The Journal of Nutrition
Nutritional Epidemiology

Animal Protein Intakes during Early Life and Adolescence Differ in Their Relation to the Growth Hormone-Insulin-Like-Growth-Factor Axis in Young Adulthood[1,2]

Gesa Joslowski,[3]* Thomas Remer,[3] Karen E. Assmann,[3] Danika Krupp,[3] Guo Cheng,[4,5] Sarah P. Garnett,[6,7] Anja Kroke,[8] Stefan A. Wudy,[9] Anke L. B. Günther,[8] and Anette E. Buyken[3]

[3]IEL-Nutritional Epidemiology, University of Bonn, DONALD Study at the Research Institute of Child Nutrition, Dortmund, Germany; [4]West China School of Public Health, and [5]Food Safety Monitoring and Risk Assessment Key Laboratory of Sichuan Province, Sichuan University, Chengdu, P.R. China; [6]Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Sydney, Australia; [7]Children's Hospital at Westmead Clinical School, University of Sydney, Sydney, New South Wales, Australia; [8]Fulda University of Applied Sciences, Department of Nutritional, Food, and Consumer Sciences, Fulda, Germany; and [9]Laboratory of Translational Hormone Analytics in Pediatric Endocrinology Center of Child and Adolescent Medicine, Justus-Liebig-University, Giessen, Germany

Abstract

Recent studies provide evidence that insulin-like-growth-factor I (IGF-I) and its binding proteins (IGFBP) IGFBP-2 and IGFBP-3 are related to the risk of several common cancers. It remains to be clarified whether their concentrations can be programmed by protein intake from different sources during growth. This study addressed the hypothesis that animal protein intakes during infancy, mid-childhood, and adolescence differ in their relevance for the growth-hormone (GH)-IGF-I axis in young adulthood. Data from the Dortmund Nutritional and Anthropometric Longitudinally Designed Study participants with at least 2 plausible 3-d weighed dietary records during adolescence (age: girls, 9–14 y; boys, 10–15 y; n = 213), around the adiposity rebound (age 4–6 y; n = 179) or early life (age 0.5–2 y; n = 130), and one blood sample in young adulthood were included in the study. Mean serum concentrations of IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 were compared between tertiles of habitual animal protein intake using multivariable regression analysis. Habitually higher animal protein intakes in females during puberty were related to higher IGF-I (P-trend = 0.005) and IGFBP-3 (P-trend = 0.01) and lower IGFBP-2 (P-trend = 0.04), but not to IGFBP-1 in young adulthood. In turn, IGF-I concentrations in young adulthood were inversely related to animal protein intakes in early life among males only (P-trend = 0.03), but not to animal protein intake around adiposity rebound (P-trend > 0.5). Our data suggest that, among females, a habitually higher animal protein intake during puberty may precipitate an upregulation of the GH-IGF-I axis, which is still discernible in young adulthood. By contrast, among males, higher animal protein intakes in early life may exert a long-term programming of the GH-IGF-I axis. J. Nutr. 143: 1147–1154, 2013.

Introduction

The growth hormone (GH)[10]-insulin-like-growth-factor I (IGF-I) axis plays a central role in cell proliferation and apoptosis (1) and has been related to different cancers (2,3). The bioavailability of IGF-I is determined by its binding proteins (IGFBP), with IGFBP-1 and IGFBP-3 limiting its acute and longer term bioavailability. Furthermore, IGFBP-3 and IGFBP-2 concentrations are inversely associated with cellular proliferation and apoptosis independently of IGF-I (4,5), whereas lower IGFBP-2 concentrations are also considered to reflect a lower long-term insulin sensitivity (4).

Several recent cross-sectional studies in adults suggest that high protein intakes, particularly animal protein, are related to higher IGF-I (6–8) and IGFBP-3 concentrations (7) and that the source of animal protein is an important determinant of IGF-I levels, with most studies pointing to dairy products or milk (6,7,9) and others to meat (8,10).

The IGF system has a central role in the regulation of fetal and childhood growth and metabolism (11). Hence, it is plausible to assume that protein intake in childhood in particular may be related to the GH-IGF-I axis. Indeed, a number of intervention studies in children showed a relation between higher

[1] Supported by the Ministry of Science and Research of North Rhine Westphalia, Germany (the DONALD Study), and this analysis was funded by the Wereld Kanker Onderzoek Fonds (grant no.2010/248).
[2] Author disclosures: G. Joslowski, T. Remer, K. E. Assmann, D. Krupp, G. Cheng, S. P. Garnett, A. Kroke, S. A. Wudy, A. L. B. Günther, and A. E. Buyken, no conflicts of interest.
[10] Abbreviations used: DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; FFMI, fat-free mass index; GH, growth hormone; IGFBP, insulin-like-growth-factor binding protein; IGF-I, insulin-like-growth-factor I.
* To whom correspondence should be addressed. E-mail: joslowski@fke-do.de.

Manuscript received February 14, 2013. Initial review completed March 9, 2013. Revision accepted April 16, 2013.
First published online May 22, 2013; doi:10.3945/jn.113.175877.

milk intake and higher IGF-I (12–14) and IGFBP-3 (13) concentrations. However, not all studies confirmed the association with IGF-I concentrations (14,15). Only 2 prospective studies have addressed the long-term relevance of (animal) protein intake during growth and/or its sources in relation to the GH-IGF-I axis. Ben-Shlomo et al. (16) and Martin et al. (17) found an inverse association between milk intakes in early childhood and IGF-I concentrations in young adulthood (16) and older age (17). The authors proposed that this inverse relation reflects an early programming of the GH-IGF-I axis in response to higher (animal) protein intakes in early life. Hence, early pituitary resetting in response to higher ambient IGF-I concentrations may occur, which would ultimately result in an inverse association between animal protein in early life and IGF-I concentrations in young adulthood. However, prospective evidence covering different, potentially critical, developmental periods is lacking to unravel whether such an inverse association between animal protein intake and the GH-IGF-I axis is confined to early life.

