

Dietary predictors of the insulin-like growth factor system in adolescent females: results from the Dietary Intervention Study in Children (DISC)^{1–3}

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ABSTRACT

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

Objective: We examined associations between dietary food intake [fat, protein (animal and vegetable), carbohydrate, lactose, dietary fiber, calcium, zinc, and sodium] and serum IGF-I, IGF binding protein 1 (IGFBP-1), IGF binding protein 3 (IGFBP-3), and the IGF-I:IGFBP-3 molar ratio in adolescent females.

Design: One hundred fifty-nine adolescent females in the Dietary Intervention Study in Children (age range: 14–18 y; 0.2–6.3 y post-menarche) were included. The dietary intake was assessed via three 24-h dietary recalls. IGF-related biomarkers were determined by using radioimmunoassays. Associations between dietary intakes and biomarkers were assessed with Pearson's correlations and multivariable linear regression. Dietary intakes and biomarkers were logarithmically transformed; thus, β coefficients represented percentages.

Results: In analyses adjusted for energy, age, and time since menarche, significant correlations ($P < 0.05$) were as follows: 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 ($\beta = 0.14$, $P < 0.05$) and calcium ($\beta = 0.14$, $P < 0.01$), the significant predictor of IGFBP-3 was calcium ($\beta = 0.07$, $P < 0.05$), and significant predictors of IGFBP-1 were vegetable protein ($\beta = 0.49$, $P < 0.05$) and body mass index–for-age percentile ($\beta = -0.01$, $P < 0.001$).

Conclusion: This study provides evidence that dietary intake affects IGF-related biomarkers—particularly elevated calcium with IGF-I and IGFBP-3 and elevated vegetable protein with IGFBP-1—and, to our knowledge, is novel in reporting these associations in adolescent females. The Dietary Intervention Study in Children was registered at clinicaltrials.gov as NCT00000459. *Am J Clin Nutr* 2010;91:643–50.

INTRODUCTION

Elevated concentrations of insulin-like growth factor I (IGF-I) and IGF binding protein 3 (IGFBP-3) have been associated with an increased risk of breast, prostate, colorectal, and lung cancers in adults (1), possibly through the advancement of cell cycle progression and/or the inhibition of apoptosis (2). In contrast, some evidence has suggested that low concentrations of IGF-I

may be associated with an increased risk of osteoporosis (3), impaired glucose tolerance (4), and coronary artery disease (5). Because the IGF axis regulates growth and development, and the highest rates of IGF-I production occur during the pubertal growth spurt (6), adolescence may be a critical window of time in affecting lifetime concentrations 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 the relative concentrations of the IGFBPs (6). Potential mechanisms by which dietary intake may affect circulating concentrations of IGF-I include the inhibition of hepatic synthesis or indirectly through effects on IGFBPs (7).

IGFBP-3 is the binding protein that binds the majority of circulating IGF-I (>90%), and it is thought to inhibit IGF-I action (6). 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 was proposed as an index of bioavailable IGF-I (8, 9). IGF binding protein 1 (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 (8, 9).

In adults, positive associations were 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 the effects of these highly correlated dietary constituents (7). In children, severe energy restriction lowers IGF-I concentrations, and nutrient repletion increases IGF-I concentrations in growth-retarded children (6). However, few studies (10–13) examined dietary correlates of components of the IGF system in healthy children. In the 4 studies (10–13) we identified,

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high intakes of animal protein and/or dairy affected serum concentrations of IGF-I and IGFBP-3. All of the studies were conducted in early childhood and prepuberty before peak IGF-I concentrations at menarche.

In this study, we used data from the last visit of the Insulin-Related Biomarkers Study (IRBS; DAMD 17-03-1-0504; EMV, principal investigator), 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 (ie, reduced total fat, saturated fat, and cholesterol) in children who were 8–10 y old at baseline (14). 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 were observed in adults (7) and younger children (10–13) were also present in adolescent females.

