Associations of serum insulin-like growth factor-I and insulin-like growth factor-binding protein 3 levels with biomarker-calibrated protein, dairy product and milk intake in the Women's Health Initiative

Jeannette M. Beasley*, Marc J. Gunter, Andrea Z. LaCroix, Ross L. Prentice, Marian L. Neuhouser, Lesley F. Tinker, Mara Z. Vitolins and Howard D. Strickler

Albert Einstein College of Medicine, 1300 Morris Park Avenue, Belfer 1312c, Bronx, NY 10461, USA

Abstract

It is well established that protein–energy malnutrition decreases serum insulin-like growth factor (IGF)-I levels, and supplementation of 30 g of whey protein daily has been shown to increase serum IGF-I levels by 8 % after 2 years in a clinical trial. Cohort studies provide the opportunity to assess associations between dietary protein intake and IGF axis protein levels under more typical eating conditions. In the present study, we assessed the associations of circulating IGF axis protein levels (ELISA, Diagnostic Systems Laboratories) with total biomarker-calibrated protein intake, as well as with dairy product and milk intake, among postmenopausal women enrolled in the Women's Health Initiative (n 747). Analyses were carried out using multivariate linear regression models that adjusted for age, BMI, race/ethnicity, education, biomarker-calibrated energy intake, alcohol intake, smoking, physical activity and hormone therapy use. There was a positive association between milk intake and free IGF-I levels. A three-serving increase in milk intake per d (approximately 30 g of protein) was associated with an estimated average 18·6 % higher increase in free IGF-I levels (95 % CI 0·9, 39·3 %). However, total IGF-I and insulin-like growth factor-binding protein 3 (IGFBP-3) levels were not associated with milk consumption and nor were there associations between biomarker-calibrated protein intake, biomarker-calibrated energy intake, and free IGF-I, total IGF-I or IGFBP-3 levels. The findings of the present study carried out in postmenopausal women are consistent with clinical trial data suggesting a specific relationship between milk consumption and serum IGF-I levels, although in the present study this association was only statistically significant for free, but not total, IGF-I or IGFBP-3 levels.

Key words: Insulin-like growth factor-I: Insulin-like growth factor-binding protein-3: Dairy products: Milk: Biomarker-calibrated protein intake

Recent research carried out by our group and others suggests that higher protein intake may be associated with the preservation of lean body mass[1] and reduced frailty[2] in postmenopausal women. The characterisation of mechanisms through which higher protein intake may be related to successful ageing phenotypes could inform dietary guidelines. The insulin-like growth factor (IGF) axis constitutes an evolutionarily conserved system involved in the regulation of cell growth, proliferation and survival that affects nearly every organ system in the body. The axis consists of two phylogenetically conserved peptide ligands, IGF-I and IGF-II, with potent anabolic effects and six high-affinity binding proteins (insulin-like growth factor-binding protein (IGFBP)-1 to IGFBP-6)[3].

IGF-I is the primary mediator of the effects of growth hormone and is thought to be the major IGF affecting growth, health and disease following fetal development[4,5]. IGF-I shares extensive sequence homology and downstream signalling pathways with insulin and has insulin-like effects on glucose and fat uptake in peripheral tissues. However, IGF-I exhibits stronger mitogenic and anti-apoptotic activity than insulin[6]. Previous research has suggested that circulating IGF-I levels may influence the risk of cancer[4], diabetes[5] and other conditions related to healthy ageing[7]. Most of the IGF-I in the circulation is produced by the liver and is bound to IGFBP, with IGFBP-3 binding 75 % or more of all IGF-I in the blood. Only approximately 1 % of total serum IGF-I is unbound, and this free fraction may be the most biologically active component of total IGF-I[8].