The main hypothesis of this study is that consumption of animal protein and its components (i.e., meat and dairy protein) during puberty, a period when the GH-IGF-I axis undergoes major changes, is of prospective relevance for IGF-I and its binding proteins in young adulthood. Because a reversal in the long-term relation between animal protein and the GH-IGF-I axis has been proposed, an additional aim was to examine whether animal protein intakes in early life or around the adiposity rebound are inversely related to IGF-I concentrations in young adulthood.

Methods

Study population. The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study is an ongoing, open cohort study conducted at the Research Institute of Child Nutrition in Dortmund, Germany. Details on this study were previously described (18). In brief, since recruitment began in 1985, detailed data on diet, growth, development, and metabolism from infancy to adulthood have been collected from >1300 healthy children. Every year, a mean of 40 infants are newly recruited and first examined at the age of 3 mo. Each child returns for 3 more visits in the first year, 2 in the second, and then once annually until adulthood. Since 2005, participants ≥18 y of age are invited for subsequent examinations with fasting blood withdrawal. The study was approved by the Ethics Committee of the University of Bonn and all examinations are performed with parental and adult participants' consent.

Because of the open cohort design, many participants have not yet reached young adulthood. Among those who did, age varied from 18 to 36 y. For 308 participants who were term (36–43 wk gestation) singletons with a birth weight ≥2500 g, one measurement of IGF-I and IGFBP-3 was available. Of these, 222 participants had provided at least 2 plausible 3-d weighed dietary records during adolescence (age: girls, 9–14 y; boys, 10–15 y), describing habitual dietary intake during puberty. Participants who had consistently underreported energy intake during puberty (i.e., all food records were implausible or they had provided more implausible than plausible food records) were excluded from the analysis (n = 18). A 3-d weighed dietary record was considered plausible when the total recorded energy intake was adequate in relation to the estimated BMR using modified age-dependent cutoffs from Goldberg et al. (19). For boys and girls aged 14 y and older, a ratio between reported energy intake and basal metabolic rate <1.07 and 0.97, respectively, was considered implausible. For boys and girls younger than 14 y, the cutoffs were 1.04 and 1.01, respectively (20). Furthermore, participants had to have anthropometric data available at the beginning of puberty and young adulthood and information on relevant covariates such as early life and socioeconomic factors. This resulted in final samples of 213 (55.4% females). Overall analyses are based on 1131 records (i.e., 2–6 records/participant; mean = 5).

Among participants with a blood sample available in young adulthood, 130 and 179 had provided a minimum of 2 plausible, 3-d weighed dietary records during early life (age 0.5–2 y) and around adiposity rebound (age 4–6 y), respectively. IGFBP-1 and IGFBP-2 measurements were missing for a few individuals, resulting in slightly lower sample sizes for these outcomes (see tables).

Clinical measurements and calculations. Venous blood samples were drawn after an overnight fast. Blood samples were frozen at −80°C and then shipped to the Laboratory for Translational Hormone Analytics in Pediatric Endocrinology at the University of Giessen. Serum samples were analyzed for IGF-I and IGFBP-3 using an RIA according to Blum et al. (21) and for IGFBP-2 and IGFBP-1 with an ELISA (Mediagnost, Germany, lot 061010 and lot 050910), respectively.

Anthropometric measurements and calculations. Participants are measured at each visit according to standard procedures with the participants dressed in underwear only and barefoot. From the age of 2 y onward, standing height is measured to the nearest 0.1 cm using a digital stadiometer (Harpenden). Body weight is measured to the nearest 100 g using an electronic scale (Seca 753E; Seca Weighing and Measuring Systems). Skinfold thicknesses are measured from the age of 6 mo onward at 4 different sites (supra-iliacal, subscapular, biceps, and triceps) on the right side of the body to the nearest 0.1 mm using a Holtain caliper (Holtain). The 3 trained nurses who perform the measurements undergo an annual quality control conducted in 6–8 healthy young adult volunteers.

Sex- and age-independent SD scores were calculated for BMI (kg/m^2) at baseline using the German reference curves for BMI (22). Percentage body fat was derived using equations of Slaughter et al. (23) for pubescent children, which includes triceps and subscapular skinfolds. From this, fat mass index and fat-free mass index (FFMI) were calculated as weight × percentage body fat/height^2 and [weight − weight × percentage body fat]/height^2, respectively.

Nutritional assessment. Food consumption in the DONALD Study is assessed annually using 3-d weighed dietary records. All foods and beverages as well as leftovers consumed are weighed and recorded to the nearest 1 g for 3 d using electronic food scales (initially Soehnle Digita 8000, Leifheit; now WEDO digi 2000, Werner Dorsch). For this analysis, dietary variables were calculated as individual means of the 3-d weighed dietary records using LEBTAB (18), the in-house database that is continuously updated to include all recorded food items. LEBTAB is based on the German standard food tables (24) and data obtained from commercial food products (25). With regard to breastfeeding, test weighing is performed (i.e., weighing the infant before and after each meal) to the nearest 10 g with the use of an infant-weighing scale (Soehnle multina 8300) (25). In this analysis, 5% was added to the test weighing results to account for insensible water losses (26).

To examine food groups providing animal protein in more detail, all recorded foods were assigned to the respective food groups, i.e., meat products, dairy products, and miscellaneous. Animal protein did not include protein from human milk.

Statistical analysis. All statistical analyses were carried out using SAS procedures (version 9.1.3, SAS Institute). $P < 0.05$ was considered significant.

Baseline characteristics are presented in sex-specific and energy-adjusted tertiles of dietary animal protein intake (T1–T3). Tests for differences were performed across the tertiles of dietary animal protein intake using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables, chi-square test for categorical variables, and Fisher's exact test for categorical variables if 50% of cells had expected counts less than frequencies <5.

