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Diet, serum insulin-like growth factor-I and IGF-binding protein-3 in European women

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Objective: The aim of this study was to examine the relationship of diet with serum insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in women.
Design: Cross-sectional study.
Setting and subjects: The population are 2109 women who were control subjects in a case–control study of breast cancer nested in the European Prospective Investigation into Cancer and Nutrition. Control subjects were randomly chosen among risk sets consisting of female cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. Matching criteria were age at enrolment, follow-up time, time of the day of blood collection and study centre. Diet was measured through validated questionnaires. Serum hormone concentrations were measured by enzyme-linked immunosorbent assays. The relationship between serum IGF-I, IGFBP-3, and intake of nutrients and foods was explored by linear regression in models adjusted for energy intake, age, body mass index, smoking, physical activity, centre and laboratory batch.
Results: Serum IGF-I levels were positively related to protein intake ($P_{trend}<0.001$), but not related to energy, fat or carbohydrate intake. Positive relationships were observed with the intake of milk ($P_{trend}=0.007$), calcium ($P_{trend}<0.001$), magnesium ($P_{trend}=0.003$), phosphorus ($P_{trend}<0.001$), potassium ($P_{trend}=0.002$), vitamin B6 ($P_{trend}=0.03$), vitamin B2 ($P_{trend}=0.001$) and inverse relationships with vegetables ($P_{trend}=0.02$) and beta-carotene ($P_{trend}=0.02$). IGFBP-3 was not related with most of the nutrients and foods in this study.

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Contributors: RK is the principal investigator of the study. TN and LD carried out data analysis. SR conducted the laboratory analyses. The writing team was integrated by TN, LD, SR and RK. All authors contributed to the collection of data, interpretation of results and to the writing of the paper.

Conclusions: In this population, circulating IGF-I is modestly related with the intake of protein and minerals, and with milk and cheese, while IGFBP-3 does not appear to be related with diet.

Introduction

Insulin-like growth factor-I (IGF-I), together with insulin, is central to the regulation of anabolic (growth) processes as a function of available energy and essential nutrients (e.g. amino acids) from body reserves and diet (Thissen *et al.*, 1994; Estivariz and Ziegler, 1997; Yu and Berkel, 1999; Kaaks and Lukanova, 2001). More than 90% of IGFs in the circulation are bound to the IGF-binding proteins (IGFBPs) (binding proteins), mainly IGFBP-3 (Yu and Rohan, 2000). Circulating levels of IGF-I and IGFBP-3 vary considerably between normal individuals, but are relatively constant in each individual. Circulating IGF-I levels are largely determined by genetic factors and age (Harrela *et al.*, 1996; Hong *et al.*, 1996), but are also affected by sex, anthropometric indices, physical activity, exogenous sex hormones, smoking, alcohol consumption (Yu and Rohan, 2000) and nutritional status (Thissen *et al.*, 1994). Energy and/or protein deprivation markedly lower IGF-I concentrations (Clemmons *et al.*, 1981; Douyon and Schteingart, 2002). Excess energy intake may increase IGF-I but this does not appear to affect IGF-I levels as strongly as nutritional restriction (Thissen *et al.*, 1994).

Epidemiologic studies on the relationship of circulating IGF-I with diet have provided inconsistent results. The most consistent are the positive associations observed with the intake of protein (Devine *et al.*, 1998; Allen *et al.*, 2002; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Gunnell *et al.*, 2003; Heald *et al.*, 2003; Larsson *et al.*, 2005), minerals (Devine *et al.*, 1998; Ma *et al.*, 2001; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Gunnell *et al.*, 2003; DeLellis *et al.*, 2004; Larsson *et al.*, 2005; Maskarinec *et al.*, 2005) and milk (Ma *et al.*, 2001; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Gunnell *et al.*, 2003). Less consistently, positive relationships has been observed with the intake of energy (Holmes *et al.*, 2002; Heald *et al.*, 2003), carbohydrate (Gunnell *et al.*, 2003), fat (Kaklamani *et al.*, 1999; Heald *et al.*, 2003; DeLellis *et al.*, 2004), saturated fat (Heald *et al.*, 2003), polyunsaturated fat (Baibas *et al.*, 2003; Gunnell *et al.*, 2003), dietary fibre (Maskarinec *et al.*, 2005), some vitamins (Holmes *et al.*, 2002; Maskarinec *et al.*, 2005), red meat (Kaklamani *et al.*, 1999; Mucci *et al.*, 2001; Larsson *et al.*, 2005), fish (Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Larsson *et al.*, 2005) and poultry (Giovannucci *et al.*, 2003) . In contrast with the previous results, circulating IGF-I has been found inversely related with intake of carbohydrates (Heald *et al.*, 2003), fat from meat (DeLellis *et al.*, 2004) and fish (Maskarinec *et al.*, 2005), not related with diet (Vrieling *et al.*, 2004) and inversely related with the intake of vegetables (Gunnell *et al.*, 2003) and tomatoes (Mucci *et al.*, 2001; Gunnell *et al.*, 2003). IGFBP-3 has been found inversely related with fat intake (Kaklamani *et al.*, 1999; Holmes *et al.*, 2002), energy intake (Kaklamani *et al.*, 1999) and positively related with dietary zinc (Maskarinec *et al.*, 2005). The ratio IGF-I to IGFBP-3 has been found inversely related with fish intake and positively with dietary iron (Maskarinec *et al.*, 2005).