It is well known that diets deficient in energy and/or protein lead to substantive reductions in serum IGF-I levels[9]; however, the role of protein and other dietary factors under conditions of weight maintenance is not well characterised. Significant increases in serum IGF-I levels (51·5 (95 % CI 18·6, 84·4) %

after 6 months and 8 % ($P=0.016$) after 2 years, respectively) have been reported by two randomised, controlled trials of 20 g[10] and 30 g[11] of daily protein supplementation compared with isoenergetic placebo. A randomised trial comparing three daily servings of milk with control has reported a 10 % increase in IGF-I levels ($P<0.001$)[12]. Observational studies have suggested that animal protein[13], particularly from dairy sources[14], is specifically associated with higher serum IGF-I levels; such studies can help to discern whether the putative increases in IGF-I levels are sustained with habitually higher protein intake than with lower protein intake.

We are unaware of any published study that has addressed the relationship of protein intake with free IGF-I levels, and a few dietary studies have examined that with IGFBP-3 levels, though some data from two cross-sectional studies indicate that IGFBP-3 levels are not associated with protein consumption[13,15]. The present study also has the potential advantage of having data regarding biomarker-calibrated total protein intake, thought to be a better measure of dietary intake compared with self-report alone, from the Women's Health Initiative (WHI). Therefore, the present study examined the cross-sectional associations of total IGF-I, free IGF-I and IGFBP-3 levels with biomarker-calibrated total protein intake, along with uncalibrated dairy product and milk consumption, among 747 women in the WHI – Observational Study (WHI-OS)[16,17].

Methods

Study population

The WHI-OS is a prospective cohort study that enrolled 93 676 women aged 50–79 years at forty US clinical centres between 1993 and 1998, as has been described in detail elsewhere[18]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the institutional review boards at each of the WHI clinical centres and at the Clinical Coordinating Center at the Fred Hutchinson Cancer Research Center (Seattle, Washington, USA). Written informed consent was obtained from all the participants. The participants of the present study were controls from an ancillary nested case–cohort study designed to evaluate the relationship between the IGF axis and colorectal, breast and endometrial cancers, as reported previously[19–21]. Of the 816 randomly selected controls from the WHI-OS, the analytical sample consisted of 747 women who reported plausible energy intake in FFQ (i.e. excluding values <2510.4 or $>20 920 \text{ kJ/d}$) and having complete information on covariates needed to calculate biomarker-calibrated protein intake (age, BMI, smoking, race/ethnicity, education, income and physical activity).

Measurement of insulin-like growth factor levels

Total IGF-I, free IGF-I and IGFBP-3 levels were determined in serum samples collected at baseline using commercially available ELISA kits (Diagnostic Systems Laboratories)[19–21]. Inter-assay CV were 8·2, 11·2 and 3·6 % for total IGF-I, free IGF-I and IGFBP-3, respectively.

Dietary protein assessment

All the participants completed the WHI FFQ at WHI-OS baseline. The self-administered FFQ included 122 items for individual foods/food groups, nineteen adjustment items and summary questions[22]. Protein intake was characterised as absolute energy intake (total intake in grams) and relative to energy intake (as a percentage of total energy intake) and relative to body weight (as a ratio of 0.1 g of daily protein intake per kg of body weight)[23]. Milk consumption was captured from FFQ questions of frequency and portion of milk as beverage, on cereals and in coffee or tea. Dairy product consumption was computed by summing milk consumption and twenty items from the FFQ querying weekly servings of cheese, yogurt and combination foods containing these items (i.e. quesadilla and cream soups).

Calibrated energy and protein estimation

As has been described previously[17], the WHI Nutritional Biomarkers Study used objective biomarkers of total energy expenditure (equivalent to energy intake in weight-stable persons) and protein intake to assess the measurement properties of the FFQ. Briefly, 544 women from twelve clinical centres of the Dietary Modification Trial were enrolled in a doubly labelled water protocol to estimate total energy expenditure over a 2-week period and a urinary N protocol to estimate protein consumption over a 24 h period to be compared with concurrent self-reported dietary intake data. Calibrated energy and protein estimates were obtained by introducing baseline FFQ consumption estimates and other participant characteristics obtained at baseline from the WHI-OS into linear regression calibration equations[19]. For example:

log-calibrated percentage of energy obtained from protein = $2.66 + 0.439 \times$ log FFQ percentage of energy obtained from protein $- 0.004 \times$ BMI $- 0.005 \times$ age $- 0.129 \times$ current smoker (1 = yes, 0 = no).