Multiple linear regression analysis was used to analyze the potential relation of dietary animal intake during puberty to concentrations of IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 in young adulthood. Because the outcome variables were not normally distributed, IGF-I and IGFBP-2 were transformed prior to analysis using the square root, and IGFBP-1 and

TABLE 1 Demographic, anthropometric, birth, and socioeconomic characteristics by energy-adjusted tertiles of animal protein during puberty (n = 213) (DONALD Study, Germany)[1]

	Males			Females		
	T1	T2	T3	T1	T2	T3
Animal protein intake, *g/d*	38.4 (30.7, 46.4)	42.9 (36.9, 49.4)	49.8 (46.9, 57.6)*	26.2 (21.8, 31.7)	32.8 (30.9, 36.2)	41.6 (37.8, 48.7)*
All, *n*	31	32	32	39	40	39
Age, *y*	10.00 (9.97, 10.18)	10.02 (9.98, 10.09)	9.99 (9.97, 10.05)	8.99 (8.97, 9.02)	9.03 (8.98, 9.07)	8.99 (8.98, 9.07)
BMI-SDS	−0.27 ± 0.93	0.13 ± 0.83	0.34 ± 0.59*	−0.27 ± 0.95	−0.16 ± 0.88	0.28 ± 0.97*
BMI, *kg/m²*	15.7 (15.3, 18.8)	17.7 (16.1, 18.8)	18.0 (16.8, 18.7)	15.7 (15.0, 17.2)	15.9 (15.0, 17.4)	17.6 (15.4, 19.0)*
FMI,[2] *kg/m²*	2.0 (1.6, 3.7)	3.0 (2.0, 4.4)	2.7 (2.3, 3.2)	2.6 (2.2, 3.3)	2.7 (2.2, 3.6)	3.7 (2.3, 4.6)
FFMI,[3] *kg/m²*	13.8 (13.3, 14.6)	14.3 (13.3, 15.2)	15.2 (14.1, 15.8)*	13.2 (12.6, 13.9)	13.3 (12.6, 14.0)	13.7 (13.0, 14.7)*
Birth weight, *g*	3558 ± 403	3487 ± 461	3549 ± 470	3490 ± 489	3395 ± 393	3381 ± 425
Birth length, *cm*	53 (52, 54)	52 (50, 53)	52 (50, 54)	52 (50, 53)	51 (50, 53)	51 (49, 52)
Pregnancy duration, *wk*	40 (39, 41)	40 (39, 40)	40 (39, 40)	40 (40, 41)	40 (40, 41)	40 (39, 40)
Birth weight and length appropriate for gestational age,[4] *n (%)*	25 (80.6)	23 (71.9)	27 (84.4)	29 (74.4)	35 (87.5)	29 (74.4)
Breast feeding >2 wk,[5] *n (%)*	23 (74.2)	20 (62.5)	22 (68.8)	29 (74.4)	29 (72.5)	26 (66.7)
Maternal overweight,[6] *n (%)*	7 (22.6)	8 (25.0)	14 (43.8)	10 (25.6)	11 (27.5)	17 (43.6)
Maternal education,[7] *n (%)*	16 (51.6)	9 (28.1)	21 (65.6)*	21 (53.8)	22 (55.0)	12 (30.8)
Maternal employment,[8] *n (%)*	15 (48.4)	14 (43.8)	19 (59.4)	18 (46.2)	24 (60.0)	17 (43.6)
Paternal overweight,[6] *n (%)*	12 (52.2)	12 (48.0)	20 (74.1)	16 (50.0)	20 (55.6)	14 (45.2)
Paternal education,[7] *n (%)*	16 (55.2)	15 (46.9)	17 (53.1)	22 (59.5)	22 (57.9)	14 (36.8)
Paternal employment,[8] *n (%)*	28 (96.6)	30 (93.8)	29 (90.6)	35 (94.6)	38 (100)	37 (97.4)
Smokers in the household, *n (%)*	6 (19.4)	17 (53.1)	10 (31.3)*	14 (35.9)	17 (42.5)	9 (23.1)

[1] Values are means ± SDs or median (25th and 75th percentiles). Differences between the tertiles were tested using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for not normally distributed continuous variables, chi-square test for categorical variables, and Fisher's exact test for categorical variables if 50% of cells had expected counts less than frequencies <5. *$P < 0.05$ for differences between tertiles. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; FMI, fat mass index; FFMI, fat-free mass index; SDS, SD score; T, tertile.

[2] FMI calculated as weight × percentage of body fat/height².

[3] FFMI calculated as [weight − weight × percentage of body fat)]/height².

[4] That is, birth weight and length were between the 10th and 90th percentiles of the German sex-specific birth weight-for-gestational-age curves (28).

[5] Breast feeding categories: ≤2 wk or >2 wk of full breastfeeding.

[6] Maternal and paternal BMI ≥25 kg/m²; paternal BMI: n = 39 missing.

[7] School education for at least 12 y; paternal education: n = 7 missing.

[8] Maternal and paternal employment (yes/no); paternal employment: n = 7 missing.

IGFBP-3 were log-transformed to obtain normal distribution. Back-transformed data are presented for ease of interpretation. All dietary variables were energy adjusted using the residual method (27). To account for age-dependent changes in intake, all variables were standardized by age group and sex (mean = 0, SD = 1).

For this analysis, the following covariates were considered as potentially confounding factors: birth weight and length appropriate for gestational [yes/no, defined as birth weight and birth length between the 10th and 90th percentiles of the German sex-specific birth weight and height-for-gestational age curves (28)], full breastfeeding for >2 wk (yes/no), maternal or paternal overweight status (BMI ≥25 kg/m², yes /no), high maternal or paternal educational status (≥12 y of schooling, yes /no), maternal or paternal occupation (whether parents were employed, yes/no), smokers in the household (yes/no), and FFMI at the beginning of puberty. The basic model considers age in young adulthood only (model A). In the next step, each potential confounder was initially considered separately, yet only covariates that substantially affected the associations between animal protein intakes and parameters of the GH-IGF-axis (by ~10% or more) were included in model B. To explicitly assess the effect of substituting animal for plant protein, we ran an additional model that included total energy and all energy-bearing macronutrients except plant protein (i.e., total fat, total carbohydrate, animal protein in percent energy). The coefficient (β) obtained for animal protein then reflects the effect of substituting animal for plant protein, because total energy, fat, and carbohydrates are held constant (27). Similar models were run to address substitution of animal protein for carbohydrates or fats.