Recent evidence suggest that the risk of some common cancers that could be associated in a variable manner with diet, such as colorectal, prostate and pre-menopausal breast cancer, might be associated with higher circulating levels of IGF-I (Pollak *et al.*, 2004; Renehan *et al.*, 2004). The mechanisms by which IGF-I could play a role in cancer risk have not been fully elucidated. IGF-I could favour tumour growth by enhancing cell proliferation and inhibiting apoptosis in normal and neoplastic cells of various tissue origins (Khandwala *et al.*, 2000; Werner and Le Roith, 2000). Other mechanisms that could favour tumour growth are the induction of vascular epithelial growth factor (Fukuda *et al.*, 2002) and the increase of cell migration (Playford *et al.*, 2000) by IGF-I. It is not clear if the IGF–cancer associations seen for a range of different cancer sites are likely to be explained in terms of common dietary influences on the growth factor axis.

In this study, we examine the cross-sectional relationship of diet with serum concentrations of IGF-I and its main binding protein IGFBP-3 in a large study in 2109 women participating in the European Prospective Investigation into Cancer and Nutrition (EPIC). To our knowledge, this is the biggest cross-sectional study on diet and blood IGF-I levels conducted in women. Our main objective is to determine the relationship of serum IGF-I levels with the intake of protein, and between food groups, with the intake of dairy products, for which previous associations has been observed. Secondarily, we will present results of the relationship of serum IGF-I and IGFBP-3 with the other nutrients and main food groups available in the EPIC study.

Materials and methods

The population for this cross-sectional study are 2109 women from eight European countries who were control subjects in a case–control study of breast cancer risk and endogenous hormones nested in the EPIC.

EPIC is a large prospective study, designed to investigate the relationships among diet, lifestyle, genetic and environmental factors, and the incidence of different forms of cancer (Riboli *et al.*, 2002; Bingham and Riboli, 2004) in almost half a million men and women from 10 European countries. The participants in EPIC, mostly aged 35–70 years at enrolment, were recruited from the general population and resided in defined areas in each country, with some exceptions (women who were members of a health insurance scheme for state school employees in France; women attending breast cancer screening in Utrecht, the Netherlands; components of the Italian and Spanish cohorts included members of local blood donors associations). A large number of subjects who did not eat meat were enrolled in the Oxford 'Health conscious' cohort. Eligible participants completed questionnaires on their diet, lifestyle and medical history. Blood samples were collected according to a standardized protocol. All participants had given their written consent for future analysis of their blood samples.

A detailed description of the nested case–control study on breast cancer and endogenous hormones has been published elsewhere (Kaaks *et al.*, 2005). Case and control subjects were selected among women who did not use any hormone replacement therapy at the time of blood donation, and who had no previous diagnosis of cancer (except non-melanoma skin cancer). Case subjects were women who developed breast cancer after their recruitment into the EPIC study and blood donation, and before the end of the study period, for each study centre. For each case subject, up to two control subjects were chosen at random among appropriate risk sets consisting of all female cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. Matching criteria were the study centre where the subjects were enrolled in the cohort, age at enrolment (± 6 months), follow-up time since blood donation (± 3 months) and time of the day of blood collection (± 1 h). In this cross-sectional analysis, we excluded women with insulin treatment for diabetes and women in the lowest and highest 1% of the cohort distribution of the ratio of reported total energy intake:energy requirement (Ferrari *et al.*, 2002) and we included only women who had both IGF-I and IGFBP-3 measurements. Approval for this study was obtained from the ethical review boards of the International Agency for Research on Cancer and all local institutions where subjects had been recruited for the EPIC study.

Diet over the 12 months before enrolment was measured between 1992 and 1998 by country-specific validated questionnaires. The validity of methods used was established in prior studies using 24-h urine and blood samples as source of biomarkers (Slimani *et al.*, 2002). Most centres adopted a self-administered dietary questionnaire of 88–266 food items. In Greece, all centres in Spain, and Ragusa, Italy, the questionnaire was administered at personal interview. In Malmö, Sweden, a questionnaire method combined with a food record was used. Data on height and weight, alcohol use, smoking status, occupational and non-occupational physical activity, menstrual and reproductive history, current and past use of oral contraceptives and previous illnesses were also collected. Descriptions of the questionnaires used can be found on web sites of the participating cohorts (Bingham and Riboli, 2004).

