Circulating IGF-axis protein levels and their relation with levels of plasma adipocytokines and macronutrient consumption in women

Jeannette M. Beasley [a,*], Nicole M. Wedick [b], Swapnil N. Rajpathak [c], Xiaonan Xue [a], Michelle D. Holmes [d,g], Marc J. Gunter [e], Judith Wylie-Rosett [a], Thomas E. Rohan [a], Michael Pollak [f], Robert C. Kaplan [a], Frank B. Hu [d,g], Qi Sun [d,1], Howard D. Strickler [a,1]

[a] Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, United States
[b] Department of Medicine, University of Massachusetts Medical School, Worcester, MA, United States
[c] US Outcomes Research, US Medical Affairs, Merck & Co., North Wales, PA, United States
[d] Department of Epidemiology, Harvard School of Public Health, Boston, MA, United States
[e] Department of Epidemiology Biostatistics, School of Public Health, Imperial College, London, UK
[f] Department of Medicine and Oncology, Cancer Prevention Research Unit, Lady Davis Research Institute of Jewish General Hospital, McGill University, Montreal, Quebec, Canada
[g] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States

A B S T R A C T

Objective: Circulating free insulin-like growth factor (IGF)-I and its binding proteins, most notably, IGFBP-1 and IGFBP-2, have been prospectively associated with incident type 2 diabetes in women. However, little is known regarding the factors that may influence these IGF-axis protein levels. The aim is to study the relation of IGF-axis protein levels with adipcytokines, macronutrient consumption, and other factors related to diabetes.
Design: Fasting plasma from 558 controls enrolled in a nested case–control study within the Nurses' Health Study of incident type 2 diabetes in women was tested for: IGF-axis proteins (free and total IGF-I, IGFBP-1, IGFBP-2, IGFBP-3), adipocytokines (leptin, adiponectin, resistin), soluble leptin receptor (sOB-R), inflammatory factors (IL-18 and C-reactive protein (CRP)), insulin, and glycated hemoglobin (HbA1C).
Results: In multivariate models, each 1% increase in sOB-R (mean 34.9 ng/mL, standard deviation (SD) ± 11.3) was associated with −0.20% total IGF-I ($P = 0.0003$) and −0.42% free IGF-I ($P = 0.002$), as well as 0.73% higher IGFBP-1 ($P < 0.0001$) and 0.27% IGFBP-2 ($P = 0.003$). For example, a one SD change from the mean sOB-R level was associated with 11% lower free IGF-I. Insulin levels (mean 6.8 μU/mL ± 5.3) were inversely and adiponectin (mean 18.3 μg/mL ± 7.4) positively associated with IGFBP-1 and IGFBP-2 (all $P < 0.01$). Consumption of dairy protein, monounsaturated fats, and saturated fats, was also correlated with IGF-axis protein levels (all $P < 0.05$).
Conclusions: Several molecular factors and macronutrients were independently associated with plasma IGF-axis protein levels. Which of these, if any, reflect biologic relationships that can be intervened upon to influence IGF-axis protein concentrations warrants further investigation.

1. Introduction

The factors that influence circulating levels of insulin-like growth factor (IGF)-axis proteins are only partly understood. Most prior studies have focused on total IGF-I and its major binding protein, IGFBP-3 [1–3]. However, laboratory data have shown that free (i.e., unbound) IGF-I may be the most bioactive component of total IGF-I [4], and increasing clinical/epidemiologic evidence indicates that free IGF-I plays an important biologic role in normal growth, healthy aging, and the risk of disease, including diabetes [5]. In addition to IGF-I, the IGF-axis also includes IGF-II, a related growth factor, and six IGFBPs (IGFBP-1 to -6) [6,7]. While these IGFBPs were originally considered passive transport proteins, they are now understood to have important IGF-I-independent effects [8]. IGFBP-1 and -2, for example, can inhibit preadipocyte proliferation and differentiation independent of IGF-I, and overexpression of these binding proteins in rodent models was found to decrease the risk of obesity and insulin resistance [9,10]. The most abundant IGFBP in circulation, IGFBP-3, binds >90% of all circulating IGF-I. A large prospective cohort study of women found strong inverse associations between baseline serum levels of IGFBP-1 and -2 with risk of incident diabetes, whereas IGFBP-3 was positively related to diabetes risk, and the relation of free IGF-I and diabetes varied by insulin level [11].

