Determinants of Circulating Insulin-like Growth Factor I and Insulin-like Growth Factor Binding Protein 3 Concentrations in a Cohort of Singapore Men and Women[1]

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Abstract

Variation in the circulating concentrations of the insulin-like growth factor (IGF) system has been implicated in the etiology of chronic diseases including cancer (prostate, breast, colon, and lung), heart disease, type 2 diabetes, and osteoporosis. We searched for sociodemographic, anthropometric, reproductive, lifestyle, and dietary determinants of IGF-I and insulin-like growth factor binding protein (IGFBP) -3 serum concentrations. Serum samples were collected in a Singapore Chinese cohort with a mean age of 61 years. Subject information was assessed during an in-person interview. Radioimmunometrically measured IGF-I and IGFBP-3 concentrations were available for 312 men and 326 postmenopausal women ages 50 years or older. Mean IGF-I concentrations were 144 ng/ml and 121 ng/ml for men and women, respectively (gender difference, $P < 0.0001$), and mean IGFBP-3 concentrations were 3710 ng/ml and 4147 ng/ml for men and women, respectively (gender difference, $P < 0.0001$). IGF-I and IGFBP-3 decreased with age (P for trend <0.0001); the age-related decrease in the IGF-I:IGFBP-3 molar ratio was stronger in women than men. IGF-I concentrations were higher among physically inactive subjects and among women with an early age at menarche. Consumption of saturated fat was found to decrease, and intake of ω-3 polyunsaturated fatty acids and of dietary fiber was found to increase circulating IGFBP-3 concentrations. Intake of calcium from food and supplement was associated positively with circulating IGF-I, IGFBP-3, and molar ratio. Intake of soy was associated positively with IGF-I and molar ratio concentrations, but only in men. The results of this study lend additional support to the hypothesis that circulating IGF-I concentrations increase the risk of prostate, bladder, colorectal, and breast cancer.

Introduction

IGF-I[3] regulates growth and metabolism in an autocrine, paracrine, or endocrine manner (1). IGF-I in the blood circulates freely or is bound to BPs. IGFBP-3 accounts for ~95% of the IGF total binding activity in serum. Both IGF-I and IGFBP-3 are mainly secreted by the liver, under the control of growth hormones and influenced by nutritional status (2, 3). Variation in the circulating levels of the IGF system has been implicated in the etiology of chronic diseases including cancer (prostate, breast, colon, and lung; Ref. 4), heart disease, type 2 diabetes (5), and osteoporosis (6). Blood concentrations of IGF-I, IGF-2, and IGFBP-3 are strongly genetically determined in the fetus, and to a lesser degree in children and adults (7–10). Environmental factors are believed to play a role as well (11–19). Most of the previous studies were conducted among Caucasian populations. We investigated sociodemographic, anthropometric, reproductive, lifestyle, as well as dietary predictors of circulating IGF-I and IGFBP-3 concentrations in an Asian population.

Subjects and Methods

Subjects. Subjects were participants of the Singapore Chinese Health Study, a population-based prospective investigation of diet and cancer risk. During April 1993 through December 1998, 63,257 Chinese men and women ages 45–74 years who were residents of government housing estates (86% of the Singapore population reside in such facilities) enrolled in the study. At recruitment, an in-person interview was conducted in subject homes by a trained interviewer using a structured questionnaire, which included a validated dietary component (including coffee, tea, and alcoholic beverages) soliciting current intake pattern (20). There are seven common soyfoods (all are nonfermented) in the Singapore Chinese diet, including plain-tofu, taupok, taukwa, foopei, foojook, tofu-far, and soybean drink. We used an algorithm to estimate an overall soy intake per study subject in units of plain-tofu equivalents as described previously. Total soy isoflavone intake for a given subject was estimated from the summation of genistein, daidzein, and glycitein contents of all seven soyfoods. Likewise, total soy protein

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[3] The abbreviations used are: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; CV, coefficient of variation; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein.

intake per study subject was calculated from the protein contents of soyfoods listed in the Singapore Food Composition Table (21). The questionnaire also requested demographic information, lifetime use of tobacco (cigarettes or water-pipe), current physical activity profile, reproductive history (women only), occupational exposure, medical history, and family history of cancer.