SUBJECTS AND METHODS

Subjects

Participants were adolescent females who were in the DISC, a dietary intervention study initiated in 1988 in 8–10-y-old children (girls and boys) followed through childhood for a median duration in the study of 7.0 y. The National Heart, Lung, and Blood Institute 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 (14–16). In brief, the DISC was a multicenter, randomized, controlled clinical trial, wherein 663 children were enrolled in 1 of 6 clinical centers between 1988 and 1990 (the Children's Hospital, New Orleans, LA; the Johns Hopkins University Hospital, Baltimore, MD; the Kaiser Permanente Center for Health Research, Portland, OR; the University of Medicine and Dentistry of New Jersey, Newark, NJ; the Northwestern University Medical School, Chicago, IL; and the 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 National Heart, Lung, and Blood Institute.

Children were recruited through schools, a health maintenance organization, and pediatric practices and were eligible if they had a serum LDL cholesterol in the 80th to 98th age- and sex-specific percentiles of the Lipid Research Clinics population (17), were at least in the 5th percentile for height, and were in the 5th to 90th percentiles for weight-for-height (18). The LDL-cholesterol eligibility criteria were established to exclude children with severe hypercholesterolemia for whom medication may have been clinically indicated. Exclusion criteria included children with medical conditions or medications that could affect growth or the serum cholesterol, behavioral problems, or onset of pubertal maturation. Children were randomly assigned to receive a dietary intervention or usual care on the basis of a designation from the study coordinating center (Maryland Medical Research Institute, Baltimore, MD).

IRBS

A DISC ancillary study, the 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 females. The IRBS included 274 adolescent females 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 postmenarcheal adolescent females at the last DISC visit.

Of the 301 adolescent females in the DISC, 269 individuals attended the last visit. IGF-related biomarker data were available for 191 of these adolescent females, 172 of whom also had complete information on diet. After excluding adolescent females 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 adolescent females 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 adolescent female was excluded because of implausible dietary intake (3-d average zinc intake of 84 mg/1000 kcal), resulting in a final sample of 159 adolescent females. The final sample ($n = 159$) was compared with the full baseline sample ($n = 301$) in terms of socio-demographic characteristics, and no significant differences (tested by the chi-square test) were shown for race, income, maternal education, or treatment group.

Because the only dietary intake differences between treatment groups in our study of adolescent females were for saturated fat intake (data not shown), and saturated fat intake was not associated with the IGF system in adults (7) or hypothesized to be associated in healthy children (6), 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 (Baltimore, MD), and provided to investigators at Michigan State University for analyses.

Data collection

Sociodemographic, physical, biochemical, and lifestyle data were collected at baseline, postrandomization years 1, 3, and 5, and at the last visit. Data were collected via a questionnaire and medical examination by project personnel trained specifically for the DISC who were blinded to the treatment assignments of the participants. Height and weight were measured at baseline and annually thereafter. Body mass index (BMI; in kg/m^2) was calculated. All adolescent females were at Tanner stage 1 at baseline, and Tanner staging was performed to assess sexual maturation annually (19) until Tanner stage 5 was reached. The date of onset of menses was ascertained annually until menarche.

Diet assessment

A detailed description of the 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 who were blinded to assignments of study

groups by using 3 nonconsecutive 24-h dietary recalls. Nutrient analyses were performed by the Nutrition Coordinating Center (Nutrition Data System, version 20; University of Minnesota, Minneapolis, MN) (20). Data from the 3 recalls at each visit were averaged to estimate the mean nutrient intake. Because information about added salt was not ascertained, reported sodium values reflected the intake from food and not the total sodium intake.

Biochemical analyses

Blood samples were collected by venipuncture after an overnight fast. Blood samples were kept at room temperature for ≥ 45 min to allow complete clotting, and serum was separated by centrifugation. An aliquot of serum was removed and stored in glass vials at -80°C until it was analyzed for hormone, lipid, and micronutrient concentrations for use in DISC analyses (14, 16). Serum samples for the measurement of IGF-related biomarkers were stored for a total of 14–16 y, during which time they were thawed twice under controlled conditions to allow removal of additional aliquots of serum, and each time they were refrozen immediately at -80°C . IGF-related biomarkers were shown to remain stable after long-term storage and repeated freeze-thaw cycles (21, 22). Laboratory measurements for analytes assayed for this study were conducted in the laboratory of Cliff J Rosen at the Maine Center for Osteoporosis Research and Education (Scarborough, ME). IGF-I concentrations were measured with the IGF-I (IGFBP-blocked) radioimmunoassay (ALPCO, Windham, NH). Serum IGFBP-1 concentrations were measured with the Total IGFBP-1 IRMA kit (DSL, Webster, TX). Serum IGFBP-3 concentrations were measured with the “Active” IGFBP-3 IRMA kit (DSL, Webster, TX). Samples were assayed with 3 randomly inserted laboratory-masked quality-control samples included per batch. The external interassay CVs 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.