All analyses were stratified by sex based on the following considerations: *1*) the association between animal protein intake during puberty and our main outcome, i.e., IGF-I concentrations, differed between males and females (P-interaction < 0.1); 2) stratified analyses revealed that the relevance of the investigated exposure-outcome relations consistently differed between genders; and *3*) both growth and IGF-I concentrations are known to differ between genders (29,30), supporting the biological plausibility of stratification. A similar approach was used to analyze the potential relation of dietary animal protein intake in early life and around adiposity rebound to IGF-I concentrations in young adulthood. All models conform to the assumptions of linear regression models (linearity, normality and homoscedasticity of residuals, absence of multicollinearity).

Results

The characteristics of participants in this study at the beginning of puberty are presented in tertiles of dietary animal protein intake during puberty (**Table 1**). Females and males in the highest tertile of dietary animal protein intake were more likely to have had a higher BMI-SD score and a higher FFMI at the beginning of puberty. Males in the middle tertile of dietary animal protein intake were least likely to have a mother with a high educational level and most likely to live in a household with smokers (Table 1).

By definition, higher animal protein intakes were related to higher total protein, meat, and dairy intakes, but not to plant protein. Higher animal protein intakes in both males and females were also related to lower carbohydrate intakes, but not to fiber intakes. Of note, higher intakes of animal protein were associated with higher intakes of total fat and MUFAs in females only (**Table 2**).

TABLE 2 Nutritional data during puberty by energy-adjusted tertiles of animal protein intake during puberty (n = 213) (DONALD Study, Germany)[1]

	Males			Females		
	T1	T2	T3	T1	T2	T3
Animal protein intake, *g/d*	38.4 (30.7, 46.4)	42.9 (36.9, 49.4)	49.8 (46.9, 57.6)*	26.2 (21.8, 31.7)	32.8 (30.9, 36.2)	41.6 (37.8, 48.7)*
All, *n*	31	32	32	39	40	39
Total energy, *MJ/d*	9.1 (8.1, 10.5)	8.62 (7.3, 9.6)	8.8 (8.2, 9.7)	7.3 (6.4, 8.1)	7.1 (6.3, 7.6)	7.2 (6.5, 8.1)
Fat, *%en*	35.3 ± 4.3	35.9 ± 3.5	35.8 ± 3.5	34.9 ± 3.9	35.7 ± 3.5	37.5 ± 4.3*
SFA, *%en*	15.5 ± 2.6	16.0 ± 1.9	15.5 ± 1.5	15.7 ± 2.2	15.9 ± 1.9	16.4 ± 2.6
PUFA, *%en*	5.3 ± 1.3	5.1 ± 0.9	5.2 ± 1.2	5.2 ± 1.1	5.2 ± 0.9	5.6 ± 1.1
MUFA, *%en*	10.9 ± 1.3	11.2 ± 1.4	11.5 ± 1.4	10.6 ± 1.6	11.1 ± 1.3	11.8 ± 1.6*
Protein, *%en*	11.8 ± 1.1	13.2 ± 0.8	14.5 ± 0.9*	11.2 ± 0.9	12.8 ± 0.8	14.5 ± 1.2*
Animal protein, *%en*	7.0 ± 0.9	8.3 ± 0.4	9.8 ± 0.9*	6.2 ± 0.9	7.9 ± 0.4	9.8 ± 1.0*
Meat protein, *%en*	2.7 (2.0, 3.2)	3.1 (2.6, 3.9)	3.5 (2.7, 5.4)*	1.8 (1.2, 2.3)	3.2 (2.2, 4.1)	3.9 (2.7, 4.6)*
Dairy protein, *%en*	3.5 ± 1.2	4.4 ± 0.7	4.6 ± 1.3*	3.5 ± 0.9	3.7 ± 1.1	4.6 ± 1.4*
Plant protein, *%en*	4.9 ± 0.8	4.8 ± 0.8	4.6 ± 0.7	5.0 ± 0.7	4.9 ± 0.7	4.7 ± 0.7
Consumers of alcohol, *n (%)*	5 (16.1)	2 (6.3)	2 (6.3)	2 (5.1)	1 (2.5)	3 (7.7)
Carbohydrate, *%en*	52.9 ± 4.3	50.9 ± 3.6	49.7 ± 3.5*	53.9 ± 4.1	51.5 ± 3.3	48.0 ± 4.4*
Added sugar, *%en*	16.8 ± 5.6	14.8 ± 4.6	13.9 ± 3.9	16.4 ± 4.9	15.1 ± 4.2	12.6 ± 4.6*
Fiber, *g/d*	21.3 (17.2, 26.9)	20.1 (17.6, 22.5)	20.6 (16.3, 24.4)	17.8 (16.2, 21.2)	16.8 (15.4, 19.9)	18.4 (16.0, 19.8)

[1] Values are means ± SDs or median (25th and 75th percentiles). Differences between the tertiles were tested using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for not normally distributed continuous variables, and Fisher's exact test for categorical variables if 50% of cells had expected counts less than frequencies under 5. *$P < 0.05$ for differences between tertiles. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; T, tertile; %en, percent energy.

Puberty. Among females, a habitually lower animal protein intake during puberty was associated with lower concentrations of IGF-I and IGFBP-3, but not with IGFBP-1 (**Table 3**, model A). Similar results were seen after additional adjustment for socioeconomic factors and FFMI at the beginning of puberty (Table 3, model B). Conversely, a higher animal protein intake was related to lower levels of IGFBP-2 (model B). Animal protein was not related to IGF-I, IGFBP-3, IGFBP-1, or IGFBP-2 in males.

Substitution models revealed similar associations for a substitution of animal protein intake for total fat ($\beta_{\text{animal protein}} = 0.5137$; P-trend = 0.01) or total carbohydrate intake ($\beta_{\text{animal protein}} = 0.4812$; P-trend = 0.03) with respect to its association with IGF-I concentrations. Substitution of animal protein intake for plant protein intake was related to slightly lower, albeit nonsignificant increases in IGF-I concentrations ($\beta_{\text{animal protein}} = 0.4131$; P-trend = 0.4).