In April 1994, 1 year after commencement of the cohort study, we began collecting blood and spot urine specimens from a random 3% sample of study enrollees. Most blood samples were collected in the morning with no requirement for fasting. However, information on time of last meal was obtained. All of the blood specimens were processed and separated into their various components (serum, plasma, red cells, and buffy coat) before storage at −70°C. The present study included men and postmenopausal women ages 50 years or older; the first 638 (312 men and 326 women) eligible cohort participants on whom blood was available constituted our study subjects. The study was approved by the Institutional Review Boards of the University of Southern California and the National University of Singapore.

Laboratory Measurements. Blood (nonfasting) samples were obtained from the study subjects by venipuncture. The number of hours elapsed since the last meal was recorded. Blood samples were transported immediately to the laboratory, where they were allowed to stand for 2 h, spun at 2500 *g* for 15 min, after which serum was extracted and stored at −70°C until analysis.

Measurements of serum IGF-I and IGFBP-3 concentrations were carried out using immunoradiometric assay kits (Diagnostic Systems Laboratories, Inc., Webster, TX). The assays were performed following the instructions of the manufacturer, and serum specimens were identified only by code numbers that were not linked to any characteristics of the study subjects. Serum samples from individual subjects were batched into 16 sets. Each batch contained 40 specimens with approximately equal numbers of males and females within 5-year age groups. The order of the 16 batches was randomized for analysis. Each batch contained 6 standards and 2 control specimens.

Duplicate aliquots from each blood sample were analyzed for each individual, and the average of the two measurements was used for data analyses. The CV for duplication was <10%. The ranges for interbatch-assay CV were 3.6–4.5% for serum IGF-I and 6–9% for serum IGFBP-3. For each batch we also calculated within-person CV and between-person CV. The within-person CV values for IGF-I ranged from 2.4 to 4.9%; for IGFBP-3 were 2 to 3.6%. The between-person CV were 37.4–77.7% *versus* 26.1–41.0% for IGF-I and IGFBP-3, respectively.

The methods used to analyze blood concentrations of vitamin B6, B12, folate, homocysteine, and lipid fractions have been described previously (22).

Statistical Analysis. Statistical analysis was conducted with the help of SAS software, Version 8. The molar ratio, which may estimate the biologically active fraction of IGF-I, was calculated based on the following conversion: for IGF-I, 1 ng/ml is equal to 0.130 nM, and for IGFBP-3, 1 ng/ml is equal to 0.036 nM. Spearman's correlation coefficients were calculated to investigate the associations among various serum markers. Analyses of variance and covariance methods were used to compare mean IGF-I, IGFBP-3, and molar ratio levels between different categories of potential predictors, and to derive *P*s for linear trend. Multiple linear regression models were used to examine levels of serum IGF-I, IGFBP-3, and their molar ratio in relation to dietary nutrients. Energy intake adjustment was achieved by entering nutrient densities into the models (23). Adjustment for potential confounding factors was achieved by entering age and BMI as continuous variables, and gender and physical activity (no weekly vigorous or strenuous sports and >9 h of sitting per day *versus* others) as dichotomous variables into the models. A gender-covariate product term was included in different models to assess possible gender differences in the covariate-IGF relationships. All of the *P*s quoted are two-sided; they are considered statistically significant when values are <0.05 (24).

