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DIETARY PREDICTORS OF COMPONENTS OF THE INSULIN-LIKE GROWTH FACTOR SYSTEM IN ADOLESCENT GIRLS, AGED 14-18 YEARS: RESULTS FROM THE DIETARY INTERVENTION STUDY IN CHILDREN

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ABSTRACT

DIETARY PREDICTORS OF COMPONENTS OF THE INSULIN-LIKE GROWTH FACTOR SYSTEM IN ADOLESCENT GIRLS, AGED 14-18 YEARS: RESULTS FROM THE DIETARY INTERVENTION STUDY IN CHILDREN

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Evidence suggests that diet may affect risk for chronic diseases through its effect on the insulin-like growth factor (IGF) system. While dietary factors have been associated with the IGF system in adults, there is limited information available to describe dietary correlates of IGF-related biomarkers in healthy children. The objective of this thesis was to explore associations between dietary intake and IGF-I, IGF binding protein (BP)-1, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio in post-pubertal girls (aged 14-18 y) who participated in the Dietary Intervention Study in Children, a multicenter randomized controlled trial (n=159). Cross-sectional associations between dietary intake (total fat, protein, carbohydrate, animal and vegetable protein, lactose, fiber, calcium, zinc, and sodium) and the IGF-related biomarkers were assessed using correlation and multivariate linear regression analyses. It was hypothesized that animal protein, lactose, and calcium intake would be associated with increased levels of IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio. In multivariable analyses, significant predictors of IGF-I were energy and calcium; of IGFBP-3 was calcium; and of IGFBP-1 were vegetable protein and BMI for age percentile. This study provides evidence that dietary intake affects IGF-related biomarkers – particularly increased calcium with IGF-I and IGFBP-3 and increased vegetable protein with IGFBP-1 – and is novel in reporting these associations in adolescent girls.

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CHAPTER 1: INTRODUCTION

1.1 Overview

Abnormal levels of insulin-like growth factor (IGF)-I and its binding proteins are used in pediatric medicine as indicators of growth hormone deficiency and other specific disorders (1). However, there is now growing evidence to suggest that IGF-I levels that fall within normal limits are associated with chronic diseases in adults (2). While variation within normal limits of IGF-I levels have not been associated with childhood diseases, IGF-I levels in childhood may track into adulthood. Furthermore, it has been suggested that the childhood environment may influence levels of components of the IGF system (3, 4), suggesting that potentially modifiable lifestyle factors during phases of growth and development could affect lifetime disease risk.

Both high normal and low normal IGF-I levels in adults have been associated with different chronic diseases (2, 5). High normal plasma IGF-I and IGF binding protein (IGFBP)-3 concentrations have been suggested as potential markers for increased risk of breast cancer, prostate, colorectal, and lung cancers in adults (6). It has been postulated that components of the IGF system may advance cell cycle progression and/or inhibit apoptosis either directly or indirectly through interactions with other hormones such as insulin (7). Diet and other lifestyle factors may affect cancer risk through their effects on serum insulin concentrations and/or levels of components of the IGF system (8).

Furthermore, in contrast to the possible increased risk of certain cancers with *high* levels of IGF-I, some evidence implies that *low* normal serum IGF-I concentrations may be associated with increased risk of osteoporosis (5), impaired glucose tolerance (9), and coronary artery disease (10). The IGF system is critically important in normal growth

and development and accordingly, components of the IGF system change considerably during puberty when children undergo a period of rapid growth (11). IGF-I and IGFBP-3 reach a peak during puberty and then begin a slow decline throughout adulthood whereas IGFBP-1 reaches a nadir during puberty. Taken together, the evidence of associations of adult levels of IGF-I with varied chronic diseases and the fact that the pubertal time period may set the stage for lifetime levels of components of the IGF system, it is important to pursue an understanding of the correlates of components of the IGF system during adolescence.

In adults, a large body of evidence has accumulated to suggest that specific dietary components may affect IGF-related biomarkers. The most consistent associations have been intakes of animal protein, dairy products, and some minerals with IGF-I and IGFBP-3, although it is difficult to disentangle effects of single minerals from other nutrients or food groups (12). However, even as it is well-established that severe energy restriction lowers IGF-I levels and nutrient repletion increases IGF-I levels in growth retarded children (13), there is very little information on dietary correlates of components of the IGF system in *healthy* children. In the few studies conducted in healthy children, evidence indicates that early childhood and pre-pubertal dietary intakes, especially high intakes of animal protein and/or dairy intakes, may affect the serum concentration of IGF-I and IGFBP-3 during childhood (14-17). We are aware of no studies exploring possible dietary determinants of IGF-related biomarkers during late adolescence in healthy children, but because the IGF axis regulates growth and development and the highest rates of IGF-I production occur during the pubertal growth spurt (13), it may be a critical window of time in affecting lifetime levels of IGF-related biomarkers.

This study utilizes existing data from the Dietary Intervention Study in Children (DISC) to explore associations between specific dietary constituents and components of the IGF system in adolescent girls. The DISC was a longitudinal randomized controlled trial designed to test the safety and efficacy of a cholesterol-lowering diet in children and is described in detail in Chapter 4 of this thesis. A DISC ancillary study, the Insulin-Related Biomarkers Study (IRBS) was conducted with sera from girls (n=274) making serum IGF-I, IGFBP-1 and IGFBP-3 data available for analysis.

The goals of this study are to explore the associations between the more subtle effects of diet on the IGF system in healthy adolescent girls and determine if those associations are still present after consideration of potential confounding variables. Toward that end, the purpose of this exploratory study is to examine the effect of specified dietary exposures on post-pubertal measures of the IGF system in girls who participated in the DISC/IRBS regardless of study arm assignment. This study will focus on the dietary correlates of total IGF-I, IGFBP-1, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio.

1.2 Study Aim

The specific aim of this study is to cross-sectionally explore the crude association between major dietary constituents and serum concentrations of IGF-I, IGFBP-1, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio in post-pubertal girls (last DISC visit, ages 14-18 y) among girls in the DISC/IRBS. Major dietary constituents to be examined include the macronutrients (i.e. total fat, protein, and carbohydrate as percentages of energy), and animal and vegetable protein, lactose, fiber, calcium, zinc, and sodium in g or mg/1000 kcal.

Hypothesis: Animal protein, lactose, and calcium intake will be associated with increased levels of IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio.

Cross-sectional analyses will be conducted to explore associations between diet and the system at the last DISC visit (ages 14-17 y). In order to ensure the girls are post-pubertal at last visit, we will assess the relationship between age and IGF-I at last DISC visit, as well as the relationship between time since menarche and IGF-I at last DISC visit. If there is significant variation in IGF-I by age or time since menarche at the last DISC visit, we will consider limiting the analyses to girls who are at least 2 years post-menarche at the time of last DISC visit. Crude correlations between each dietary exposure and each biomarker will be assessed using Pearson correlation analysis. Where necessary, dietary exposures and biomarker outcomes will be transformed (e.g. to the natural logarithm) in order to reduce skewness in the distribution and the effect of extreme outliers will be evaluated using the studentized residuals. Median levels of each biomarker within quartiles of each dietary exposure will also be assessed. After evaluating results from the crude analyses, multivariable linear regression analyses will be conducted to test the strength of the relationships between dietary exposures and biomarker outcomes while including potential confounding variables in the model. Covariates to be considered will include age, race, household income, maternal education level, physical activity, treatment group, and age at menarche. Sample size permitting, we will stratify on vitamin/mineral supplement use.

1.3 Organization of thesis

This thesis includes a detailed review of the literature in chapter two. The third chapter includes the methods and results of the study and is presented as a stand-alone manuscript to be submitted for publication. The fourth and final chapter includes conclusions and public health implications of the results. Because of this organizational format, there will be some redundancy between the third chapter and the rest of the thesis.

CHAPTER 2: BACKGROUND

2.1 Biologic organization of the IGF system

IGF-I is part of a complex system that is under control of the larger hypothalamic-pituitary axis. The IGF system is comprised of three ligands (IGF-I, IGF-II, and insulin) that share peptide homology, three receptors (IGF-I receptor, IGF-II receptor, and insulin receptor), and six binding proteins (IGFBP-1-6) (18). The synthesis and bioavailability of IGF-I is primarily regulated by growth hormone (GH) and insulin. IGF-I has autocrine and paracrine functions as well as the ability to regulate cellular proliferation via mitogenic actions. These myriad functions may explain how IGF-I could be involved in multiple and distinct disease processes. IGF-I action is determined by the availability of IGF-I to interact with the IGF-I receptor, which is dependent on IGF-I concentrations and also on the relative concentrations of the IGFBPs (13). Total serum IGF-I includes both free IGF-I and that which is bound to the binding proteins (IGFBP-1-6) and an acid labile subunit (ALS) and has been shown to change throughout life in a pattern similar to free serum IGF-I (19).

Each IGFBP has a different structure and is thought to have different roles in regulating the action of IGF-I (20). IGFBP-1 only binds a relatively small percentage of IGF-I but it is regulated by the portal supply of insulin and is thought to regulate IGF-I bioavailability in relation to energy intake. IGFBP-1 has been shown to inhibit IGF-I action by limiting the availability of free IGF-I (21, 22). IGFBP-1 concentrations vary rapidly in response to food intake/insulin levels; decreasing with eating and increasing dramatically under even short conditions of fasting (i.e. during the night) (20).