In all countries contributing to the present study, 30 ml or larger blood samples were drawn, kept at 5–10°C and protected from light, and transferred to a local laboratory for further processing and aliquoting, except in Oxford, UK, where samples were sent to a central laboratory by post at ambient temperature. Blood fractions (serum, plasma, red cells, buffy coat) were aliquoted in straws of 0.5 ml each, except in Denmark, where samples were aliquoted in 1 ml tubes, and frozen within 2 h at −20°C. At the end of the day, all samples were stored under liquid nitrogen (−196°C). Half of the aliquots were stored locally and the other half centrally at the International Agency for Research on Cancer (IARC), Lyon, France.

All hormone assays were performed at the laboratory of Hormones and Cancer, at IARC. IGF-I and IGFBP-3 were measured by enzyme-linked immunosorbent assays (ELISA) from Diagnostic System Laboratories (DSL, Webster, TX, USA). IGF-I assays included an acid–ethanol precipitation step to eliminate IGF-I-binding proteins, to avoid their interference with the IGF-I measurement. Measurements were performed on never-thawed serum sample aliquots. The mean intra-batch and inter-batch coefficients of variation were 6.2 and 16.2%, respectively, for IGF-I, and 7.2 and 9.7%, respectively, for IGFBP-3.

We estimated least-square means of serum IGF-I and IGFBP-3 across quintiles of dietary variables using linear regression. We tested the heterogeneity between group means using the *F*-test statistics from the ANOVA. A test for linear trend was performed to assess statistical significance across dietary categories by scoring the categories according to their median values, and entering the variable as a continuous term in the ANOVA. *P*s are reported for the linear trend across categories. The hormone measurements were transformed via the natural log to best approximate normal distribution. The mean values are presented back-transformed in the tables. Total energy intake was included in all models to partially control for error in estimated diet intake. We included age (<40, 40–44, 45–49,50–54,55–59,60–64, >65 years), body mass index (BMI) (<22; 22–23; 24–25; 26–27; 28–29, $\geqslant 30\,kg/m^2$), current alcohol intake (g/day), smoking status (never smoker, current smoker, former smoker) and physical activity (low, moderately inactive, moderately active, active) as covariates, based on previous reports on potential lifestyle determinants of IGF-I. Multivariate models were adjusted for centre to control for differences in dietary questionnaire and other centre effects. Indicator terms for laboratory batch were included in all the models. A *P*-value of less than 0.05 was considered statistically significant. All the analyses were conducted using SAS Statistical Software, version 8 (SAS Institute, Cary, NC, USA).

Results

Selected characteristics of the participants in this cross-sectional study by country are presented in Table 1. Overall, mean age was 54.5 years (standard deviation, 8.7), and mean BMI 26.1 kg/m^2 (standard deviation, 4.6). The percentage of post-menopausal women was 55.9%.

Mean serum IGF-I concentrations increased across increasing levels of energy intake, but the association was not statistically significant ($P_{trend} = 0.07$) (Table 2). Serum IGF-I was significantly positively related with intake of protein ($P_{trend} < 0.001$). The differences in mean IGF-I concentrations when comparing women in the fifth quintile of protein intake (more than 98.6 g/day) with women in the first quintile (less than 61.5 g/day) were 12%.

No significant relationships were observed with intake of carbohydrates and fats. No relationship was observed with the intake of saturated, polyunsaturated or monounsaturated fats, or with the ratios of saturated, monounsaturated and polyunsaturated fats with respect to total fat intake (results not shown). Adjustment of mean serum IGF-I for IGFBP-3 did not modify the observed relationships, with the exception of the inverse association with polyunsaturated fats and with the ratio of polyunsaturated:saturated fats that became statistically significant ($P_{trend} = 0.02$ and 0.04, respectively). Serum IGFBP-3 was not related with intake of calories, protein, carbohydrate or fats in this study.

Serum IGF-I was positively related with the dietary intake of beta-carotene ($P_{trend} = 0.02$), vitamin B2 (riboflavin) ($P_{trend} = 0.001$), vitamin B6 ($P_{trend} = 0.03$), calcium ($P_{trend} < 0.001$), magnesium ($P_{trend} = 0.003$), phosporus ($P_{trend} < 0.001$) and potassium ($P_{trend} = 0.002$), and not related with vitamin B1 (thiamine), vitamin B12, vitamin C, vitamin D, vitamin E, retinol and iron. The difference of the adjusted mean IGF-I levels of women in the fifth quintile of dietary intake compared with the mean of women in the first quintile of intake were 3.8% for beta-carotene, 7.3% for calcium, 5.8% for magnesium, 9.5% for phosphorus, 8.4% for potassium, 6.3% for vitamin B6 and 7.9% for riboflavin. Serum IGFBP-3 was positively related with dietary intake of calcium ($P_{trend} = 0.01$) and phosphorus ($P_{trend} = 0.03$), but not with any other of the micronutrients analysed in this study.