Of the 638 subjects, 21% ($n = 133$) offered fasting blood samples (≥12 h after meal), whereas 32% ($n = 203$) were collected within 2 h after meal. We examined blood IGF components by time since last meal and observed a decrease of IGF-I concentrations with increasing time since last meal. The age- and gender-adjusted mean IGF-I concentrations for <2 h, 2–11 h, and ≥12 h of fasting time were 138, 133, and 124 ng/ml, respectively (P for linear trend = 0.02). No statistically significant association between fasting status and circulating IGFBP-3 concentrations was observed ($P = 0.73$). Results of analyses with or without adjustment for fasting status were similar. Analysis involving circulating IGF components and lipid parameters were restricted to fasting blood samples. The mean difference between age at interview and age at blood draw was 2.1 years (range, 0–6 years). Restriction of the analysis to a time difference between age at interview and age at blood draw of at most 2 years did not materially alter the results.

Results

The study population had a mean age of 61 years. Mean weight (kg), height (cm), and BMI (kg/m^2) for men and women were 61 and 55 kg; 165 and 155 cm; and 22.5 and 22.9 kg/m^2, respectively. Thirty-three percent and 5% of men and women, respectively, were smoking at the time of interview. Sixty-seven percent and 92% of men and women, respectively, did not consume alcoholic beverages. Among women, 6% were nulliparous, and 51% reported a menarchal age of 15 years or later. Mean concentrations (SD, range) for IGF-I were 144 (53, 15–354) for men and 121 (55, 18–393) for women, respectively. Comparable figures for IGFBP-3 were 3710 (808, 1869–6298) and 4147 (883, 1772–8424), respectively. In both gender groups, IGF-I and IGFBP-3 were positively correlated (Spearman's correlation coefficient $r = 0.64$ and $r = 0.63$ in men and women, respectively; both $P < 0.0001$).

Table 1 shows concentrations of IGF-I, IGFBP-3, and the molar ratio by age and sex. Women had lower total IGF-I and IGF-I:IGFBP-3, but higher IGFBP-3 levels than men, irrespective of age (all three of the *P*s for gender difference = 0.0001). In both sexes IGF-I and IGFBP-3 decreased with increasing age. The inverse association with age was also present for the molar ratio, being somewhat stronger in women than men (P for linear trend 0.05 and 0.13 in women and men, respectively).

The association of IGF-I, IGFBP-3, and their molar ratio with anthropometric and lifestyle factors is presented in Table 2. In all of the subjects combined, BMI was not associated with IGF-I or molar ratio, but a positive association was observed between BMI and IGFBP-3 ($P = 0.006$). There was some indication that the BMI:IGF-I association may be modified by gender (P for interaction = 0.09). Among men only, BMI was positively associated with IGF-I ($\beta = 2.44$; $P = 0.01$). Neither alcohol nor cigarette smoking, alone or in combination, had a substantial impact on the IGF system variables. IGF-I and

Table 1 Means of serum IGF-I, IGFBP-3, and molar ratio (IGF-I: IGFBP-3), by sex and age, Singapore Chinese Health Study

Sex	Age (years)	Number of subjects	IGF-I (ng/ml)	IGFBP-3 (ng/ml)	Molar ratio (IGF-I:IGFBP-3)*1000
Men	All[a]	312	144	3714	140
	50–54	77	161	4047	144
	55–64	133	144	3732	141
	65–74	102	131	3425	135
P for linear trend by age			0.0001	0.0001	0.13
Women	All[a]	326	121	4143	103
	50–54	70	130	4335	108
	55–64	163	123	4153	105
	65–74	93	112	3995	97
P for linear trend by age			0.04	0.02	0.05
P by sex[a]			0.0001	0.0001	0.0001

[a] Adjusted for age at specimen collection as a continuous variable.