IGFBP-3 is the binding protein that binds the majority of circulating IGF-I (over

90% in combination with the ALS) and similarly to IGFBP-1, it is thought to inhibit IGF-I action. The IGF-I:IGFBP-3 molar ratio is often estimated in order to get a better understanding of the relative concentrations of IGF-I and IGFBP-3 levels and has been proposed as an index of bioavailable IGF-I (23, 24).

2.2 Major determinants of the components of the IGF system in children

2.2.1 Age, sex, and pubertal maturation

There is an abundance of research on the effects of age, sex, and pubertal maturation on the IGF system. Most of the published research utilizes cross-sectional data, with several studies including over 1,000 subjects throughout the lifecycle (25-27). Although results have been reported from diverse study populations including large samples from Germany (n=1584) (25), Japan (n=1,075; n=354) (27, 28), Denmark (n=1,030; n=907) (26, 29), Turkey (n=807) (30), Spain (n=600; n=354; n=121) (31-33), India (n=434) (34), Thailand (n=260; n=244) (35, 36), Israel (n=217) (37), England (n=333; n=102) (38, 39), and the United States (n=110) (40), results of the *patterns* of components of the IGF system with age, sex, and pubertal maturation have been remarkably similar among populations even as differences exist in the absolute numbers.

Cross-sectional studies show that total **IGF-I** levels increase steadily throughout childhood, with a more pronounced increase during puberty and a peak level between 11-15 years of age, after which time there is a slow decline (23, 43-60). The magnitude of the increase is on the order of 8-9 times from infancy to midpuberty in those studies that report data throughout the entire spectrum of childhood (25-28, 37). The peak in IGF-I generally occurs about 1-2 years earlier in girls than in boys in most (23, 44-52, 54, 55, 57, 59), but not all (25), of the studies in which it was assessed. In the one study where

concentrations of serum IGF-I peaked in both boys and girls at age 15 y, IGF-I levels were measured by a newer assay technique (automated chemiluminescent) (25).

The earlier peak in IGF-I levels generally seen in girls is consistent with the earlier pubertal maturation of girls vs. boys (13). Accordingly, IGF-I increases with increased pubertal maturation and peak levels have been shown in Tanner stages III-V in girls and Tanner stages IV-V in boys (26, 30, 32, 35, 37, 38, 40, 41). Although overall there is considerable consistency among all of the many published studies regarding the increase in IGF-I levels with age, gender, and pubertal maturation, there is also a considerable amount of variation in serum IGF-I concentrations between children and with age within the Tanner stages (13). In the early stages of puberty, IGF-I has been shown to increase with age, while in the later stages of puberty it decreases with age (26). Additionally, because these attributes change at different rates in different individuals, the diagnostic cut-offs for growth disorders for an individual must concurrently take into account age, gender, and pubertal maturation. To this end, reference models (regression) have been developed to make comparisons of IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio possible between children and thus improve the diagnostic utility of IGF-I in suspected growth disorders (42, 43) but there are no published reports assessing the reliability and validity of this technique as of yet.

Given the abundance of data from cross-sectional studies of IGF-I, there are surprisingly few studies with **longitudinal** data on measures of the IGF-I system in children. There are only two published studies with longitudinal measures of IGF-I throughout the pubertal growth spurt and both were focused on growth and height/height velocity (44, 45). Briefly, both studies reported results on age, sex, and pubertal

maturation similar to the body of cross-sectional literature. An increase in IGF-I (formerly termed somatomedin-C) with age and pubertal maturation was reported, with the peak in IGF-I occurring earlier in girls than boys, prior to decreasing to adult levels (44, 45).

In the largest (n=907) published study to provide reference values of **IGFBP-1** levels in children, IGFBP-1 decreased with age in prepubertal and pubertal children, with lowest values during puberty (29). No significant differences in IGFBP-1 were found between males and females (29). In a study designed to assess the correlation between IGFBP-1 and fasting insulin (n=116; ages 5-48 y), Holly et al. show a decrease in IGFBP-1 from childhood throughout puberty with multiple regression analyses attributing these changes more to pubertal status than age (46). Results from Holly et al. also show no significant difference between males and females (P=0.06), although there was a tendency for prepubertal boys to have higher peak levels of IGFBP-1 than prebubertal girls (46).

IGFBP-3 concentrations also increase with age, achieving maximal levels in puberty (28-30, 32, 34-36, 43). In studies that report IGFBP-3 throughout the entire spectrum of childhood, IGFBP-3 levels increased almost linearly from infancy to 2-3 times as high in puberty (25, 28, 29, 43). The peak level of IGFBP-3 in adolescence is similar in boys and girls and occurs between 11-13 y (25, 28, 29).

IGF-I has been shown to increase to relatively higher levels than IGFBP-3 during puberty in the studies that have measured both, and therefore there is an increased **IGF-I/IGFBP-3 molar ratio** (29, 43, 47). In a Japanese population (n=354), the concomitant finding that free IGF-I increased in parallel to the increase in the IGF-I/IGFBP-3 molar

ratio during puberty supports the hypothesis that the increased IGF-I/IGFBP-3 molar ratio in puberty reflects an increase in free, biologically active IGF-I. This suggests that the IGF-I/IGFBP-3 molar ratio may be indicative of active IGF-I (28).

2.2.2 Diet/nutritional status

Nutrition is considered to be one of the main regulators of circulating IGF-I (48). Severe energy, protein, and zinc deficits have all been shown to decrease serum IGF-I concentrations, while energy and/or nutrient repletion is able to restore normal serum IGF-I (49, 50). Until recently, however, the relationship between nutrition and the IGF system has primarily been focused on disorders of nutrition (e.g. malnutrition, anorexia, obesity), rather than normal nutritional states. More recently, however, evidence suggests that variation in nutrient intakes even within the normal range is associated with variation in IGF-I levels. The most consistent associations reported are positive associations of IGF-I with dietary intakes of animal protein, consumption of milk or dairy products, and with mineral intakes, including zinc, potassium, calcium, phosphorus, and magnesium (12). Nearly all of the studies of dietary determinants of components of the IGF system are in adults with relatively few published studies of normal dietary intakes in healthy children in relation to components of the IGF system. Table 1 summarizes the cross-sectional literature on dietary associations with IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio in children. To our knowledge, there are no published reports assessing the relationship between dietary intake and IGFBP-1 in children.

2.2.2.1 Energy and Macronutrients

There have been four cross-sectional reports from three studies of the association between energy and macronutrient intakes of healthy prepubertal children and IGF-I (16,

51, 52) and IGFBP-3 (16, 53) with conflicting results. The earliest results were from two reports of healthy girls (mean age=12.3 ± 0.7 y; n=324) by Wilson et al., where diet was assessed by a semiquantitative food frequency questionnaire (FFQ) (52, 53). These reports showed no associations between dietary intake (energy, protein, fat, or carbohydrate) and IGF-I (52) or IGFBP-3 (53). In agreement with these results is a recent study of European American (mean age=7.9 ± 2.0 y; n=29) and African American (AA) children (mean age=8.3 ± 1.4 y; n=26) in which none of the dietary intake variables (energy, protein, fat, or carbohydrate; assessed by an average of three 24-hour dietary recalls) were significantly correlated with IGF-I (51). Because dietary protein intake was higher in AA children, it was included as an independent predictor of IGF-I in a multiple regression model along with genetic admixture, fat mass, socioeconomic status, adjusted energy expenditure, and IGFBP-3, however, the dietary protein intake was not found to independently predict IGF-I levels.

In contrast to these three reports, cross-sectional data derived from the Avon Longitudinal Study of Parents and Children (ALSPAC) (ages 7-8 y; n=521) (16), showed positive associations between IGF-I and dietary intake (assessed by 3-day unweighed dietary records) of energy-adjusted total protein ($r=0.19$; $P<0.001$) and animal protein ($r=0.16$; $P<0.001$), (but not vegetable protein), and negative associations with energy-adjusted total fat ($r= -0.12$; $P<0.01$), monounsaturated fat ($r= -0.11$; $P<0.01$), and polyunsaturated fat ($r= -0.09$; $P<0.05$). Additionally, IGFBP-3 was positively associated with energy intake ($r= 0.13$; $P<0.01$), and negatively associated with energy-adjusted total fat ($r= -0.09$; $P<0.05$) and monounsaturated fat ($r= -0.11$; $P<0.05$). Lastly, the IGF-I/IGFBP-3 molar ratio was positively associated with energy-adjusted intakes of total

protein (r= 0.14; P<0.01) and animal protein (r= 0.13; P<0.01), and negatively associated with polyunsaturated fat (r= -0.10; P<0.05). Most of these associations remained consistent whether adjusting for energy, age, and sex only or the additional confounders of maternal education and housing tenure, BMI and birthweight-for-gestational age (16).

