ENERGY BALANCE-RELATED FACTORS AND INSULIN-RELATED BIOMARKERS THROUGHOUT PUBERTY: A POTENTIAL LINK BETWEEN CHILDHOOD ADIPOSITY AND BREAST CANCER

By

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ABSTRACT

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Background: Childhood adiposity has been shown to be inversely associated with both pre- and post-menopausal breast cancer (BC), potentially mediated through insulin-related biomarkers (insulin, C-peptide, glucose, and HOMA). Childhood energy balance-related factors including dietary and anthropometric factors have been shown to be associated with insulin-related biomarkers in adults, and have also been implicated in the development of BC. The overall goal of this dissertation research is to examine associations of dietary and anthropometric factors with insulin-related biomarkers among girls during puberty development. **Methods:** We conducted a systematic review of epidemiological studies that examined associations of dietary factors and fasting insulin-related biomarkers in healthy children and adolescents through PUBMED. We also conducted secondary analyses of data from the Dietary Intervention Study in Children (DISC), a multicenter randomized clinical trial. We performed cross-sectional data analyses to examine associations between dietary intake of selected nutrients as well as BMI-for-age percentile (BMIPCT) and insulin-related biomarkers, among 176 postmenarcheal girls (age 14-18 years) who attended the last visit. We then examined associations between baseline childhood anthropometric indices and longitudinal measures of insulin-related biomarkers among girls aged 8-10 years at baseline of the DISC and followed for a median duration of 7 years. Dietary intake was assessed via 3

averaged, nonconsecutive 24-hr dietary recalls. Anthropometric indices were measured at multiple visits by trained interviewers. Biomarkers were determined by immunoassay (insulin, C-peptide), enzymatic reaction (glucose) and calculation (HOMA). Stepwise linear regression and multivariable linear mixed-effect models were used for statistical analyses. **Results:** Literature review identified 13 studies that examined associations between dietary factors and insulin-related biomarkers during childhood. The evidence suggests total sugar, sugar-sweetened beverage, added sugar, simple carbohydrate, fructose, white bread and milk are positively associated with insulin-related biomarkers, whereas a healthy dietary pattern, whole grain and fiber are negatively associated with insulin-related biomarkers. Our cross-sectional data analyses suggest dietary fiber or vegetable protein intake is inversely associated with C-peptide, and starch intake is inversely associated with HOMA. BMIPCT measured in these adolescents is positively associated with insulin, C-peptide and HOMA levels. Our longitudinal analyses indicate greater BMIPCT measured at baseline is associated with higher insulin-related biomarkers premenarche; greater baseline waist circumference (WC) is associated with higher insulin-related biomarkers both pre- and postmenarche; and greater baseline height is associated with higher insulin-related biomarkers postmenarche. **Conclusions:** Limited data is available that examine dietary factors and the insulin system in children. This study suggests dietary factors including fiber, vegetable protein and starch intake as well as anthropometric factors including baseline BMIPCT, WC and height are associated with insulin-related biomarkers among girls during puberty. Since the insulin system has been hypothesized to be in the biological pathway in the development of BC, pubertal changes in these biomarkers could affect later BC risk.

This dissertation is dedicated to my beloved parents, Zhongsuo Zhang and Xinmei Liu;
and my wonderful husband Zhuang Feng.
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KEY TO ABBREVIATIONS

BC: Breast Cancer

BMI: Body Mass Index

BMIPCT: Body Mass Index-for-age Percentile

CV: Coefficient of Variation

DISC: Dietary Intervention Study in Children

HOMA: Homeostasis Model Assessment of Insulin Resistance

IRBS: Insulin-Related Biomarkers Study

SD: Standard deviation

T2D: Type 2 Diabetes

WC: Waist Circumference

WHR: Waist-to-Hip Ratio

WHtR: Waist-to-Height Ratio

CHAPTER 1: INTRODUCTION AND AIMS

1.1. Introduction

Breast cancer is the most common cause of death from cancer in women worldwide and the most prevalent cancer in women in the United States as well as several other industrialized countries.[1] Chronic diseases, including obesity and type 2 diabetes, have increased to epidemic levels worldwide.[2,3,4-6] These conditions also appear to be associated with breast cancer. The associations between adiposity and breast cancer risk however are complex. Epidemiological studies have consistently shown adult obesity to be positively associated with postmenopausal, but negatively associated with premenopausal breast cancer risk.[7] Childhood adiposity, on the other hand, has been shown to be inversely associated with both pre- and post-menopausal breast cancer.[8-15] These associations have been hypothesized to be mediated through circulating insulin-related biomarker levels (e.g. insulin, C-peptide, glucose) and insulin sensitivity (homeostasis model assessment of insulin resistance [HOMA]), though the biologic mechanisms are not well understood.[16,17]

One hypothesized biologic mechanism underlying the association between childhood adiposity and breast cancer is that insulin-related biomarkers could re-program mammary morphogenesis during puberty. These biomarkers could indirectly stimulate mammary gland differentiation through decreasing sex-hormone binding globulin (SHBG) and increasing bioavailable estrogen levels.[18] Early breast differentiation could then reduce the sensitivity of the breast to malignant carcinogenic exposures in later life, providing life-long protective effects to the breast against carcinogenesis.[19,20] Since the association of adiposity and

breast cancer is complex and varies over the life course, the effect of insulin-related biomarkers on breast tissue may be different between childhood, early adulthood and late adulthood.

Childhood adiposity is determined by energy balance-related factors including nutritional intake and could affect several anthropometric indices. Nutritional and anthropometric factors have also been shown to be associated with breast cancer risk. Specifically, childhood adiposity is inversely associated with adolescent peak height velocity,[9,21] which has been found to be positively associated with breast cancer.[8,22,23] In addition, higher childhood adiposity and an energy-dense diet have been associated with early age at menarche, which has been associated with increased breast cancer risk.[24] The latter is however associated with shorter adult height,[25,26] which in turn is associated with a decreased risk for breast cancer.[27,28] Shorter adult height is also associated with childhood undernutrition, which is associated with lower adiposity, an increased risk factor for breast cancer. All these associations between nutritional and anthropometric factors through puberty do not seem readily reconcilable in terms of their risks with breast cancer. However, they have all been shown to be associated with levels of adult insulin-related metabolic biomarkers,[29] and potentially childhood insulin-related biomarker levels which may further affect breast cancer development.[30-34] Childhood biomarker levels may track into adulthood,[31-34] and higher adult insulin-related biomarker levels have been associated with increased breast cancer risk.[35]

The associations between dietary or anthropometric factors with insulin-related biomarkers may be primarily through their effects on carbohydrate metabolism and insulin secretion from pancreatic β-cells.[36,37] The seemingly irreconcilable associations between childhood adiposity related factors and breast cancer risk underlie the need to study insulin-related biomarkers during puberty.[38,39] Since insulin promotes growth during puberty, and since the breast is undergoing substantial anatomical changes during pubertal development, it may be particularly sensitive to changes in circulating levels of insulin-related biomarkers.[38,40] Childhood and adolescence are transitional periods accompanied by tremendous growth and development, in particular breast tissue maturation.[41,42] At menarche, insulin or insulin resistance level peaks whereas insulin sensitivity decreases.[38,43,44] Amplified insulin resistance during puberty is offset by increased insulin secretion to maintain glucose homeostasis.[45] Therefore, puberty is a critical time period when the risk for breast cancer may be 'set', or may modify later exposures associated with the eventual development of breast cancer.[46]

Given that potentially modifiable childhood energy balance-related factors could be associated with circulating insulin levels and insulin sensitivity, a better understanding of how dietary and anthropometric factors affect serum insulin-related biomarkers among healthy children and adolescents during puberty may well help us to understand the development of breast cancer and other chronic diseases such as type 2 diabetes. Since elevated levels of insulin-related biomarkers during childhood may potentially decrease the susceptibility of breast tissues to various carcinogens, in later life, even a small decrease in biomarker levels

at the population level could have a significant public health impact. Identification of modifiable childhood energy balance-related factors that may affect insulin-related biomarkers may therefore be important in the prevention of breast cancer.

In this dissertation, we conducted secondary data analyses to examine the association between childhood dietary as well as anthropometric factors and blood levels of insulin-related biomarkers (insulin, glucose, C-peptide) and insulin sensitivity measured by HOMA. We used data from the longitudinal Dietary Intervention Study in Children (DISC), a multicenter randomized clinical trial. Subjects include adolescent girls aged 8-10 years at baseline of the DISC and followed for a median duration of 7 years. Among repeated fasting blood samples of the 301 girls in the DISC, 270 had data on insulin-related biomarkers collected during at least one of the four visits: baseline, study years 3, 5 and last visit (total blood samples n=579). Dietary intake was assessed via 3 averaged, nonconsecutive 24–hour dietary recalls with nutrient intakes estimated using Nutrition Data System v20. Anthropometric indices (weight, height, waist circumference and hip circumference) were measured at multiple visits by trained interviewers.

This dissertation examines the association of dietary and anthropometric factors with fasting serum levels of insulin-related biomarkers throughout puberty among children and adolescents in the DISC study, which may provide further insights into the mechanisms underlying the puzzling protection effect of childhood adiposity on breast cancer. **Figure 1.1** shows the framework of this dissertation. Results from this prospective study may contribute to a better understanding of the biological regulatory mechanisms of the insulin system throughout puberty and its possible role in the etiology of breast cancer and other chronic diseases. The results also may have important public health implications on disease

prevention by providing evidence that may inform dietary or other lifestyle recommendations during childhood and adolescence that may help prevent breast cancer and other chronic diseases.

1.2. Aims and Hypotheses

The specific aims and hypotheses of this dissertations are:

Aim 1): Literature Review on Dietary Correlates of Insulin-related Biomarkers in Healthy Children and Adolescents:

To systematically identify and review all published literature on dietary correlates of insulin and its related biomarkers (glucose, C-peptide), and insulin sensitivity as calculated homeostasis model assessment (HOMA) level in healthy children and adolescents.

AIM 2): Association between Dietary and Anthropometric Factors and Insulin-related Biomarkers among Postmenarcheal Adolescent Girls:

To examine the effect of specific dietary factors and Body Mass Index (BMI)-for-age-percentile (BMIPCT) on insulin-related biomarkers (Insulin, C-peptide, glucose, HOMA) in adolescent girls who attended the last visit of the Dietary Intervention Study in Children (DISC)

Hypotheses: 1) Higher dietary intake of specific dietary constituents such as saturated fat, and animal protein is associated with increased levels of insulin-related biomarker (Insulin, C-peptide, glucose, HOMA). 2) Higher dietary intake of fiber and vegetable protein is associated with decreased levels of insulin-related biomarker. 3) The association between dietary factors and biomarkers is more apparent with C-peptide than insulin since C-peptide has a longer half-life than insulin. 4) BMIPCT is positively associated with insulin-related biomarker components.

AIM 3): Longitudinal Analysis of Anthropometric Factors and Insulin-related Biomarkers throughout Pubertal Development in Girls:

To prospectively examine the association between baseline anthropometric parameter (BMIPCT, height and waist circumference) and longitudinal measures of serum insulin-related biomarkers (Insulin, C-peptide, glucose, HOMA) among girls in the DISC.

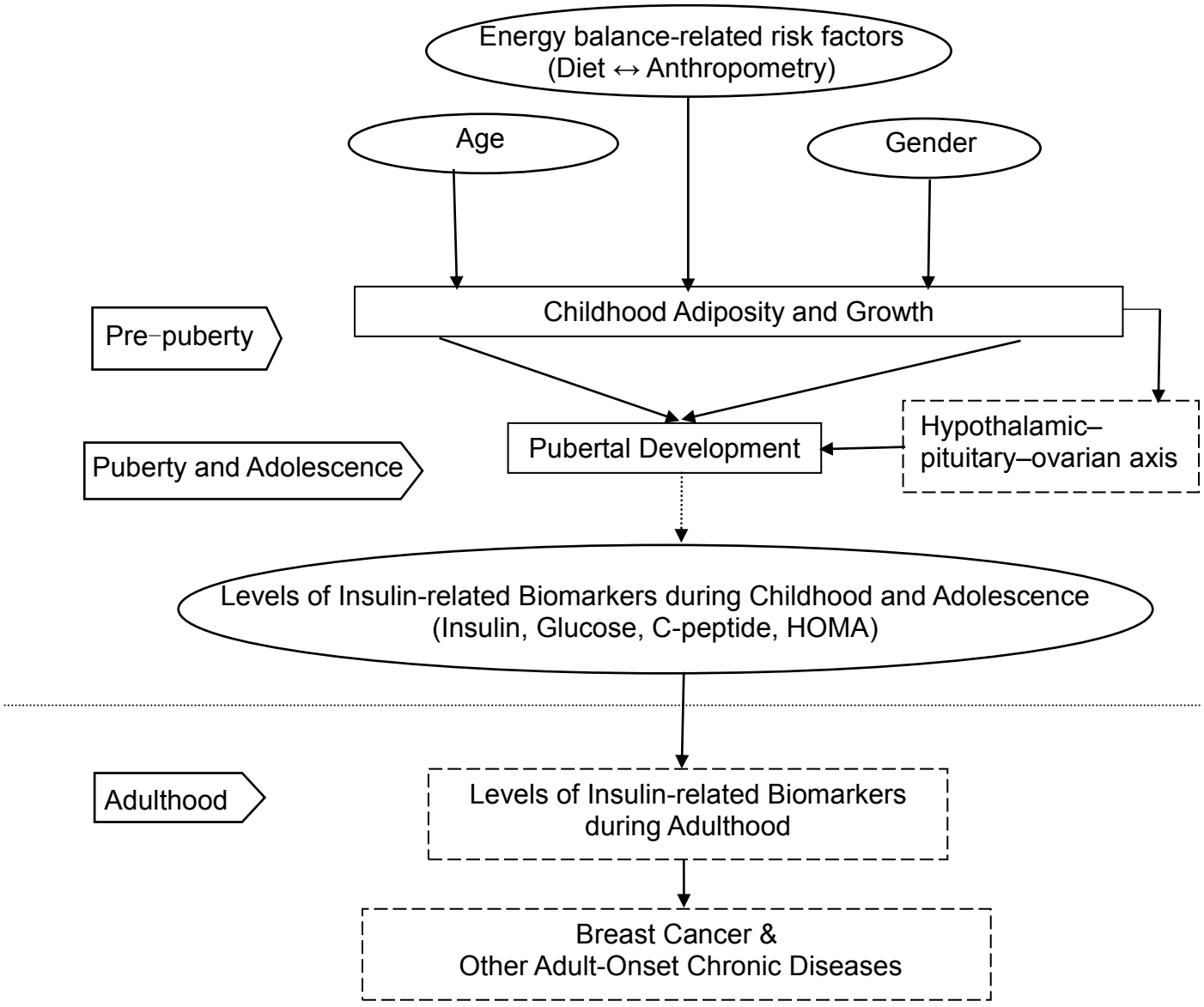
Hypotheses: 1) Baseline childhood adiposity measured by BMIPCT and waist circumference is positively associated with insulin-related biomarker levels during puberty. 2) Baseline childhood growth measured by height is positively associated with insulin-related biomarker levels during puberty. 3) The association is stronger with C-peptide than other insulin-related biomarkers since C-peptide has a longer half-life than insulin.

1.3. Organization of Dissertation

This dissertation is organized into four additional chapters. In **Chapter 2**, 'Background', the burden of obesity, insulin resistance, type 2 diabetes and breast cancer in the United States is briefly described, followed by a discussion of the epidemiological associations between childhood adiposity and adult breast cancer, definitions and biological mechanisms linking adiposity and insulin-related biomarkers, and epidemiological associations between anthropometric factors and insulin-related biomarkers in healthy children and adolescents. In **Chapter 2 part 2.5,** a systematic review (**Aim 1**) is presented in the format of a stand-alone manuscript entitled "A review of dietary correlates of insulin-related biomarkers in healthy children and adolescents". This review summarizes all published epidemiologic studies that examine the association of dietary factors (dietary patterns, several foods or food groups, and macronutrient or micronutrient) with insulin-related biomarkers in healthy children and adolescents. In **Chapter 3,** an analytic study (**Aim 2**) is presented in the format of a stand-

alone manuscript entitled “The association of dietary and anthropometric factors with insulin-related biomarker levels in adolescent girls: results from the DISC”. In this manuscript, analyses are presented that examine the association between selected dietary factors as well as BMIPCT and insulin-related biomarkers in adolescents among participants who attended the last visit of the DISC. In **Chapter 4,** a second analytic study (**Aim 3**) is presented in the format of a stand-alone manuscript entitled “Anthropometric indices and insulin-related biomarkers in girls: Results from the DISC”. In this manuscript, analyses are presented that prospectively examine the association between baseline childhood anthropometric factors and longitudinal measures of serum insulin-related biomarkers among girls in the DISC. Chapter 2 part 2.5, Chapter 3 and Chapter 4 are independent manuscripts and thus there may be some overlap in the materials presented in each chapter. In **Chapter 5,** the findings of the dissertation are summarized along with their implications for public health research and recommendations for future research directions.

Figure 1.1. Framework for the association between childhood adiposity, its associated insulin-related biomarkers and later chronic disease development



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CHAPTER 2: BACKGROUND

Chronic diseases are among the leading causes of death and have been increasing dramatically in recent decades.[1] Worldwide, in 2010 compared to one decade ago, twice as many people died from diabetes, 25% more from cardiovascular disease (CVD) and stroke, and 38% more from cancer.[2] The morbidity attributed to major chronic diseases contributed to 43% of the global burden of diseases.[3] The impact of chronic diseases on health related quality of life overall was similar roughly across countries, but different chronic conditions have different levels of quality of life impact in the general population.[4] Breast cancer is the most prevalent female cancer and the primary cause of death among women worldwide, the incidence of breast cancer has been stabilized in the United States but continues to grow in countries with historical low rates.[5] A migration study has shown that the breast cancer incidence rate of Asian women from countries with lower breast cancer occurrence increased to near the rate of the U.S. women within two generations.[6] The substantially increased burden of chronic diseases including breast cancer reflects longer life-expectancies, but also adverse changes in lifestyles that increase risk for adult obesity,[7] a well-established risk factors for postmenopausal breast cancer.[8,9]

Obesity results from chronic excessive energy intake and is closely related to insulin resistance as well as altered insulin-related biomarker levels.[10] The steady increase in the prevalence of obesity in the past 25 years among children and

adolescents,[11] as well as among adults,[12] has been accompanied by an increase in several insulin-related metabolic abnormalities, such as impaired glucose tolerance,[13] hyperinsulinemia and insulin resistance.[14] These insulin-related metabolic changes, which may start from childhood and could track to adulthood,[15-18] have been found to be associated with several adult chronic diseases.[19-22] Although studies that directly examine childhood insulin-related biomarkers and adult breast cancer are not available, a growing body of literature has examined the association between childhood adiposity and breast cancer. Unlike the positive association between adult obesity and postmenopausal breast cancer, childhood adiposity has been shown to be inversely associated with both pre- and post-menopausal breast cancer development.[23-30] Childhood adiposity may potentially affect metabolic factors including insulin-related biomarker levels, which could re-program mammogenesis during puberty. Early breast differentiation can then reduce the sensitivity of the breast to malignant carcinogenic exposures in later life, providing life-long protective effects to the breast against carcinogenesis.[31,32] Many epidemiological studies have shown adult level insulin-related biomarkers are associated with breast cancer,[33,34] which may suggest childhood insulin-related biomarker levels are also associated with later breast cancer risk, although the effect of the insulin-related biomarkers on breast tissue may vary across life.

Childhood adiposity is determined by energy balance-related factors, including nutritional intake, and could affect several anthropometric indices. Both childhood nutritional factors and anthropometric factors have been found to be associated with insulin-related biomarker levels and have been associated with later chronic diseases including breast cancer risk.[30,35] Higher energy-dense diet intake is associated with higher adiposity and early menarche, and overweight girls may experience slower pubertal growth and sex maturation despite their early menarche, which could be associated with decreased breast cancer risk.[36] How energy balance-related factors, including dietary and anthropometric factors, affect insulin-related biomarker levels among healthy children and adolescents remains largely unknown.

In this chapter, I will introduce the current burden of obesity, insulin resistance, type 2 diabetes, and breast cancer. I will also summarize the epidemiological evidence between childhood adiposity and breast cancer, as well as discuss its potential underlying biological mechanisms. I will then discuss the potential biological mechanisms linking adiposity and insulin-related biomarkers, and summarize the current findings of the association between anthropometric factors and insulin-related biomarkers in healthy children and adolescents. In addition, I will conduct a systematic review of potential dietary correlates of insulin-related biomarkers among healthy children and adolescents.

2.1. Burdens of obesity, insulin resistance, type 2 diabetes and breast cancer

Obesity has become an epidemic affecting all ages of the population worldwide.[37] In the United States, the prevalence of obesity had grown in 2009-2010 to

a widespread rate of 35.7% among adults aged ≥ 20 years[12] and 16.9% among children and adolescents aged 2-19 years.[38] A study published in 2009 showed obesity rates among children and adolescents in the U.S. tripled in the previous 25 years.[11] Worldwide, the prevalence of childhood overweight and obesity increased from 4.2% in 1990 to 6.7% in 2010.[39] Annual medical expenditures attributable to obesity have doubled within a decade from 1998-2008, estimated to be as high as $147 billion per year in the U.S..[40] The World Health Organization coined a term "Globesity" to emphasize this problem of increasing obesity.[37] The burden of obesity is closely associated with the increasing prevalence of insulin resistance and type 2 diabetes.

The burdens of insulin resistance and diagnosed type 2 diabetes are also growing significantly.[41] American Heart Association statistics show that insulin resistance affects more than 60 million individuals in the United States.[42] More than half of the obese adolescents in the United States have insulin resistance, with the prevalence rate at 52.1% (95% CI: 44.5-59.8).[43] The age-adjusted prevalence of diagnosed Type 2 diabetes (T2D) among the U.S. adults aged 18 years or older increased from 4.5% in 1995 to 8.2% in 2010.[44] T2D among children and adolescents was also rising from 0.13% in 1988-1994 to 0.3% in 1999-2002.[45] Adult T2D has been estimated to be associated with a 20% increased risk of breast cancer from a meta-analysis of 20 studies.[46]

In the United States, breast cancer is the most common cancer in women and the second leading cause of cancer-related death among women.[47] The estimated incident invasive breast cancer cases is 226,870 in the United States in 2012.[47] The 5-year relative survival rates based on SEER historic stage were 98.6% for women diagnosed with local stage breast cancer, 83.8% for regional stage and 23.3% for distant stage.[48] According to data from the National Cancer Institute's Surveillance Epidemiology and End Results (SEER), since 1975 when the population-based cancer registries were available, invasive breast cancer incidence rates increased until 1999 with more pronounced increases occurring between 1980 and 1987.[48] This increase was primarily attributed to increased use of mammography screening for early detection.[49] The rates dropped by 7% annually from 2002-2003 and incidence rates have remained steady since then without any further decrease among all age groups of women,[50] partially because of the cessation of Hormone Replacement Therapy (HRT) use after Women's Health Initiative's findings on the adverse effect of HRT in 2002.[51] Economic costs for breast cancer care were around $45.6 billion per year in the United States with the highest costs at the first 6-12 months of treatment and the last 6-12 months of life.[52]

In summary, the existing literature suggests there has been an increasing burden of conditions and diseases (obesity, insulin resistance and T2D) that are associated

with metabolic dysregulation, but a relatively stable burden of breast cancer in the United States.

2.2. Association between childhood adiposity and breast cancer

Childhood obesity is a well-established risk factor for early atherosclerosis,[53,54] hyperlipidemia,[55] and hyperinsulinemia[56] in children and also a risk factor for adult T2D and metabolic syndrome.[20,42] It has been hypothesized that childhood obesity could set an early stage for adult-onset chronic diseases including breast cancer; however, a growing number of epidemiological studies have shown childhood adiposity is not a risk factor, but a protective factor for adult breast cancer.

2.2.1. Epidemiological evidence

Through searching the electronic database PUBMED, we have identified 53 studies[23-30,36,57-100] that examined the association between childhood or adolescence adiposity and breast cancer among children and adolescents aged 5-20 years old. Childhood and adolescence adiposity levels were assessed by perceived body size/weight compared to peers, somatotype, recalled anthropometric parameters or measured anthropometric parameters. Twenty-one studies reported significant inverse associations between increased childhood adiposity and premenopausal breast cancer risk,[24,30,36,62,64-66,68,72-74,77,79,81,82,84,93,94,99,100] and 22 studies reported null associations.[57,59,61,63,64,68,71-76,81,83-87,91,92,97] Thirteen studies reported significant inverse associations between increased childhood adiposity and postmenopausal breast cancer,[30,58,60-6324,57,63,80-82,94] and 20 studies reported null

associations.[57,60,64,69,71-73,80,81,83,85-87,89,90,92,93,97-99] For overall breast cancer risk without menopause status specified, nine studies reported significant inverse associations[23-30] and five study reported null associations.[27,28,67,70,95]

The results from these studies, although not entirely consistent, have shown, in general, that higher adiposity in children appears to be associated with decreased breast cancer risk in both pre- and post-menopausal women. The biological mechanisms underlying the inverse association between childhood adiposity and breast cancer are currently unknown. However, childhood adiposity may be interrelated with insulin-related biomarkers, as well as inflammatory and hormone biomarkers, which together would affect later breast cancer risk.