Table 2 Age- and sex-adjusted means of serum IGF-I, IGFBP-3, and molar ratio (IGF-I:IGFBP-3), by lifestyle and anthropometric variables, Singapore Chinese Health Study

Predictor	Number of subjects	IGF-I (ng/ml)	IGFBP-3 (ng/ml)	Molar ratio (IGF-I:IGFBP-3)*1000
BMI (kg/m^2)				
Quartile				
1 <20.95	159	127	3800	120
2 <23.05	158	137	3935	124
3 <24.25	161	130	3899	121
4 ≥24.25	160	136	4080	121
P for linear trend		0.25	0.006	0.95
Current cigarette smoking and alcohol intake[a]				
No/no	429	134	3963	122
No/yes	89	127	3921	115
Yes/no	79	138	3875	130
Yes/yes	41	119	3694	117
P for alcohol effect[b]		0.06	0.35	0.04
P for smoking effect[b]		0.96	0.14	0.11
P for interaction[c]		0.30	0.45	0.46
Physical activity				
No weekly vigorous or strenous sports and ≥9 h of sitting/day	94	145	4068	128
Others	544	131	3904	120
P for physical activity		0.02	0.08	0.08
Weekly intake of vitamin/mineral supplements				
No	589	131	3915	120
Yes	49	152	4097	132
P for supplement intake		0.01	0.14	0.04

[a] Combined effect of current cigarette smoking and current intake of at least 1 alcoholic drink/month.
[b] *P*s for current smoking and alcohol intake, respectively, based on three-way analysis of covariance with current alcohol intake and smoking status as independent variables, and gender and age as covariates.
[c] *P* for interaction term, based on four-way analysis of covariance with current alcohol intake and smoking status, as well as their interaction terms as independent variables, and gender and age as covariates.

molar ratio levels were somewhat lower among subjects who reported consumption of at least one alcoholic drink/month ($P = 0.06$ and 0.04, respectively). Number of cigarettes smoked did not reveal an association between current smoking, and circulating IGF-I and IGFBP-3 concentrations. The association between current smoking and endocrine IGF-I and IGFBP-3 levels was not confounded by dietary, anthropometric, or additional lifestyle factors. IGF-I, IGFBP-3, and molar ratio levels were higher among physically less active subjects, a difference that reached statistical significance for IGF-I ($P = 0.02$). Subjects who reported a weekly intake of vitamin and mineral supplements had higher IGF-I concentrations paralleled by an increase in the IGF-I:IGFBP-3 molar ratio ($P = 0.01$ and 0.04, respectively).

Table 3 summarizes the association of reproductive and hormonal factors with circulating IGF components among women in the study. Women with a later age at menarche, or whose periods never became regular or only at a late age, had low concentrations of both IGF-I (both $P = 0.06$) and IGFBP-3 ($P = 0.04$ and 0.003, respectively), but no statistically significant difference was observed with regard to the molar ratio ($P = 0.21$ and 0.47, respectively). Parity or age at first birth were not associated with circulating IGF components, nor was use of oral contraceptives.

Table 4 shows the relationship between circulating IGFs and a series of serum markers of cardiovascular risk, including vitamins B6 and B12, folate, homocysteine, cholesterol, HDL, LDL, triacylglycerol, and total cholesterol/HDL. Vitamin B6 was associated positively with IGFBP-3. Cholesterol, triacylgycerol, and the total cholesterol:HDL ratio were all corre-

Table 3 Adjusted[a] means of serum IGF-I, IGFBP-3, and molar ratio (IGF-I:IGFBP-3), by reproductive history[b] and hormonal variables, Singapore Chinese Health Study

Predictor	Number of subjects	IGF-I (ng/ml)	IGFBP-3 (ng/ml)	Molar ratio (IGF-I:IGFBP-3)*1000
Age at menarche				
<17 years	274	128	4233	107
≥17 years	52	112	3956	100
P		0.06	0.04	0.21
Age when periods became regular				
<17 years	256	128	4254	106
≥17 years	60	113	3912	103
Never became regular	10	116	3831	102
P for <17 yrs *vs.* ≥17 yrs		0.06	0.003	0.47
Age at first birth				
Nulliparous	20	125	4174	107
≤30 yrs	276	124	4189	104
>30 yrs	30	138	4240	116
P for (nullip. or ≤30 yrs *vs.* >30 yrs)		0.26	0.85	0.13
Use of oral contraceptives[c]				
Never	241	128	4213	108
Ex, >25 years at start	64	137	4236	114
Ex, ≤25 years at start	20	113	4109	98
P		0.09	0.69	0.09

