THE ASSOCIATIONS BETWEEN DIET QUALITY, TOTAL AND REGIONAL ADIPOSITY, AND METABOLIC RISK IN HISPANIC AND NON-HISPANIC ADOLESCENT GIRLS

by

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FINAL EXAMINING COMMITTEE APPROVAL PAGE

As members of the dissertation committee, we certify that we have read the dissertation prepared by Danielle M. Vassallo entitled, “ The Associations between Diet Quality, Total and Regional Adiposity, and Metabolic Risk in Hispanic and Non-Hispanic Adolescent Girls” and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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DEDICATION

To my family and friends for their endless love and support.

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ABSTRACT

Nutrient deprived diets are major contributors to the development of childhood obesity and metabolic diseases. Total and site-specific adiposity, such as visceral and skeletal muscle fat, have been associated with an increased risk of metabolic syndrome, insulin resistance, and other cardiometabolic risk factors in youths. C-reactive protein (CRP), a circulating inflammatory biomarker, is an established risk factor for cardiovascular disease and is associated with adiposity even at a young age. Diet quality indexes have been developed for use in adolescents and have evaluated the relationships between diet quality and selected health outcomes. Studies that assess relationships between diet quality, adiposity measured using direct methods, and metabolic risk are lacking in youth, particularly Hispanic Americans. Therefore, the objective of this dissertation was to evaluate the relationships between diet quality, assessed by the Youth Healthy Eating Index (YHEI), measures of total and site-specific adiposity, by dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT), and inflammation, assessed by high-sensitivity CRP (hs-CRP) in a cross-sectional study at baseline of 576 healthy Hispanic American and non-Hispanic girls aged 8-13 years. Diet was assessed using the validated semi-quantitative Harvard Youth/Adolescent Food Frequency Questionnaire (YAQ). Diet quality was assessed from the YHEI, developed based on the YAQ. Muscle density (mg/cm^3), a surrogate for fat infiltration, was measured at tibial and femoral sites using pQCT. Total body and android fat (surrogate for visceral fat) were measured by DXA. Serum hs-CRP concentrations were measured by nephelometry. Multiple linear and logistic regression analyses were employed to assess these relationships. Total YHEI score was inversely associated with total body fat percent ($p= 0.01$) and android percent fat ($p= 0.02$),

but not body mass index (BMI) or muscle density. Lower "margarine and butter use" and higher "meat ratio" were associated with higher leg muscle density. Higher "meat ratio" was inversely associated with BMI and greater "multivitamin use" was inversely associated with visceral adiposity. In a subsample of 113 Hispanic girls, over 50% of study participants demonstrated non-detectable serum hs-CRP levels. In adjusted models, there was no significant relationship between overall YHEI score and hs-CRP in this sample of adolescent girls. For every 1-unit increase in "whole grains" score there was a 44% increase in odds of being in the "high" category compared to the "undetectable" category of hs-CRP, after adjusting for maturity offset, PYPAQ score, total energy intake, total body fat, and all other individual YHEI components (OR: 1.44, 95% CI: 1.06, 1.97). The odds of being in the "high" category of hs-CRP were 38% higher compared to those with "non-detectable" hs-CRP for every 1-kg/m^2 increase in BMI (OR: 1.33, 95% CI 1.16, 1.53, $p<0.0001$). The odds of being in the "high" hs-CRP category increased with every 5 percent unit increase in total body percent fat (OR: 2.38, 95% CI 1.58, 3.58, $p<0.0001$) and android percent fat (OR: 1.89, 95% CI 1.39, 2.57, $p<0.0001$), compared to those with "non-detectable" concentrations. Calf muscle density was associated with lower odds of elevated hs-CRP compared to the "non-detectable" group (OR: 0.58, 95% CI 0.35, 0.75, $p<0.001$). The findings suggest that higher diet quality scores are associated with lower total and visceral body fat in adolescent girls. While greater total and regional adiposity are associated with increased inflammation, independent of biological and lifestyle factors, diet quality is not significantly associated with inflammation, as assessed by concentrations of hs-CRP, in Hispanic American girls.

CHAPTER 1

INTRODUCTION

Background and Significance

Cardiovascular disease (CVD) remains the leading cause of adult death in the U.S., resulting in 1 in 3 deaths reported annually (1, 2). Obesity, another substantial public health concern, is considered a primary modifiable risk factor in the CVD risk profile, and is impacted greatly by obesogenic behaviors including the consumption of energy dense diets and physical inactivity (3). Not only is obesity linked with marked excess mortality in the U.S. population but also excess morbidity related to cardiovascular risk factor development, incidence of type-2 diabetes mellitus (T2DM), CVD end points, and other health conditions (3). The prevalence of childhood obesity has grown to over 20% during the last three decades (3) and is the most predominant cardiovascular risk factor observed in adolescents (1, 4). Current estimates indicate that 1 in 3 American children are either overweight or obese and 20% of obese youths are at risk for cardiometabolic diseases (4). Most recent estimates from NHANES data indicate that 37%, 49%, and 61% of normal weight, overweight, and obese adolescents, respectively, have at least one cardiovascular disease risk factor (1).

Visceral adiposity, dyslipidemia, hypertension, insulin resistance, and hyperglycemia are risk factors that when clustered are collectively known as “metabolic syndrome” and independently increase the risk of T2DM, CVDs, morbidity and mortality (5, 6). Sun et al. reported that older adolescent girls with BMI or waist circumference values $\geq 60^{th}$ percentile of Centers of Disease Control and Prevention (CDC) growth charts, had greater odds (odds ratio (OR) 0.8-2.8, 1.7-2.5, respectively) of developing metabolic syndrome 25-

30 years later compared to their leaner peers (7). Additionally, research has shown that 19.4% of adults who had metabolic syndrome as children developed CVD after 25 years compared to 1.5 % of their peers who did not have metabolic syndrome as children (8). It is estimated that by 2035, the prevalence of coronary heart disease in young adults will be nearly 16% spurred by the current youth obesity epidemic (9).

Obesogenic behaviors, obesity, and its comorbidities, track into adulthood such that obese adolescents tend to become the heaviest adults with the most adverse risk profiles (10-12). Hispanics and Latinos, the largest minority group in the U.S., experience the highest burden of cardiovascular risk factors (13). Indeed, data from National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999-2006 indicated that Mexican Americans have the highest prevalence of metabolic syndrome compared to non-Hispanic White and non-Hispanic Black adults (14). Moreover, data from NHANES 2003-2004 suggested that Mexican American women are 1.5 times more likely to meet the criteria for metabolic syndrome than non-Hispanic White females (15). High rates of obesity and metabolic syndrome are prevalent among Hispanics, yet studies assessing risk factors related to obesity and CVD risk in adult and adolescent Hispanic American samples are limited (16). There is a considerable clinical and public health need to understand the relationships between eating habits, excess weight, and metabolic risk in Hispanic youths because childhood obesity has apparent cardiovascular consequences that have the potential to accelerate the onset and increase the incidence of CVD in adulthood, a disease already prominent in this population (13, 17-20).

Total and Regional Adiposity and Inflammation

Obesity has been widely acknowledged as an inflammatory condition (21) that develops when energy intake chronically exceeds the capacity of energy storage in white adipose tissue. Substantial evidence indicates that while excess adiposity increases CVD risk, much of the threat is facilitated by mechanisms associated with related cardiometabolic risk factors (17). Specifically, the obesity-CVD risk profile has been largely attributable to insulin resistance (22) and inflammation (23). While both subcutaneous and visceral adipose tissues have been associated with metabolic risk profiles, excess intra-abdominal fat, an established cardiometabolic rick factor, is associated with metabolic disturbances without the manifestation of obesity and has a more profound influence on CVD risk than subcutaneous fat (24-29). This is due to fundamental characteristic dissimilarities in subcutaneous and visceral adipocytes including phenotypic, physiological, and functional differences whereby visceral adipose tissue has a more pro-inflammatory profile than subcutaneous fat (5, 30-34).

Obesity-related insulin resistance is initiated by two main mechanisms; first via endoplasmic reticulum (ER) and mitochondrial oxidative stress; and second, through lipotoxicity (35). During weight gain leading to obesity, adipocytes exhibit hypertrophy and hyperplasia, thereby promoting adipocyte function abnormalities including ER and mitochondrial oxidative stress, a process that is exacerbated in visceral adipocytes (36). M1 macrophages, free-fatty acids, and pro-inflammatory cytokines [e.g. Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6)] are released during obesity and states of ER stress. TNF-α, produced by adipocytes and macrophages within adipose tissue, promotes the release of IL-6 and activates c-Jun N-terminal kinases (JNK) in muscle, liver, and adipose cells, which in turn reduces insulin receptor substrate action and ultimately increases insulin resistance in adipocytes (5, 31, 37). IL-6 promotes the production of acute phase reactants in the liver, including C-reactive protein (CRP) (38-40). CRP is a circulating marker of inflammation measured at high concentrations during a state of acute infection or systemic inflammation. In the absence of infection or systemic inflammation, CRP is considered a stable long-term biomarker of adipocyte-generated chronic low-grade inflammation (36, 41, 42). It has been suggested that CRP may be the strongest biological predictor of risk of CVD and is more cost-effective compared to most other cardiovascular screening methods (36, 41, 42). Indeed, one study reported that in adults, CRP measured from initial blood collection was a strong predictor of risk 20 years later, suggesting that CRP has a long-term predictive value (43). In terms of clinical application, findings from a prospective, nested case-control study including baseline data from 28,263 participants from the Women's Health Study showed that CRP was the strongest predictor of cardiovascular events compared to homocysteine, serum amyloid A, soluble intercellular adhesion molecule type 1, IL-6, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, apolipoprotein A-I, apolipoprotein B-100, L (a) lipoprotein, and the ratio of total cholesterol to HDL cholesterol (44). Even at an early age, CRP concentrations are associated with adiposity, which may be explained by the fact that CRP synthesis is regulated by adipocyte-produced IL-6. Furthermore, elevated concentrations of CRP during childhood are associated with elevated concentrations in adulthood (38, 45-61). NHANES data have also shown that CRP concentrations are higher in children who demonstrate the presence of more metabolic syndrome components (21, 62). Although CRP and IL-6 concentrations are moderately correlated (r_s= 0.45 p<0.0001), CRP appears to be a more stable and reliable indicator of inflammation (63). This is due to the fact that CRP has a longer half-life compared to IL-6 and because IL-6 is susceptible to diurnal variation (64).

Abdominal adiposity, an independent cardiovascular risk factor, is predominantly high in Hispanic/Latino women (19) and youth, compared to other race/ethnic groups (65). For example, in a group of 55 obese Hispanic, Caucasian, and African American adolescents, Hispanics had the highest abdominal visceral adiposity (83.0 cm^2, [range 70.7-95.0]) as measured by MRI, compared to Caucasian (75.4 cm^2, [range 65.0 - 86.0]) and African American adolescents (49.8 cm^2, [range 38.0 - 64.7]); although significant differences were only observed between Hispanics and African American adolescents after controlling for age, gender, and percent fat ($p=0.003$) (66). Similarly, data from NHANES 1999-2000 revealed that CRP values in Mexican American girls ages 8-11 years and 12-17 years are almost 2-fold higher than in non-Hispanic White girls ($p=0.03$) independent of BMI, age, smoking status, total cholesterol, homocysteine, systolic and diastolic blood pressure, and glycosylated hemoglobin (38). It is possible that in Hispanics, genetic factors play a role in metabolic processing of excess fat that contributes to the heightened levels of CRP. This is supported by the increased risk of insulin resistance, T2DM, and other metabolic dysfunctions related to CVD, seen in this population (13, 20, 67).

There is sufficient evidence from cross-sectional studies to suggest that anthropometric indexes of adiposity are significantly and positively associated with CRP concentrations in adolescents (38, 45-60). These data suggest that preclinical inflammatory processes are detectable in youths; therefore, it is possible that measuring inflammatory biomarkers from a young age may be an accurate method of tracking the progression of cardiovascular related risk into adulthood (68). However, these data are from cross-sectional studies and better conclusions regarding inflammation may be drawn from longitudinal studies, given that chronic low-grade inflammation occurs over extended time periods.

Most research that has investigated the relationships between adiposity and inflammatory biomarkers in youths has been conducted in non-Hispanic samples. The majority of studies that have assessed the relationships between adiposity and inflammatory biomarkers in Hispanic adolescents have been limited to use of anthropometric indexes of adiposity and cross-sectional designs (46, 51, 58, 59, 69-74). CRP was the most frequently assessed biomarker of inflammation. Similar to findings from non-Hispanic samples, studies in Hispanic or Mexican American adolescents have consistently reported that adiposity including BMI, weight, total body fat, and waist circumference have a significant positive association with concentrations of CRP. For example, Warnberg et al. reported that weight ($\rho = 0.235$), BMI ($\rho = 0.265$), waist circumference ($\rho = 0.220$), and body fat percentage ($\rho = 0.281$) were all significantly associated ($p<0.01$) with CRP in 472 Spanish adolescents, after controlling for age and tanner stage maturation (59).

A few intervention studies have also assessed the relationship between adiposity and concentrations of CRP (75-78). The time spans of these intervention studies ranged up to a 1 year and reported that lifestyle changes including healthy eating and activity behaviors resulted in weight loss (between 2.6 and 5.3 kilograms) and significant decreases in CRP levels (75, 77). One randomized-controlled trial conducted in a small sample of 21 adolescents (15 obese) reported that the intervention arm significantly reduced CRP levels ($p= 0.02$) while maintaining weight after a 3-month diet and physical activity intervention (76) suggesting that improving health related behaviors have potential to directly impact cardiometabolic risk without weight loss.

Anthropometric and direct techniques are used to measure visceral adiposity, each with different strengths and limitations regarding accuracy, cost effectiveness, safety, and portability of device (79). Waist circumference is the most common anthropometric index used to indirectly measure visceral adiposity. It is a cost effective method that has be validated against other more direct methods, e.g. dual-energy x-ray absorptiometry (DXA), computed tomography (CT), and magnetic resonance imaging (MRI) (27, 79-85). However, waist circumference measurements are limited in that they are prone to low accuracy and high intra- and inter-personal variability.

Apart from biopsy, the most direct and invasive method of measuring adiposity, CT and MRI are the most accurate techniques for distinguishing fat compartments. However, these techniques are expensive and time consuming. CT technology has excellent precision and accuracy (>99% for both) in the reconstruction of adipose tissue and organ masses based on scans (86, 87). CT also poses a risk of radiation hazards; therefore this method is not primarily used in adolescents and is not appropriate for repetitive measurements. MRI is a safer alternative since it does not use radiation, however this technique is not advantageous for certain populations, specifically morbidly obese individuals, and is very costly. Researchers typically quantify visceral adipose tissue from one abdominal scan using MRI and CT in order to save time and costs (27). This raises the potential concern for over- and under-estimation of fat mass measured at one level compared to total fat volume measurements from multiple scans (27). There is a lack of accuracy in measuring visceral adipose tissue from one scan given that fat loss or distribution is not uniform. Multiple scans would minimize variability in scans and more accurately assess fat volume (79).

Few studies that have used MRI or CT to assess the relationship between CRP concentrations and adiposity in youths have reported a positive and significant association of moderate effect (88-90). These studies were limited to small sample sizes and did not control for potential confounders in their analyses. In a study of 30 overweight and obese adolescents assessed the cross-sectional relationship between visceral and subcutaneous abdominal adipose tissue estimated by MRI, and concentrations of CRP (88). Spearman correlation coefficients were reported and average CRP was significantly associated with visceral adipose tissue (r_s=0.55, $p<0.01$) and increased across tertiles of subcutaneous abdominal adipose tissue (1.44 ± 0.88 mg/L, 3.45 ± 2.65 mg/L, 5.08 ± 3.79 mg/L, $p<0.05$, respectively) (88). Similarly, in 59 lean and overweight adolescents, aged 7-12 years, fasting CRP was significantly higher in overweight adolescents (1.10 ± 1.11 mg/L) compared to their leaner peers (0.17 ± 0.21) ($p<0.0001$) and was also significantly correlated (all $p<0.001$) with total abdominal fat (r=0.59), intra-abdominal fat (r=0.59), and subcutaneous fat (r=0.63) (45). The participants in this study were between 7 and 12 years old, therefore maturation should have been considered as a potential confounder as early age at puberty onset is a risk factor that has been linked to insulin resistance (91) and adiposity (92), thereby potentially modulating CRP levels. Similarly, traditional coronary risk factors including gender, blood lipids, blood pressure, smoking status, physical activity, glucose tolerance, and insulin sensitivity have been found to modulate CRP levels, in adults (46, 51). As such, these risk factors should also be considered as potential confounders when assessing CRP related relationships, in adolescents. Lastly, given that these studies assessed the relationship between regional adiposity and inflammation it would be important for the investigators to adjust for total adiposity in order to fully understand if the relationships with inflammation are directly attributed to regional adiposity or confounded by overall fatness.

DXA is the method most commonly used to quantify total body fat *in vivo* (65). It is a non-invasive and indirect technique that measures indexes of body composition using x-rays at two different energies. This technology is relatively affordable and measures full and regional body composition with high precision, reliability, and minimal time, making it the most suitable alternative to CT and MRI (85, 86, 93). DXA is appropriate for use in children as it is capable of repetitive measures with minimal radiation exposure. With respect to measuring visceral adiposity, DXA is limited in that it is not capable of differentiating between visceral and subcutaneous adipose tissues. Truncal fat, as assessed by DXA, can be partitioned into android (upper body or central) and gynoid (lower body or peripheral) areas. Android fat is known to be associated with greater cardiometabolic risk than gynoid fat and it correlates strongly with visceral adipose tissue ($r=0.813$ $p<0.01$), as measured by CT (27, 94, 95). Indeed, in a study of 565 elderly Koreans, android fat was strongly and significantly associated with clustering of metabolic syndrome risk factors, after adjusting for visceral adipose tissue (27). There are a limited number of studies that have investigated the implications of android fat, as measured by DXA, on cardiometabolic risks in adults and even less evidence in children and adolescents (27, 96).

The few studies that have assessed cross-sectional relationships between inflammation and adiposity using DXA to measure total and central adiposity in adolescents have consistently revealed significant positive associations (45, 49, 89, 97, 98). One study also assessed the association between risk factors associated with CVD and percent leg fat, measured by DXA in 391 White and African American youths, ages 5-18 years, and found a lower odds of low HDL, high triglycerides, and insulin resistances were associated with percent leg fat, after controlling for age, sex, race, maturation, total body fat, and physical activity (97). Higher odds of CRP was associated with percent trunk fat, however there was no significant association observed between CRP and percent leg fat, after controlling for age, sex, race, maturation, total body fat, and physical activity (97). In a small sample of 74 obese teens, aged 12-17 years, body fat percentage was assessed by DXA, visceral adiposity was measured using MRI, and at baseline, CRP was strongly correlated with body fat percent ($r= 0.61$, $p\leq0.0001$) and visceral adiposity ($r=0.47$, $p\leq0.0001$) (89). Similarly, in a large sample of 7,589 healthy adolescents, simple Pearson correlation coefficients were reported and data showed that CRP was positively correlated with total fat ($r= 0.44$ $p<0.001$), as measured by DXA (49).

Findings from longitudinal studies have also confirmed a positive relationship between adiposity and concentrations of CRP. A 7.5-year observational study was conducted in a large sample of 396 girls, aged 11.2 ± 0.8 years in which the relationship between fat mass, measured by DXA, and hs-CRP concentrations was assessed, along with associated developmental trajectories from pre-puberty to young adulthood (99). Results were stratified by maturation status (before and after menarche) and multiple linear regression analyses were employed to assess the relationships between fat mass, hsCRP, estradiol, serum-hormone binding globulin, testosterone, leptin, adiponectin, diet quality index score, and leisure-time physical activity. Only fat mass was significantly ($p<0.01$) associated with hsCRP both before ($\beta=1.058$) and after menarche ($\beta=1.121$)(99).

Although findings are consistent and suggest that adiposity is positively associated with CRP concentrations in adolescents, to my knowledge there are no studies that have shown this relationship using more direct measures of adiposity, such as DXA, MRI, or CT in Hispanic American youth. This dissertation will fill the gap by assessing the relationship between total and android fat, as assessed by DXA, and concentrations of hs-CRP in a large sample of healthy normal weight, overweight, and obese Hispanic American girls.

In addition to total and visceral adiposity, skeletal muscle adiposity is also of concern because fat stored in this region is also strongly associated with chronic low-grade inflammation, impaired glucose tolerance, and increased total cholesterol, independent of total adiposity in adults (5, 100). In a study of obese Hispanic, Caucasian, and African American adolescents, ethnicity was found to play a role in the storage of fat in myocytes accounting for 10% of the difference in intramyocellular fat, independent of age, gender, and body fat percent (66). Findings from this study also revealed that obese Hispanic adolescents had higher fat storage within the soleus muscle (1.71% [range 1.43% - 2.0%]) compared to both Caucasians (1.2% [range 0.94% -1.5%] p=0.04) and African Americans (1.04% [range 0.75% - 1.34%] p=0.01), after controlling for covariates (66). Critical to insulin resistance development, venous return from visceral adipose tissue promotes increased circulation of free fatty acids and pro-inflammatory cytokines via the portal system to ectopic depots (i.e., liver and skeletal muscle). Increased storage of lipids in ectopic depots contributes to chronic cellular dysfunction and ultimately lipotoxicity (22). High intramyocellular triglycerides activate the production of ceramides and diacylglycerol, which promote the development of insulin resistance (22) via mitochondrial dysfunction and impaired glucose uptake.

In the skeletal muscle, TNF-α increases glucose uptake while inhibiting the activity of lipoprotein lipases thereby increasing lipolysis and resulting in lipotoxicity and ultimately insulin resistance (36). Heightened levels of TNF-α and IL-6 have been found in obese youths (41, 101) and research has shown that TNF-α messenger RNA is overproduced in obese adipocytes, thereby increasing the JNK inflammatory cascade (47, 102). It is unclear as to how early in life accretion of lipids occurs in the skeletal muscle to the degree of metabolic consequence, however, it is likely that limiting fat accumulation in skeletal muscle in youths would minimize the risk of insulin resistance and T2DM. Limited studies in adolescents have investigated the relationships between skeletal muscle fat and risk factors associated with increased risk of CVD, including Interleukin-1, IL-6, Interleukin-8, TNFα, monocyte chemoattractant protein-1, insulin sensitivity, insulin resistance, and fasting insulin (103-107) as many studies have been restricted to measuring total body and abdominal adiposity, due to the invasive or expensive methods required to measure skeletal muscle fat. To my knowledge, no studies have assessed the relationship between skeletal muscle fat and CRP concentration in adolescents.

This dissertation is unique in that it utilizes a technique known as peripheral quantitated computed tomography (pQCT) to measure muscle density as a surrogate for skeletal muscle fat. It does so by differentiating tissues based on attenuation characteristics that are directly related to tissue density reflecting soft tissue composition (108, 109). In a study of 471 adults of Afro-Caribbean decent, skeletal muscle density was measured at 66% tibia length (proximal to the terminal end of the tibia) using pQCT with the aim of assessing the cross-sectional relationship between regional adiposity and serum markers of inflammation (110). Higher concentrations of CRP were associated with greater fat infiltration within skeletal muscle, as reflected by lower muscle density ($r= -0.10$, $p<0.05$), after adjusting for age, gender, height, DXA total body fat percent, current anti-inflammatory medication use, and skeletal muscle area (110), suggesting that greater fat accumulated within skeletal muscle increases inflammation.

Dietary Quality

An energy dense diet is a major contributor and modifiable determinant of the development of childhood obesity and associated metabolic impairment that increase CVD risk including metabolic syndrome and T2DM (111, 112). Studies have shown that food habits and behaviors established during adolescence are likely to be maintained in adulthood (113-117). In nutritional epidemiology, most diet-related research in adults and youth has successfully evaluated the effects of isolated food groups or nutrients with regards to the risk of developing various chronic diseases and associated risk factors (118). However, since individuals consume complex combinations of foods and nutrients, assessing the effects of single nutrients or foods on the risk of developing chronic diseases or associated risk factors disregards the complexity of a whole diet, thereby making the effect of total dietary intake a potential confounder (119). There is growing interest in evaluating dietary intake as a multidimensional exposure, i.e., multiple dietary or behavioral components operationalized as a single variable. This method is known as dietary pattern assessment and is capable of examining a broader scope of food and nutrient consumption. Foods, nutrients, and their respective metabolism are interrelated and as such interpreting their relationship in isolation with a health outcome does not adequately account for their complicated interactions (i.e. enhanced absorption of iron in the presence of vitamin C) (119, 120). Individual nutrients are commonly correlated with each other and also with overall dietary intake, therefore including multiple individual nutrients in analyses may reveal misleading results due to confounding effects between variables (120, 121). The cumulative effects of various nutrients and foods in one score characterize patterns based on actual real life eating behaviors (119) and also minimize the effect of confounding (120).

Dietary guidelines align with recommendations for prevention of overweight and obesity, and dietary indexes are derived directly from or based on dietary guidelines, therefore, it is suggested that diet indexes are suitable for understanding the relationship between diet quality and risk of overweight and obesity (122). Although the dietary quality approach cannot specifically distinguish independent contributions of various biological pathways, from a public health standpoint, assessing diet quality rather than single foods or nutrients is becoming recognized as a more relevant and complementary approach of identifying relationships between nutrition and health outcomes (120, 123). To date, diet quality has been assessed through statistical methods based on either *a priori* parameters or *a posteriori* explorative methods (120). Both of these methods have their limitations. *A priori* methods are limited in that they are constructed on the basis of established dietary recommendations and nutritional knowledge. Research in nutritional epidemiology is frequently updated as new evidence emerges and dietary recommendations are not necessarily updated in the same timely manner, therefore new evidence may not be best represented by each index. Incomparability is a common limitation of all dietary quality assessment methods. Regarding dietary indexes, many have not been validated or assessed longitudinally. Diet quality can be assessed by indices with different component quantities, cut-off values, and scores derived from various diet assessment tools with limitations (119). Variability of population groups, length of follow-up, and approaches to adjusting confounders add to the difficulty of inter-comparisons (124). For example, a study by Jennings et al. (125) assessed the relationships between diet quality, using three different dietary indexes, and measures of adiposity in 1700 adolescents, after adjusting for the gender, parental educational attainment, under-reporting (ratio of energy intake to estimated energy requirements), energy density, and physical activity. Higher Diet Quality Index (DQI) and Healthy Diet Indicator (HDI) scores were associated lower waist circumference (-3.0%, p=0.005 and -2.5%, p=0.033, respectively) and lower percent body fat (-5.1%, p=0.023 and -4.9%, p=0.026, respectively). The DQI was also associated with lower weight and BMI (-5.9%, p=0.002 and -4.2%, p=0.004, respectively). The third index, The Mediterranean Diet Score was not significantly associated with adiposity. While not all index scores of dietary quality are equally associated with adiposity, it is suggested that diet quality is an independent predictor of adiposity during adolescence.

A posterior methods are data-driven and use factor or cluster analysis in order to determine dietary or behavioral patterns within a sample. Data-driven patterns are specific to the sample from which they are derived, and therefore are not restricted by adherence to nutritional recommendations. As a result, they may not present optimal patterns and may not represent general eating habits of a population (119). Since dietary patterns developed *a posteriori* are simply based on eating behaviors of a specific group, comparability across studies is sometimes not possible. Currently, both methods of assessing diet quality have limitations. However, *a priori* methods may be a more accurate measure in that they assess overall diet quality and variety by using a numerical index score to represent how closely an individual's intake is adherent to current national dietary recommendations or pre-determined nutritional knowledge (124). As such, *a priori* methods were used in this dissertation in order to expand on the sparse research that has assessed diet quality in adolescents using the Youth Healthy Eating Index (YHEI).

Dietary indexes have been designed as easy tools of assessing diet quality for better use by consumers, non-dietetic health professionals, and policymakers in order to evaluate overall quality of diet (126). As such, easy interpretation of dietary indexes could result in more direct public health implications (127). According to Kant (128), there are three major methodologies that can be used to construct a diet index, based on 1. Either foods or food groups; 2. Nutrient intakes; or 3. Combination of both foods and nutrient intakes (124). The inclusion of index components vary and to a degree are arbitrary, however, there are specific nutrients, e.g., fat and cholesterol, and food groups, e.g., fruits, vegetables, whole cereals/grains, and sometimes meat, that are widely measured in dietary indices based on their established impact on health outcomes (121). Dietary index scores have correlated highly with several macro- and micro-nutrient intakes, thereby suggesting that existing indexes accurately assess diet quality (121, 129-135).

Diet quality has been assessed in adults in epidemiological studies to evaluate its association on risk factors related to heart disease, cancer, and all cause-mortality (124, 128, 136, 137). Similar studies are needed in children and youth. Madruga et al. (113) suggests that dietary habits adapted in childhood may persist until adolescence, and then may change or be discontinued throughout adolescence. As a result, it is important to understand dietary habits established during adolescence, as these are the behaviors that will most likely track into adulthood and contribute to risk or prevention of disease. Three recent review articles have identified 80 diet indices that have either been developed for, or used in, child and adolescent samples (123, 137, 138). Nevertheless, more research is needed to better understand the relationships between the diet quality of adolescents and risk factors related to CVD, an area of research that is currently understudied (123, 137, 138). For example, most studies assessing the relationship between diet quality and risk factors related to CVD, including CRP, serum total cholesterol, serum HDL, systolic and diastolic blood pressure, and HbA1C, in adolescents have been restricted to cross-sectional designs and have not controlled for potential confounders (118, 139-145). Studies have suggested that there are a variety of demographic factors that are associated with diet quality. In a review by Lazarou and Newby, 54% of the studies reported significant associations between indexes and socio-demographic variables in youths, including but not limited to socio-economic status (146-152), age (146, 148, 149, 151, 152), gender (147, 148), and ethnic or migration background (147-149). Cheng et al. also suggests that maturation is a factor associated with dietary quality in that healthy children with lower dietary quality appear to enter puberty 0.4 years earlier compared to children with higher diet quality, independent of gender, maternal overweight, baseline energy intake, and baseline BMI z-score (153). As a result, it is important to control for socio-demographic variables when assessing relationships with dietary quality in youths.