2.2.2. Proposed biological mechanisms

Although less evidence is available, some hypotheses have been proposed to explain the inverse association between childhood adiposity and breast cancer risk. One hypothesized biological mechanism is that childhood adiposity may affect circulating levels of metabolic factors including insulin-related biomarker levels, the adiposity induced insulin production will, in turn, decrease sex-hormone binding globulin (SHBG) and increase bioavailable estrogen, and then will directly stimulate breast differentiation.[32] Hilakivi-Clarke has reviewed studies on estrogens and BRCA1 and suggested that estrogen exposures in early life may up-regulate the activity of tumor suppressor gene BRCA1, helping to maintain genetic stability and induce differentiation among girls whose breasts haven't accumulated malignant or transformed cells, therefore preventing breast cancer development.[101,102] Early breast differentiation can

then reduce the sensitivity of the breast to malignant carcinogenic exposures in later life, providing life-long protective effects to the breast against carcinogenesis.[31,32]

Another possible mechanism may be through the insulin growth factor (IGF) pathway altered by childhood adiposity level. IGF-I is a systemic hormone with mitogenic and anti-apoptotic properties, which are implicated in breast cancer development.[103] In the Nurses' Health Study, being heavier at age 10 years and 18 years were found to be associated with lower adult IGF-I level and IGF binding protein IGFBP-3 level, independent of adult adiposity,[104] suggesting that childhood adiposity is associated with lower breast cancer risk through decreasing IGF-related biomarker levels.

2.3. Definitions and potential biological mechanisms linking adiposity and insulin-related biomarkers

The biological mechanisms linking childhood adiposity and breast cancer are complex and incompletely understood. However, taking a step back, we may need to consider the associations between childhood adiposity and insulin-related biomarkers. Among both children and adults, increased general adiposity measured by body mass index and central adiposity measured by waist circumference or waist-to-hip-ratio are associated with increased insulin-related biomarker levels. These biomarkers interact with other elevated adipose-derived inflammatory biomarkers and adipocytokines, all of which may be involved in etiologies of many obesity-related diseases including breast cancer,[105] although increased insulin-related biomarkers may desensitize breast tissue

to later carcinogenesis at early life.[106] Except the known association between adiposity and insulin-related biomarker levels, pubertal development also affects insulin-related biomarker levels among children and adolescents undergoing puberty. Part 2.3.1—part 2.3.3 describe the definitions of obesity and its associated metabolic conditions and diseases, patterns of insulin-related biomarker changes during puberty, and some other important biomarkers that are associated with the insulin-related biomarker levels in childhood.

2.3.1. Definitions

Obesity is defined as excess body fat or adipose tissue and results from an imbalance of energy intake (diet) with energy expenditure (primarily physical activity).[107 108] Body Mass Index (BMI), defined as weight (kg)/height^2 (m^2) is the most frequently used method to define obesity. In adults, BMI=30-34.9 is defined as obese class I, BMI=35-39.9 is defined as obese class II and BMI>40 is defined as obese class III.[108] In children and adolescents, BMI percentile based on a particular population is commonly used to define obesity with 95 percentile the most adopted cut-off point for obesity.

Insulin is a polypeptide hormone synthesized and secreted by β-cells of the pancreatic Langerhans islets. This process of insulin generation involves two inactive precursors, preproinsulin and proinsulin, that are sequentially cleaved in the rough endoplasmic reticulum (rER) and then proinsulin is transported from rER to the Golgi complex and processed to form insulin and C-peptide. C-peptide is essential for proper insulin folding and is secreted in equal molar amounts as insulin.[109,110] Upon release,

C-peptide has a longer half-life than insulin and is less active,[111] therefore, it is a good indicator of insulin production and secretion and is often used to monitor β cell function in diabetic patients who are receiving exogenous insulin.

Blood glucose is the major factor that regulates insulin secretion and insulin is a key regulator of blood glucose metabolism.[112] Ingestion of glucose or a carbohydrate-rich meal can lead to a rise in blood glucose concentration, which is an important stimulus for insulin secretion. Insulin regulates glucose metabolism mainly in three tissues: liver, muscle and adipose.[113]

Insulin resistance is a disorder where the cells in target tissues, including liver, adipose tissue and muscle, cannot respond to normal circulating levels of insulin.[113] To compensate for the increased glucose, increased insulin will be produced by the pancreatic β-cell to maintain glucose in a normal range and insulin resistance per se could not lead to diabetes with normal β-cell compensation function. If the β-cell function is impaired and insulin cannot be produced increasingly to compensate the increased glucose production, T2D will develop. To date, the homeostasis model assessment of insulin resistance (HOMA-IR) is the most commonly used parameter to quantify insulin resistance in epidemiological studies. The HOMA-IR is derived from fasting insulin (uU/mL) x fasting glucose (mmol/L)/22.5. It was developed by Matthew et al in 1988 as an indirect index of insulin resistance.[114] HOMA was validated with the hyperinsulinemic euglycemic clamp to be a good method of assessing insulin resistance in epidemiologic studies.[115]

Hyperinsulinemia initially occurs in people with insulin resistance or hyperglycemia. Insulin levels rise to compensate for increased blood glucose levels to maintain normal blood glucose levels.[113]

Hyperglycemia is defined as a fasting plasma glucose level ≥100 mg/dl or drug treatment for elevated glucose.[116]

Type 2 diabetes (T2D) is characterized by diminished insulin secretion, increased insulin resistance and increased glucose production in the liver. It is an end result of a combination of insulin resistance and dysfunctional pancreatic β-cell.[113] Unlike type 1 diabetes, treatment of T2D does not require exogenous insulin to sustain life, although insulin may be helpful to control hyperglycemia in some T2D patients.[113] According to the American Diabetes Association 2011 criteria,[117] individuals who had HbA1c ≥ 6.5%, or fasting plasma glucose **(**FPG) ≥ 126 mg/dl, or 2-hour oral glucose tolerance test (OGTT) plasma glucose ≥ 200 mg/dl were considered as T2D.

2.3.2. Patterns of insulin-related biomarker changes during puberty

Despite the fact that adiposity level could affect insulin-related biomarker levels in childhood, age and puberty development could affect insulin-related biomarker levels independently. Insulin is well known to promote growth during puberty.[118] Fasting insulin levels were found to be positively associated with height velocity during puberty.[119] After linear growth, insulin loses its growth hormone role but still functions as a metabolic hormone.[118] The increase of insulin levels with age was first reported by

Grant DB in 1967,[120] and it was confirmed by many later studies showing that insulin-related biomarkers (Insulin, C-peptide, glucose and HOMA-IR) significantly increased with age and pubertal stage, whereas insulin sensitivity decreased with puberty stage.[118,121-125] The peak of fasting insulin levels was observed early in puberty and reduced gradually in both boys and girls.[126] Insulin resistance can be seen in normal children during puberty[127] and possibly arises before the onset of puberty.[128] The highest values of HOMA were detected during puberty, at the age of 13 - 14.9 years.[129] Accordingly, the prevalence of hyperinsulinemia is highest among adolescents undergoing pubertal development.[129,130] Prepubertal hyperinsulinemia is hypothesized to precede early menarche and the onset of puberty.[131]

The mechanisms for the physiological change of insulin-related biomarker levels with age and pubertal stage are still unknown. Specific hypotheses that have been proposed to explain the elevated levels of insulin and insulin resistance during puberty include: (1) permitting insulin's role as a growth promoting hormone to accommodate the need for rapid growth during puberty;[118] (2) reflecting the puberty-associated increase in the amount of growth hormone and insulin growth factor;[125] (3) revealing the gender-dependent changes in body composition during puberty, and facilitating glucose homeostasis by increasing insulin secretion.[132]

2.3.3. Other major biomarkers associated with insulin-related biomarkers in children

Insulin-related biomarker levels during puberty may be influenced by many other circulating hormones, including growth hormone, sex hormone, adipokines and their binding proteins.[118,133] There are reciprocal regulations between these hormones under the control of the neuroendocrine system including the Hypothalamic-pituitary-adrenal (HPA) axis to promote growth. Growth Hormone and Insulin growth factor (IGF): Both growth hormone and IGF levels have been found to be higher during adolescence than pre-puberty and adulthood[134] and have been viewed as contributors to insulin resistance of puberty.[135] Growth hormone is a major hormone that promotes growth into adulthood; it is secreted in response to insulin to prevent an insulin-induced lower glucose level. It also generates IGF production in the liver and can directly down-regulate glucose uptake into cells.[136] Insulin and IGF systems are interrelated systems that regulate the human body's normal physiological functions.[137] Sex hormones: Sex hormone levels are low in early childhood but could increase to adult levels during puberty, while insulin-related biomarker levels could return to normal after puberty.[135] Neither testosterone nor estradiol have been found to be associated with insulin resistance in early pubertal children.[132] However, the sex hormone binding globin (SHBG), which regulates the bioavailability of the circulating sex hormone,[138] was found to be negatively associated with insulin levels among both boys and girls during puberty development.[139] Adipokines: Adipokines are a collection of various biologically active polypeptides produced by adipose tissue, of which adiponectin and leptin are of

particular interest as links between adipose tissue and insulin resistance. Leptin has been found to increase and adiponectin has been found to decrease with the progression of puberty.[133] Leptin is important in the initiation and progression of puberty[140] and is associated with increased insulin resistance.[141] On the contrary, higher level of adiponectin may decrease insulin resistance.[142] Also, adiponectin has anti-inflammatory, anti-atherogenic and potential anti-diabetic roles.[142] Childhood adiposity is associated with these interrelated biomarkers either directly or indirectly, which together take part in the pathoetiology of adult chronic diseases including breast cancer.

2.4. Associations between anthropometric factors and insulin-related biomarkers in healthy children and adolescents

Many epidemiological studies have found childhood anthropometric factors are associated with breast cancer risk.[23-30] Specifically, height reflects energy balance and adolescent peak height velocity has been found to be positively associated with breast cancer.[23,143,144] On the contrary, general adiposity measured by body mass index during childhood has been found to be inversely associated with breast cancer,[23,24] however, central adiposity measured by waist circumference or waist-to-hip ratio during childhood and breast cancer risk, has not yet been studied. These anthropometric factors may affect breast cancer development through their effects on serum insulin-related biomarkers. A growing number of epidemiological studies have investigated the associations between anthropometric factors and insulin-related biomarkers (insulin, C-

peptide, glucose, and HOMA). We identified a total of 33 studies that examined the association between anthropometric indices [body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) and height] and insulin-related biomarkers in healthy children and adolescents (See Appendix: Table 2.2).

Among the 33 studies, 28 studies examined the association between anthropometric factors and insulin levels, with the majority reporting significant associations between anthropometric factors and insulin levels: BMI (19 of 20), BMI change (three of three), WC (15 of 18), WC change (one of two), WHR (eight of 12), WHtR (all six), height (one study) reporting significant association with insulin concentration. Only one study was identified that examined an association between BMI and C-peptide; it reported a significant positive association between C-peptide levels measured at 11-18 years old and BMI measured at 11-18 years old. In terms of anthropometric predictors of glucose levels, 16 studies were identified with the following indices reporting positive associations with glucose levels: BMI (13 of 16), WC (six of 12), WC change (one of one), WHR (three of six), WHtR (three of six), and height (one of one). Similar to the findings on insulin, most studies have reported a positive relationship between HOMA-IR and anthropometric indices including BMI (12 of 13 papers), BMI change (three of three), WC (11 of 12), WC change (one of two), WHR (five of eight), WHtR (three of three), and height (two of two).

Most, although not all, anthropometric factors examined (BMI, WC, WHR, WHtR and height) were found to be significantly associated with insulin-related biomarkers. Usually, higher general adiposity measured by BMI and central adiposity measured by

WC, WHR or WHtR were positively associated with insulin-related biomarker levels. In particular, WC, which was a measure of central adiposity, has been consistently found to be an independent predictor of HOMA-IR after adjusting for dietary intake and/or physical activity[145,146] and WC has been suggested to be a better independent predictor of insulin resistance among adolescents.[145,147] Manios et al. proposed the reason for this could be pertaining to the higher exposure of the liver to abdominal adipocytes, which will result in lower hepatic insulin clearance.[146] However, another study in China found BMI, WC and WHtR were similarly associated with insulin (r=0.22-0.23) and HOMA (r=0.21-0.23).[148] Whether WC is a better predictor of the insulin-related biomarker levels than BMI among children and adolescence still needs further study.

2.5. Systematic review of dietary correlates of insulin-related biomarkers in healthy children and adolescents

2.5.1. Abstract

INTRODUCTION: Growing evidence supports the theory that nutritional exposures in children and adolescents are associated with chronic diseases in adulthood. It is hypothesized that nutritional factors affect the insulin system and insulin-related biomarker levels, which in turn are associated with later risk of development of chronic disease. The purpose of this review is to summarize the literature examining dietary correlates of insulin-related biomarkers during childhood and adolescence.

MATERIALS AND METHODS: We conducted a systematic review of epidemiological studies that examined the associations of fasting insulin-related biomarkers (insulin, C-peptide, glucose and homeostasis model assessment of insulin resistance [HOMA]) and dietary factors in healthy children (6-18 years). We searched the electronic database PUBMED through May 2013 to identify all published studies.

RESULTS: A total of 13 studies of dietary factors (11 cross-sectional and two intervention studies) examined during childhood were identified and included in this review. From the generally sparse literature on associations between dietary factors and insulin-related biomarkers in healthy children and adolescents, whole grain or fiber intake were found to be inversely associated with C-peptide or HOMA levels in girls, not in boys; fruits intake was positively associated with glucose level; white bread, simple carbohydrate, fructose, total sugar or sugar-sweetened beverage intake were positively associated with insulin or HOMA levels; soft drinks/sweetened beverage or fructose intake were also positively associated with glucose level; red processed meat intake was negatively associated with glucose level, but general meat intake was not associated with any of the insulin-related biomarkers; milk intake or whey protein intake from milk was positively associated with insulin, C-peptide or HOMA levels, not glucose level; western dietary pattern was positively and healthy dietary pattern was inversely associated with glucose level.

CONCLUSIONS: Total sugar, sugar-sweetened beverage, added sugar, simple carbohydrate, fructose, white bread, milk and whey protein from milk intakes have been suggested to be positively associated with insulin-related biomarkers; on the contrary, a

healthy dietary pattern, whole grain, fiber, red processed meat intakes have been suggested to be negatively associated with insulin-related biomarkers.

2.5.2. Introduction

Elevated levels of insulin-related biomarkers (insulin, C-peptide, glucose, and HOMA) measured in adulthood, are established risk factors for chronic diseases including T2D, metabolic syndrome, cardiovascular diseases and various common types of cancer.[149-157] Childhood insulin-related biomarker levels may track into adulthood,[15-18] and childhood insulin response to oral glucose intake could predict adult acute insulin response, suggesting that the capacity of pancreatic β-cell to compensate for a glucose challenge is set in childhood.[158] Therefore, higher levels of insulin-related biomarkers during childhood may increase or be indicative of the risk of chronic disease in later life, and even a small decrease in the insulin-related biomarker levels could have a significant public health impact at the population level.

In both children and adolescents, many factors including, age, puberty stage, obesity and adiposity, physical activity have been found to affect insulin-related biomarker levels. A number of epidemiological studies have shown that insulin-related biomarker (Insulin, C-peptide, glucose and HOMA) levels significantly increase with age and pubertal stage.[118,121-125] Peak levels of fasting insulin have been observed to occur at menarche and reduce gradually after puberty in both boys and girls.[126] Obesity results from a chronic imbalance between energy intake, a component of dietary intake, and energy expenditure, which is primarily determined by physical activity levels, and is

closely correlated with altered insulin-related biomarker levels.[10] It has been suggested that measures of central adiposity, such as waist circumference or waist-to-hip ratio, may be better markers than general adiposity measures, such as body mass index, in predicting insulin-related biomarker levels.[159] Higher physical activity levels have been found to be associated with lower glucose and insulin levels consistently,[160,161] through increasing glucose uptake into exercising muscles and increasing post-receptor insulin signaling.[162]

It is well established in adults that dietary factors[163-165] play a major role in determining insulin-related biomarker levels. It is hypothesized that childhood exposures to various dietary factors may affect later chronic disease development through their effects on serum insulin-related biomarkers. The biological mechanisms of the effects of dietary intake, adiposity and physical activity on insulin-related biomarker levels are mainly through affecting carbohydrate metabolism, since blood glucose is the major factor that regulates insulin secretion,[112] and insulin regulates glucose metabolism mainly in three tissues: liver, muscle and adipose.[113] However, what and how dietary factors affect insulin-related biomarker levels during childhood and adolescence are not well understood. Among children, the association between nutrient intake, obesity, insulin-related biomarkers and chronic diseases is mostly limited to cross-sectional data and non-healthy populations.

The objective of this review is to summarize the published literature to identify gaps in our understanding of the associations between energy balance-related factors

(dietary factors) and insulin-related biomarker levels in childhood. We will review epidemiological evidence investigating the association between dietary factors (eg. specific dietary patterns, food or food groups, macronutrients or micronutrients) and insulin-related biomarkers.

2.5.3. Methods

Literature search

A systematic literature search for studies of dietary correlates of insulin-related biomarkers in healthy children and adolescents published in peer-reviewed journals from January, 1966 up to May, 2013 was conducted, using the PUBMED electronic database.

To be included in our systematic review, a study had to be from a peer-reviewed published epidemiological study based on population-based or school-based cross-sectional or prospective study designs, and reporting quantitative estimates of the association between dietary factors (dietary patterns, several foods or food groups, and macronutrient or micronutrient) and at least one of the four insulin-related biomarkers (Insulin, C-peptide, glucose, HOMA) of our interest in healthy children and adolescents. This review is focused on the associations of various dietary factors with HOMA-IR and three other insulin-related biomarkers: insulin, C-peptide and glucose. HOMA index includes HOMA-IR and HOMA-beta, which are used separately to quantify insulin resistance and pancreatic beta-cell function.[114]

In order to identify the relevant literature on dietary correlates with insulin-related biomarkers from 1966 through May 2013, the following terms were used: 1) "diet" or "nutrition" or “low-fat diet” or “dietary intervention”, 2) "insulin" or "glucose" or "C-

peptide" or "HOMA" or “insulin status” or “homeostasis model assessment” and 3) "fasting". From a review of titles and abstracts, we excluded studies that did not meet our search criteria (see ‘‘Search Strategy’’ for specific criteria). In addition, citations of all relevant articles were tracked in Google Scholar to identify additional studies. Full texts were reviewed if studies reported the association between dietary correlates and insulin-related biomarkers in healthy children and adolescents.

Study selection and data extraction

To be included in this review, a study had to report quantitative estimates of the association between dietary correlates and at least one of the four insulin-related biomarker (Insulin, C-peptide, glucose and HOMA) levels among healthy children and adolescents aged 6-18 years. The following search limits were first applied:1) English; 2) Humans; 3) Child Adolescents (age 6-18 years); and 4) particular after 1966.

For dietary correlates, the search strategy identified 886 possible articles. After a review of titles and abstracts, 64 full-text references were reviewed. We then excluded studies if they: 1) did not have original data (case report or review articles) (n=4); 2) included only overweight and/or obese children in the study (n=12); 3) included only college students or adolescents and young adults combined (n=3); 4) did not report quantitative estimates of dietary factors and insulin-related biomarker levels (n=22); 5) had only children with special conditions (type1 diabetes, metabolic syndrome, nonalcoholic fatty liver disease, anorexia nervosa or protein deficiency) (n=6); 6) did not appropriately consider or model at least either age, menarche or pubertal status (n=2)[166,167]; and 7) did not consider gender in the analyses (n=3). Thirteen publications from the 64 full text articles met the inclusion criteria and were included in

this systematic review, including 11 cross-sectionals and two intervention studies (**Figure 1**).

Data were extracted following a standardized protocol. Information on country of study, population, participants' characteristics, measurement of dietary factors, sample size, estimate of the associations, potential confounding factors adjusted in the analyses, as well as other relevant methodological issues were extracted.

Methodological issues

Several primary methodological issues need to be considered. First, the selection of the study population and its age distribution need to be considered in interpreting study results. Insulin-related biomarker levels in childhood and adolescence are known to vary with age and pubertal status.[118,121-125] The age distribution of the study population may affect the overall insulin-related biomarker levels. Second, the age at measurement of dietary factors, the age at measurement of insulin-related biomarkers and timeline between these two ages need to be considered. With remarkable growth and development, children could have different levels of dietary factors and insulin-related biomarker levels; therefore, age at measurements of exposures and outcomes needs to be considered in examining their associations. Third, when both pre- and postmenarcheal girls are included in the study, age needs to be modeled as quadratic rather than linear distribution or stratified by menarcheal status, because peak levels of fasting insulin level have been observed to occur at menarche.[126] There is a non-linear association between age and insulin-related biomarker levels during childhood and adolescence. Fourth, fasting status of the biomarker needs to be considered. Glucose

levels were shown to decrease significantly in children during fasting,[168] which triggers a decline in insulin secretion and an increase in glucagon release.[113] Therefore, fasting-based indices for insulin-related biomarkers are recommended for epidemiologic studies.[169]

2.5.4. Results

We identified 13 studies that examined associations between dietary factors and insulin-related biomarkers and the dietary factors examined are summarized in this review as dietary patterns, specific food or food groups, and macronutrients or micronutrients (**Table 2.1**.).

The sample sizes in these studies ranged from 24 to 5,267 children and/or adolescents with ages ranging from 6-19 years old. The majority of the studies (11 studies) were cross-sectional in design and two were randomized double-blind placebo controlled trials. The percentages of girls in the study population among these 13 studies ranged from 0%-55%. Correspondingly, the percentages of boys ranged from 45%-100%. Seven studies reported the results on dietary factors and insulin levels, three studies on C-peptide, eight studies on glucose, and nine studies on HOMA. Results of potential dietary predictors of insulin-related biomarkers are summarized as follows.

Dietary patterns

A review of the literature identified two studies[170,171] that examined the association between dietary patterns and insulin-related biomarkers during childhood and adolescence that met our selection criteria. Among these, a cross-sectional study

using a population-based multistage random cluster sampling procedure by Shang et al., collected dietary data from three consecutive 24-hour recalls among Chinese children aged 6-13 years. They found that a western dietary pattern characterized by higher intake of red meat, eggs, and refined grain was associated with higher plasma glucose levels compared to the healthy dietary pattern characterized by higher intake of milk/yogurt, eggs, fruits/vegetables and lower intake of meat, after adjusting for gender, age and school in the study center.[170] Another cross-sectional study, the Raine Study, was conducted in Australia and examined dietary pattern data collected from a food frequency questionnaire. It found that a healthy dietary pattern dominated by higher intake of vegetables, fruits, legumes and whole grains was significantly inversely associated with serum glucose levels, but had no association with insulin or HOMA levels. This was true in both girls and boys at 14 years of age, after adjusting for total energy intake, aerobic fitness, single parent status, maternal education, television viewing, BMI and WC.[171]

Specific food or food group

The associations between food or food groups and insulin-related biomarker levels among children and adolescents have also been investigated by several studies. A study published in 2012, using data from the National Health and Nutrition Examination Survey (NHANES) 1999-2004 in the U.S., found higher whole-grain intake was associated with lower fasting insulin levels in boys, and with lower C-peptide levels in only girls.[172]

Several studies examined sugar-related intake and insulin-related biomarker levels among children and adolescents. Using NHANES data 1999-2004, Bremer et al. found sugar-sweetened beverage intake was positively associated with HOMA among US adolescents 12-19 years for girls, but not for boys.[173] Two other papers both published in 2010 among children in Denmark[174] and Mexico[175] reported positive associations of either total sugar intake[174] or sugar sweetened drink intake[175] with insulin-related biomarkers. In the study among Mexican school-age (9-13 years) boys and girls, soft drinks/sweetened beverage intake as well as intake of fruits was positively associated with glucose levels, while vegetable oils/avocado and red/processed meat intake were negatively associated with glucose levels.[175] The same study also found white bread intake was positively associated with insulin levels.[175]

The association between milk and/or meat and insulin-related biomarkers has been examined in three selected studies – as well as components of milk intake in a clinical trial. In a cross-sectional study conducted in Argentina that included 365 low-income students aged 10 ± 2.3 years, increased milk intake measured by a five-level index according to daily intake of milk was associated with lower HOMA levels (β=-0.135, $p<0.05$), after adjustment for multiple key potential confounders, suggesting its potential role in decreasing insulin resistance and later T2D risk.[176] The authors also suggested the findings might reflect an overall healthy behavior associated with higher milk intake. In contrast, Hoppe et al. conducted a small scale intervention study to

examine whether an increase in animal protein intake as either from milk or meat could affect insulin-related biomarker levels that included 24 eight-year old boys and found that short-term high elevated skim milk intake increased insulin, C-peptide and HOMA levels-- though it did not increase glucose levels.[177] In contrast, meat intake did not increase levels of any of these biomarkers.[177] A cross-sectional study among Mexican children aged 9-13 years old found red and processed meat intake was significantly positively associated with glucose level, after adjusting for several key potential confounders.[175] A double-blinded randomized study with a 2x2 factorial design studied the associations between milk protein content (whey and casein) and insulin-related biomarker levels among 57 eight-year old boys followed for seven days and showed that whey protein could increase insulin levels and HOMA values,[178] suggesting different milk proteins have different effects on the insulin system. Thus, the relationship between milk intake and insulin-related biomarkers among children and adolescents is still not well understood.