While all of these cross-sectional studies were in prepubertal children, the ALSPAC study differed from the other studies in two important respects. First, the subjects in the studies that found no association between diet and the IGF system (51-53) included a more heterogeneous subject population in terms of race/ethnicity, which has shown to be associated with variation in IGF-I levels (54). A wider variation in study-specific IGF-I levels may lead to a lower ability to detect subtle associations between IGF-I concentrations and measures of dietary intake. Secondly, the ALSPAC study is the only study of the three reported that adjusted for total energy intake. Because total energy intake reflects to a large extent body size, physical activity, and metabolic efficiency, the effect of not controlling for energy intake weakens evidence of any association between nutrient intake and the outcome of interest (i.e. IGF system components) if the outcome is independent of the factors influencing total energy intake (55). Furthermore, many of the associations observed in the ALSPAC data are similar to findings from the literature on adults. Positive associations were found between measures of the IGF system and energy, protein, and mineral intakes (reviewed below) and negative associations with fat intakes (16). The inverse association between IGF-I and fat intakes is consistent with associations seen in adults between both high fat intakes and low IGF-I concentrations and the risk of coronary heart disease. Taken together, these cross-sectional data suggest that variation in dietary intakes, even within normal ranges,

may be associated with variation in concentrations of components of the IGF system in childhood, which may confer risk for chronic diseases in adulthood.

2.2.2.2 Micronutrients

The only report of associations between micronutrient intakes and components of the IGF system in healthy children comes from the cross-sectional analysis of the ALSPAC study (described previously) (16). In that study, IGF-I was positively associated with energy-adjusted intakes of zinc ($r=0.14$; $P<0.01$), magnesium ($r= 0.15$; $P<0.01$), calcium ($r= 0.12$; $P<0.01$), potassium ($r= 0.14$; $P<0.01$), phosphorus ($r= 0.16$; $P<0.001$), and the vitamin, folate ($r= 0.11$; $P<0.01$). IGFBP-3 had no linear relations with any of the minerals (or vitamins) studied. The IGF-I/IGFBP-3 molar ratio was positively associated with energy-adjusted zinc ($r= 0.09$; $P<0.05$), and phosphorus ($r= 0.10$; $P<0.05$) intakes (16). Thus variation in IGF-I within a range of normal dietary intakes in healthy children was shown to be associated with mineral intakes, including zinc, which is consistent with observations in adults (12).

2.2.2.3 Dairy/Milk

Cow's milk is a source of animal protein that is associated with a higher childhood linear growth velocity in developing countries and to a lesser extent in well-nourished populations (56). Because IGF-I is a growth regulator, and decreased IGF-I concentrations are associated with deficits in protein intakes, it has been hypothesized that IGF-I may mediate the positive association between protein and growth (15). There have been only three cross-sectional studies and two clinical trials studying the effects of milk or dairy intake on the IGF system in healthy children and all five reflect higher IGF-I levels with higher milk consumption (14, 15, 57, 58) (17).

The first cross-sectional study to report effects of milk intake on the IGF system was in 2.5 y old Danish children (n=90) (15). Serum IGF-I concentrations were significantly associated with animal protein and milk intakes ($P<0.05$ for both), but not vegetable protein or meat intakes even after adjusting for sex and body weight (body weight was included in the model to control for the influence of body size on dietary intake, instead of adjusting for energy intake) (15). An increase in milk intake from 200 to 600 mL/d (240 mL=1 Cup) was associated with a 30% higher serum IGF-I concentration. The same research group recently reported somewhat similar results in a cross-sectional study of 56 healthy, prepubertal boys (mean age=8.1 ± 0.1 y) (17). In multiple linear regression models adjusted for age and BMI, free serum IGF-I was positively associated with energy-adjusted total protein ($P=0.01$) calcium ($P<0.01$) and milk ($P=0.03$) intakes, but not with meat, dairy, or plant protein.

The other cross-sectional study to report effects of milk intake on the IGF system in healthy children is from the ALSPAC cohort (described previously; n=538) (58). Cows' milk and total dairy products (which included milk) were both positively associated with IGF-I ($P<0.05$ for both) and IGFBP-3 ($P=0.08$, and $P=0.07$, respectively) in boys in fully adjusted models (including maternal education, housing tenure, birth weight and the child's BMI). There were no significant associations between cows' milk or dairy products and the molar ratio IGF-I/IGFBP-3, or between cows' milk or dairy products and any measure of the IGF system in girls.

Two clinical trials of milk consumption in children verified the positive associations seen between milk consumption and serum IGF-I concentrations in cross-sectional data. A one week intervention study was conducted in 8 yr old Danish boys

(n=12 with increased milk consumption; n=12 with increased low-fat meat consumption) (14). The milk intervention was significantly associated with a 19% increase in IGF-I (P=0.001) and a 13% increase in the IGF-I/IGFBP-3 ratio (P>0.0001), whereas the meat group showed no significant increases in either IGF-I or IGFBP-3. These results suggest that milk consumption and not simply high protein consumption, affects the IGF system, at least in the short-term. In a longer randomized controlled trial with English adolescent girls (n=82), where the experimental group received one pint (568 mL or ~16 oz) of milk (fat content chosen by subject) daily for 18 months and the control group was asked to continue their usual milk intake, IGF-I increased significantly more in the experimental group compared with the control group (35% vs. 25%; P=0.02) (57).

Altogether, the few data available regarding associations between measures of dietary intake and measures of the IGF system in healthy children indicate that variation in normal dietary intakes may be associated with levels of IGF-I and possibly IGFBP-3 in healthy children.

2.2.3 Physical Activity

Most of the studies conducted on the effects of physical activity on the IGF system during childhood have been small clinical trials conducted to answer the following questions: 1) is physical fitness associated with the IGF system? And; 2) does strenuous exercise, such as that experienced by child athletes, have an adverse effect on pubertal development and growth?

Addressing the first question, two studies conducted in late pubertal adolescent males (59, 60) report no association between measures of fitness and IGF-I, whereas three studies in pre- or early pubertal males (61), or girls (both pubertal and pre-pubertal)

(62, 63) report positive associations between measures of fitness and IGF-I. More studies relating measures of fitness to measures of the IGF system in healthy children will be needed to address the question of whether or not the IGF system differs by level of fitness and these studies may need to include longitudinal measures of the GH-IGF system in order to fully elucidate the adaptations that may occur in response to physical activity.

Regarding strenuous activity, results are also conflicting. Because the GH-IGF axis is comprised of growth hormones and other mediators of growth, its role in growth responses to exercise (e.g. muscle hypertrophy) is widely accepted (64). However, as noted by Nemet and Cooper (64), results from studies of exercise training in children have shown that exercise training (5 weeks duration) leads to reductions in IGF-I, instead of the expected increases (61-63, 65). Eliakim was the first to hypothesize that initiation of an endurance training program may first lead to hormonal adaptations such as decreased IGF-I that are suggestive of a catabolic state, but at some point an anabolic rebound will likely occur as suggested by the higher levels of IGF-I seen in fitter individuals (62). The effect of regular physical activity on measures of the IGF system in adolescents, if any, is largely unstudied.

2.2.4 Body size

2.2.4.1 Height/Height Velocity

IGF-I is strongly associated with both height and height velocity. A 2006 study assessed the longitudinal relation between IGF-I and height velocity in girls (n=309) and boys (n=359) between 5 and 10 years of age who were participating in the ALSPAC cohort in southwest England (66). IGF-I was associated with subsequent height velocity in both girls and boys ($P<0.001$) and all the associations were only slightly attenuated

after adjusting for maternal education, housing tenure, BMI, and birth weight as potential confounders. This recent report on prepubertal children is in agreement with earlier longitudinal studies and a large body of cross-sectional literature (3, 31, 37, 44, 45, 66-74), although two studies reported no association between IGF-I and height or height velocity (52, 75).

2.2.4.2 BMI/Body Composition

Most of the studies that have assessed the association between BMI or other measures of body fatness and IGF-I in *normal weight* children have found a positive relationship (3, 31, 67, 69, 70, 72, 74, 76). However, IGF-I was unassociated with BMI in normal weight prepubertal (26, 52) and pubertal children (75) in three cross-sectional studies. Two of the studies that did find a positive relationship between IGF-I and BMI or weight for height and age only found it in prepubertal subjects, and not in pubertal subjects (31, 67), suggesting that discrepancies in the literature may result from various degrees of statistical control over pubertal stage.

It appears clear that the IGF system is altered by states of overnutrition. Of 22 studies exploring associations of obesity with IGF-I or other components of the IGF system (77-98), 17 were cross-sectional (79-81, 83-95, 97) and 4 were trials that involved a weight loss component and thus IGF-I was reported before and after an intervention (78, 82, 96, 98). Of the 14 descriptive studies that assessed IGF-I in relation to BMI (79-81, 83, 84, 86-92, 94, 97), 9 report higher IGF-I in obese children (79, 81, 83, 86-89, 92, 97), and 5 report no difference in IGF-I by obesity status (80, 84, 90, 91, 94). Of the 9 studies that report higher IGF-I in obese children, two found higher IGFBP-3 in obese children vs. normal weight controls (81, 88), one found lower IGFBP-3 (79), and the rest

did not measure IGFBP-3. All three of the nine studies reporting higher IGF-I in obese children that measured IGFBP-1 found lower IGFBP-1 with obesity (79, 86, 95).