Twenty-three studies conducted in adolescent samples have assessed the relationship between diet quality and health-related outcomes using 22 different indices. Several of which were observational and cross-sectional in design and assessed relationships between diet quality and measures of adiposity. The majority of these studies reported small inverse associations between diet quality and adiposity while the remaining found associations that were not significant, and only one found a positive association. BMI or BMI category was the most reported outcome, and significant inverse (125, 152, 154, 155), null (156-161), or positive (152, 162, 163) relationships were observed with diet quality. Golley et al. (152) reported a weak positive relationship between diet quality and BMI z-score (b=0.004, 95%

CI: 0.001, 0.008) in adolescents aged 12-16 years, suggesting a 0.04 increase in BMI z-score for every 10-point increase in diet quality score, and a non-significant association was observed in youths aged 8-11 years (152). This may be explained by an increase in lean tissue with better diet quality given that these are growing girls and BMI is limited in that it does not indicate what type of tissue composition is being lost or gained. Overall, findings from studies in adolescents correspond with results from studies in adults that have shown an inconsistent relationship between BMI and diet quality (164). Although BMI is an easy-to-obtain index, a cheap practical tool for indirect assessment of body composition, having shown to be moderately associated with body fatness, it cannot distinguish between fat and lean mass and assumes that fat distribution is constant (165). Additionally, due to variations related to age, gender, and race/ethnicity, BMI is limited in the BMI-body fat relationship (165). Consequently, differences in BMI among individuals do not reflect differences in adiposity alone and studies have shown a twofold range of variation in fatness for a given BMI value in individual children (79, 165, 166). BMI is an index derived from a ratio of total body mass and height, two variables that are frequently changing during periods of rapid bone and muscle growth, such as puberty, therefore it may not be the best index for body fat for adolescents.

Some studies have assessed the cross-sectional relationships between measures of adiposity including percent body fat, waist circumference, percent abdominal fat, and overweight and obesity, and total diet quality scores (125, 152, 167-171). All studies were conducted in large samples (>600 adolescents), six of which assessed body composition from anthropometric measures (125, 152, 167-171). Only one study evaluated the association between dietary quality scores and direct measures of adiposity by DXA and only in a subsample of their participants (n=196) (167). All seven studies found either significant inverse (125, 167, 171) or null (152, 167-170) associations between adiposity and diet quality scores, except for one which found a very weak positive significant association between diet quality scores and waist circumference z-score (ß =0.006, 95% CI: 0.002, 0.010, p=0.001) in 12-16 year olds after adjusting for age, gender, energy intake, socio-economic characteristics, and family circumstance (129).

Results from studies assessing the longitudinal relationships between diet quality and adiposity, using anthropometric indexes of adiposity, suggest that diet quality is not significantly associated with anthropometric indexes of total body adiposity (153, 172). In a study of 222 adolescents, diet quality was not significantly associated with BMI z-score, fat mass/height^2 z-score, or fat-free mass/height^2 z-score after adjusting for sex, maternal overweight, baseline energy intake, and pre-pubertal body composition (153). To my knowledge, only one randomized controlled trial has been conducted in adolescents regarding diet quality and metabolic risk. The investigators assessed the relationship between change in diet quality and BMI, plasma total cholesterol, triglycerides, and HDL and weight in 198 hypercholesterolaemic children and adolescents who were randomized into two intervention groups, home-based, self-instructed, parent-child nutrition education program or nutrition counseling from a registered dietician promoting lower dietary fat, for 10 weeks (173). While diet quality scores improved after 3 months in the intervention groups versus the controls, simple bivariate correlations revealed no significant association between change in diet quality scores and change in BMI, weight, or plasma lipids (173). The relationship between dietary quality and adiposity among youth remains unclear. This is potentially attributed to the lack of precision of adiposity estimation from anthropometric measures and the limited understanding of the association using more direct methods of adiposity. Similarly, most studies assessing diet quality and adiposity in youth did not control for potential confounders. This dissertation will fill this gap by evaluating the relationship between diet quality and measures of adiposity as assessed by methods of DXA and pQCT, and using multivariate models in order to control for potential confounders.

The Healthy Eating Index (HEI) is a dietary scoring index that was created by the USDA in order to assess how eating habits of American adults adhere to the Dietary Guidelines of America (DGA) (131). The DGA were designed to recommend healthy dietary patterns that could reduce risk of chronic disease in children and adults (167). Although created for adults, the HEI is a validated diet index that has been used extensively to assess diet quality and relationships between diet quality and health-outcomes in children and adolescents. The Youth Healthy Eating Index (YHEI) is a modified version of the HEI, developed in order to assess the eating habits and behaviors of children and adolescents (154). Rather than estimating specific nutrient intakes, the YHEI focuses on food choices to assess fat, sugar, fiber and sodium intake, including high trans-fat snack foods and sugar-sweetened beverages. It also accounts for behaviors associated with scholastic performance, such as frequency of eating breakfast, and healthy dietary intake patterns. YHEI scores are modified from those of the HEI to reflect the 5-A-Day serving size recommendations developed by the National Cancer Institute (174, 175). Both scoring indices are acceptable for assessing diet quality in children and adolescents although they are different. HEI scores are partially driven by energy intake and assess' the diet-related risk of disease whereas YHEI scores reflect diet quality (167). While the HEI was created to assess diet-related risk of disease, some components of the HEI, such as alcohol intake, are not a valuable variable to measure in older children and adolescents. Similarly, the HEI does not differentiate between whole and refined grains: rather it clusters both together so that high consumption infers better diet quality. Current dietary habits of adolescents include higher consumption of snack foods, comprised greatly of refined grains and sugar-sweetened beverages. The YHEI accounts for these dietary trends by incorporating them as individual components in the index to more accurately assess dietary habits of this age group. For these reasons, among others, the YHEI was used to measure diet quality in this dissertation.

Only three studies have used the YHEI to measure diet quality (154, 167, 176). All three assessed the relationship between diet quality and measures of adiposity in adolescents. Feskanich et al. (128) developed the YHEI and then used both the YHEI and HEI to assess the relationship between diet quality scores and BMI in 16,452 adolescents. Simple Pearson correlations were reported and revealed inverse relationships between BMI and HEI score (r= -0.08) and YHEI score (r= -0.12). Although this study utilized a very large sample that was comprised of 95% Caucasian adolescents, ages 9 to 14, it was limited in that no potential confounders were controlled during the analysis stage. The participants in this study were at the age of puberty onset. As stated previously, in addition to many socio-geographic factors, maturation is associated with dietary quality and adiposity, therefore should be considered as a potential confounder. Feskanich et al. (128) also reported that HEI scores (r= 0.67) were more closely associated with energy intake than YHEI scores (r= 0.12). Therefore, in the model that assessed the relationship between YHEI scores and BMI, it would have been important to control for the influence of energy intake, as it is known that greater energy intake is associated with greater adiposity. It was also reported that HEI scores were correlated with activity to an extent (r= 0.14) while YHEI scores were not correlated with activity. Since physical activity plays a role in the prevention of overweight and obesity, it should be considered as a potential confounder in studies measuring adiposity (177).

Hurley et al. (140) repeated this assessment, using both YHEI and HEI in two samples totalling 317 African American adolescents. No significant relationships between HEI and YHEI scores and the risk of being overweight or obese were reported. The investigators had the means to collect data on percent body fat and percent abdominal fat, as measured by DXA, in only a sub-sample (n= 196) in this study. Investigators used simple Pearson correlations to quantify the relationship between YHEI and HEI total scores and absorptiometric measures of adiposity. Significant inverse associations between HEI total score and body fat percent (r= -0.17) and percent abdominal fat (r= -0.19) were reported. The relationships between YHEI scores and DXA measures of adiposity were not significant. This study utilized a large sample of low socio-economic status, older African American adolescents. As a result, although no potential confounders were controlled during the analysis stage, some potential confounders including ethnicity, socioeconomic status, and maturation were restricted by study design. Since the HEI and YHEI were used, physical activity and energy intake should have been considered as possible confounders during the analysis stage as they may have influenced the relationship between diet quality and BMI. Finally, Vitale et al. (176) assessed the relationship between YHEI scores, BMI, and salivary nitric oxide concentrations in 45 adolescents, aged 9.4 ± 0.6 years, and reported a positive relationship between total YHEI score and BMI (r= 0.936, p= 0.2290), however no confounders were controlled. This is the only study to report a positive relationship between total YHEI score and adiposity and was conducted in a small sample. Since total energy intake was not controlled in this analysis, it is possible that the positive relationship between diet quality and BMI may be confounded by total energy intake (176). To date, the YHEI has not been used to measure diet quality in order to assess the relationship between diet quality and adiposity in Hispanic American girls, a group that has significant differences in biological characteristics, described previously, compared to other races/ethnicities. This dissertation utlizes a large sample of Hispanic American and non-Hispanic girls and evaluates the relationship between diet quality, as measured by YHEI, and direct measures of adiposity, while adjusting for multiple influencing factors such as ethnicity, maturation, physical activity, energy intake, and cohort assignment, in multivariate models.

In relation to CVD risk factors, to date, the YHEI has been limited to measuring diet quality in order to assess its relationship with measures of adiposity. Two studies have evaluated the effects of dietary quality on serum CRP concentrations in adolescents suggesting significant inverse and null relationships (68, 95). In 5198 German adolescents, ages 12 to 17 years, Truthmann et al. used three different dietary indices (the Healthy Food Diversity-Index (HFI), the Healthy Nutrition Score for Kids and Youth (HuSKY), and the Indicator Food Index (IFI)) to measure diet quality and assess its relationship with CRP (95). Mean values of CRP decreased signficantly across increasing tertiles of IFI (β= -14.005 p=0.007) in 2,438 girls and across increasing tertiles of HuSKY in girls (β= -8.215 p=0.05) and 2,554 boys (β=-8.205 p=0.03) after controlling for age, energy intake, BMI, alcohol consumption, season, physical activity, smoking status, and family socio-economic status (95). No significant associations were observed between HFI scores and concentrations of CRP. In a different study, Lazarou et al. used two indexes to measure diet quality, the Mediterranean Diet Quality Index for children and adolescents (KIDMED) and the Dietary Inflammation Index (DII). Results did not reveal significant associations between KIDMED scores and stratified concentrations of hs-CRP (<0.10 mg/dL and ≥ 0.10 mg/dL) in 83 children aged 6 to 12 years (68). It is postulated that specific inflammatory foods, including fried and fatty foods and sweets, may be the foods which increase CRP levels and these foods were not captured well by the KIDMED index (68). Therefore, the researchers decided to use the DII after results revealed a non-significant association between KIDMED scores and hs-CRP levels. Three logististic regression models were used to evaluate the relationships between BMI, WC, and body fat pecent with hs-CRP and DII had a borderline statistically significant positive correlation with concentrations of hs-CRP. This study is of specific interest because the main objective was to assess the relationship between anthropometric measures of overall and central obesity with concentrations of hs-CRP while controlling for age, gender, physical activity, and diet quality as measured by KIDMED and DII. Although limited to a small sample of children and the use of anthropometric indexes of adiposity, this was the second study to account for diet quality as a potential confounder in adolescent populations. In doing so, DII only explained an estimated 5% of the variance in hs-CRP while the whole model explained up to 30% of the variance in hs-CRP (68). These findings suggest that diet-related inflammation is a minor player in comparison to adiposity-associated inflammatory exposure. The use of dietary indexes to measure foods associated with the targeted health outcome is critical to understanding the true nature of these relationships in adolescents. With so few exitsing studies, more work is needed in order to evaluate the association between dietary quality and inflammation. To date, no studies have evaluated this relationship in Hispanic American girls. As a result, the YHEI was used in this dissertation as it measure dietary quality and assess the relationship between diet quality and inflammation in otherwise healthy Hispanic American adolescents.

It is important to note that healthy adolescents typically do not experience chronic low-grade inflammation unless they are prone to acute infection. Chronic low-grade inflammation is more commonly observed in adults. Nonetheless, to date no longitudinal analyses have been conducted to evaluate the relationship of diet quality on inflammatory biomarkers in adolescents at multiple time points which would provide more detail on the lasting effects of this relationship. Several studies in adults have confirmed an inverse association between diet quality and biomarkers of cardiovascular status, using a variety of dietary indexes (178-184). As a result, it is likely that attempts to observe strong relationships between diet quality and biomarkers of inflammation in adolescents may best be assessed by longitudinal analyses in order to collect more consistent measures of low-grade inflammation in youths. Identifying relationships between diet quality and CVD risk factors in adolescents will be useful for the future development of clinical trials and ultimately clarify the health implications of diet quality in this population. Only cross-sectional data was available for this dissertation, however, there is a capacity to assess these relationships longitudinally in the future once the parent study has concluded.

Purpose and Aims

The primary aims of the current study were:

Specific Aim 1: To assess the relationship between diet quality and adiposity (total and regional body fat).

Hypothesis: Higher diet quality (higher total YHEI score) will be associated with lower adiposity, as measured by DXA and pQCT.

Specific Aim 2: To assess the relationship between diet quality and inflammation.

Hypothesis: Higher diet quality (higher total YHEI score) will be associated with lower inflammation.

Specific Aim 3: To assess the relationships between inflammation and total and regional adiposity, as measured by pQCT and DXA.

Hypothesis: Lower adiposity will be associated with lower inflammation.

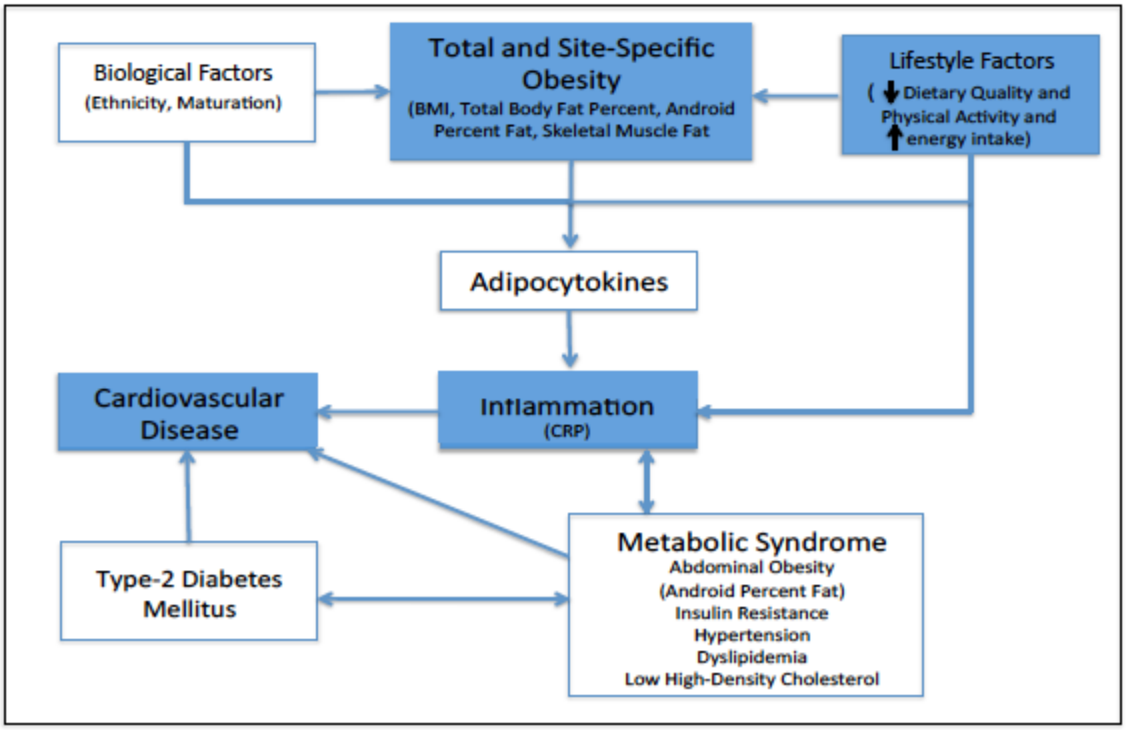

Figure 1. The role of lifestyle and biological factors, obesity and inflammation in cardiovascular disease risk (*adapted from* Sypniewska G. ***Laboratory assessment of cardiometabolic risk in overweight and obese children.*** **Clinical biochemistry 2015.)**

The obesity epidemic is multifactorial, influenced greatly by lifestyle and biological factors. Lifestyle factors are modifiable determinants of obesity and its associated metabolic risks. Obesity has been acknowledge as an inflammatory disease characterized by the expression of pro-inflammatory cytokines. The obesity-cardiovascular disease profile has been largely attributable to low-grade chronic inflammation. Total and regional adiposity, including visceral and skeletal muscle fat have been associated with metabolic impairment and dysfunction. Visceral adiposity has been found to pose the greatest risk for metabolic disturbances, characterized by a greater pro-inflammatory profile compared to subcutaneous fat. Visceral adiposity, in combination with other risk factors for disease, have been coined with the term "metabolic syndrome" and have independently and collectively been associated with increased risk of type-2 diabetes mellitus and cardiovascular disease. (Parentheses indicate factors assessed in the present dissertation)

This dissertation is designed to address many of the limitations of past work. For example, this is the first study to evaluate relationships between dietary quality, total and regional adiposity, and inflammation (concentrations of hs-CRP) in a large sample of Hispanic American and non-Hispanic girls across all BMI categories. DXA and pQCT technologies were used to obtain multiple measures of total and regional adiposity for all participants making this is the first study to have multiple direct measures of adiposity, including both total and regional areas in a large sample of adolescents. This dissertation will also expand on the sparse work that has used the YHEI to assess dietary quality in youths. This will be the first study to assess the relationship between total score and individual component scores of the YHEI and inflammation and multiple direct measures of adiposity. Multivariate analyses that control for potential confounders will also be utilized in this dissertation, a major limitation of many previous studies. This is the first study to adjust for diet quality, BMI, ethnicity, maturity, total energy intake, and physical activity when evaluating the relationships between total and regional adiposity and inflammation in a sample of Hispanic American and non-Hispanic adolescents. Additionally, this will be one of few studies to control for diet quality when assessing the relationships between adiposity and inflammation in youths. When assessing relationships between regional adiposity and hs-CRP, BMI will additionally be controlled in order to adjust for the influence of overall adiposity. These results will enhance our understanding of the relationships between diet quality and cardiometabolic risk factors in Hispanic American and non-Hispanic adolescent girls.

CHAPTER 2

METHODS

Study Design

This was a cross-sectional study using pooled baseline data from 576 adolescent girls enrolled in one of two studies, The Jump-In: Building Better Bones study (176) or the Soft Tissue and Bone Development in Young Girls study (STAR) were included in the present analysis. Jump-In was a group-randomized, controlled trial evaluating the effects of a 2-year structured jumping intervention on bone development in pre-pubertal and early pubertal girls (185). Jump-In participants were recruited from elementary and middle schools within two school districts in Tucson, Arizona.

STAR is an ongoing prospective cohort study in normal weight (BMI 5th to 85th percentile), over-weight (BMI 85^{th} to 95th percentile), and obese (BMI>95^{th} percentile) pre-menarcheal girls. STAR participants were recruited from schools, pediatric offices, and community groups in Tucson, Arizona. The University of Arizona Human Subjects Protection Committee approved both Jump-In and STAR studies; written consent was obtained for all participants.

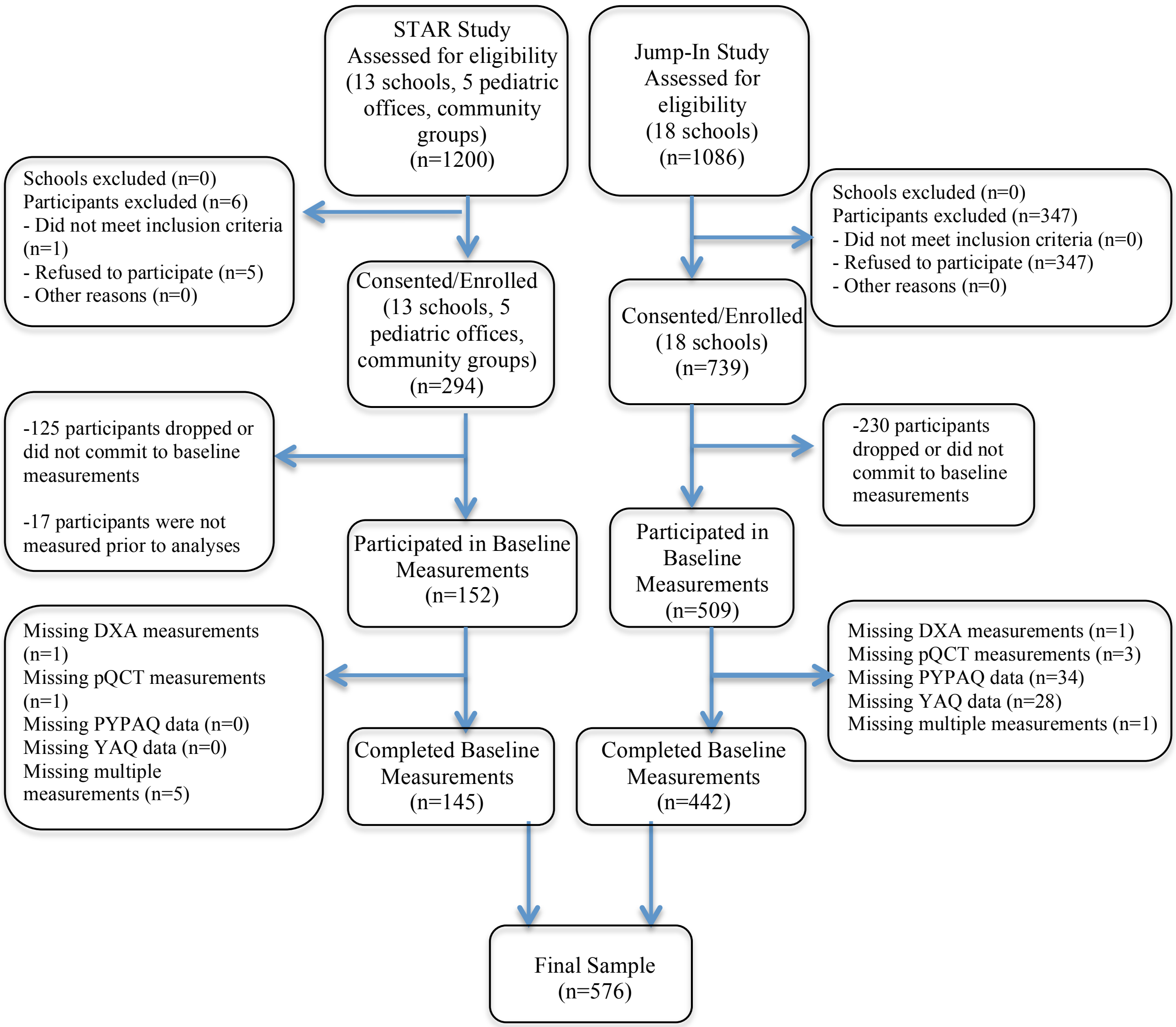

Figure 2. CONSORT flowchart describing the progress of participants through the STAR and Jump-In studies. [*adapted from* **Farr.** ***Influences of soft tissue composition and physical activity n bone volumetric density, bone geometry, and fracture prevalence in young girls.*** **2011. Retrieved from ProQuest Dissertations and Theses. (Accession Order No. AAT 3450728)]**

Participants

This dissertation included data for 434 healthy girls, aged 8-13 years, who were participants in the Jump-In study and 142 healthy girls, aged 9 to 13 years, who were participants in STAR. Jump-In exclusion criteria included the inability to read and understand English, learning disabilities identified by the school that made it impossible to complete questionnaires, medications, medical conditions, or a disability that limited physical exercise participation. Pre-pubertal females aged 9 to 12 years who had a BMI greater than or equal to the 5^{th} percentile were eligible for STAR. Girls were excluded if they had a previous diagnosis of type 1 or type 2 diabetes, used medications (i.e. growth hormone, albuterol) that alter body composition and bone mineral accrual; had a physical disability that could limit participation in physical activity; or diagnosis of a learning disability that would limit completion of questionnaires. A CONSORT flowchart showing the sample of participants is presented in **Figure 2.** Overall, the average age of participants was 10.7±1.1 years, with a range of 8.8-13.2 years, and 37.5% of participants reported Hispanic ethnicity. On average, the girls were 1.37±1.1 years from achieving peak height velocity, with no difference detected between Hispanic and non-Hispanic girls.

Dietary Assessment

Dietary intake was estimated using the semi-quantitative Youth Adolescent Questionnaire (YAQ), a food frequency questionnaire validated by Rockett et al. in adolescents (174, 175). Jump-In participants used the 1995 version of the YAQ which asks respondents to report their usual frequency of consumption of 132 food items during the past year including specified serving sizes. STAR participants used the 2012 version of the YAQ, which included an additional 22 food items, for a total of 154 items as compared to

132 on the 1995 version. The 2012 YAQ also excluded some of the behavioral frequency questions that evaluated the frequency of preparing dinner for oneself and/or other family members and the frequency of consuming breakfast, lunch, snacks, and dinner outside of the home. Participants could receive assistance to complete the form from trained study technicians or a parent/guardian, if needed. YAQs were evaluated for completeness, coded by trained staff following a standard protocol and were subsequently sent to Channing Laboratories (Boston, MA) for food and nutrient analyses that have been described elsewhere (186).

Youth Healthy Eating Index

Feskanich et al. modified the validated and widely used Healthy Eating Index (HEI), created by the USDA, in order to develop the YHEI to accurately assess the dietary habits specific to children and adolescents (154). Rather than calculating specific nutrient intakes, the YHEI, which is described in detail elsewhere (35), focuses on food choices to assess fat, sugar, fiber and sodium intake, including high trans-fat snack foods and sugar-sweetened beverages (components 6 and 7), and also includes behaviors associated with scholastic performance and healthy dietary intake patterns (components 12 and 13). YHEI scores were modified from those of the HEI to reflect the 5-A-Day serving size recommendations developed by the National Cancer Institute (174, 175). Frequency factors developed by Feskanich et al. (154) were used to determine the number of daily serving sizes for each component. These serving sizes were then used to determine the YHEI score for each component.

A modified version of the YHEI scoring was used in this study. Specifically, three components of the original YHEI could not be accurately assessed or calculated in this study due to restricted food and frequency questions on both the 1995 and 2012 versions of the YAQ. These components included the consumption of visible fat and skin (component 11), a second measure of saturated fat intake with lower weight (5 points) versus the 10-point meat ratio (component 5), and two behavioral components including the frequency of eating dinner with family (component 12) and the frequency of breakfast consumption (component 13). Seven components of the modified and original versions of the YHEI were scored from 0 to 10 points and were derived from multiple foods and questions from the YAQ. These included fruit, vegetables, whole grains, dairy, meat ratio (higher lean protein: higher fat protein), snack foods (high in salt or sugar), and soda and drinks (i.e. regular soda, fruit punch, and sweetened iced tea). Three components of the YHEI, including multivitamin use, margarine and butter, and fried foods were scored from 0 to 5 points, consistent with previous literature on scoring parameters and reflecting the limited number of foods and questions from the YAQ that captured these exposures (154). Feskanich et al. previously demonstrated that 85% of the variation in YHEI scores in a sample of 16,452 adolescents was due to 5 of the 7 most heavily weighted components (whole grains, fruits, meat ratio, snack foods, soda and drinks), which we assessed, while the 3 components excluded from the modified YHEI used here accounted for only 5% of the variation in the total YHEI score. Thus, the modified YHEI score used here with a maximum score of 85 (compared to the original 100-point YHEI) is adequate to capture the diet quality of our sample of adolescent girls. The scoring criteria for the original and modified versions of the YHEI are found in **Table 1.** A higher total YHEI score reflects better diet quality; therefore, higher values for components 6, 7, 9, and 10 reflect lower consumption, given that those components represent energy dense foods.

Table 1. Youth Healthy Eating Index scoring criteria

		YHEI scoring criteria	
Original YHEI Components	**Modified YHEI Components**	**Requirement for minimum score of 0**	**Requirements for maximum score of 10**
		servings/day[1]	
1. Whole Grains	1. Whole Grains	0	≥3
2. Vegetables	2. Vegetables	0	≥3
3. Fruits	3 Fruits	0	≥3
4. Dairy	4. Dairy	0	≥2
5. Meat Ratio[2]	5. Meat Ratio[2]	0	≥2
6. Snack Foods[3]	6. Snack Foods[3]	≥3	0
7. Soda and Drinks	7. Soda and Drinks	≥3	0
		Requirement for minimum score of 0	**Requirements for maximum score of 5**
		servings/day[1]	
8. Multivitamin use	8. Multivitamin use	Never	Daily
9. Margarine and butter use	9. Margarine and butter use	≥2 pats/day	Never
10. Fried foods outside home	10. Fried foods outside home	Daily	Never
11. Visible animal fat[4]	-	None	All
13. Dinner with family	-	≥5 times/week	Never
14. Eat breakfast	-	Daily	Never
Total YHEI score (0-100)	Total YHEI score (0-85)		

[1]Jump-In and STAR studies used the Youth/Adolescent Questionnaire (YAQ) to assess habitual dietary intake in adolescent girls ages 8-13 years. Serving sizes are based on definitions of the YAQ.