[a] Adjusted for age, BMI, and physical activity.
[b] Women who reportedly stopped bleeding, but had undergone a hysterectomy without double oophorectomy and an age at specimen collection <55 years were assigned an undetermined menopausal status.
[c] Only a single woman reported current use of oral contraceptives. She was excluded from the analysis.

Table 4 Partial Spearman correlation (r) between serum IGF-I, IGFBP-3, and molar ratio (IGF-I:IGFBP-3) and serum markers adjusting for age, BMI, gender, and physical activity, Singapore Chinese Health Study

Serum component	*n*	IGF-I ng/ml		IGFBP-3 ng/ml		Molar ratio (IGF-I:IGFBP-3)*1000	
		r	(p)	r	(p)	r	(p)
Vitamin B6	375	0.075	(0.15)	0.169	(0.001)	−0.010	(0.86)
Vitamin B12	375	−0.028	(0.59)	0.079	(0.13)	−0.09	(0.10)
Folate	375	0.011	(0.83)	0.074	(0.15)	0.018	(0.73)
Homocysteine	375	0.009	(0.86)	−0.016	(0.76)	0.002	(0.97)
Cholesterol[a]	128	0.250	(0.005)	0.240	(0.007)	0.172	(0.06)
HDL[a]	128	−0.088	(0.33)	−0.047	(0.60)	−0.062	(0.49)
LDL[a]	128	0.209	(0.02)	0.221	(0.01)	0.110	(0.23)
Triacylglycerol[a]	128	0.384	(<0.0001)	0.332	(0.0002)	0.314	(0.0004)
Total cholesterol/HDL[a]	128	0.241	(0.007)	0.219	(0.02)	0.156	(0.08)

[a] Restricted to serum samples collected after at least 12 h of fasting.

lated positively with IGF-I, IGFBP-3, and the molar ratio, whereas for LDL positive associations were only observed with IGF-I and IGFBP-3. None of the IGF components were associated with HDL or homocysteine.

Associations between dietary nutrients and circulating IGF components are presented in Table 5. Among macronutrients, ω3-polyunsaturated fat was positively, whereas saturated fat was inversely associated with IGFBP-3. Among micronutrients, statistically significant associations were observed for dietary fiber, calcium, and soy protein. Intake of dietary fiber was associated positively with IGFBP-3 (β = 32.969; P = 0.006), calcium intake was associated positively with IGF-I (β = 0.044; P = 0.007), IGFBP-3 (β = 0.790; P = 0.002), and the molar ratio (β = 0.006; P = 0.06), and soy protein intake among men was associated positively with IGF-I (β = 6.532, P = 0.02) and the molar ratio (β = 1.216; P = 0.05). No corresponding soy protein-IGF associations were evident in women (P for gender interaction = 0.01 for IGF-I and molar ratio, respectively). Mutual adjustment for different micronutrients in the models did not materially alter the results presented in the table.

Table 6 presents additional analysis of soy intake in relation to circulating levels of IGFs. Nutrient density of soy protein and isoflavones, and food density of total soy, respectively, as well as urinary levels of soy isoflavones were all positively associated with circulating IGF-I and the molar ratio in men. No corresponding associations were evident in women.