Statistical analyses

All statistical analyses were performed with 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 with Pearson's correlations in exploratory analyses and subsequently with multivariable linear regression.

The 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 on the basis of those associated in the literature with IGF-related biomarkers (7, 12) and those available in our data set. All dietary intakes and IGF-related biomarkers were logarithmically transformed to stabilize variances and mitigate skewness in distributions. The intakes of dietary variables were adjusted for energy by using the multivariate nutrient-density approach (23, 24). Because of the known associations between energy and nutrient intake (24) and between age and time since menarche and IGF-related biomarkers (25), it was determined

a priori that correlation analyses should be assessed before and after energy intake, age, and time since menarche were controlled for. Therefore, crude correlations were assessed, as well as partial correlations, after the effect of energy intake, age (y), and time since menarche (y) was controlled for. In addition, all correlation results were assessed before and after stratification on vitamin and/or mineral supplement usage (yes/no), and results were not appreciably different (data not shown); therefore, further analyses did not consider vitamin and/or mineral supplement usage. Geometric mean concentrations of each biomarker (adjusted for energy, age, and time since menarche) within quartiles of each dietary exposure were assessed 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 together into the regression model only if univariate correlations were less than $r = 0.60$. Thus, 4 dietary variables were not included in the analysis: total protein (% kcal) was removed to retain animal protein (g/1000 kcal) ($r = 0.89$), total carbohydrate (% kcal) was removed to retain total fat (% kcal) ($r = -0.81$), fiber (g/1000 kcal) was removed to retain vegetable protein (g/1000 kcal) ($r = 0.72$), and lactose (g/1000 kcal) was removed to retain calcium (mg/1000 kcal) ($r = 0.71$). The decision as to which dietary variable to remove or retain was subjectively based on the nutrient of greater interest in the IGF-related literature (7). Additional regression analyses, including the removed nutrients, were conducted to test the significance of these nutrients in the presence of identified confounders; none of these nutrients were significant and therefore none were retained in the final model-building process.

Initial univariate models included the following—dietary variables: energy (kcal), total fat (% energy), animal protein (g/1000 kcal), vegetable protein (g/1000 kcal), calcium (mg/1000 kcal), zinc (mg/1000 kcal), and sodium (mg/1000 kcal); and potential confounders: age, time since menarche, BMI percentile for age, physical activity (hours of moderate and intense activity per week), maternal educational level (high school or less, some college, and college or graduate degree), and treatment group. Covariates were entered in a multivariable model by 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 and/or ethnicity; however, because of reported differences in associations between covariates and IGF-related biomarkers by race and/or ethnicity in adolescent females (26), analyses restricted to only white adolescent females (92%) were also conducted. Results were similar for models including all adolescent females compared with only white adolescent females (data not shown), and therefore analyses are presented that include all races and/or ethnicities combined.

RESULTS

Sociodemographic characteristics of participants are presented in **Table 1**. Approximately 94% of adolescent females in our

TABLE 1

Sociodemographic characteristics of adolescent females in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study ($n = 159$)¹

	<i>n</i>	Percentage 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 are presented for subjects with complete data on diet, menarche, and insulin-like growth factor I at the last visit.

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

sample were between ages 15 and 17 y at the last visit, with a mean age at menarche of 12.9 ± 1.1 y. Participants were predominantly white and spanned a broad socioeconomic background on the basis of household income and mother's education. Adolescent females in our study sample were equally balanced between treatment and control groups in the DISC.

Summary statistics for covariates and exposures of interest for adolescent females in the DISC/IRBS at the last study visit are shown in **Table 2**. Mean (\pm SD) statistics are reported to describe sample distributions. Reported nutrient intakes include a mean (\pm SD) energy intake of 1633 ± 492 kcal and total fat of $28.0 \pm 6.7\%$ energy, reflecting estimates similar to those seen in a nationally representative sample of US female adolescents (age range: 12–19 y) (27). Geometric means (95% CI) for each IGF-related biomarker are shown in Table 2 and are within published reference ranges for adolescent females (28).