Macronutrient and Micronutrient Intake

Specific micronutrients and macronutrients have also been suggested to be associated with insulin-related biomarker levels among children and adolescents. In a cross-sectional study among 202 children aged 7-12 years old in the U.S, energy percentage from fat and protein was negatively correlated with blood glucose, while energy percentage from carbohydrate intake was positively correlated with blood glucose.[179] Total sugar intake was positively and fiber intake was negatively associated

with HOMA among girls, not boys, in the Danish part of the European Youth Heart Studies.[174] In a cross-sectional study among 559 US adolescents aged 14-18 years, fructose was positively correlated with both glucose and HOMA.[180] Similarly, simple carbohydrate intake was found to be significantly positively correlated with both insulin and HOMA values among 10-12 year old children in the Children Study in Greece.[146] Potential associations between other kinds of micronutrients and insulin-related biomarkers have not been studied.

2.5.5. Discussion

Thirteen studies have been identified for this review and significant associations of specific dietary pattern, food or food group, macronutrients or micronutrients were reported. Specifically, dietary fiber and whole grain intakes are inversely associated with insulin-related biomarker levels; carbohydrate/sugar-related dietary intakes are positively associated with insulin-related biomarkers; healthy dietary pattern with higher intake of milk/yogurt, eggs, fruits/vegetables and lower intake of meat is inversely but western dietary pattern with higher intake of red meat, eggs, and refined grain is positively associated with glucose level; findings on milk or meat are not consistent. These findings on the associations between dietary intake and insulin-related biomarker levels among children and adolescence, however, are sparse and not well-defined, and the earliest study that met our inclusion criteria was published in 2008. To date, data among children examining dietary factors and insulin-related biomarkers are limited to cross-sectional data. More studies are needed to replicate the findings of dietary factors and insulin-related biomarkers, given this sparse literature.

The results from the studies on fiber or whole grain consumption, although not entirely consistent between boys and girls, have shown, in general, that increased whole grain or fiber intake is associated with decreased insulin, C-peptide or HOMA levels. Many mechanisms have been proposed to explain fiber's observed effects on insulin secretion and action, e.g. promoting bowel movement regularity, delaying nutrient absorption, increasing gastrointestinal hormone secretion and colonic fermentation which suppress hepatic gluconeogenesis, eliminating toxic and carcinogenic compounds.[105,181]

The results of sugar/carbohydrate-related diets are consistent in showing positive associations with insulin-related biomarkers. Although insulin secretion depends on the type and physical form of the carbohydrates, food that contains high sugar or carbohydrate components could increase glucose level, which stimulates insulin secretion from pancreatic β-cells.

Although less evidence is available on the associations between milk or meat consumption and insulin-related biomarkers among children and adolescents, a study in 3,042 adults aged 18-89 years old has found increased red meat and milk intake was associated with increased insulin resistance.[182] The finding on milk intake and HOMA is consistent with the intervention study among 8-year old boys included in this review.[177]

A recent review of insulin resistance in children has suggested higher levels of energy intake rather than specific macronutrient components are associated with insulin sensitivity levels among children.[183] Although none of the studies included in this

review specifically examined total energy intake and insulin-related biomarkers, four of these studies have adjusted energy intake as a confounding factor. Further studies are needed to investigate energy intake as a main exposure of interest and insulin-related biomarker levels among healthy children and adolescents.

There are several issues that need to be considered in interpreting the results of the studies examining dietary factors and insulin-related biomarker levels among children and adolescents.

First, the effect of menarche status on insulin-related biomarker levels needs to be considered. Few studies selected in this review have adequately adjusted for growth and pubertal development in examining the associations between dietary intake and insulin-related biomarkers. Menarche status for girls was only controlled in one study, and one study included Tanner stage as a covariate while another study included sexual maturity as a covariate. Not taking into account menarche status may bias the results toward null findings given that the insulin-related biomarker levels peak during puberty and declines after puberty. Biologic mechanisms for the physiological changes associated with insulin-related biomarker levels with age and pubertal stage are still unknown. Specific hypotheses that have been proposed include: (1) permitting insulin's role as a growth promoting hormone to accommodate the need for rapid growth during puberty;[118] (2) reflecting the puberty-associated increase in the amount of growth hormones and insulin growth factors;[125] and (3) revealing the gender-dependent changes in body composition during puberty, and facilitating glucose homeostasis by increasing insulin secretion.[132]

Second, many potential confounders such as physical activity and adiposity have not been considered and/or well managed in many of the published studies. Whether or not potential confounding factors (e.g., physical activity) should be included needs to be evaluated in the study analyses. Stratified analyses by confounding factors in the statistical models are ways to test the existence of confounding factors. Since physical activity and adiposity have been found to be associated with insulin-related biomarkers, considering these factors in the analyses of dietary factors in association with insulin-related biomarkers may generate more accurate results. Physical activity was controlled in six out of the 13 studies included in this review and six out of the 13 studies have controlled adiposity level. However, none of these studies used a standardized measurement such as BMI-for-age percentile for children. Since the percentage of body fat differs with age and sex in children, and BMI percentile points the relative position of the child's BMI among their peers of the same age and gender, using the standardized measurement for adiposity may more accurately reflect children's adiposity level.

Third, the selection and representation of the study population for epidemiological studies among children and adolescents could affect the results' generalizability. Results from epidemiological studies with participants randomly selected from the general population have better generalization. Therefore, in studies of children, it is not easy to randomly select participants from a general population making convenience samples commonly selected. Four of the 13 studies in this review selected participants from convenience samples. In this case, the study design may have selection bias, and the study population may not well represent the general population, which restricts the generalization of the results.

Fasting insulin levels have been found to be significantly related with lipids, and blood pressure in adolescent.[184] Therefore, studying the associations between other biomarkers and insulin-related biomarkers may add to our current knowledge of biological mechanism of associations between dietary factors and insulin-related biomarkers in children and adolescents. Besides, dietary nutrient intake and nutrient blood circulating levels need to be compared since dietary nutrient intake may not accurately represent the plasma/serum/red blood cell level nutrient. Bioavailability of nutrients is determined or affected by different transporting abilities of nutrients from dietary intake to blood or tissues and is determined by food structure.[185] Therefore, measuring blood nutrient levels may better reflect the amount of nutrients absorbed by the human body. For example, in a study examining the effect of fish intake on the metabolic profile, red blood cell (RBC)-DHA level was examined instead of direct fish intake on insulin level and a significant correlation between high DHA status and increased fasting insulin levels ($p=0.018$) was demonstrated in Danish adolescent girls and boys.[186] Additional research is needed to clarify associations between bioavailable nutrients and insulin-related biomarker levels.

2.5.6. Conclusions

No definitive conclusions pertaining to potential associations between specific dietary factors and insulin-related biomarkers in childhood and adolescence as a whole can be made, and studies examining healthy children over time with follow-up visits are scarce. However, some studies have provided a glimpse of evidence for associations. This limited evidence can be used to better design epidemiological studies to examine

predictors of insulin-related biomarkers in children, which can help inform guidelines for how children and adolescents may maintain normal insulin-related biomarker levels. However, early interventions on individual dietary counseling in childhood and adolescence will help individuals prevent later chronic diseases. Additional epidemiologic research with lengthier follow-up time and randomly selected participants from the general population will provide more definitive evidence of the association between dietary factors and insulin-related biomarker levels among healthy children and adolescents.

2.5.7. Search Strategy

Search terms used to conduct searches in PubMed

Used keywords and medical subject headings in PUBMED:

Overall terms for dietary correlates and insulin-related biomarkers: ("diet" or "nutrition" or “low-fat diet” or “dietary intervention”) and ("insulin" or "glucose" or "C-peptide" or "HOMA" or “insulin status” or “homeostasis model assessment”) and ("fasting"). As of 05/09/2013, the searching generate 886 publications.

MESH field: ("diet"[All Fields] OR "nutrition"[All Fields] OR "low-fat diet"[All Fields] OR "dietary intervention"[All Fields]) AND ("insulin"[All Fields] OR "glucose"[All Fields] OR "C-peptide"[All Fields] OR "HOMA"[All Fields] OR "insulin status"[All Fields] OR "homeostasis model assessment"[All Fields]) AND "fasting"[All Fields] AND (("1966/01/01"[PDAT] : "3000/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND "female"[MeSH Terms] AND ("adolescent"[MeSH Terms] OR "child"[MeSH Terms:noexp]))

Search Limits

Articles published in English, studies of humans, the fields title/abstract., Child Adolescents (age 6-18 years), 1966 and after

Inclusion Criteria

1) A study had to be from a peer-reviewed published epidemiological study based on population-based or school-based cross-sectional or prospective study designs; 2) a study has to report quantitative estimate of the association between dietary factors (dietary patterns, several foods or food groups, and macronutrient or micronutrient) and at least one of the four insulin-related biomarkers (Insulin, C-peptide, glucose, HOMA) of our interest in healthy children and adolescents; 3) measurements of insulin-related biomarkers must be on fasting blood sample; 4) children and adolescents are aged 6-18 years old; 5) publication dates are from 1966 through May 2013; 6) publication is based on English language.

Exclusion Criteria

Studies 1) with only overweight and/or obese children or with only cluster of risk scores; 2) with insulin resistance syndrome or insulin sensitivity or metabolic syndrome or hyperglycemia or impaired fasting glucose as outcome; 3) using school based intervention; 4) using pediatric outpatients; 5) on glucose induced insulin level as the biomarker assessment; 6)with insulin resistance assessed by insulin sensitivity check index (QUICKI); 7) with study population of special medical condition (type 1 diabetes , nonalcoholic fatty liver disease HIV, epilepsy, polycystic ovary syndrome, bipolar disorder or other psychosocial problems, psychosis, cystic fibrosis, anorexis nervosa, hypertension, sleep-disordered breathing, obstructive sleep apnea, acanthosis nigricans,

Turner syndrome, Prader Willi syndrome, precocious puberties, intellectual disabilities, premature adrenarch, low birth weight).

Figure 2.1. Flow diagram for literature search and study selection for dietary correlates and insulin-related biomarkers.

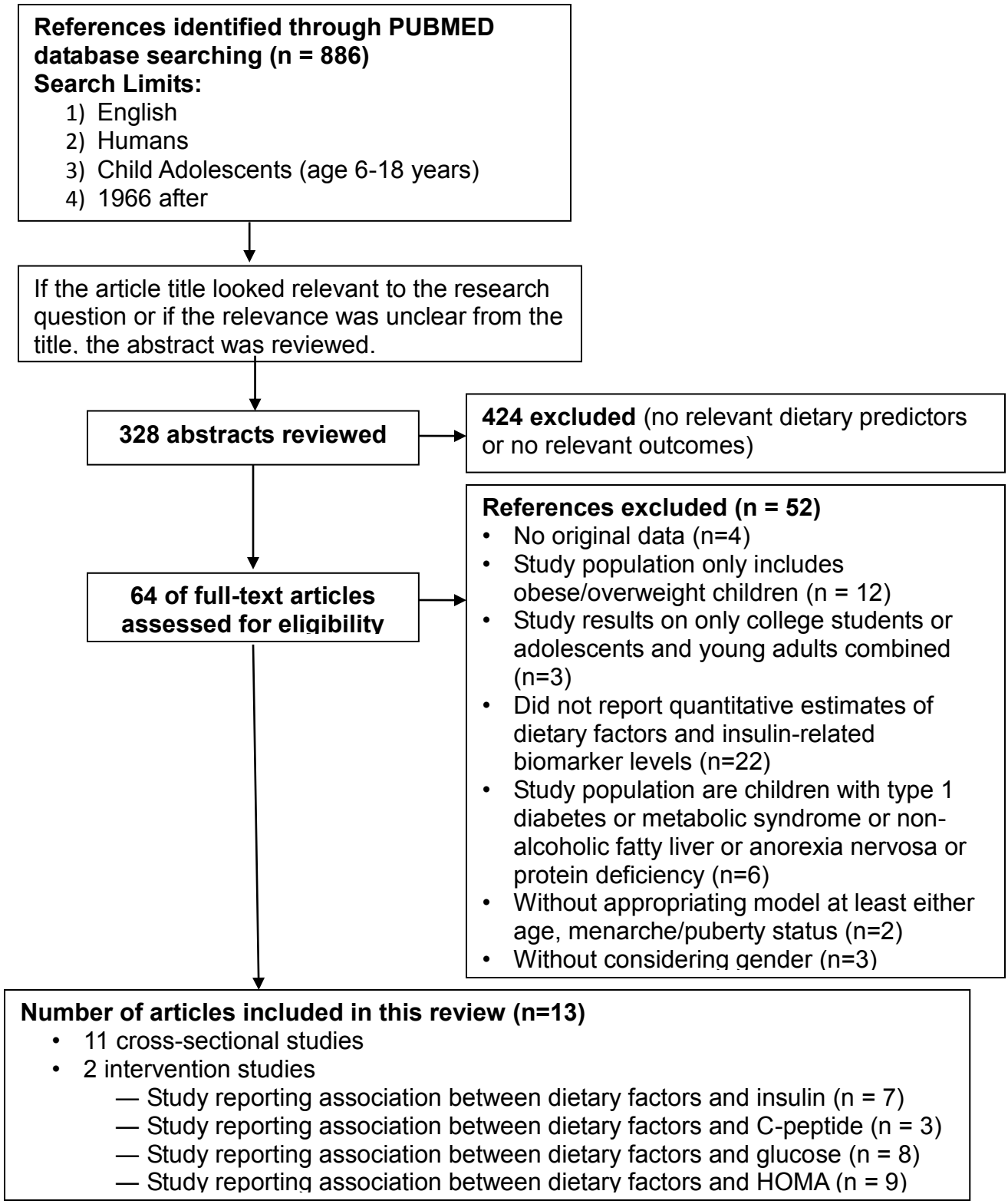

Table 2.1. Summary table of studies on the association between diet/nutrition and levels of insulin-related biomarkers among children and adolescents

Study	Study design	Pop.	Diet/Nutrition Exp. (Source)	Fasting Insulin	Fasting C-peptide	Fasting Glucose	HOMA	Adjusted covariate / Other Comments
Hur, 2012[172] the U.S NHANES 1999-2004	CS	Nationally representative non-institutionalized population Total N (%girls): 4,928 (49%) Age:12-19 y	Whole Grain (WG) (24-hr food recall)	Insulin by WG consumption Category None: 13.6±0.7 (Girls) 13.2±0.5 (Boys) Low: 12.6±0.5 (Girls) 10.1±0.6 (Boys) High 11.8±0.5 (Girls) 12.4±0.5 (Boys) (p_{trend} =0.06) (Girls) (p_{trend} =0.002) (Boys)	C-peptide by WG consumption category None: 0.74±0.02 (Girls) 0.70±0.02 (Boys) Low: 0.74±0.02 (Girls) 0.65±0.02 (Boys) High 0.69±0.02 (Girls) 0.69±0.02 (Boys) (p_{trend}=0.02) (Girls) (p_{trend}=0.701) (Boys)	N/A	N/A	Age, race/ethnicity, family income, energy intake, smoking, & PA

Table 2.1. (cont'd)

Kynde, 2010[174] Denmark Danish part of the European Youth Heart Studies (EYHS) I and II.	CS & prospective	Girls and boys at 8–10 and 14–16 years from EYHS I (n=651) and 8–10-years from baseline followed up 6 years later in EYHS II (n=233). Total N (%girls): CS: 651 (52%) Prospective: 233 (58%)	Total sugar, fiber (a single 24 h recall)	N/A	N/A	N/A	**Sugar**: CS [β (SE):0.23 (0.11); p=0.03] (Girls) [β (SE):<-0.01(0.12); p=0.97] (Boys) Prospective [β (SE):-0.07 (0.23); p=0.74] (Girls) [β (SE):0.18 (0.29); p=0.53] (Boys) **Fiber:** CS [β (SE): -1.28 (0.58); p=0.03] (Girls) [β (SE): -0.50 (0.71); p=0.48] (Boys) Prospective [β (SE):-1.68 (1.20); p=0.17] (Girls) [β (SE):-1.18 (1.42); p=0.41] (Boys)	Age, BMI, sexual maturity (both years at prospective analyses), PA and mother's education at baseline and school (random effect).

Table 2.1. (cont'd)

Ambrosini, 2010[171] Australia Western Australian Pregnancy Cohort (Raine) Study	CS	Children of participants in the Raine Study Total N (%girls): 1,139 (48%) Age:14 y	Dietary Pattern (FFQ) Log insulin, log HOMA	Healthy Dietary Pattern p_{trend}=0.67 (Girls) p_{trend}=0.43 (Boys)	N/A	Healthy Dietary Pattern Inverse p_{trend}=0.04 (Girls) Inverse p_{trend}=0.01 (Boys)	Healthy Dietary Pattern p_{trend}=0.97 (Girls) p_{trend}=0.08 (Boys)	Total energy intake, PA, single parent status, maternal education, television viewing, BMI & WC
Bremer, 2009[173] the U.S NHANES 1999-2004	CS	Nationally representative non-institutionalized population Total N (%girls): 6,967 (49%) Age:12-19 y	Sugar Sweetened Beverage (24-hr food recall)	N/A	N/A	N/A	[β (SE):0.07 (0.03); p<0.05] (Girls) [β (SE):0.04 (0.02); p>0.05] (Boys)	PA, age, race, menarche (for female adolescents), & energy intake.
Hoppe, 2009[178] Denmark	Double-blinded randomized	Caucasian boys were randomly selected from the National	540 ml of one of the following drinks: (1) whey	Baseline correlations: Milk: r=0.19 (NS) Total protein: r=0.20 (NS)	Baseline correlations: Milk: r=0.17 (NS) Total protein: r=0.27 (NS)	Baseline correlations: Milk: r=0.05 (NS) Total protein: r=0.05 (NS)	Baseline correlations: Milk: r=0.17 (NS) Total protein: r=0.17 (NS)	None

Table 2.1. (cont'd)

	2X2 factorial design	Danish Civil Registry Total N (%girls): 57 (0%) Age: 8 y	with low milk mineral (Ca and P); (2) whey with high milk mineral; (3) casein with low milk mineral (4) casein with high milk mineral daily for 7 days	Total energy: r=0.37 (p<0.05) Whey group (N=27) Baseline 33 ±11.6 Day 7:39.93 ± 14.5 (p=0.006) Casein group (N=29) Baseline: 37.27 ± 12.4 Day 7: 40.9 ± 23.9.9 (p=0.36) Treatment p=0.049	Total energy: r=0.26 (NS) Whey group (N=27) Baseline 328 ±99.7 Day 7:351 ± 116 (p=0.11) Casein group (N=29) Baseline: 327 ± 149 Day 7: 390 ± 194 (p=0.46) Treatment p=0.898	Total energy: r=0.23 (NS) Whey group (N=27) Baseline 4.52 ± 0.38 Day 7:4.58 ± 0.29 (p=0.37) Casein group (N=29) Baseline: 4.47 ± 0.26 Day 7: 4.53 ± 0.24 (p=0.28) Treatment p=0.99	Total energy: r=0.37 (NS) Whey group (N=27) Baseline 1.12 ±0.42 Day 7:1.37 ± 0.52 (p=0.006) Casein group (N=29) Baseline: 1.25 ± 0.01 Day 7: 1.39 ± 0.31 (p=0.36) Treatment p=0.58	
Hoppe, 2005[177] Denmark	Intervention Study	Boys born Oct-Dec 1992 were drawn randomly from the Central	53 g protein daily, 12 boys as 1. 5 l of skimmed milk, and	Milk group (N=12) Baseline: 22.4 ± 6.6 Day 7:45.0 ± 25.8 (p<0.01)	Milk group (N=12) Baseline: 237.8 ± 52.5 Day 7:299.4 ± 86.5 (p<0.001)	Milk group (N=12) Baseline 4.8 ± 0.4 Day 7:4.7 ± 0.4 (p: NS)	Milk group (N=12) Baseline 0.8 ± 0.3 Day 7:1.4 ± 0.7 (p<0.01)	None

Table 2.1. (cont'd)

		Personal Register, & were invited to the study. Total N (%girls): 24 (0%) Age:8 y	12 other boys as 250 g low fat meat	Meat group (N=12) Baseline: 25.1 ± 9.1 Day 7: 26.7 ± 6.9 (p: NS)	Meat group (N=12) Baseline: 250.4 ± 49.3 Day 7: 271.8 ± 48.3 (p: NS)	Meat group (N=12) Baseline: 4.8 ± 0.3 Day 7: 4.5 ± 0.3 (p: NS)	Meat group (N=12) Baseline: 0.9 ± 0.4 Day 7: 0.9 ± 0.2 (p: NS)	
Shang, 2012[170] China	CS	Subjects randomly selected from 5 provincial capital cities Total N (%girls): 5,267 (50%) Age: 6-13 y	Dietary pattern (3 consecutive 24-hr recall: 2 weekdays and 1 weekend day)	N/A	N/A	Western dietary pattern vs. Healthy dietary pattern: 4.53±0.55 mmol/L vs 4.46±0.49 mmol/L, P=0.0082)	N/A	Gender, age were fixed effect variable, school was a random effect variable in the general linear reg model
Pollock, 2012[180] the U.S	CS	Subjects recruited from local high school in Augusta, Georgia	Fructose (4-7 24-h recalls) Log HOMA	N/A	N/A	β=0.13; p_{trend} =0.024	β=0.11; p_{trend} =0.038	Age, sex, race, Tanner stage, FFST mass, fat mass, PA, SES,

Table 2.1. (cont'd)

		Total N (%girls): 559 (50%) Age:14–18 y						energy & fiber intake
Welsh, 2011[187] the U.S NHANES 1999-2004	CS	Nationally representative non-institutionalized population Total N (%girls): 2,157 (50%) Age:12-18 y	Added sugar (24-hr food recall)	≥85th percentile BMI (n=817): positive linear p_{trend} = 0.006 <85th percentile BMI (n=1340): positive linear p_{trend} = 0.33	N/A	≥85th percentile BMI: p_{trend} = 0.16 <85th percentile BMI: p_{trend} = 0.54	≥85th percentile BMI: positive linear p_{rend} = 0.004 <85th percentile BMI: p_{trend} = 0.41	Sex, race, age, education, BMI, PA, total energy intake, the energy-adj. nutrient residuals for protein, sodium, cholesterol, & fiber
Perichart-Perera , 2010[175] Mexico	CS	A convenience sample from three public urban schools Total N (%girls): 228 (48%)	Soft drinks /sweetened beverages, fruits, red and processed meat, vegetable	White bread: β=3.88 p_{trend}=0.02	N/A	Soft drinks/sweetened beverage: β=6.01 p_{trend} =0.004 Fruits: β=0.71 p_{trend} =0.04	N/A	Sex; age; BMI; PA; sedentary activities; energy intake of white bread, corn tortilla, pasta/rice,

Table 2.1. (cont'd)

		Age:9-13 y	oil /avocado, white bread (two multiple pass 24-hour recalls)			Red and processed meat: β=-7.75 p_{trend} =0.02 Vegetable oil.avocado: β=-3.34 p_{trend} =0.02		refined CHO, red/processed meat, poultry/fish, all veg, all fruits, soft drinks/sweetened beverages, high-fat dairy, veg oils/avocado, other added fats, &sugars/desserts (adj.by total energy intake).
Casazza, 2009[188] the U.S	CS	Participants were recruited as a part of an ongoing cross-sectional Study. Total N	% fat %CHO %Protein (two 24-hour recalls)	N/A	N/A	%fat β =−0.35803 (p<0.05) % CHO β =0.48834 (p<0.05) %Protein β =−0.43221	N/A	Total body fat, age, sex, SES

Table 2.1. (cont'd)

		(%girls): 202 (47%) Age:7-12 y				(p<0.05)		
Hirschler, 2009[176] Argentina	CS	Participants were from 2 poor suburbs of Buenos Aires. Total N (%girls): 365 (52%) Age:10±2 y	Milk (5-level index Based on daily intake of milk: 1, 2, 3, 4, ≥5)	N/A	N/A	N/A	β =-0.135 (p<0.05)	Sex, age, blocks walked/d, TV viewing, soft drink intake, parental educational level, HDL, & systolic BP
Manios, 2008[146] Greece The Children Study	CS	Participants were recruited from primary schools, the island of Crete. Total N (%girls): 248 (55%) Age:10-12 y	Simple CHO (A combination of a 24-h recall and a 3-day food diary)	r=0.143 p<0.05	N/A	N/A	β (SE): 0.003 (0.001) p=0.008	Sex, WC

Table 2.1. (cont'd)

Abbreviation: BMI: body mass index; BP: blood pressure; CHO: carbohydrate; CS: cross-sectional: FFQ: food frequency questionnaire; FFST: fat free soft tissue; HDL: high-density lipoprotein cholesterol; HOMA: Homeostatic model assessment; PA: physical activity; SES: socioeconomic status; SE: standard error; WC: waist circumference.