Discussion

This is the first study to investigate the combined effect of sociodemographic, lifestyle, anthropometric, and dietary factors on circulating IGF-I and IGFBP-3 levels in an Asian population. We found associations between circulating IGF-I and/or IGFBP-3 levels with age, gender, physical activity, fat, fiber, calcium, and soy intake that are consistent with current hypotheses regarding the involvement of the IGF system in the

Table 5 Adjusted[a] regression coefficients based on regression of IGF-I, IGFBP-3, and molar ratio (IGF-I:IGFBP-3) on dietary nutrient densities, Singapore Chinese Health Study

Nutrient density	IGF-I ng/ml		IGFBP-3 ng/ml		Molar ratio (IGF-I:IGFBP-3)*1000	
	β	P	β	P	β	P
Energy (kcal/day)[b]	−5.714	0.41	−156.670	0.14	−1.750	0.73
Carbohydrates						
Model 1[b,c]	0.206	0.47	−0.674	0.88	0.160	0.44
Model 2[b,d]	0.647	0.35	−1.572	0.88	0.808	0.11
Protein						
Model 1[b,c]	0.235	0.78	4.985	0.71	0.304	0.63
Model 2[b,d]	1.837	0.18	8.898	0.67	1.723	0.09
Total fat						
Model 1[b,c]	−0.242	0.51	−0.243	0.97	−0.112	0.68
Model 2[b,d]	−0.049	0.95	−4.735	0.70	0.570	0.60
Saturated fat						
Model 1[b,c]	−1.896	0.03	−27.114	0.04	−0.770	0.23
Model 3[b,e]	−2.401	0.16	−71.762	0.007	0.263	0.83
Monounsaturated fat						
Model 1[b,c]	−0.846	0.39	−0.793	0.96	−0.577	0.42
Model 3[b,e]	−0.914	0.69	46.730	0.18	−0.651	0.70
ω3-Polyunsaturated fat						
Model 1[b,c]	18.818	0.10	422.171	0.02	8.563	0.30
Model 3[b,e]	21.937	0.18	618.224	0.01	4.122	0.73
ω6-Polyunsaturated fat						
Model 1[b,c]	1.730	0.13	32.624	0.06	0.869	0.29
Model 3[b,e]	0.476	0.81	−27.308	0.35	1.530	0.27
Dietary fiber	0.469	0.55	32.969	0.006	−0.147	0.80
Calcium from food and supplement	0.044	0.007	0.790	0.002	0.023	0.06
Soy protein[f]	1.498	0.47	43.461	0.17	0.761	0.61
Men	6.532	0.02	59.549	0.15	4.390	0.05
Women	−3.442	0.26	32.223	0.51	−3.005	0.14

[a] All models adjusted for age, BMI, gender, and physical activity.
[b] No statistically significant interaction was observed between nutrient density and gender.
[c] Model 1: unadjusted for other nutrient densities.
[d] Model 2: adjusted for other macronutrient densities.
[e] Model 3: adjusted for carbohydrate, protein, saturated fat, monounsaturated fat, ω3-polyunsaturated fat, and ω6-polyunsaturated fat density.
[f] *P* for interaction between soy protein density and gender: 0.01 for IGF-I; 0.51 for IGFBP-3; 0.01 for molar ratio.

etiology of several chronic diseases including various types of cancer.

The observed positive association between circulating IGF-I concentrations and calcium intake from food and supplements is consistent with the hypothesis of calcium impacting on prostate cancer risk through the modulation of IGF-I concentrations. Frequent consumption of calcium and dairy products has been associated with elevated prostate cancer risk in several (25–28) but not all of the studies (29). Positive associations between circulating IGF-I and the incidence of prostate cancer have been reported rather consistently by several prospective studies (30–32). Previous studies had found elevated circulating IGF-I levels among subjects with frequent milk consumption (15, 33). In a randomized intervention study among 204 healthy men and women ages 55–85 years, individuals who consumed three servings per day of nonfat or 1% milk for 12 weeks had a statistically significant 10% increase in serum IGF-I, whether or not the cows were treated with recombinant bovine somatotropin for milk production (34). The positive association between calcium intake from food and supplements with circulating concentrations of IGF-I and IGF-I:IGFBP-3 molar ratio in our study population is independent of dairy milk intake. The primary sources of calcium in a Singapore Chinese diet are soy products.