Unadjusted and adjusted correlations (Pearson's *r*) between nutrients and IGF-related biomarkers at last visit are shown in **Table 3**. 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 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), whereas after adjustment, only the association between calcium and the IGF-I:IGFBP-3 molar ratio remained significant ($P < 0.05$).

Geometric mean levels of IGF-related biomarkers by quartiles of nutrient intake after adjustment for energy intake, age, and time since menarche are shown in **Table 4**. Biomarker values are

TABLE 2

Characteristics of adolescent females in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit ($n = 159$)¹

	Value
Age (y)	16.6 ± 0.9 ²
Time since menarche (y)	3.7 ± 1.2
Height (cm)	164.1 ± 6.2
Weight (kg)	60.9 ± 11.3
BMI (kg/m^2)	22.6 ± 3.7
BMI-for-age percentile	59.8 ± 27.8
Physical activity (h/wk) ³	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	
IGF-I (ng/mL)	345.5 (332.8, 358.7) ⁴
IGFBP-3 (ng/mL)	5009 (4912, 5109)
IGF-I:IGFBP-3 molar ratio	0.38 (0.37, 0.39)
IGFBP-1 (ng/mL) ⁵	31.2 (27.5, 35.4)

¹ Data are presented for subjects with complete data on diet, menarche, and insulin-like growth factor I (IGF-I) at the last visit. IGFBP-1, IGF binding protein 1; IGFBP-3, IGF binding protein 3.

² Mean \pm SD (all such values).

³ Hours of moderate and intense physical activity per week; data missing for 15 subjects.

⁴ Geometric mean; 95% CI in parentheses (all such values).

⁵ Data missing for 7 subjects.

shown only for those nutrients where the crude or adjusted association with a measure of the IGF system was significant at $P < 0.05$ in the correlation analysis (ie, those footnoted in Table 3). Significant linear trends were present in IGF-I concentrations across increasing quartiles of total protein, calcium, and sodium intakes. Similarly, there were 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 was 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 4 IGF-related biomarkers are presented in **Table 5**. With IGF-I as the dependent variable, 2 dietary variables remained significant in the most parsimonious model: energy ($\beta = 0.14 \pm 0.06$, $P = 0.029$) and calcium ($\beta = 0.14 \pm 0.05$, $P = 0.007$), whereas 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. Last, 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, β coefficients were interpreted as percentages. For

TABLE 3

Pearson's correlations between dietary intakes and insulin-like growth factor (IGF)-related biomarkers among adolescent females in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit¹

Nutrient	IGF-I (ng/mL) (n = 159)		IGFBP-1 (ng/mL) (n = 1522)		IGFBP-3 (ng/mL) (n = 1592)		IGF-I:IGFBP-3 molar ratio (n = 1592)	
	r ²	Adjusted r ³	r ²	Adjusted r ³	r ²	Adjusted r ³	r ²	Adjusted r ³
Total fat (% kcal)	-0.09	-0.13	-0.01	-0.02	-0.16 ⁴	-0.17 ⁴	-0.01	-0.05
Total protein (% kcal)	0.16 ⁴	0.17 ⁴	-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 ⁴	0.18 ⁴	0.10	0.11	0.01	0.04
Lactose (g/1000 kcal)	0.22 ⁴	0.19 ⁴	-0.04	-0.05	0.18 ⁴	0.17 ⁴	0.16 ⁴	0.12
Fiber (g/1000 kcal)	0.10	0.13	0.11	0.12	0.17 ⁴	0.18 ⁴	0.01	0.04
Calcium (mg/1000 kcal)	0.25 ⁴	0.24 ⁴	-0.07	-0.07	0.20 ⁴	0.20 ⁴	0.18 ⁴	0.18 ⁴
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 ⁴	0.19 ⁴	-0.01	0.00	0.13	0.13	0.13	0.16

¹ Data are presented for subjects with complete data on diet, menarche, and IGF-I at the last visit. IGFBP-1, IGF binding protein 1; IGFBP-3, IGF binding protein 3.