APPENDIX

Table 2.2. Summary table of studies on the association between anthropometric factors and levels of insulin-related biomarkers among children and adolescents

Study	Study design	Pop.	Anthr-opmetric Measures	Insulin	C-peptide	Glucose	HOMA	Adj. covariates /Other Comments
Androutsos, 2012[189] Greece sub-cohort of the Healthy Growth Study	CS	Participants were recruited from primary schools in Greece Total N (%girls): 324 (48.5%) Age: 9-13 yrs	BMI z-score, WC, WHR WHtR	WC (ln insulin): [β (SE):0.025 (0.003); p<0.001] BMI z-score (ln insulin): [β (SE):0.174 (0.025); p<0.001] WHR (ln insulin): [β (SE):2.633 (0.549); p<0.001] WHtR (ln insulin): [β (SE):3.463 (0.485); p<0.001	N/A	WC: [β (SE):-0.039 (0.053); p=0.465] BMI z-score: [β (SE):-0.436 (0.419); p=0.300] WHR: [β (SE):-1.723 (8.773); p=0.844] WHtR: [β (SE):-7.251 (8.086); p=0.371	WC (ln HOMA): [β (SE):0.025 (0.003); p<0.001] BMI z-score (ln HOMA): [β (SE):0.170 (0.026); p<0.001] WHR (ln HOMA): [β (SE):2.624 (0.558); p<0.001] WHtR (ln HOMA): [β (SE):3.388 (0.496); p<0.001	Age, gender, Tanner stage, protein intake carbohydrate intake, fat intake and PA. Other comments: girls had higher insulin level than boys. Neck circumference was also found to be associated with insulin and HOMA.

Table 2.2 (cont'd)

Jiménez-Pavón, 2011[190] Europe The HELENA-Cross-sectional Study	CS	Participants were recruited from 10 cities in nine different European countries. Total N (%girls): 1053 (53%) Age: 12.5-17.5 yrs 14.9 ± 1.2 yrs	BMI WC	BMI Underwt β=-0.31, p=0.34 (Girls) β=-0.26, p=0.23 (Boys) Normal weight β=-0.34, p<0.001 (Girls) β=-0.08, p=0.22 (Boys) Over weight β=-0.054, p=0.69 (Girls) β=-0.44, p<0.01 (Boys) Obese β=-0.04, p=0.94 (Girls) β=-0.14, p=0.51 (Boys) WC Lowest tertile β=-0.30, p=0.001(Girls) β=-0.16, p=0.16 (Boys) Middle tertile β=-0.36,	N/A	BMI Underwt β=-0.04, p=0.89 (Girls) β=-0.57, p<0.05 (Boys) Normal weight β=-0.16, p<0.05 (Girls) β=-0.14, p<0.05 (Boys) Over weight β=-0.28, p<0.05 (Girls) β=-0.06, p=0.72 (Boys) Obese β=-0.04, p<0.01 (Girls) β=-0.02, p=0.94 (Boys) WC Lowest tertile β=-0.19, p<0.05 (Girls) β=-0.28, p<0.05 (Boys) Middle tertile β=-0.09,	BMI Underwt β=-0.32, p=0.32 (Girls) β=-0.32, p=0.19 (Boys) Normal weight β=-0.32, p<0.001 (Girls) β=-0.09, p=0.19 (Boys) Over weight β=-0.081, p=0.54 (Girls) β=-0.76, p<0.01 (Boys) Obese β=-0.43, p=0.44 (Girls) β=-0.11, p=0.63 (Boys) WC Lowest tertile β=-0.28, p<0.01 (Girls) β=-0.18, p=0.10 (Boys) Middle tertile β=-0.36,	Age, center and pubertal status

Table 2.2 (cont'd)

				p=0.001 (Girls) β=-0.03, p=0.77 (Boys) High tertile β=-0.31, p<0.01 (Girls) β=-0.34, p<0.001 (Boys)		p=0.44 (Girls) β=-0.09, p<0.05 (Boys) High tertile β=-0.28, p<0.01 (Girls) β=-0.11, p=0.21 (Boys)	p=0.001 (Girls) β=-0.02, p=0.82 (Boys) High tertile β=-0.33, p=0.001 (Girls) β=-0.34, p<0.001 (Boys)	
Labaye, 2011[191] Sweden and Estonia the European Youth Heart Study	Longitudinal	Participants in the Estonian and Swedish part of the European Youth Heart Study were included Total N (%girls): 659 (53%) Age at baseline: 9 yrs Age at follow up: 15 yrs	6-yr ΔBMI ΔWC	N/A	N/A	N/A	Girls ΔBMI: [β (SE): 0.326(0.159) p=0.041 ΔWC: [β (SE): 1.011(0.349) p=0.004 Boys ΔBMI: [β (SE): 0.176(0.167) p=0.292 ΔWC: [β (SE): 0.477(0.401) p=0.235	Change in pubertal status, center and the corresponding baseline adiposity value

Table 2.2 (cont'd)

Tybor, 2011[192] the U.S.	Longitudinal	Girls enrolled in 1987–1988 recruited from the Richmond School district near Berkeley, CA, Cincinnati, OH and a large HMO, Washington, DC area. Total N (%girls): 2379 (100%) Age at baseline: 9–10 yrs	ΔWC ΔBMI z-score Biomarker data available for annual visits7 &10 log transformed insulin	White girls ΔWC β= 4.9 x10-4, NS ΔBMI z-score β= 0.1895, p<0.05 Black girls ΔWC β= 5.5 x10-3, NS ΔBMI z-score β= 0.09, NS	N/A	White girls ΔWC β= 8.1 $x10^{-2}$, NS ΔBMI z-score β= -0.56, NS Black girls ΔWC β= 0.65, p<0.05 ΔBMI z-score β= -5.76, NS	White girls ΔWC β= 2.1 $x10^{-4}$, NS ΔBMI z-score β= 0.46, p<0.05 Black girls ΔWC β= 0.16, p<0.05 ΔBMI z-score β=- 0.32, NS	Age, age x waist, age x BMI z-score
Kondaki, 2011[193] Europe Helena-CSS	CS	Participants were recruited from 10 cities in nine different	BMI WC WHR WHtR Log	Girls: BMI [β (SE): 0.058(0.009) R^2 =0.231 WC	N/A	Girls: BMI r=-0.059 NS WC r=-0.028 NS WHR	Girls: BMI [β (SE): 0.057(0.009) R^2 =0.224 WC	For insulin and HOMA: anthropometric indices were entered into different

Table 2.2 (cont'd)

Study		European countries. Total N (%girls): 1089 (54%) Age: 12.5-17.5 yrs	transformed insulin and HOMA (not glucose)	[β (SE): 0.028(0.004) R^2 =0.269 WHR [β (SE): 1.93(0.52) R^2 =0.152 WHtR [β (SE): 4.59(0.62) R^2 =0.269 Boys: BMI [β (SE): 0.083(0.01) R^2 =0.28 WC [β (SE): 0.033(0.004) R^2 =0.245 WHR [β (SE): 2.73(0.82) R^2 =0.069 WHtR [β (SE): 6.49(0.78) R^2 =0.27		r=0.01 NS WHtR r=0.01 NS Boys: BMI r=0.10 p ≤ 0.05 WC r=0.071 NS WHR r=-0.071 NS WHtR r=0.079 NS	[β (SE): 0.028(0.004) R^2 =0.267 WHR [β (SE): 1.97(0.54) R^2 =0.119 WHtR [β (SE): 4.64(0.64) R^2 =0.257 Boys: BMI [β (SE): 0.086(0.010) R^2 =0.274 WC [β (SE): 0.034(0.005) R^2 =0.237 WHR [β (SE): 2.56(0.86) R^2 =0.061 WHtR [β (SE): 6.61(0.83) R^2 =0.259	multiple reg models adjusting age, sex, Tanner stage, total energy intake, simple CHO and fat intake, and PA. (adjusted R^2 is variance explained by each one model) For glucose, results from univariate correlations are reported

Table 2.2. (cont'd)

Metcalf, 2011[194] U.K. EarlyBird 48	Longitudinal	Participants were recruited at school entry Jan 2000-Jan 2001 from 54 Plymouth primary schools. Total N (%girls): 280 (44%) Age at baseline: 5 yrs	Height	N/A	N/A	N/A	Correlation (r) **Girls:** 7 yr: 0.36 (p<0.001) 8 yr: 0.44 (p<0.001) 9 yr: 0.48 (p<0.001) 10 yr: 0.51 (p<0.001) 11 yr: 0.46 (p<0.001) 12 yr: 0.49 (p<0.001) **Boys:** 7 yr: 0.18 (p<0.05) 8 yr: 0.20 (p<0.05) 9 yr: 0.36 (p<0.001) 10 yr: 0.41 (p<0.001) 11 yr: 0.27 (p<0.01) 12 yr: 0.33 (p<0.001)	None
Huang, 2011[195]	CS	14-year-old Caucasian	WC WHR	Girls BMI	N/A	N/A	Girls BMI	Stepwise linear reg

Table 2.2 (cont'd)

Australia		children of the participants in the West Australian Pregnancy Cohort (Raine) Study Total N (%girls): 1149 (47%) Age: 13.8 ± 0.4 yrs	WHtR BMI Log transformed biomarkers	β (95% CI)=0.03 (0.01, 0.05), p=0.01 WHtR β (95% CI)= 1.43 (-0.06, 2.93), p= 0.06 Boys BMI β (95% CI)=0.03 (0.01, 0.06), p=0.002 WC β (95% CI)= 0.67 (-0.17, 1.51), p= 0.12			β (95% CI)=0.05 (0.03, 0.06), p<0.001 WHR β (95% CI)= 0.65 (-0.18, 1.48), p= 0.13 Boys BMI β (95% CI)=0.03 (0.00, 0.07), p=0.01 WC β (95% CI)= 1.55 (0.38, 2.73), p= 0.01	model adjusting for age, family income, PA, energy/day, pubertal stage At least one measure of central adiposity (WC for boys or WHtR for girls) was an independent predictor for insulin and HOMA
Vuksan, 2010[196] Canada	CS	Participants recruited from 62 Greater Toronto Area secondary schools. Total N (%girls): 182 (70%)	BMI WC WHR WHtR	N/A	N/A	BMI: Positive linear trend p<0.001 WC: Positive linear trend p<0.001 WHR: Positive linear trend p=0.011 WHtR: Positive linear trend	N/A	Sex, age and blood pressure parameters

Table 2.2 (cont'd)

		Age: 15-19 yrs				p=0.005		
Jago, 2010[197] the U.S, HEALTHY Study	CS	Participants recruited from 42 middle schools at 7 field centers Total N (%girls): 4955 (53%) Age: 11.3 ± 0.6 yrs	BMI	Girls: BMI<85th pct: [(7.7 (7.3–8.0)] BMI 85th–94th pct: [(12.0 (11.3–12.7)] BMI ≥95th pct [18.2 (17.3–19.2)] p < 0.0001 Boys: BMI<85th pct: [(6.1 (5.8–6.4)] BMI 85th–94th pct: [(9.6 (9.0–10.2)] BMI ≥95th pct [16.0 (15.2–16.9)] p < 0.0001	N/A	Girls: BMI<85th pct: [91.6 (91.0–92.2)] BMI 85th–94th pct: [(92.0 (91.3–92.7)] BMI≥95th pct: [93.2 (92.5–93.9)], p<0.0001 Boys: BMI<85th pct: [(93.5 (92.9–94.2)] BMI 85th–94th pct: [(94.3 (93.5–95.1)] BMI ≥95th pct [94.7 (94.0–95.4)], p=0.0003	N/A	None
Ouyang, 2010[198] China	Longitudinal	Twins were recruited in 8 counties of the Anqing region in	WC BMI Log	Girls: WC: Positive trend:p < 0.0001; Partial	N/A	N/A	N/A	Tanner stage, age ,zygosity and PA

Table 2.2 (cont'd)

		Anhui Province in 1998–2000 at baseline, & the follow-up study conducted since 2005. Total N (%girls): 1613 (45%) Age baseline ≥6 yrs; Age follow up≤ 20 yrs	transformed insulin	R^2: 0.097 BMI: Positive trend: p < 0.0001; Partial R^2: 0.077 Boys: WC: Positive trend: p < 0.0001; Partial R^2: 0.119 BMI: Positive trend: p < 0.0001; Partial R^2: 0.096				
Lawlor, 2010[199] U.K. Avon Longitudinal Study of Parents and Children	Longitudinal	Total N (%girls): 5235 (52%) Age: Anthropometric indices assessed at 9-12 yrs and 15-16 yrs Insulin-	BMI WC	Prospective associations among Girls: BMI at 9-12 1.08 (1.06 to 1.10) WC at 9-12 1.08 (1.06 to 1.10) Boys: BMI at 9-12	N/A	Prospective associations among Girls: BMI at 9-12 0.01 (−0.02 to 0.04) WC at 9-12 0.01 (−0.02 to 0.04) Boys: BMI at 9-12	N/A	Age, height, & height^2. maternal age, parity, family social class, maternal & paternal, education, birth weight, gestational age, maternal & paternal

Table 2.2 (cont'd)

		related biomarkers assessed at 15-16 yrs		1.20 (1.18 to 1.21) WC at 9-12 1.22 (1.19 to 1.24)		0.06 (0.05 to 0.07) WC at 9-12 0.05 (0.04 to 0.06)		BMI, & puberty

Denney-Wilson, 2010[200]
 	
Australia
 	
New South Wales (NSW) Schools Physical Activity and Nutrition Survey, 2004
	CS	Participants were students attending Grades K, 2, 4, 6, 8 and 10 in schools in NSW. Blood samples were collected from Grade 10 students
		
		Total N (%girls): 496 (41%)
			BMI
			WC
			WHtR
				Girls:
				BMI
				r=0.35 (p<0.001);
				WC
				r=0.40 (p<0.001);
				WHtR
				r=0.42 (p<0.001);
				
				Boys:
				BMI
				r=0.53 (p<0.001);
				WC
				r=0.49 (p<0.001);
				WHtR
					N/A	Girls not overweight/obese (BMI<25)
						Median: 4.5 mmol/L
						Girls overweight (BMI: ≥25 and BMI<30)
						Median: 4.5 mmol/L
						Girls obese (BMI≥30)
						Median: 4.4 mmol/L
						
						Boys not overweight/obese (BMI<25)
							N/A	None

Table 2.2 (cont'd)

		Age: 15.3 ± 0.03 yrs		r=0.51 (p<0.001);		Median: 4.6 mmol/L Boys overweight (BMI: ≥25 and BMI<30) Median: 4.7 mmol/L Boys obese (BMI≥30) Median: 4.8 mmol/L		
Zeelie, 2010[201] South Africa Physical Activity in the Young Study (PLAY)	CS	Participants selected from students of grade 9 attending two schools at risk of undernutrition Total N (%girls): 232 (57%) Age:15-19 yrs	BMI WC WHR Log transformed insulin & HOMA	BMI r=0.20, p=0.01 WC r=0.15, p=0.057 WHR r=0.004, p=NS		BMI r=0.16, p=0.04 WC r=0.09, p=NS WHR r=0.004, p=NS	BMI r=0.21, p=0.007 WC r=0.06, p=0.05 WHR r=0.01, p=NS	Gender and Tanner stage
Hirschler,	CS	Total N	BMI	N/A	N/A	N/A	BMI, Positive	Age and sex

Table 2.2 (cont'd)

2009[202] Argentina		(%girls): 625 (49%) Age: 6-14 yrs 9.6 ± 2.0 yrs	WC				trend p<0.001 across HOMA quartiles WC, Positive trend p<0.001 across HOMA quartiles	ROC for IR (the upper 3rd quartile of HOMA): BMI 0.77 (0.73–0.82) WC 0.782 (0.74–0.82) WC/ht 0.67 (0.62–0.72)
Arngrimsson, 2008[203] Iceland	CS	9-yr-old & 15-yr-old students from 18 schools, Iceland Total N (%girls): 9-yr-old : 103 (54%) 15-yr-old: 104 (49%) Age: 9-yr-old 9.4 ± 0.3 yrs 15-yr-old 15.3 ± 0.3 yrs	BMI WC adj. for height	9-yr-old BMI r = 0.36; p<0.001 WC adjusted for height r = 0.33; p<0.001 15-yr-old BMI r = 0.17; p=0.096 WC adjusted for height r = 0.24; p=0.025	N/A	N/A	N/A	Sexual maturity, gender, fitness

Table 2.2 (cont'd)

Gardner, 2008[204] U.K. EarlyBird 29	CS analyses of longitudinal study	Participants recruited 2000-2001, from randomly selected schools, stratified by SES status. Children were examined annually between 5 and 8 yr. Total N at baseline (%girls): 307 (44.6%) Total N with complete data at 4 time points (% girls) 231 (43.3%) Age at baseline: mean 4.8 yrs	BMI WC	N/A	N/A	N/A	BMI Correlation Girls: 5 yr: 0.28 (p<0.01) 6 yr: 0.25 (p=0.01) 7 yr: 0.50* 8 yr: 0.49* Boys: 5 yr: 0.20 (p=0.02) 6 yr: 0.17 (p=0.05) 7 yr: 0.30* 8 yr: 0.38* WC Correlation Girls: 5 yr: 0.32* 6 yr: 0.23 (p=0.02) 7 yr: 0.48* 8 yr: 0.49* Boys: 5 yr: 0.25 (p<0.01) 6 yr: 0.17 (p=0.06) 7 yr: 0.37* 8 yr: 0.40* Note: * means p<0.01	N/A

Table 2.2. (cont'd)

Manios, 2008[205] Greece The Children Study	CS	Participants were recruited from primary schools in island Crete, Greece. Total N (%girls): 522 (52%) Age: 10-12 yrs 10.46± 0.36 yrs	BMI WC WHR WHtR	BMI [β (SE): 0.07 (0.01) p<0.001, R^2=0.235 WC [β (SE): 0.03 (0.004) p<0.001, R^2=0.256 WHR [β (SE): 2.66 (0.79) p=0.001, R^2=0.084 WHtR [β (SE): 4.18 (0.64) p<0.001, R^2=0.214	N/A	BMI [β (SE): 0.26 (0.16) p=0.117, R^2=0.001 WC [β (SE): 0.10 (0.06) p=0.109, R^2=0.001 WHR [β (SE): -6.21(10.6) p=0.559, R^2=0.004 WHtR [β (SE): 15.8 (9.90) p=0.112, R^2=0.001	BMI [β (SE): 0.07 (0.01) p<0.001, R^2=0.200 WC [β (SE): 0.03 (0.004) p<0.001, R^2=0.221 WHR [β (SE): 2.78 (0.86) p=0.001, R^2=0.066 WHtR [β (SE): 4.29 (0.69) p<0.001, R^2=0.186	Age, sex, pubertal stage, total energy, carbohydrate and fat intake
He, 2007[206] China	CS	Tanner stage 1 children recruited from local schools or children of hospital employees Total N (%girls): 247 (40%)	WC Height	WC β =0.01 p=0.19 Height β =0.03 p=0.01	N/A	N/A	N/A	Initial model included age, sex, waist, weight, height, sex x waist, sex x weight, & sex x height

Table 2.2 (cont'd)

		Age: Girls 6.0 ± 1.9 yrs Boys 7.1 ± 2.2 yrs						
Ramachandran, 2007[207] India	CS	Participants recruited from a school-based survey in 16 schools in Chennai, India. Total N (%girls): 2,640 (50%) Age: 12-19 yrs	BMI WC	N/A	N/A	BMI [β (SE): 0.14 (0.02) p<0.0001 WC [β (SE): 0.02 (0.008) p<0.004	BMI [β (SE): 0.14 (0.02) p<0.0001 WC [β (SE): 0.02 (0.008) p<0.004	Age, sex, fat percentage
Sung, 2007[208] China	CS	Participants were recruited from 90 classes randomly sampled in eight primary schools participating in University-	BMI WC WHR WHtR Log insulin	BMI r=0.54, p<0.001 (Girls) r=0.57, p<0.001 (Boys) WC r=0.55, p<0.001 (Girls) r=0.61, p<0.001 (Boys)	N/A	BMI r=0.18, p=0.01 (Girls) r=0.002, p=0.96 (Boys) WC r=0.16, p=0.004 (Girls) r=-0.023, p=0.60 (Boys)	N/A	Age-adjusted BMI

Table 2.2 (cont'd)

		based health promotion activities in different districts of Hong Kong between 2002 and 2004 Total N (%girls): 2593 (47%) Age: 6-12 yrs		WHR r=0.06, p=0.24 (Girls) r=0.28, p<0.001 (Boys) WHtR r=0.38, p<0.001 (Girls) r=0.51, p<0.001 (Boys)		WHR r=-0.11, p=0.03 (Girls) r=-0.099, p=0.02 (Boys) WHtR r=-0.09, p<0.001 (Girls) r=-0.12, p=0.005 (Boys)		
Yan, 2006[148] China	CS	Participants selected from a school-based CS study in Xinjiang Autonomous Region stratified by age, sex, and ethnicity. Total N (%girls):	BMI WC WHR	BMI, WC, WHR have similar partial correlations with fasting insulin (r=0.224-0.312). (Corresponding r is not reported)		WC had the highest correlation with fasting glucose (r=0.105) compared to BMI and WHR. (r for BMI or WHR is not reported)	BMI, WC, WHR have similar partial correlations with HOMA (r=0.210-0.229). (Corresponding r is not reported)	Age, sex and ethnicity

Table 2.2 (cont'd)

		661 (N/A): 389 Han & 272 Uygur children Age: 7-18 yrs						
Thorsdottir, 2006[209] Iceland	CS	Participants were randomly selected 9- and 15-year-old students from 18 randomly selected schools. Total N (%girls): 262 (49%) 9-yr: 134 (55%) 15-yr: 133 (48%) Age: 9 yr and 15 yr	Height BMI WC WHR	Height positive trend: p=0.008 (9-yr girls) p=0.292 (15-yr girls) p=0.104 (9-yr boys) p=0.113 (15-yr boys) BMI positive trend: p<0.001 (9-yr girls) p=0.013 (15-yr girls) p<0.001 (9-yr boys) p=0.015(15-yr boys) WC positive trend: p<0.001 (9-yr girls) p=0.006 (15-yr girls) p=0.002 (9-yr boys) p=0.014 (15-yr boys) WHR positive trend: p<0.001(9-yr girls) p=0.072 (15-yr girls) p=0.432 (9-yr boys) p=0.001 (15-yr boys)	N/A	N/A	N/A	None

Table 2.2 (cont'd)

Garcés, 2005[210] Spain	CS	Participants were randomly selected from public&private schools. Total N (%girls): 1048 (50%) Age: 6-8 yrs	BMI	Girls: BMI: r=0.36, p<0.01 Boys: BMI: r=0.37, p<0.01	N/A	Girls: BMI: r=0.17, p<0.01 Boys: BMI: r=0.13, p<0.01	Girls: BMI: r=0.356, p<0.01 Boys: BMI: r=0.36, p<0.01	None
Wilson, 2004[211] the U.S The Girls Health Enrichment Multisite Study (GEMS)	CS	AA girls recruited from four US study centers: Minnesota, Memphis, Baylor and Stanford. Total N (%girls): 119 (100%) Age: 9.2 ± 0.9 yrs	BMI WC Height	WC r=0.33, p<0.0001 Height r=0.16, p=0.08	N/A	BMI r=0.54, p<0.0001 WC r=0.67, p<0.0001 Height r=0.53, p<0.0001	N/A	None
Misra, 2004[212]	CS	Participants were taken randomly from	BMI WC WHR	BMI r=0.39, p<0.001 (Girls)	N/A	N/A	BMI r=0.39, p<0.001 (Girls)	Age

Table 2.2 (cont'd)

India		Epidemiological Study of Adolescents and Young adults (ESAY study) Total N (%girls): 250 (38%) Girls: 17.2 ± 1.2 yrs Boys: 16.2 ± 1.2 yrs		r=0.62, p<0.001 (Boys) WC r=0.25, p<0.05 (Girls) r=0.63, p<0.001 (Boys) WHR r=0.01, NS (Girls) r=0.38, p<0.001 (Boys)			r=0.56, p<0.001 (Boys) WC r=0.28, p<0.01 (Girls) r=0.58, p<0.001 (Boys) WHR r=0.008, NS (Girls) r=0.38, p<0.001 (Boys)	
Klein, 2004[213] the U.S Growth and Health Study (NGHS)	Longitudinal	Black & white girls enrolled in the NGHS in Cincinnati, Washington D.C during Yr 1 and Yr 10. Total N (%girls): 955 (100%) Age at baseline: 9 and 10 yrs	the 10-year Δ BMI	r = 0.26, p< 0.0001	N/A	r = 0.16, p< 0.0001	r=0.24, p <0.0001	N/A for adjusted covariates. In white girls, the rate of BMI increase, not baseline BMI, was associated w. Year 10 glucose level

Table 2.2. (cont'd)