[2] Total servings per day of white meat including chicken, fish, seafood, eggs, soy, tofu, beans, and nuts were divided by servings per day of dark meat including beef, pork, and lamb.

[3]Snack foods include salty snacks (e.g., potato chips, corn chips, popcorn, pretzels, and crackers) and snacks with added sugar (e.g., cake, snack cake, toaster pastry, sweet roll/Danish/pastry, doughnut, brownie, cookies, pie, chocolate, candy bar with chocolate, candy without chocolate, fruit rollup, popsicle, and flavored gelatin).

[4]Visible animal fat includes the visible fat on meat and the skin on chicken or turkey.

Laboratory Methods

After an overnight 12-h fast, blood samples were collected from all subjects through venous puncture by a trained phlebotomist. Blood for serum samples were collected in serum-separating tubes and allowed to clot. The serum was separated by centrifugation at 3,000 rpm for 15 minutes at 20°C. The serum was then divided into aliquots, frozen, and stored at−80°C until assayed. Serum hs-CRP concentrations were measured by latex-enhanced nephelometry at the University of Washington Department of Laboratory Medicine [BN-II nephelometer, Siemens]. Low and high inter-assay quality control procedures were used and the coefficient of variations were 3.75% to 4.64%, respectively. The assay could detect a minimal concentration of 0.2 mg/L, and values below this level were classified as “non-detectable”. Approximately half of participants had “non-detectable” concentrations of hs-CRP. Greater than 50% of adolescent participants with non-detectable CRP concentrations has been reported in other studies thereby justifying the inclusion of the participants in the current analyses (38).

Anthropometry

Anthropometric data were collected on all participants using identical methods. Body weight and height were measured by technicians following standard protocols as described in the Anthropometric Standardization Reference Manual (187). Weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Model 881; Seca, Hamburg, Germany). Standing height, measured to the nearest 0.1 cm, was assessed at full inhalation using a stadiometer (Shorr Height Measuring Board, Olney). The mean of two measurements was taken, for each anthropometric variable. BMI (kg/m^2) was calculated from height and weight.

Body Composition

Dual-energy x-ray absorptiometry (DXA) was used to quantify total fat mass, percent body fat (ratio of fat mass to whole body mass), and android percent fat as a surrogate of visceral fat, using the GE Lunar PRODIGY [GE Lunar Radiation Corp; Madison, WI, USA] (software version 5.60.003). Participants were positioned for whole body scans using standard manufacturer protocols. DXA coefficients of variation and precision in our laboratory have been reported elsewhere (188). All scans were completed and analyzed by a certified technician following a standard protocol using the extended research mode software. Android fat was estimated for the area enclosed by demarcations immediately above the iliac crest and at 20% of the total distance between the iliac crest and the base of the skull (approximately the bottom rib), using the following equation: [(Android fat mass) / (Android Bone Mineral Content + Android fat mass + Android lean mass)] x 100.

Peripheral quantitative computed tomography (pQCT), a low-dose radiation technique, was used to measure muscle density, a surrogate for skeletal muscle fat (108). This technique is not capable of distinguishing intra-myocellular from extra-myocellular fat and therefore cannot directly measure fat infiltration in skeletal muscle. Thus, muscle density reflects both intra- and extra-myocellular fat stores and is inversely related to muscle fat content such that a lower muscle density is indicative of higher skeletal muscle fat content (108).

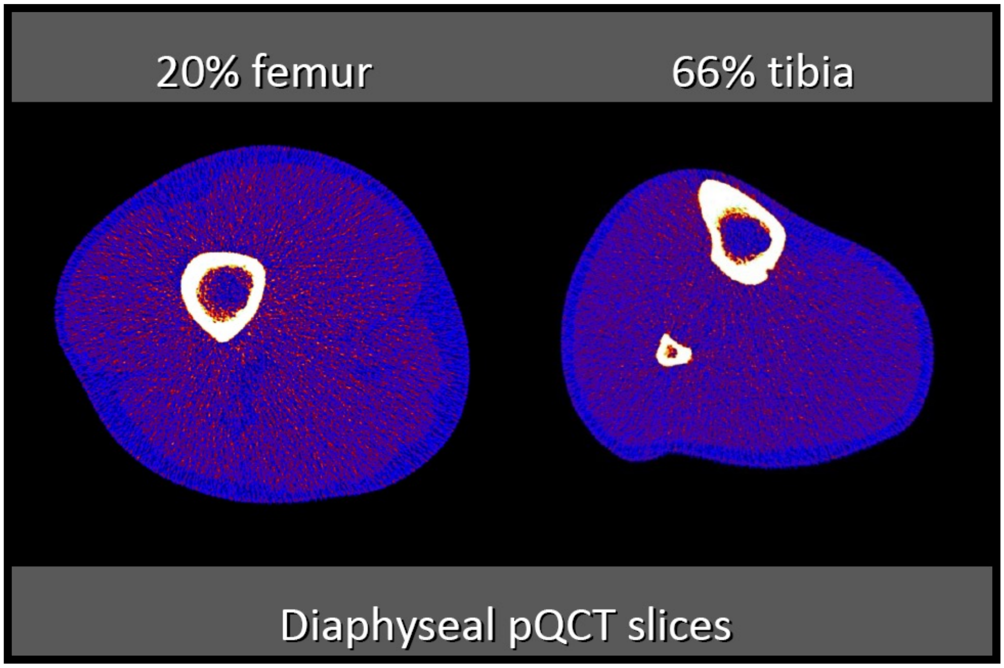

Figure 3. Representative pQCT images of diaphyseal regions of the femur and tibia.

pQCT was used to measure thigh and leg soft tissue composition, including muscle density (mg/cm^3), at the 20% femur (thigh) and 66% tibia (188) sites relative to the respective distal growth plates of the non-dominant limb **Figure 3**. Scans were performed using a Stratec XCT 3000 scanner [Stratec Medical GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY, USA] and analyzed using Stratec software, Version 5.50. All pQCT scans and scan analyses were performed by trained technicians using the guidelines of Bone Diagnostics, Inc. [Fort Atkinson, WI, USA]. Adipose tissue, skeletal muscle, and bone are distinguishable as independent tissues based on attenuation characteristics, which are directly related to tissue composition and density, and can be estimated using edge detection and threshold techniques (109, 189). Images were filtered prior to being analyzed using contour mode 3 (-101 mg/cm^3) and peel mode 2 (40 mg/cm^3) to separate adipose (<40 mg/cm^3) and muscle/bone (-40 mg/cm^3), respectively (109).

Images were filtered subsequently with a 7x7 image filter that clearly defined the edge of the muscle and eliminated all bone above 120 mg/cm^3 (190). This method ensures that muscle density was estimated from only soft tissue within the edge of the muscle.

Physical Maturation

Maturation was assessed using the validated gender-specific equation developed by Mirwald et al. to estimate maturity offset, an estimate of years from peak height velocity (PHV), from chronological age, weight, and ratios of skeletal lengths (191). Mirwald's equation was derived using data from a longitudinal study in boys and girls (192). In Mirwald's sample, the maturity offset equation explained 89% of the variance of years from PHV for girls (191). A negative maturity offset value represents years before PHV, whereas a positive maturity offset value represents years after PHV. Maturity offset was estimated using the following equation: Maturity offset (y) = -9.376 + 0.0001882*Leg Length (cm) and Sitting Height (cm) interaction + 0.0022*Age (y) and Leg Length (cm) interaction + 0.005841*Age (y) and Sitting Height (cm) interaction – 0.002658*Age (y) and Weight (kg) interaction + 0.07693*Weight (kg) by Height (cm) ratio.

Physical Activity

The validated Past Year Physical Activity Questionnaire (PYPAQ) was used to assess physical activity (193). PYPAQ assesses participation in 41 common sport and leisure-time activities performed ≥10 times in the past year (194). Average duration, average weekly frequency, number of months of participation for each activity were obtained and total PYPAQ score was computed using a modified algorithm which has been reported elsewhere (194).

Statistical Analysis

Data were checked for outliers and distributions of all continuous variables were assessed for normality using histograms and all variables were tested for skewness and kurtosis. Distributions of total energy intake, PYPAQ score, and BMI were moderately skewed and therefore were log transformed. For descriptive purposes, untransformed means and standard deviations for total energy intake, PYPAQ score, and BMI are reported. Independent t-tests were used to assess differences in characteristics between Hispanic and non-Hispanic participants (Aim 1) and between normal weight and overweight/obese BMI categories (Aim 3). A Chi-square test was used to evaluate differences in BMI percentiles by ethnicity (Aim1). To identify relationships between measures of adiposity, hs-CRP, diet quality, and potential covariates, bivariate correlations were computed using Pearson's r for continuous and Spearman's rho for categorical variables. Multiple linear regression was used to regress measures of adiposity on each independent variable of interest, total YHEI score and individual YHEI component scores, after adjusting for covariates based on known biological relations (Aim 1). Prior to multiple linear regression analyses, all variables were checked for normality, linearity, and homoscedasticity using residual plots. All models assessed the effect of interaction by ethnicity, in which the interaction term was added as a covariate. Partial correlation coefficients from multiple linear regression models were computed to examine the associations between individual YHEI components, YHEI total score, and measures of adiposity, including BMI, total body fat percent, android percent fat, thigh muscle density, and calf muscle density. Variables assessed for confounding that were included in the final models were maturity offset, PYPAQ score, ethnicity, cohort assignment, and total energy intake. Further analyses also controlled for BMI and total body fat percent in the models that had android percent fat and skeletal muscle density as outcome variables, respectively.

Multinomial logistic regression was employed to examine the associations between each independent variable and categories of serum hs-CRP levels in a sub-sample of 113 Hispanic American adolescent girls (Aims 2 and 3). Concentrations of hs-CRP were categorized as “non-detectable” (<0.2 mg/L), “middle”, and “high”. The “middle” and “high” categories were derived based on the median from all detectable concentrations in order to establish equal sample sizes. Sensitivity analyses were conducted by running all analyses excluding observations with hs-CRP concentrations >10 mg/L (n=3), since concentrations greater than >10 mg/L may indicate acute infection or illness (42). There were substantive differences in the results and subsequently these observations were excluded from the final dataset. Adjusted odds ratios (ORs) were computed and respective 95% confidence intervals (95%CIs) and p-values were reported to examine the associations between individual YHEI components, YHEI total score, and categories of serum hs-CRP levels (Aim 2). In order to facilitate the interpretation of the OR, total YHEI score was transformed to represent a 10-unit difference (1 SD change). Potential confounders included in the final models were maturity offset, PYPAQ score, BMI, and total energy intake. In addition to these covariates, when assessing the associations between each individual YHEI components and categories of hs-CRP concentrations, all other individual YHEI components were controlled. Adjusted ORs were computed and respective 95% confidence intervals and p-values were reported to examine the associations between measures of adiposity and categories of serum hs-CRP levels (aim 3). In order to facilitate the interpretation of the OR, total body fat percent and android percent fat were transformed by a 5 percent unit difference. Specifically, due to collinearity between measures of adiposity, adjusted OR from five separate multinomial logistic regression models were computed to examine the associations between BMI, total body fat percentage, android percent fat, and skeletal muscle density, and categories of serum hs-CRP concentrations. Variables assessed for confounding that were included in the final models were maturity offset, PYPAQ, total energy intake, and total YHEI score. In models with outcomes including android percent fat or muscle density, BMI was added into the model to control for total adiposity. A significance level of $p \leq 0.05$ (two-tailed) was used in all tests. All analyses were performed using The Statistical Package for the Social Sciences for Windows, Version 22.0 [SPSS, Chicago, IL, USA].

CHAPTER 3

MAIN FINDINGS

The primary goal of this dissertation was to assess the relationships between diet quality, total and regional adiposity, and metabolic risk factors in Hispanic American and non-Hispanic adolescent girls. Analyses for Aim 1 were based on a combined sample of 576 Hispanic American and non-Hispanic girls who were participants in either Jump-In or STAR. Analyses for Aims 2 and 3 were based on data for 113 Hispanic American girls in the STAR study. The main findings are summarized in this chapter and in three manuscripts included in Appendices 1-3.

Specific Aim 1: To assess the relationship between diet quality and adiposity (total and regional body fat). Hypothesis 1: Higher diet quality (higher total YHEI score) will be associated with lower adiposity, as measured by DXA and pQCT.

Diet quality was assessed by the YHEI and total and regional adiposity was measured using DXA and pQCT in Hispanic American and non-Hispanic girls. It was hypothesized that higher quality diets, represented by a higher total YHEI score, would be associated with lower adiposity at all sites. No statistically significant effect of interaction by ethnicity was detected when the interaction term was included as a covariate; therefore, the data from all participants were collapsed into a single group and examined for influences of dietary quality on measures of adiposity. Descriptive characteristics of Hispanic American, non-Hispanic, and the total sample of girls are shown in **Table 2.**

Overall, the average age was 10.7±1.1 years, with a range of 8.8-13.2 years, and 37.5% of participants reported Hispanic ethnicity. On average, the girls were 1.37±1.1 years from achieving peak height velocity, with no significant difference detected between

Hispanic and non-Hispanic girls. Physical activity was not significantly different between Hispanic and non-Hispanic girls.

The average YHEI score of the total sample was 50.7 ± 8.9 and reported total energy intake was 1868 ± 752 kcals. Hispanic girls had an estimated total energy intake that was 16.0% higher than non-Hispanic girls. The average BMI and total body fat percent for all participants were 19.0 kg/m^2 and 29.0 ± 9.1%, respectively. Based on the U.S. National Center for Health Statistics/Centers for Disease Control and Prevention percentiles for body mass index (BMI, kg/m^2) (17) 3.0% of the sample were underweight (BMI<5^{th} percentile), 69.0% were classified as normal (BMI 5th to 85th percentile), 15.0% were overweight (BMI 85^{th} to 95th percentile), and 13.0% were obese (BMI>95^{th} percentile). A Chi-square test revealed significant differences ($p \leq 0.0001$) in BMI categories between ethnicities. While most girls fell in the normal BMI category for both non-Hispanics (74.0%) and Hispanics (62.0%), with a similar proportion of overweight girls, obesity was more common in Hispanic (21.0%) vs. non-Hispanic girls (7.0 %). Independent t-tests indicated significantly ($p \leq 0.05$) greater scores in Hispanic compared to non-Hispanic girls for the following body composition variables: weight (+10.0%), BMI (+11.0%), total body fat percent (+11.0%), android percent fat (+13%), and thigh muscle density (+1.0%), which is inversely related to muscle fat.

Table 2. Descriptive characteristics of 576 adolescent girls

Characteristics	Non-Hispanic n=360 Mean ± SD	Hispanic n=216 Mean ± SD	Total Sample n=576 Mean ± SD
Age (years)	10.7±1.1	10.8±1.0	10.7±1.1
Maturational Status			
Maturity offset (years)[1]	-1.1±1.0	-1.5±0.5	-1.37±1.1
Dietary Intake/Assessment			
Total energy (kcals)	1762±649	2048±870	1868 ± 752
Total YHEI score [2]	51.9±8.4	51.0±9.5	51.6 ± 9.0
Physical Activity Assessment			
PYPAQ score [3]	5046.5±4536	5381.5±5825.3	5152.8 ± 5053.8
Body Composition			
Height (cm)	144.7±9.9	145.6±9.2	145.1± 9.6
Weight (kg)	39.2±10.8	43.1±13.0	40.9±12.2
BMI (kg/m^2)	18±3	20±4	19 ± 4
BMI Category (%)			
Underweight	4	3	3
Normal weight	74	62	69
Overweight	15	14	15
Obese	7	21	13
Total body percent fat	28±9	31±10	29 ± 9.1
Android percent fat	30±12	34±14	31.6 ± 12.9
Thigh muscle density (mg/cm^3)	76.5±1.6	77.3±2.0	76.8 ± 1.8
Calf muscle density (mg/cm^3)	79.1±1.2	79.1±1.3	79.1 ± 1.2

[1]Maturity offset= estimated years from peak height velocity (PHV)

[2] YHEI score: Youth Healthy Eating Index (modified) 0 (non-adherent) to 85 (full adherence)

[3] PYPAQ score: Past year physical activity

Table 3 shows the descriptive statistics for individual YHEI components and total YHEI score for study participants. Compared to Hispanic girls, despite lower consumption of vegetables ($p \leq 0.03$) and fruit intake ($p = 0.005$), non-Hispanic girls consumed a higher quality diet in regards to sources of protein ($p \leq 0.0001$), multivitamin use ($p \leq 0.0001$), less fried food intake ($p = 0.006$), and fewer sugar-sweetened beverages consumed ($p \leq 0.03$).

Table 3. Youth Healthy Eating Index scores among 576 non-Hispanic and Hispanic American adolescent girls

	YHEI scores, mean (range)		
YHEI component	**Non-Hispanic**	**Hispanic**	**Total Sample**
1. Whole grains	2.9 (0-10)	2.9 (0-10)	2.9 (0-10)
2. Vegetables	4.9 (0.21-10)	5.5 (0.21-10)	5.1 (0.21-10)
3. Fruit	4.9 (0.53-10)	5.7 (0.4-10)	5.2 (0.40-10)
4. Dairy	7.2 (1-10)	7.2 (1.13-10)	7.2 (1-10)
5. Meat ratio[1]	9.9 (0.83-10)	9.3 (0.61-10)	9.6 (0.61-10)
6. Snack foods[1]	5.5 (0-9.87)	5.3 (0-9.73)	5.5 (0-9.87)
7. Soda and drinks	7.9 (0-10)	7.6 (0-10)	7.8 (0-10)
8. Multivitamin use	1.8 (0-5)	1.2 (0-5)	1.6 (0-5)
9. Margarine and butter use	3.5 (0-5)	3.6 (0-5)	3.5 (0-5)
10. Fried foods	3.3 (0-5)	2.9 (0-5)	3.1(0-5)
Total YHEI score	51.9 (28.2-74.6)	51.0 (27.8-78.9)	51.5 (27.8-78.9)

[1] Refer to **Table 1** for variable criteria

Pearson correlation coefficients were computed in order to assess the bivariate relationships between individual YHEI component scores and total YHEI score **Table 4**. All individual YHEI component scores were significantly associated with total YHEI score. There was a small correlation between “Margarine and butter use score” and total YHEI score ($p \leq 0.05$, $r = 0.08$) while all other individual YHEI components were moderately correlated with total YHEI score (all $p \leq 0.0001$ range $r = 0.2 - 0.56$). Results from multiple linear regression analysis showed that all individual YHEI components explained 96.9% of the variation in total YHEI score, after adjustments.

Table 4. Pearson correlation coefficients from bivariate correlations between individual YHEI component and total YHEI score

	YHEI Score
1. Whole Grains	**0.56**[a]
2. Vegetables	**0.55**[a]
3. Fruit	**0.48**[a]
4. Dairy	**0.40**[a]
5. Meat ratio	**0.37**[a]
6. Snack foods	**0.20**[a]
7. Soda and drinks	**0.34**[a]
8. Multivitamin use	**0.41**[a]
9. Margarine and butter use	**0.08**[b]
10. Fried Foods	**0.43**[a]

[a] sig. at $p \leq 0.0001$
[b] sig. at $p \leq 0.05$

Pearson correlation coefficients were computed to assess the bivariate relationships between total YHEI score and individual YHEI component scores and measures of adiposity **Table 5**. Total YHEI score was significantly and inversely associated with BMI ($p \leq 0.0001$, $r = -0.12$), total body fat percent ($p \leq 0.0001$, $r = -0.15$), and android percent fat ($p \leq 0.0001$, $r = -0.15$). Total YHEI score was not significantly associated with muscle density. “Dairy” score was inversely associated with BMI ($p \leq 0.05$, $r = -0.09$) and android percent fat ($p \leq 0.01$, $r = -0.10$). “Meat ratio” score was inversely associated with BMI ($p \leq 0.0001$, $r = -0.17$) total body fat percent ($p \leq 0.0001$, $r = -0.16$), android percent fat ($p \leq 0.0001$, $r = -0.17$) and thigh muscle density ($p \leq 0.01$, $r = -0.11$). “Multivitamin use” score was significantly and inversely associated with BMI ($p \leq 0.01$, $r = -0.12$) total body fat percent ($p \leq 0.001$, $r = -0.14$) and android percent fat ($p \leq 0.001$, $r = -0.15$). Finally, “margarine and butter use” score was significantly and positively associated with thigh muscle density ($p \leq 0.0001$, $r = 0.15$).

Table 5. Pearson correlation coefficients from bivariate relationships between individual YHEI component scores, total YHEI score, and measures of adiposity

	BMI (m/kg^2)	Total Body Percent Fat	Android Percent Fat	Thigh Muscle Density (mg/cm^3)	Calf Muscle Density (mg/cm^3)
Total YHEI score	**-0.12**[d]	**-0.15**[d]	**-0.15**[d]	-0.06	-0.01
Individual YHEI components					
Whole Grains	0.01	0.0001	0.005	-0.003	-0.02
Vegetables	0.01	0.0001	0.01	0.06	-0.02
Fruit	0.03	0.03	0.04	0.001	-0.07
Dairy	**-0.09**[a]	-0.08	**-0.10**[b]	-0.04	0.02
Meat ratio	**-0.17**[d]	**-0.16**[d]	**-0.17**[d]	**-0.11**[b]	0.06
Snack foods	0.007	-0.05	-0.03	0.003	0.05
Soda and drinks	-0.08	**-0.09**[a]	**-0.08**[a]	0.08	0.04
Multivitamin use	**-0.12**[b]	**-0.14**[c]	**-0.15**[c]	-0.04	0.01
Margarine and butter use	0.01	-0.03	-0.003	**0.15**[d]	0.04
Fried Foods	-0.009	-0.03	-0.03	0.0001	0.07

[a]sig. at p≤ 0.05
[b]sig. at p≤ 0.01
[c]sig. at p≤ 0.001
[d]sig. at p≤ 0.0001

Pearson (r) and Spearman (r_s) correlation coefficients were computed to evaluate the bivariate relationships between covariates and measures of adiposity **Table 6.** Total PYPAQ score was significantly and inversely associated with BMI ($p \leq 0.001$, $r = -0.12$), total body fat percent ($p \leq 0.0001$, $r = -0.19$), and android percent fat ($p \leq 0.0001$, $r = -0.18$). Maturity offset was positively associated with BMI ($p \leq 0.0001$, $r = 0.22$), total body fat percent ($p \leq 0.05$, $r = 0.08$), and inversely associated with thigh muscle density ($p \leq 0.001$, $r = -0.13$). Cohort assignment was positively and significantly associated with all measures of adiposity (all $p \leq 0.001$, range $r_s =$ 0.20 - 0.44). Similarly, ethnicity was positively and significantly associated with all measures of adiposity, except for calf muscle density (all $p \leq 0.0001$, r_s range 0.15-0.18). With regard to muscle density, when thigh and calf muscle density were regressed in two separate models with ethnicity and controlling for total muscle area without muscle fat, thigh and calf muscle densities were significantly and inversely associated with ethnicity, however these relationship was attenuated after controlling for total body fat (data not shown).

Table 6. Pearson and Spearman correlation coefficients from bivariate relationships between controlled covariates and measures of adiposity

	BMI (m/kg^2)	**Total Body Percent Fat**	**Android Percent Fat**	**Thigh Muscle Density (mg/cm^3)**	**Calf Muscle Density (mg/cm^3)**
Total PYPAQ score[1]	**-0.12[b]**	**-0.19[c]**	**-0.18[c]**	0.07	0.05
Maturity offset[1]	**0.22[c]**	**0.08[a]**	0.06	**-0.13[b]**	-0.02
Total energy intake[1](kcals)	0.002	0.03	0.03	0.02	-0.04
Cohort assignment[2,3]	**0.24[c]**	**0.24[c]**	**0.26[c]**	**0.44[c]**	**0.20[c]**
Ethnicity[2,4]	**0.15[c]**	**0.16[c]**	**0.17[c]**	**0.18[c]**	0.03

[1]Pearson correlation coefficient
[a]sig. at $p \leq 0.05$
[b]sig. at $p \leq 0.001$
[c]sig. at $p \leq 0.0001$
[2]Spearman correlation coefficient
[3] Category assignment: 1- Jump-In, 2-STAR
[4] Category assignment: 1- non-Hispanic, 2- Hispanic

Partial correlation coefficients from multiple linear regression models were computed to evaluate the association between total YHEI score and measures of adiposity, after controlling for total energy intake, ethnicity, maturity offset, PYPAQ score, and cohort assignment **Table 7.** Total YHEI score was significantly and inversely associated with android percent fat ($p= 0.03$, $\rho = -0.09$). This association remained significant after further controlling for BMI ($p= 0.02$, $\rho = -0.09$). The final model accounted for 74.7% of the variability in android percent fat in which BMI accounted for 70% of the variance. BMI ($p \leq 0.0001$, $\rho= 0.84$), maturity offset ($p \leq 0.0001$, $\rho= -0.22$), and PYPAQ score ($p \leq 0.0001$, $\rho= -0.11$) were all significant contributors in the final model. Additionally, total YHEI score was significantly and inversely associated with total body percent fat ($p \leq 0.01$, $\rho = -0.10$) but not with BMI or muscle density (before or after controlling for total body fat percent). In the model where total body percent fat was the outcome variable, cohort assignment ($p \leq 0.0001$, $\rho = 0.22$), PYPAQ score ($p \leq 0.0001$, $\rho = -0.16$), and maturity offset ($p \leq 0.0001$, $\rho = 0.18$), were also significant contributors. Together, these variables explained 10.4 % of the variance, while YHEI score explained 1% of the variance in total body fat percent.

Table 7: Partial correlations from multiple linear regressions between YHEI total score and measures of adiposity

	BMI (m/kg^2)	**Total Body Percent Fat**	**Android Percent Fat[1]**	**Thigh Muscle Density[2] (mg/cm^3)**	**Calf Muscle Density[2] (mg/cm^3)**
Total YHEI Score	-0.05	**-0.14[a]**	**-0.09[b]**	0.05	0.04

All models controlled for total energy intake, PYPAQ Score, maturity offset, cohort assignment, and ethnicity
[1]Additionally controlled for BMI
[2]Additionally controlled for total body fat percent
[a]sig. at $p \leq 0.01$
[b]sig. at $p \leq 0.05$

Table 8 displays partial correlation coefficients from multiple linear regression models regressing individual YHEI components on measures of adiposity after controlling for maturity offset, total energy intake, PYPAQ score, cohort assignment, and ethnicity. Of the 10 individual components, only 3 were statistically related to measures of adiposity; "margarine and butter use", "meat ratio" score, and "multivitamin use". "Margarine and butter use" and "meat ratio score" were components that were derived to represent fat intake. "Meat ratio score" was inversely associated with BMI ($p \leq 0.05$, $\rho = -0.08$) and positively associated with calf muscle density (i.e. low skeletal muscle fat content) before ($p \leq 0.001$, $\rho = 0.16$) and after controlling for total body fat percent ($p \leq$

0.001, $\rho = 0.14$). In the model with BMI as the outcome variable, cohort assignment ($p \leq 0.0001$, $\rho = 0.24$), PYPAQ score ($p \leq 0.001$, $\rho = -0.12$), and maturity offset ($p \leq 0.0001$, $\rho = 0.33$), were all significant contributors. Similarly, in the model with calf muscle density as the outcome variable, cohort assignment ($p \leq 0.0001$, $\rho = 0.28$), PYPAQ score ($p \leq 0.01$, $\rho = 0.11$), and total body fat percent ($p \leq 0.0001$, $\rho = -0.42$), were significant contributors. The final model accounted for 23.8% of the variability in calf muscle density in which total body fat accounted for 17.6% of the variance. Lower "margarine and butter use" was positively associated with muscle density (i.e. low fat content) of the thigh before ($p \leq 0.001$, $\rho = 0.15$) and after controlling for total body fat percent ($p \leq 0.001$, $\rho = 0.14$). In the model with thigh muscle density as the outcome variable PYPAQ score ($p \leq 0.0001$, $\rho = 0.20$) and maturity offset ($p \leq 0.0001$, $\rho = 0.44$) were also significant contributors. The final model accounted for 31.2% of the variability in calf muscle density in which cohort assignment accounted for 23% of the variance. "Multivitamin use" was inversely associated with android percent fat before and after controlling for BMI ($p \leq 0.05$, $\rho = -0.08$). The final model accounted for 74.6% of the variability in android percent fat in which BMI accounted for 70% of the variance. Maturity offset ($p \leq 0.0001$, $\rho = -0.23$) and PYPAQ score ($p \leq 0.001$, $\rho = -0.11$) were also significant contributors.