The results of the analyses of the association of serum IGF-I and IGFBP-3 with foods are shown in Table 3. Serum IGF-I was positively related with the intake of milk ($P_{trend} = 0.007$), cheese ($P_{trend} = 0.03$), but not significantly with yogurt ($P_{trend} = 0.07$). The difference in mean IGF-I concentrations when comparing women in the fifth quintile of milk intake (more than 400 g/day) with women in the first quintile of intake (less than 25.1 g/day) was 8%, while the difference was 3% for the same comparison for cheese intake.

In order to examine if the association with milk intake could be explained by its content in protein and calcium, we included these variables in the regression models. The relationship of milk intake with serum IGF-I was attenuated after adjustment for protein intake ($P_{trend} = 0.04$; correlation

Table 1 Main characteristics of 2109 women from the European prospective investigation into cancer and nutrition included in the cross-sectional study

Centre	*No*	*Age (years)*	*BMI (kg/m^2)*	*Alcohol intake (g/day)*	*Smoking status (%)*			*Physical activity (%)*			*IGF-I (ng/ml)*	*IGFBP-3 (ng/ml)*
		Mean (s.d.)			*Never*	*Smoker*	*Former*	*Low*	*Moderate*	*Active*	*(least-square means; 95% CI)*	
Denmark	59	52.3 (1.4)	25.1 (3.7)	12.5 (11.7)	59.3	16.9	23.7	39.0	49.1	11.9	316 (291–344)	4987 (4537–5481)
France	130	56.1 (8.0)	24.1 (3.6)	11.7 (14.2)	60.8	4.6	25.4	17.7	80.7	1.5	249 (236–263)	4940 (4642–5257)
Heidelberg, Germany	51	56.2 (8.3)	26.2 (3.9)	12.8 (14.1)	58.8	15.7	25.5	7.8	76.4	15.7	232 (214–253)	2648 (2407–2914)
Potsdam, Germany	61	57.0 (8.9)	27.2 (5.3)	6.8 (9.0)	67.2	13.1	19.7	16.4	82	1.6	229 (212–247)	3125 (2863–3411)
Greece	66	54.9 (10.3)	30.3 (5.5)	2.1 (3.5)	80.3	9.1	6.1	3.0	87.9	9.1	210 (195–227)	3335 (3065–3629)
Italy	543	52.3 (7.7)	25.5 (4.4)	8.5 (12.1)	58.0	21.2	20.8	9.0	79.8	11.2	227 (221–234)	3337 (3237–3441)
Bilthoven, The Netherlands	55	49.1 (8.3)	25.7 (4.9)	6.5 (10)	29.1	30.9	38.2	5.5	50.9	9.1	246 (227–267)	4216 (3845–4624)
Utrecht, The Netherlands	412	59.1 (6.0)	26.1 (4.2)	8.3 (11.0)	41.7	21.4	36.9	3.9	73.8	22.3	241 (233–249)	3166 (3052–3285)
Spain	332	49.8 (7.7)	28.1 (4.5)	5.6 (10.5)	74.4	15.4	9.9	3.6	40.3	56.0	221 (214–229)	3156 (3036–3281)
Cambridge, UK	215	61.5 (8.7)	26.3 (4.4)	5.6 (9.7)	62.3	5.1	31.6	6.5	81.4	11.2	240 (230–251)	3198 (3044–3360)
Oxford Health Conscious, UK	100	51.0 (10.4)	23.6 (3.5)	7.1 (8.7)	58.0	5.0	37.0	19.0	73	7.0	215 (202–228)	3425 (3199–3668)
Oxford General Population, UK	85	51.2 (7.2)	25.4 (4.3)	6.9 (8.3)	62.4	8.2	29.4	14.1	72.9	12.9	228 (213–243)	3320 (3081–3577)

Percentages do not add 100% owing to missing values.
Abbreviations: BMI, body mass index; CI, confidence interval; IGF-I, insulin-like growth factor-I; IGFBP, insulin-like growth factor-I-binding protein.

Table 2 Geometric mean of serum concentrations of IGF-I and IGFBP-3 in relation to quintiles of increasing dietary intake of nutrients