Covariate measurements

Data pertaining to demographic characteristics, medical history and other health-related characteristics were obtained by self-report at WHI-OS baseline. BMI was computed using measured height and weight at baseline (kg/m^2).

Statistical analysis

IGF levels and protein, dairy product and milk intake were ln-transformed both to better approximate a normal distribution and to allow coefficients of regression models to be interpreted as percentage difference. Spearman's correlations were calculated between the levels of total IGF-I, free IGF-I and IGFBP-3. A series of linear regression models were examined to evaluate the influence of energy intake, BMI and other potential confounders on observed associations between IGF axis component levels and protein, dairy product and milk intake. Age, energy intake and BMI were modelled as continuous variables[24]. Exposures of interest were modelled both

Table 1. Demographic and health characteristics by free insulin-like growth factor-I tertiles (n 747), Women's Health Initiative – Observational Study
(Mean values and standard deviations; number of participants and percentages)

Characteristics	Overall Mean	Overall SD	Tertile 1 Mean	Tertile 1 SD	Tertile 2 Mean	Tertile 2 SD	Tertile 3 Mean	Tertile 3 SD	P
Age (years)	62·9	7·4	62·5	7·1	63·0	7·5	63·1	7·6	0·34
BMI (kg/m^2)	27·4	5·7	27·2	6·0	27·4	5·7	27·7	5·5	0·37
Ethnicity									0·08
White									
n	638								
%	85		35		34		32		
Black									
n	58								
%	8		21		29		50		
Hispanic									
n	25								
%	3		28		44		28		
Other									
n	26								
%	4		31		27		42		
Education									0·36
≤ High school									
n	156								
%	21		31		32		37		
School after high school									
n	273								
%	37		38		33		29		
College degree									
n	91								
%	12		29		31		41		
Graduate school									
n	227								
%	30		31		36		33		
Hormone therapy use									<0·001
Never									
n	216								
%	29		23		32		45		
Past									
n	158								
%	22		30		34		37		
Current									
n	360								
%	49		41		34		25		
Smoking									0·56
Never									
n	401								
%	54		31		35		34		
Past									
n	298								
%	40		35		31		34		
Current									
n	48								
%	6		40		33		27		
Alcohol intake									0·59
Non-drinker									
n	78								
%	11		23		37		40		
Past drinker									
n	138								
%	19		36		32		33		
< 1 drink/week									
n	237								
%	32		35		33		32		
≥ 1 drink/week									
n	292								
%	39		33		33		34		
Physical activity (MET-h/week)	13·9	14·6	13·8	15·2	12·7	12·6	15·2	15·9	0·27
Dietary intake									
Total energy, biomarker calibrated (kJ/d)	8657	858	8661	100	8632	812	8657	837	0·96
Protein, biomarker calibrated (% energy/d)	14·3	1·4	14·2	1·3	14·3	1·4	14·3	1·5	0·42
Protein, biomarker calibrated (g/d)	76·3	11·3	76·7	11·3	76·2	11·2	75·8	11·4	0·40

Table 1. *Continued*

Characteristics	Overall Mean	SD	Tertile 1 Mean	SD	Tertile 2 Mean	SD	Tertile 3 Mean	SD	P
Protein, biomarker calibrated (g/kg body weight per d)	1·09	0·20	1·11	0·19	1·09	0·21	1·07	0·20	0·04
Dietary Ca (diet alone) (mg/d)	847	461	842	445	841	436	859	501	0·68
Dietary Ca (with supplements) (mg/d)	1264	815	1303	917	1234	760	1255	762	0·51
Dairy products (servings/d)	1·9	1·4	1·9	1·3	1·9	1·3	1·9	1·5	0·53
Milk (servings/d)	0·83	0·98	0·80	0·92	0·79	0·93	0·88	1·08	0·35

MET, metabolic equivalent; % energy, percentage of total energy intake.