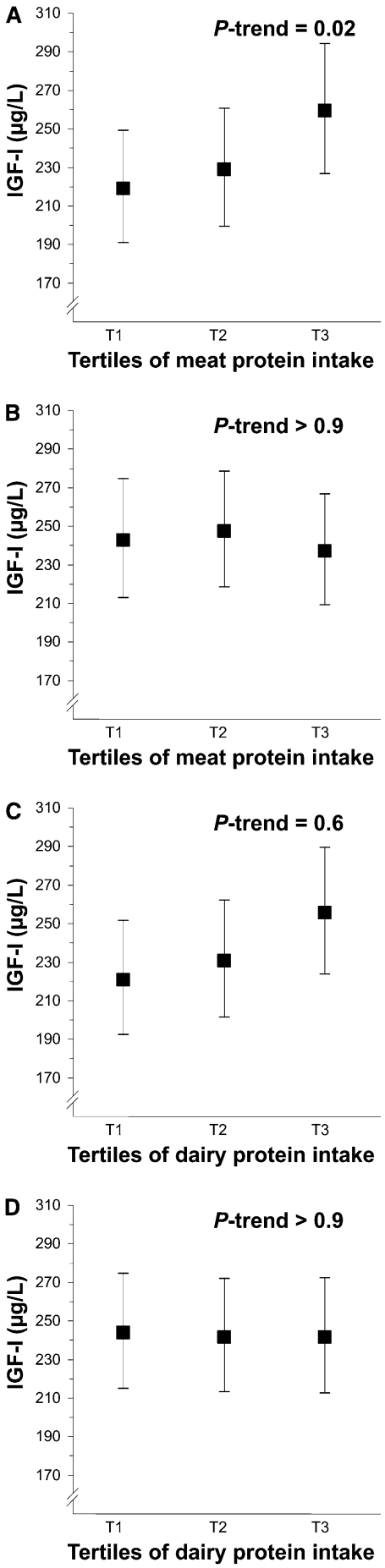
Analysis of animal protein sources revealed that intake of dietary meat protein only was significantly associated with adult IGF-I concentrations (**Fig. 1***A*). No associations were found in males for any protein source. Plant protein was not related to the IGF-I axis among females or males (data not shown).

Additional consideration of early-life characteristics (e.g., birth weight, full breastfeeding) or other nutritional variables (e.g., monounsaturated fat or plant protein intake) did not affect the results (data not shown).

TABLE 3 Relation of dietary animal protein intake during puberty to serum IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 concentrations in young adulthood (DONALD Study, Germany)[1]

	Males				Females			
	T1	T2	T3	P-trend	T1	T2	T3	P-trend
Animal protein intake,[2] *g/d*	38.4 (30.7, 46.4)	42.9 (36.9, 49.4)	49.8 (46.9, 57.6)	<0.0001	26.2 (21.8, 31.7)	32.8 (30.9, 36.2)	41.6 (37.8, 48.7)	<0.0001
IGF-I, $g \cdot L^{-1}$								
Model A	237 (210, 267)	266 (237, 297)	218 (192, 245)	0.7	213 (185, 244)	268 (236, 302)	241 (211, 273)	0.01
Model B	242 (212, 273)	261 (232, 293)	222 (194, 253)	0.8	204 (177, 232)	261 (231, 292)	241 (209, 275)	0.005
IGFBP-1, $\mu g \cdot L^{-1}$								
Model A	6.5 (4.4, 9.6)	4.1 (2.8, 6.0)	6.6 (4.5, 9.7)	0.7	12.6 (9.4, 17.0)	7.8 (5.9, 10.5)	9.92 (7.40, 13.30)	0.7
Model B	5.4 (3.6, 8.1)	4.4 (3.0, 6.4)	5.6 (3.7, 8.3)	0.6	12.3 (9.1, 16.7)	7.8 (5.8, 10.4)	10.1 (7.31, 13.95)	0.9
IGFBP-2, $\mu g \cdot L^{-1}$								
Model A	193 (158, 232)	185 (151, 222)	219 (182, 260)	0.9	155 (130, 184)	136 (112, 162)	123 (100, 149)	0.09
Model B	183 (145, 225)	175 (139, 214)	224 (183, 268)	0.8	155 (128, 183)	130 (106, 157)	113 (90, 138)	0.04
IGFBP-3, $mg \cdot L^{-1}$								
Model A	3.3 (3.0, 3.6)	3.2 (2.9, 3.5)	3.5 (3.1, 3.8)	0.4	3.5 (3.3, 3.8)	3.6 (3.3, 3.8)	3.7 (3.4, 4.0)	0.02
Model B	3.3 (2.9, 3.6)	3.2 (2.9, 3.6)	3.3 (3.0, 3.7)	>0.9	3.5 (3.3 3.8)	3.5 (3.3, 3.8)	3.7 (3.4, 4.0)	0.01

[1] Values are means and 95% CIs unless otherwise indicated; n = 213. For IGF-I and IGFBP-3: 118 females/95 males; for IGFBP-1 and IGFBP-2: 109 females/92 males. Model A: adjusted for age in adulthood. Model B for IGF-I, IGFBP-1, and IGFBP-3: model A + socioeconomic factors (maternal education, smokers in the household) and FFMI at the beginning of puberty. Model B for IGFBP-2: model A + socioeconomic factors (maternal education, maternal overweight) and FFMI at the beginning of puberty. P-trend refers to the P value obtained in linear regression models with dietary animal protein as continuous variable. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; FFMI, fat-free mass index; IGF-I, insulin-like-growth-factor I; IGFBP, insulin-like-growth-factor binding protein; T, tertile.
[2] Values are medians (25th and 75th percentiles).



Early life (age 0.5–2 y). Among females, animal protein was not related to IGF-I (**Fig. 2***A*). Among males, a habitually higher animal protein intake in early life was associated with lower concentrations of IGF-I in young adulthood, after controlling for early life and socioeconomic factors (Fig. 2*B*). Plant protein intake in early life was not associated with IGF-I levels in young adulthood, neither among males nor among females (data not shown). Again, additional consideration of other nutritional variables (e.g., total energy intake) did not affect the results (data not shown).

Adiposity rebound (age 4–6 y). No associations were seen between animal protein intake around adiposity rebound and IGF-I in young adulthood in either females or males (Fig. 2*C*,*D*). Similarly, there was no association with plant protein (data not shown).