Molero-Conejo , 2003[214] Venezuela	CS	Participants were recruited from an urban school in Maracaibo-Venezuela Total N (%girls): 167 (59%) Age: 14-17 yrs	BMI WC WHR	BMI r=0.17, NS (Girls) r=0.38, NS (Boys) WC r=0.16, NS (Girls) r=0.36, NS (Boys) WHR r=-0.01, NS (Girls) r=0.15, NS (Boys)	N/A	N/A	BMI r=0.14, NS (Girls) r=0.37, NS (Boys) WC r=0.17, NS (Girls) r=0.36, NS (Boys) WHR r=0.0002, NS (Girls) r=0.315, NS (Boys)	None Boys and girls in the highest BMI quartile had significantly higher levels of insulin.
Lindsay, 2001[215] the U.S	CS	Participants recruited from a population study of T2D in an Indian community, Arizona Total N (%girls): 5-9 yr 373 (53%) 10-14 yr 379 (51%)	BMI	r= 0.31–0.67 across all the age and sex groups; all P <0.05	N/A	BMI was significantly correlated with fasting glucose in all the age and sex groups (data shown in the figures)	N/A	Age

Table 2.2 (cont'd)

		15-19 yr 233 (56%) Age:5-19 yrs						
Bavdekar, 1999[216] India	CS	Participants from newborns in the King Edward Memorial Hospital, Oct.1987 to Apr. 1989 Total N (%girls): 477 (46%) Age: 8.47 ± 0.11 yrs	Height at 8 Log HOMA	N/A	N/A	N/A	β (95% CI) =2.7 (1.5 to 3.3), P<0.001	Age, sex, fat mass, birth weight, father's height, mother's height
Freedman, 1999[217] the U.S Bogalusa Heart Study 1992-1994	CS	Children in Ward 4 of Washington Parish, LA Total N (%girls): 2996 (51%) Age: 5-17 yrs	WC WHR	WC Predicted change (pmol/L)=6, t statistic=5 WHR Predicted change (pmol/L)=7, t statistic=6	N/A	N/A	N/A	Race, sex, age, height, and weight

Table 2.2 (cont'd)

Jiang, 1996[218] the U.S the Bogalusa Heart Study 1992-1993	CS	Children in Ward 4 of Washington Parish, LA Total N (%girls): 1157 (52%) Age: 11-18 yrs	BMI Log transformed biomarkers	White girls: BMI R^2: 0.351, p<0.05 Black girls: BMI R^2: 0.24 (adj body fat), p<0.05 White boys: BMI R^2: 0.44, p<0.05 Black boys: BMI R^2:0.23, p<0.05	White girls: BMI R^2: 0.04 (adj SSSF) Black girls/White & Black boys: BMI was not selected in the reg model	N/A	N/A	Independent variables (each measure of obesity) were assessed by a stepwise regression analysis performed for race-sex specific groups.
Mo-Suwan, 1996[219] Thailand	CS	Children living in Hat Yai municipality Total N (%girls): 239(49%) Age: Normal weight: 10.2 ± 1.8 yrs Obese: 10.5 ± 1.8 yrs	WHR Log insulin	r=0.06; p=0.42	N/A	N/A	N/A	Age, sex, Systolic BP, obesity (defined by weight-for-height values > 1205), LDL, HDL, TG
Rönnemaa, 1991[220]	CS analyses of	Among Finnish children and	BMI	Age=9 r=0.49; p<0.001 (Girls)	N/A	N/A	N/A	None Results for age=21 and

Table 2.2 (cont'd)

Finland	longitudinal study	young adults, 3 field exams were conducted in 1980,1983, &1986 in five university hospital cities and their surrounding rural areas. Total N (%girls): 2433 (53%) Age in 1980: 3-18 yrs Age in 1986: 9-24 yrs		r=0.32; p<0.001 (Boys) Age=12 r=0.52; p<0.001 (Girls) r=0.48; p<0.001 (Boys) Age=15 r=0.23; p<0.001 (Girls) r=0.33; p<0.001 (Boys) Age=18 r=0.38; p<0.001 (Girls) r=0.25; p<0.01 (Boys)				age=24 were not reported here.

Note: AA: African American; CS: cross-sectional; NHANES: National Health and Nutrition Examination Survey; BMI: body mass index; IR: insulin resistance; PA: physical activity; PCT: percentile; NC: Neck circumference; TG: triglycerides; WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; WFH: weight-for-height; NS: non-significant; SSSF: subscacuplar skinfold thickness; Underwt: Underweight.

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CHAPTER 3: THE ASSOCIATION OF DIETARY AND ANTHROPOMETRIC FACTORS WITH INSULIN-RELATED BIOMARKERS IN ADOLESCENT GIRLS: RESULTS FROM THE DIETARY INTERVENTION STUDY IN CHILDREN (DISC)

3.1. Abstract

Background: Dietary and anthropometric factors during adolescence have been hypothesized to impact future chronic disease risk through their effect on insulin-related biomarker levels. Information on the associations between diet, anthropometry and insulin-related biomarkers in adolescence, however, are limited.

Objectives: To examine associations of dietary intake of selected nutrients and body mass index (BMI)-for-age percentile (BMIPCT) with serum insulin-related biomarkers (insulin, C-peptide, glucose) and insulin sensitivity (homeostasis model assessment of insulin resistance [HOMA]) in adolescent girls who took part in the DISC study.

Design: The current study included 176 postmenarcheal adolescent girls (mean age: 16.6 ± 0.9 yrs) who attended the last visit of the DISC. Dietary intake was examined through 3 averaged, nonconsecutive 24–hour dietary recalls. Biomarkers were determined by immunoassay (insulin, C-peptide), enzymatic reaction (glucose) or HOMA calculation (insulin sensitivity). BMI was examined as BMIPCT based on Centers for Disease Control growth charts 2000. Associations of dietary and anthropometric factors with biomarkers were estimated using crude and adjusted Pearson's correlation analyses and

multivariable linear regression models. To mitigate skewness, insulin, HOMA and nutrients were logarithmically transformed; C-peptide and glucose were modeled untransformed.

Results: In adjusted correlation analyses, the following dietary factors were found to be significantly associated with the biomarkers of interest ($p<0.05$): total fat, vegetable protein, starch and fiber with C-peptide; sucrose with insulin; sucrose with HOMA; no significant dietary factor for glucose was found. BMIPCT was significantly associated with C-peptide, insulin and HOMA, not with glucose in adjusted correlation analyses. In stepwise linear regression multivariable models with forward selection that included confounding variables such as age, time since menarche, treatment, and energy intake, the most significant dietary or anthropometric factors associated with C-peptide were fiber [$\beta=-174.13 \pm 60.44$, $p=0.005$], vegetable protein ($\beta= -207.5 \pm 79.4$, $p=0.01$) and BMIPCT ($\beta=2.90 \pm 0.76$, $p=0.0002$); the most significant factor associated with insulin was BMIPCT ($\beta=0.007 \pm 0.001$, $p<0.0001$); the most significant factors associated with HOMA were starch ($\beta=-0.31 \pm 0.15$, $p=0.04$) and BMIPCT ($\beta=0.008 \pm 0.001$, $p<0.0001$); no significant factor was associated with glucose.

Conclusions: After adjustment for BMIPCT and energy intake, this study suggests dietary fiber or vegetable protein intake in adolescent girls is inversely associated with C-peptide, and starch intake is inversely associated with HOMA. BMIPCT is also positively and independently associated with insulin, C-peptide and HOMA levels.

3.2. Introduction

Chronic diseases, such as type 2 diabetes, cancer, and cardiovascular diseases, are among the top ten leading causes of death in the United States.[1] The prevalence of the associated condition, obesity, has steadily increased in the past 25 years among children and adolescents,[2] as well as among adults.[3] This increase has been accompanied by an increase in several insulin-related metabolic abnormalities.[4,5] Higher adult insulin-related biomarker levels have been associated with increased chronic disease risk[6-16] and childhood biomarker levels may track into adulthood.[17-20] Adolescence is a transitional period accompanied by tremendous growth and development, and could be a critical time in affecting future metabolic control as reflected by changes in insulin-related biomarker levels (insulin, C-peptide, glucose, insulin sensitivity [HOMA]) in later life.[21,22] Therefore, levels of insulin-related biomarkers during adolescence may reflect changes in lifetime risk for chronic diseases.

Dietary intake has been found to be associated with changes in insulin-related biomarkers in adults, children and adolescents. Among adults, in a meta-analysis of 15 cohort studies mainly in non-diabetic populations, diets high in whole grains, fish, fruits, vegetables and nuts/seeds are associated with lower fasting insulin and glucose levels regardless of insulin or glucose associated genetic loci identified by genome-wide association studies.[23] In healthy girls, four studies[24-27] have specifically reported the associations between dietary factors

and components of insulin-related biomarker levels. They reported that higher intake of whole grains was associated with lower insulin and C-peptide levels.[24] Lower total sugar intake,[25] lower sugar-sweetened beverage intake[27], a healthy dietary pattern,[26] and higher fiber intake[25] were associated with lower HOMA levels. Information on associations between dietary nutrient factors and insulin-related biomarker levels in adolescent girls, therefore, is limited.

Adiposity generally results from a chronic imbalance between energy intake and energy expenditure and is closely associated with insulin resistance, insulin action, and glucose homeostasis.[28] General adiposity is measured by body mass index (BMI) and reflects primarily subcutaneous fat deposition. Whether general adiposity is associated with changes in insulin-related biomarkers among adolescents, however, still needs further study.

Dietary intake and adiposity have been hypothesized to be associated with insulin-related biomarker levels primarily through their effect on carbohydrate metabolism. It is well known that blood glucose is the major factor that regulates insulin secretion,[29] and insulin regulates glucose metabolism mainly in three tissues: liver, muscle and adipose.[30] C-peptide and insulin are both cleavage products from proinsulin and are secreted in equal molar amounts.[31,32] C-peptide has a longer half-life than insulin and therefore may more accurately reflect insulin secretion.[33]

The aim of this study was to examine whether or which dietary or anthropometric factors are related to changes in fasting serum insulin-related biomarkers (insulin, C-peptide, glucose) and insulin sensitivity (HOMA) in adolescent girls. We used data from girls 14-18 years of age participating the Dietary Intervention Study in Children (DISC). The DISC was a multicenter, randomized controlled clinical trial to examine the safety and efficacy of a dietary intervention to reduce serum low-density lipoprotein cholesterol (LDL-C) levels in children.[34] Understanding dietary or anthropometric factors associated with the insulin-related biomarkers among healthy, postmenarcheal adolescent girls, may shed light on the development of dietary or anthropometry related intervention recommendations for this population.

3.3. Methods

Study Design of the DISC and the insulin related biomarkers study (IRBS)

The current study included adolescent girls who were originally participants in the DISC. The design of the DISC has been well described in previous literature.[34-40] Briefly, the DISC was a longitudinal multicenter randomized controlled clinical trial that was initiated at 6 U.S. centers in 1988 and included 663 (301 girls, 362 boys) healthy children aged 8-10 years at baseline with a median follow-up of 7 years. The original aim of the DISC was to determine the efficacy of a low-fat dietary intervention to decrease serum low-density lipoprotein (LDL) cholesterol levels and its safety to promote growth and development. Participants had serum LDL cholesterol levels in the 80th-98th

percentile,[41] and were randomized to either a dietary intervention group or usual care control group. The DISC behavioral intervention successfully promoted decreased consumption of red meats and whole milk dairy products and increased consumption of fruits, vegetables and whole grains in order to limit total and saturated fat intakes to 28%, and less than 8%, respectively, of daily caloric intake and to increase dietary fiber intake in children during puberty.[37] The dietary intake differences were most pronounced between girls in the intervention vs. usual care group at year 3, and there was generally no dietary intake difference at the last visit. The National Heart, Lung, and Blood Institute (NHLBI) sponsored the DISC study and an NHLBI-appointed independent data and safety monitoring committee provided oversight.

The IRBS was an ancillary study of the DISC, designed to examine the effect of the DISC dietary intervention and nutritional factors on insulin- and insulin-related growth factor-axes biomarkers analyzed in blood samples collected from girls.[39] The IRBS was approved by the DISC steering committee, the Department of Defense, Michigan State University and the Fox Chase Cancer Center human subject review boards. The current analyses were focused on the associations between reported dietary intakes and insulin-related biomarker (insulin, C-peptide, glucose and HOMA) levels among adolescent girls who attended the last DISC visit and had serum samples available. The last visit took place between 6.4–9.1 years (median=7 years) after girls' randomization in DISC when they were between 14.6 years and 18.9 years old.[34] The DISC

dietary intervention did not lead to detectable differences in the insulin-related biomarkers overall.

Of a total 301 girls participating in the original DISC study, 269 girls attended the last visit and 198 of them had serum available to measure levels of insulin-related biomarkers. After excluding girls with missing dietary data (n=10) or missing data on time since menarche (n=9) or girls who were pregnant (n=2), 177 girls had complete data on dietary intake, insulin-related biomarkers, and time since menarche at the last visit of the DISC. One additional girl was excluded due to implausible insulin (48.0 uU/mL) and HOMA (11.6) values, leaving a total of 176 girls between 14.6 years to 18.9 years of age for analysis. No statistically significant differences were found between girls in the final sample (n=176) compared with the excluded sample (n=125) in terms of socio-demographic characteristics (race, mothers' education, family income, or treatment group), total energy intake, BMIPCT or LDL cholesterol levels.

Data Collection and Assessment

Dietary Assessment

Dietary assessment procedures for the DISC study have been previously documented.[38,39,42] Briefly, dietary intakes were measured using 3 nonconsecutive 24-hour dietary recalls at baseline (before randomization), post-randomization years 1, 3, 5, and at the last visit. The first recall was obtained through a face-to-face interview at the clinic and the following 2 recalls were obtained by telephone interview. Nutrient analyses were performed by the Nutrition Coordinating Center, University of Minnesota, using Nutrition Data

System version 20.[42] The mean intake from the three recalls at each visit was used to estimate each girl's nutrient intake.

Insulin-related Biomarker Measures

Blood samples were collected by venipuncture in the morning following an overnight fast at baseline, year 1, 3, 5 and the last visit. After blood was completely clotted, samples were centrifuged and serum was aliquoted and stored at around –70°C or colder until it was thawed for analyses for hormone, lipid and micronutrient levels for the DISC.[39] Serum samples for the insulin-related biomarker measurements had been thawed twice to allow removal of additional aliquots of serum, and were refrozen immediately at around –70°C or colder. Glucose, insulin and C-peptide assays were conducted at Boston Children's Hospital (Dr. Nader Rifai's laboratory, Department of Laboratory Medicine, Harvard University). Glucose was measured by the glucose oxidase reaction using Roche Diagnostics reagents (Indianapolis, IN). C-peptide was measured by an ultra-sensitive electrochemiluminescence immunoassay using Elecsys technology (Roche Diagnostics, Indianapolis, IN). Insulin was measured by an ultra-sensitive enzyme-linked immunosorbent assay (ELISA) (ALPCO, Windham, NH). As an indirect measure of insulin resistance status, HOMA was derived from fasting insulin (uU/mL) x fasting glucose (mmol/L)/22.5.[43]

Anthropometry

Data were collected at baseline and annually thereafter by trained personnel blinded to participants' treatment group assignments. Height and

weight were measured twice, and if the difference was within allowed tolerances (0.5 cm for height and 0.2 kg for weight), the 2 values were averaged.[40] If differences in the two measurements were larger than allowed, the measurement was taken again and the two closest values were averaged. BMI was calculated as weight (kg) divided by height^2 (m). BMI standardized measurement BMIPCT was computed based on Centers for Disease Control growth charts 2000.[44] In children and adolescents, BMIPCT points the relative position of the child's BMI among a reference population of children of the same age and gender, given that the percentage of body fat differs with age and sex in children and adolescents.[44] According to the National Center for Health Statistics (NCHS) 2007 recommendation, BMIPCT≥95th is categorized as obese and 85th ≤ BMIPCT < 95th is categorized as overweight.[45]

Covariate Measures

Covariates including demographic information (age, race/ethnicity, household income, mother's education) and lifestyle information (physical activity, smoking status, alcohol consumption, medical conditions and use of medications) were assessed by questionnaire. Information on onset of menses, pregnancy history and oral contraceptives use was collected starting with the year 3 visits.

Statistical Methods

The current study was a cross-sectional analysis using data collected at the last visit (ages 14.6-18.9 years) to examine the effect of dietary constituents

including fat, animal protein, vegetable protein, carbohydrate, lactose, fructose, glucose, sucrose, starch, fiber, calcium, zinc, iron, and sodium on insulin-related biomarker levels. The selection of nutrients examined was based on the literature that showed the association between these nutrients with insulin-related biomarkers (Insulin, C-peptide, Glucose, and HOMA) and the availability of data in our study.[25,27,46-55] The nutritional factors were energy adjusted through the multivariate nutrient-density approach.[56] To mitigate skewness, insulin, HOMA and all nutrients were log transformed. C-peptide and glucose were approximately normally distributed and were therefore not log transformed.

Descriptive statistics were calculated as N (%) for baseline categorical variables such as race/ethnicity, total household income, treatment group and mother's education. Descriptive statistics for continuous variables at the last visit included age, anthropometric measurements reported as a mean (standard deviation, SD), and nutritional intake and biomarker levels reported as median (interquartile range, IQR).

Associations between nutrients, BMIPCT and biomarkers were estimated using crude and partial correlation analyses. Because total energy intake is associated with nutrient intake,[56] age, time since menarche are associated with insulin-related biomarkers in the literature,[57-61] and treatment group is a dietary intervention factor affecting the dietary intake in our study population, we controlled for total energy intake, age, time since menarche and treatment group in the partial correlation analyses. Further, geometric mean levels (insulin and

HOMA) or least squares mean levels (C-peptide and glucose) of each biomarker were calculated within quartiles of each nutrient to fully describe the associations between the outcome (insulin-related biomarkers) and the exposure of interest (dietary factors) adjusting for total energy intake, age, time since menarche and treatment group. We tested the linear trends across quartiles by using the medians of biomarkers in each nutrient quartile category as continuous variables.

Associations were also examined using simple linear regression models and multivariable linear regression models. Initial simple linear regression models examined the effects of all selected nutrients (fat, animal protein, vegetable protein, carbohydrate, lactose, fructose, glucose, sucrose, starch, fiber, calcium, zinc, iron, and sodium) on each of the insulin-related biomarker levels. Potential confounding factors including age, time since menarche, treatment group status, BMIPCT, physical activity, batch number, mother's education (high school or less, some college, and college or graduate degree), physical activity (hours of moderate and intense activity per week) and dietary supplement intake (yes or no) were also examined in the simple linear regression models. The selection of the covariates into the initial multivariable models were based on the simple linear regression models with parameter estimate p-values less than 0.20. In order to further select covariates entered into a more parsimonious model, we used a forward regression approach with $p<0.20$ as the initial significance criterion. In order to avoid multicollinearity of dietary variables, we only include dietary variables that have bivariate correlations less than $r=0.60$. Therefore, for multivariable linear regression analyses, vegetable protein and fiber intake

(r=0.74, p<0.0001) for C-peptide was examined in separate models due to collinearity. The nutrient variables entered in the multivariable regression model for C-peptide were total energy, total fat, fiber or vegetable protein and starch; for insulin were total energy, total fat, sucrose, and zinc; for glucose were total energy, lactose, starch and dietary glucose; for HOMA were total energy, total fat, zinc, starch, sucrose, and glucose.

We also conducted the following sensitivity analyses. Due to potential interactions by racial/ethnicity[62-64] and that 91.5% of our sample either self-reported or were reported by parents as being 'white' in the last visit data, we conducted analyses subset to these girls. Secondary analyses in intervention group and usual care group were also performed to see if the dietary factors remained the same. Interaction terms between dietary factors and the other main factors including BMIPCT, age at visit, time since menarche or treatment group were evaluated to examine whether associations between dietary factors and biomarker levels varied with different levels of the other main factors.

All analyses were performed by using SAS 9.3 software (SAS Institute Inc, Cary, NC).

3.4. Results

Demographic and socioeconomic characteristics of the participants are presented in **Table 3.1**. The majority of the participants were white, had higher household income (≥$30,000) and had higher mother's education (> some college). Participants attending the last visit were equally distributed in the intervention group (50.6%) and the usual care group (49.4%).

Results of the summary statistics for the key covariates, nutrients, anthropometric factors and insulin-related biomarkers are shown in **Table 3.2**. Means and standard deviations (SD) are reported for covariates (age, time since menarche and physical activity) and anthropometric factors (height, weight, BMI, BMI-for-age percentile). Medians (IQ ranges) are reported for nutrient intakes and insulin-related biomarkers. Insulin, C-peptide, glucose and HOMA values were within published normal reference ranges for the adolescent population.[21,65]

Table 3.3 shows the results of the crude and adjusted partial correlations between various nutrients and each of the four insulin-related biomarkers. The significance level and the strength of the associations between the outcome (biomarkers) and the exposures of interest (nutrient intakes) were not changed after adjusting for total energy intake, age, time since menarche and treatment group. Total fat, vegetable protein, starch and fiber were identified as statistically significant factors of C-peptide ($p<0.05$) in both crude and adjusted correlation analyses. Adjusted Pearson's correlation coefficients (r) for the above four dietary variables were 0.17, -0.21, -0.18 and -0.21, respectively. Dietary sucrose intake was shown to be a significant factor for insulin in the adjusted correlation analyses ($r=0.17$, $p<0.05$). None of the nutrients was significantly correlated with glucose levels. Dietary sucrose intake was significantly positively correlated with HOMA in the adjusted correlation analyses ($r=0.18$, $p<0.05$). BMIPCT was significantly associated with C-peptide ($r=0.29$, $p<0.05$), insulin ($r=0.44$, $p<0.05$)

and HOMA (r=0.42, p<0.05), not with glucose (r=0.07, p=0.39) in adjusted correlation analyses.

Table 3.4 shows the least squares means of C-peptide level as well as geometric means of insulin and HOMA by quartiles of the statistically significant nutrients (ie, those nutrients shown to be significantly correlated with insulin-related biomarkers in Table 3.3) from the correlation analyses, adjusting for age, time since menarche, energy intake and treatment group. For C-peptide, among the four significant nutrients (total fat, vegetable protein, starch and fiber), trends in adjusted least squares means of C-peptide across quartiles of nutrients were significant for vegetable protein (p=0.03) and fiber (p=0.03). For insulin and HOMA, the linear trends across increasing quartiles of sucrose intake were not significant, although the crude and adjusted Pearson correlation tests showed significant correlations.

In forward selection multivariable analysis of C-peptide mutually adjusting for age, time since menarche, BMIPCT, energy intake, treatment group, starch and fiber, only BMIPCT (β=2.90 ± 0.76, p=0.0002) and fiber intake (β=-174.13 ± 60.44, p=0.005) remained as significant factors of C-peptide, as shown in **Table 3.5**. Because C-peptide and BMIPCT are not log transformed while nutrients of interest were log transformed, β coefficients can be interpreted as the expected change in the biomarkers. For example, the β coefficients indicate that a 10 unit increase of BMIPCT is associated with a 29 pmol/L increase of C-peptide level, and a 10% increase in fiber intake is associated with a 16.60 pmol/L decrease of C-peptide level. **Figure 3.1** shows the inverse association between fiber intake

and C-peptide level. Potential confounding factors including mother's education, physical activity and dietary supplement intake were not significantly associated with biomarkers in the simple linear regression models, therefore were not included in the multivariable models. In a similar model including age, time since menarche, BMIPCT, treatment group, energy intake, starch and vegetable protein, BMIPCT (β=2.81 ± 0.76, p=0.003) and vegetable protein (β=-207.5 ± 79.4, p=0.01) were identified as significant factors (data not shown in the table). In the sensitivity analysis including only white girls, the results were still the same as all girls included (data not shown). In the secondary analyses using the same multivariable linear regression model among girls in the intervention group, only fiber was identified as a significant factor for C-peptide (β=-268.3 ± 87.3, p=0.003). In contrast, only BMIPCT (β=3.38 ± 0.92, p=0.0004) was found to be a significant factor for C-peptide among girls in the usual care group. There was no statistically significant difference of either fiber or starch intake or BMIPCT between intervention and usual care groups. Observed difference in the selection of the factors in the treatment groups may be due to the reduced sample size in the secondary analyses stratified by treatment group.