Among the five studies that found no difference in IGF-I by obesity status, three found higher IGFBP-3 concentrations among obese subjects (84, 90, 91), while two found no difference (80, 94). Additionally, four of the five aforementioned studies measured IGFBP-1 (84, 90, 91, 94) and all but one reported lower IGFBP-1 concentrations in obese children (90). Two additional studies measured IGFBP-1, but no other components of the IGF system and found lower IGFBP-1 concentrations in obese children (85, 93). Thus, the preponderance of the evidence implies that components of the IGF system in children differ by obesity status.

The results of an altered IGF system in obese children in relation to normal weight control subjects are consistent in the literature despite enormous variation in important participant characteristics such as the degree of overweight (definitions for obesity status vary across studies), boys and girls analyzed together, and varying pubertal status analyzed together in the same study. Of note is that while age was controlled in most of the studies referenced above, most did not control for pubertal stage, which is an important determinant of components of the IGF system.

The hyperinsulinemia of obesity has long been hypothesized to be the cause of higher IGF-I levels in obese children (81). Recently, hyperinsulinemia in obese prepubertal children has been shown to be positively related to IGF-I and the 17β-oestradiol/sex hormone-binding globulin (SHBG) ratio and inversely related to SHBG (83). (95). Furthermore, in response to the apparent suppression of IGFBP-1 in obese children, Travers, et al. conclude that perhaps insulin-mediated suppression of IGFBP-1

in obese prepubertal children increases free IGF-I levels and contributes to somatic growth early, and in pubertal children, where insulin resistance and growth acceleration occur simultaneously (95). Accordingly, it is likely that the association between obesity and IGF-I or other components of the IGF system is confounded by pubertal status. Clearly, the effects of obesity on the complex hormonal milieu of puberty are not yet fully elucidated, but with the high prevalence of childhood obesity, this is an area that deserves further research attention.

An area of particular relevance that has scarcely been researched involves assessing the effects of weight loss on components of the IGF system. Of the four trials that involved a weight loss component and thus IGF-I was reported before and after an intervention (78, 82, 96, 98), the two shortest trials (3 and 6 wks, respectively) (96, 98) showed significant reductions in IGF-I after weight loss, although the 3 week trial only showed significant results in boys, but not girls (98). The other two trials differed considerably in design yet neither showed reductions in IGF-I after weight loss (78, 82). Most notably, the former included a 12-week intervention, while the latter reported results after a 6 and 12 month intervention. These results may imply that the short-term changes in the IGF system may result from weight loss, but these changes may be transient, or follow-up of longer than one year is required to see permanent changes. Clearly, this is an area where more research is needed.

2.2.5 Race/ethnicity

Circulating IGF-I concentrations have consistently been shown to be higher in African-American (AA) vs. white children (51, 54, 99-103), while no race/ethnic differences were seen in the one study that compared IGF-I among Caucasian, Asian and

Hispanic girls (52). Interest in determining differences in components of the IGF system by race/ethnicity is motivated by the desire to explain other physiological differences seen between African-American and white children. For instance, it is possible that the differences that have been shown in African American (AA) vs. white children in insulin sensitivity (lower in AA) (99, 104-106), growth rates and pubertal maturation in girls (higher and earlier, respectively, in AA) (107-109), and bone mineral density (higher in AA) (110-112) all may be mediated by the GH-IGF axis.

The relationships between IGF-related biomarkers and race/ethnicity and their interactions with other correlates of IGF-related biomarkers, such as body size, is an area that warrants further research attention. A significant interaction has been shown between race/ethnicity and obesity status in predicting IGF-I levels in adults (113), but to our knowledge, this has not yet been studied in children.

CHAPTER 3: METHODS AND RESULTS

3.1 Manuscript for publication

3.1.1 Abstract

Background: The insulin-like growth factor (IGF) system is associated with adult diet and chronic disease. Childhood diet may influence chronic disease through its effect on the IGF system; limited information, however, describes dietary predictors of the IGF system in adolescents.

Objective: We aimed to examine associations between dietary food intake (fat, protein [animal and vegetable], carbohydrate, lactose, dietary fiber, calcium, zinc, sodium) and serum IGF-I, IGFBP-1, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio in adolescent girls (n=159).

Design: Girls in the Dietary Intervention Study in Children (ages 14-18 y; 0.2-6.3 y post-menarche) were included. Dietary intake was assessed via three 24-hour dietary recalls. IGF-related biomarkers were determined with radioimmunoassays. Associations between dietary intakes and biomarkers were assessed with Pearson correlations and multivariable linear regression. Dietary intakes and biomarkers were all logarithmically transformed; thus β-coefficients represent percentages.

Results: In analyses adjusted for energy, age, and time since menarche, significant correlations (at $P<0.05$) were: IGF-I with total protein, lactose, calcium, and sodium; IGFBP-3 with total fat (inverse), lactose, fiber, and calcium; IGF-I/IGFBP-3 with lactose and calcium; and IGFBP-1 with vegetable protein. In multivariable analyses, significant predictors of IGF-I were energy (β= 0.14, $P<0.05$) and calcium (β= 0.14, $P<0.01$); of

IGFBP-3 was calcium (β= 0.07, P<0.05); and of IGFBP-1 were vegetable protein (β= 0.49, P<0.05) and BMI for age percentile (β= −0.01, P<0.001).

Conclusions: This study provides evidence that dietary intake affects IGF-related biomarkers – particularly increased calcium with IGF-I and IGFBP-3 and increased vegetable protein with IGFBP-1 – and is novel in reporting these associations in adolescent girls.

3.1.2 Introduction

Increased insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) concentrations have been associated with increased risk of breast, prostate, colorectal, and lung cancers in adults (6), possibly through advancement of cell cycle progression and/or inhibition of apoptosis (7). In contrast, some evidence suggests *low* levels of IGF-I may be associated with increased risk of osteoporosis (5), impaired glucose tolerance (9), and coronary artery disease (10). Because the IGF axis regulates growth and development and the highest rates of IGF-I production occur during the pubertal growth spurt (13), adolescence may be a critical window of time in affecting lifetime levels of IGF-related biomarkers.

IGF-I is part of a complex system that is under control of the larger hypothalamic-pituitary axis. IGF-I action is determined by the availability of IGF-I to interact with the IGF-I receptor, which is dependent on IGF-I concentrations and also on the relative concentrations of the IGFBPs (13). Potential mechanisms by which dietary intake may affect circulating levels of IGF-I include inhibition of hepatic synthesis or indirectly through effects on IGFBPs (12).

IGFBP-3 is the binding protein that binds the majority of circulating IGF-I (over 90%) and it is thought to inhibit IGF-I action. The IGF-I/IGFBP-3 molar ratio is often estimated to get a better understanding of the relative concentrations of IGF-I and IGFBP-3 and has been proposed as an index of bioavailable IGF-I (23, 24). IGFBP-1 only binds a relatively small percentage of IGF-I, but it is regulated by the portal supply of insulin and is thought to regulate IGF-I bioavailability in relation to energy intake (23, 24).

In adults, positive associations have been identified between intakes of animal protein, dairy products, and minerals (total or zinc) with IGF-I and IGFBP-3 concentrations, although it is difficult to disentangle effects of these highly correlated dietary constituents (12). In children, it is well-established that severe energy restriction lowers IGF-I concentrations and nutrient repletion increases IGF-I concentrations in growth retarded children (13). Few studies, however, have examined dietary correlates of components of the IGF system in *healthy* children. In the four studies we identified, high intakes of animal protein and/or dairy affected serum concentrations of IGF-I and IGFBP-3 (14-17). All were conducted in early childhood and pre-puberty, prior to peak IGF-I levels at menarche.

This study utilizes data from the last visit of the Insulin-Related Biomarkers Study (IRBS), an ancillary study of the Dietary Intervention Study in Children (DISC). The DISC was a longitudinal, randomized controlled trial designed to test the safety and efficacy of a reduced fat diet (i.e. reduced total fat, saturated fat, and cholesterol) in children who were 8-10 years old at baseline (114). The purpose of the present study was to test the hypothesis that the positive associations between intakes of animal protein,

calcium, and zinc with the IGF system that have been observed in adults (12) and younger children (14-17) are also present in adolescent girls.

3.1.3 Subjects and methods

3.1.3.1 Subjects

Participants are girls who were in the DISC, a dietary intervention study initiated in 1988 in 8-10 year old children (girls and boys) followed through childhood for a median duration in the study of 7.0 years. The National Heart, Lung, and Blood Institute (NHLBI) sponsored the study to test the efficacy and safety of a diet intervention designed to reduce serum LDL cholesterol in children. Specific details of the design and results of the DISC are described elsewhere (114-116). In brief, the DISC was a multicenter, randomized, controlled clinical trial, wherein 663 children were enrolled in one of six clinical centers between 1988 and 1990 (Children's Hospital, New Orleans, LA; Johns Hopkins University Hospital, Baltimore, MD; Kaiser Permanente Center for Health Research, Portland, OR; University of Medicine and Dentistry of New Jersey, Newark, NJ; Northwestern University Medical School, Chicago, IL; and University of Iowa Hospital and Clinics, Iowa City, IA). The study was approved by the institutional review boards of all participating centers. The protocol was reviewed by an independent data and safety monitoring committee appointed by the NHLBI. The DISC is registered in the NIH ClinicalTrials.gov registry (identifier: NCT00000459).