continuously and categorically. Standard errors for regression coefficients where total protein was the exposure variable were estimated using a bootstrapping procedure (1000 replicates) to acknowledge the uncertainty in the regression calibration coefficients as well as that due to the sampling from the study population. To compare results with those of intervention studies, for the dairy product and milk intake analyses, both the outcome and exposure variables were log-transformed and scaled to express associations in terms of percent difference in the outcome per three-serving increase in the exposure. Logistic regression models were used to measure associations between calibrated protein intake categorised into quartiles and the odds of high serum total IGF-I, free IGF-I and IGFBP-3 levels (defined as top 10%). Additional analyses were carried out modelling the percentage of energy obtained from protein sources (animal, vegetable and milk) as the exposure variable, adjusting for Ca intake, restricted to participants with no history of hormone therapy use and without prevalent diabetes (*n* 201). Analyses stratified by BMI category (<25, 25–29·9 and ≥30 kg/m²), protein source (animal/vegetable) and milk fat content (whole/2%, 1% and skimmed) were also carried out, and the *P* for interaction term was estimated by multiplying the exposure variable by the interaction term. All analyses were carried out using SAS version 9.3 (SAS Institute)

Results

The majority of the women were White (85%), completed at least high school education (79%), were current users of postmenopausal hormone therapy at baseline (49%) and were overweight (BMI 27·4 (SD 5·7) kg/m²) (Table 1). Women who were current hormone therapy users had lower free IGF-I levels (*P*<0·05), as reported previously[19–21]. Each of the IGF axis proteins was positively correlated with

one another, with the strongest association between total IGF-I and IGFBP-3 (*r* 0·46 and *P*<0·05) (Table 2).

Mean calibrated protein intake was 1·1 (SD 0·2) g/kg body weight per d, exceeding the current RDA for protein (0·8 g/kg body weight per d)[23]. Expressed as a percentage of biomarker-calibrated energy intake, median protein intake was 14·3% across a narrow range (interquartile range 13·3–15·2%). Dairy product and milk intake also did not vary widely (i.e. median of 1·6 servings of dairy products daily (interquartile range 0·9–2·6)).

Calibrated protein intake, expressed in absolute terms, relative to calibrated energy intake, or relative to body weight, was not associated with free IGF, IGF-I or IGFBP-3 levels (Table 3). However, there was a positive association between free IGF-I levels and milk intake (Table 4). Specifically, a three-serving increase in milk intake per d was associated with an estimated average 18·6% increase in free IGF-I levels (95% CI 0·9, 39·3%) (Table 4).

IGFBP-3 levels were not associated with dairy product or milk intake in continuous or categorical analyses. Results obtained from logistic regression models between calibrated protein intake, dairy product intake and milk intake categorised into quartiles and the odds of high serum total IGF-I, free IGF-I and IGFBP-3 levels (defined as top 10%) were consistent with those obtained from linear regression models (data not shown). The results of analyses that modelled the exposure variable as a categorical or continuous variable were similar, and there was no statistically significant or discernible difference in estimates restricted to (1) non-HT users and (2) women without prevalent diabetes. Nor were the results meaningfully affected by stratification by BMI category (<25, 25–29·9, and ≥30 kg/m²), protein source (percentage of energy obtained from animal vegetable and milk protein) or milk fat content (whole/2%, 1% and skimmed) (all *P*>0·05 for interaction, data not shown). Analyses including

Table 2. Geometric mean levels and Spearman correlations between insulin-like growth factor (IGF) axis proteins

(Geometric means and 95% confidence intervals)

IGF axis proteins (ng/ml)	Geometric mean	95% CI	Free IGF	Total IGF	IGFBP-3
Free IGF-I	0·30	0·28, 0·32			
Total IGF-I	117·4	114·0, 120·8	0·23*		
IGFBP-3	4041	3984, 4100	0·14*	0·46*	

IGFBP, insulin-like growth factor-binding protein.
*P < 0·05.