A positive association between soy intake and circulating IGF-I concentrations that was not confounded by calcium intake and restricted to men was also present in our dataset. Consistent with our results, a 3-month intervention randomly assigning 64 healthy men to either 40 g of casein or soy protein per day caused a statistically significant increase in circulating IGF-I levels in the soy protein consuming group (35). Circulating IGF-I concentrations in vegan women were reported recently to be elevated when intake of soy milk was frequent (36). Soy represents a rich source of dietary calcium and isoflavone, and the impact of soy intake on endocrine IGF-I may be attributable to either compound. Fermented soybeans, which contain relatively high levels of calcium, are consumed in higher quantities in eastern than western Japan, in parallel with a lower incidence of osteoporotic bone fractures in eastern than western Japan (37, 38). Isoflavones have been investigated as possible alternatives to hormone replacement therapy in the prevention of osteoporosis attributable to their estrogenic activity. The restriction of a soy but not calcium effect to men in our study suggests that isoflavones, rather than the calcium in soy, is responsible for the observed impact on IGF-I. Endogenous estrogen concentrations are known determinants of estrogenic *versus* antiestrogenic signaling of isoflavonoids through the estrogen receptor. Thus, the restriction of the soy effect to males in our study may be explained by their lower endocrine estrogen production and an associated predominance of estrogenic and thus IGF-I stimulating isoflavonoid activity (39). It is of interest that in this same cohort of Singapore Chinese we have reported recently that high intake of soyfood was associated with an elevated risk of bladder cancer, a predominantly

Table 6 Adjusted[a] mean IGF-I, IGFBP-3, and molar ratio (IGF-I:IGFBP-3) by quantiles of soy indicators and gender, Singapore Chinese Health Study

	n Male/Female	IGF-I ng/ml		IGFBP-3 ng/ml		Molar ratio (IGF-I:IGFBP-3)*1000	
		Men	Women	Men	Women	Men	Women
Soy protein density							
1	92/70	143	132	3729	4118	137	111
2	79/84	143	122	3736	4135	137	105
3	76/77	150	126	3748	4256	144	105
4	65/95	163	122	3890	4258	152	102
P for linear trend		0.01	0.39	0.21	0.23	0.02	0.18
P for gender interaction[b]		0.01		0.51		0.01	
Isoflavonoid density							
1	92/72	137	131	3714	4152	131	110
2	88/77	144	121	3780	4162	138	103
3	73/90	161	131	3742	4320	156	108
4	59/87	155	119	3865	4163	145	102
P for linear trend		0.01	0.34	0.30	0.67	0.01	0.30
P for gender interaction[b]		0.01		0.38		0.03	
Tofu product density							
1	100/71	141	130	3725	4119	135	110
2	73/77	146	125	3755	4191	140	105
3	78/87	151	128	3763	4253	145	107
4	61/91	160	120	3857	4222	150	101
P for linear trend		0.02	0.34	0.30	0.42	0.02	0.18
P for gender interaction[b]		0.01		0.41		0.01	
Urinary isoflavonoids (nmol*1000/mg*10)							
1 ≤2.93	23/15	137	131	4116	4334	120	107
2/3 >2.93	40/38	161	117	3794	4160	152	100
P for linear trend		0.06	0.48	0.07	0.60	0.004	0.46
P for gender interaction[b]		0.10		0.66		0.01	

[a] Adjusted for age, BMI, and physical activity.
[b] Based on regression models containing soy intake indicator, gender, and a gender*soy indicator interaction term.

male cancer (21). Laboratory studies involving bladder cancer cells (40) and whole animals (41) have supported a role of IGF-I in bladder carcinogenesis.