Table 8. Partial correlations from multiple linear regressions between select individual YHEI components and measures of adiposity

YHEI Components	BMI (m/kg^2)	Total Body Percent Fat	Android Percent Fat[1]	Thigh Muscle Density[2] (mg/cm^3)	Calf Muscle Density[2] (mg/cm^3)
Margarine and butter use	-0.01	-0.05	-0.05	**0.14**[b]	0.003
Meat ratio	**-0.08**[a]	-0.08	-0.02	0.07	**0.14**[b]
Multivitamin use	-0.05	-0.07	**-0.08**[a]	0.002	0.01

All models controlled for total energy intake, PYPAQ score, maturity offset, cohort assignment, ethnicity, and all other individual YHEI components when not the independent variable (fruit, vegetables, whole grains, meat ratio, dairy, soda and drinks, margarine and butter use, multivitamin use, fried foods, and snack foods)
[1]Additionally controlled for BMI
[2]Additionally controlled for total percent fat
[a] sig. at $p \leq 0.05$; [b] sig. at $p \leq 0.001$

Specific Aim 2: To assess the relationship between diet quality and inflammation. Hypothesis 2: Higher diet quality (higher total YHEI score) will be associated with lower inflammation.

Diet quality was assessed using the YHEI and inflammation was measured by hs-CRP in order to evaluate the relationship between diet quality and inflammation in Hispanic girls. It was hypothesized that diet quality, represented by a higher total YHEI score, would be associated with lower inflammation.

Descriptive characteristics of the 113 study participants are shown in **Table 9**. The average age of participants was 11.0±1.0 years, with a range of 9.0 to 13.1 years. On average, the girls were 1.9± 0.9 years from achieving peak height velocity. BMI and total body fat percent for all participants averaged 21.0 ± 5.0 kg/m^2 and 32.2 ± 9.8%, respectively. Based on the U.S. National Center for Health Statistics/Centers for Disease Control and Prevention percentiles for body mass index (BMI, kg/m^2) (17) 3.5% of the study sample was underweight (BMI<5^{th} percentile), 55.8% were normal weight (BMI 5th to 85th percentile), 13.3% were overweight (BMI 85th to 95th percentile), and 27.4% were obese (BMI>95th percentile).

The average YHEI score was 48.1 ± 9.7 and average daily energy intake was 2119±968 kcals. Serum hs-CRP concentrations in the “middle” and “high” groups ranged from 0.2 to 0.7 mg/L and 0.7 to 6.2 mg/L, respectively (data not shown). Cardiovascular risk associated with concentrations of CRP are not currently available for youths therefore the widely accepted categories of cardiovascular risk, as determined for adult Caucasians, were used to define risk in this study. There were 13 study participants with hs-CRP concentrations considered as moderate cardiovascular risk (1.0mg/L -3.0 mg/L) and 10 participants with hs-CRP concentrations considered as high cardiovascular risk (>3.0 mg/L) (196).

Table 9. Descriptive characteristics of 113 Hispanic American girls

Characteristics	Mean ± SD
Age (years)	11.0±1.0
Dietary Intake/Assessment	
Total energy (kcals)	2119 ± 968
YHEI score[1]	48.1±9.7
Body Composition	
Height (cm)	146.9±8.8
Weight (kg)	45.8±14.3
BMI (kg/m^2)	21±5
BMI Category (%)	
Underweight	3
Normal weight	55
Overweight	14
Obese	28
Total body percent fat	32.2±9.8
Android percent fat	37.1±14.0
Thigh Muscle Density (mg/cm^3)	78.2±1.8
Calf Muscle Density (mg/cm^3)	79.5±1.4
Maturational Status	
Maturity offset (years)[2]	-1.9±0.9
Physical Activity Assessment	
PYPAQ score[3]	5490.1 ± 5940.2

[1]YHEI score: Youth Healthy Eating Index (modified) 0 (non-adherent) to 85 (full adherence)
[2]Maturity offset= estimated years from peak height velocity (PHV)
[3]PYPAQ score: Past year physical activity

Table 10 shows the descriptive statistics for individual YHEI components and total YHEI score for study participants. Out of a total of ten points for each component towards the total YHEI score, average “fruit” score was 5.78, “vegetables” score was 5.87, “dairy” score was 6.98, “whole grains” score was 3.37, “meat ratio” score was 8.85, “snack foods” score was 5.31, and “soda and drinks” score was 7.75. Out of a total of five points for each component towards the total YHEI score, average “multivitamin use” score was 1.0, “margarine and butter use” score was 3.66, and “fried foods” score was 2.98.

Table 10. Youth Healthy Eating Index scores among a subsample of 113 Hispanic American girls

YHEI component	YHEI scores, mean (range)
	(Maximum total 10 points)
Fruit	5.78 (0.62-10)
Vegetables	5.87 (0.49-10)
Dairy	6.98 (1.13-10)
Whole grains	3.51 (0-10)
Meat ratio[1]	8.85 (1.31-10)
Snack foods[1]	5.31 (0-9.73)
Soda and drinks	7.75 (0-10)
	(Maximum total 5 points)
Multivitamin Use	1.0 (0-5)
Margarine and butter use	3.66 (0.25-5)
Fried foods	2.98 (0-5)
YHEI total score	48.10 (25.33-72.69)

[1]Refer to **Table 1** for variable criteria

Spearman correlation coefficients were computed to assess the bivariate relationship between total YHEI score, individual YHEI component scores, and categories of hs-CRP **Table 11**. There was no significant association between total YHEI score and categories of hs-CRP. Regarding individual YHEI components, only “margarine and butter use” showed a significant and positive association with categories of hs-CRP ($p \leq 0.05$, $r_s = 0.27$).

Table 11. Spearman correlation coefficients from bivariate correlations between individual YHEI component scores, total YHEI score, and categories of hs-CRP

	hs-CRP categories (mg/L)
Total YHEI score	0.09
Individual YHEI components	
Whole Grains	0.10
Vegetables	-0.01
Fruit	-0.04
Dairy	-0.12
Meat ratio	0.06
Snack foods	0.05
Soda and drinks	0.10
Multivitamin use	0.02
Margarine and butter use	**0.27**[a]
Fried foods	0.05

[a]sig. at $p \leq 0.05$
[b] sig. at $p \leq 0.0001$

Spearman correlation coefficients were computed to assess the bivariate relationships between measures of adiposity and categories of hs-CRP **Table 12**. Only total body fat percent showed a significant positive association with categories of hs-CRP, of moderate effect ($p \leq 0.0001$, $r_s = 0.37$).

Table 12. Spearman correlation coefficients from bivariate correlations between covariates and categories of hs-CRP

	hs-CRP categories (mg/L)
Total PYPAQ score[1]	-0.12
Total body fat percent	**0.37**[a]
Maturity offset (years)[1]	-0.04
Total energy intake (kcals)	-0.15

[a]sig. at $p \leq 0.0001$
[1]Refer to **Table 9** for definition

Adjusted odds ratios and their respective 95% CI and p-values from multinomial logistic regression models are reported in **Table 13.** Results indicate that the odds of being in the “middle” or “high” categories was not significantly associated with total YHEI score. For every 1-unit increase in “whole grains” score there was a 44% increase in odds of being in the “high” category compared to the “undetectable” category, after adjusting for maturity offset, PYPAQ score, total energy intake, total body fat, and all other individual YHEI components (OR: 1.44, 95% CI: 1.06, 1.97)

Table 13. Adjusted odds ratios from multinomial logistic regression models between YHEI total and individual component scores and categories of serum hs-CRP concentrations

	Middle[1] [hs-CRP (mg/L)]	**High[1] [hs-CRP (mg/L)]**
Total YHEI Score[2,3]	1.01 (0.96, 1.07)	1.05 (0.99, 1.12)
Fruit[4]	0.87 (0.67, 1.15)	1.18 (0.86, 1.62)
Vegetables[4]	1.06 (0.87, 1.30)	1.01 (0.78, 1.30)
Whole grains[4]	1.00 (0.77, 1.31)	**1.44 (1.06, 1.97)**[a]
Meat ratio[4]	1.11 (0.80, 1.54)	1.10 (0.77, 1.58)
Dairy[4]	1.20 (0.90, 1.61)	0.74 (0.53, 1.03)
Snacks[4]	0.76 (0.58, 1.01)	0.84 (0.60, 1.18)
Margarine and butter use[4]	1.32 (0.69, 2.56)	1.66 (0.65, 4.26)
Multivitamin use[4]	1.10 (0.78, 1.55)	1.09 (0.72, 1.65)
Soda and drinks[4]	1.21 (0.88, 1.66)	0.87 (0.62, 1,21)
Fried foods[4]	1.10 (0.74, 1.63)	0.99 (0.61, 1.59)

[1]Compared to reference group: “non-detectable”
[2]Model 1 controlled for: maturity offset, PYPAQ score, total energy intake, total body fat percent
[3] Total YHEI score was transformed to represent a 10-unit difference (1 SD change)
[a]sig. at p<0.05

[4]Model 2 controlled for Model 1 covariates and all other individual YHEI components when not the independent variable (fruit, vegetables, whole grains, meat ratio, dairy, snacks, margarine and butter use, multivitamin use, soda and drinks, fried foods)

Table 14 describes the characteristics of study participants who reported consuming <2 and ≥ 2 servings of whole grains per day. Girls who reported consuming greater than or equal to two servings of whole grains per day also reported greater energy intake (3020 ± 1070 kcals/day vs. 2068 ± 943 kcals/day) and fat intake (101.0 ± 40.0 grams/day vs. 71.0 ± 37.0 grams/day) and less physical activity, as measured by PYPAQ score (4820.2 ± 3559.6 vs. 5527.7 ± 6054.6) compared to those who reported consuming less than two servings a day. On average, girls who reported consuming greater than or equal to two servings of whole grains per day had approximately 2.5 ± 0.34 servings of whole grains a day compared to 0.63 ± 0.45 reported by those in the less than two servings per day group. Girls who reported consuming greater than or equal to two servings of whole grains per day had slightly less total percent fat (31.8 ±11.1% vs. 32.2 ± 9.8 %), android percent fat (36.0 ± 16.8% vs. 37.1 ± 13.9%) and BMI (20 ± 4 kg/m^2 vs. 21 ± 5 kg/m^2).

Table 14. Descriptive characteristics of study participants who reported < 2 or ≥ 2 servings per day of whole grains

	Whole grains < 2 servings/day (n=107)	**Whole grains ≥ 2 servings/day (n=6)**
Total energy intake (kcals/day)	2068 ± 943	3020 ± 1070
Total fat intake (grams/day)	71.0 ± 36.7	101.0 ± 39.9
YHEI total score	47.6 ± 9.5	56.6 ± 8.7
Average whole grains (servings/day)	0.63 ± 0.45	2.5 ± 0.34
PYPAQ score	5527.7 ± 6054.6	4820.2 ± 3559.6
Total body fat percent	32.2 ± 9.8	31.8 ± 11.1
Android percent fat	37.1 ± 13.9	36.0 ± 16.8
BMI (kg/m^2)	21 ± 5	20 ± 4

Specific Aim 3: To assess the relationships between inflammation and total and regional adiposity, as measured by pQCT and DXA. Hypothesis 3: Lower adiposity will be associated with lower inflammation.

DXA and pQCT technologies were used to measure total and regional adiposity and inflammation was measured by hs-CRP in order to evaluate the relationships between adiposity and inflammation in Hispanic girls, after controlling for total energy intake, total YHEI score, PYPAQ score, and maturity offset. In models with android percent fat or muscle density as independent variables, BMI or total body fat percent was added into the model, respectively, in order to control for total adiposity. It was hypothesized that lower adiposity, at all sites, would be associated with lower inflammation.

The descriptive characteristics of study participants are given for the total sample and by normal weight and overweight/obese BMI categories in **Table 15.** Characteristics for the total sample have previously been described (Aim 2). The majority of "non-detectable" hs-CRP concentrations were found in normal weight participants (70%). The number of participants with detectable hs-CRP concentrations was comparable between normal weight (n= 24) and overweight/obese participants (n= 28). The average detectable hs-CRP concentration in normal weight participants was 0.9 ± 1.5 mg/L with a range of 0.2-6.2 mg/L. The average detectable hs-CRP concentration in overweight/obese participants was 2.0 ± 1.4 mg/L with a range of 0.3 – 5.5 mg/L. Results from independent t-test identified significant differences by BMI categories. The overweight/obese group had significantly higher BMI (+47%), total body fat percent (+63%), android percent fat (+85%), and lower calf muscle density (-2%), compared to their leaner peers (all $p<0.01$).

Based on categories of hs-CRP the average serum hs-CRP concentration for the "middle" group ranged from 0.2 to 0.7 mg/L and from 0.7 to 6.2 mg/L for the "high" group.

There were 13 study participants with hs-CRP concentrations that would categorize them as moderate cardiovascular risk (1.0mg/L -3.0 mg/L) and 10 participants with hs-CRP concentrations that would categorize them at high cardiovascular risk (>3.0 mg/L) (196). Girls with "non-detectable" hs-CRP comprised 54% of the sample.

Table 15. Descriptive characteristics of 113 Hispanic American adolescent girls

Characteristics	**Normal weight BMI 5th to 85th % n=67 (58%) Mean ± SD**	**Overweight/Obese BMI >85th % n=46 (42%) Mean ± SD**	**Total Sample n=113 Mean ± SD**
Age (years)	11.0 ± 1.0	11.0 ± 1.0	11.0±1.0
C-Reactive Protein			
non-detectable/detectable (n)	43/24	18/28	61/52
Average serum hs-CRP (mg/L)[1]	0.9 ± 1.5	2.0 ± 1.4	1.5 ± 1.5
Body Composition			
Height (cm)	144.3 ± 8.4	150.7 ± 7.9	146.9 ± 8.8
Weight (kg)	36.8 ± 7.0	58.7 ± 12.1	45.8 ± 14.3
BMI (kg/m^2)	17 ± 2	25 ± 3	21 ± 5
Total body percent fat	25.6 ± 6.4	41.9 ± 4.0	32.2 ± 9.8
Android percent fat	27.5 ± 9.5	51.0 ± 4.7	37.1 ± 14.0
Thigh Muscle Density (mg/cm^3)	78.4 ± 1.7	78.1 ± 1.8	78.2 ± 1.8
Calf Muscle Density (mg/cm^3)	80.1 ± 1.0	78.6 ± 1.5	79.5 ± 1.4
Maturational Status			
Maturity offset (years)[1]	-1.7 ± 0.9	-2.0 ± 0.7	-1.9 ± 0.9
Dietary Intake Assessment			
Total energy (kcals)	2225 ± 923	1964 ± 1022	2119 ± 968
YHEI score[3]	48.8 ± 10.0	47.0 ± 9.2	48.1 ± 9.7
Physical Activity Assessment			
PYPAQ score[4]	5936.9 ± 5863.7	4839.2 ± 6055.0	5490.1 ± 5940.2

[1]Average serum hs-CRP for all detectable concentrations (n=52)
[2]Maturity offset= estimated years from peak height velocity (PHV)
[3]YHEI score: Youth Healthy Eating Index (modified) 0 (non-adherent) to 85 (full adherence)
[4]PYPAQ score: Past year physical activity

The bivariate relationships between controlled covariates and categories of hs-CRP have been previously reported **Table 12.** Spearman correlation coefficients were computed to assess the bivariate relationships between measures of adiposity and categories of hs-CRP **Table 16**. BMI ($p \leq 0.0001$, $r_s = 0.35$), total body fat percent ($p \leq 0.0001$, $r_s = 0.37$), and android percent fat ($p \leq 0.0001$, $r_s = 0.39$) were all positively and significantly associated with categories of hs-CRP, with a moderate effect. Calf muscle density was significantly and inversely associated with categories of hs-CRP ($p \leq 0.05$, $r_s = -0.21$). Thigh muscle density was not significantly associated with categories of hs-CRP.

Table 16. Spearman correlation coefficients from bivariate relationships between measures of adiposity and categories of hs-CRP

	hs-CRP categories (mg/L)
BMI (kg/m^2)	**0.35**[b]
Total Body Fat Percent	**0.37**[b]
Android Percent Fat	**0.39**[b]
Thigh Muscle Density (mg/cm^3)	0.12
Calf Muscle Density (mg/cm^3)	**-0.21**[a]

[a]sig. at $p \leq 0.05$
[b]sig. at $p \leq 0.0001$

Due to collinearity between measures of adiposity, adjusted odds ratios and their respective 95% CI and p-values from five separate multinomial logistic regression models are reported in **Table 17.** Results indicate that for every 1 kg/m^2 increase in BMI, the odds of being in the “high” hs-CRP category increases by 37% compared to the “non-detectable” category, after adjusting for total energy intake, PYPAQ score, maturity offset, and total YHEI score (OR: 1.37, 95% CI 1.18, 1.58, $p<0.0001$). Similarly, for every 5 percent unit increase in total body fat percent and android percent fat, the odds of being in the “high” hs-CRP group compared to the “non-detectable” group were 2.5 and 1.9 times larger, respectively, after adjusting for all other covariates (OR: 2.52, 95% CI 1.65, 3.86, $p<0.0001$, OR: 1.93, 95% CI 1.41, 2.64, $p<0.0001$, respectively). The association between android percent fat and categories of hs-CRP was attenuated after controlling for BMI and was no longer significant. For every 1mg/cm^3 increase in calf muscle density, there were lower odds of being in the “high” hs-CRP category compared to the “non-detectable” group (OR: 0.53, 95% CI 0.36, 0.77, $p<0.001$), although the relationship was attenuated and no longer significant after controlling for total body fat percent. No significant relationship was observed between thigh muscle density and categories of hs-CRP. Similar results were found from unadjusted models between measures of adiposity and categories of hs-CRP (data not shown). When only controlled covariates were entered in the model, total body fat percent was the only covariate that significantly increased the odds of being in the “high” category of hs-CRP compared to the “non-detectable” category (OR: 1.21, 95% CI 1.11, 1.31, $p<0.0001$) (data not shown).

Table 17: Adjusted odd ratios from multinomial logistic regression models between measures of adiposity and categories of hs-CRP concentrations in Hispanic American girls

	Middle[1,2] [hs-CRP (mg/L)] OR (95% CI)	**High[1,3] [hs-CRP (mg/L)] OR (95% CI)**
BMI (kg/m^2)	0.98 (0.87, 1.11)	**1.37 (1.18, 1.58)[a]**
Total Body Fat Percent [4]	0.97 (0.73, 1.29)	**2.52 (1.65, 3.86)[a]**
Android Percent Fat [4]	1.00 (0.84, 1.20)	**1.93 (1.41, 2.64)[a]**
Thigh Muscle Density (mg/cm^3)	1.02 (0.79, 1.33)	1.21(0.91, 1.61)
Calf Muscle Density (mg/cm^3)	1.05 (0.71, 1.55)	**0.53 (0.36, 0.77)[b]**

All models controlled for: maturity offset, PYPAQ score, total energy intake, total YHEI score
[1]Compared to reference group: “undetectable”
[2]Range of hs-CRP concentrations 0.20-0.70 mg/L
[3]Range of hs-CRP concentrations 0.70-6.2 mg/L
[4]Transformed to represent a 5 percent unit difference
[a]sig. at $p<0.0001$ [b]sig. at $p<0.001$

CHAPTER 4

SUMMARY AND CONCLUSIONS

Cross-sectional data from two large, well-characterized samples totaling 576 Hispanic American and non-Hispanic American adolescent girls were analyzed to assess relationships between dietary quality, total and regional adiposity, and metabolic risk in adolescents. Novel findings that add to the literature include:

1. **Higher diet quality scores are associated with lower total and visceral body fat in Hispanic American and non-Hispanic girls (Aim 1).**

2. **Diet quality is not significantly associated with inflammation, as assessed by concentrations of hs-CRP, in Hispanic American girls (Aim 2).**

3. **Greater total and regional adiposity, including visceral body fat and skeletal muscle fat, are associated with increased inflammation, independent of physical activity, total energy intake, maturation, and total YHEI score, in Hispanic American girls (Aim 3).**

1. Higher diet quality scores are associated with lower total and visceral body fat in Hispanic American and non-Hispanic adolescents (Aim 1). These findings are in agreement with cross-sectional studies in adolescents that have reported statistically significant inverse (154, 167, 168, 171, 197) associations between diet quality and measures of adiposity. However, overall findings regarding the relationship between diet quality and adiposity are mixed with other studies reporting null (152, 154, 167-170) or positive (152, 162, 163) associations. Only three studies have used the YHEI diet quality score to assess associations between diet quality and measures of adiposity. In these studies potentially confounding variables were not controlled, limiting their interpretation and comparisons with the present results (154, 167, 176). Only one of the three studies examined associations between YHEI and total and regional adiposity using methods more direct than anthropometry. In that

subsample of 196 African American adolescents (49% females), the YHEI score was inversely related with DXA measures of total body fat (r= -0.09) and android percent fat (r= -0.09) (45). Although these correlation coefficients were not significant (167), their magnitude was similar to the coefficients reported herein. In agreement with our multivariate findings, the YHEI score was not significantly associated with BMI in a large study of 16,452 adolescents (154) as well as in a smaller study by Vitale et al. examining relationships between YHEI total scores and BMI in 45 underweight, normal, and overweight/obese adolescents (176).

In the current analyses, the final models for android percent fat and muscle density included BMI or total body fat percent, respectively. Clearly, total body fat percent is a more accurate assessment of overall fatness compared to BMI, however, total body fat percent was highly correlated with android percent fat ($p<0.0001$ $r=0.97$), therefore, in order to minimize collinearity while still controlling for overall fatness, BMI was used as a covariate. Since total body fat was not highly correlated with thigh or calf muscle density ($p<0.0001$ $r= -0.16$, -0.35, respectively), total body fat percent was controlled in the final models for muscle density. Other significant contributors in the models explaining total body fat and android percent fat were PYPAQ score, maturity offset, and cohort assignment. PYPAQ score was inversely associated android percent fat before and after controlling for BMI, and inversely associated with total body fat (range $\rho= -0.11$ to -0.17 $p=0.008$), suggesting that greater physical activity contributes to lower total and regional body fat. Maturity offset and cohort assignment had positive associations with total body fat ($p<0.0001$ $\rho= 0.18$, $\rho= 0.20$, respectively). While maturity offset and cohort assignment also had positive associations with android percent fat ($p<0.0001$ $\rho= 0.16$, $\rho= 0.22$), the direction of the association with maturity offset changed to an inverse association after controlling for BMI ($p<0.0001$ $\rho= -0.23$) and cohort assignment was no longer significant. This finding suggests that as girls mature they gain more total body fat; girls that are less mature have more android fat independent of their BMI compared to girls who are more mature and these findings were similar between cohorts. These findings are consistent with typical changes in fat distribution in during both before and during puberty (65).

Whereas most past studies have been limited to overall estimates of diet quality, in the current work, associations between individual YHEI components and measures of adiposity were also examined. The results showed some components were significantly associated with adiposity after controlling for biological and lifestyle factors as well as the influence of all other individual components. Pearson correlation coefficients for bivariate relationships between individual YHEI components were $r \leq 0.49$. Since the YHEI components were not highly inter-correlated they were all included in the model. Similarly, bivariate correlations were used to examine the relationships between the individual YHEI components and total energy intake. Snacks and total energy intake had the strongest relationship ($r= -0.63$), however, since the magnitude was moderate, total energy intake remained in the model. The results of these analyses showed that higher intake of leaner proteins and multivitamins and lower consumption of margarine consumption were associated with lower total and/or regional adiposity. "Margarine and butter use" is a component in the YHEI that serves as a surrogate of trans-fatty acids and total fat intake in adolescents. Lower margarine and butter consumption was significantly associated with lower skeletal muscle fat. Similarly, a higher ratio of lean- to fat- protein consumption was associated with lower BMI and lower skeletal muscle fat. The proteins that contribute to fat-protein include those that are higher in fat content, including beef, pork, and lamb. Longitudinal data has shown that higher fat diets are associated with higher BMI in youths (198). This study was the first to assess the relationship between surrogates of fat intake and skeletal muscle fat in healthy adolescent girls. The findings aligned with evidence from studies in adults showing that high-fat diets are associated with higher intramuscular fat. These studies suggest high-fat diets lead to significant increases in intramyocellular lipid content of the tibialis anterior after 2 hours ($p<0.005$) (199) and the vastus lateralis after 7 days ($p=0.007$) (200), compared to a low-fat diet, in young lean adults. Results from the present study also indicate that greater multivitamin use was associated with lower android percent fat. While data in adolescents are limited, evidence in adults suggests that dietary supplement users, in general, are typically health conscious individuals seeking to adopt health-related habits, including better dietary patterns with the greatest prevalence in non-Hispanic Whites (201). In Aim 1, the majority of participants reported being non-Hispanic and compared to Hispanic participants, non-Hispanic participants reported higher multivitamin use. Therefore, a likely explanation for the findings reported in the current study may be due to health conscious parents encouraging their children to adapt health-related habits. Maturation, physical activity, and cohort assignment were additional significant predictors of adiposity. Regarding muscle density, maturation was only significantly correlated with calf muscle density ($p= 0.01$ $\rho= 0.10$) and physical activity was only significant correlated with thigh muscle density ($p<0.0001$ $\rho= 0.16$). Cohort assignment had a moderate significant correlation with muscle density of the thigh and calf ($p<0.0001$ $\rho= 0.48, 0.37$, respectively) suggesting that girls in the STAR study had higher muscle density compared to girls in the Jump-In study, independent of ethnicity, energy intake, maturation, physical activity, and total body fat percent.

2. Diet quality was not significantly associated with inflammation, as assessed by concentrations of hs-CRP, in Hispanic American girls (Aim 2). To date, only two studies have assessed the relationship between diet quality and inflammation in adolescents, using dietary quality scoring (68, 95). These studies reported significant inverse and null findings between diet quality and inflammation, respectively, using two (68) and three (95) dietary indexes. The results of these two studies suggest that dietary quality does not have a consistent relationship with inflammation, as measured by hs-CRP concentrations, even when index scores are derived from the same data, reflecting the differences in diet quality indices based on different component quantities, cut-off values, and scores derived from various diet assessment tools with their own limitations (119). Potential differences between populations, length of follow-up, and approaches to adjusting confounders add to the difficulty of inter-study comparisons (124).

The results of the present study also showed that higher “whole grains” score increase the odds of being in the “high” hs-CRP category compared to the “non-detectable” category. This finding was not anticipated. Results from observational studies collectively suggest an inverse effect between whole grain intake and inflammation (202-205), while results from intervention studies are mixed. In a recent review (206), only one of five intervention studies in adults reported a beneficial association between whole-grain intake and inflammation (207), while the remaining four studies revealed no change in inflammation with whole-grain intake (208-211). A recent randomized controlled crossover trial in 44 overweight or obese girls, aged 8-15 years, reported that the intervention group, in which half of their needed servings of grains came from whole-grains, girls had significant decreases ($p=0.03$) in hs-CRP levels (-21.8%) compared to increases in hs-CRP concentrations (+12.1%) in the control group after 6 weeks (212).

In order to further understand the results, the characteristics of participants who reported consuming < 2 vs. those who reported consuming ≥ 2 servings of whole grains per day were examined. Groups were formed based on greater/equal or less than 2 servings per day because the YHEI awards 10 points to those who report consuming 2 or more servings of whole grains per day. Only six participants reported consuming two or more servings of whole grains per day with an average of 2.5 servings per day and a range from 2 to 3 servings per day. The average daily intake reported by those who consumed less than 2 servings per day (n=107) was 0.62 with a range from 0.0 to 1.8 and an average YHEI score of 3.1. Compared to participants who reported consuming less than 2 servings of whole grains per day, those who reported consuming greater than 2 servings had slightly less total and android percent fat however they had higher hs-CRP values (+8.4%), reported higher energy intake (+46%), lower physical activity (-12%), and reported higher fat intake (+45%). In order to understand which factors of the whole grain score were driving the significant positive relationship with hs-CRP, the reported intake of cold cereal, hot cereal, brown rise, and dark bread servings per day in each group was assessed. Those who reported consuming 2 or more servings of whole grains also reported a significantly greater consumption of dark bread (+407%) compared to those who reported consuming less than 2 servings of whole grains per day. The questionnaire does not distinguish between 100% whole wheat bread and whole wheat bread, therefore the relationship between "whole grains" score and inflammation may be confounded by higher intake of refined grains from bread that is not 100% whole wheat or grains. It is important to acknowledge that, in general, girls in our sample were not consuming high intakes of whole grains. Indeed, the majority reported consuming less than 2 servings a day and the six that reported consuming above 2 servings, did not report intake much higher than 2 servings. As a result, the results reported herein are limited by a lack of distribution of data regarding whole grain serving sizes, where the higher intakes are limited to representation by six participants.

The data in the current research were initially examined in multiple ways. Given that the majority (54%) of participants had non-detectable hs-CRP values, multiple linear regression was deemed an inappropriate method, given that the data for hs-CRP was not linear. Binary and multinomial logistic regression were then used to determine whether there was a dose-dependency regarding hs-CRP concentrations. For the binary logistic regression, data were divided into "non-detectable" vs. "detectable" categories and for the multinomial logistic regression, data were divided into "non-detectable", "middle", and "high". Although the range in values for the "high" category was much greater than the range for the "middle" group, dividing the two groups based on the median of all of the detectable values was considered the most statistically objective method. The results of the binary and multinomial logistic regression models differed and therefore suggested a dose-dependent effect. Multinomial logistic regression was then employed in the final models. Regarding significant covariates, only higher total body fat percent increased the likelihood of being in the "high" category compared to the "non-detectable" category (range OR 1.20-1.23; $p<0.0001$) when assessing the relationship between diet quality and inflammation.