Table 3. Multivariate linear regression associations between free insulin-like growth factor (IGF)-I, total IGF-I and insulin-like growth factor-binding protein (IGFBP)-3 levels and calibrated protein intake†‡

(β-Coefficients and 95 % confidence intervals)

	n	Calibrated protein (% energy)		Calibrated protein (g/d)		Calibrated protein (g/kg body weight per d*)	
		β	95 % CI	β	95 % CI	β	95 % CI
Free IGF (%)	732	0·02	−0·004, 0·04	0·003	−0·003, 0·007	−0·02	−0·03, 0·02
Total IGF (%)	759	−0·34	−3·94, 1·75	−0·15	−0·91, 0·43	1·9	−2·7, 3·0
IGFBP-3 (%)	736	6·81	−43·4, 50·8	3·11	−10·3, 14·4	−7·8	−67·6, 33·7

% energy, percentage of energy intake.
* Scaled to 0·1 g/kg body weight per d.
† Adjusted for age, BMI, race/ethnicity, education, biomarker-calibrated energy intake, alcohol intake, smoking, physical activity and hormone therapy use.
‡ Primary exposure and outcome variables ln-transformed to represent the expected percentage difference in IGF levels per percentage difference in protein intake.

uncalibrated energy and protein intake instead of calibrated one were also similar (data not shown). Lastly, we assessed whether the relationship of milk consumption with free IGF-I levels might be explained by Ca intake. There was no association between free IGF-I levels and Ca intake (β = 0, 95 % CI −0·002, 0·0003) for Ca from foods alone as well as from supplements (β = 0, 95 % CI −0·001, 0).

Discussion

The present study supports evidence from clinical trials and observational studies of a specific relationship between milk consumption and IGF-I levels, although in the present study this relationship was only statistically significant for free IGF-I levels. To our knowledge, this is the first study of the relationship between protein intake and milk consumption and free IGF-I levels. Our data, however, did not support reports from other epidemiological studies suggesting a positive relationship between higher overall total protein intake and higher total IGF-I levels[10–12,25]. Biomarker calibration of protein intake did not appreciably affect the results.

Prior observational studies[13,26–28] also support a positive association between protein intake, particularly from dairy sources, and total IGF-I levels[10–12,25]. Data from the European Prospective Investigation into Cancer and Nutrition

(EPIC, n 4731 men and women) indicate 2·5 and 2·4 % increases in IGF-I levels per 3 and 2 % increase in energy derived from total protein and dairy protein, respectively[26]. Given that each serving of dairy products equates to 601 kJ[29] and mean energy intake was 9876 kJ in the EPIC, 2 % energy equates to 198 kJ or approximately 1/3 dairy product serving. Assuming a linear dose response, this would equate to a 22·5 % increase in IGF-I levels with a three-serving dairy product increase per d, which is consistent with the strength of the association observed in the present study (17·3 %) and in clinical trials (range 8–52 %). Moreover, of the protein sources examined (plant, meat, dairy products, fish and eggs) in the EPIC, only dairy products were found to be significantly associated with IGF-I levels. The Nurses' Health Study, examining a comprehensive list of dietary correlates, has reported 9·3 and 7·7 % higher IGF-I levels by comparing highest v. lowest quintiles of total protein and dairy protein, respectively[13]. Similar to the present study, prior studies examining correlates of IGFBP-3 levels[15,26,27] have reported no significant associations with either total dietary protein intake or dairy protein intake.

In prior clinical trials, an additional 20–30 g of protein, often whey protein, or two to three milk servings daily have been shown to result in increases in IGF-I levels of 8–10 % that were sustained for up to 2 years[10–12,25]. This is well above

Table 4. Percent difference in insulin-like growth factor (IGF) levels per three-serving increase in daily dairy product and milk intake estimated from linear regression models†

(Percent differences and 95 % confidence intervals)

	Total dairy products		Milk	
	Percent difference	95 % CI	Percent difference	95 % CI
Free IGF (%)	11·0	−1·3, 25·3	18·6*	0·9, 39·3
Total IGF (%)	4·7	−0·8, 10·5	4·4	−3·0, 12·3
IGFBP-3 (%)	0·7	−2·1, 3·5	−1·1	−4·7, 2·7