Children were recruited through schools, a health maintenance organization, and pediatric practices and were eligible if they had serum LDL cholesterol in the 80^{th}-98^{th} age- and sex-specific percentiles of the Lipid Research Clinics population (117), were at least in the 5^{th} percentile for height and were in the 5^{th}–90^{th} percentiles for weight-for-

height (118). The LDL cholesterol eligibility criteria were established to exclude children with severe hypercholesterolemia for whom medication might be clinically indicated. Exclusion criteria included children with medical conditions or medications that could affect growth or serum cholesterol, behavioral problems, or onset of pubertal maturation. Children were randomly assigned to receive a dietary intervention or usual care based on designation from the study coordinating center (Maryland Medical Research Institute, Baltimore, MD).

3.1.3.2 Insulin-Related Biomarkers Study (IRBS)

A DISC ancillary study, the Insulin-Related Biomarkers Study (IRBS), was conducted to determine the effects of the DISC dietary intervention, as well as specific dietary constituents, on serum concentrations of insulin-related biomarkers measured biennially in adolescent girls. The IRBS included 274 girls with stored serum available for laboratory determination of IGF-I, IGFBP-1, IGFBP-3, glucose, insulin, and C-peptide. The present analyses were designed to assess the associations between diet and IGF-related biomarkers among post-menarcheal girls at the last DISC visit.

Of the 301 girls in the DISC study, 269 attended the last visit. IGF-related biomarker data were available for 191 of these girls, 172 of whom also had complete information on diet. After excluding girls who had missing data on time since menarche at the last visit (n=8), were pregnant (n=2) or had a serious illness (n=2), there were 160 girls with complete data on diet, menarcheal status, and IGF-I at the last DISC visit. After reviewing the sample distributions of all dietary intake variables, and reviewing residual diagnostics to check for influential observations in regression models, one additional girl was excluded due to implausible dietary intake (three day average zinc intake of 84

mg/1000 kcal) resulting in a final sample of 159 girls. The final sample (n=159) was compared to the full baseline sample (n=301) in terms of sociodemographic characteristics and no significant differences (tested by chi-square) were found in race, income, maternal education, or treatment group.

Because the only dietary intake differences between treatment groups in our study of adolescent girls were for saturated fat intake (data not shown), and saturated fat intake has not been associated with the IGF-system in adults or been hypothesized to be associated in healthy children, we combined treatment groups in analyses. This study was approved by the DISC steering committee and the institutional review board of Michigan State University, East Lansing, MI. All data, except for the IGF-related biomarkers, were collected by each participating clinical center, prepared for analyses by the DISC Coordinating Center at the Maryland Medical Research Institute, and provided to investigators at Michigan State University for analyses.

3.1.3.3 Data collection

Sociodemographic, physical, biochemical, and lifestyle data were collected at baseline, post-randomization years 1, 3, and 5, and at the last visit. Data were collected via questionnaire and medical examination by project personnel trained specifically for the DISC who were blinded to participants' treatment assignments. Height and weight were measured at baseline and annually thereafter. Body mass index was calculated as weight (in kg) divided by height^2 (in m). All girls were at Tanner stage 1 at baseline and Tanner staging was performed to assess sexual maturation annually (119) until Tanner stage 5 was reached. Date of onset of menses was ascertained annually until menarche.

3.1.3.4 Diet assessment

Detailed description of dietary assessment methodology is provided elsewhere (15, 20). Because the DISC was a dietary intervention study, particular attention was paid to the quality of dietary assessment. Dietary intakes were assessed by trained, certified nutritionists, blinded to study group assignment using 3 nonconsecutive 24-hour dietary recalls. Nutrient analyses were performed by the Nutrition Coordinating Center, (version 20; University of Minnesota, Minneapolis, MN) (120). Data from the three recalls at each visit were averaged to estimate mean nutrient intake. Because information about added salt was not ascertained, reported sodium values reflect intake from food and not total sodium intake.

3.1.3.5 Biochemical analyses

Blood samples were collected by venipuncture after an overnight fast. Blood samples were kept at room temperature for at least 45 minutes to allow complete clotting and then serum was separated by centrifugation. Serum was then aliquoted and stored in glass vials at −80°C until it was analyzed for hormone, lipid, and micronutrient levels for use in DISC analyses (114, 116). Serum samples for measurement of IGF related biomarkers reported here were stored for a total of 14-16 years during which time they were thawed twice under controlled conditions to allow re-aliquoting and each time were re-frozen immediately at −80°C. IGF related biomarkers have been shown to remain stable after long-term storage and repeated freeze-thaw cycles (121, 122). Laboratory measurements for analytes assayed for this study were conducted in the laboratory of Dr. Cliff J. Rosen at the Maine Center for Osteoporosis Research and Education. IGF-I levels were determined using the IGF-I (IGFBP-blocked) radioimmunoassay (ALPCO,

Windham, NH). Serum IGFBP-1 levels were determined using the Total IGFBP-1 IRMA kit (DSL, Webster, TX). Serum IGFBP-3 levels were determined using the "Active" IGFBP-3 IRMA kit (DSL, Webster, TX). Samples were assayed with three randomly inserted laboratory-masked quality control samples included per batch. The external interassay coefficients of variation for IGF-I, IGFBP-3, and IGFBP-1 were 4%, 3%, and 10%, respectively. The IGF-I/IGFBP-3 molar ratio was estimated as a possible index of bioavailable IGF-I.

3.1.3.6 Statistical analysis

All statistical analyses were performed using SAS software (version 9.1.3; SAS Institute, Inc, Cary, NC). Descriptive statistics were calculated for age at study entry and other sociodemographic variables to describe the sample at baseline, and for anthropometric, physical activity, dietary and biochemical measures at the last DISC visit to describe the exposures and outcomes of interest. Associations between dietary intakes and IGF-I, IGFBP-1, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio were assessed first with Pearson correlations in exploratory analyses and subsequently with multivariable linear regression.

Dietary variables examined were energy, total fat, total protein, total carbohydrate, animal protein, vegetable protein, lactose, dietary fiber, calcium, zinc, and sodium. These nutrients were selected based on those shown to be associated with IGF-related biomarkers in the literature (12, 16) and those available in our dataset. All dietary intakes and IGF-related biomarkers were logarithmically transformed to stabilize variances and mitigate skewness in distributions. Intake of dietary variables were adjusted for energy using the multivariate nutrient density approach (55, 123). Because of

the known associations between energy and nutrient intake (123) and also between age and time since menarche and IGF-related biomarkers (29), it was determined *a priori* that correlation analyses should be assessed before and after controlling for energy intake, age and time since menarche. Therefore, crude correlations were assessed, as well as partial correlations, after controlling for the effect of energy intake, age (in years), and time since menarche (in years). Additionally, all correlation results were assessed before and after stratification on vitamin/mineral supplement usage (yes/no) and results were not appreciably different (data not shown); therefore, further analyses did not consider vitamin/mineral supplement usage. Geometric mean concentrations of each biomarker (adjusted for energy, age, and time since menarche) within quartiles of each dietary exposure were also assessed in order to fully describe the outcome (IGF-related biomarkers) in relation to the exposure of interest (dietary intake).

Multivariable linear regression analyses were conducted to examine associations between dietary exposures and biomarker outcomes, while controlling for potential confounders. To avoid multicollinearity, dietary exposures were entered into the regression model together only if univariate correlations were less than r= 0.60. Thus, four dietary variables were not included in the analysis: total protein (% kcal) was dropped in order to retain animal protein (g/1000 kcal) (r= 0.89); total carbohydrate (% kcal) was dropped in order to retain total fat (% kcal) (r= − 0.81); fiber (g/1000 kcal) was dropped in order to retain vegetable protein (g/1000 kcal) (r= 0.72); and lactose (g/1000 kcal) was dropped in order to retain calcium (mg/1000 kcal) (r= 0.71). The decision as to which dietary variable to drop/retain was subjectively based on the nutrient of greater interest in the IGF-related literature. Additional regression analyses, including the

dropped nutrients, were also conducted to test the significance of these nutrients in the presence of identified confounders; none were significant and therefore retained in the final model building process described below.

Initial univariate models included the following dietary variables: energy (kcals), total fat (% energy), animal protein (g/1000 kcals), vegetable protein (g/1000 kcals), calcium (mg/1000 kcals), zinc (mg/1000 kcals), and sodium (mg/1000 kcals); and the following potential confounders: age, time since menarche, BMI percentile for age, physical activity (hours of moderate and intense activity/week), maternal education level (high school or less; some college; college or graduate degree), and treatment group. Covariates were entered in a multivariable model using a forward regression approach with $P < 0.30$ as the initial significance criterion for entry. A parsimonious model was derived from the forward multivariate model by retaining variables that were then significant in the presence of other covariates at $P < 0.10$. An F test was used to assess significance.

The sample was not large enough to justify stratification by race/ethnicity, however, because of reported differences in associations between covariates and IGF related biomarkers by race/ethnicity in girls (54), analyses restricted to only White girls (92%) were also conducted. Results were similar for models including all girls vs. only White girls (data not shown) and therefore analyses including all race/ethnicities combined are presented.

