogle ML, Tucker KL. Serum carotenoid and tocopherol concentrations vary by dietary pattern among African Americans. *J Am Diet Assoc.* Dec 2008;108(12):2013-2020.
403. Gao X, Martin A, Lin H, Bermudez OI, Tucker KL. alpha-Tocopherol intake and plasma concentration of Hispanic and non-Hispanic white elders is associated with dietary intake pattern. *The Journal of nutrition.* Oct 2006;136(10):2574-2579.
404. Jacobsen BK, Heuch I, Kvale G. Association of low age at menarche with increased all-cause mortality: a 37-year follow-up of 61,319 Norwegian women. *American journal of epidemiology.* Dec 15 2007;166(12):1431-1437.
405. Jacobsen BK, Oda K, Knutsen SF, Fraser GE. Age at menarche, total mortality and mortality from ischaemic heart disease and stroke: the Adventist Health Study, 1976-88. *International journal of epidemiology.* Feb 2009;38(1):245-252.
406. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol.* Feb 2002;13(1):3-9.
407. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Archives of disease in childhood.* Jun 1969;44(235):291-303.
408. de Ridder CM, Thijssen JH, Bruning PF, Van den Brande JL, Zonderland ML, Erich WB. Body fat mass, body fat distribution, and pubertal development: a longitudinal study of physical and hormonal sexual maturation of girls. *The Journal of clinical endocrinology and metabolism.* Aug 1992;75(2):442-446.
409. Reynolds EL, Wines JV. Individual differences in physical changes associated with adolescence in girls. *Am J Dis Child.* Mar 1948;75(3):329-350.
410. Wilson ME, Fisher J, Chikazawa K, et al. Leptin administration increases nocturnal concentrations of luteinizing hormone and growth hormone in juvenile female rhesus monkeys. *The Journal of clinical endocrinology and metabolism.* Oct 2003;88(10):4874-4883.
411. Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Honjo H. Leptin directly stimulates aromatase activity in human luteinized granulosa cells. *Mol Hum Reprod.* Aug 1999;5(8):708-713.
412. Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC. The insulin-related ovarian regulatory system in health and disease. *Endocrine reviews.* Aug 1999;20(4):535-582.