IGFBP-3, insulin-like growth factor-binding protein-3.
* P<0·05.
† Estimated from ln-transformed outcome and primary exposure variables in multivariate linear regression models adjusted for age, BMI, race/ethnicity, education, energy intake, alcohol intake, smoking, physical activity and hormone therapy use. Estimates were scaled (base = 2·58 and 4·3 for dairy products and milk, respectively) to reflect intervention amount of three servings (average dairy product intake is 1·9 servings per d, so a three-serving increase per d is equal to 158 % increase, and average milk intake is 0·8 servings per d, so a three-serving increase is equal to 375 % increase).

typical average daily milk consumption of 0·7 servings for the US population aged 2 years and above (National Health and Nutrition Examination Survey (NHANES) 2003–8 data)[29]. In the present study, mean daily milk consumption was 0·8 (SD 1·0) servings, less than 25 % of women reported consuming three milk servings per d, and the upper limit of intake was 7·2 servings per d. Thus, the magnitude of the effect size observed in the present study is in keeping with that found in clinical trials and, for the first time, indicates that dairy product intake within the upper range of the typical American diet increases free IGF-I levels. Furthermore, a meta-analysis of eight randomised trials has reported a weighted mean difference of circulating IGF-I levels between milk intervention and control groups of 13·8 ng/ml (95 % CI 6·1, 21·5), and postulated components of milk that may increase IGF-I levels include its nutrient composition of high protein, fat, and Ca and/or contaminants or hormones such as oestrogens and IGF[30].

Collectively, the present study and prior reports are supportive of a particular association between IGF-I levels and milk consumption, which may involve components of milk other than protein. That is, the consistent positive association between IGF-I levels and milk consumption might be explained by a single non-protein nutrient that, nonetheless, may be correlated with total protein or milk protein (since these two factors were variably significant in some prior studies, but not in the present study or several others) or possibly a combination of milk components that include protein. For example, the Singapore Chinese Health Study has reported a positive association between Ca intake from foods and supplements with IGF-I and IGFBP-3 levels[31]. However, Ca intake, both from foods alone and in combination with Ca supplement use, was not significantly associated with free IGF-I, total IGF-I or IGFBP-3 levels in the present study.

The limitations of the present study should be considered while interpreting the data. Diet was characterised using a FFQ, which is limited by both differential and non-differential measurement errors[32]. For example, the participants were not queried about some items that could contribute to protein intake, such as protein supplements. However, using biomarkers of energy and protein intake, we were able to mitigate some important sources of measurement errors associated with energy and total protein intake[17]. Similar to representative samples of the US population[33], the range of protein, and particularly dairy product and milk, intake was not wide in this population. Since the dairy, cheese and yogurt food group variables are a summary of several FFQ items having different weights and protein content, we could not compute with certainty the protein intake for dairy, cheese and yogurt groups. However, our analyses of IGF axis protein levels and their relationship with protein consumption were null even when examined separately by source (animal, vegetable or milk protein), suggesting that protein consumption alone is not responsible for the observed positive association between milk consumption and free IGF-I levels.

In conclusion, the study data suggest that a three-serving increase in milk consumption per d is associated with an 18·6 % (95 % CI 0·9, 39·3 %) increase in free IGF-I levels. These data provide new insight into the factors that influence free IGF-I, presumably the most bioactive component of IGF-I, a hormone peptide that is the primary mediator of the effects of growth hormone and has been reported to be associated with several major health conditions, including diabetes, CVD and cognitive function in the elderly.