3.1.4 Results

Sociodemographic characteristics of participants are presented in Table 2. Approximately 94% of girls in our sample were between ages 15 and 17 y at last visit

with a mean age at menarche of 12.9 ± 1.1 y. Participants were predominantly White spanning a broad socioeconomic background based on household income and mother's education. Girls in our study sample were equally balanced among treatment and control groups in the DISC.

Summary statistics for covariates and exposures of interest for girls in the DISC/IRBS at the last study visit are shown in Table 3. Mean (± SD) statistics are reported to describe sample distributions. Reported nutrient intakes include a mean energy intake of 1633 ± 492 kcal and total fat of 28.0 ± 6.7 % energy, reflecting estimates similar to those seen in a nationally representative sample of US female adolescents (age 12-19) (124). Geometric means (95% CI) are shown for each IGF-related biomarker in Table 3 and are within published references ranges for adolescent girls (26).

Both the unadjusted and adjusted correlations (Pearson's r) between nutrients and IGF-related biomarkers at last visit are shown in Table 4. Adjustment for energy intake, age, and time since menarche did not substantially change the significance or the strength of the associations between dietary variables and IGF-I, IGFBP-1, or IGFBP-3. In unadjusted and adjusted correlation analyses, total protein, lactose, calcium, and sodium were all positively associated with IGF-I; lactose, fiber and calcium were positively associated with IGFBP-3 whereas total fat was negatively associated with IGFBP-3 ($P<0.05$ for all); and vegetable protein was positively associated with IGFBP-1. In unadjusted correlation analyses of dietary variables and the IGF-I/IGFBP-3 molar ratio, lactose and calcium were positively associated with the IGF-I/IGFBP-3 molar ratio ($P<0.05$ for both), while after adjustment only the association between calcium and the IGF-I/IGFBP-3 molar ratio remained significant ($P<0.05$).

Table 5 shows geometric mean levels of IGF-related biomarkers by quartiles of nutrient intake after adjustment for energy intake, age, and time since menarche. Biomarker values are only shown for those nutrients where the crude or adjusted association with a measure of the IGF system was significant at $P<0.05$ in correlation analysis (i.e. those shown in bold in table 4). Significant linear trends are present in IGF-I concentrations across increasing quartiles of total protein, calcium, and sodium intakes. Similarly, there are significant linear trends in IGFBP-3 concentrations across increasing quartiles of fiber and calcium intake and across decreasing quartiles of total fat intake. In parallel analyses, a significant linear trend is seen in the IGF-I/IGFBP-3 molar ratio across increasing quartiles of calcium intake.

Results from regression analyses including mutually adjusted dietary factors as predictors of the four IGF-related biomarkers are presented in Table 6. With IGF-I as the dependent variable, two dietary variables remained significant in the most parsimonious model: energy ($\beta=0.14 \pm 0.06$, $P=0.029$) and calcium ($\beta=0.14 \pm 0.05$, $P=0.007$), while for IGFBP-3 calcium was also significant ($\beta=0.07 \pm 0.03$, $P=0.011$), but for the IGF-I/IGFBP-3 molar ratio as the dependent variable, there were no significant dietary predictors in the final model. Lastly, with IGFBP-1 as the dependent variable, vegetable protein ($\beta=0.49 \pm 0.24$, $P=0.041$) and BMI for age percentile ($\beta= -0.01 \pm 0.00$, $P<0.001$) remained significant in the final model. Because the IGF-related biomarkers and dietary variables were log-transformed, the β -coefficients are interpretable as percentages. For example, in the model where IGF-I is the dependent variable and energy and sodium are held constant, a one percent increase in calcium yields a 0.14% increase in IGF-I (ng/ml).

3.1.5 Discussion

This study examined the associations between dietary intakes of specific nutrients and serum IGF-I, IGFBP-3, the IGF-I/IGFBP-3 molar ratio, and IGFBP-1 in post-menarcheal girls (aged 14-18 y) who participated in the DISC/IRBS. We found that IGF related biomarker concentrations were associated with several nutrient intakes in this group of adolescent girls. Calcium was a significant predictor of both IGF-I and IGFBP-3 concentrations, whereas vegetable protein and BMI for age percentile were significant predictors of IGFBP-1 concentrations.

The positive correlations in the present analyses between IGF-I and total protein and calcium intakes and the negative correlation between IGFBP-3 and total fat are consistent with results from the ALSPAC analyses in 7-8 year old children (16), as well as multiple reports in adults (12). Unlike the ALSPAC study (16), in our analyses, both IGFBP-3 and the IGF-I/IGFBP-3 molar ratio were also significantly correlated with calcium. In other ALSPAC analyses energy-adjusted milk and dairy intake were significantly and positively associated with IGF-I and positively, though not significantly, associated with IGFBP-3 concentrations before but not after controlling for protein intake (58).

Our study differs from the ALSPAC analyses in several ways: our analyses included post-menarcheal girls, while the ALSPAC data included pre-pubertal (age 7-8 years) girls and boys (analyses adjusted for gender). Perhaps a more salient difference, however, is in the diet composition between the two cohorts. Although median energy intakes, total protein, vegetable protein and calcium intakes in girls in the ALSPAC analyses (1638 kcal, 52 g (13% energy), 22 g, and 738 mg, respectively) were very

similar to the DISC girls in these analyses (1603 kcal, 58 g (14% energy), 19 g, and 752 mg, respectively), the diet composition differed markedly in median total fat and animal protein intakes with 68 g total fat (37% energy) and 31 g animal protein in the ALSPAC analyses and 50 g total fat (28% energy) and 38 g animal protein in these DISC analyses. These differences in dietary composition likely reflect different sources of nutrients (e.g. different sources of protein), which may underlie the differences seen in the relationships between calcium and IGFBP-3 and the IGF-I/IGFBP-3 molar ratios between the two cohorts.

In final parsimonious multivariable linear regression models, which account for all nutrients and potential confounders simultaneously, calcium was a significant dietary predictor of both IGF-I and IGFBP-3, although it was not a significant predictor of the IGF-I/IGFBP-3 molar ratio. These findings for the independent effects of IGF-1 and IGFBP-3 are consistent with the literature. There have been three cross-sectional studies and three clinical trials studying the effects of milk or dairy intake on the IGF system in healthy children (ranging in age from 2-12 y) and all six reflect higher IGF-I levels with even short term higher milk consumption (14, 15, 17, 57, 58, 125). Although we did not look at food level data in the present analyses, milk is a major source of both calcium and protein in the diets of US children. At the time of the DISC study, nationally representative estimates indicated that milk contributed 43% of the total calcium intake among females aged 12-18 y and 15% of the total protein intake (CSFII 1988-1991) (126). Milk is associated with a higher childhood linear growth velocity in developing countries and to a lesser extent in well-nourished populations (56). Because IGF-I is a growth regulator, and decreased IGF-I concentrations are associated with deficits in

protein intakes, it has been hypothesized that IGF-I may mediate the positive association between protein intake and growth (15). Since milk is a significant source of both calcium and protein, it is difficult to disentangle the effects of one from the other in observational studies.

Sodium was also a borderline significant predictor of IGF-I (P=0.06), which may indicate the importance of sodium per se, or may point to the need to consider the potential effects of dietary patterns on IGF-related biomarkers given salty snacks are a primary contributor to sodium intake among adolescents (127). As previously noted, information about added salt was not ascertained, thus reported sodium values reflect intake from food and not total sodium intake. However, the majority of sodium consumed by Americans is added during commercial processing and preparation (128). In a nationally representative sample of US children, 53% of 12-19 year old females never or rarely added table salt to their foods (128), thus having only sodium from food may bias estimates slightly, but this is not likely to be a major factor.

In both correlation and multivariable analyses, a significant positive association was seen between IGFBP-1 and vegetable protein. In addition, BMI for age percentile was a weak but highly significant negative predictor of IGFBP-1 in regression analyses. We believe the present study is the first to assess the relationship between IGFBP-1 and dietary predictors in adolescents. In adults, IGFBP-1 has been shown to be positively associated with energy and carbohydrate intake in adult men (129) and plant lignans in postmenopausal women (130, 131), and negatively correlated with regular soda intake in adult men and women (132). Although we did not include carbohydrate in our regression models because it was highly correlated with fat intake (r= − 0.81), IGFBP-1 was not

correlated with carbohydrate intake in adjusted correlation analyses (r=0.02, *P*=0.82). Our finding of a negative association between BMI for age percentile and IGFBP-1, is consistent with previous studies in adults (133), but we again did not identify any studies for comparison in adolescents.

In this study, we make the assumption that three nonconsecutive 24-hour dietary recalls represents the usual intake of the girls. Three nonconsecutive 24-hour dietary recalls have been shown to be a valid estimator of usual intake for nutrients that do not have a high day to day variation (55). As two of our primary nutrients of interest are protein and calcium intake, this is likely to be a valid assumption – particularly in children given milk intake is more consistent from day to day (134). Furthermore, results of the Observing Protein and Energy Nutrition (OPEN) Study show that multiple 24-hour recalls provide better estimates of energy and protein intakes than a semiquantitative food frequency questionnaire (135). An additional limitation of this study is the cross-sectional design, which prevents any assumption of dietary intake as a causal factor in observed levels of IGF-related biomarkers. Dietary intake was assessed via multiple 24-hour dietary recalls around the time of the blood draw [the first recall was obtained concurrent to the blood draw and the 2 subsequent recalls were obtained within 2 weeks after the blood draw(114)] and thus our assumption is that the dietary assessment represents the usual intake of the girls at the time the biomarkers are measured.