Nutrients	*IGF-I (ng/ml)*			*IGFBP-3 (ng/ml)*		
	First quintile	*Fifth quintile*	P-*trend*	*First quintile*	*Fifth quintile*	P-*trend*
Total energy intake	231	236	0.07	3490	3487	0.94
Protein	225	252	<0.001	3511	3555	0.07
Carbohydrate	239	231	0.69	3493	3483	0.53
Total fat	244	226	0.19	3577	3473	0.81
Saturated fat	241	234	0.80	3484	3557	0.76
Polyunsaturated fat	234	232	0.05	3557	3461	0.74
Monounsaturated fat	240	228	0.41	3501	3480	0.93
Retinol	228	231	0.46	3530	3550	0.47
Beta-carotene	238	229	0.02	3542	3467	0.55
Vitamin B1 (thiamine)	223	238	0.09	3540	3498	0.38
Vitamin B2 (riboflavin)	220	237	0.001	3462	3557	0.17
Vitamin B6	224	238	0.03	3540	3523	0.82
Vitamin B12	225	289	0.25	3530	3595	0.16
Vitamin C	233	234	0.84	3521	3448	0.37
Vitamin D	228	225	0.42	3538	3452	0.59
Vitamin E	234	235	0.82	3501	3496	0.69
Calcium	229	246	<0.001	3477	3514	0.01
Iron	232	241	0.29	3545	3508	0.82
Magnesium	226	239	0.003	3510	3534	0.49
Phosphorus	227	248	<0.001	3488	3556	0.03
Potassium	230	249	0.002	3535	3527	0.83

Nutrients adjusted for total energy intake (except energy). All results are adjusted for age (<40, 40–44, 45–49, 50–54, 55–59, 60–64, >65 years), BMI (<22; 22–23; 24–25; 25–26; 26–27; 28–29; ⩾30 kg/m^2), physical activity (low, moderately inactive, moderately active, active), smoking (never smoker, current smoker, ex-smoker), centre and laboratory batch. Data on calcium, iron, potassium and phosphorus were missing in 66 participants. Data on magnesium, retinol, riboflavin, thiamine, vitamin B6, Vitamin B12 and Vitamin D were missing in 125 participants.
Abbreviations: BMI, body mass index; IGF-I, insulin-like growth factor-I; IGFBP, insulin-like growth factor-I-binding protein.

Table 3 Geometric mean of serum concentrations of IGF-I and IGFBP-3 in relation to quintiles of increasing intake of foods

	IGF-I (ng/ml)			*IGFBP-3 (ng/ml)*		
	1st quintile	*5th quintile*	P-*trend*	*1st quintile*	*5th quintile*	P-*trend*
Red meat	223	230	0.27	3528	3448	0.44
Processed meat	225	252	0.51	3537	3442	0.01
Poultry	225	231	0.18	3476	3533	0.14
Fish and shellfish	219	225	0.84	3416	3515	0.45
Eggs and egg products	224	233	0.56	3517	3455	0.22
Milk and milk beverages	216	233	0.007	3480	3537	0.06
Cheese and fromage blanc	224	230	0.03	3430	3579	0.05
Yogurt	220	228	0.07	3444	3488	0.78
Vegetables	232	222	0.02	3517	3519	0.74
Fruits	220	231	0.06	3500	3455	0.24
Legumes	226	229	0.48	3439	3526	0.53
Potatoes and other tubers	288	228	0.15	3504	3485	0.84
Cereal and cereal products	230	226	0.34	3460	3509	0.96
Sugar and confectionery	227	230	0.92	3549	3432	0.10
Cakes	229	227	0.50	3459	3472	0.57
Non-alcoholic beverages	230	229	0.68	3632	3505	0.42
Added vegetable oil	227	220	0.28	3450	3568	0.43
Butter	227	220	0.27	3551	3457	0.73
Margarines	222	224	0.99	3524	3486	0.95

All results are adjusted for total energy intake (continuous), age (<40, 40–44, 45–49, 50–54, 55–59, 60–64, >65 years), BMI (<22; 22–23; 24–25; 25–26; 26–27; 28–29; ⩾30 kg/m^2), physical activity (low, moderately inactive, moderately active, active), smoking (never smoker, current smoker, ex-smoker), centre and laboratory batch.
Abbreviations: BMI, body mass index; IGF-I, insulin-like growth factor-I; IGFBP, insulin-like growth factor-I-binding protein.

coefficient of milk and protein = 0.26) and disappeared after adjustment for dietary calcium ($P_{trend} = 0.10$; correlation coefficient of milk and dietary calcium = 0.61).

Although mean serum IGF-I values tended to increase across quintiles of red and processed meat, poultry, fish and shellfish, and eggs, all protein rich foods, none of the

relationships attained statistically significance. Serum IGF-I was inversely related to vegetable intake ($P_{trend} = 0.02$). The differences of adjusted mean IGF-I concentration in women in the fifth quintile (more than 295.9 g/day) compared with women in the first quintile (less than 105.7 g/day) was only 4%. No association was observed with the intake of fruits, legumes, potatoes and other tubers. Further adjustment for IGFBP-3 did not substantially modify the results.

Serum IGFBP-3 was inversely related with intake of processed meat ($P_{trend} = 0.01$), but not related with the intake any of the other foods investigated.