² Unadjusted.

³ Adjusted for age, time since menarche, and energy intake.

⁴ Significant correlation at $P < 0.05$.

example, in the model in which IGF-I was the dependent variable, and energy and sodium were held constant, a 1% increase in calcium yielded a 0.14% increase in IGF-I (ng/mL).

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 postmenarcheal adolescent females (aged 14–18 y) who participated in the DISC/IRBS. We showed that IGF-related biomarker concentrations were associated with several nutrient intakes in this group of adolescent

females. Calcium was a significant predictor of IGF-I and IGFBP-3 concentrations, whereas vegetable protein and the 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-y-old children (12) and from multiple reports in adults (7). Unlike the ALSPAC (Avon Longitudinal Study of Parents and Children) study (12), in our analyses, IGFBP-3 and the IGF-I:IGFBP-3 molar ratio were significantly correlated with calcium. In other ALSPAC analyses

TABLE 4

Insulin-like growth factor (IGF)-related biomarkers by quartile of dietary intake among adolescent females in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit¹

	Quartile of dietary intake				P ²
	1	2	3	4	
IGF-I (ng/mL) (n = 159)					
Total protein (% kcal)	323 (300, 347)	347 (323, 373)	354 (329, 381)	360 (334, 388)	0.035
Lactose (g/1000 kcal)	331 (308, 356)	337 (313, 362)	361 (335, 388)	354 (329, 381)	0.104
Calcium (mg/1000 kcal)	314 (293, 338)	343 (319, 369)	362 (336, 389)	366 (340, 392)	0.002
Sodium (mg/1000 kcal)	331 (308, 356)	328 (304, 353)	357 (332, 384)	368 (341, 397)	0.020
IGFBP-3 (ng/mL) (n = 159)					
Total fat (% kcal)	5186 (4985, 5396)	5055 (4863, 5256)	4926 (4738, 5121)	4880 (4693, 5074)	0.020
Lactose (g/1000 kcal)	4845 (4658, 5039)	4986 (4792, 5187)	5135 (4937, 5340)	5077 (4882, 5279)	0.058
Fiber (g/1000 kcal)	4805 (4621, 4997)	5063 (4868, 5267)	4964 (4776, 5159)	5209 (5010, 5416)	0.013
Calcium (mg/1000 kcal)	4850 (4666, 5042)	4941 (4749, 5140)	5082 (4884, 5287)	5173 (4976, 5377)	0.013
IGF-I:IGFBP-3 molar ratio (n = 159)					
Lactose (g/1000 kcal)	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 (mg/1000 kcal)	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 (g/1000 kcal)	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 All values are geometric means with 95% CIs in parentheses, adjusted for age, time since menarche, and energy intake. IGFBP-1, IGF binding protein 1; IGFBP-3, IGF binding protein 3. Biomarker values are shown for nutrients for which the crude or adjusted association with an IGF-related biomarker in the correlation analysis (see Table 3) was significant at $P < 0.05$.

² For adjusted biomarker trend across quartiles of dietary intake.

TABLE 5

Major factors predicting insulin-like growth factor (IGF)-related biomarkers among adolescent females in the Dietary Intervention Study in Children/Insulin-Related Biomarkers Study at the last visit assessed by multiple linear regression analysis¹

	Final models (<i>n</i> = 159) ²		
	β	SEE	<i>P</i>
IGF-I (ng/mL)			
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)			
Calcium (mg/1000 kcal)	0.07	(0.03)	0.011
IGFBP-1 (ng/mL) ³			
Vegetable protein (g/1000 kcal)	0.49	(0.24)	0.041
BMI-for-age percentile	-0.01	(0.00)	<0.001

¹ Biomarker and nutrient values were log transformed (ln). IGFBP-1, IGF binding protein 1; IGFBP-3, IGF binding protein 3.

² Initial model contained the following: energy (kcal); fat (% kcal); animal and vegetable protein (g/1000 kcal); calcium, zinc, and sodium (mg/1000 kcal); maternal education (high school or less, some college, college or graduate degree); physical activity (hours of moderate and intense activity per week); and treatment group.

³ Data were missing for 7 subjects (*n* = 152).