A major strength of this study is that it is the first step in understanding the associations of dietary intake with IGF-related biomarkers in healthy post-pubertal adolescent girls. Because the DISC study collected detailed information on age at menarche—a factor highly associated with the lifetime peaks and nadir of IGF-related biomarkers (26)—we

can be sure that all girls in these analyses were post-menarcheal. The results presented here are in agreement with the larger body of literature in adults (12) and the smaller body of literature in pre-pubertal children (14-16, 136) that suggest IGF-related biomarkers are associated with several aspects of diet, most notably protein and calcium intakes. Overall, this study provides support to the speculation that variation in dietary intakes, even within normal ranges in healthy adolescents, is associated with variation in concentrations of components of the IGF system. Thus, childhood dietary intake, through mediation of the IGF system, may affect risk for cancer and other chronic diseases in adulthood, but longitudinal studies are needed to address the multiple complexities inherent in lifetime dietary intake and growth.

CHAPTER 4: CONCLUSIONS

4.1 Summary of findings

This thesis provides a summary of the published literature of the major determinants of the components of the IGF system in children and a report of original analyses of diet and IGF-related biomarkers in adolescent girls. Age, sex, and pubertal maturation clearly are the major predictors IGF-related biomarkers at any given time during childhood and therefore studies relating to those characteristics were reviewed first to put the entire system into some biological context. Secondly, because lifestyle factors have been shown to be associated with adult levels of IGF-related biomarkers, the literature pertaining to lifestyle factors in children as those factors relate to the IGF system was reviewed.

Lifestyle factors reviewed here include dietary intake, physical activity, and the related measures of body size because body weight and BMI are influenced by dietary and physical activity behaviors. The literature in children regarding diet is sparse but is emerging in agreement with results from studies in adults. Namely, the dietary components most associated with IGF-related biomarkers appear to be protein/animal protein, dairy intakes and the intakes of some minerals such as calcium, magnesium, and zinc, which are highly correlated with animal protein and dairy intakes. However, this is a very young body of literature and there are many hypotheses that have yet to be explored. The results from the original analyses reported here exploring associations between diet and IGF-related biomarkers in adolescent girls appears to support the concept that diet, specifically intakes of nutrients found in meat and/or dairy products may contribute to the variation in IGF-related biomarkers. The literature on both

physical activity and body size in relation to IGF-related biomarkers is also growing and overall appears to support a role for lifestyle factors in influencing the IGF system.

4.2 Public health implications

Especially because the IGF system has been shown to be associated with different chronic diseases in adults *in different directions* [i.e. high IGF-I has been associated with cancer (6), while low IGF-I has been associated with coronary heart (10) disease and osteoporosis (5)], it is imperative to carefully think through the public health implications of this body of work. If the literature were to strongly support effects of lifestyle on IGF-related biomarkers, for example, would it be prudent to suggest lifestyle modifications that would likely *increase* or *decrease* IGF-I? We must pursue an understanding of correlates of the IGF system firstly to help understand the etiology of chronic diseases that manifest themselves in adulthood but may be initiated in adolescence or even earlier in life. Secondly, once we gain a more complete understanding of the etiology, we may find intervention strategies that are supportive of good health in general as opposed to targeting prevention of specific diseases.

4.3 Plans for Related Research

Related research to be completed in the near future will include longitudinal modeling of the effect of diet on IGF-related biomarkers while stratifying on body size. Toward that end, I will be developing a system to categorize usual dietary intake of key nutrients over time to predict IGF-related biomarkers among girls who participated in the DISC/IRBS. Our first step will be to assess the strength of the relationship between habitual longitudinal nutrient intake of a few key nutrients on IGF-related biomarkers at the last DISC visit. Secondly, we will model the entire curve of specific dietary

components on IGF-related biomarkers at all available time points from baseline to last visit. As mentioned, more emphasis will be placed on stratification by body size. Finally, I have a particular interest in the effects of alcohol use on IGF-related biomarkers in adolescence, and will pursue an exploratory analysis assessing that relationship among girls who participated in the DISC/IRBS.

This thesis represents one part of a series of analyses regarding lifecycle impacts on lifetime health and disease in general, and specifically relating dietary intake to insulin-related biomarkers in adolescent girls. This work is being carried out under the direction of Dr. Ellen Velie and will continue with further analyses to more comprehensively explore dietary intake and body size as predictors of insulin-related biomarkers (IGF-I, IGFBP-1, IGFBP-3, IGF-I/IGFBP-3, insulin, c-peptide, and glucose). In addition to the data available to our research team at this time, we will continue to pursue opportunities to collect and analyze longitudinal data in children, such as that which may be available from the National Children's Study.

APPENDIX

Table 1. Cross-sectional studies on the association between diet and IGF-I, IGFBP-3, or IGF-I/IGFBP-3 in healthy children by publication year[1]

Author (year)	n	Race/ Ethnicity	Mean Age (y)	Sex	Dietary Assessment	Diet Components	Adjustments	IGF-I		IGFBP-3		IGF-I/IGFBP-3	
								r^2	*P*	r	*P*	r	*P*
Wilson (1991)	243	White Af-Amer Asian Hispanic	12	F	FFQ	Energy (kcals/d) Protein (g/d) Fat (g/d) Carbohydrate (g/d)	Pubertal stage	0.04 0.05 0.08 0.02	0.52 0.43 0.22 0.75	- - - -	- - - -	- - - -	- - - -
Wilson (1992)[3]	242	Caucasian Af-Amer Asian Hispanic	12	F	FFQ	Energy (kcals/d) Protein (g/d) Fat (g/d) Carbohydrate	Pubertal stage	- - - -	- - - -	NR NR NR NR	NS NS NS NS	- - - -	- - - -
Hoppe (2004)	90	White	2.5	M/F	7-day diet diary	**Animal protein (g/d)** Vege protein (g/d) **Milk (g/d)** Meat (g/d)	Sex, weight, height	1.4 0.12 0.05 0.15	**0.01** 0.91 **0.05** 0.34	- - - -	- - - -	- - - -	- - - -
Higgins (2005)	29 26	Euro-Amer Af-Amer	8	M/F M/F	3 24-hour diet recalls	Energy (kcals/d) Carbohydrate (g/d) Protein (g/d) Fat (g/d) Saturated fat (g/d)	Unadjusted	0.02 -0.09 0.09 0.14 0.02	0.89 0.52 0.49 0.31 0.91	- - - - -	- - - - -	- - - - -	- - - - -
Rogers (2005)	521	White	8	M/F	3-day diet diary	**Energy** **Total Fat** Saturated Fat **PUFA** **MUFA** **Protein** **Animal protein** Vegetable protein Carbohydrate Sugar NSP **Zinc**	Age, sex for all variables Energy intake for all except energy	0.07 -0.12 -0.06 -0.09 -0.11 0.19 0.16 0.01 0.04 0.03 0.08 0.14	0.10 **<0.01** 0.10 **0.03** **<0.01** **<0.01** **<0.01** 0.90 0.40 0.40 0.06 **<0.01**	0.13 -0.09 -0.05 0.00 -0.11 0.07 0.04 0.04 0.05 0.03 0.04 0.06	**<0.01** **0.05** 0.20 0.90 **0.01** 0.10 0.40 0.40 0.20 0.60 0.30 0.20	-0.04 -0.05 -0.02 -0.10 -0.02 0.14 0.13 -0.03 -0.01 0.01 0.05 0.09	0.30 0.20 0.70 **0.02** 0.60 **<0.01** **<0.01** 0.60 0.80 0.70 0.30 **0.03**

Table 1. Continued[1]

Author (year)	n	Race/ Ethnicity	Mean Age (y)	Sex	Dietary Assessment	Diet Components	Adjustments	IGF-I		IGFBP-3		IGF-I/IGFBP-3	
								r^2	*P*	r	*P*	r	*P*
Rogers (2005) (continued)						**Phosphorus**		0.16	**<0.01**	0.07	0.10	0.10	**0.02**
						Magnesium		0.15	**<0.01**	0.08	0.06	0.08	0.07
						Calcium		0.12	**<0.01**	0.06	0.20	0.07	0.09
						Potassium		0.14	**<0.01**	0.08	0.07	0.08	0.07
						Selenium		0.04	0.30	0.03	0.40	0.02	0.70
						Folate		0.11	**0.02**	0.05	0.30	0.07	0.10
						Vitamin C		0.06	0.10	0.02	0.70	0.05	0.20
						Retinol		<-0.01	0.90	0.01	0.80	-0.01	0.80
						Carotene		0.07	0.10	0.05	0.20	0.03	0.50
						Vitamin D		-0.02	0.60	0.01	0.90	-0.03	0.50
						Vitamin E		-0.07	0.10	0.02	0.60	-0.09	**0.04**
Rogers (2006)	538	White	8	M/F	3-day diet diary	**Cow's milk**	Energy intake	NR	**0.04**	NR	0.08	-	-
						Dairy products		NR	**0.03**	NR	0.07	-	-
Budek (2007)	56	White	8	M	3-day diet diary	**Total protein**	Energy intake, Age, BMI	1.41	**0.01**	12.51	0.15	<0.01	**<0.01**
						Plant protein		0.15	0.85	-2.04	0.85	<0.01	0.93
						Dairy protein		0.91	0.12	9.61	0.30	<0.01	0.06
						Meat protein		0.46	0.48	-5.03	0.53	<0.01	0.28
						Milk intake		0.05	**0.03**	0.51	0.15	<0.01	**0.03**
						Calcium intake		0.05	**<0.01**	0.62	**0.03**	<0.01	**<0.01**