Discussion

In this cross-sectional study in women, the main dietary correlates of serum IGF-I are the intake of protein and minerals, specifically, calcium, magnesium, potassium, phosphorus and riboflavin. Dairy products (milk, cheese, but not yogurt) were positively related to circulating IGF-I. Positive associations were observed with the intake of riboflavin and vitamin B6, and inverse associations were observed with the intake of vegetables and beta-carotene. No significant relationship was observed with the intake of energy, carbohydrates and fats or with other nutrients and foods. Serum IGFBP-3 levels were not related with most of the dietary items investigated in this study, with the exception of calcium, phosphorus and processed meat.

Strengths of the present study are its large size, which reduce the possibility that bias influenced our results, the use of detailed and validated dietary questionnaires and the inclusion of women from eight European countries Dietary questionnaires were validated and standardized in the core data set of the EPIC study. All the analyses were adjusted for centre to control for differences in dietary questionnaires and other centre effects. Another strength of this study is the exclusion of women who were using exogenous hormones at the time of blood donation – a factor that can substantially influence IGF-I levels (Weissberger *et al.*, 1991).

Our study has some limitations. We could not examine the temporality of the association between diet and serum IGF-I and IGFBP-3 because of the cross-sectional nature of this study. Our results are based on a single measurement of IGF-I and IGFBP-3, but the intra-individual variation in IGF-I and IGFBP-3 appears to be small and a single measurement might be adequate to represent long-term circulating levels (Goodman-Gruen and Barrett-Connor 1997). Measurement error in the laboratory assays and in dietary measurements could have biased the results. However, we adjusted our analyses for energy intake, which is thought to partially correct for measurement error of diet (Willett, 2001). We did sensitivity analyses excluding laboratory batch and centre, as variable for adjustment and the results remained essentially the same.

In this analysis, we conducted multiple comparisons, and some significant associations could have emerged only by chance. The association we observed with protein intake is supported by previous studies (Clemmons *et al.*, 1981; Isley *et al.*, 1984; Devine *et al.*, 1998; Allen *et al.*, 2002; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Gunnell *et al.*, 2003; Heald *et al.*, 2003; Larsson *et al.*, 2005). The increase in mean-adjusted IGF-I levels across increasing quintiles of protein intake in our study was 12%, similar to the 13% increase observed in 1037 women in the Nurses Health Study (Holmes *et al.*, 2002) and slightly lower than the 17% observed in 226 free-living healthy middle-aged and elderly men in Sweden (Larsson *et al.*, 2005). In the Health Professionals Follow-Up Study, men with relatively high intakes of total protein and minerals had a 25% higher mean plasma level of IGF-I compared with those in the low quintiles simultaneously (Giovannucci *et al.*, 2003). No association with protein intake was found in a large multi-ethnic American population (DeLellis *et al.*, 2004) and in studies with smaller number of participants (Kaklamani *et al.*, 1999; Mucci *et al.*, 2001; Baibas *et al.*, 2003; Gunnell *et al.*, 2003; Vrieling *et al.*, 2004).

The association of IGF-I with protein intake has been observed mainly with animal but not with vegetable protein (Allen *et al.*, 2002; Holmes *et al.*, 2002). We could not estimate the intake of protein by source, because this information has not yet been standardized across centres in EPIC. In our study, from the food groups rich in animal protein, dairy products were significantly associated with serum IGF-I. Mean values of IGF-I increased across increasing levels of red meat, poultry, fish and eggs, all sources of animal protein, but none of the relationships were statistically significant. Legumes and cereals, both sources of vegetable proteins, were not associated with serum IGF-I, whereas the association with vegetable intake was inverse.

The relationship of mineral intake with circulating IGF-I levels has been observed in several studies (Devine *et al.*, 1998; Ma *et al.*, 2001; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Gunnell *et al.*, 2003). An independent effect of zinc has been previously suggested (Devine *et al.*, 1998), but we could not include zinc in this study because standardized estimates across countries participating in EPIC are not yet available. It was not possible to assess the independent association of each mineral and vitamin, because these nutrients are often present in the same food and tend to be highly correlated (the correlation coefficients between calcium, magnesium, riboflavin, potassium, and phosphorus were all above 0.51).

The positive association of circulating levels of IGF-I with dietary calcium and milk intake in our study is also in agreement with the results of two trials (Heaney *et al.*, 1999; Hoppe *et al.*, 2004) and several cross-sectional studies (Ma *et al.*, 2001; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003; Gunnell *et al.*, 2003). The association of milk with IGF-I was attenuated after adjustment for protein intake, suggesting that protein in milk might partially explain the modest association of serum IGF-I with milk intake observed in this study. Milk is the main source of calcium in this population and the association of IGF-I with milk disappeared after

adjustment for calcium. It is not known if bovine IGF-I in milk plays a role in the observed association, but there is no strong evidence that bovine IGF-I in cows milk could be absorbed intact by adults (Juskevich and Guyer 1990; Daxenberger *et al.*, 1998).