(29), energy-adjusted milk and dairy intakes were significantly and positively associated with IGF-I and positively, although not significantly, associated with IGFBP-3 concentrations before, but not after, protein intake was controlled for.

Our study differs from the ALSPAC analyses in several ways: our analyses included postmenarcheal adolescent females, whereas the ALSPAC data included prepubertal (age range: 7–8 y) girls and boys (analyses adjusted for sex). However, perhaps a more salient difference is in the diet composition between the 2 cohorts. Although median energy, 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 intakes in DISC adolescent females in our 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 our DISC analyses. These differences in dietary composition likely reflect different sources of nutrients (eg, different sources of protein), which may underlie the differences in the relations between calcium and IGFBP-3 and the IGF-I:IGFBP-3 molar ratios between the 2 cohorts.

In final parsimonious, multivariable linear regression models, which account for all nutrients and potential confounders simultaneously, calcium was a significant dietary predictor of 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-I and IGFBP-3 are consistent with the literature. There were 3 cross-sectional studies (11, 13, 29) and 3 clinical trials (10, 30, 31) that studied the effects of milk or dairy intake on the IGF system in healthy children ranging in age from 2 to 12 y, and all 6 studies reflected higher IGF-I concentrations with even short-term higher milk consumption. Although we did not look at food-level data in the current analyses, milk is a major source of

calcium and protein in the diets of US children. At the time of the DISC, nationally representative estimates indicated that milk contributed 43% of the total calcium intake and 15% of the total protein intake among US females aged 12–18 y [Continuing Survey of Food Intakes by Individuals (CSFII) 1988–1991] (32). Milk is associated with a higher childhood linear growth velocity in developing countries and, to a lesser extent, in well-nourished populations (33). Because IGF-I is a growth regulator, and decreased IGF-I concentrations are associated with deficits in protein intakes, it was hypothesized that IGF-I may mediate the positive association between protein intake and growth (11). Because milk is a significant source of 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 that salty snacks are a primary contributor to sodium intake among adolescents (34). 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 (35). In a nationally representative sample of US children, 53% of 12–19-y-old females never or rarely added table salt to their foods (35); thus, consumption of sodium only 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, the BMI-for-age percentile was a weak but highly significant negative predictor of IGFBP-1 in regression analyses. To our knowledge, the current study is the first to assess the relation between IGFBP-1 and dietary predictors in adolescents. In adults, IGFBP-1 was shown to be positively associated with energy and carbohydrate intake in adult men (36) and plant lignans in postmenopausal women (37, 38) and negatively correlated with regular soda intake in adult men and women (39). 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 the BMI-for-age percentile and IGFBP-1 is consistent with previous studies in adults (40), but, again, we did not identify any studies for comparison in adolescents.

In this study, we make the assumption that 3 nonconsecutive 24-h dietary recalls represent the usual intake of adolescent females. Three nonconsecutive 24-h dietary recalls were shown to be a valid estimator of usual intake for nutrients that do not have a high day-to-day variation (23). Because 2 of our primary nutrients of interest were protein and calcium intake, this is likely to be a valid assumption, particularly in children, given that milk intake is more consistent from day to day (41). Furthermore, results of the Observing Protein and Energy Nutrition (OPEN) Study showed that multiple 24-h recalls provided better estimates of energy and protein intakes than a semiquantitative food-frequency questionnaire (42). An additional limitation of the current study is the cross-sectional design, which prevented any assumption of dietary intake as a causal factor in observed

concentrations of IGF-related biomarkers. Dietary intake was assessed via multiple 24-h 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 wk after the blood draw (14)]; thus, our assumption was that the dietary assessment represented the usual intake of the adolescent females at the time the biomarkers were measured.

A major strength of this study is that, to our knowledge, it is the first step in understanding the associations of dietary intake with IGF-related biomarkers in healthy postpubertal adolescent females. Because the DISC collected detailed information on age at menarche—a factor highly associated with the lifetime peaks and nadir of IGF-related biomarkers (28)—we can be sure that all adolescent females in these analyses were postmenarcheal. Our results are in agreement with the larger body of literature in adults (7) and the smaller body of literature in prepubertal children (10–12, 43) 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 a variation in dietary intakes, even within normal ranges in healthy adolescents, is associated with a variation in concentrations of components of the IGF system. Thus, childhood dietary intake, through mediation of the IGF system, may affect the risk of cancer and other chronic diseases in adulthood, but longitudinal studies are needed to address the multiple complexities inherent in lifetime dietary intake and growth.