3. Greater total and regional adiposity, including visceral body fat and skeletal muscle fat, is associated with increased inflammation, independent of physical activity, total energy intake, maturation, and total YHEI score, in Hispanic American girls (Aim 3). Our findings are in agreement with many other cross-sectional studies showing that anthropometric measures of adiposity are significantly and positively associated with levels of CRP in non-Hispanic and Mexican American adolescents (38, 45-60, 195). Similarly, our results are also consistent with research in international samples of Hispanic youths, suggesting similar relationships (46, 59). Our findings are novel due to the assessment of regional adiposity through more direct measures of adiposity. The current study employed the use of DXA to assess abdominal adiposity and pQCT to measure skeletal muscle density, which to our knowledge, have not yet been used in a sample of Hispanic American girls and have been limited to minimal or no use in other race/ethnic groups of adolescents.

The metabolic risks associated with greater visceral adiposity have been previously discussed. Our findings suggest that greater abdominal adiposity is associated with increased odds of higher hs-CRP concentrations in Hispanic girls after controlling for biological and lifestyle factors. These results are consistent with the few studies that have assessed the cross-sectional relationships between CRP values and measures of adiposity using DXA to assess total and central adiposity in non-Hispanic adolescents, and have reported positive associations (45, 49, 98). These findings are similar in small and large samples of non-Hispanic adolescents, however these studies have been limited to bivariate relationships or have not controlled for many potential confounders.

As previously discussed, research has shown that Hispanic adolescents have greater abdominal adiposity compared to other race/ethnic groups, including Caucasian and African

American youth (65). However, significant differences have only been observed between Hispanics and African American adolescents after controlling for age, gender, and percent fat (p= 0.003) (66). Data from the current study similarly showed that on average, android percent fat was greater than total body percent regardless of BMI category, suggesting that Hispanic girls have a high distribution of central fat. The association between truncal fat and inflammation was no longer significant after controlling for BMI. Therefore, it is possible that hs-CRP concentrations are most strongly driven by overall fatness instead of a specific regional influence.

Our findings suggest that greater calf muscle fat is associated with increased odds of higher hs-CRP concentrations. This is the first study to assess this relationship in youths however our results are consistent with limited findings in adults (110). Previous work has suggested that obese Hispanic adolescents have higher fat storage within the calf muscle compared to both Caucasians and African Americans after controlling for age, gender, and total body fat (66). This is of great concern given the associated metabolic impairment related to increase storage of lipids in ectopic depots (22). Using our total sample (n=576), thigh and calf muscle fat was found to be higher in Hispanic girls compared to non-Hispanic girls after controlling for muscle area excluding muscle fat. However, these relationships were attenuated after controlling for total body fat (data not shown). Given that only 37.5% of our total sample reported Hispanic ethnicity, it is possible that with a better distribution of ethnicity, that these results would remain consistent with other findings. Our data also suggested that overweight and obese Hispanic girls had, on average, significantly more calf muscle fat than normal weight Hispanic girls, with no significant difference in thigh muscle density. There was no significant association between thigh muscle density and categories of hs-CRP.

Research has also shown that Mexican American girls from NHANES 1999-2000 ages 8 to 17 years, had average CRP concentrations that were 2-fold greater (geometric mean of 0.76 mg/L) ($p<0.05$) than non-Hispanic white girls (213). The assay and location of assessment used to measure hs-CRP in the current study is the same as that is used for NHANES data. While 54% of our participants had non-detectable hs-CRP concentrations, research has shown this to not be uncommon. This is not unexpected, as healthy youth generally do not have elevated levels of inflammation. The average detectable hs-CRP value reported herein (1.5 ± 1.5 mg/L) was double the geometric mean reported in other Mexican American girls (213). This is explained by the fact that our sample consisted of 27% obese girls compared to the 19% obese Mexican American girls from NHANES 1999-2000 with a similar percentage from NHANES 2011-2012 (214, 215).

The results of the current study align with findings in the literature, however unlike previous work, the current findings confirmed these relationships after adjusting for multiple covariates including total energy, diet quality score, physical activity, and maturation. Previous research has suggested that total YHEI score and energy intake are not highly correlated (154). Research assessing diet quality has also demonstrated the importance of adjusting for total energy intake, especially when the dietary quality score is not highly correlated with energy intake. In the current research, total YHEI score and energy intake were only modestly correlated ($p<0.001$ $r= 0.14$), therefore both YHEI and energy intake were included in the final model. However, no covariates were significant in any of the models assessing the relationship between measures of adiposity and inflammation. Our findings support the larger body of evidence suggesting higher total and regional body fat increases inflammation in otherwise healthy youths.

Limitations and Future Directions

The current study was not without limitations. The primary limitation was that all analyses were restricted to cross-sectional associations and prospective studies are required to more robustly evaluate these relationships. This is important relative to measuring dietary intake and long-term inflammatory status in youths, both of which are more accurately assessed from multiple measures over extended durations of time. Most research assessing the relationships between dietary quality, adiposity, and inflammation in youths has been limited to a cross-sectional design. Future work should include longitudinal data with multiple assessments of direct measures of adiposity, dietary intake, and biomarkers of inflammation. To date, only one randomized controlled trial has assessed the relationships between diet quality, adiposity, and biomarkers of inflammation including total serum cholesterol, HDL, and triglycerides and revealed non-significant associations. Additional randomized trials are needed to test the effect of diet quality on modifying adiposity and inflammation in adolescents.

Only a single biomarker of inflammation was used in the current research. Measures of multiple inflammatory/anti-inflammatory biomarkers closely associated with adiposity, such as IL-6, TNFα, IL-10, would have improved the overall assessment of inflammatory status and may have shown stronger and/or different associations between dietary quality, adiposity and inflammation. Most studies in adolescents have assessed inflammation by measures of CRP, given that it is a strong biomarker and risk factor of increased risk of

CVD that is reliable and cost effect compared to other assessment tools. In addition to its association with increased risk of CVD, CRP is associated with acute infection and illness. Although youths are not prone to infection and illness as frequently as adults, multiple measures of CRP are necessary in order to confirm whether elevated CRP levels are attributed to low-grade inflammation or acute infection. The current research did not survey recent illness; therefore it was not possible to determine whether elevated hs-CRP values of three participants were due to illness. Future work should include a comprehensive assessment of inflammatory status in youth through the use of multiple biomarkers at multiple time points.

The prevalence of nonalcoholic fatty liver disease (i.e. hepatic steatosis; accumulation of fat within liver cells) is increasing in children and adolescents and it has been postulated that the main reason may be greater childhood obesity (216). In adults, nonalcoholic fatty liver disease has been shown to moderately increase the risk of CVD and has been associated with increased odds of high CRP, independent of and additive to obesity and metabolic syndrome (217, 218). As a result, this research would have been enriched if liver fat could have been measured by noninvasive technologies including abdominal ultrasound, MRI, or magnetic resonance spectroscopy.

The limitations of questionnaires for dietary assessment are well known. Energy intake is commonly misreported and while the YAQ has been validated, reporting bias is a concern with food frequency questionnaires (219, 220). Under-reporting of energy intake is common among older children and adolescents and it is strongly associated with overweight and obesity (219). Different versions of the YAQ were used in the Jump-In and STAR studies necessitating the use of a modified version of the YHEI scoring for this analysis.

Further refinement of the YHEI diet quality score is warranted as additional study of the diet quality, adiposity, and inflammation relationship is undertaken.

The foods that comprise the “whole grains” component in the YHEI include cooked breakfast cereals, cold breakfast cereal, other grains (brown rice), and dark bread (whole wheat or whole grain). These items are multiplied by a factor of 1.0 if >=50% of the item is whole grain, 0.5 if >25% and <50% of the item is whole grain, or 0.0 if <=25% of the item was whole grain. Due to the limitation of the food frequency questionnaire, it was impossible to identify whether participants consumed 100% whole wheat or whole grain bread instead of bread containing some whole wheat. Given that those who reported consuming two or more servings of whole grains per day reported consuming on average 407% greater servings of dark bread than those who reported consuming less than 2 servings of whole grains per day, more specific information regarding bread intake may have altered this relationship. Similarly, it was not possible to distinguish between instant and rolled oats. de Punder and Pruimboom recently suggested that cereal grains that are not “whole grains” contain “anti-nutrients” that may contribute to the inflammatory pathway (221); as a result, it is possible that items that may not have met the definition of whole grain or contain very little whole grains may have been included in the estimate of whole grains.

Unfortunately, socioeconomic status information was not collected in either the Jump-In or STAR studies. It has been reported that Hispanic youth have higher obesity rates compared to non-Hispanic Whites and differences in socioeconomic status have been shown to contribute to these racial/ethnic disparities (214, 222-224). Results from Fradkin et al. indicate that seventh grade non-Hispanic White girls and Hispanic girls in the lower socioeconomic stratas (< high school graduate, high school graduate, and some college) had significantly higher risk of obesity, (OR range 2.37-7.14, 2.48-2.67, respectively) compared to those in the highest socioeconomic strata (>4 year college degree) (222). It has been postulated that differences in dietary intake and physical activity by socioeconomic status strata may contribute to the relationship between socioeconomic status and obesity in youth. Although socioeconomic status was not captured in this study, total energy intake and physical activity were collected for all participants and controlled in all analyses.

Physical inactivity is a common risk factor for CVD in adolescents and it has been suggested that physical/sexual maturation contributes to disparities in adiposity and inflammation (76, 225). Given that the PYPAQ is a questionnaire that measures physical activity in youth, it is limited by self-report. Although guardian assistance was encouraged, the recall of past year physical activity and sport participation is susceptible to reporting errors. The parent studies used the method of Mirwald et al. (191) to estimate maturity offset by predicting years from peak height velocity based on gender specific anthropometric equations While this method was used to assess maturity offset from cross-sectional data, peak height velocity is more appropriately captured from data from serial measurements for multiple years surrounding the occurrence of PHV, and as such the use of maturity offset was a limitation of the current research (226).

Although muscle fat content is a valid surrogate for muscle fatty infiltration, the use of pQCT technology to reflect muscle fatty infiltration is another potential limitation since pQCT cannot distinguish between intra and extra-myocellular fat compartments (108). The use of MRI may have been more appropriate as it would have provided a precise measure of intramyocellular fat. Only single slices were obtained from the thigh and calf at the 20% femur and 66% tibia sites, relative to the respective distal growth plates on the non-dominant limb. The regions measured may be limited in that they typically have smaller depots of adipose tissue compared to the mid-thigh. However, results by computed tomography have indicated a significant correlation between muscle attenuation of the mid thigh with the whole calf ($r=0.62$ $p<0.05$) (227). Similarly, DXA does not provide a direct measure of visceral fat (27), although DXA android fat is highly correlated with visceral fat, While MRI may provide better contrast resolution than pQCT and DXA, it is limited for use in large samples due to its high costs, time restraints, and restrictions regarding obese individuals. Most studies assessing the relationships between dietary quality and adiposity have been limited to anthropometric indexes of adiposity. Future work is needed to assess the relationships between diet quality and adiposity using more direct measures of adiposity. This was the first study to assess the relationship between diet quality and skeletal muscle fat. Given the known risks associated with higher skeletal muscle fat, more work is needed to add to the knowledge base regarding dietary quality, inflammation, and regional fat depots other than abdominal fat.

There is a large body of evidence suggesting that enhanced diet quality may improve risk factors associated with CVD in adults, including but not limited to lower adiposity and inflammation. Less is known of these relationships in adolescents. Previous work assessing the relationship between dietary quality and adiposity in youths has been limited to anthropometric measures of adiposity, smaller sample sizes, lack of multivariate analyses, and many studies have been restricted to non-Hispanic White adolescents. Current literature concerning associations between diet quality scores and inflammation in youth is scant; therefore no conclusive relationships can be described. The use of dietary indexes to measure dietary quality and its association with targeted health outcomes is critical to understanding the true nature of these relationships in adolescents. Based on the results of the current research, greater total and regional adiposity is associated with inflammation in Hispanic adolescent girls, and while better diet quality is associated with lower total and regional adiposity in Hispanic and non-Hispanic girls, these findings suggest that diet quality, as assessed by the YHEI, is not associated with inflammation in Hispanic youths. The range in concentrations of hs-CRP was small and because the majority of the sample had non-detectable concentrations, the sample sizes of the "middle" and "high" groups were also very small. It is possible that with a larger sample of detectable CRP concentrations that the relationship between diet quality and inflammation may have been altered. Dietary reporting error may have largely affected the outcome of these results. However, it is also possible that inflammation in otherwise healthy Hispanic girls is not at a degree that is worrisome. Assessing the relationship between multiple dietary index scores with inflammation in this sample would help to elucidate this relationship. The majority of girls in the current study were prepubescent and research has shown that inflammation is associated with maturation, therefore stronger relationships may potentially be observed in older adolescent girls. Futhermore, it is clear that more work needs to be done to improve dietary quality indexes to better evaluate the role of dietary quality on metabolic risk factors in youths.

APPENDIX A. Diet quality is associated with lower total and visceral adiposity in Hispanic and non-Hispanic adolescent girls

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ABSTRACT

Background: Evaluation of diet quality may provide a better assessment of diet and health relationships, including body composition, than individual foods or nutrients. Despite the potential to intervene, the association between diet quality and absorptiometric measures of adiposity in Hispanic and non-Hispanic adolescent girls remains understudied.

Objective: To evaluate the relationship between diet quality, as measured by the Youth Healthy Eating Index (YHEI), and absorptiometric measures in ethnically diverse adolescent girls.

Design: Dietary intake was estimated using the validated semi-quantitative food frequency questionnaire (FFQ), Harvard Youth/Adolescent Questionnaire (YAQ), in a cross-sectional study of 576 healthy Hispanic and non-Hispanic adolescent girls ages 8-13 years. Diet quality was calculated using a modified version of the YHEI, derived from YAQ components. Total body and android fat, a surrogate for visceral fat, were measured by dual energy x-ray absorptiometry (DXA). Muscle density (mg/cm^3) was measured at tibial and femoral sites using peripheral quantitative computed tomography (pQCT). Linear regression was employed to assess the relationships between diet quality and total body fat, android fat, skeletal muscle density, and body mass index (BMI).

Results: Total YHEI score was inversely associated with total body fat percent ($p= 0.01$) and android percent fat ($p= 0.02$), but not BMI or muscle density. Lower “margarine and butter use” and higher “meat ratio” were associated with higher leg muscle density. Higher “meat ratio” was inversely associated with BMI and greater “multivitamin use” was inversely associated with visceral adiposity.

Conclusions: Higher quality diets were associated with lower total and visceral body fat in girls aged 8-13 years.

INTRODUCTION

Several dietary indices have been used extensively in adults to assess diet quality, usually with the aim of evaluating the effect of diet quality on the risk of health related outcomes (1-3). Characterizing dietary intake using diet patterns has been supported in epidemiological research as diet patterns are thought to better capture the total dietary exposure, including the interplay of the multiple dietary components potentially associated with disease risk (4-6). Both dietary indices and empirical methods of measuring diet quality, including factor or cluster analyses, have been widely used in adult populations to evaluate the relationship between diet and obesity (7-13). Results of many cross-sectional and a few longitudinal analyses in adult populations seem inconsistent in that some show clear beneficial effects (7-10) while others do not provide clear evidence of protection (11-13) of diet quality on overweight or obesity.

Currently, 1 in 3 children are overweight or obese (14). Energy dense diets are key contributors to obesity in childhood. It has been suggested that dietary patterns adapted in childhood may persist until adolescence, and then may change throughout adolescence (15). As a result, it is important to understand diets of adolescents, as dietary choices during adolescence are likely to track into adulthood and parallel the risk of obesity and its comorbidities (15-17).

The study of dietary quality in adolescents is not new. Although multiple cross-sectional studies have applied diet indices to examine bivariate relationships between diet quality and anthropometric measures of adiposity, such as BMI and waist circumference (18-23), only a few studies have been conducted in Hispanic American adolescents (24, 25). Other gaps in the literature also exist; for example, few studies have assessed the relationship between diet quality and total or visceral adiposity using direct methods as quantified by DXA and none have evaluated the association between diet quality and skeletal muscle fat in adolescents (26).

The present study is designed to fill these gaps by assessing the relationship between diet quality, evaluated by the YHEI, and direct absorptiometric measures of total and regional body fat in a large sample of Hispanic and non-Hispanic girls, aged 8-13 years. Our hypothesis was that higher YHEI scores (i.e. diet quality) would be associated with lower total and regional adiposity in both Hispanic and non-Hispanic girls.

SUBJECTS AND METHODS

Study Design

Cross-sectional baseline data from 576 adolescent girls enrolled in The Jump-In: Building Better Bones study (27) or the "Soft Tissue and Bone Development in Young Girls" study (STAR) were included in the present analysis. Jump-In has been described in detail elsewhere; briefly, it was a group-randomized, controlled trial evaluating the effects of a 2-year structured jumping intervention on bone development in pre-pubertal and early pubertal girls (28). Jump-In participants were recruited from elementary and middle schools within two school districts in Tucson, Arizona.

STAR is an ongoing prospective cohort study in normal weight (BMI 5th to 85th percentile), over-weight (BMI 85th to 95th percentile), and obese (BMI>95th percentile) pre-menarcheal girls. STAR participants were recruited from schools, pediatrician's offices, and community groups in Tucson, Arizona. The University of Arizona Human Subjects Protection Committee approved both Jump-In and STAR studies; written, informed consent was obtained for all participants.

Participants

The sample included 434 healthy girls, aged 8-13 years, who were participants in Jump-In and 142 healthy girls, aged 9 to 13 years, who were participants in STAR. Jump-In exclusion criteria included the inability to read and understand English; learning disabilities identified by the school that made it impossible to complete questionnaires; medications, medical conditions, or a disability that limited physical exercise participation. Pre-pubertal females aged 9 to 12 years who had a BMI greater than or equal to the 5th percentile were eligible for STAR. Girls were excluded if they had a previous diagnosis of type 1 or type 2 diabetes, used medications (i.e. growth hormone, albuterol) that alter body composition including bone mineral accrual, had a physical disability that could limit participation in physical activity, or diagnosis of a learning disability that would limit completion of questionnaires.

Dietary assessment

Dietary intake was estimated using the semi-quantitative YAQ, a FFQ validated by Rockett et al. in adolescents (29). Jump-In participants used the 1995 version of the YAQ which asks respondents to report their usual frequency of consumption of 132 food items during the past year including specified serving sizes. STAR participants used the 2012 version of the YAQ, which included an additional 22 food items, for a total of 154 items as compared to 132 on the 1995 version. The 2012 YAQ also excluded some of the behavioral frequency questions that evaluated the frequency of preparing dinner for oneself and/or other family members and the frequency of consuming breakfast, lunch, snacks, and dinner outside of the home. Participants could receive assistance to complete the form from trained study technicians or a parent/guardian, if needed. YAQs were evaluated for completeness and coded by trained staff following a standard protocol and were subsequently sent to Channing Laboratories (Boston, MA) for food and nutrient analyses that have been described in detail elsewhere (30).

Youth Healthy Eating Index (YHEI)

Feskanich et al. modified the validated and widely used Healthy Eating Index (HEI), created by the USDA, in order to develop the YHEI to accurately assess the dietary habits specific to children and adolescents (31). Rather than calculating specific nutrient intakes, the YHEI, focuses on food choices to assess fat, sugar, fiber and sodium intake, including high trans-fat snack foods and sugar-sweetened beverages (components 6 and 7), and also includes behaviors associated with scholastic performance and healthy dietary intake patterns (components 12 and 13). YHEI scores were modified from those of the HEI to reflect the 5-A-Day serving size recommendations developed by the National Cancer Institute. Frequency factors developed by Feskanich et al. (31) were used to determine the number of daily serving sizes for each component. These serving sizes were then used to determine the YHEI score for each component.

A modified version of the YHEI scoring was used in this study. Specifically, three components of the original YHEI could not be calculated due to modifications to both the

1995 and 2012 versions of the YAQ. These components included the consumption of visible fat and skin (component 11), a second measure of saturated fat intake with lower weight (5 points) versus the 10-point meat ratio (component 5), and two behavioral components, the frequency of eating dinner with family (component 12) and the frequency of breakfast consumption (component 13). Seven components of the modified and original versions of the YHEI were scored from 0 to 10 points and were derived from multiple foods and questions from the YAQ, including fruit, vegetables, whole grains, dairy, meat ratio (lean protein: fat protein), snack foods (high in salt or sugar), and soda and drinks (i.e. regular soda, fruit punch, and sweetened iced tea). Three components of the YHEI, multivitamin use, margarine and butter use, and fried foods, were scored from 0 to 5 points, consistent with previous literature on scoring parameters and reflecting the limited number of foods and questions from the YAQ that captured these exposures (31). Feskanich et al. previously demonstrated that 85% of the variation in YHEI scores in a sample of 16,452 adolescents was due to 5 of the 7 most heavily weighted components (whole grains, fruits, meat ratio, snack foods, soda and drinks), which we assessed, while the 3 components excluded from the modified YHEI used here accounted for only 5% of the variation in the total YHEI score. Thus, the modified YHEI score used here with a maximum score of 85 (compared to the original 100-point YHEI) is adequate to capture the diet quality of our sample of adolescent girls. The scoring criteria for the original and modified versions of the YHEI are given in **TABLE 1.** A higher total YHEI score reflects better diet quality, therefore, higher values for components 6,7,9, and 10 reflect lower consumption, given that those components represent energy dense foods.

Anthropometry

Anthropometric data were collected on all participants using identical methods. Body weight and height were measured by technicians following standard protocols as described in the Anthropometric Standardization Reference Manual (32). Weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Model 881; Seca, Hamburg, Germany). Standing height, measured to the nearest 0.1 cm, was assessed at full inhalation using a stadiometer (Shorr Height Measuring Board, Olney). The mean of two measurements was used for each anthropometric variable. BMI (kg/m^2) was calculated from height and weight.

Body composition

DXA was used to quantify total fat mass, percent body fat (ratio of fat mass to whole body mass), and android percent fat as a surrogate of visceral fat, using the GE Lunar PRODIGY [GE Lunar Radiation Corp; Madison, WI, USA] (software version 5.60.003). Participants were positioned for whole body scans using standard manufacturer protocols. DXA coefficients of variation and precision in our laboratory have been reported elsewhere (33). All scans were completed and analyzed by a certified technician following a standard protocol using the extended research mode software. Android fat was estimated for the area enclosed by demarcations immediately above the iliac crest and at 20% of the total distance between the iliac crest and the base of the skull (approximately the bottom rib), using the following equation: [(Android fat mass) / (Android Bone Mineral Content + Android fat mass + Android lean mass)] x 100.

pQCT, a low-dose radiation technique, was used to measure muscle density, a surrogate for skeletal muscle fat (34). This technique is not capable of distinguishing intra-myocellular from extra-myocellular fat and therefore cannot directly measure fat infiltration in skeletal muscle. Thus, muscle density reflects both intra- and extra-myocellular fat stores and is inversely related to muscle fat content such that a lower muscle density is indicative of higher skeletal muscle fat content (34). pQCT was used to measure thigh and leg soft tissue composition, including muscle density (mg/cm^3), at the 20% femur (thigh) and 66% tibia (33) sites relative to the respective distal growth plates of the non-dominant limb. Scans were performed using a Stratec XCT 3000 scanner [Stratec Medical GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY, USA] and analyzed using Stratec software, Version 5.50. All pQCT scans and scan analyses were performed by trained technicians using the guidelines of Bone Diagnostics, Inc. [Fort Atkinson, WI, USA]. Adipose tissue, skeletal muscle, and bone are distinguishable as independent tissues based on attenuation characteristics, which are directly related to tissue composition and density, and can be estimated using edge detection and threshold techniques (35, 36). Images were filtered prior to being analyzed using contour mode 3 (-101 mg/cm^3) and peel mode 2 (40 mg/cm^3) to separate adipose ($<$40 mg/cm^3) and muscle/bone (-40 mg/cm^3), respectively (36). Images were filtered subsequently with a 7x7 image filter that clearly defined the edge of the muscle and eliminated all bone above 120 mg/cm^3 (37). This method ensures that muscle density was estimated from only soft tissue within the edge of the muscle.

Physical maturation

Maturation was assessed using the validated gender-specific equation developed by Mirwald et al. (38) to estimate maturity offset, an estimate of years from peak height velocity (PHV), from age, weight, and ratios of skeletal lengths. A negative maturity offset value represents years before PHV, whereas a positive maturity offset value represents years after PHV.

Physical activity

The validated Past Year Physical Activity Questionnaire (PYPAQ) was used to assess physical activity (39), which assesses participation in 41 common sport and leisure-time activities performed ≥10 times in the past year (40). Average duration, average weekly frequency, and number of months of participation for each activity were obtained and total PYPAQ score was computed using a modified algorithm that has been reported elsewhere (40).

Statistical analysis

Data were analyzed using SPSS for Windows statistical software, Version 22.0 [Chicago, IL, USA]. A combined sample (n=576) of Jump-In and STAR participants who had complete data for all variables were used in this study. Means, standard deviations, and ranges were calculated by ethnicity and for the total sample. Distributions of all continuous variables were assessed for normality and skewness. Distributions of total energy intake, PYPAQ score, and BMI were skewed and therefore were log transformed. For descriptive purposes, untransformed means and standard deviations for total energy intake, PYPAQ score, and BMI were reported. Independent t-tests were used to assess differences between Hispanic and non-Hispanic participants. A Chi-square test was used to evaluate differences in BMI percentiles by ethnicity.

Partial correlation coefficients from multiple linear regression models were computed to examine the associations between individual YHEI components, YHEI total score, and measures of adiposity, including BMI, total body fat percent, android percent fat, thigh muscle density, and calf muscle density. Variables assessed for confounding that were included in the final models were maturity offset, PYPAQ score, ethnicity, cohort assignment, and total energy intake. Further analyses additionally controlled for BMI or total body fat percent in models that had android percent fat and skeletal muscle density as outcome variables, respectively. All models assessed the effect of interaction by ethnicity, in which the interaction term was included as a covariate. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Descriptive statistics of the 576 study participants are shown in **TABLE 2**. Overall, the average age was 10.7±1.1 years, with a range of 8.8-13.2 years, and 37.5% of participants reported Hispanic ethnicity. On average, the girls were 1.37±1.1 years from achieving peak height velocity, with no difference detected between Hispanic and non-Hispanic girls. Physical activity was not different between Hispanic and non-Hispanic girls.

The average YHEI score of the total sample was 50.7 ± 8.9 and reported total energy intake was 1868 ± 752 kcals. Hispanic girls had an estimated total energy intake was 16.0% higher than non-Hispanic girls. The average BMI and total body fat percent for all participants were 19.0 kg/m^2 and 29.0 ± 9.1%, respectively. Based on the U.S. National Center for Health Statistics/Centers for Disease Control and Prevention percentiles for body mass index (BMI, kg/m^2) (17) 3.0% of the sample were underweight (BMI<5th percentile), 69.0% were classified as normal (BMI 5th to 85th percentile), 15.0% were overweight (BMI 85th to 95th percentile), and 13.0% were obese (BMI>95th percentile). A Chi-square test revealed significant differences ($p \leq 0.0001$) in BMI categories between ethnicities. While most girls fell in the normal BMI category for both non-Hispanics (74.0%) and Hispanics

(62.0%), with a similar proportion of overweight girls, obesity was more common in Hispanic (21.0%) vs. non-Hispanic girls (7.0 %). Independent t-tests indicated significantly ($p \leq 0.05$) greater scores in Hispanic compared to non-Hispanic girls for the following body composition variables: weight (+10.0%), BMI (+11.0%), total body fat percent (+11.0%), android percent fat (+13%), and thigh muscle density (+1.0%), which is inversely related to muscle fat.

TABLE 3 shows the descriptive statistics for individual YHEI components and total YHEI score for Hispanics, non-Hispanics, and the total sample. Compared to Hispanic girls, despite lower consumption of vegetables ($p \leq 0.03$) and fruit intake ($p = 0.005$), non-Hispanic girls consumed higher quality in regards to sources of protein ($p \leq 0.0001$), multivitamin use ($p \leq 0.0001$), less fried food intake ($p = 0.006$), and fewer sugar-sweetened beverages consumed ($p \leq 0.03$).

Total YHEI score was significantly and inversely associated with android percent fat after controlling for total energy intake, ethnicity, maturity offset, PYPAQ score, and cohort assignment ($p = 0.03$, $R = -0.09$). This association remained significant after further controlling for BMI ($p = 0.02$, $R = -0.09$). The final model accounted for 74.7% of the variability in android percent fat. Additionally, total YHEI score was significantly and inversely associated with total body fat ($p = 0.01$, $R = -0.10$) but not with muscle density or BMI (data not shown). No statistically significant interactions by ethnicity were detected.

TABLE 4 displays partial correlation coefficients from multiple linear regression models between individual YHEI components and measures of adiposity after controlling for maturity offset, total energy intake, PYPAQ score, cohort assignment, ethnicity, and either BMI or total body fat percent in models predicting android percent fat or muscle density, respectively. Of the 10 individual components, only 3 were statistically related to measures of adiposity; “margarine and butter use”, “meat ratio” score, and “multivitamin use”. “Margarine and butter use” and “meat ratio score” were components that were derived to represent fat intake. “Meat ratio score” was inversely associated with BMI ($p \leq 0.05$, R= -0.08) and positively associated with calf muscle density (i.e. low skeletal muscle fat content) before ($p \leq 0.001$, R= 0.16) and after controlling for total body fat percent ($p \leq 0.001$, R= 0.14). Lower “margarine and butter use” was positively associated with muscle density (i.e. low fat content) of the thigh before ($p \leq 0.001$, R= 0.15) and after controlling for total body fat percent ($p \leq 0.001$, R= 0.14). “Multivitamin use” was inversely associated with android percent fat before and after controlling for BMI ($p \leq 0.05$, R= -0.08). The final model accounted for 74.6% of the variability in android percent fat.