Multivariable liner regression analyses with forward selection ($p<0.05$) were also performed for insulin, glucose and HOMA adjusting for age, time since menarche, BMIPCT, treatment group and energy intake as shown in **Table 3.5**. For insulin, BMIPCT (β=0.007 ± 0.001, p<0.0001), age (β=0.11 ± 0.04, p=0.005) and time since menarche (β=-0.11 ± 0.04, p=0.0004) showed significant associations. Because insulin, HOMA and nutrients of interest were log

transformed, β coefficients can be interpreted as proportional changes. For example, the β coefficients indicate a 10% increase in BMIPCT is associated with a 0.07% increase in insulin, a 10% increase in age is associated with a 1% increase in insulin, and a 10% increase in time since menarche is associated with a 1% decrease in insulin. For glucose, none of the variables was selected to be a significant factor. For HOMA, starch (β=-0.31 ± 0.15, p=0.04), BMIPCT (β=0.008 ± 0.001, p<0.0001), age (β=0.12 ± 0.04, p=0.006) and time since menarche (β=-0.12 ± 0.03, p=0.0003) were identified to be significant. This indicates a 10% increase in BMIPCT is associated with a 0.08% increase in HOMA, a 10% increase in age is associated with a 1% increase in HOMA, a 10% increase in time since menarche is associated with a 1% decrease in HOMA, and a 10% increase in starch intake is associated with a 3% decrease in HOMA. Secondary analyses for HOMA by treatment group showed BMIPCT was a significant factor for HOMA in the intervention group (β=0.005 ± 0.002, p=0.01); in contrast, starch (β=-0.52 ± 0.24, p=0.03), BMIPCT (β=0.008 ± 0.002, p<0.0001), and time since menarche (β=-0.08 ± 0.04, p=0.04) remained as significant factors of HOMA in the usual care group. Tests for interaction did not indicate that the treatment group assignment, age, time since menarche, or BMIPCT modified the associations of dietary factors and insulin-related biomarkers (data not shown). Sensitivity analyses were also conducted including BMIPCT and BMIPCT change (BMIPCT at the last visit - BMIPCT at the baseline visit) in the same multivariable linear regression model (data not shown). The results showed the BMIPCT rather than BMIPCT change had a significantly

positive association with insulin, C-peptide and HOMA levels, indicating the BMIPCT around the time of the blood draw had more impact on the biomarker levels compared to the BMIPCT change.

3.5. Discussion

In our study of 176 postmenarcheal adolescent girls (aged 14-18 years) who attended the last visit of the DISC, we showed several nutrient intakes were associated with insulin-related biomarker levels. Dietary fiber or vegetable protein intake was inversely associated with serum C-peptide level, and starch intake was potentially inversely associated with HOMA value. Our results also showed BMIPCTs were significantly positively associated with insulin, C-peptide and HOMA values.

Our results of BMIPCT as a highly significant positive factor for insulin, C-peptide and HOMA levels are consistent with other studies that showed higher BMI was associated with higher levels of insulin-related biomarkers (insulin, C-peptide, or HOMA) in adults and adolescents.[66,67] The positive associations between BMIPCT and insulin, C-peptide and HOMA levels remained significant after controlling for dietary factors, physical activity, and time since menarche, which suggests an independent effect of BMIPCT on these insulin-related biomarker levels. In addition, our results showed the BMIPCTs rather than BMIPCT changes had significant positive associations with insulin, C-peptide and HOMA levels, which indicated that the BMIPCT around the time of the blood draw had more impact on the biomarker levels compared to the BMIPCT change.

In contrast, three other studies[68-70] reported significant relations between BMI change and insulin or HOMA levels in children and adolescents with age 9-10 years at baseline and 7-10 years of follow-up, although BMIPCTs were not used in these studies.

In children, the relationship between dietary factors and insulin biomarker levels, especially C-peptide, has been less investigated. To date, there is only one study reporting dietary factors on C-peptide level among adolescents.[24] The study, based on data collected from participants aged 12-19 years in NHANES 1999-2004, reported an inverse association between whole grain intake and C-peptide levels (P_{trend}=0.019).[24] Since whole grain is a major source of fiber, our finding of an inverse association between fiber intake and serum C-peptide level is consistent with their results. For other insulin-related biomarkers (insulin, glucose or HOMA), we identified several studies including both boys and girls that studied specific dietary factors on these biomarkers among children and adolescents.[24-27,47,48,50,51,71-77] A healthy dietary pattern was reported to be inversely associated with glucose, insulin and HOMA levels among children 14 years of age.[26] Specific food or food groups such as whole-grains,[24] soluble fiber,[71] milk[72] and nuts[73] were found to be inversely associated with insulin, glucose, and HOMA levels. In contrast, total sugar,[25] soft drinks/sweetened beverages intake,[74] sugar-sweetened beverage intake[27] and simple

carbohydrate[77] were found to be positively related with insulin, glucose, and HOMA levels. Specific macro- or micro-nutrients such as total energy, fat, saturated fat, protein intakes,[50] energy percentage from fat, protein,[75] and fructose[48] were reported to be positively associated with insulin, glucose, and HOMA levels; calcium[51] and zinc[47] intakes were negatively associated with insulin, glucose, and HOMA levels. All of these studies were based on cross-sectional study designs to evaluate the relationship between certain dietary or nutritional intakes and at least one of the four insulin-related biomarkers (insulin, C-peptide, glucose and HOMA).

In contrast to the significant findings of total energy, fat, saturated fat, protein intakes,[50] energy percentage from fat, protein,[75] fructose[48], calcium[51] and zinc[47] and insulin-related biomarker levels from previous literature among children and adolescents, our study did not find significant associations. There are several methodological considerations that must be considered when interpreting the previous published studies examining associations between nutritional and insulin-related biomarkers. Most of the studies examining dietary factors and insulin-related biomarkers either did not fully adjust for the confounding factors such as physical activity, or examine the interaction terms. In both children and adults, higher physical activity is consistently associated with lower insulin levels,[46,78] therefore, studies that have not controlled for physical activity may generate false positive results. When both pre- and post-menarcheal

girls were included, few studies conducted stratified analyses by menarcheal status. Given that the insulin-related biomarker levels peak during puberty and declines after puberty, not taking into account menarche status may bias the results toward null findings.

The inverse association in the present analyses between dietary fiber intake and C-peptide is consistent with a previous study that found higher dietary fiber intake was associated with lower C-peptide levels in adult women.[79] We did not find a significant association between dietary fiber and fasting insulin level among adolescent girls, although an inverse association among adults was reported by others.[80] C-peptide measured in blood is a good indicator of insulin production,[81] and therefore may more accurately reflect the true association between diet and insulin production. The major source of fiber comes from dietary intake of vegetables, fruits, unrefined whole grains and legumes.[82] Dietary fiber can be soluble which dissolves in water or insoluble which does not dissolve in water. In prospective cohort studies, it has been found that insoluble fiber instead of soluble fiber is consistently associated with decreased risk for type 2 diabetes.[83] Our results (data not shown) also showed that insoluble fiber was significantly associated with decreased C-peptide levels whereas soluble fiber was not significantly associated with C-peptide levels. Unknown mechanisms between the two types of fiber need to be further disentangled.

The relationship between overall starch intake and insulin-related biomarkers among adolescents are still unclear. In our analyses, starch was not shown to be significantly correlated with HOMA in crude and adjusted correlation analyses (p=0.09), but was significantly negatively associated with HOMA in multivariable regression models adjusting for BMIPCT, age and time since menarche. Since our study is the first to examine the effect of dietary starch on the HOMA value in postmenarcheal adolescent girls, this result requires replication. Starch is a type of carbohydrate, with the glucose units arranged either in a straight chain called amylose or a branched chain called amylopectin.[84] Some starch is highly digestible but some, called resistant starch, is unable to be digested in the small intestine and has a similar action as a fiber in the large intestine.[84] Both resistant starch and soluble fiber have been found to decrease insulin-related biomarker levels in adults.[85] We speculate that the uncertain relationship between overall starch intake and HOMA value in our study is because we could not differentiate resistant starch from digestible starch, and other types of carbohydrate such as fiber intake. Therefore, different statistical models may generate different significance levels of associations between starch intake and HOMA.

Sucrose is associated with insulin and HOMA in correlation analysis model, but is only associated with HOMA in forward linear regression model. The non-significant finding between sucrose and C-peptide in either correlation analysis or linear regression model could be due to the stronger associations

between fiber intake and C-peptide. Since our study is also the first to examine the effect of dietary sucrose on the HOMA value in postmenarcheal adolescent girls, these results require replication as well.

We also speculate a plausible reason for the null results on the findings of dietary or anthropometric factors on fasting serum glucose level is that the range of glucose level in our study population was narrow with median (IQR) values at 78.0 (73.5-82.0) mg/dL. These values are at the lower end of the recommended normal range of fasting serum glucose value for adolescents.

Our study has several strengths. First, in evaluating the association between nutritional factors and insulin-related biomarkers, our study had critical information available on time since menarche. Adolescence is characterized by many biological changes such as height, weight, sex maturation, skeletal growth and changes in location and quantity of body fat.[21,22] During childhood, fasting insulin, glucose and HOMA index levels have been consistently shown to peak in normal weight children around the age of menarche,[57,86] and therefore it's critically important to consider time since menarche when examining factors of biomarker levels. We ensured that our current study population included only postmenarcheal girls who had passed the peak of their insulin and HOMA levels during the normal pubertal growth and considered time since menarche as a confounder in analyses. Second, all the measurements of the insulin-related biomarker levels were performed on fasting blood samples, which minimized the measurement bias in our study. Glucose levels are well known to decrease

significantly, particularly in children, during fasting,[87] and therefore fasting-based indices for insulin secretion are recommended for epidemiologic studies.[88] Third, we employed energy-adjusted nutrient intake values to reduce the measurement errors caused by misreporting of dietary energy intake, since a previous study showed the adolescents were more likely to misreport their energy intake.[89] Fourth, we use BMIPCT instead of BMI to account for the considerable variation of BMI with age among children and adolescents.

Out study has some limitations. First, cross-sectional analyses prevent us from making causal inference, though biomarker levels are unlikely to determine dietary intake. Second, participants were selected to the DISC because they had serum LDL cholesterol levels within the normal range at baseline, but that were relatively elevated during age 8-10 years, which may limit the generalizability of our study results. However, when these girls reached age 14-18 years during the last visit, the average LDL cholesterol levels were not elevated. According to the cutoff points by sex and age in adolescents defined from the National Health and Nutrition Education Survey (NHANES),[90] only 33% of our included girls had LDL cholesterol levels above borderline high levels (≥119 mg/dl, 83rd percentile). Last, insulin-related biomarker levels were shown to have racial/ethnic difference; however, most of our study population was reported by parents to be 'white', which may also restrict the generalization of our results.[91,92]

3.6. Conclusions

Our study results indicate that higher fiber or vegetable protein intake is significantly associated with lower fasting serum C-peptide levels, indicative of lower insulin production; higher intake of starch is significantly associated with lower HOMA value in adolescent girls; higher BMIPCT is consistently significantly associated with higher insulin, C-peptide and HOMA values. The association of a high fiber/vegetable protein or a high starch intake with lower levels of insulin-related biomarkers highlights an importance of a dietary pattern that is high in fiber intake, especially soluble fiber and resistant starch. The association of BMIPCT and insulin-related biomarkers emphasizes the effects of adiposity on components of insulin-related biomarkers. This study supports dietary recommendations for children to eat high fiber diets such as whole grains and fruits and vegetables, it also supports the importance of disease prevention starting from childhood and adolescence. Future longitudinal studies are needed to determine the most effective dietary pattern to promote fiber-rich food among children and adolescents, as well as healthy behaviors to control obesity among children and adolescents, which may help decrease risk of chronic diseases later in life.

Table 3.1. Sociodemographic characteristics of adolescent girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit (n=176)

	n[1]	%[2]
Race/ethnicity		
White	161	91.5
Black	8	4.5
Other	7	4.0
Total household income		
<$20,000	17	9.6
$20,000 to <$30,000	29	16.5
$30,000 to <$50,000	65	36.9
$50,000+	64	36.4
Missing	1	0.6
Mother's education		
High school or less	40	22.7
Some college	42	23.9
College degree	44	25.0
Graduate degree	33	18.8

Table 3.1. (cont'd)

Missing	17	9.7
Treatment group		
Intervention	89	50.6
Usual care	87	49.4

[1]Data presented for girls with complete data on diet, menarche, and C-peptide at the last visit.

[2]Percentages may not add up to 100 because of rounding or missing values.

Table 3.2. Anthropometric, nutritional and biomarker characteristics of adolescent girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit (n=176)

Variables[1]	Mean (SD)
Age (y)	16.6 (0.9)
Time since menarche (y)	3.7 (1.2)
Height (cm)	164.1 (6.1)
Weight (kg)	60.7 (11.1)
BMI	22.5 (3.6)
BMI for age (percentile)	59.5 (27.3)
Moderate-to-vigorous Physical Activity (hr/wk)[2]	17.0 (12.3)
Energy/nutrient	Median (IQR)
Energy (kcal)	1572.1 (671.8)
Total fat (% kcal)	28.0 (9.7)
Total protein (% kcal)	14.3 (3.9)
Total carbohydrate (% kcal)	58.0 (10.6)
Animal protein (g/1000 kcal)	23.2 (8.9)
Vegetable protein (g/1000 kcal)	12.2 (4.4)
Lactose (g/1000 kcal)	9.6 (9.8)
Fructose (g/1000 kcal)	15.7 (10.7)
Glucose (g/1000 kcal)	15.4 (9.7)

Table 3.2. (cont'd)

Sucrose (g/1000 kcal)	24.3 (14.1)
Starch (g/1000 kcal)	60.0 (17.9)
Fiber (g/1000 kcal)	6.2 (2.9)
Calcium (mg/1000 kcal)	497.0 (272.3)
Zinc (mg/1000 kcal)	4.7 (1.6)
Iron (mg/1000 kcal)	6.4 (2.1)
Sodium (mg/1000 kcal)	1623.4 (454.8)
Insulin-related biomarker	Median (IQR)
Insulin (uU/mL)[3]	8.2 (6.5, 11.1)
C-peptide (pmol/L)[4]	633.3 (458.3, 854.9)
Glucose (mg/dL)	78.0 (73.5, 82.0)
HOMA[5]	1.6 (1.2, 2.1)

[1]Data presented for girls with complete data on diet, menarche, and at least one of the insulin-related biomarkers at the last visit.

[2]Data were missing for 19 girls.

[3]Data were missing for 18 girls.

[4]Data were missing for 20 girls.

[5]Data were missing for 18 girls.

Table 3.3. Pearson correlations between dietary intakes and Insulin-related biomarker levels among adolescent girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit

	Fasting Insulin (uU/mL) n=158[2,3,4]		C-peptide (pmol/L) n=156[2,3,4]		Glucose (mg/dL) n=176[2,3,4]		HOMA n=158[2,3,4]	
Nutrient[1]	Crude Corr r	Partial Corr r	Crude Corr r	Partial Corr r	Crude Corr r	Partial Corr r	Crude Corr r	Partial Corr r
Total Calories	0.10		0.05		-0.13		0.07	
Total fat (% kcal)	0.06	0.05	**0.17**	**0.17**	-0.05	-0.03	0.05	0.04
Total protein (% kcal)	-0.12	-0.13	0.04	0.05	-0.005	-0.02	-0.11	-0.12
Total carbohydrate (% kcal)	0.02	-0.02	-0.12	-0.12	0.03	0.03	0.02	-0.02
Animal protein (g/1000 kcal)	-0.03	-0.05	0.14	0.14	-0.01	-0.01	-0.03	-0.04
Vegetable protein (g/1000 kcal)	-0.09	-0.09	**-0.21**	**-0.21**	-0.02	-0.04	-0.09	-0.09
Lactose (g/1000 kcal)	-0.02	-0.03	-0.04	-0.03	-0.13	-0.12	-0.04	-0.05
Fructose (g/1000 kcal)	0.07	0.08	0.02	0.02	0.05	0.04	0.07	0.08
Glucose (g/1000 kcal)	0.08	0.10	0.07	0.07	0.13	0.13	0.09	0.11

Table 3.3. (cont'd)

Sucrose (g/1000 kcal)	**0.19**	**0.17**	-0.05	-0.05	0.07	0.08	**0.19**	**0.18**
Starch (g/1000 kcal)	-0.10	-0.12	**-0.18**	**-0.18**	-0.11	-0.11	-0.11	-0.13
Fiber (g/1000 kcal)	-0.08	-0.07	**-0.21**	**-0.21**	0.07	0.05	-0.06	-0.06
Calcium (mg/1000 kcal)	-0.06	-0.08	0.03	0.03	-0.09	-0.09	-0.08	-0.10
Zinc (mg/1000 kcal)	-0.13	-0.14	0.03	0.04	0.03	0.02	-0.11	-0.12
Iron (mg/1000 kcal)	-0.11	-0.13	-0.13	-0.13	0.01	0.004	-0.10	-0.12
Sodium (mg/1000 kcal)	-0.09	-0.09	0.04	0.06	-0.05	-0.07	-0.09	-0.10

[1]Data presented for girls with complete data on diet, menarche, and at least one of the available insulin-related biomarker at the last visit. Data analyses were performed on log transformed insulin, HOMA and nutrients. C-peptide and glucose were not log transformed.

[2]First column is unadjusted.

[3]Second column is adjusted for age, time since menarche, energy intake, and treatment status (additional adjust physical activity, the results are the same).

[4]Correlations that are significant at the $p<0.05$ level are highlighted in bold.

Table 3.4. Adjusted Least squares means (95% CI) of fasting serum C-peptide levels and geometric means (95% CI) of fasting serum insulin levels and HOMA values by quartile of nutrient intake among adolescent girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit

	Quartile of Dietary Intake[3]				
	1	2	3	4	P[4]
C-peptide (pmol/L)[1] (N=156)					
Total fat (% kcal)	604 (516, 691)	658 (566, 750)	640 (549, 730)	675 (589, 760)	0.30
Vegetable protein (g/1000 kcal)	669 (582, 757)	714 (627, 800)	644 (555, 733)	552 (465, 638)	**0.03**
Fiber (g/1000 kcal)	704 (616, 792)	652 (562, 742)	654 (566, 742)	567 (479, 655)	**0.03**
Starch (g/1000 kcal)	686 (597, 775)	703 (614, 791)	589 (505, 674)	604 (517, 692)	0.08
Insulin (uU/mL)[2] (N=158)					
Sucrose (g/1000 kcal)	7.78 (6.74, 8.97)	9.08 (7.87, 10.47)	8.49 (7.41, 9.73)	9.18 (8.05, 10.48)	0.18
HOMA[2] (n=158)					
Sucrose (g/1000 kcal)	1.49 (1.28, 1.74)	1.70 (1.46, 1.98)	1.67 (1.44, 1.93)	1.78 (1.54, 2.05)	0.15

[1]All values are least squares means (95% CI) adjusted for age, time since menarche, energy intake, and treatment status.

Table 3.4. (cont'd)

[2]All values are geometric means (95% CI) adjusted for age, time since menarche, energy intake, and treatment status.

[3]Biomarker values are shown for nutrients where the adjusted association with an insulin-related biomarker in correlation analyses (see Table 3.3) was significant at $p<0.05$.

[4]p is for adjusted biomarker trend across quartiles of dietary intake

Table 3.5. Major predicting factors of insulin-related biomarker levels among adolescent girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit assessed by multiple linear regression analysis

	Final Models (n=176) [1]		
	β	SE	p
Insulin (uU/mL)[2] (N=158)			
Age	0.11	0.04	0.005
Time since menarche	-0.11	0.04	0.0004
BMI for age percentile	0.007	0.001	<0.0001
C-peptide (pmol/L)[3] (N=156)			
BMI for age percentile	2.90	0.76	0.0002
Fiber (g/1000 kcal)	-174.13	60.44	0.005
Glucose (mg/dL)[4] (N=176) None of the variables was selected to significantly predict glucose levels			
HOMA[5,6] (N=158)			
Age	0.12	0.04	0.006
Time since menarche	-0.12	0.03	0.0003
BMI for age percentile	0.008	0.001	<0.0001
Starch (g/1000 kcal)	-0.31	0.15	0.04

[1] Insulin, HOMA & nutrient values logarithmically transformed (ln). C-peptide and glucose are not logarithmically transformed.

Table 3.5. (cont'd)

[2]Initial model for log(Insulin) contained energy (kcal), age, time since menarche, energy intake (kcal), treatment group, BMI-for age percentile, total fat (% kcal), sucrose (g/1000kcal), zinc (mg/1000kcal), mother's education, and batch number. Data were missing for 18 subjects (n=158).

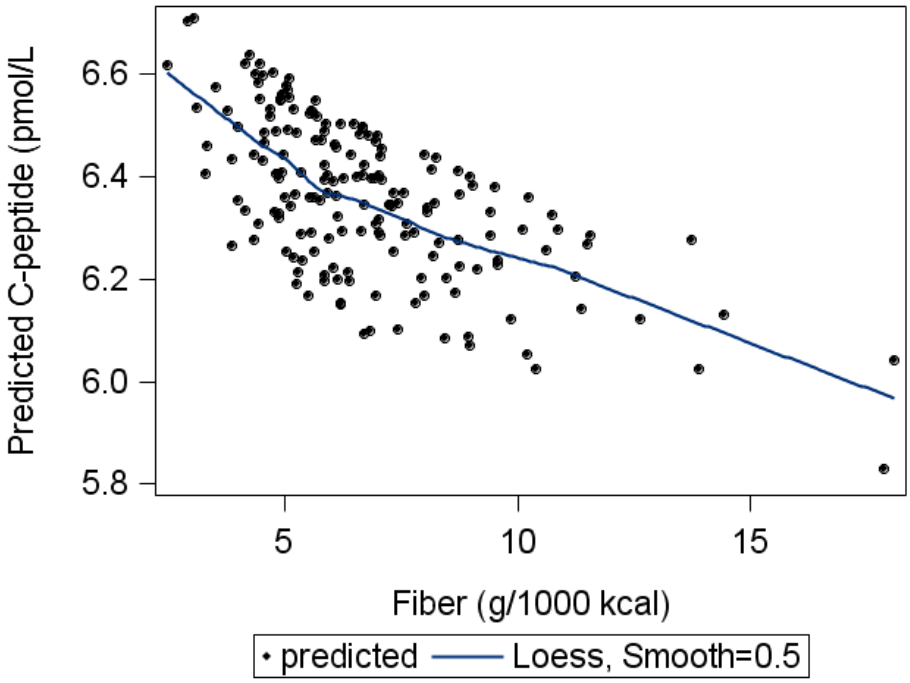
[3]Initial model for C-peptide contained: age, time since menarche, energy intake (kcal), treatment group, BMI-for age percentile, physical activity, mother's education, batch number; fat (% kcal); fiber (g/1000kcal) or vegetable protein (g/1000 kcal), and starch (g/1000kcal). Data were missing for 20 subjects (n=156). Vegetable protein and fiber intake were examined in separate models due to collinearity.

[4]Initial model for glucose contained age, time since menarche, energy intake (kcal), treatment group, BMI-for age percentile, lactose (g/1000kcal), starch (g/1000kcal), and glucose (g/1000kcal).

[5]HOMA was derived from fasting insulin (uU/mL) x fasting glucose (mmol/L)/22.5. Data were missing for 18 subjects (n=158).

[6]Initial model for log(HOMA) contained age, time since menarche, energy intake (kcal), treatment group, BMI-for age percentile, mother's education, batch number, total fat (g/1000kcal), zinc (mg/1000kcal), starch (g/1000kcal), sucrose (g/1000kcal), and glucose (g/1000kcal).

Figure 3.1. Serum C-peptide levels and dietary fiber intake among adolescent girls in the DISC at last visit (n=156).



Note: Predicted C-peptide levels are adjusted for BMI percentile-for-age. Figure was obtained through SGPLOT procedure in SAS 9.3.

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CHAPTER 4: ANTHROPOMETRIC INDICES AND INSULIN-RELATED BIOMARKERS IN GIRLS: RESULTS FROM THE DIETARY INTERVENTION STUDY IN CHILDREN (DISC)

4.1. Abstract

Background: Higher adiposity during childhood has consistently been shown to be associated with reduced risk for both pre- and post-menopausal breast cancer. It has been hypothesized that childhood adiposity affects circulating insulin-related biomarker levels, which in turn affect later breast cancer risk.

Objectives: To prospectively examine associations between baseline childhood anthropometric indices [body mass index (BMI)-for-age percentile (BMIPCT), waist circumference (WC)] as well as height and longitudinal measures of serum insulin-related biomarkers (insulin, C-peptide, glucose) and insulin sensitivity (homeostasis model assessment of insulin resistance [HOMA]) among girls in the Dietary Intervention Study in Children (DISC).

Methods: The present study includes 270 of the 301 girls who participated in the DISC (median follow-up: 7 yrs). Among these girls, 224 had premenarcheal and 203 had postmenarcheal fasting blood samples. Adjusted geometric means of insulin-related biomarkers were calculated within quintiles of baseline anthropometric indices. Baseline anthropometric indices were also modeled as continuous variables to estimate their associations with longitudinal measures of insulin-related biomarkers using multivariable linear mixed-effect models. All adjusted analyses included age, age squared, treatment group, and physical activity.

Results: Premenarcheal girls in the lowest quintile of baseline BMIPCT had the lowest

geometric means of insulin (lowest vs. highest=6.56 vs 9.77 uU/mL), C-peptide (lowest vs. highest=330.73 vs. 524.65 pmol/L), and HOMA (lowest vs. highest= 1.25 vs.1.41). Both pre- and post-menarcheal girls in the lowest quintile of baseline WC had the lowest geometric means of insulin [lowest vs. highest=6.44 vs. 9.63 uU/mL (premenarche) and 8.75 vs. 11.19 uU/mL (postmenarche)], C-peptide [lowest vs. highest=302.97 vs. 505.71 pmol/L (premenarche) and 571.73 vs. 665.81 pmol/L (postmenarche)], and HOMA [lowest vs. highest=1.24 vs. 1.89 (premenarche) and 1.71 vs. 2.20 (postmenarche)]. Postmenarcheal girls in the lowest quintile of baseline height had the lowest geometric means of C-peptide (lowest vs. highest=509.12 vs. 766.10 pmol/L), and glucose (lowest vs. highest=77.79 vs. 82.34 mg/dL).