Our results on other dietary and lifestyle predictors of IGF components are consistent in some but not all of the aspects with previous reports investigating IGF-determinants (reviewed in Refs. 15, 16) and with a role of a high IGF-I:IGFBP-3 molar ratio in the blood in the etiology of colorectal cancer as suggested by some but not all of the previous studies (42–47). We noted an inverse association between saturated fat intake and IGFBP-3, positive associations between IGFBP-3 and ω-3-polyunsaturated fatty acid (PUFA) or fiber intake as well as between IGF-I and physical inactivity. Diets high in saturated fat or low in ω-3-PUFA and dietary fiber, as well as physical inactivity are established risk factors for colorectal cancer. These factors may act, in part, through elevating IGF-I exposure of the intestinal epithelial cells (48). Vegan men and women, who eat more PUFA and nonstarch polysaccharides than meat eaters, were found to have, on average, 9% lower IGF-I concentrations than meat eaters and levels 8% lower than vegetarians; they also have a lower incidence of colorectal cancer (36, 49).

The finding of higher IGF-I concentrations in men and higher IGFBP-3 concentrations in women is consistent with many previous epidemiological studies (11, 13, 50–52). The association of reproductive and hormonal history with circulating IGF-I and IGFBP-3 concentrations in women has been investigated in the Nurses' Health Cohort (16). High parity was associated with lower circulating IGF-I concentrations, a history of ever breastfeeding was associated with a modest increase in IGF-I and IGFBP-3. Together with our finding of lower circulating IGF-I concentrations among women with late ages at menarche or those whose periods never became regular, all recognized protective factors against breast cancer, the results of these studies are interesting in light of recent report of a positive association between circulating IGF-I concentrations and breast cancer risk (53). However, questions about the relevant time window for an IGF-I impact on breast cancer risk remain open given that associations have thus far been restricted mainly to premenopausal women (53–55).

Results on the association between anthropometric and behavioral correlates with IGF system components have been very inconsistent in the past (16, 17). It is of interest to note that the positive association between BMI and IGFBP-3 and IGF-I, with the latter being restricted to men, is consistent with previous findings in lean Asian male populations (17, 47). This result, which is in contrast with many studies in heavier Caucasian populations, may suggest that the impact of BMI on the IGF system in healthy, normally fed populations is restricted to lean subjects.

Consistent with previous studies in Caucasian populations and a study of postmenopausal Japanese women (56) we observed a strong decline of IGF-I with increasing age; previous findings on the inverse association between IGFBP-3 and age are inconsistent (16, 43, 57). Age has been identified as the most consistent predictor of blood IGF-I in previous studies, with peak levels during puberty followed by a continuous decline with increasing age (2, 16, 11, 13, 14, 58, 59). This age-related decline parallels the profound decrease in growth hormone output with aging, which is associated with a decrease in muscle mass and bone mineral density, and an increase in body fat. The observed association of blood IGF-I levels with age, calcium, and soy intake, the positive correlations between IGF-I and/or the IGF-I:IGFBP-3 molar ratio with established

cardiovascular risk factors, including blood cholesterol, LDL, triacylglycerol, and the ratio of total cholesterol to HDL, as well as the observed associations between the cardiovascular disease (CVD) predictors saturated fat and ω-3-PUFA intake with IGFBP-3 levels support a role of the IGF-system in osteoporosis (6) and heart disease (5). As is true for all of the associations described, the cross-sectional nature of this study does not allow for the determination of whether these predictors in fact determine concentrations of IGF components in the blood or are in fact determined by them.

In summary, the results of the present study lend additional support to the hypothesis of a role of circulating IGF-I in the etiology of prostate, bladder, colorectal, and breast cancer, as well as osteoporosis and possibly heart disease as suggested by epidemiological studies on the association between endocrine IGF-I levels and these chronic diseases. Endocrine IGF-I and IGFBP-3 concentrations are potentially modifiable by dietary components such as calcium, soy, dietary fiber, and specific types of fat. The discussion of a positive association of calcium and soy intake with circulating IGF-I concentrations must consider the complex public health background of this issue with regard to different chronic diseases.

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