DISCUSSION

The current study reports novel findings of the relationship between diet quality, as assessed by YHEI, and body composition in a large sample of Hispanic and non-Hispanic adolescent girls. The findings demonstrate that better diet quality, represented by a higher total YHEI score, is associated with lower total and visceral body fat, as determined by x-ray absorptiometry in adolescence, independent of physical activity, total energy intake, maturation, cohort assignment, and ethnicity. These findings are in agreement with cross sectional studies in adolescents that have reported statistically significant inverse (22, 31, 41-43) associations between diet quality and measures of adiposity. Only three studies have used the YHEI diet quality score to assess associations between diet quality and measures of adiposity. In these studies potentially confounding variables were not controlled, limiting interpretation as well as comparisons with the present results (27, 31, 41). Only one of the three studies examined associations between YHEI and total and regional adiposity using methods more direct than anthropometry. In that study of 196 African American adolescents (49% females), the YHEI score was inversely related with DXA measures of total body fat (r= -0.09) and android percent fat (r= -0.09) (45). Although these correlation coefficients were not significant (41), their magnitude was similar to the coefficients reported herein. In agreement with our findings, the YHEI score was not significantly associated with BMI in both a large study of 16, 452 adolescents (31) as well as in a smaller study by Vitale et al. examining relationships between YHEI total scores and BMI in 45 underweight, normal, and overweight/obese adolescents (27).

With regard to diet intake, participants in the current study reported an average energy intake that was within the national recommendation range of 1800-2000 kcals for girls this age (41). In our sample, average energy intake was statistically different by ethnicity. With regard to comparability of YHEI scores from the current sample with other samples, when YHEI total score was expressed as the percent of total points possible to account for the different maximum score attainable using the modified versus the original YHEI, the average modified YHEI score for our cohort was 60.58 $\pm$ 12.4. This average YHEI score agrees well with the YHEI score reported by Feskanich et al. for 8,980 girls aged 9 to 14 using the original 100-point YHEI (59.6 $\pm$ 10.8) (31).

“Margarine and butter use” is a component in the YHEI that serves as a surrogate of trans-fatty acids and total fat intake in adolescents. Lower margarine and butter consumption was significantly associated with lower skeletal muscle fat. Similarly, a higher ratio of lean-to fat- protein consumption was associated with lower BMI and lower skeletal muscle fat. The proteins that contribute to fat-protein include those that are higher in fat content, including beef, pork, and lamb. Although this was the first study to assess the relationship between surrogates of fat intake and skeletal muscle fat in healthy adolescent girls, these findings aligned with evidence from studies in adults showing that high-fat diets are associated with higher intramuscular fat. These studies suggest high-fat diets sustained between 2 hours to 7 days, lead to significant increases in intramyocellular lipid content of the tibialis anterior ($p<0.005$) (44) and compared to a low-fat diet, in the vastus lateralis ($p=0.007$) (45), in young lean adults. Results from the present study also indicate that greater multivitamin use was associated with lower android percent fat. While data in adolescents is limited, evidence suggests that dietary supplement users, in general, are typically health conscious individuals seeking to adopt health-related habits, including better dietary patterns (49); a likely explanation for the findings reported in the current study.

This study has several strengths and limitations. An important strength was the control of several potential confounders in the analyses. Indeed, Lazarou and Newby (26) in their review of 22 studies in adolescent samples, which assessed cross-sectional relationships between diet indices and anthropometric measures of adiposity, reported lack of adjustment for confounders as a major limitation in interpretation of the data. The use of multiple direct measures of adiposity rather than anthropometry was another strength of the study. Although pQCT technology cannot distinguish between intra and extra-myocellular fat compartments, muscle fat content is a valid surrogate for muscle fatty infiltration (34). Similarly, DXA does not provide a direct measure of visceral fat but android fat is highly correlated with visceral fat (46). This study was the first to assess the relationships between diet quality and direct measures of total and regional adiposity in a large sample of Hispanic and non-Hispanic adolescent girls.

The limitations of questionnaires for dietary assessment are well known. Energy intake is commonly misreported and while the YAQ has been validated, reporting bias is a concern with FFQs (47, 48). Under-reporting of energy intake is common among older children and adolescents and is strongly associated with overweight and obesity (47). Different versions of the YAQ were used in the Jump-In and STAR studies necessitating the use of a modified version of the YHEI scoring for this analysis. Further refinement of the YHEI diet quality score is warranted as additional study of the diet quality and adiposity relationship is undertaken. Finally, the present analyses were limited to cross-sectional associations and prospective longitudinal studies are required to more robustly evaluate these relationships.

In conclusion, higher diet quality scores were associated with lower total and visceral body fat in Hispanic and non-Hispanic adolescents. More specifically, the results of this study suggested that higher intake of leaner proteins and lower consumption of margarine and butter were the diet quality score components associated with lower total and/or regional adiposity. While additional studies are needed, including randomized trials testing the effect of diet quality on modifying adiposity in youths, these findings support the larger body of evidence suggesting improvements in body composition may be achieved with enhanced diet quality.

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TABLE 1. Youth Healthy Eating Index (YHEI) scoring criteria

		YHEI scoring criteria	
Original YHEI Components	**Modified YHEI Components**	**Requirement for minimum score of 0**	**Requirements for maximum score of 10**
		servings/day[1]	
1. Whole Grains	1. Whole Grains	0	≥3
2. Vegetables	2. Vegetables	0	≥3
3. Fruits	3 Fruits	0	≥3
4. Dairy	4. Dairy	0	≥2
5. Meat Ratio[2]	5. Meat Ratio[2]	0	≥2
6. Snack Foods[3]	6. Snack Foods[3]	≥3	0
7. Soda and Drinks	7. Soda and Drinks	≥3	0
		Requirement for minimum score of 0	**Requirements for maximum score of 5**
		servings/day[1]	
8. Multivitamin use	8. Multivitamin use	Never	Daily
9. Margarine and butter use	9. Margarine and butter use	≥2 pats/day	Never
10. Fried foods outside home	10. Fried foods outside home	Daily	Never
11. Visible animal fat[4]	-	None	All
13. Dinner with family	-	≥5 times/week	Never
14. Eat breakfast	-	Daily	Never
Total YHEI score (0-100)	Total YHEI score (0-85)		

[1]Jump-In and STAR studies used the Youth/Adolescent Questionnaire (YAQ) to assess habitual dietary intake in 576 adolescent girls ages 8-13 years. Serving sizes are based on definitions of the YAQ.

[2] Total servings per day of white meat including chicken, fish, seafood, eggs, soy, tofu, beans, and nuts were divided by servings per day of dark meat including beef, pork, and lamb.

[3]Snack foods include salty snacks (e.g., potato chips, corn chips, popcorn, pretzels, and crackers) and snacks with added sugar (e.g., cake, snack cake, toaster pastry, sweet roll/Danish/pastry, doughnut, brownie, cookies, pie, chocolate, candy bar with chocolate, candy without chocolate, fruit rollup, popsicle, and flavored gelatin).

[4]Visible animal fat includes the visible fat on meat and the skin on chicken or turkey.

TABLE 2. Descriptive characteristics of 576 adolescent girls

Characteristics	Non-Hispanic n=360 Mean ± SD	Hispanic n=216 Mean ± SD	Total Sample n=576 Mean ± SD
Age (years)	10.7±1.1	10.8±1.0	10.7±1.1
Maturational Status			
Maturity offset (years)[1]	-1.1±1.0	-1.5±0.5	-1.37±1.1
Dietary Intake/Assessment			
Total energy (kcals)	1762±649	2048±870	1868 ± 752
Total YHEI score [2]	51.9±8.4	51.0±9.5	51.6 ± 9.0
Physical Activity Assessment			
PYPAQ score [3]	5046.5±4536	5381.5±5825.3	5152.8 ± 5053.8
Body Composition			
Height (cm)	144.7±9.9	145.6±9.2	145.1± 9.6
Weight (kg)	39.2±10.8	43.1±13.0	40.9±12.2
BMI (kg/m^2)	18±3	20±4	19 ± 4
BMI Category (%)			
Underweight	4	3	3
Normal weight	74	62	69
Overweight	15	14	15
Obese	7	21	13
Total body percent fat (%)	28±9	31±10	29 ± 9.1
Android percent fat (%)	30±12	34±14	31.6 ± 12.9
Thigh muscle density (mg/cm^3)	76.5±1.6	77.3±2.0	76.8 ± 1.8
Calf muscle density (mg/cm^3)	79.1±1.2	79.1±1.3	79.1 ± 1.2

[1]Maturity offset= estimated years from peak height velocity (PHV)

[2] YHEI score: Youth Healthy Eating Index (modified) 0 (non-adherent) to 85 (full adherence)

[3] PYPAQ score: Past year physical activity

TABLE 3. Youth Healthy Eating Index (YHEI) scores among Non-Hispanic and Hispanic adolescent girls

	YHEI scores, mean (range)		
YHEI component	**Non-Hispanic**	**Hispanic**	**Total Sample**
1. Whole grains	2.88 (0-10)	2.92 (0-10)	2.90 (0-10)
2. Vegetables	4.95 (0.21-10)	5.46 (0.21-10)	5.14 (0.21-10)
3. Fruit	4.93 (0.53-10)	5.66 (0.4-10)	5.20 (0.40-10)
4. Dairy	7.23 (1-10)	7.21 (1.13-10)	7.22 (1-10)
5. Meat ratio[1]	9.87 (0.83-10)	9.25 (0.61-10)	9.64 (0.61-10)
6. Snack foods[1]	5.54 (0-9.87)	5.29 (0-9.73)	5.45 (0-9.87)
7. Soda and drinks	7.98 (0-10)	7.55 (0-10)	7.82 (0-10)
8. Multivitamin use	1.81 (0-5)	1.16 (0-5)	1.57 (0-5)
9. Margarine and butter use	3.49 (0-5)	3.62 (0-5)	3.54 (0-5)
10. Fried foods	3.25 (0-5)	2.91 (0-5)	3.12 (0-5)
Total YHEI score	51.92(28.21-74.63)	51.03 (27.85-78.97)	51.50 (27.85-78.97)

[1] Refer to Table 1 for variable criteria

TABLE 4. Partial correlations from multiple linear regressions between YHEI components and measures of adiposity

YHEI Components	BMI (m/kg^2)	Total Body Percent Fat (%)	Android Percent Fat[1] (%)	Thigh Muscle Density[2] (mg/cm^3)	Calf Muscle Density[2] (mg/cm^3)
Margarine and butter use	-0.01	-0.05	-0.05	0.14[b]	0.003
Meat ratio	-0.08[a]	-0.08	-0.02	0.07	0.14[b]
Multivitamin use	-0.05	-0.07	-0.08[a]	0.002	0.01

All models controlled for total energy intake, PYPAQ score, maturity offset, cohort assignment, ethnicity, and all other individual YHEI components when not the independent variable (fruit, vegetables, whole grains, meat ratio, dairy, soda and drinks, margarine and butter use, multivitamin use, fried foods, and snack foods)

[1]Additionally controlled for BMI

[2]Additionally controlled for total percent fat

[a] sig. at $p \leq 0.05$; [b] sig. at $p \leq 0.001$

APPENDIX B. The association between diet quality and inflammation in Hispanic American adolescent girls.

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ABSTRACT

Background: C-reactive protein (CRP), a circulating biomarker for risk of cardiovascular disease, is used to evaluate the presence and level of sub-clinical inflammation. Diet quality indexes have been developed for use in adolescents and have evaluated the relationships between diet quality and selected health outcomes. Little is known of the relationships between diet quality and inflammation in Hispanic American adolescent girls.

Objective: We assessed relationships between total and individual component scores of diet quality, as measured by the Youth Healthy Eating Index (YHEI), and inflammation, measured by serum hs-CRP concentrations, in Hispanic American adolescent girls.

Design: Dietary intake was estimated using the validated semi-quantitative food frequency questionnaire, Harvard Youth/Adolescent Questionnaire (YAQ) in a cross-sectional sample of 113 healthy Hispanic American adolescent girls aged, 9-13 years. Diet quality was assessed using a modified version of the YHEI, derived from YAQ-derived components. Serum hs-CRP concentrations were measured by nephelometry. Multinomial logistic regression was employed to assess the relationships between diet quality and inflammation.

Results: YHEI scores ranged from 25.3 to 72.7. Over 50% of girls demonstrated non-detectable serum hs-CRP levels. In adjusted models, there was no significant relationship between overall YHEI score and hs-CRP in this sample of adolescent girls. For every 1-unit increase in “whole grains” score there was a 44% increase in odds of being in the “high” category compared to the “undetectable” category of hs-CRP, after adjusting for maturity

offset, PYPAQ score, total energy intake, total body fat, and all other individual YHEI components (OR: 1.44, 95% CI: 1.06, 1.97).

Conclusions: Diet quality, as assessed by YHEI, is not significantly associated with hs-CRP, a circulating biomarker of inflammation in Hispanic adolescents girls.

INTRODUCTION

Behavioral risk factors, including the consumption of energy dense diets, are modifiable determinants that contribute to the development of childhood obesity. Obesogenic behaviors, obesity, and its comorbidities, track into adulthood such that obese adolescents tend to become the heaviest adults with the most adverse health risk profiles (1-3). Research in adults (4-9) and adolescents (40, 47-63) have consistently shown that excess adiposity is associated with elevated concentrations of inflammatory biomarkers, including C-reactive protein (CRP), and that these biomarkers are highly predictive of cardiovascular disease (CVD), type 2 diabetes mellitus, and other chronic diseases in adults (10-13). Consumption of specific energy dense foods such as fatty and fried foods and sweets has been associated with inflammation (14). Characterizing dietary intake using diet patterns has been supported in epidemiological research since these approaches provide estimates of total diet exposure. Further, the characteristic diet pattern may be more predictive of health outcomes associations than individual nutrients or even foods or food groups (15-17). Studies in adults have shown that higher diet quality scores are related to lower concentrations of inflammatory biomarkers, particularly CRP (18-25), while far less is known of this relationship in adolescents (26).

In the absence of infection or acute inflammation, CRP is considered the closest and most accurate biomarker of adipocyte-generated chronic low-grade inflammation (27, 28). Even at an early age, CRP concentrations increase with adiposity (29-45) and data from the National Health and Nutrition Examination

Survey (NHANES) show that CRP concentrations are higher in children who demonstrate the presence of more metabolic syndrome components (46, 47).

In the U.S., Hispanics and Latinos experience the highest burden of cardiovascular risk factors (48, 49) and among youth, data from NHANES 2011-2012 estimated that the prevalence of overweight and obesity [body mass index (BMI) > 85th percentile] is the highest in Hispanic youths ages 6-11 years (46.2%) compared to both non-Hispanic black (38.1%) and non-Hispanic white youths (29.4%) (50). Similarly, in a study of 1358 adolescent girls from NHANES 1999-2000, results from multiple linear regression analyses revealed that CRP values in Mexican American girls ages 8 to 11 years and 12-17 years were almost 2-fold higher than in non-Hispanic white girls ($p=0.03$), independent of BMI, age, smoking status, total cholesterol, homocysteine, systolic and diastolic blood pressure, and glycosylated hemoglobin (35).

To date, no studies have assessed the relationship between diet quality and inflammation in Hispanic American adolescents. The present study assessed the relationship between diet quality, evaluated by the Youth Healthy Eating Index (YHEI), and inflammation, measured by hs-CRP from serum in a large sample of Hispanic American adolescent girls, ages 9-13 years. It was hypothesized that diet quality would be associated with lower inflammation in Hispanic American girls.

SUBJECTS AND METHODS

Study Design

For the present analysis, cross-sectional baseline data from Hispanic adolescent girls enrolled in the “Soft Tissue and Bone Development in young girls” study (STAR) were used. STAR is an ongoing prospective cohort study in normal weight (BMI 5th to 85th percentile), over-weight (BMI 85^{th} to 95th percentile), and obese (BMI>95^{th} percentile) pre-menarcheal girls. STAR participants were recruited from schools, pediatrician's offices, and community groups in Tucson, Arizona. The University of Arizona Human Subjects Protection Committee approved the STAR study and written, informed consent was obtained for all participants.

Participants

The sample included 113 healthy Hispanic girls, aged 9-13 years. Girls were excluded if they had a previous diagnosis of type 1 or type 2 diabetes, used medications that alter body composition including bone mineral accrual (i.e. growth hormone, albuterol), had a physical disability that could limit participation in physical activity, or diagnosis of a learning disability that would limit completion of questionnaires. Smoking status and use of anti-inflammatory medication have been found to be associated with altering CRP concentrations in adults and adolescents (51-55) however data regarding these two variables were not collected in the STAR study.

Dietary assessment

Dietary intake was estimated using the semi-quantitative 2012 Youth Adolescent Questionnaire (YAQ), a food frequency questionnaire validated by Rockett et al. in adolescents (56, 57). The YAQ was completed at home unless the questionnaire was incomplete when the participant arrived to the lab for measurements. Participants could receive assistance to complete the form from trained study technicians or a parent/guardian, if needed. YAQs were evaluated for completeness and coded by trained staff following a standard protocol and were subsequently sent to Channing Laboratories (Boston, MA) for food and nutrient analyses that have been described in detail elsewhere (58).

Youth Healthy Eating Index (YHEI)

Feskanich et al. modified the validated and widely used Healthy Eating Index (HEI), created by the USDA, in order to develop the YHEI to accurately assess the dietary habits specific to children and adolescents (59). Rather than calculating specific nutrient intakes, the YHEI focuses on food choices to assess fat, sugar, fiber and sodium intake, including high trans-fat snack foods and sugar-sweetened beverages (components 6 and 7), and also includes behaviors associated with scholastic performance and healthy dietary intake patterns (components 12 and 13). YHEI scores were modified from those of the HEI to reflect the 5-A-Day serving size recommendations developed by the National Cancer Institute (56, 57). Frequency factors developed by Feskanich et al. (59) were used to determine the number of daily serving sizes for each component. These serving sizes were then used to determine the YHEI score for each component.

A modified version of the YHEI scoring was used in this study. Specifically, three components of the original YHEI could not be accurately calculated in this study due to modifications to the YAQ since the development of the YHEI. These components included the consumption of visible fat and skin (component 11), a second measure of saturated fat intake with lower weight (5 points) versus the 10-point meat ratio (component 5), and two behavioral components, the frequency of eating dinner with family (component 12) and the frequency of breakfast consumption (component 13). Seven components of the modified and original versions of the YHEI were scored from 0 to 10 points and were derived from multiple foods and questions from the YAQ, including fruit, vegetables, whole grains, dairy, meat ratio (lean protein: fat protein), snack foods (high in salt or sugar), and soda and drinks (i.e. regular soda, fruit punch, and sweetened iced tea). Three components of the YHEI, multivitamin use, margarine and butter use, and fried foods, were scored from 0 to 5 points, consistent with previous literature on scoring parameters and reflecting the limited number of foods and questions from the YAQ that captured these exposures (59). Feskanich et al. previously demonstrated that 85% of the variation in YHEI scores in a sample of 16,452 adolescents was due to 5 of the 7 most heavily weighted components (whole grains, fruits, meat ratio, snack foods, soda and drinks), which we assessed, while the 3 components excluded from the modified YHEI used here accounted for only 5% of the variation in the total YHEI score. Thus, the modified YHEI score used here with a maximum score of 85 (compared to the original 100-point YHEI) is adequate to capture the diet quality of our sample of adolescent girls. The scoring criteria for the original and modified versions of the YHEI are given in **Table 1.** A higher total YHEI score reflects better diet quality; therefore, higher values for components 6,7,9, and 10 reflect lower consumption, given that those components represent energy dense foods.

Laboratory Methods

After an overnight 12-h fast, blood samples were collected from all subjects through venous puncture by a trained phlebotomist. Blood for serum samples were collected in serum-separating tubes and allowed to clot. The serum was separated by centrifugation at 3,000 rpm for 15 minutes at 20°C. The serum was then divided into aliquots, frozen, and stored at−80°C until assayed. Serum hs-CRP concentrations were measured by latex-enhanced nephelometry at the University of Washington Department of Laboratory Medicine [BN-II nephelometer, Siemens]. Low and high inter-assay quality control procedures were used and the coefficient of variations were 3.75% to 4.64%, respectively. The assay could detect a minimal concentration of 0.2 mg/L, and values below this level were classified as "non-detectable". Approximately half of participants had "non-detectable" concentrations of hs-CRP. Greater than 50% of adolescent participants with non-detectable CRP concentrations has been reported in other studies thereby justifying the inclusion of the participants in the current analyses (35).

Anthropometry

Anthropometric data were collected on all participants. Body weight and height were measured by technicians following standard protocols as described in the Anthropometric Standardization Reference Manual (60). Weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Model 881; Seca, Hamburg, Germany) (61). Standing height, measured to the nearest 0.1 cm, was assessed at full inhalation using a stadiometer (Shorr Height Measuring Board, Olney). The mean of two measurements was used for each anthropometric variable. The two measurements were repeated if the measures differed by more than 4 mm for height and sitting height, and 0.3 kg for body weight. If repeat measures were required, the mean of the repeated measures was used. BMI (kg/m^2) was calculated from height and weight.

Body composition

Dual-energy x-ray absorptiometry (DXA) was used to quantify total fat mass, percent body fat (ratio of fat mass to whole body mass), and android percent fat as a surrogate of visceral fat, using the GE Lunar PRODIGY [GE Lunar Radiation Corp; Madison, WI, USA] (software version 5.60.003). Participants were positioned for whole body scans using standard manufacturer protocols. DXA coefficients of variation and precision in our laboratory have been reported elsewhere (62). All scans were completed and analyzed by a certified technician following a standard protocol using the extended research mode software.

Physical maturation

Maturation was assessed using the validated gender-specific equation developed by Mirwald et al. to estimate maturity offset, an estimate of years from peak height velocity (PHV), from chronological age, weight, and ratios of skeletal lengths (63). Mirwald's equation was derived using data from a longitudinal study in boys and girls (64). In Mirwald's sample, the maturity offset equation explained 89% of the variance of years from PHV for girls (63). A negative maturity offset value represents years before PHV, whereas a positive maturity offset value represents years after PHV.

Physical activity

The validated Past Year Physical Activity Questionnaire (PYPAQ) was used to assess physical activity (65). PYPAQ assesses participation in 41 common sport and leisure-time activities performed ≥10 times in the past year (66). Average duration, average weekly frequency, and number of months of participation for each activity were obtained and total PYPAQ score was computed using a modified algorithm, which has been reported elsewhere (66).

Statistical analysis

Data were analyzed using SPSS for Windows statistical software, Version 22.0 [Chicago, IL, USA]. Participants with complete data for all variables were used in this analysis. Means, standard deviations, and ranges were calculated. Concentrations of hs-CRP were categorized into categories: “non-detectable”, “middle”, and “high”. hs-CRP concentrations below 0.2 mg/L were “non-detectable”. The “middle” and “high” groups were derived based on the median number of remaining concentrations in order to establish equal groups.

Multinomial logistic regression was employed to examine the associations between individual YHEI components, YHEI total score, and categories of serum hs-CRP levels. In order to facilitate the interpretation of the OR, total YHEI score was transformed to represent a 10-unit difference (1 SD change). Covariates included in the final models were maturity offset, PYPAQ score, BMI, and total energy intake. In addition to these covariates, when assessing the associations between each individual YHEI components and categories of hs-CRP concentrations, all other individual YHEI components were controlled.

Sensitivity analyses were conducted by running all analyses excluding observations with hs-CRP concentrations >10 mg/L (n=3), as it is suggested that concentrations greater than >10 mg/L may indicate acute infection or illness (67). There were substantive differences in the results and as such these observations were excluded from the final dataset. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics of the 113 study participants are shown in **Table 2**. The average age of participants was 11.0±1.0 years, with a range of 9.0 to 13.1 years. On average, the girls were 1.9± 0.9 years from achieving peak height velocity. BMI and total body fat percent for all participants averaged 21.0 ± 5.0 kg/m^2 and 32.2 ± 9.8%, respectively. Based on the U.S. National Center for Health Statistics/Centers for Disease Control and Prevention percentiles for body mass index (BMI, kg/m^2) (17), 3.5% of the study sample was underweight (BMI<5th percentile), 55.8% were normal weight (BMI 5th to 85th percentile), 13.3% were overweight (BMI 85th to 95th percentile), and 27.4% were obese (BMI>95th percentile).

The average YHEI score was 48.1 ± 9.7 and average daily energy intake was 2119±968 kcals. Serum hs-CRP concentrations in the "middle" and "high" groups ranged from 0.2 to 0.7 mg/L and 0.7 to 6.2 mg/L, respectively (data not shown). There were 13 study participants with hs-CRP concentrations considered as moderate cardiovascular risk (1.0mg/L -3.0 mg/L) and 10 participants with hs-CRP concentrations considered as high cardiovascular risk (>3.0 mg/L) (11).

Table 3 shows the descriptive statistics for individual YHEI components and total YHEI score for study participants. Out of a total of ten points for each component towards the total YHEI score, average "fruit" score was 5.78, "vegetables" score was 5.87, "dairy" score was 6.98, "whole grains" score was 3.51, "meat ratio" score was 8.85, "snack foods" score was 5.31, and "soda and drinks" score was 7.75. Out of a total of five points for each component towards the total

YHEI score, average “multivitamin use” score was 1.0, “margarine and butter use” score was 3.66, and “fried foods” score was 2.98.

Adjusted odds ratios and respective 95% CI and p-values from multinomial logistic regression models are reported in **Table 4.** Results indicate that for every 10-unit increase in total YHEI score, the odds of being in the “middle” or “high” categories were not significantly different compared to the “undetectable” category, after adjusting for maturity offset, PYPAQ score, total energy intake, and total body fat percent. For every 1-unit increase in “whole grains” score there was a 44% increase in odds of being in the “high” category compared to the “undetectable” category, after adjusting for maturity offset, PYPAQ score, total energy intake, total body fat, and all other individual YHEI components (OR: 1.44, 95% CI: 1.06, 1.97).

DISCUSSION

The current study is novel in that it is the first to assess the relationship between diet quality and individual component scores and inflammation in a large group of Hispanic American girls. Our results revealed non-significant findings, suggesting that a 10-unit increase in diet quality score, as assessed by the YHEI scoring index, does not increase the odds of being in the “middle” or "high” categories of inflammation, as measured by hs-CRP, compared to the “non-detectable” category. To date, only two studies have assessed the relationship between diet quality and inflammation, by measures of hs-CRP, in adolescents, using dietary quality scoring (26, 68). These studies found both significant inverse and null findings between diet quality and inflammation, each using two (68) and three (26) dietary indexes.

For example, in 5198 German adolescents, ages 12 to 17 years, Truthmann et al. used three different dietary indices [the Healthy Food Diversity-Index (HFD), the Healthy Nutrition Score for Kids and Youth (HuSKY), and the Indicator Food Index (IFI)] to measure diet quality and assess the relationship between diet quality and inflammation (26). Mean values of CRP decreased signficantly (20.5 mg/L, 16.4 mg/L, 15.5 mg/L, respectively) across increasing quintiles 1,3, and 5 of IFI scores (β=-14.005 p=0.007) and decreased significantly (19.4 mg/L, 16.6 mg/L, 15.5 mg/L, respectively) across increasing quintiles of HuSKY scores (β=-8.215 p=0.05) after controlling for age, energy intake, BMI, alcohol consumption, season, physical activity, smoking status, and family socioeconomic status, in 2,438 girls (26). CRP concentrations did not significantly decrease across increasing quintiles of HFD scores (19.6 mg/L, 15.2 mg/L, 16.2 mg/L, respectively).

Compared to the study by Truthmann et al., our sample size was much smaller (n= 113 vs. 2,438) therefore significant relationships may have been observed in their study due to sample size. On average, our girls were younger (9-13 years vs. 12-17 years). In our study, age was not found to be significantly associated with concentrations of hs-CRP (data not shown), however, age and maturation are both risk factors associated with increased risk of CVD. Given than maturation was controlled in our analyses and that most girls were pre-pubertal, it is possible that higher values of CRP were observed in the Truthmann et al. study because the age group of participants included those who have reached puberty. Although, age was controlled in their analyses, maturation was not. However, compared to Mexican American girls from NHANES 1999-2000, average CRP scores reported in the current study are comparable (0.76 mg/L vs. 0.68 mg/L, respectively). Although the method used to calculate obesity differed, we had a higher percentage of obese participants (28% vs. 17.2%). Results from the current study indicated that total body fat was the only significant covariate in the models in that the odds of being in the "high" category of hs-CRP were 21% higher than being in the "non-detectable" category (OR: 1.21, 95% CI 1.11, 1.31, $p<0.0001$) (data not shown). As a result, it is clear from our findings that adiposity is more closely associated with hs-CRP concentrations than diet quality scores. In the current study it is possible that a significant association was not observed between diet quality scores and hs-CRP concentrations because so many participants were obese and adiposity was a much strong contributor to hs-CRP concentrations. Data from NHANES 2011-2012 reported that the prevalence of obesity in Mexican American girls, ages 6 to 11 years and 12-19 years is 23.4% and 21.3%, respectively (50).