Conclusions: Results show higher baseline BMIPCT in girls is associated with higher levels of insulin-related biomarkers only pre-menarche, higher baseline WC is associated with higher insulin-related biomarkers both pre- and post-menarche, while higher baseline height is associated with higher insulin-related biomarkers only post-menarche.

4.2. Introduction

A growing body of literature suggests that childhood exposures may impact the development of chronic diseases. In particular, childhood adiposity has been found to be associated with decreased breast cancer risk in pre- and post-menopausal women.[1-3] It has been suggested that earlier adiposity, perhaps before puberty, had stronger association with later breast cancer risk than later adiposity.[4] On the other hand, adult obesity has been found to be positively associated with postmenopausal, but negatively

associated with premenopausal breast cancer risk.[5,6] Childhood adiposity has been inversely associated with adolescent peak height velocity,[7] and similarly childhood growth index-height is positively associated with childhood adiposity.[8] Epidemiology studies have suggested greater childhood height, growth rate, and final height are generally associated with increased breast cancer risk.[9-12] Both adiposity and height are associated with insulin system. For example, bone growth is stimulated by insulin as well as insulin growth factors;[13] excess adiposity can lead to insulin resistance where cells fail to adequately metabolize glucose as a result of unresponsiveness to normal circulating insulin levels.[14,15] These associations suggest a potential role of insulin-related biomarkers as mediators of the association between childhood adiposity or growth and breast cancer.

Biologic mechanisms underlying the observed association of childhood adiposity or growth with adult breast cancer risk are currently unknown. Insulin-related biomarkers and their associated insulin-like growth factors, however, have been hypothesized to be hormonal mediators underlying the puzzling association, since these hormones and growth factors promote growth during puberty.[16,17] In adults, the associations of insulin-related biomarker levels with breast cancer have been studied extensively with inconclusive results.[18-32] A meta-analysis of five case-control studies published in 2008 found upper categories of insulin/ C-peptide were associated with increased breast cancer risk; however, no association was found from prospective

cohort studies identified in this meta-analysis.[33] In children, there has been no study examining childhood insulin-related biomarkers and breast cancer. However, insulin-related biomarkers are related to adiposity with higher adiposity having higher levels of insulin-related biomarkers, perhaps due to increased insulin secretion from pancreatic β-cells in response to peripheral insulin resistance.

A few studies have examined the association between childhood anthropometric indices with insulin-related biomarker levels. Most, although not all, anthropometric factors examined were found to be significantly associated with insulin-related biomarkers. Generally, higher general adiposity measured by BMI and central adiposity measured by WC were positively associated with insulin-related biomarker levels among healthy children and adolescents.[34-38] Results from the Bogalusa Heart Study found that childhood height was associated with increased insulin levels.[8] However, information on anthropometric predictors of repeated fasting serum levels of insulin-related biomarkers measured at multiple times throughout pubertal development among healthy children and adolescents is limited. Peak levels of fasting insulin or insulin resistance have been observed to occur at menarche.[16,39,40] Few previous studies have incorporated information on menarche status, which could significantly influence insulin-related biomarker levels.[41-47]

The purpose of this study was to prospectively examine associations between baseline anthropometric indices and longitudinal measures of insulin-related biomarkers

(Insulin, C-peptide, glucose, HOMA) in girls participating in the Dietary Intervention Study in Children (DISC) by menarcheal status.

4.3. Methods

Study Design of the DISC and the Insulin-Related Biomarkers Study (IRBS)

The DISC was a multi-center randomized controlled clinical trial to assess the safety and efficacy of a low fat dietary intervention to decrease serum low-density lipoprotein (LDL) cholesterol in healthy children. It was initiated in 1988 among 663 (301 girls) pre-pubertal children 8-10 years of age, who had an elevated serum LDL cholesterol level (80th-98th percentile for age and sex).[48] The design of DISC has been previously described.[49-55] In brief, children were randomized to either a dietary intervention group or usual care control group at one of six clinical centers (Children's Hospital, New Orleans, LA; Johns Hopkins University Hospital, Baltimore, MD; Kaiser Permanente Center for Health Research, Portland, OR; University of Medicine and Dentistry of New Jersey, Newark, NJ; Northwestern University Medical School, Chicago, IL; and University of Iowa Hospital and Clinics, Iowa, IA) between 1988 and 1990.[55] The planned intervention was carried out until 1997 when the girls were on average 16.7 years old and were followed for a median period of 7 years. The DISC dietary behavioral intervention successfully promoted adherence to a reduced fat diet (<28% kcal from total fat) in children during puberty. Differences in dietary intake were most pronounced between girls in the intervention vs. usual care group at year 3, and there was generally no dietary intake difference at the last visit. The DISC dietary intervention did not lead to detectable differences in the insulin-related biomarkers at any time

period. The National Heart, Lung, and Blood Institute (NHLBI) sponsored the DISC study and an NHLBI-appointed independent data and safety monitoring committee provided oversight.

The Insulin-Related Biomarkers Study (IRBS) was an ancillary study using data collected from the DISC. It was approved by the DISC steering committee, the Department of Defense, the Michigan State University and the Fox Chase Cancer Center human subject review boards. The investigators at the DISC blood repository Biotech Research Laboratories (BBI) provided blood serum samples and the Maryland Medical Research Institute (MMRI) (Baltimore, MD) provided questionnaire information from the DISC study to IRBS researchers. IRBS researchers performed insulin-related biomarker analyses and integrated this information with existing DISC data.

The current analyses were focused on the association between anthropometric indices and insulin-related biomarker (insulin, C-peptide, glucose and HOMA) levels among adolescent girls who attended the DISC and had serum samples available. Because there were no differences of insulin-related biomarker levels and anthropometric indices between the treatment groups at any time period, data from the intervention and control groups were combined and intervention status was included as a covariate in adjusted analyses.

Study Population

There were 301 girls participating in the DISC study. These girls were enrolled in the DISC if they were 8-10 years old with normal weight and elevated LDL level, had no major illness or were not taking medications that could affect their growth or serum lipid

levels.[55] Throughout the DISC, none of the girls took cholesterol-lowering medications.[55]

Figure 4.1 shows the number of girls who attended the DISC and who had each of the four insulin-related biomarkers (Insulin, C-peptide, Glucose and HOMA) by visit. Measures of insulin-related biomarker levels (Insulin, C-peptide, glucose, and HOMA) were available for 270 girls with a total of 579 fasting blood samples collected on at least one of the four visits: baseline, years 3, 5 and last visit. Girls who were pregnant or had taken oral contraceptives in the past 4 months or had a serious illness were excluded. Among these 270 girls, 224 girls had premenarcheal fasting blood samples with 151 girls having one visit, 66 girls having two visits and 6 girls having three visits data; and 203 girls had postmenarcheal fasting blood samples, with 139 girls having one visit, 54 girls having two visits and 10 girls having three visits data.

Data Collection

Data were collected at baseline, years 3, 5, and at the last visit for the duration of the study. Basic demographic information including age, and lifestyle information including physical activity, smoking status, alcohol consumption, dietary intake as well as medical conditions and use of medications were collected at every visit. Dietary intake was assessed through three non-continuous 24-hour food recalls over a 2-week period before each visit where blood samples were drawn. Participants had physical examinations at each visit to evaluate their Tanner staging and sexual development. Information about use of oral contraceptives and pregnancy were collected starting from the year 3 visit. Trained interviewers were blinded to participants' treatment assignment.

Anthropometric Measurements

Anthropometric indices were measured by trained clinical staff who were certified and recertified by the DISC study. Standing height was measured as the perpendicular distance between the top of the head and the bottom of the feet with stadiometers provided by the University of Iowa. Weight was measured using either electronic or beam balancing scales that were calibrated regularly. Height and weight were measured twice, and if the difference was within allowed tolerances (0.5 cm for height and 0.2 kg for weight), the two values were averaged. If difference in two measurements was larger than allowed, the measurement was taken again and the two closest values were averaged. BMI was calculated as weight (kg) divided by height^2 (m). BMIPCT were computed based on Centers for Disease Control growth charts 2000.[56] WC was measured at the mid-point between the lower border of the 10th rib and the level of the top of the iliac crest, perpendicular to the long axis of the trunk. Hip circumference (HC) was measured as the widest point around the hip. Waist-to-hip ratio (WHR) was calculated as WC divided by HC.

Insulin-related Biomarker Measurement

Overnight fasting blood samples were drawn by venipuncture in the morning at all visits. After blood was completely clotted, samples were centrifuged and serum was aliquoted and stored at around –70 °C or colder until it was thawed for analyses for hormone, lipid and micronutrient levels for the DISC.[53] Serum samples for the insulin-related biomarker measurements had been thawed twice to allow removal of additional aliquots of serum, and were refrozen immediately at around –70 °C or colder.

Insulin is a key regulator of blood glucose metabolism and blood glucose is the major factor that regulates insulin secretion.[57] Insulin and C-peptide are both cleavage products from proinsulin.[58,59] C-peptide has a longer half-life compared to insulin and thus is a better long-term indicator for insulin secretion.[60] Glucose, insulin and C-peptide assays were conducted at the Clinical and Epidemiologic Research Laboratory at Boston Children's Hospital (Dr. Nader Rifai's laboratory). Glucose was measured using Roche Diagnostics reagents (Indianapolis, IN). Ultra-sensitive electrochemiluminescence immunoassay through Elecsys technology (Roche Diagnostics, Indianapolis, IN) was used for C-peptide analyses and ultra-sensitive ELISA assay (ALPCO, Windham, NH) was used for insulin analyses. The coefficients of variations (CVs) within-visit were estimated from quality control samples. The external interassay CV for glucose was 3%; the internal CVs for insulin and C-peptide were 4% and 3% respectively. HOMA was derived from fasting insulin (uU/mL) x fasting glucose (mmol/L)/22.5.[61] The use of HOMA as a proxy measure for insulin resistance has been validated in both adults and adolescents.[62,63]

Menstrual Status Assessment

To determine the timing of the postmenarcheal girls' menstrual cycle at the time of the blood collection, menstrual cycle calendars were given to postmenarceal girls and they were asked to complete the information about their menstrual cycle 6 weeks before and 6 weeks after their blood collections.

Statistical Methods

We conducted longitudinal analyses using baseline anthropometric indices, when the girls were 8-10 years old, to predict insulin-related biomarker levels during the follow-up period.[64] The insulin-related biomarker values were examined according to quintiles of baseline BMIPCT, WC and height. In order to assess potential linear cross-sectional associations of anthropometric factors (BMIPCT, WC, and height) and insulin-related biomarkers (insulin, C-peptide, Glucose, and HOMA), crude and adjusted Spearman rank correlation analyses by visit were computed. There is no requirement for normal distribution to conduct Spearman rank correlation and all the correlation analyses were conducted adjusting for energy intake (kcals), age (for all girls) and time since menarche (for postmenarcheal girls).

Median age for menarche was estimated by the product-limit method (Kaplan-Meier method) taking into account censoring of girls who had not had a first menstrual period (menarche) at their last visits, as well as different entry ages into the study. The log-rank test was used to compare the age for menarche according to different anthropometric groups.

For longitudinal data analyses, after reviewing the sample distributions of all the insulin-related biomarkers in order to obtain approximate normality, biomarkers were log transformed. Residual diagnostics were also reviewed to check for influential observations in regression models. To compare the distributions of insulin-related biomarkers across age, Kernel smoothing was used to plot the distribution of the biomarkers using SAS procedure “PROC GPLOT” shown in **Figure 4.2**.

To assess the associations between the baseline anthropometric factors and longitudinal insulin-related biomarkers by pre- and post-menarche status, mixed linear

regression models using SAS procedure “PROC MIXED” were used. These models were used to calculate the geometric mean concentrations and 95% confidence intervals (95% CI) of insulin-related biomarkers during follow-up according to quintiles of BMIPCT at baseline. These analyses accounted for repeated anthropometric and biomarker measurements within the same girl in follow-up visits. The mixed effects model has the advantage of exploring the correlation between observations across years and will generate an estimate through reducing variance. The PROC MIXED procedure appropriately handles missing values.[65] Trend tests were performed on continuous anthropometric measures to estimate percentage changes in insulin-related biomarkers during follow-up. As shown in **Figure 4.2**, age at the time of blood collection was included in all models and it was included as a quadratic term in all models to take into account the nonlinear associations between age and insulin-related biomarkers levels. Other covariates included in the mixed regression models include physical activity (hours of moderate and intense activity/week in the past year), treatment group (intervention or usual care), baseline height (if the exposure of interest is one of the adiposity measures) or baseline BMIPCT (if the exposure of interest is height).

Interaction terms between age and baseline anthropometric measures (BMIPCT, WC, height) were included to examine whether associations between these baseline anthropometric measures and insulin-related biomarkers varied by age during follow-up. Interaction between baseline anthropometric indices and treatment group were examined as well.

Sensitivity analyses were also conducted to examine associations among postmenarcheal girls between those in the follicular phase (days 15-33 before/on the

day of onset of the next menses), and luteal phase (days 1-14 before the onset of next menses) of the menstrual cycle. Previous investigations suggested that the effect of adiposity on indexes of insulin secretion differs by race/ethnicity,[66] therefore, sensitivity analyses were conducted subsetting analyses to 'white' girls (90.2% of the sample) only.

All data analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) with $p<0.05$ as the significance level.

4.4. Results

Table 4.1 describes the baseline characteristics of the 270 girls with insulin-related biomarkers data according to quintiles of baseline BMIPCT. The mean values of anthropometric indices including weight, WC and height increased with higher BMIPCT quintiles. The Spearman correlation coefficients (p values) between baseline BMIPCT with WC, height, weight and WHR were 0.84 ($p<0.0001$), 0.27 ($p<0.0001$), 0.82 ($p<0.0001$) and 0.22 ($p<0.0001$). There was also a significant difference of physical activity across BMIPCT quintiles. However, there was no statistically significant difference in age, treatment group, race/ethnicity, annual household income across baseline BMIPCT quintiles. Median age at menarche decreased with an increase of BMIPCT quintile, indicating that girls with higher baseline adiposity had earlier age at menarche ($p=0.0011$).

Geometric means of insulin-related biomarkers among premenarcheal and postmenarcheal girls according to quintiles of adiposity measurements are shown in **Table 4.2** (BMIPCT) and **Table 4.3** (WC). The geometric means of insulin-related biomarkers concentrations across quintiles of BMIPCT and WC, were generally similar in premenarcheal girls but different in postmenarcheal girls. For the associations

between BMIPCT and insulin-related biomarkers, premenarcheal girls in the lowest quintile of baseline BMIPCT had the lowest geometric means. In continuous analyses, each 10% increment increase in baseline BMIPCT was associated with a 3.9% increase (95% CI: 2.1%, 5.7%) in insulin (P_{trend}<0.0001), a 4.2% increase (95% CI: 1.4%, 7.1%) in C-peptide (P_{trend}=0.003), a 0.5% increase (95% CI: 0.0007%, 1.1%) in glucose (P_{trend}=0.05) and a 4.4% increase (95% CI: 2.4%, 6.5%) in HOMA (P_{trend}<0.0001). No significant associations were observed for baseline BMIPCT and insulin-related biomarkers among postmenarcheal girls. In addition, there was a statistically significant interaction between BMIPCT and age in predicting insulin levels among premenarcheal girls (p<0.0001), suggesting that the increase in insulin levels with higher BMIPCT was greater at older ages among premenarcheal girls.

For the associations between WC and insulin-related biomarkers, both premenarcheal and postmenarcheal girls in the lowest quintile of baseline WC had the lowest geometric mean of insulin, C-peptide and HOMA. Among premenarcheal girls, each 1 cm increment in baseline WC was associated with a 2.6% increase (95% CI: 1.6%, 3.6%) in insulin (P_{trend}<0.0001), a 3.1% increase (95% CI: 1.6%, 4.7%) in C-peptide (P_{trend}=0.0001), a 2.8% increase (95% CI: 0.0007%, 1.1%) in HOMA (P_{trend}<0.0001). Among postmenarcheal girls, each 1cm increment in baseline WC was associated with a 2.0% increase (95% CI: 0.8%, 3.2%) in insulin (P_{trend}=0.0009), a 1.5% increase (95% CI: 0.1%, 2.8%) in C-peptide (P_{trend}=0.03), a 2.1% increase (95% CI: 0.8%, 3.4%) in HOMA (P_{trend}=0.002). There was no statistically significant association between WC and glucose for both pre- and post-menarcheal girls. There was no statistically significant interaction between age and WC in predicting any of the insulin-

related biomarkers levels, indicating that the increase in insulin-related biomarkers with higher BMIPCT was similar across the examined ages.

Table 4.4 shows the geometric means of insulin-related biomarkers according to quintiles of baseline height. The geometric means of insulin-related biomarker concentrations across quintiles of baseline height were different between pre- and post-menarcheal girls. There were no statistically significant linear trends for any of the insulin-related biomarkers among premenarcheal girls. Among postmenarcheal girls however, each 1 cm increment in baseline height was associated with a 1.9% increase (95% CI: 0.7%, 3.1%) in C-peptide (P_{trend}=0.002) and a 0.26% increase (95% CI: 0.08%, 0.44%) in glucose (P_{trend}=0.006). Similar to the interaction between WC and age, no significant interaction between height and age was observed.

In secondary analyses, the pattern of insulin-related biomarkers across baseline WHR, another adiposity index, was similar to the pattern of insulin-related biomarkers across baseline WC for postmenarcheal girls but there was no statically significant linear trend found for premenarcheal girls (data not shown).

Results from cross-sectional Spearman correlation analyses that examine the associations between BMIPCT and WC at the time of blood draw with insulin-related biomarkers concentrations were similar to results from longitudinal analyses (data not shown). In addition, little evidence for an effect of time in menstrual cycle (ie. luteal phase or follicular phase) on the associations between baseline anthropometric indices and insulin-related biomarkers was observed (data not shown).

For analyses among white girls only, results from examination of associations between anthropometric indices (BMIPCT, WC) and insulin-related biomarkers were

very similar as results from participants combining these two treatment groups. For secondary analyses by treatment group, results for the association between baseline BMIPCT and insulin-related biomarkers during follow-up in usual care group was very similar to combined results. There was no significant association between baseline BMIPCT and insulin-related biomarkers in the intervention group (data not shown); decreased significance levels among girls in the intervention group may indicate the effect of reduced sample size on the analyses. In addition, among premenarcheal girls there were statistically significant interactions between treatment and associations of BMIPCT with insulin and HOMA levels. Among postmenarcheal girls, there was also a statistically significant interaction between treatment and BMIPCT with C-peptide level among postmenarcheal girls. There was also a statistically significant interaction between treatment and WC in predicting premenarceal insulin levels (data not shown). No interaction between treatment group and height was observed.

4.5. Discussion

In general, our results suggest that higher adiposity at baseline, between ages 8-10 years, is associated with higher levels of insulin-related biomarkers in girls. BMIPCT at baseline had stronger associations with insulin-related biomarkers among premenarcheal girls than postmenarcheal girls. WC at baseline was significantly associated with insulin-related biomarkers in both premenarcheal and postmenarcheal girls. Height at baseline was not associated with insulin-related biomarkers among premenarcheal girls but was associated with significantly increased levels of C-peptide and glucose among postmenarcheal girls. Therefore, different baseline anthropometric indices may have different associations with insulin-related biomarkers based on

menarche status. WC at age 8-10 years is more consistently positively associated with insulin-related biomarkers throughout pubertal development than BMIPCT and height.

We had hypothesized that there might be positive associations between BMIPCT and insulin-related biomarkers in postmenarcheal girls as well. A possible reason why we did not observe these associations in our findings is that general adiposity measured by BMIPCT may affect insulin-related biomarker levels to a less extent after menarche. Sensitivity analyses examining the original baseline BMI and insulin-related biomarkers showed similar results as the associations between BMIPCT and insulin-related biomarkers among pre-menarcheal girls. However, among postmenarcheal girls, BMI at baseline was significantly associated with HOMA while BMIPCT at baseline wasn'significantly associated with HOMA. The original BMI measurement did not account for age-related differences, therefore, the discrepancy of the results between BMI vs BMIPCT in association with insulin-related biomarkers is expected.

Although our study found different associations between BMIPCT with insulin-related biomarkers among pre- vs. post-menarcheal girls, WC was consistently associated with insulin-related biomarker levels regardless of menarche status. This may suggest that WC is a more reliable anthropometric measure than BMI in predicting the effect of adiposity on the insulin system and health risk, which has also been suggested by several studies in adults.[67-69] A proposed mechanism for this could be that higher exposure of the liver to abdominal adipocytes will result in lower hepatic insulin clearance.[70] In cross-sectional analyses of adolescents, however, BMI and WC were found to have similar correlations with serum insulin and HOMA levels among

participants in the National Health and Nutrition Examination Survey (NHANES).[71] A cross-sectional study in Australia also did not find WC to be a better predictor of insulin-related metabolic risk factors compared to BMI among Grade 10 students in Sydney.[72]

Our study showed the associations between height and insulin-related biomarkers differed by menarche status. Specifically, we found a significant association between height at baseline and both C-peptide and glucose among postmenarcheal girls, but did not observe a significant association between height and either insulin or HOMA in these girls. We also did not observe significant associations between height and any of the insulin-related biomarkers examined in premenarcheal girls. Three studies that examined the association between height with at least one of the insulin-related biomarkers in adolescent girls and all consistently reported a positive association.[73-75] Those previous studies were either focused on insulin resistance measured by HOMA or insulin levels as outcome. The reason why we only observed positive associations between height and C-peptide or glucose among postmenarcheal girls is perhaps because postmenarcheal girls have dramatically increase in energy intake and growth spurt, which drive big changes of the glucose and C-peptide levels. In addition, C-peptide is a better indicator for insulin secretion, given it has longer half life time.[60]

Although we could not directly examine the association between childhood adiposity and breast cancer, our findings on their associations with insulin-related biomarkers during puberty may shed light on the biological mechanisms of the inverse association between childhood adiposity and breast cancer. Childhood adiposity reflects

the energy balance status and energy balance-related factors during childhood have been hypothesized to be associated with later breast cancer risk through the insulin pathway. As our data showed, childhood adiposity could well affect insulin levels throughout puberty. It has been hypothesized that insulin-related biomarkers could re-program mammary morphogenesis during puberty, a critical time in breast development. These biomarkers could also indirectly stimulate mammary gland differentiation through decreasing sex-hormone binding globulin (SHBG) levels and increasing bioavailable estrogen levels.[34] Early breast differentiation could then reduce the sensitivity of the breast to malignant carcinogenic exposures in later life, providing life-long protective effects to the breast against carcinogenesis.[54,76]

Similarly, our study may also shed light on the biological mechanisms of the positive association between childhood height and breast cancer risk. A recent study from the Women's Circle of Health Study found greater childhood heights during childhood or adolescence were associated with increased risk for breast cancer among White women. However, higher stature at age 7-8 years was found to be associated with decreased breast cancer risk among African American women.[77] Our study specifically examined height at age 8-10 years in association with insulin-related biomarker levels, and the null findings between height at age 8-10 years with insulin or HOMA in both premenarcheal and postmenarcheal girls but significant findings with C-peptide and glucose in postmenarcheal girls may contribute to our understanding of the height influence on these biomarkers, and the prolonged exposure to these biomarkers in the effect of breast development.

Our study has several strengths. First, similar to other studies with longitudinal design, our study has blood samples of repeated measurements, which allowed us to examine the associations between anthropometric indices with insulin-related biomarkers over time. Second, we had information on girls' menarche status and day of the menstrual cycle, these factors could affect insulin-related biomarkers levels since levels of fasting insulin, glucose and HOMA index are known to vary by age relative to menarche status.[42,78]

Our study has some limitations that also warrant mention. The HOMA index is not as sensitive as the gold standard measurement (hyperinsulinemic-euglycemic clamp[79]) for assessing insulin resistance. Our study is also limited by the decreased generalizability because all the girls included in the study had elevated LDL cholesterol and were in the 5th-90th percentiles for weight-for-height when they were first enrolled into the study at baseline. When these girls reached age 14-18 years during the last visit however, the average LDL cholesterol levels were not elevated. Most of our study participants were also white and had higher socioeconomic status. In addition, we did not include BMIPCTs at the time of blood collection together with BMIPCT at baseline because they are too highly correlated with each other.

In conclusion, our findings suggest that that higher BMIPCT in girls at baseline is associated with higher levels of insulin, C-peptide and HOMA premenarche, higher WC is associated with higher levels of insulin, C-peptide and HOMA both pre- and post-menarche, while height is associated with higher levels of C-peptide and glucose postmenarche. Additional studies are needed to examine how these insulin-related

biomarkers may possibly modify breast tissue development and potentially influence later breast cancer risk.

Figure 4.1. Flowchart for the number of girls who attended the Dietary Intervention Study in Children (DISC) and who had available each of the four insulin-related biomarkers (Insulin, C-peptide, Glucose and HOMA) at each visit.