Both, milk or calcium intake and circulating levels of IGF-I have been found related with the risk of several diseases, although not always in the same direction. Recent evidence suggests that cardiovascular disease risk may be elevated among apparently healthy individuals who have serum IGF-I levels in the low-normal range (Kaplan *et al.*, 2005), and that milk drinking may be associated with a small but worthwhile reduction in heart disease (Elwood *et al.*, 2004). The intakes of calcium and dairy products have been found associated with lower prevalence of the metabolic syndrome in middle-aged and older women (Liu *et al.*, 2005). IGF-I concentrations were significantly lower in subjects with metabolic syndrome compared with subjects without metabolic syndrome in a recent study (Sesti *et al.*, 2005). Higher plasma concentrations of IGF-I have been found associated with a reduced risk of osteoporosis (Rudman *et al.*, 1994). Prostate cancer risk has been found positively associated with intake of dairy products, and with circulating IGF-I (Chan and Giovannucci, 2001). Colorectal cancer risk might be positively associated with IGF-I levels (Renehan *et al.*, 2004), but it has been consistently found inversely associated with intake of milk and dietary calcium (Cho *et al.*, 2004). In a prospective study, the inverse association of low-fat milk with colorectal cancer was stronger in men with high IGF-I/IGFBP-3 ratio compared to subjects with low ratio (Ma *et al.*, 2001).

These evidence indicate that, despite the moderate positive potential influence of milk or dietary calcium on circulating IGF-I levels, the possibility that the milk or calcium–disease associations varies according to IGF-I levels deserves further investigation.

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References

Allen NE, Appleby PN, Davey GK, Kaaks R, Rinaldi S, Key TJ (2002). The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev* **11**, 1441–1448.

Baibas N, Bamia C, Vassilopoulou E, Sdrolias J, Trichopoulou A, Trichopoulos D (2003). Dietary and lifestyle factors in relation to plasma insulin-like growth factor I in a general population sample. *Eur J Cancer Prev* **12**, 229–234.

Bingham S, Riboli E (2004). Diet and cancer – the European prospective investigation into cancer and nutrition. *Nat Rev Cancer* **4**, 206–215.

Chan JM, Giovannucci EL (2001). Dairy products, calcium, and vitamin D and risk of prostate cancer. *Epidemiol Rev* **23**, 87–92.

Cho E, Smith-Warner SA, Spiegelman D, Beeson WL, van den Brandt PA, Colditz GA *et al.* (2004). Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. *J Natl Cancer Inst* **96**, 1015–1022.

Clemmons DR, Klibanski A, Underwood LE, McArthur JW, Ridgway EC, Beitins IZ *et al.* (1981). Reduction of plasma immunoreactive somatomedin C during fasting in humans. *J Clin Endocrinol Metab* **53**, 1247–1250.

Daxenberger A, Breier BH, Sauerwein H (1998). Increased milk levels of insulin-like growth factor 1 (IGF-1) for the identification of bovine somatotropin (bST) treated cows. *Analyst* **123**, 2429–2435.

DeLellis K, Rinaldi S, Kaaks RJ, Kolonel LN, Henderson B, LeMarchand L (2004). Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): the multiethnic cohort. *Cancer Epidemiol Biomarkers Prev* **13**, 1444–1451.

Devine A, Rosen C, Mohan S, Baylink D, Prince RL (1998). Effects of zinc and other nutritional factors on insulin-like growth factor I and insulin-like growth factor binding proteins in postmenopausal women. *Am J Clin Nutr* **68**, 200–206.

Douyon L, Schteingart DE (2002). Effect of obesity and starvation on thyroid hormone, growth hormone, and cortisol secretion. *Endocrinol Metab Clin North Am* **31**, 173–189.

Elwood PC, Pickering JE, Hughes J, Fehily AM, Ness AR (2004). Milk drinking, ischaemic heart disease and ischaemic stroke II. Evidence from cohort studies. *Eur J Clin Nutr* **58**, 718–724.

Estivariz CF, Ziegler TR (1997). Nutrition and the insulin-like growth factor system. *Endocrine* **7**, 65–71.

Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C *et al.* (2002). Evaluation of under- and overreporting of energy intake in the 24-h diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* **5**, 1329–1345.

Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL (2002). Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. *J Biol Chem* **277**, 38205–38211.

Giovannucci E, Pollak M, Liu Y, Platz EA, Majeed N, Rimm EB *et al.* (2003). Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. *Cancer Epidemiol Biomark Prev* **12**, 84–89.

Goodman-Gruen D, Barrett-Connor E (1997). Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. *Am J Epidemiol* **145**, 970–976.

Gunnell D, Oliver SE, Peters TJ, Donovan JL, Persad R, Maynard M *et al.* (2003). Are diet–prostate cancer associations mediated by the IGF axis? A cross-sectional analysis of diet, IGF-I and IGFBP-3 in healthy middle-aged men. *Br J Cancer* **88**, 1682–1686.

Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J *et al.* (1996). Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J Clin Invest* **98**, 2612–2615.