In the second study, Lazarou et al.(68) used two indexes to measure diet quality, the Mediterranean Diet Quality Index for children and adolescents (KIDMED) and the dietary inflammation index (DII). Results did not reveal significant associations between KIDMED scores and stratified concentrations of hs-CRP (<0.01 mg/L and ≥ 0.01 mg/L) in 83 children of Greek ancestry, aged 6 to 12 years (68). Fifty percent of the sample reported that they consume delicatessen-type meat, and 40% of participants reported that they consume soft drinks (68). Therefore, although numerous foods modulate inflammation, it is apparent that many participants reported consuming "inflammatory" foods despite the non-significant relationship between KIDMED score and CRP. The investigators also evaluated the relationships between intake of specific "inflammatory" foods, measured by questions on the food frequency questionnaire, and concentrations of CRP and found significant ($p<0.05$) positive relationships between CRP concentrations and potato chip consumption (rho=0.265) and delicatessen meats (rho=0.286). Although not signficant, several other "inflammatory" food items were found to be positively associated with CRP, with a range in rho of 0.100 to 0.150 (68). Inflammatory foods are not measured well by the KIDMED index which may explain why a non-significant relationship between KIDMED score and hs-CRP concentrations was observed. KIDMED is a 16-component index that awards +1 points to foods or behaviors that are favorable to the Mediterranean diet and -1 points to those that have a negative conotation. Since there are only 16 components, each category is fairly general (i.e. takes a fruit or fruit juice every day) (69). As a result, it was not designed to adequately assess detailed consumption of foods that are associated with inflammation and the scoring criteria does not allow for much diversity. Consequently, in a second analysis wherein the relationship between measures of adiposity and hs-CRP concentrations were assessed, diet quality as measured by DII was included as one of the covariates. DII was positively correlated ($p<0.05$) with concentrations of hs-CRP. In other words, DII explained as estimated 5% of the variability in hs-CRP while the whole model, including age, gender, KIDMED score, and physical activity, and adiposity variables explained as much as 30% of the variability in hs-CRP. Of the 30%, adiposity variables, BMI, WC, and body fat percent explained 18.5% of the variance (68).

The results of these two studies suggest that dietary quality does not have a consistent relationship with hs-CRP even when index scores are derived from the same data, reflecting the differences in diet quality indices based on different component quantities, cut-off values, and scores derived from various diet assessment tools with their own limitations (70). Potential differences between population groups, length of follow-up, and approaches to adjusting confounders add to the difficulty of inter-study comparisons (71).

The results of the present study also showed that higher “whole grains” score increase the odds of being in the “high” hs-CRP category compared to the “non-detectable” category. This finding was not anticipated and could be a chance finding. Results from observational studies collectively suggest an inverse effect between whole grain intake and inflammation (72-75), while results from intervention studies are mixed. In a recent review (76), only one of five intervention studies in adults reported a beneficial association between whole-grain intake and inflammation (77), while the remaining four studies revealed no change in inflammation with whole-grain intake (78-81). A recent randomized controlled crossover trial in 44 overweight or obese girls, aged 8-15 years, reported that the intervention group, in which half of their needed servings of grains came from whole-grains, girls had significant decreases (p=0.03) in hs-CRP levels (-21.8%) compared to increases in hs-CRP concentrations (+12.1%) in the control group after 6 weeks (82). de Punder and Pruimboom recently suggested that cereal grains that are not “whole grains” contain “anti-nutrients” that may contribute to the inflammatory pathway (83). In the current study, points were awarded towards the total “whole grains” score if greater than 25% of the food contained whole grains, as a result it is possible that “anti-nutrients” from items containing less than 100% whole grains may be contributing to the relationship between “whole grains” score and categories of hs-CRP. Similarly, girls who reported consuming two or more servings of whole grains per day on average reported consuming servings of dark bread that were 407% greater than those who reported consuming less than 2 servings of whole grains per day (data not shown). Due to the limited information from the food frequency questionnaire, we were unable to identify if participants consumed 100% whole wheat or whole grain bread instead of bread containing some whole wheat.

This study has several strengths and limitations. An important strength was the effort to robustly characterize the girls in the sample in such a way as to be able to control for several confounders in the analyses. Additionally, the sample, by design, recruited girls of diverse BMI and thus the capacity to evaluate total body fat as an effect modifier of these relationships. The use of DXA to measure total body fat percent was another strength of the study as this was the first study to control for adiposity using a more direct method than anthropometry. Finally, this is the first analysis that represents Hispanic American girls, a group that may be more susceptible to early age of onset adiposity and related inflammatory exposures. The limitations of questionnaires for dietary assessment are well known. Energy intake is commonly misreported and while the YAQ has been validated in adolescents (56, 57), reporting bias remains a concern with FFQs (84-86). The criteria of inclusion for the “whole grains component” in the YHEI includes cooked breakfast cereals, cold breakfast cereal, other grains (brown rice), and dark bread (whole wheat or whole grain). These items were then multiplied by a factor of 1.0 if >=50% of the item was whole grain, 0.5 if >25% and <50% of the item was whole grain, or 0.0 if <=25% of the item was whole grain. As a result, it is possible items that may not have meet the definition of whole grain or contain very little whole grains may have been misclassified in the estimate of whole grains. The 2012 YAQ used in the STAR study varied from the original YAQ used to derive the YHEI, necessitating the use of a modified version of the YHEI scoring for this analysis. Further refinement of the YHEI diet quality score is warranted, as additional study of the diet quality and inflammation relationship is undertaken. Finally, the present analyses were limited to cross-sectional associations and prospective studies are required to more robustly evaluate these relationships.

In conclusion, this study suggested that diet quality was not significantly associated with inflammation in Hispanic American adolescents. More specifically, our findings suggested that higher whole grain intake, possibly from consumption of whole-wheat bread that was not comprised of 100% whole grains, is associated with higher hs-CRP concentrations. To date, literature concerning associations between diet quality scores and inflammation in youth is scant. Therefore, currently, no conclusive relationships can be drawn. Future studies are needed to further elucidate these relationships. The use of dietary indexes to measure dietary quality and its association with targeted health outcomes is critical to understanding the true nature of these relationships in adolescents. However, it is clear that more work needs to be done to improve dietary quality indexes to better evaluate the role of dietary quality on inflammation.

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Table 1. Youth Healthy Eating Index (YHEI) scoring criteria

		YHEI scoring criteria	
Original YHEI Components	**Modified YHEI Components**	**Requirement for minimum score of 0**	**Requirements for maximum score of 10**
		servings/day[1]	
Whole Grains	Whole Grains	0	≥3
Vegetables	Vegetables	0	≥3
Fruits	Fruits	0	≥3
Dairy	Dairy	0	≥2
Meat Ratio[2]	Meat Ratio[2]	0	≥2
Snack Foods[3]	Snack Foods[3]	≥3	0
Soda and Drinks	Soda and Drinks	≥3	0
		Requirement for minimum score of 0	**Requirements for maximum score of 5**
		servings/day[1]	
Multivitamin Use	Multivitamin Use	Never	Daily
Margarine and butter	Margarine and butter	≥2 pats/day	Never
Fried foods outside home	Fried foods outside home	Daily	Never
Visible animal fat[4]	-	None	All
Dinner with family	-	≥5 times/week	Never
Eat breakfast	-	Daily	Never
Total YHEI score (0-100)	Total YHEI score (0-85)		

[1] STAR used the Youth/Adolescent Questionnaire (YAQ) to assess habitual dietary intake. Serving sizes are based on definitions of the YAQ.

[2] Total servings per day of white meat including chicken, fish, seafood, eggs, soy, tofu, beans, and nuts were divided by servings per day of dark meat including beef, pork, and lamb.

[3]Snack foods include salty snacks (e.g., potato chips, corn chips, popcorn, pretzels, and crackers) and snacks with added sugar (e.g., cake, snack cake, toaster pastry, sweet roll/Danish/pastry, doughnut, brownie, cookies, pie, chocolate, candy bar with chocolate, candy without chocolate, fruit rollup, popsicle, and flavored gelatin).

[4]Visible animal fat includes the visible fat on meat and the skin on chicken or turkey.

Table 2. Descriptive characteristics of 113 adolescent girls

Characteristics	Mean ± SD
Age (years)	11.0±1.0
Dietary Intake/Assessment	
Total energy (kcals)	2119 ± 968
YHEI score[1]	48.1±9.7
Body Composition	
Height (cm)	146.9±8.8
Weight (kg)	45.8±14.3
BMI (kg/m^2)	21±5
BMI Category (%)	
Underweight	3
Normal weight	55
Overweight	14
Obese	28
Total body percent fat	32.2±9.8
Android percent fat	37.1±14.0
Thigh Muscle Density (mg/cm^3)	78.2±1.8
Calf Muscle Density (mg/cm^3)	79.5±1.4
Maturational Status	
Maturity offset (years)[2]	-1.9±0.9
Physical Activity Assessment	
PYPAQ score[3]	5490.1 ± 5940.2

[1]YHEI score: Youth Healthy Eating Index (modified) 0 (non-adherent) to 85 (full adherence)

[2]Maturity offset= estimated years from peak height velocity (PHV)

[3]PYPAQ score: Past year physical activity

Table 3. Youth Healthy Eating Index (YHEI) scores

YHEI component	YHEI scores, mean (range)
	(Maximum total 10 points)
Fruit	5.78 (0.62-10)
Vegetables	5.87 (0.49-10)
Dairy	6.98 (1.13-10)
Whole grains	3.51 (0-10)
Meat ratio[1]	8.85 (1.31-10)
Snack foods[1]	5.31 (0-9.73)
Soda and drinks	7.75 (0-10)
	(Maximum total 5 points)
Multivitamin Use	1.0 (0-5)
Margarine and butter use	3.66 (0.25-5)
Fried foods	2.98 (0-5)
YHEI total score	48.10 (25.33-72.69)

[1]Refer to **Table 1** for variable criteria

Table 4. Adjusted odds ratios from multinomial logistic regression models between YHEI total and individual component scores and categories of serum hs-CRP concentrations

	Middle[1] [hs-CRP(mg/L)]	**High[1] [hs-CRP(mg/L)]**
Total YHEI Score[2,3]	1.01 (0.96, 1.07)	1.05 (0.99, 1.12)
Fruit[4]	0.87 (0.67, 1.15)	1.18 (0.86, 1.62)
Vegetables[4]	1.06 (0.87, 1.30)	1.01 (0.78, 1.30)
Whole grains[4]	1.00 (0.77, 1.31)	**1.44 (1.06, 1.97)**[a]
Meat ratio[4]	1.11 (0.80, 1.54)	1.10 (0.77, 1.58)
Dairy[4]	1.20 (0.90, 1.61)	0.74 (0.53, 1.03)
Snacks[4]	0.76 (0.58, 1.01)	0.84 (0.60, 1.18)
Margarine and butter use[4]	1.32 (0.69, 2.56)	1.66 (0.65, 4.26)
Multivitamin use[4]	1.10 (0.78, 1.55)	1.09 (0.72, 1.65)
Soda and drinks[4]	1.21 (0.88, 1.66)	0.87 (0.62, 1,21)
Fried foods[4]	1.10 (0.74, 1.63)	0.99 (0.61, 1.59)

[1]Compared to reference group: "non-detectable"
[2]Model 1 controlled for: maturity offset, PYPAQ score, total energy intake, total body fat percent
[3] Total YHEI score was transformed to represent a 10-unit difference (1 SD change)
[a]sig. at p<0.05

[4]Model 2 controlled for Model 1 covariates and all other individual YHEI components when not the independent variable (fruit, vegetables, whole grains, meat ratio, dairy, snacks, margarine and butter use, multivitamin use, soda and drinks, fried foods)

APPENDIX C. Total and regional adiposity are positively associated with inflammation in Hispanic American adolescent girls.

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ABSTRACT

Background: C-reactive protein (CRP), a circulating inflammatory biomarker, is an established risk factor for cardiovascular disease and is associated with adiposity even at a young age. Research addressing these relationships in Hispanic American girls is sparse.

Objective: To assess the relationship between total and regional adiposity and serum concentrations of high-sensitivity CRP (hs-CRP) in Hispanic American adolescent girls.

Design: Total body and android fat (a surrogate for visceral fat) were measured by dual energy x-ray absorptiometry in a cross-sectional study of 113 healthy Hispanic girls, ages 9-13 years. Muscle density (mg/cm^3), a surrogate for skeletal muscle fat content was measured at tibial and femoral sites using peripheral quantitative computed tomography. Serum hs-CRP concentrations were measured by nephelometry. Multinomial logistic regression was employed to assess the relationships between total body percent fat, android percent fat, skeletal muscle density, and body mass index (BMI) with categories of serum hs-CRP concentrations.

Results: The odds of being in the "high" category of hs-CRP were 38% higher compared to those with "non-detectable" hs-CRP for every 1 kg/m^2 increase in BMI (OR: 1.33, 95% CI 1.16, 1.53, $p<0.0001$). The odds of being in the high hs-CRP category increased with every 5 percent unit increase in total body percent fat (OR: 2.38, 95% CI 1.58, 3.58, $p<0.0001$) and android percent fat (OR: 1.89, 95% CI 1.39, 2.57, $p<0.0001$), compared to those with undetectable concentrations. Calf muscle density was associated with lower odds of elevated hs-CRP compared to the non-detectable group (OR: 0.58, 95% CI 0.35, 0.75, $p<0.001$). **Conclusions:** The findings suggest that greater total and visceral body fat and calf skeletal muscle fat contributes to inflammation among Hispanic American girls aged 9-13 years.

INTRODUCTION

Childhood obesity is the most prevalent cardiovascular (CV) risk factor observed in adolescents. Currently, 61% of obese youths have at least one cardiovascular disease (CVD) risk factor, thereby increasing future risk of CVD (1, 2). Obesogenic behaviors, obesity, and its comorbidities track into adulthood such that obese adolescents tend to become the heaviest adults with the most adverse health risk profiles (3-5). Further, obesity has been classified as a low-grade inflammatory condition (6) in that excess adiposity increases the production and release of inflammatory cytokines generated within the adipose tissue. Thus, the pathophysiology of obesity durthers the risk for an adverse CV risk profile (1).

C-reactive protein (CRP) is considered the closest and most accurate biomarker of adipocyte-generated chronic low-grade inflammation (7, 8). CRP is a strong biological predictor of risk of CVD (7, 8), and its concentrations are related to adiposity, even at an early age (9-25). While considerable work has been done that shows adiposity is significantly and positively associated with CRP concentrations in non-Hispanic adolescents (9-25), less is known of this relationship in Hispanic American youths. Hispanics and Latinos are the largest minority group in the U.S., despite this group experiencing the highest burden of CV risk factors (26). Indeed, data from National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999-2006 indicated that Mexican Americans have the highest prevalence of metabolic syndrome compared to non-Hispanic Whites and non-Hispanic Blacks (27). Moreover, results from NHANES 2003-2004 suggests that Mexican American women are 1.5 times more likely to meet the criteria for metabolic syndrome as compared to non-Hispanic white females (28). NHANES data showed that CRP concentrations are higher in children who present with metabolic syndrome components (6, 29). In fact, the obesity-CVD risk profile has been largely attributable to insulin resistance and inflammation (30-37). Research has suggested that abdominal adiposity, a risk factor of metabolic syndrome, is higher in Hispanic youth, compared to Caucasian and African American youths (38, 39) with significant differences only between Hispanic and African American youths after controlling for total adiposity (40, 41). Similarly, obese Hispanic adolescents have higher skeletal muscle fat compared to Caucasian and African American adolescents, despite a similar degree of overall adiposity (41). Most studies that have assessed the relationships between adiposity and CRP concentrations in Hispanic American adolescents have been restricted to anthropometric measures of adiposity, and skeletal muscle fat has not been studied.

The present study was designed to address the gap in the literature related to adiposity, inflammation in a sample of well-characterized adolescent Hispanic girls. We also sough to and improve upon past studies by assessing the relationship between inflammation and total, truncal, and skeletal muscle adiposity. We hypothesized a positive association between adiposity and hs-CRP, similar to the relationships reported in studies in other populations samples of divers ages and race.

SUBJECTS AND METHODS

Study Design

Cross-sectional baseline data from Hispanic adolescent girls enrolled in the "Soft Tissue and Bone Development in young girls" study (STAR) were used. STAR is an ongoing prospective cohort study in normal weight, over-weight, and obese pre-menarcheal girls. STAR participants were recruited from schools, pediatrician's offices, and community groups in Tucson, Arizona. The University of Arizona Human Subjects Protection Committee approved the STAR study and written, informed consent was obtained for all participants.

Participants

The sample included 113 healthy Hispanic girls, aged 9-13 years. Girls were excluded if they had a previous diagnosis of type 1 or type 2 diabetes, used medications (i.e. growth hormone, albuterol) that alter body composition including bone mineral accrual, had a physical disability that could limit participation in physical activity, or diagnosis of a learning disability that would limit completion of questionnaires. Study participants had a wide range of BMIs including normal weight (BMI 5th to 85th percentile), over-weight (BMI 85^{th} to 95th percentile), and obese (BMI>95^{th} percentile).

Laboratory Methods

After an overnight 12-h fast, blood samples were collected from all subjects through venous puncture by a trained phlebotomist. Blood for serum samples were collected in serum-separating tubes and allowed to clot. The serum was separated by centrifugation at 3,000 rpm for 15 minutes at 20°C. The serum was then divided into aliquots, frozen, and stored at−80°C until assayed. Serum hs-CRP concentrations were measured by latex-enhanced nephelometry at the University of Washington Department of Laboratory Medicine [BN-II nephelometer, Siemens]. Low and high inter-assay quality control procedures were used and the coefficient of variations were 3.75% to 4.64%, respectively. The assay could detect a minimal concentration of 0.2 mg/L; values below this level were classified as non-detectable. Approximately half of participants had "non-detectable" concentrations of hs-CRP. Greater than 50% of adolescent participants with non-detectable CRP concentrations has been reported in other studies thereby justifying the inclusion of the participants in the current analyses (15).

Anthropometry

Anthropometric data were collected on all participants. Body weight and height were measured by technicians following standard protocols as described in the Anthropometric Standardization Reference Manual (42). Weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Model 881; Seca, Hamburg, Germany) (43). Standing height, measured to the nearest 0.1 cm, was assessed at full inhalation using a stadiometer (Shorr Height Measuring Board, Olney). The mean of two measurements was used for each anthropometric variable. The two measurements were repeated if the measures differed by more than 4 mm for height and sitting height, and 0.3 kg for body weight. If repeat measures were required, the mean of the repeated measures was used. BMI (kg/m^2) was calculated from height and weight.

Body composition

Dual-energy x-ray absorptiometry (DXA) was used to quantify total fat mass, percent body fat (ratio of fat mass to whole body mass), and android percent fat as a surrogate of visceral fat, using the GE Lunar PRODIGY [GE Lunar Radiation Corp; Madison, WI, USA] (software version 5.60.003). Participants were positioned for whole body scans using standard manufacturer protocols. DXA coefficients of variation and precision in our laboratory have been reported elsewhere (44). All scans were completed and analyzed by a certified technician following a standard protocol using the extended research mode software. Android fat was estimated for the area enclosed by demarcations immediately above the iliac crest and at 20% of the total distance between the iliac crest and the base of the skull (approximately the bottom rib), using the following equation: [(Android fat mass) / (Android Bone Mineral Content + Android fat mass + Android lean mass)] x 100.

Peripheral quantitated computed tomography (pQCT), a low-dose radiation technique, was used to measure muscle density, a surrogate for skeletal muscle fat (45). This technique is not capable of distinguishing intra-myocellular from extra-myocellular fat and therefore cannot directly measure fat infiltration in skeletal muscle. Thus, muscle density reflects both intra- and extra-myocellular fat stores and is inversely related to muscle fat content such that a lower muscle density is indicative of higher skeletal muscle fat content (45). pQCT was used to measure thigh and leg soft tissue composition, including muscle density (mg/cm^3), at the 20% femur (thigh) and 66% tibia (44) sites relative to the respective distal growth plates of the non-dominant limb. Scans were performed using a Stratec XCT 3000 scanner [Stratec

Medical GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY, USA] and analyzed using Stratec software, Version 5.50. All pQCT scans and scan analyses were performed by trained technicians using the guidelines of Bone Diagnostics, Inc. [Fort Atkinson, WI, USA]. Adipose tissue, skeletal muscle, and bone are distinguishable as independent tissues based on attenuation characteristics, which are directly related to tissue composition and density, and can be estimated using edge detection and threshold techniques (46, 47). Images were filtered prior to being analyzed using contour mode 3 (-101 mg/cm^3) and peel mode 2 (40 mg/cm^3) to separate adipose (<40 mg/cm^3) and muscle/bone (-40 mg/cm^3), respectively (47). Images were filtered subsequently with a 7x7 image filter that clearly defined the edge of the muscle and eliminated all bone above 120 mg/cm^3 (48). This method ensures that muscle density was estimated from only soft tissue within the edge of the muscle.

Dietary assessment

Total energy intake was estimated using the semi-quantitative 2012 Youth Adolescent Questionnaire (YAQ), a food frequency questionnaire validated by Rockett et al. in adolescents (49, 50). Participants could receive assistance to complete the form from trained study technicians or a parent/guardian, if needed. YAQs were evaluated for completeness, coded by trained staff following a standard protocol and were subsequently sent to Channing Laboratories (Boston, MA) for food and nutrient analyses that have been described elsewhere (51).

Youth Healthy Eating Index (YHEI)

Dietary quality was assessed using a modified version of the YHEI (52). The original YHEI was developed by Feskanich et al. represented a modified version of the validated and widely used Healthy Eating Index (HEI), created by the USDA. The YHEI was developed to accurately assess the dietary habits specific to children and adolescents (52).

Physical maturation

Maturation was assessed using the validated gender-specific equation developed by Mirwald to estimate maturity offset, an estimate of years from peak height velocity (PHV), from chronological age, weight, and ratios of skeletal lengths (53). Mirwald's equation was derived using data from a longitudinal study in boys and girls (54). In Mirwald's sample, the maturity offset equation explained 89% of the variance of years from PHV for girls (53). A negative maturity offset value represents years before PHV, whereas a positive maturity offset value represents years after PHV.

Physical activity

The validated Past Year Physical Activity Questionnaire (PYPAQ) was used to assess physical activity (55). PYPAQ assesses participation in 41 common sport and leisure-time activities performed ≥10 times in the past year (56). Average duration, average weekly frequency, and number of months of participation for each activity were obtained and total PYPAQ score was computed using a modified algorithm that has been reported elsewhere (56).

Statistical analysis

Data were analyzed using SPSS for Windows statistical software, Version 22.0 [Chicago, IL, USA]. Participants with complete data for all variables were used in this analysis. Means, standard deviations, and ranges were calculated. For descriptive purposes, data was categorized according to BMI categories: normal weight and overweight/obese. Independent t-tests were used to assess statistically significant differences by BMI categories. Concentrations of hs-CRP were categorized into three groups: “non-detectable”, “middle”, and “high”. hs-CRP concentrations below 0.2 mg/L were “non-detectable”. All detectable concentrations were divided at the median into two equal groups to create the middle and high categories of hs-CRP.

Due to collinearity between measures of adiposity, adjusted odds ratios (OR) from five separate multinomial logistic regression models were computed to examine the associations between BMI, total body fat percent, android percent fat, and skeletal muscle density, and categories of serum hs-CRP concentrations. In order to facilitate the interpretation of the OR, total body fat percent and android percent fat were transformed to represent a 5% difference. Covariates included in the final models were maturity offset, PYPAQ, total energy intake, and total YHEI score. In models with android percent fat or muscle density as independent variables, BMI was added into the model to control for total adiposity.

Sensitivity analyses were conducted by running all analyses excluding observations with hs-CRP concentrations >10 mg/L (n=3), as it is suggested that concentrations greater than >10 mg/L may indicate acute infection or illness (57).

There were substantive differences in the results and as such these observations were excluded from the final dataset. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics of the 113 study participants are given for the total sample and also by normal weight and overweight/obese BMI categories in **Table 1**. The average age of participants was 11.0±1.0 years, with a range of 9.0 to 13.1 years. On average, the girls were 1.9± 0.9 years from achieving peak height velocity. The average YHEI score was 47.8 ± 9.9 and mean daily energy intake was 2119±968 kcals. BMI and total body fat percent for all participants averaged 21.0 ± 5.0 kg/m^2 and 32.2 ± 9.8%, respectively. Based on the U.S. National Center for Health Statistics/Centers for Disease Control and Prevention percentiles for BMI (kg/m^2) (17) 3.5% of the study sample were underweight (BMI<5th percentile), 55.8% were normal weight (BMI 5th to 85th percentile), 13.3% were overweight (BMI 85th to 95th percentile), and 27.4% were obese (BMI>95th percentile) (data not shown).

Results from independent t-test identified significant differences by BMI categories. The overweight/obese group had significantly higher BMI (+47%), total body fat percent (+63%), android percent fat (+85%), and lower calf muscle density (-2%), compared to their leaner peers (all $p<0.01$). The majority of "non-detectable" hs-CRP concentrations were found in normal weight participants (70%). The number of participants with detectable hs-CRP concentrations was comparable between normal weight (n=24) and overweight/obese participants (n=28). The average detectable hs-CRP concentration in normal weight participants was 0.9 ± 1.5 mg/L with a range of 0.2-6.2 mg/L. The average detectable hs-CRP concentration in overweight/obese participants was 2.0 ± 1.4 mg/L with a range of 0.3 – 5.5 mg/L.

Based on categories of hs-CRP, the serum hs-CRP concentration for the “middle” group ranged from 0.2 to 0.7 mg/L and from 0.7 to 6.2 mg/L for the “high” group. There were 13 study participants with hs-CRP concentrations that would categorize them as moderate cardiovascular risk (1.0mg/L -3.0 mg/L) and 10 participants with hs-CRP concentrations that would categorize them at high cardiovascular risk (>3.0 mg/L) (58). Girls with “non-detectable” hs-CRP comprised 54% of the sample (data not shown).

Adjusted odds ratios and respective 95% CI and p-values from multinomial logistic regression models are reported in **Table 2.** Results indicate that for every 1 kg/m^2 increase in BMI, the odds of being in the “high” hs-CRP category was 33% higher compared to the “non-detectable” category, after adjusting for total energy intake, PYPAQ score, maturity offset, and total YHEI score (OR: 1.37, 95% CI 1.18, 1.58, p<0.0001). Similarly, for every 5% increase in total body fat percent or android percent fat, the odds of being in the “high” hs-CRP group compared to the “non-detectable” group were 2.5 or 1.9 times larger, respectively, after adjusting for all other covariates (OR: 2.52, 95% CI 1.65, 3.86, p<0.0001, OR: 1.93, 95% CI 1.41, 2.64, p<0.0001, respectively). The association between android percent fat and categories of hs-CRP was attenuated after controlling for BMI and was no longer significant. For each 1 mg/cm^3 higher calf muscle density, there were 47% lower odds of being in the “high” hs-CRP category compared to the “non-detectable” group

(OR: 0.53, 95% CI 0.36, 0.77, p<0.001), although the relationship was attenuated and no longer significant after controlling for total body fat percent.

DISCUSSION

The findings of the current study are in agreement with many other cross sectional studies showing that anthropometric measures of adiposity are significantly and positively associated with levels of CRP in non-Hispanic adolescents (9-24, 59). Our results are novel because these findings suggest that in addition to greater BMI, greater total and android adiposity, as determined by x-ray absorptiometry, and higher calf skeletal muscle fat, as determined by pQCT, are associated with greater odds of being in the "high" category of hs-CRP compared to those with "non-detectable" concentrations of hs-CRP in early adolescence. These findings were independent of physical activity, total energy intake, maturation, and total YHEI score. The relationship between android percent fat and hs-CRP was attenuated after controlling for BMI with similar attenuations between calf muscle density and hs-CRP after controlling for total body fat percent.

Research has shown that Hispanic adolescents have greater abdominal adiposity compared to other race/ethnic groups (38). For example, in a group of 55 obese Hispanic, Caucasian, and African American adolescents, Hispanics had the highest abdominal visceral adiposity [83.0 cm^2, [range 70.7, 95.0]] as measured by MRI, compared to Caucasian [75.4 cm^2, [range 65.0, 86.0]] and African American adolescents [49.8 cm^2, [range 38.0, 64.7]]; although significant differences were only observed between Hispanics and African American adolescents after controlling for age, gender, and percent fat (p=0.003) (41). Data from the current study similarly showed that android percent fat was greater that total body percent regardless of BMI category, suggesting that Hispanic girls have a high distribution of central fat. Research has also shown that Mexican American girls from NHANES 1999-2000 ages 8 to 17 years had average CRP concentrations that were 2-fold greater ($p<0.05$) than non-Hispanic white girls with the geometric mean CRP concentration of 0.76 mg/L in Mexican American girls (25). The assay and location of assessment used to measure hs-CRP in the current study was the same that is used for NHANES data. While 54% of our participants had non-detectable hs-CRP concentrations, 90% of participants in NHANES 1988-1994 had non-detectable concentrations of CRP (15). This was not unexpected, as healthy youth generally do not have elevated levels of inflammation. The average detectable hs-CRP value reported herein (1.5 ± 1.5 mg/L) was almost double the geometric mean reported in other Mexican American girls (25). This is explained by the fact that our sample consisted of 27% obese girls compared to the 19% obese Mexican American girls from NHANES 1999-2000 with a similar percentage from NHANES 2011-2012 (60, 61).