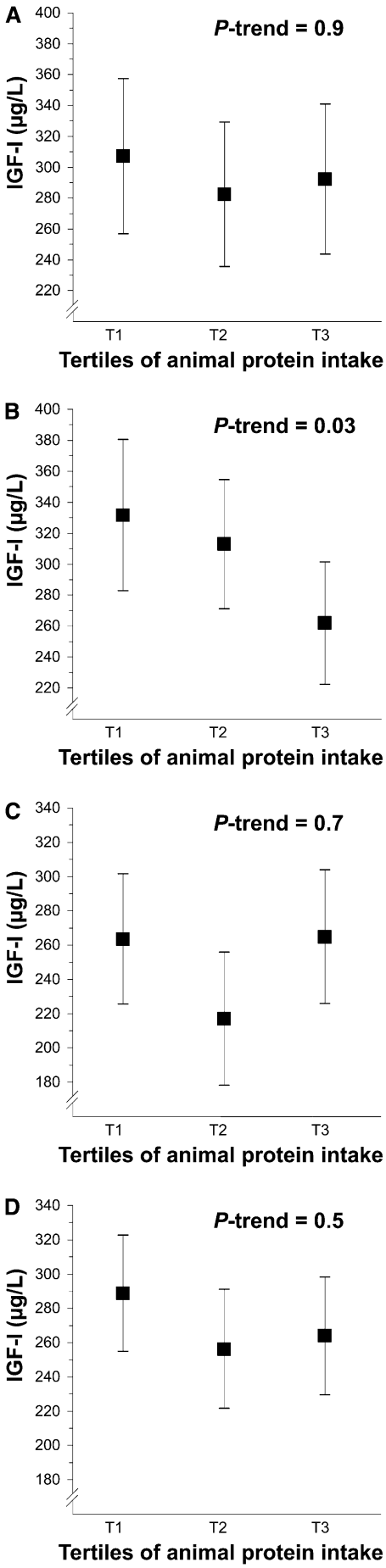
Discussion

This study provides epidemiological evidence for a prospective association between a habitually higher animal protein intake during puberty and higher concentrations of IGF-I and IGFBP-3 as well as lower IGFBP-2 concentrations in females only, suggesting an upregulation of the GH-dependent components of the GH-IGF-I axis in young adulthood. By contrast, higher animal protein intakes in early life may yield a long-term downregulation of the GH-IGF-I axis in males.

Potential mechanisms by which a higher animal protein intake may contribute to higher IGF-I levels may relate to specific amino acids, e.g., arginine (31) or combinations of amino acids (lysine and arginine) (32), which increase GH concentrations and lead to an increase in hepatic IGF-I production. Dairy and meat both contain glutamine, lysine, and arginine, but their concentrations are ~10 times higher in meat than in milk. Alternatively, it has been proposed that components in milk itself rather than animal protein as such stimulates IGF-I secretion (33). Most intervention studies in infants (34), children (12–15), and also adults (35,36) suggest that milk and dairy products are important upregulators of IGF-I concentrations. However, some (8,10) but not all (6,7,9) cross-sectional studies in adults report associations of a higher consumption of red meat with higher IGF-I concentrations. Our study supports a relevance of animal protein in general rather than dairy protein intake per se. In addition, among females, the difference between the intake levels in the lowest and highest tertiles was higher for meat protein (12.4 g) than for dairy protein (9.6 g), which may partly explain why we found an association with meat but not dairy protein. Finally, our substitution analyses revealed broadly similar effect sizes when simulating substitutions of animal protein for carbohydrate, fat, or plant protein.

We furthermore observed lower IGFBP-2 and higher IGFBP-3, but not higher IGFBP-1 concentrations, in young adulthood among females who had consumed more animal protein during

FIGURE 1 Relations of dietary meat (*A*,*B*) and dairy protein (*C*,*D*) intake during puberty to IGF-I in young adulthood among 118 females (*A*,*C*) and 95 males (*B*,*D*) in the DONALD Study, Germany. Data are means (95% CI) adjusted for age in adulthood, socioeconomic factors (maternal education, smokers in the household), and FFMI at the beginning of puberty. *P*-trend refers to the *P* value obtained in linear regression models with dietary meat or dairy protein as the continuous variable. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; FFMI, fat-free mass index; IGF-I, insulin-like-growth-factor I; T, tertile.



puberty. In contrast to the most abundant IGFBP-3, IGFBP-1 concentrations are responsive to acute dietary stimuli (37). The fact that the association with pubertal animal protein intake was confined to adult IGFBP-3 concentrations hence supports the underlying hypothesis of long-term nutritional influences on the adult GH-IGF-axis. Lower IGFBP-2 concentrations in turn are considered to also reflect a lower insulin sensitivity (4). In line with this, we also observed a tendency toward higher HOMA levels among females with a higher animal protein intake (*P*-trend = 0.1; data not shown). In addition, prospective cohort studies in adults suggest that a higher consumption of total protein as well as animal protein or red and processed meat is related to an increased risk of type 2 diabetes (38,39). One explanation for a corresponding increase in insulin resistance could be an amino acid-induced upregulation of the serine kinase 6–1 pathway, which has been shown to result in lower insulin sensitivity (40). A lower insulin sensitivity may in turn decrease IGFBP-2 concentrations over the long-term (41) and explain our findings of lower IGFBP-2 concentrations associated with higher animal protein intake in puberty.

Our results indicate a relation between pubertal animal protein intake and GH-IGF-I axis among females only. Most studies in adults or children were conducted in one gender only (12,14,15) and others did not report gender differences (14,33). During puberty, boys have higher testosterone levels than girls, which have been found to increase IGF-I concentrations among healthy men (42). Thus, higher testosterone levels in boys may have overridden a potential effect of animal protein intake on IGF-I. Furthermore, girls have a higher degree of physiological insulin resistance during puberty (43), which may make them more vulnerable than boys to dietary effects on the GH-IGF-I axis. Finally, the smaller variation in animal protein intake levels could partly explain the lack of discernible associations among males.