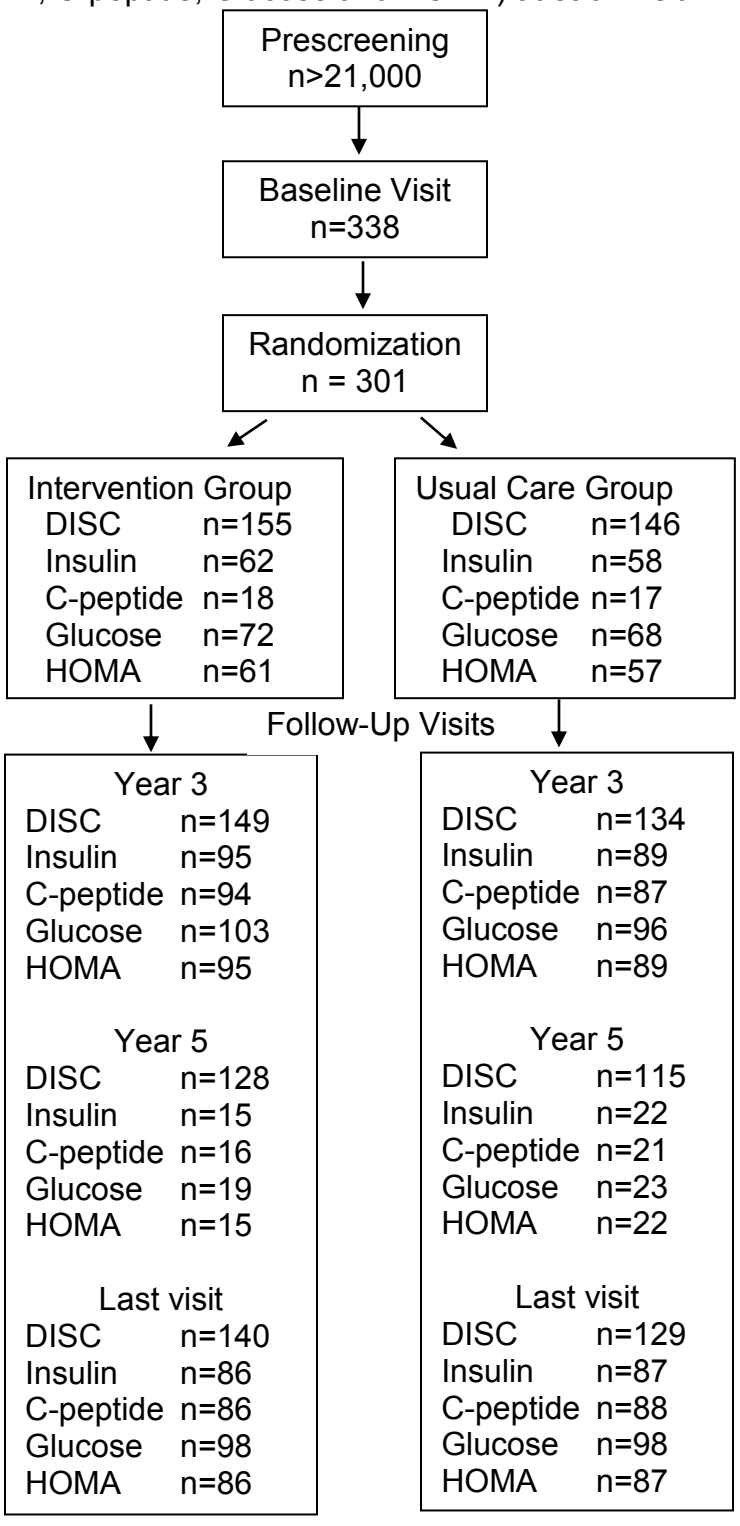

Table 4.1.Characteristics of girls who participated in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study according to quintile for BMI-for-age percentile at baseline (ages 8-10 y; n=270 girls)

	Quintile for BMI-for-age percentile (median)					P
	Q1 (14.62) n = 54	Q2 (37.86) n = 54	Q3 (58.38) n = 54	Q4 (77.85) n = 54	Q5 (90.87) n = 54	
BMI-for-age range (%)	2.99–24.51	25.36–47.94	48.02–69.51	69.72–84.54	84.55-96.80	
Means (SD)						
Age, y	9.19 (0.65)	9.07 (0.63)	8.99 (0.57)	9.06 (0.61)	9.01 (0.60)	0.47[a]
Weight, kg	25.17 (2.48)	27.26 (2.65)	29.68 (3.20)	32.92 (3.79)	38.22 (5.36)	<0.0001[a]
Waist circumference, cm	50.46 (2.69)	53.17 (2.97)	55.13 (3.01)	58.99 (3.79)	64.22 (4.99)	<0.0001[a]
Height, cm	131.55 (5.34)	131.58 (5.48)	132.70 (6.08)	133.99 (6.45)	136.16 (6.28)	0.0002[a]
Moderate and intense physical activity, h/wk	12.59 (9.46)	14.95 (11.61)	8.49 (6.70)	8.78 (7.47)	10.51 (8.81)	0.0007[a]
Percentage						
Treatment group						
Intervention	44.44	53.70	59.26	50.00	48.15	0.60[b]
Usual care	55.56	46.30	40.74	50.00	51.85	
Race / ethnicity						
White	90.74	92.59	96.30	87.04	90.74	0.72[b]
Black	7.41	5.56	1.85	7.41	3.70	
Other	1.85	1.85	1.85	5.56	5.56	
Annual household income						
<$20,000	5.56	9.26	12.96	5.56	9.26	0.20[b]
$20,000-$49,999	51.85	53.70	37.04	70.37	51.85	
>=$50,000	42.59	35.19	46.30	24.07	37.04	
Missing	0.00	1.85	3.70	0.00	1.85	
Age at menarche, y, median[c,d] (95% CI)	13.40 (13.07-13.90)	12.88 (12.52-13.26)	12.68 (12.24-13.10)	12.56 (12.37-12.84)	12.66 (12.37-12.79)	0.0011[e]

Table 4.1. (cont'd)

Note: except for age at menarche, all characteristics were assessed at baseline.

[a.] P value from ANOVA.

[b.] P value from Chi-square test, p-value is computed after dropping the missing values in each category.

[c.] Median menarcheal age was estimated for 11 girls who were pre-menarche at the end of study (SAS LIFETEST was conducted to determine the median).

[d.] Eight girls reached menarche during the study but with unknown date, and 8 girls were pre-menarche and dropped out of the study before last visit.

[e.] P value from Log-Rank test.

Table 4.2.Geometric mean serum insulin-related biomarker concentrations among premenarcheal and postmenarcheal girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study according to quintile of BMI-for-age percentile at baseline (n=270 girls)

	Baseline Quintile for BMI-for-age percentile (median) [a]					P_{trend} [*]
	Q1 (14.62)	Q2 (37.86)	Q3 (58.38)	Q4 (77.85)	Q5 (90.87)	
	Geometric Mean (95% CI)					
Premenarcheal[b]						
n	48	48	44	42	42	
Insulin (uU/mL)	6.56 (5.92-7.27)	7.39 (6.66-8.19)	6.97 (6.26-7.76)	7.28 (6.46-8.20)	9.77 (8.74-10.93)	**<0.0001**
C-peptide (pmol/L)	330.73 (282.47-387.25)	400.40 (337.43-475.13)	313.58 (265.00-371.08)	398.11 (321.22-493.39)	524.65 (443.62-620.48)	**0.003**
Glucose (mg/dL)	76.94 (74.67-79.28)	77.27 (74.89-79.72)	77.52 (75.11-80.00)	78.15 (75.47-80.92)	81.00 (78.29-83.78)	0.05
HOMA	1.25 (1.11-1.40)	1.41 (1.25-1.58)	1.34 (1.19-1.51)	1.41 (1.23-1.61)	1.94 (1.71-2.21)	**<0.0001**
Postmenarcheal[c]						
n	43	37	40	42	41	
Insulin (uU/mL)	9.17 (7.96-10.57)	8.60 (7.49-9.88)	8.07 (7.03-9.24)	8.59 (7.55-9.76)	10.82 (9.45-12.38)	0.12
C-peptide (pmol/L)	584.16 (500.05-682.42)	580.37 (496.17-678.86)	583.76 (500.59-680.76)	531.56 (459.32-615.17)	659.90 (568.80-765.60)	0.61
Glucose (mg/dL)	79.27 (77.36-81.23)	80.27 (78.31-82.27)	77.11 (75.28-78.99)	77.58 (75.85-79.35)	79.20 (77.36-81.09)	0.55
HOMA	1.80 (1.55-2.10)	1.70 (1.47-1.98)	1.53 (1.32-1.77)	1.64 (1.43-1.88)	2.13 (1.84-2.46)	.17

[*] For adjusted biomarker trend across quintiles of BMI-for-age percentile at baseline.

[a.] Geometric means adjusted for age, age x age, treatment group, baseline height and physical activity.

Table 4.2. (cont'd)

[b.] Among 224 girls with premenarcheal blood samples at baseline, year3, year5 and last visit.

[c.] Among 203 girls with postmenarcheal blood samples at baseline, year3, year5 and last visit.

Table 4.3.Geometric mean serum insulin-related biomarker among premenarcheal and postmenarcheal girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study according to quintile of waist circumference (cm) at baseline (n=270 girls)

	Quintile of Waist Circumference at baseline (median, cm) [a]					P_{trend} [*]
	Q1 (49.55) n=54	Q2 (52.73) n=54	Q3 (55.65) n=54	Q4 (58.80) n=54	Q5 (64.50) n=54	
	Geometric Mean (95% CI)					
Premenarcheal[b]						
n	50	46	46	42	40	
Insulin (uU/mL)	6.44 (5.79-7.16)	7.24 (6.50-8.08)	6.91 (6.18-7.71)	7.64 (6.78-8.60)	9.63 (8.48-10.95)	**<0.0001**
C-peptide (pmol/L)	302.97 (255.42-359.36)	366.35 (302.46-443.73)	341.97 (283.66-412.26)	423.02 (343.92-520.31)	505.71 (412.12-620.56)	**0.0001**
Glucose (mg/dL)	77.02 (74.68-79.43)	77.94 (75.45-80.51)	77.24 (74.79-79.77)	79.56 (76.82-82.40)	78.10 (75.19-81.12)	0.45
HOMA	1.24 (1.10-1.39)	1.39 (1.23-1.57)	1.29 (1.14-1.47)	1.50 (1.31-1.72)	1.89 (1.63-2.18)	**<0.0001**
Postmenarcheal[c]						
n	42	39	40	43	39	
Insulin (uU/mL)	8.75 (7.53-10.17)	7.60 (6.62-8.72)	8.75 (7.64-10.03)	8.88 (7.83-10.06)	11.19 (9.66-12.97)	**0.0009**
C-peptide (pmol/L)	571.73 (486.47-671.95)	592.24 (506.68-692.25)	518.54 (444.29-605.20)	587.46 (509.23-677.72)	665.81 (564.75-784.96)	**0.03**
Glucose (mg/dL)	79.04 (77.03-81.11)	78.79 (76.86-80.78)	78.47 (76.58-80.40)	78.59 (76.81-80.41)	79.33 (77.29-81.42)	0.82
HOMA	1.71 (1.45-2.01)	1.47 (1.27-1.71)	1.70 (1.47-1.97)	1.73 (1.51-1.98)	2.20 (1.88-2.58)	**0.0015**

[*] For adjusted biomarker trend across quintiles of height percentile at baseline.

Table 4.3. (cont'd)

a. Geometric means adjusted for age, age x age, treatment group, baseline height and physical activity.
b. Among 224 girls with premenarcheal blood samples at baseline, year3, year5 and last visit.
c. Among 203 girls with postmenarcheal blood samples at baseline, year3, year5 and last visit.

Table 4.4.Geometric mean serum insulin-related biomarker concentrations among premenarcheal and postmenarcheal girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study according to quintile of height (cm) at baseline (n=270 girls)

	Quintile of Height at baseline (median, cm) [a]					P_{trend} [*]
	Q1 (124.65) n=54	Q2 (130.33) n=55	Q3 (133.05) n=55	Q4 (136.50) n=52	Q5 (141.30) n=54	
	Geometric Mean (95% CI)					
Premenarcheal[b]						
n	51	44	42	45	42	
Insulin (uU/mL)	7.06 (6.40-7.80)	6.35 (5.69-7.10)	7.72 (6.91-8.63)	6.96 (6.18-7.83)	7.87 (7.01-8.83)	0.078
C-peptide (pmol/L)	345.69 (293.72-406.84)	294.59 (243.25-356.77)	384.85 (318.10-465.62)	326.73 (265.80-401.62)	370.00 (308.05-444.41)	0.39
Glucose (mg/dL)	76.97 (74.79-79.21)	76.54 (74.11-79.04)	79.63 (77.03-82.32)	76.38 (73.89-78.96)	77.42 (74.73-80.20)	0.43
HOMA	1.35 (1.21-1.51)	1.21 (1.07-1.37)	1.52 (1.34-1.72)	1.30 (1.14-1.49)	1.49 (1.30-1.70)	0.12
Postmenarcheal[c]						
n	42	43	43	38	37	
Insulin (uU/mL)	8.54 (7.41-9.83)	9.10 (7.98-10.37)	8.47 (7.39-9.71)	7.95 (6.92-9.13)	10.76 (9.35-12.38)	0.17
C-peptide (pmol/L)	509.12 (433.11-598.47)	570.19 (490.25-663.17)	579.69 (500.43-671.52)	529.51 (455.38-615.72)	766.10 (650.20-902.67)	**0.002**
Glucose (mg/dL)	77.79 (75.88-79.74)	77.64 (75.85-79.47)	79.78 (77.94-81.66)	77.80 (75.97-79.68)	82.34 (80.29-84.45)	**0.0057**
HOMA	1.64 (1.41-1.91)	1.74 (1.51-2.00)	1.68 (1.45-1.95)	1.54 (1.32-1.78)	2.19 (1.88-2.55)	0.074

[*] For adjusted biomarker trend across quintiles of height percentile at baseline.

Table 4.4. (cont'd)

a. Geometric means adjusted for age, age x age, treatment group, baseline bmi-for age percentile and physical activity.
b. Among 224 girls with premenarcheal blood samples at baseline, year3, year5 and last visit.
c. Among 203 girls with postmenarcheal blood samples at baseline, year3, year5 and last visit.

Figure 4.2. Insulin-related biomarker concentrations by age among premenarcheal and postmenarcheal girls in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study

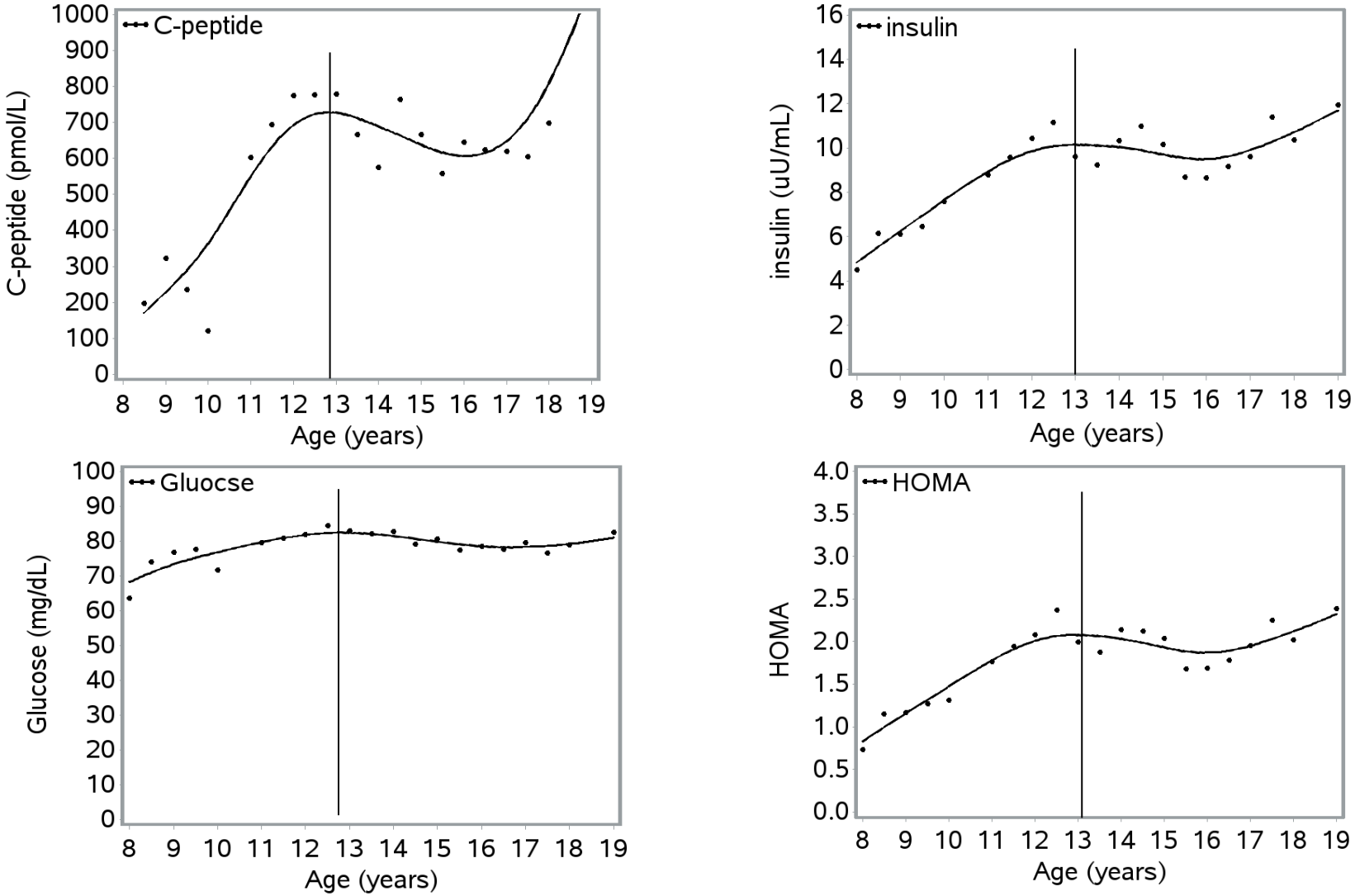

Figure 4.2. (Cont'd)

Note: Figures represent participants with age distributions within 1.5-SD; the straight line shows mean age at menarche

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CHAPTER 5 CONCLUSIONS, PUBLIC HEALTH RECOMMENDATIONS AND FUTURE DIRECTIONS

5.1. Conclusions

Childhood adiposity has been hypothesized to be associated with reduced breast cancer risk through affecting insulin-related biomarker levels. In order to shed light on the mechanisms underlying the observed decreased risk of breast cancer associated with childhood adiposity, this dissertation examined the associations between dietary or anthropometric factors and insulin-related biomarker levels in children and adolescents. This dissertation included a literature review of epidemiological studies to gain greater knowledge on what and how dietary factors affect insulin-related biomarker levels among healthy children and adolescents; and secondary data analyses examining the association between childhood dietary as well as anthropometric factors and fasting blood levels of insulin-related biomarkers, as well as the derived HOMA value.

Chapter 2 part 2.5 systematically reviewed the evidence for dietary factors in association with insulin-related biomarker levels during childhood and adolescence. Thirteen epidemiological studies that examined dietary factors and met inclusion criteria were identified and reviewed. Whole grain intake was inversely associated with insulin level in boys and C-peptide level in girls; fiber intake was inversely associated with HOMA in girls; white bread intake was positively associated with insulin level; fruit intake was positively associated with glucose level; total sugar or sugar-sweetened beverage intake was positively associated with HOMA in girls; soft drinks/sweetened beverage intake was positively associated with glucose level; simple carbohydrate intake was positively associated with insulin; fructose was positively associated with

glucose and HOMA; red processed meat intake was negatively associated with glucose level, but general meat intake was not associated with any of the insulin-related biomarkers; milk intake was positively associated with insulin and C-peptide level, not glucose level among boys; whey protein intake from milk was positively associated with insulin and HOMA; and the western dietary pattern was positively and healthy dietary pattern was inversely associated with glucose level.

In Chapter 3, the results of the association between dietary factors and insulin-related biomarker levels in adolescent girls from the Dietary Intervention Study in Children (DISC) showed that dietary fiber or vegetable protein intake in adolescent girls were inversely associated with C-peptide level, and starch intake was inversely associated with HOMA after adjustment for body mass index-for-age percentile (BMIPCT) and energy intake. BMIPCT was also a significant independent predictor for insulin, C-peptide and HOMA levels. No significant predictors were identified for glucose.

In Chapter 4, our findings in the DISC on baseline anthropometric factors and insulin-related biomarkers indicated that higher baseline BMIPCT was associated with higher levels of insulin-related biomarkers in premenarche girls, higher baseline waist circumference (WC) was associated with higher insulin-related biomarkers both pre- and post-menarche girls, while higher baseline height was associated with higher insulin-related biomarkers in postmenarche girls. Therefore, different baseline anthropometric indices may have different associations with insulin-related biomarkers based on menarche status. These may suggest general adiposity, central adiposity and growth height may have different effects on insulin-related biomarker levels during

pubertal development, which may be implicated in their different effects on breast tissue development and future breast cancer risk.

5.2. Public Health Recommendations

In our study, adolescent girls who ate diets higher in fiber had lower fasting serum C-peptide levels, indicative of lower insulin production; moreover, adolescent girls who ate diets higher in starch had lower HOMA levels, an index of insulin resistance. Both fiber and starch are complex carbohydrates that could reduce glucose absorption. This study supports the dietary recommendations for children to increase consumption of high fiber diets such as whole grains and fruits and vegetables, which may help decrease risk of breast cancer later in life.

Our studies from cross-sectional and longitudinal data analyses show that both BMIPCT and WC, which are indicators of adiposity levels, are positively associated with insulin, C-peptide and HOMA levels. We hypothesized that the inverse association between childhood adiposity and breast cancer risk may be potentially mediated through insulin-related biomarkers, which stimulate mammary gland differentiation by decreasing sex-hormone binding globulin (SHBG). Early breast differentiation can desensitize breast tissue to later carcinogenic exposure. Therefore, there might be positive associations between adiposity and insulin-related biomarker levels as well as inverse associations between adiposity and SHBG levels in girls during pubertal development. The inverse association between adiposity and SHBG has been confirmed by our colleagues using data from the DISC. They observed that higher BMI at baseline was associated with lower SHBG consistently among both pre- and post-menarche girls.[1] Our findings of the positive association between adiposity and insulin-

related biomarkers and proposed theory may shed new light on the intriguing hypothesis that insulin-related biomarkers are key components linking childhood adiposity and breast cancers. However, the degrees of the associations may be variable among different adiposity measurements, and menarche status may also affect the significance of the associations.

5.3. Future Directions

Literature investigating energy balance-related factors and insulin-related biomarkers among healthy children and adolescents is limited. Most of the current literature did not adequately consider the effect of menarche status on insulin-related biomarker changes, which may generate inaccurate results. Also, there is no study directly investigating the association between childhood insulin-related biomarker levels and subsequent breast cancer risk. Given the long latent period of breast cancer,[2] the cancer reducing effect may be greatest with prevention and intervention efforts initiated at early life. The study of childhood nutritional risk factors and biomarkers that are implicated in breast cancer development is of particular importance. Longitudinal study design is optimal but is hard to be carried out, especially among children, which further warrants the need to study potential intermediate biomarkers of breast cancer risk. Evidence from out studies shows both selected dietary factors and anthropometric factors are associated with insulin-related biomarkers during childhood and adolescence. These biomarkers may be intermediates between early life risk factors and breast cancer that are worth to study. When studies are conducted among girls during pubertal development, menarche status should be considered jointly with other potential confounding factors, as did in our study. Additional epidemiologic research

with longer follow-up time than was available in our study, as well as randomly selected participants from the general population would also provide more definitive evidence to elucidate the association between early life nutritional factors and insulin-related biomarker levels among healthy children and adolescents.

In the longitudinal data analyses of our study, we observe WC, an index of central adiposity, is more consistently associated with insulin-related biomarkers throughout puberty than BMIPCT and height. Further study is warranted to confirm these findings, as well as to address the effect of the changes of these anthropometric indices during growth on insulin-related biomarkers levels and potential risk for breast cancer.

Our current study is focused on only insulin-related biomarkers, although many other circulating hormones, including growth hormone, sex hormone, adipokines and their binding proteins could also influence insulin-related biomarker levels and have been hypothesized to be implicated in breast cancer etiology.[3,4] To expand our knowledge of biological mechanisms of early life risk factors and breast cancer, future work is also needed to investigate 1) the associations of the nutritional factors and other hormonal and metabolic biomarkers during pubertal development, 2) the associations between insulin-related biomarkers and other hormonal and metabolic biomarkers during pubertal development, 3) these intertwined biomarkers' joint associations with breast cancer development in later life; 4) and how these biomarkers measured during childhood and adolescence may possibly modify breast tissue development and potentially affect later breast cancer risk.

More research is needed to further explore the mechanisms for the associations between childhood energy balance-related factors and later breast cancer risk, as well as the protective effect of childhood adiposity on breast cancer, especially through insulin-related biomarkers as intermediates.

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