Our results are consistent with data from international samples of Hispanic youths which have consistently reported that measures of adiposity including BMI, weight, and waist circumference have a significant positive association with CRP (10, 23). Warnberg et al., for example, reported that weight ($\rho = 0.235$), BMI ($\rho = 0.265$), waist circumference ($\rho = 0.220$), and body fat percentage ($\rho = 0.281$) were all significantly associated ($p<0.01$) with CRP in 472 Spanish adolescents, after controlling for age and tanner stage maturation (23). Similar positive relationships were reported in the current study between hs-CRP and BMI, total body fat percent, and android percent fat. However, in the present study, in addition to restricting by age in our study design and controlling for maturation, the relationship between CRP, BMI, total body fat percent, and android percent fat remained significant after controlling for total energy intake, dietary quality, and physical activity, and the relationship between android percent fat and hs-CRP remained significant after further controlling for BMI.

The few studies that have identified relationships between adiposity and biomarkers of inflammation in Mexican American adolescents have been limited to anthropometric measures of adiposity (25, 62). Ford et al. conducted a study in 1358 adolescent girls from NHANES 1999-2000. Results from multiple linear regression analyses revealed that BMI was the most consistent and the strongest predictor of CRP in boys and girls ages 3 to 17 years, compared to age, systolic and diastolic blood pressure, total cholesterol, triglycerides, glucose, HbA1c, and homocysteine (25). Similar to results reported herein, results from multiple linear regression models showed that BMI percentile was significantly ($p<0.05$) and positively associated with CRP in girls of three age groups ranging from 3-17 years (ρ values ranged between 0.26 and 0.52), after adjusting for age, BMI percentile, smoking status, total cholesterol, homocysteine, systolic and diastolic blood pressure, and glycosylated hemoglobin percent (25).

A few studies have assessed the cross-sectional relationships between CRP values and measures of adiposity using DXA to measure total and central adiposity in non-Hispanic adolescents, (9, 13, 63). Two of these studies were limited to small sample sizes, between 28 and 59 adolescents, and did not adjust for confounders in their analyses (9, 63). Alvarez et al. reported a significant ($p<0.001$) and strong relationship between CRP and total body fat percent ($r= 0.71$) and total body fat mass ($r=0.67$) in 59 adolescents (9). Similarly, in a large sample of 7589 adolescents, aged 8.8-11.7 years, CRP was significantly and positively correlated with total body fat ($p<0.001$, $r=0.44$) (13), similar to the relationship between total body fat and CRP reported herein. The investigators also assessed the linear relationship between measures of adiposity and CRP concentrations in a subsample of 2331 adolescent girls after adjusting for age. Linear regression coefficients showed significant ($p<0.05$) and linear associations between CRP concentrations and DXA total fat ($R= 0.58$) and DXA central fat ($R= 0.58$) (13). Similar trends were reported herein.

The current study has several strengths and limitations. An important strength was the use of a large healthy sample with a vast distribution across BMI categories. Secondly, several potential confounders were controlled in our analyses. Previous studies have been limited by small sample sizes and the failure to control potential confounders. This was the first study to our knowledge to evaluate the relationship between multiple direct measures of adiposity and hs-CRP in Hispanic American adolescent girls while adjusting for total energy intake and diet quality among other potential confounders. Studies have shown that poor diet quality and consumption of "inflammatory" foods, such as fatty foods, sweets and junk food can contribute to levels of inflammation in adults and children (62, 63-65). In the current study, both total energy intake and total YHEI score were included in the final models in order to control for energy density and the complexity of food consumption operationalized as a single variable, which has not been done in past studies. However, our findings showed that total YHEI score was not a significant independent predictor in models that assessed the relationships between categories of hs-CRP and measures of adiposity.

The use of multiple direct measures of adiposity rather than anthropometry was another strength of the study. Although pQCT technology cannot distinguish between intra and extra-myocellular fat compartments, muscle fat content is a valid surrogate for muscle fatty infiltration (45). Similarly, although DXA does not provide a direct measure of visceral fat, android fat is highly correlated with visceral fat (33). Studies investigating the relationships between adiposity and CVD risk in adolescents have almost exclusively been restricted to total body and abdominal adiposity, due to the invasive or expensive methods required to measure skeletal muscle fat. To our knowledge, our study is the first to assess the relationship between skeletal muscle fat and hs-CRP in a large sample of Hispanic American adolescent girls. Our results suggest that lower calf skeletal muscle fat (higher muscle density) is protective against the odds of being in the "high" hs-CRP category compared to the "non-detectable" hs-CRP group. Similar to our results, findings from a study in 471 Afro-Caribbean adults also suggested that greater fat accumulated within the calf skeletal muscle increases inflammation. Calf skeletal muscle density was measured using pQCT, and higher concentrations of CRP were associated with greater fat infiltration within skeletal muscle, as reflective of lower muscle density ($r=-0.10$, $p<0.05$), after adjusting for anti-inflammatory medication, age, gender, DXA total body adipose tissue percent, skeletal muscle area, and height (64).

The liver is the main production site of CRP. Nonalcoholic fatty liver disease (i.e. hepatic steatosis; accumulation of fat within liver cells) is increasing in children and adolescents and it has been postulated that the main driver may be childhood obesity (65). In adults, nonalcoholic fatty liver disease has been shown to moderately increase the risk of CVD and has been associated with increased odds of high CRP, independent of and additive to obesity and metabolic syndrome (66, 67). As a result, this research would have been enriched if liver fat could have been measured by noninvasive technologies including abdominal ultrasound, MRI, or magnetic resonance spectroscopy.

The present analyses were limited to cross-sectional associations and prospective studies are required to more robustly evaluate these relationships and the long-term inflammatory status in youths. Additionally, we only used a single biomarker of inflammation; the investigation of multiple biomarkers of inflammation known to be closely related with adiposity would have provided more information regarding overall inflammatory status.

In conclusion, greater total and truncal adiposity were associated with higher concentrations of hs-CRP suggesting that adiposity contributes to inflammation in otherwise healthy Hispanic American adolescent girls. Future studies should utilize adolescents from minority populations, specifically Hispanic Americans, who have the greatest risk for CVD development. Additionally, diet quality among other potential confounders should be considered in future analyses in order to more accurately assess the relationship between adiposity and inflammation in both adolescent and adult populations. While longitudinal studies are needed to confirm these results, these findings support the larger body of evidence suggesting higher total and regional body fat increases inflammation in otherwise healthy youths.

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Table 1. Descriptive characteristics of 113 Hispanic American adolescent girls, ages 9-13 years

Characteristics	**Normal weight BMI 5th to 85th % n=67 (58%) Mean ± SD**	**Overweight/Obese BMI >85th % n=46 (42%) Mean ± SD**	**Total Sample n=113 Mean ± SD**
Age (years)	11.0 ± 1.0	11.0 ± 1.0	11.0±1.0
C-Reactive Protein			
non-detectable/detectable (n)	43/24	18/28	61/52
Average serum hs-CRP (mg/L)[1]	0.9 ± 1.5	2.0 ± 1.4	1.5 ± 1.5
Body Composition			
Height (cm)	144.3 ± 8.4	150.7 ± 7.9	146.9±8.8
Weight (kg)	36.8 ± 7.0	58.7 ± 12.1	45.8±14.3
BMI (kg/m^2)	17 ± 2	25 ± 3	21±5
Total body percent fat	25.6 ± 6.4	41.9 ± 4.0	32.2±9.8
Android percent fat	27.5 ± 9.5	51.0 ± 4.7	37.1±14.0
Thigh Muscle Density (mg/cm^3)	78.4 ± 1.7	78.1 ± 1.8	78.2±1.8
Calf Muscle Density (mg/cm^3)	80.1 ± 1.0	78.6 ± 1.5	79.5±1.4
Maturational Status			
Maturity offset (years)[1]	-1.7 ± 0.9	-2.0 ± 0.7	-1.9±0.9
Dietary Intake/Assessment			
Total energy (kcals)	2225 ± 923	1964 ± 1022	2119 ± 968
YHEI score[3]	48.8 ± 10.0	47.0 ± 9.2	48.1±9.7
Physical Activity Assessment			
PYPAQ score[4]	5936.9 ± 5863.7	4839.2 ± 6055.0	5490.1 ± 5940.2

[1]Average serum hs-CRP for all detectable concentrations (n=52)
[2]Maturity offset: estimated years from peak height velocity (PHV)
[3]YHEI score: Youth Healthy Eating Index (modified) 0 (non-adherent) to 85 (full adherence)
[4]PYPAQ score: Past year physical activity

Table 2: Adjusted odd ratios from multinomial logistic regression models between measures of adiposity and categories of hs-CRP concentrations in Hispanic American girls ages 9-13 y (n=113)

	Middle[1,2] [hs-CRP (mg/L)] OR (95% CI)	**High[1,3] [hs-CRP (mg/L)] OR (95% CI)**
BMI (kg/m^2)	0.98 (0.87, 1.11)	**1.37 (1.18, 1.58)[a]**
Total Body Fat Percent (%)[4]	0.97 (0.73, 1.29)	**2.52 (1.65, 3.86)[a]**
Android Percent Fat (%)[4]	1.00 (0.84, 1.20)	**1.93 (1.41, 2.64)[a]**
Thigh Muscle Density (mg/cm^3)	1.02 (0.79, 1.33)	1.21(0.91, 1.61)
Calf Muscle Density (mg/cm^3)	1.05 (0.71, 1.55)	**0.53 (0.36, 0.77)[b]**

All models controlled for: maturity offset, PYPAQ score, total energy intake, total YHEI score
[1]Compared to reference group: “undetectable”
[2]Range of hs-CRP concentrations 0.20-0.70 mg/L
[3]Range of hs-CRP concentrations 0.70-6.2 mg/L
[4]Transformed to represent a 5 percent unit difference
[a]sig. at $p<0.0001$ [b]sig. at $p<0.001$

APPENDIX D- ADDITIONAL PUBLICATION- Relationships of dairy and non-dairy calcium with adiposity in adolescent girls.

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Key Words: calcium, skeletal muscle fat content, central adiposity, total adiposity, obesity, adolescence

Running Title: Calcium and adolescent adiposity

ABSTRACT:

BACKGROUND: Insufficient calcium intake is common in adolescents. Higher intake of dairy sources of calcium has been linked to lower total body, visceral and subcutaneous adiposity. Any differential associations between dairy calcium or non-dairy calcium and skeletal muscle fat have yet to be determined, particularly in adolescents wherein the relationship between these dietary components and whole body and regional adiposity remains unclear.

SOURCE OF DATA: Baseline and three-year follow-up data from 165 girls who were participants in the "Jump-In: Building Better Bones Study".

DESIGN: Prospective, observational measures repeated at a three year interval.

OBJECTIVE: Examine the relationships of dairy calcium and non-dairy calcium with skeletal muscle fat, android fat, and total body fat in healthy girls aged 8–13 years.

METHODS: Muscle density (mg/cm^3), an index of skeletal muscle fat, was measured at the calf and thigh on the non-dominant limb using peripheral quantitative computed tomography (pQCT). Whole body and central (android) percent fat were measured using dual energy x-ray absorptiometry (DXA). Dairy calcium and non-dairy calcium intake were assessed using the Harvard Youth/Adolescent Questionnaire (YAQ).

RESULTS: Dairy calcium intake was inversely correlated with android percent fat ($r= -0.13$, $p=0.09$), and positively correlated with calf muscle density ($r=0.14$, $p=0.07$). In longitudinal analyses based on change (three years – baseline), average non-dairy calcium intake was significantly associated with greater positive changes in

thigh skeletal muscle density ($r = 0.18$, $p=0.02$) and calf muscle density ($r=0.17$, $p=0.03$).

CONCLUSIONS: Higher dairy calcium consumption is associated with lower skeletal muscle fat and lower android fat. Higher non-dairy calcium is correlated with increases in skeletal muscle fat of the thigh and calf over three years.

Background

Childhood and adolescent obesity is a major public health concern and while multi-factorial, the critical predictor is a calorie surfeit, relative to energy expenditure (1). Dietary trends contributing to this imbalance also lead to lower intakes of nutrient dense foods. In addition to its role as an essential nutrient for bone and muscle growth, as well as protecting against fractures (2), dietary calcium has also been shown to regulate energy metabolism; thereby having potential to lower obesity risk (3). During stages of rapid physical growth, children have the potential of absorbing up to 75% of dietary calcium due to increased absorption efficacy (2, 4). An adequate intake of calcium is crucial to accommodate healthy growth and minimize risk of disease.

Dairy products, contribute approximately 70% of the dietary calcium intake in Western diets (2, 5) with milk being the leading provider in adolescents (6). Epidemiological and clinical studies have reported that dairy calcium promotes more pronounced effects on fat mass reduction than non-dairy sources do (7). Dairy calcium is likely to have this effect due to the high calcium content per serving and the synergistic bioactive absorption promoters (lactose and caseinophosphopeptides), which contribute to high absorption (~ 40%) (1, 7). Certain non-dairy dietary calcium sources contain the calcium absorption inhibitors, oxalic or phytic acid, therefore limiting overall absorption (8).

Most observational studies in adults have found significant inverse relationships between dairy consumption and body mass index (BMI), insulin resistance, and visceral adiposity (9). While data in children are limited, cross-sectional studies support the hypothesis that a dairy-rich diet is either associated with decreased fat mass in children or has a neutral effect, although longitudinal observational and randomized-controlled trials have given mixed results (10).

Although adipose tissue is the primary site for fat accumulation, skeletal muscle is another storage depot where fat oxidation occurs. High fat intake has been related to dysfunctional beta oxidation and increased intramuscular triglyceride stores. Metabolic inhibition of fat oxidation and intramuscular triglyceride stores have been linked to metabolic inflexibility and insulin resistance in overweight and obese adolescents (11, 12). In a study that restricted energy intake in mice, milk and whey were found to preserve skeletal muscle mass more so than calcium alone (6). Since the synergistic effects of dairy calcium minimize fat gain and preserve skeletal muscle mass, it is possible that a dairy-rich diet may minimize the development of fat deposition within skeletal muscle as well as the metabolic inhibition of fat oxidation.

While the relationships of visceral and subcutaneous fat depots with dietary factors have been studied, the relationship between skeletal muscle fat deposition and dietary factors has not been widely explored. Skeletal muscle fat deposition is important in that it is altered in obesity and is associated with impaired glucose tolerance and insulin resistance (13-16). The objective of this study was to assess the relationships of dairy and non-dairy calcium with muscle fat content and central adiposity, and whole body adiposity in adolescent girls over a three year period.

SUBJECTS and METHODS

Study Design and Participants

Data from 165 fourth and sixth grade girls, ages 8-13y, participating in the "Jump-In: Building Better Bones" study was used to examine both cross-sectional and longitudinal associations between dietary factors and measures of adiposity over three years. The "Jump-In" (JI) study design has been described elsewhere (17). Briefly, JI was a randomized, controlled trial evaluating the effects of a structured jumping intervention on bone development in young girls (17). The study was approved by the University of Arizona Human Subjects Protection Committee; child assent and parental consent were obtained for all participants.

Physical Maturation

Maturation was assessed using gender specific equations developed by Mirwald (18) to estimate maturity offset as an estimate of years from peak height velocity. Maturity offset was estimated using the following equation:

Maturity offset (y) = -9.376 + 0.0001882*Leg Length (cm) and Sitting Height (cm) interaction + 0.0022*Age (y) and Leg Length (cm) interaction + 0.005841*Age (y) and Sitting Height (cm) interaction – 0.002658*Age (y) and Weight (kg) interaction + 0.07693*Weight (kg) by Height (cm) ratio.

Dietary Assessment

Total energy, dairy calcium, and non-dairy calcium intakes were assessed at baseline, year one, year two, and year three using the validated semi-quantitative Harvard Youth/Adolescent Food Frequency Questionnaire (YAQ) (19). Participants completed the YAQ with assistance from trained technicians or a parent/guardian. YAQs were evaluated for completeness and coded by trained staff following the standard protocol (20).YAQs were subsequently sent to Channing Laboratories (Boston, MA) for nutrient analysis.

Physical Activity

The validated past year physical activity questionnaire (PYPAQ) was used to assess physical activity at baseline, year one, year two, and year three (21). The PYPAQ was modified to assess engagement in 41 common sport and leisure-time activities performed by youth (≥10 times) in the past year apart from physical education class (22). Participants were asked to record the average duration, average weekly frequency, and the number of months of participation for each activity (22). Total PYPAQ score was computed using a modified algorithm that has been reported elsewhere (22).

Anthropometry

Anthropometric measures were obtained following standard protocols as described in the Anthropometric Standardization Reference Manual (23). Standing and sitting height (measured to the nearest 0.1 cm) were assessed at full inhalation using a stadiometer (Shorr Height Measuring Board, Olney, MD). Body mass was measured to the nearest 0.1 kg with a calibrated digital scale (Seca, Model 881; Hamburg, Germany) and body mass index (kg/m^2) was calculated from height and weight.

Body Composition

Dual energy x-ray absorptiometry (DXA) was used to quantify total body fat, percent body fat (ratio of fat mass to whole body mass), and android percent fat using the GE Lunar PRODIGY (software version 5.60.003). The android region was delineated using the regions of interest (ROIs) described in the manufacturer's manual. Android percent fat was calculated from the following equation:

[(Android fat mass) / (Android BMC + Android fat mass + Android lean mass)] x 100

Participants were positioned for whole body scans following standard manufacturer protocols. The densitometer (GE Lunar Radiation Corp; Madison, WI,USA) was calibrated and quality assurance was performed daily. All scans were completed and analyzed by a certified technician following standard protocol using the extended research mode software.

Peripheral quantitative computed tomography (pQCT), a low-dose radiation technique, was used to measure thigh and leg soft tissue composition, including muscle density (mg/cm^3), at the 20% femur and 66% tibia sites relative to the respective distal growth plates of the non-dominant limb. Scans were performed using a Stratec XCT 3000 scanner (Stratec Medical GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY, USA) and analyzed using Stratec software, Version 5.50. All pQCT scans and scan analyses were performed by trained technicians using the guidelines of Bone Diagnostics, Inc. (Fort Atkinson, WI, USA). Adipose tissue, skeletal muscle, and bone are distinguishable as independent tissues based on attenuation characteristics, which are directly related to tissue composition and density, and are estimated using edge detection and threshold techniques (24, 25). Images were filtered prior to being analyzed using contour mode 3 (-101 mg/cm^3) and peel mode 2 (40 mg/cm^3) to separate adipose (<40 mg/cm^3) and muscle/bone (-40 mg/cm^3), respectively (25). Images were filtered subsequently with a 7x7 image filter that clearly defined the edge of the muscle and eliminated all bone above 120 mg/cm^3 (26). This method ensures that muscle density was estimated from only soft tissue within the edge of the muscle. This technique is not capable of distinguishing intra-myocellular from extra-myocellular fat, and therefore cannot directly measure fat infiltration in skeletal muscle. Thus, as a surrogate for fat infiltration, muscle density reflects both intra- and extra-myocellular fat stores and is inversely related to muscle fat content such that a lower muscle density is indicative of higher skeletal muscle fat content (27).

Statistical analysis

Data were analyzed using SPSS for Windows statistical software, Version 19.0 (Chicago, IL, USA). Twenty - one individuals were missing one mean value at either 1- year or 2-year for one or more of the following variables: PYPAQ, dairy calcium, non-dairy calcium, and total energy. The missing mean was calculated by imputing the average of the other 3 time points. Repeated measures ANOVA analyses and bonferroni post hoc tests were used to check for significant differences between all four time points (baseline, year one, year two, and year three) of PYPAQ, total energy, dairy calcium, and non-dairy calcium before and after the averages for the missing time points were imputed.

Means, standard deviations, and ranges were calculated. Dietary variables included: total energy, total calcium, total dairy calcium, and total non-dairy calcium. The distributions of dairy calcium, non-dairy calcium, thigh muscle density, and BMI were skewed and were therefore square root transformed. Partial correlation coefficients from multiple linear regression analyses were computed in a cross-sectional manner at baseline to examine the association of dairy and non-dairy calcium, to body composition (BMI, % total body fat, % android fat, thigh muscle density, and calf muscle density) after controlling for important confounders, which maintained a parsimonious model (i.e. ethnicity, maturity offset, PYPAQ, and total energy).

Longitudinal analyses examined the association of dairy and non-dairy calcium, to the change (year three minus baseline) in body composition (Δ BMI, Δ % total body fat, Δ % android fat, Δ thigh muscle density, and Δ calf muscle density). Repeated measures ANOVA and bonferroni post hoc tests were used to check for significant differences between all four time points of the following independent and controlled variables: PYPAQ, total energy, dairy calcium, and non-dairy calcium. Repeated measures ANOVA and bonferroni post hoc tests showed that values of both dairy and non-dairy calcium were significantly different in which the difference was small over time. As a result, in order to capture a more accurate view of dietary intake over time and to maintain parsimony of the model; average values of dairy and non-dairy calcium, PYPAQ, and total energy were used in the subsequent analyses. Average values of dairy, non-dairy calcium, total energy, and PYPAQ were then computed using all four time points. Change variables were computed for BMI, total body fat percentage, calf muscle density, thigh muscle density, and android percent fat. Partial correlation coefficients were computed from multiple linear regression models between change variables and average dairy and non-dairy calcium in order to evaluate relationships between dietary variables and changes in whole body and regional adiposity after controlling for baseline body composition variables (i.e. BMI,

% total body fat, % android fat, thigh muscle density, and calf muscle density), average PYPAQ, average total energy, baseline maturity offset, and ethnicity. All assumptions of the multiple linear regression analysis were assessed. A *p* value of ≤ 0.05 was considered statistically significant.

Results

Baseline and three-year follow-up descriptive statistics are shown in **Table 1**. Participant average age at baseline was approximately 11 years (range of 8 -12 years). On average, at baseline the girls were one year from achieving peak height velocity (PHV) with a range of three years before PHV to one year after PHV. As defined by U.S. National Center for Health Statistics/Centers for Disease Control and Prevention percentiles for body mass index (BMI, kg/m^2) (17), two percent of the sample was underweight (BMI<5th percentile), 79% of the sample was in the healthy weight range (BMI 5th to 85th percentile), 13% of the sample was overweight (BMI 85th to 95th percentile), and six percent of the sample was obese (BMI>95th percentile).

The baseline average energy intake of this sample was 1613 kcals, below the NHANES 2007-2008 (28) national average of 1824 kcals, and lower than the recommended range of 1800-2000 kcals for girls of this age. Seventy-six percent did not meet the recommended 1300 mg/d RDA value of calcium for adolescent girls (29, 30), but that was similar to the national average (NHANES 2003-2006, 988 mg/d).The majority of the calcium consumed was derived from dairy products (728 mg/day).

Cross-sectional analyses with baseline data showed that dairy calcium was inversely correlated and approaching significance with android percent fat (r = -0.13, p= 0.09), and positively associated with calf muscle density, approaching significance after controlling for ethnicity, maturation, PYPAQ, and total energy (r = 0.14, p = 0.07), as given in **Table 2**.

The results of the longitudinal analyses examining the relationship between average dairy and non-dairy calcium with changes in adiposity after three years are shown in **Table 3**. Average dairy calcium showed no significant correlations with either whole body or regional changes in adiposity. There was a significant correlation observed between average non-dairy calcium and increases in thigh muscle density after three years (r = 0.18, p=0.02) and calf muscle density (r = 0.17, p=0.03).

Discussion

This study examined cross-sectional and longitudinal relationships of dairy and non-dairy calcium with adiposity measured by pQCT and DXA in adolescent girls. The findings suggest that dairy and non-dairy sources of calcium may modulate total body, skeletal muscle, and abdominal fat as early as adolescence, independent of physical activity, energy intake, maturation, and ethnicity. The outcomes suggest that various dietary sources of calcium may affect regional body fat independently of total body fat. To our knowledge, this is the first study to examine the relationship between dairy and non-dairy calcium with skeletal muscle fat content.

Our cross-sectional analysis revealed that higher intakes of dairy calcium are associated with lower android percent fat and higher skeletal calf muscle density, indicative of lower skeletal fat. Similarly, a study of 315 adolescent girls concluded that higher intake of dairy calcium was associated with lower adiposity while non-dairy calcium intake showed no association with adiposity (31). Previous cross-sectional studies have reported positive associations of calcium, milk, and dairy intake with lower BMI, body weight, and body fat (31-33). Other studies have failed to demonstrate an association between calcium, milk, or other dairy products on BMI or adiposity in adolescent girls (34-37).

Our longitudinal analyses, although not statistically significant, showed that higher average intakes of dairy and non-dairy calcium were associated with an increase in total body, and android percent fat over three years. Higher dairy calcium intakes also showed higher changes in BMI, although not significant. Similar results were observed in the longitudinal Growing-Up Today Study, in which those who drank ≥3 servings of milk a day compared to those who drank 1-2 servings had significantly higher BMIs from year to year in a sample of 12,829 adolescents (38). However, the association no longer existed after adjusting for total energy. Although not statistically significant, higher intakes of dairy calcium predicted lower change in skeletal muscle fat. Similarly, higher intakes of non-dairy calcium significantly predicted lower change in thigh and calf muscle fat. With regards to longitudinal analyses in this field, it is apparent that no clear relationship between dairy and non-dairy calcium and adiposity in youth has been established. For example, studies examining higher intakes of calcium or dairy products in children and adolescents have either concluded a neutral relationship with adiposity (39-45) or decreased body fat (46, 47).

This study has several limitations. Similar to the girls in this sample (76%), adolescents do not meet the RDA for dietary calcium on average. As such, it is difficult to determine a clear relationship between these recommended values and their effect on adiposity. Secondly, dietary data was collected through the use of a past year semi-quantitative food frequency questionnaire (FFQ). While the YAQ has been validated previously in youth (19), reporting bias is a major concern with FFQs and must be acknowledged. Another limitation of this study was a small sample size. Finally, we acknowledge that the pQCT technology is not capable of distinguishing between intra- and extra-myocellular fat compartments and DXA is not capable of distinguishing visceral from subcutaneous adiposity. It is possible that more precise measures of adiposity, such as CT scans or MRI, would have revealed more accurate and stronger relationships with dietary factors. However, as a faster, less expensive, low-dose radiation technique, the use of pQCT in cohort studies is more feasible.

Since this is the first study to examine the relationship between dairy and non-dairy calcium with skeletal muscle fat content, additional studies are needed in order to confirm these findings. The longitudinal results did not support the cross-sectional associations. It is unclear as to why dairy and nondairy sources of calcium have opposing effects on adiposity. Further research is needed in order to identify possible mechanisms associated with different sources of dietary calcium and adiposity, specifically to elucidate the mechanisms related to skeletal muscle fat infiltration.

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Table 1: Descriptive Statistics at Baseline and Follow-up (n=165)		
	Baseline	**3-year Follow-Up**
	Mean ± SD	Mean ± SD
Age (y)	10.6 ± 1.0	13.7 ± 1.2
Maturational Status		
Maturity Offset (y)*	-1.2± 1.0	1.6 ± 0.9
Body Composition		
BMI (kg/m^2)	18.1 ± 2.9	20.7 ± 3.5
PYPAQ Score**	5490 ± 4438	6290 ± 5956
Total Body Fat (%)	26.7 ± 8.5	29.8 ± 7.9
Android Fat (%)	28.4 ± 11.8	32.5 ± 10.4
Thigh Muscle Density (mg/cm^3)	76.4 ± 1.6	78.3 ± 1.4
Calf Muscle Density (mg/cm^3)	79.1 ± 1.2	81.1 ± 1.1
Dietary Intake		
Total Energy Intake (kcals/ day)	1613 ± 493	1650 ± 566
Total Calcium (mg/day)	1003 ± 413	969 ± 452
Dairy Calcium (mg/day)	728 ± 380	650 ± 390
Non-Dairy Calcium (mg/day)	278 ± 111	319 ± 191

*Maturity Offset = estimated years from peak height velocity (PHV)
**PYPAQ= past year physical activity questionnaire

Table 2: Partial Correlations from Multiple Linear Regression Analysis with Baseline Data

	BMI (m/kg^2)	Total Body Fat Percentage (%)	Android Percent Fat (%)	Thigh Muscle Density (mg/cm^3)	Calf Muscle Density (mg/cm^3)
Baseline Dairy Calcium (mg/day)	-0.13	-0.09	-0.13*	0.12	0.14*
Baseline Non-Dairy Calcium (mg/day)	-0.03	-0.02	0.02	-0.09	-0.11

* approaching sig. at p≤0.10, **sig. at p≤0.05

Controlled for ethnicity, PYPAQ, maturity offset, and total energy.

Table 3: Partial Correlations from Multiple Linear Regression Analysis: Average Dairy and Non-dairy Calcium intakes predicting the change in Adiposity after 3 years

	ΔBMI (m/kg^2)	Δ Total Body Fat Percentage (%)	Δ Android Percent Fat (%)	Δ Thigh Muscle Density (mg/cm^3)	Δ Calf Muscle Density (mg/cm^3)
Average Dairy Calcium (mg/day)	0.10	0.12	0.08	0.07	0.09
Average Non-Dairy Calcium (mg/day)	-0.02	0.03	0.01	0.18**	0.17**

* approaching sig. at $p \leq 0.10$, **sig. at $p \leq 0.05$

Averages computed from baseline, 1 year, 2 year, and 3 year measurements. Controlled for baseline body composition variables, ethnicity, average PYPAQ, baseline maturity offset, and average total energy.

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