Heald AH, Cade JE, Cruickshank JK, Anderson S, White A, Gibson JM (2003). The influence of dietary intake on the insulin-like growth factor (IGF) system across three ethnic groups: a population-based study. *Public Health Nutr* **6**, 175–180.

Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS *et al.* (1999). Dietary changes favorably affect bone remodeling in older adults. *J Am Diet Assoc* **99**, 1228–1233.

Holmes MD, Pollak MN, Willett WC, Hankinson SE (2002). Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomark Prev* **11**, 852–861.

Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U (1996). Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. *J Clin Endocrinol Metab* **81**, 1791–1797.

Hoppe C, Molgaard C, Juul A, Michaelsen KF (2004). High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. *Eur J Clin Nutr* **58**, 1211–1216.

Isley WL, Underwood LE, Clemmons DR (1984). Changes in plasma somatomedin-C in response to ingestion of diets with variable protein and energy content. *J Parenteral Enteral Nutr* **8**, 407–411.

Juskevich JC, Guyer CG (1990). Bovine growth hormone: human food safety evaluation. *Science* **249**, 875–884.

Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C *et al.* (2005). Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* **97**, 755–765.

Kaaks R, Lukanova A (2001). Energy balance and cancer: the role of insulin and insulin-like growth factor-I. *Proc Nutr Soc* **60**, 91–106.

Kaklamani VG, Linos A, Kaklamani E, Markaki I, Koumantaki Y, Mantzoros CS (1999). 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* **17**, 3291–3298.

Kaplan RC, Strickler HD, Rohan TE, Muzumdar R, Brown DL (2005). Insulin-like growth factors and coronary heart disease. *Cardiol Rev* **13**, 35–39.

Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE (2000). The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. *Endocr Rev* **21**, 215–244.

Larsson SC, Wolk K, Brismar K, Wolk A (2005). Association of diet with serum insulin-like growth factor I in middle-aged and elderly men. *Am J Clin Nutr* **81**, 1163–1167.

Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM (2005). Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U. *S Women Diabetes Care* **28**, 2926–2932.

Ma J, Giovannucci E, Pollak M, Chan JM, Gaziano JM, Willett W *et al.* (2001). Milk intake, circulating levels of insulin-like growth factor-I, and risk of colorectal cancer in men. *J Natl Cancer Inst* **93**, 1330–1336.

Maskarinec G, Takata Y, Kaaks R (2005). The relation between nutritional factors and insulin-like growth factor-I in premenopausal women of different ethnicity. *Eur J Nutr* **44**, 105–113.

Mucci LA, Tamimi R, Lagiou P, Trichopoulou A, Benetou V, Spanos E *et al.* (2001). Are dietary influences on the risk of prostate cancer mediated through the insulin-like growth factor system? *BJU Int* **87**, 814–820.

Playford MP, Bicknell D, Bodmer WF, Macaulay VM (2000). Insulin-like growth factor 1 regulates the location, stability, and transcriptional activity of beta-catenin. *Proc Natl Acad Sci USA* **97**, 12103–12108.

Pollak MN, Schernhammer ES, Hankinson SE (2004). Insulin-like growth factors and neoplasia. *Nat Rev Cancer* **4**, 505–518.

Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M (2004). Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* **363**, 1346–1353.

Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M *et al.* (2002). European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* **5**, 1113–1124.

Rudman D, Drinka PJ, Wilson CR, Mattson DE, Scherman F, Cuisinier M *et al.* (1994). Relations of endogenous anabolic hormones and physical activity to bone mineral density and lean body mass in elderly men. *Clin Endocrinol (Oxf)* **40**, 653–661.

Sesti G, Sciacqua A, Cardellini M, Marini MA, Maio R, Vatrano M *et al.* (2005). Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. *Diabetes Care* **28**, 120–125.

Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G *et al.* (2002). European prospective investigation into cancer and nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutr* **5**, 1125–1145.

Thissen JP, Ketelslegers JM, Underwood LE (1994). Nutritional regulation of the insulin-like growth factors. *Endocr Rev* **15**, 80–101.

Vrieling A, Voskuil DW, Bueno De Mesquita HB, Kaaks R, Van Noord PA, Keinan-Boker L *et al.* (2004). 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* **15**, 787–796.

Weissberger AJ, Ho KK, Lazarus L (1991). Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-h growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. *J Clin Endocrinol Metab* **72**, 374–381.

Werner H, Le Roith D (2000). New concepts in regulation and function of the insulin-like growth factors: implications for understanding normal growth and neoplasia. *Cell Mol Life Sci* **57**, 932–942.

Willett W (2001). Dietary diaries versus food frequency questionnaires – a case of undigestible data. *Int J Epidemiol* **30**, 317–319.

Yu H, Berkel H (1999). Insulin-like growth factors and cancer. *J La State Med Soc* **151**, 218–223.

Yu H, Rohan T (2000). Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst* **92**, 1472–1489.