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REFERENCES

- LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. *Cancer Lett* 2003;195:127–37.
- Moschos SJ, Mantzoros CS. The role of the IGF system in cancer: from basic to clinical studies and clinical applications. *Oncology* 2002;63:317–32.
- Niu T, Rosen CJ. The insulin-like growth factor-I gene and osteoporosis: a critical appraisal. *Gene* 2005;361:38–56.
- Yakar S, Wu Y, Setser J, Rosen CJ. The role of circulating IGF-I: lessons from human and animal models. *Endocrine* 2002;19:239–48.
- Kaplan RC, Strickler HD, Rohan TE, Muzumdar R, Brown DL. Insulin-like growth factors and coronary heart disease. *Cardiol Rev* 2005;13:35–9.
- Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Hormone & IGF Research* 2003;13:113–70.
- Voskuil DW, Vrieling A, van't Veer LJ, Kampman E, Rookus MA. The insulin-like growth factor system in cancer prevention: potential of dietary intervention strategies. *Cancer Epidemiol Biomarkers* 2005;14:195–203.
- Juul A, Main K, Blum WF, et al. The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. *Clin Endocrinol (Oxf)* 1994;41:85–93.
- Laursen T, Flyvbjerg A, Jørgense JO, Baxter RC, Christiansen JS. Stimulation of the 150-kilodalton insulin-like growth factor-binding protein-3 ternary complex by continuous and pulsatile patterns of growth hormone (GH) administration in GH-deficient patients. *J Clin Endocrinol Metab* 2000;85:4310–4.
- Hoppe C, Mølgaard C, Juul A, et al. High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. *Eur J Clin Nutr* 2004;58:1211–6.
- Hoppe C, Udam TR, Lauritzen L, et al. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. *Am J Clin Nutr* 2004;80:447–52.
- Rogers IS, Gunnell D, Emmett PM, Glynn LR, Dunger DB, Holly JM. Cross-sectional associations of diet and insulin-like growth factor levels in 7- to 8-year-old children. *Cancer Epidemiol Biomarkers Prev* 2005;14:204–12.
- Budek AZ, Hoppe C, Michaelsen KF, Bøugel S, Mølgaard C. Associations of total, dairy, and meat protein with markers for bone turnover in healthy, prepubertal boys. *J Nutr* 2007;137:930–4.
- Obarzanek E, Kimm SY, Barton BA, et al. Long-term safety and efficacy of a cholesterol-lowering diet in children with elevated low-density lipoprotein cholesterol: seven-year results of the Dietary Intervention Study in Children (DISC). *Pediatrics* 2001;107:256–64.
- DISC Collaborative Research Group. Dietary Intervention Study in Children (DISC) with elevated low-density-lipoprotein cholesterol. Design and baseline characteristics. *Ann Epidemiol* 1993;3:393–402.
- The Writing Group for the DISC Collaborative Research Group. Efficacy and safety of lowering dietary intake of fat and cholesterol in children with elevated low-density lipoprotein cholesterol. The Dietary Intervention Study in Children (DISC). *JAMA* 1995;273:1429–35.
- Lipid Research Clinics. Population studies data book, I: the prevalence study. Washington, DC: US Public Health Service, 1980.
- Dorgan JF, Hunsberger SA, McMahon RP, et al. Diet and sex hormones in girls: findings from a randomized controlled clinical trial. *J Natl Cancer Inst* 2003;95:132–41.
- Tanner JM. Growth at adolescence. 2nd ed. Oxford, United Kingdom: Blackwell Scientific Publications Ltd, 1962.
- Horn LV, Stumbo P, Moag-Stahlberg A, et al. The Dietary Intervention Study in Children (DISC): dietary assessment methods for 8- to 10-year-olds. *J Am Diet Assoc* 1993;93:1396–403.
- Holl K, Lundin E, Kaasila M, et al. Effect of long-term storage on hormone measurements in samples from pregnant women: the experience of the Finnish Maternity Cohort. *Acta Oncol* 2008;47:406–12.
- Ito Y, Nakachi K, Imai K, et al. Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor beta, soluble Fas, and superoxide dismutase activity for the JACC study. *J Epidemiol* 2005;15(suppl 1):S67–73.
- Willett W. Nutritional epidemiology. 2nd ed. New York, NY: Oxford University Press, 1998.
- Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;65(4 suppl):1220S–8S; discussion 1229S–31S.
- Juul A, Dalgaard P, Blum W, et al. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. *J Clin Endocrinol Metab* 1995;80:2534–42.
- Wong WW, Copeland KC, Hergenroeder AC, et al. Serum concentrations of insulin, insulin-like growth factor-I and insulin-like growth factor binding proteins are different between white and African American girls. *J Pediatr* 1999;135:296–300.
- Briefel RR, McDowell MA, Alaimo K, et al. Total energy intake of the US population: the Third National Health and Nutrition Examination Survey, 1988–1991. *Am J Clin Nutr* 1995;62:1072S–80S.
- Juul A, Bang P, Hertel NT, et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J Clin Endocrinol Metab* 1994;78:744–52.
- Rogers I, Emmett P, Gunnell D, Dunger D, Holly J. Milk as a food for growth? The insulin-like growth factors link. *Public Health Nutr* 2006;9:359–68.

30. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. *BMJ* 1997;315:1255–60.
31. Rich-Edwards JW, Ganmaa D, Pollak MN, et al. Milk consumption and the prepubertal somatotrophic axis. *Nutr J* 2007;6:28.
32. Subar AF, Krebs-Smith SM, Cook A, Kahle LL. Dietary sources of nutrients among US children, 1989–1991. *Pediatrics* 1998;102:913–23.
33. Hoppe C, Mølgaard C, Michaelsen KF. Cow's milk and linear growth in industrialized and developing countries. *Annu Rev Nutr* 2006;26:131–73.
34. Nielsen SJ, Siega-Riz AM, Popkin BM. Trends in food locations and sources among adolescents and young adults. *Prev Med* 2002;35:107–13.
35. Loria CM, Obarzanek E, Ernst ND. Choose and prepare foods with less salt: dietary advice for all Americans. *J Nutr* 2001;131:536S–51S.
36. Wolk K, Larsson SC, Vessby B, Wolk A, Brismar K. Metabolic, anthropometric, and nutritional factors as predictors of circulating insulin-like growth factor binding protein-1 levels in middle-aged and elderly men. *J Clin Endocrinol Metab* 2004;89:1879–84.
37. Lukanova A, Toniolo P, Akhmedkhanov A, et al. A cross-sectional study of IGF-I determinants in women. *Eur J Cancer Prev* 2001;10:443–52.
38. Vrieling A, Voskuil DW, Bueno de Mesquita HB, et al. Dietary determinants of circulating insulin-like growth factor (IGF)-I and IGF binding proteins 1, -2 and -3 in women in the Netherlands. *Cancer Causes Control* 2004;15:787–96.
39. DeLellis Henderson K, Rinaldi S, Kaaks R, Kolonel L, Henderson B, Le Marchand L. Lifestyle and dietary correlates of plasma insulin-like growth factor binding protein-1 (IGFBP-1), leptin, and C-peptide: the Multiethnic Cohort. *Nutr Cancer* 2007;58:136–45.
40. Sandhu MS, Gibson JM, Heald AH, et al. Association between insulin-like growth factor-I: insulin-like growth factor-binding protein-1 ratio and metabolic and anthropometric factors in men and women. *Cancer Epidemiol Biomarkers Prev* 2004;13:166–70.
41. Field AE, Peterson KE, Gortmaker SL, et al. Reproducibility and validity of a food frequency questionnaire among fourth to seventh-grade inner-city school children: implications of age and day-to-day variation in dietary intake. *Public Health Nutr* 1999;2: 293–300.
42. Subar AF, Kipnis V, Troiano RP, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *Am J Epidemiol* 2003;158:1–13.
43. Budek AZ, Hoppe C, Michaelsen KF, Mølgaard C. High intake of milk, but not meat, decreases bone turnover in prepubertal boys after 7 days. *Eur J Clin Nutr* 2007;61:957–62.