References

1. Houston DK, Nicklas BJ, Ding J, et al. (2008) Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 87, 150−155.
2. Beasley JM, LaCroix AZ, Neuhouser ML, et al. (2010) Protein intake and incident frailty in the Women's Health Initiative observational study. J Am Geriatr Soc 58, 1063−1071.
3. Froesch ER, Schmid C, Schwander J, et al. (1985) Actions of insulin-like growth factors. Annu Rev Physiol 47, 443−467.
4. 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.
5. Rajpathak SN, Gunter MJ, Wylie-Rosett J, et al. (2009) The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. Diabetes Metab Res Rev 25, 3−12.
6. Zapf J, Schmid C & Froesch ER (1984) Biological and immunological properties of insulin-like growth factors (IGF) I and II. Clin Endocrinol Metab 13, 3−30.
7. Kawai M & Rosen CJ (2010) The IGF-I regulatory system and its impact on skeletal and energy homeostasis. J Cell Biochem 111, 14−19.
8. Juul A, Holm K, Kastrup KW, et al. (1997) Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. J Clin Endocrinol Metab 82, 2497−2502.
9. Thissen JP, Ketelslegers JM & Underwood LE (1994) Nutritional regulation of the insulin-like growth factors. Endocr Rev 15, 80−101.

10. Schurch MA, Rizzoli R, Slosman D, et al. (1998) Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* **128**, 801–809.

11. Zhu K, Meng X, Kerr DA, et al. (2011) The effects of a two-year randomized, controlled trial of whey protein supplementation on bone structure, IGF-1, and urinary calcium excretion in older postmenopausal women. *J Bone Miner Res* **26**, 2298–2306.

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

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

14. Ma J, Giovannucci E, Pollak M, 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.

15. Kaklamani VG, Linos A, Kaklamani E, et al. (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.

16. Prentice RL, Mossavar-Rahmani Y, Huang Y, et al. (2011) Evaluation and comparison of food records, recalls, and frequencies for energy and protein assessment by using recovery biomarkers. *Am J Epidemiol* **174**, 591–603.

17. Neuhouser ML, Tinker L, Shaw PA, et al. (2008) Use of recovery biomarkers to calibrate nutrient consumption self-reports in the Women's Health Initiative. *Am J Epidemiol* **167**, 1247–1259.

18. The Women's Health Initiative Study Group (1998) Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* **19**, 61–109.

19. Gunter MJ, Hoover DR, Yu H, et al. (2008) A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. *Cancer Epidemiol Biomarkers Prev* **17**, 921–929.

20. Gunter MJ, Hoover DR, Yu H, et al. (2008) Insulin, insulin-like growth factor-I, endogenous estradiol, and risk of colorectal cancer in postmenopausal women. *Cancer Res* **68**, 329–337.

21. Gunter MJ, Hoover DR, Yu H, et al. (2009) Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* **101**, 48–60.

22. Patterson RE, Kristal AR, Tinker LF, et al. (1999) Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* **9**, 178–187.

23. Food and Nutrition Board, Institute of Medicine of the National Academies (2005) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academies Press.

24. Harrell FE (1991) *Regression Modeling Strategies*. New York, NY: Springer.

25. Hoppe C, Kristensen M, Boiesen M, et al. (2009) 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* **102**, 1047–1051.

26. Crowe FL, Key TJ, Allen NE, et al. (2009) The association between diet and serum concentrations of IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev* **18**, 1333–1340.

27. Vrieling A, Voskuil DW, Bueno de Mesquita HB, 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.

28. Norat T, Dossus L, Rinaldi S, et al. (2007) Diet, serum insulin-like growth factor-I and IGF-binding protein-3 in European women. *Eur J Clin Nutr* **61**, 91–98.

29. Fulgoni VL 3rd, Keast DR, Auestad N, et al. (2011) Nutrients from dairy foods are difficult to replace in diets of Americans: food pattern modeling and an analyses of the National Health and Nutrition Examination Survey 2003–2006. *Nutr Res* **31**, 759–765.

30. Qin LQ, He K & Xu JY (2009) Milk consumption and circulating insulin-like growth factor-I level: a systematic literature review. *Int J Food Sci Nutr* **60**, Suppl. 7, 330–340.

31. Probst-Hensch NM, Wang H, Goh VH, et al. (2003) 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. *Cancer Epidemiol Biomarkers Prev* **12**, 739–746.

32. Subar AF, Kipnis V, Troiano RP, et al. (2003) Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *Am J Epidemiol* **158**, 1–13.

33. Fulgoni VL 3rd (2008) Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. *Am J Clin Nutr* **87**, 1554S–1557S.