Our results further indicate that there may be a reversal in the association between animal protein intake and adult IGF-I levels between early life and adolescence. Although no relation was observed with intakes in the period around the adiposity rebound, dietary animal protein intake in early life was inversely associated with IGF-I concentrations in young adulthood among males. This finding is in accordance with the long-term follow-up of a milk intervention (16) and a prospective cohort study (17). In our purely observational study, the association was confined to males. The absence of an association among females may be partly attributable to the small overall sample with consumption data in early life. It has been proposed that higher animal protein intakes in early life may cause an acute increase in hepatic IGF-I production, which then negatively feedbacks to the pituitary GH output, possibly leading to a long-term pro-

FIGURE 2 Relation of dietary animal protein intake in early life (*A*,*B*) (n = 68 females, 62 males) and around adiposity rebound (*C*,*D*) (n = 94 females, 85 males) to IGF-I in young adulthood among females (*A*,*C*) and males (*B*,*D*) in the DONALD Study, Germany. Data are means (95% CI); model in early life: adjusted for early life (breast feeding) and socioeconomic factors (maternal education and smokers in the household); adiposity rebound: adjusted for age in adulthood, early life (birth weight and length appropriate for gestational age), and socioeconomic factors (maternal education and smoking in the household). Additional consideration of body composition in early life or at adiposity rebound yielded similar results. *P*-trend refers to the *P* value obtained in linear regression models with dietary animal protein as continuous variable. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; IGF-I, insulin-like-growth-factor I; T, tertile.

gramming of the pituitary with lower IGF-I concentrations in adulthood (17).

It remains to be determined whether our data may reflect adaptive responses of the GH-IGF-I axis to intakes of animal protein and/or whether these associations indicate higher or lower risks of future disease. Higher intakes of animal protein during puberty appear to upregulate the entire GH-IGF-I axis, because we observed higher concentrations of both IGF-I and IGFBP-3, but not of the IGF-I:IGFBP-3 ratio (data not shown). In terms of disease risk, a higher concentration of IGF-I has been linked to an increased risk of breast cancer (2), whereas a direct association between IGFBP-3 and breast cancer is currently questioned (3). On the other hand, higher IGF-I concentrations are prospectively related to lower risks of cardiovascular disease (44), osteoporosis (45), and impaired glucose tolerance (46), further complicating a public health appraisal of our results.

A clear strength of our study is its prospective nature, carefully collected repeated dietary data, and the availability of data on several possible confounders. By contrast, the analysis is based on a single measurement of the GH-IGF-I axis in young adulthood to represent long-term circulating levels. However, IGF-I values were reported to have a low intra-individual variation (47). The study sample is relatively small and the DONALD population is characterized by a relatively high socioeconomic status (18). Therefore, extremes of diet or behavior might not be represented in this healthy sample, which is, however, likely to result in an underestimation of the true associations. In addition, the homogeneity of our sample might have reduced our vulnerability to residual confounding.

In conclusion, our data suggest that among females, a habitually higher animal protein intake during puberty may precipitate an upregulation of the GH-IGF-I axis that is discernible in the long-term in young adulthood. By contrast, inverse associations between higher animal protein intakes in early life and IGF-I concentrations among adult males support the idea that habitually higher animal protein intakes in this period may trigger an early programming of the GH-IGF-I axis.

Acknowledgments

A.E.B. and A.L.B.G. conceived the research project; all variables of the GH-IGF-I axis and plasma insulin concentrations were measured in the laboratory of S.A.W.; G.J. conducted the statistical analysis; G.J. and A.E.B. wrote the manuscript; and A.E.B. supervised the study and had primary responsibility for final content. All authors made substantial contributions the interpretation of the results and read and approved the final manuscript.

Literature Cited

1. Pollak M. Insulin-like growth factor physiology and cancer risk. Eur J Cancer. 2000;36:1224–8.
2. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet. 2004;363:1346–53.
3. Endogenous Hormones and Breast Cancer Collaborative Group, Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol. 2010;11:530–42.
4. Wheatcroft SB, Kearney MT. IGF-dependent and IGF-independent actions of IGF-binding protein-1 and -2: implications for metabolic homeostasis. Trends Endocrinol Metab. 2009;20:153–62.
5. Jogie-Brahim S, Feldman D, Oh Y. Unraveling insulin-like growth factor binding protein-3 actions in human disease. Endocr Rev. 2009;30:417–37.
6. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. Cancer Epidemiol Biomarkers Prev. 2002;11:852–61.
7. Giovannucci E, Pollak M, Liu Y, Platz EA, Majeed N, Rimm EB, Willett WC. Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. Cancer Epidemiol Biomarkers Prev. 2003;12:84–9.
8. Larsson SC, Wolk K, Brismar K, Wolk A. Association of diet with serum insulin-like growth factor I in middle-aged and elderly men. Am J Clin Nutr. 2005;81:1163–7.
9. Norat T, Dossus L, Rinaldi S, Overvad K, Gronbaek H, Tjonneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Boeing H, et al. Diet, serum insulin-like growth factor-I and IGF-binding protein-3 in European women. Eur J Clin Nutr. 2007;61:91–8.
10. Kaklamani VG, Linos A, Kaklamani E, Markaki I, Koumantaki Y, Mantzoros CS. Dietary fat and carbohydrates are independently associated with circulating insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 concentrations in healthy adults. J Clin Oncol. 1999;17:3291–8.
11. Cohen P. Overview of the IGF-I system. Horm Res. 2006;65:3–8.
12. 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.
13. Hoppe C, Molgaard C, Juul A, Michaelsen KF. 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.
14. Rich-Edwards JW, Ganmaa D, Pollak MN, Nakamoto EK, Kleinman K, Tserendolgor U, Willett WC, Frazier AL. Milk consumption and the prepubertal somatotropic axis. Nutr J. 2007;6:28.
15. Zhu K, Du X, Cowell CT, Greenfield H, Blades B, Dobbins TA, Zhang Q, Fraser DR. Effects of school milk intervention on cortical bone accretion and indicators relevant to bone metabolism in Chinese girls aged 10–12 y in Beijing. Am J Clin Nutr. 2005;81:1168–75.
16. Ben-Shlomo Y, Holly J, McCarthy A, Savage P, Davies D, Davey Smith G. Prenatal and postnatal milk supplementation and adult insulin-like growth factor I: long-term follow-up of a randomized controlled trial. Cancer Epidemiol Biomarkers Prev. 2005;14:1336–9.
17. Martin RM, Holly JMP, Middleton N, Davey Smith G, Gunnell D. Childhood diet and insulin-like growth factors in adulthood: 65-year follow-up of the Boyd Orr Cohort. Eur J Clin Nutr. 2007;61:1281–92.
18. Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U, Lentze MJ. The DONALD Study. History, current status and future perspectives. Eur J Nutr. 2004;43:45–54.
19. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, Prentice AM. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr. 1991;45:569–81.
20. Sichert-Hellert W, Kersting M, Schoch G. Underreporting of energy intake in 1 to 18 year old German children and adolescents. Z Ernahrungswiss. 1998;37:242–51.
21. Blum WF, Ranke MB, Bierich JR. Isolation and partial characterization of six somatomedin-like peptides from human plasma Cohn fraction IV. Acta Endocrinol (Copenh). 1986;111:271–84.
22. Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß H, Hesse V, von Hippel A, Jaeger U, Johnsen D, Korte W, et al. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. Monatsschr Kinderheilkd. 2001;149:807–18.
23. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, Bemben DA. Skinfold equations for estimation of body fatness in children and youth. Hum Biol. 1988;60:709–23.
24. Souci S, Fachmann W, Kraut H. Food composition and nutrition tables. Stuttgart: Medpharm Scientific Publishers; 2008.
25. Sichert-Hellert W, Kersting M, Chahda C, Schäfer R, Kroke A. German food composition database for dietary evaluations in children and adolescents. J Food Compost Anal. 2007;20:63–70.
26. Reilly JJ, Wells JC. Duration of exclusive breast-feeding: introduction of complementary feeding may be necessary before 6 months of age. Br J Nutr. 2005;94:869–72.

27. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol. 1999;149:531–40.
28. Voigt M, Friese K, Schneider KTM, Jorch G, Hesse V. Kurzmitteilung zu den Perzentilwerten für die Körpermaße Neugeborener. [A short communication on the reference values for body weight and height in newborns] Geburtshilfe Frauenheilkd. 2001;61:700–6.
29. Clark PA, Rogol AD. Growth hormones and sex steroid interactions at puberty. Endocrinol Metab Clin North Am. 1996;25:665–81.
30. Barrett-Connor E, Goodman-Gruen D. Gender differences in insulin-like growth factor and bone mineral density association in old age: the Rancho Bernardo Study. J Bone Miner Res. 1998;13:1343–9.
31. Collier SR, Casey DP, Kanaley JA. Growth hormone responses to varying doses of oral arginine. Growth Horm IGF Res. 2005;15:136–9.
32. Isidori A, Lo Monaco A, Cappa M. A study of growth hormone release in man after oral administration of amino acids. Curr Med Res Opin. 1981;7:475–81.
33. Hoppe C, Udam TR, Lauritzen L, Molgaard C, Juul A, Michaelsen KF. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. Am J Clin Nutr. 2004;80:447–52.
34. Larnkjaer A, Hoppe C, Molgaard C, Michaelsen KF. The effects of whole milk and infant formula on growth and IGF-I in late infancy. Eur J Clin Nutr. 2009;63:956–63.
35. Hoppe C, Kristensen M, Boiesen M, Kudsk J, Fleischer Michaelsen K, Molgaard C. Short-term effects of replacing milk with cola beverages on insulin-like growth factor-I and insulin-glucose metabolism: a 10 d interventional study in young men. Br J Nutr. 2009;102:1047–51.
36. Manios Y, Moschonis G, Trovas G, Lyritis GP. Changes in biochemical indexes of bone metabolism and bone mineral density after a 12-mo dietary intervention program: the Postmenopausal Health Study. Am J Clin Nutr. 2007;86:781–9.
37. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res. 2003;13:113–70.
38. Sluijs I, Beulens JW, van der AD, Spijkerman AM, Grobbee DE, van der Schouw YT. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. Diabetes Care. 2010;33:43–8.
39. Song Y, Manson JE, Buring JE, Liu S. A prospective study of red meat consumption and type 2 diabetes in middle-aged and elderly women: The Women's Health Study. Diabetes Care. 2004;27:2108–15.
40. Weickert MO, Roden M, Isken F, Hoffmann D, Nowotny P, Osterhoff M, Blaut M, Alpert C, Gogebakan O, Bumke-Vogt C, et al. Effects of supplemented isoenergetic diets differing in cereal fiber and protein content on insulin sensitivity in overweight humans. Am J Clin Nutr. 2011;94:459–71.
41. Arafat AM, Weickert MO, Frystyk J, Spranger J, Schofl C, Mohlig M, Pfeiffer AF. The role of insulin-like growth factor (IGF) binding protein-2 in the insulin-mediated decrease in IGF-I bioactivity. J Clin Endocrinol Metab. 2009;94:5093–101.
42. Hobbs CJ, Plymate SR, Rosen CJ, Adler RA. Testosterone administration increases insulin-like growth factor-I levels in normal men. J Clin Endocrinol Metab. 1993;77:776–9.
43. Moran A, Jacobs DR Jr, Steinberger J, Cohen P, Hong CP, Prineas R, Sinaiko AR. Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. J Clin Endocrinol Metab. 2002;87:4817–20.
44. Conti E, Carrozza C, Capoluongo E, Volpe M, Crea F, Zuppi C, Andreotti F. Insulin-like growth factor-1 as a vascular protective factor. Circulation. 2004;110:2260–5.
45. Yamaguchi T, Kanatani M, Yamauchi M, Kaji H, Sugishita T, Baylink DJ, Mohan S, Chihara K, Sugimoto T. Serum levels of insulin-like growth factor (IGF); IGF-binding proteins-3, -4, and -5; and their relationships to bone mineral density and the risk of vertebral fractures in postmenopausal women. Calcif Tissue Int. 2006;78:18–24.
46. Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. Lancet. 2002;359:1740–5.
47. Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am J Epidemiol. 1997;145:970–6.