[1]Nonstandard abbreviations: Af-Amer=African-American; Euro-Amer=European American; Vege=vegetable; NSP=Nonstarch Polysaccharide FFQ=food frequency questionnaire; PUFA=polyunsaturated fat; MUFA=monounsaturated fat

[2]All values are r (Spearman's or Pearson's correlation coefficient) except Hoppe et al, 2004 and Budek, et al, 2007, where a β coefficient is reported; and Rogers et al, 2006 where a *P* for trend across increasing quartiles of dietary intake are reported; " - " indicates unmeasured; Bold indicates $P<0.05$

[3]Wilson et al, 1992, report no relevant data (NR=not reported), but indicate in the text that the relationship is not significant (NS=nonsignificant)

Table 2. Sociodemographic characteristics of girls in the DISC/IRBS (n=159)[1]

	n	%[2]
Race/ethnicity		
White	146	92
Black	8	5
Other	5	3
Total household income		
<$20,000	15	9
$20,000 to <$30,000	25	16
$30,000 to <$50,000	58	36
$50,000+	60	38
Mother's education		
High school or less	38	24
Some college	33	21
College or graduate degree	74	47
Treatment group		
Intervention	76	48
Usual care	83	52

[1]Data presented for girls with complete data on diet, menarche, and IGF-I at last visit

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

Table 3. Characteristics of girls in the DISC/IRBS at last visit (N=159)[1]

	Mean (SD)		
Age (y)	16.6 (	0.9)	
Time since menarche (y)	3.7 (	1.2)	
Height (cm)	164.1 (	6.2)	
Weight (kg)	60.9 (	11.3)	
BMI	22.6 (	3.7)	
BMI for age (percentile)	59.8 (	27.8)	
Physical Activity (hr/wk)[2]	17.4 (	12.2)	
Energy/nutrient			
Energy (kcal)	1633 (	492)	
Total fat (% kcal)	28.0 (	6.7)	
Total protein (% kcal)	14.9 (	3.2)	
Total carbohydrate (% kcal)	57.8 (	7.5)	
Animal protein (g)	39.8 (	17.0)	
Vegetable protein (g)	20.1 (	7.6)	
Lactose (g)	17.5 (	12.9)	
Fiber (g)	10.8 (	4.6)	
Calcium (mg)	852 (	433)	
Zinc (mg)	7.9 (	3.5)	
Sodium (mg)	2718 (	861)	
IGF-related biomarker	**Geometric mean (95% CI)**		
IGF-I (ng/mL)	345.5 (	332.8 ,	358.7)
IGFBP-3 (ng/mL)	5009 (	4912 ,	5109)
IGF-I/IGFBP-3 molar ratio	0.38 (	0.37 ,	0.39)
IGFBP-1 (ng/mL)[3]	31.2 (	27.5 ,	35.4)

[1]Data presented for girls with complete data on diet, menarche, and IGF-I at last visit

[2]Hours of moderate and intense physical activity per week; data missing on 15 girls

[3]Data missing on 7 girls

Table 4. Pearson correlations between dietary intakes and IGF-related biomarkers among girls in the DISC/IRBS at last visit[1]

	IGF-I (ng/mL) n=159[2,3,4]		IGFBP-1 (ng/mL) n=152[2,3,4]		IGFBP-3 (ng/mL) n=159[2,3,4]		IGF-I/IGFBP-3 molar ratio n=159[2,3,4]	
Nutrient	r	Adjusted r	r	Adjusted r	r	Adjusted r	r	Adjusted r
Total fat (% kcal)	–0.09	–0.13	–0.01	–0.02	**–0.16**	**–0.17**	–0.01	–0.05
Total protein (% kcal)	**0.16**	**0.17**	–0.03	–0.02	0.10	0.10	0.14	0.15
Total carbohydrate (% kcal)	0.04	0.05	0.02	0.02	0.13	0.13	–0.03	–0.02
Animal protein (g/1000 kcal)	0.14	0.13	–0.11	–0.11	0.05	0.04	0.15	0.14
Vegetable protein (g/1000 kcal)	0.06	0.09	**0.16**	**0.18**	0.10	0.11	0.01	0.04
Lactose (g/1000 kcal)	**0.22**	**0.19**	–0.04	–0.05	**0.18**	**0.17**	**0.16**	0.12
Fiber (g/1000 kcal)	0.10	0.13	0.11	0.12	**0.17**	**0.18**	0.01	0.04
Calcium (mg/1000 kcal)	**0.25**	**0.24**	–0.07	–0.07	**0.20**	**0.20**	**0.18**	**0.18**
Zinc (mg/1000 kcal)	0.04	0.04	–0.09	–0.09	0.10	0.09	–0.02	–0.02
Sodium (mg/1000 kcal)	**0.17**	**0.19**	–0.01	0.00	0.13	0.13	0.13	0.16

[1]Data presented for girls with complete data on diet, menarche, and IGF-I at last visit

[2]First column is unadjusted

[3]Second column is adjusted for age, time since menarche, and energy intake

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

Table 5. Adjusted geometric means (95% CI) of IGF-related biomarkers by quartile of dietary intake among girls in the DISC/IRBS at last visit[1,2,3]

	Quartile of Dietary Intake			
	1	2	3	4	
IGF-I (ng/mL)	n=159				P^4
Total protein	323 (300 , 347)	347 (323 , 373)	354 (329 , 381)	360 (334 , 388)	0.035
Lactose	331 (308 , 356)	337 (313 , 362)	361 (335 , 388)	354 (329 , 381)	0.104
Calcium	314 (293 , 338)	343 (319 , 369)	362 (336 , 389)	366 (340 , 392)	0.002
Sodium	331 (308 , 356)	328 (304 , 353)	357 (332 , 384)	368 (341 , 397)	0.020
IGFBP-3 (ng/mL)	n=159				
Total fat	5186 (4985 , 5396)	5055 (4863 , 5256)	4926 (4738 , 5121)	4880 (4693 , 5074)	0.020
Lactose	4845 (4658 , 5039)	4986 (4792 , 5187)	5135 (4937 , 5340)	5077 (4882 , 5279)	0.058
Fiber	4805 (4621 , 4997)	5063 (4868 , 5267)	4964 (4776 , 5159)	5209 (5010 , 5416)	0.013
Calcium	4850 (4666 , 5042)	4941 (4749 , 5140)	5082 (4884 , 5287)	5173 (4976 , 5377)	0.013
IGF-I/IGFBP-3 molar ratio	n=159				
Lactose	0.37 (0.35 , 0.40)	0.37 (0.35 , 0.39)	0.38 (0.36 , 0.41)	0.38 (0.36 , 0.40)	0.430
Calcium	0.35 (0.33 , 0.37)	0.38 (0.36 , 0.40)	0.39 (0.37 , 0.41)	0.39 (0.36 , 0.41)	0.026
IGFBP-1 (ng/mL)	n=152				
Vegetable protein	27.8 (21.5 , 36.1)	28.3 (22.0 , 36.4)	30.5 (23.8 , 39.2)	39.9 (30.6 , 52.1)	0.057

[1] Adjusted for age, time since menarche, and energy intake

[2] Biomarker values are shown for nutrients where the crude or adjusted association with an IGF-related biomarker in correlation analyis (see Table 3) was significant at $P<0.05$

[3] Units for dietary variables are as follows: total protein, total fat (% kcal); vegetable protein, lactose, fiber (g/1000 kcal); calcium, sodium (mg/1000 kcal)

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

Table 6. Major factors predicting IGF-related biomarkers among girls in the DISC/IRBS at last visit assessed by multiple linear regression analysis

	Final Models (n=159)[1,2]		
IGF-I (ng/mL)	β	SEE	*P*
Energy intake (kcal)	0.14	(0.06)	0.029
Calcium (mg/1000 kcal)	0.14	(0.05)	0.007
Sodium (mg/1000 kcal)	0.17	(0.09)	0.055
IGFBP-3 (ng/mL)	β	SEE	*P*
Calcium (mg/1000 kcal)	0.07	(0.03)	0.011
IGFBP-1 (ng/mL)[3]	β	SEE	*P*
Vegetable protein (g/1000 kcal)	0.49	(0.24)	0.041
BMI for age %tile	−0.01	(0.00)	<0.001

[1]Biomarker & nutrient values log transformed (ln)

[2]Initial model contained: energy (kcal); fat (% kcal); animal & vegetable protein (g/1000 kcal); calcium, zinc, sodium (mg/1000 kcal); Maternal education: high school or less; some college; college or graduate degree; Physical activity: hrs of moderate & intense activity/wk; and treatment group

[3]IGFBP-1 data is missing on 7 girls (n=152)

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