* Corresponding author at: Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, United States. Tel.: +1 718 430 3089; fax: +1 718 430 8780.
E-mail address: jeannette.beasley@einstein.yu.edu (J.M. Beasley).
[1] The two senior authors contributed equally to this study.

While correlates of total IGF-I and IGFBP-3 levels have been previously reported, little is known regarding the factors that may influence circulating IGFBP-1 and -2, or free IGF-I levels. Furthermore, no studies have concurrently assessed the relative strength of these correlates with IGF-axis protein levels, adjusted for one another. Therefore, this study for the first time presents an analysis that addresses a wide range of the major factors that have been reported to influence levels of the IGF-axis across multiple categories to determine their relative independent associations with IGF-axis protein levels. These factors included demographic and behavioral variables, macronutrient consumption, and levels of adipocytokines, soluble leptin receptor (sOB-R), insulin, glycated hemoglobin (HbA1C%), IL-18, and C-reactive protein (CRP) with IGF-axis protein levels.

These association data represent an important step in determining the major biologic determinants of the levels of IGF-axis — proteins that have been associated with risk of diabetes (as well as other conditions, such as cancer and heart disease). The findings should help inform additional efforts, including laboratory studies, to identify the relevant molecular pathways. The results are also important in determining which factors may act as confounders in molecular epidemiologic studies and therefore should be measured along with IGF-axis proteins.

2. Methods

2.1. Study population and specimens

The Nurses' Health Study (NHS) cohort was established in 1976 with 121,700 female registered nurses. The subjects were aged 30–55 years and completed a mailed questionnaire that addressed their medical history and lifestyle characteristics at baseline, and since then on a biennial basis. During 1989–1990, per request, blood samples were returned from 32,826 women who had in the prior follow-up period reported that they were free of diabetes, coronary heart disease, stroke or cancer. The majority (97%) of blood samples were received within 26 h of blood draw. Immediately upon arrival, whole blood samples were centrifuged and aliquoted into cryotubes as plasma, buffy coat, and erythrocytes, which were then stored in liquid nitrogen freezers at ≤ -130 °C. The current analysis included 558 controls from a nested case–control study of incident type 2 diabetes in the NHS who had complete data on IGF-axis proteins, lifestyle, and molecular factors and had fasted ≥ 8 h. The study was restricted to controls since they are more representative of the cohort as a whole than incident cases. Controls were selected by individual matching to participants who reported a diagnosis of diabetes during follow-up; they were matched on age (± 1 year), date of blood draw (± 3 months), and race. In keeping with standard nested case–control design, while the controls had not developed type 2 diabetes, cardiovascular disease, or cancer by the time the matched case was diagnosed, there was no restriction regarding whether or not the control might later have developed diabetes. Each participant completed informed consent, and the study protocol was approved by the institutional review and human subject boards of the Brigham and Women's Hospital, Harvard School of Public Health, and the Albert Einstein College of Medicine.

2.2. Laboratory assays

Total IGF-I, free IGF-I, and IGFBP-1, -2 and -3 (both intact and fragmented) levels were measured using ELISAs from Diagnostic Systems Laboratories (Webster, TX). The average intra-assay coefficient of variation was 5% for total IGF-I and the three IGFBPs and 13% for free IGF-I [11]. As previously reported, the coefficients of variation for the additional assays were: 3.8% for CRP, 9.5% for fasting insulin, 3.8% for HbA1c, 8.9% for adiponectin, 7.7% for the leptin, 7.3% for the sOB-R assay, 2.5% for resistin, 7.7% for leptin, 7.3% for sOB-R, and 7.3% for IL-18 [12–14]. All assays were conducted using well established commercial assays, per manufacturer recommendations. CRP levels were measured via a high-sensitivity latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Newark, DE). Insulin levels were measured with a double antibody system with <0.2% cross-reactivity between insulin and its precursors (Linco Research, St. Louis, MO); HbA1c using the well-established Hitachi 911 Analyzer (Roche Diagnostics, Indianapolis, IN); resistin by enzyme-linked immunosorbent assay (ELISA) (Linco Research, St. Charles, Missouri); total leptin by radioimmunoassay (Millipore, Billerica, MA); adiponectin (ALPCO Diagnostics, Salem, New Hampshire), sOB-R (R&D Systems, Minneapolis, MN), and IL-18 concentration (MBL, Naka-ku Nagoya, Japan) were each measured by ELISA.

2.3. Assessment of lifestyle factors

Major lifestyle risk factors for chronic diseases, such as current weight, cigarette smoking, physical activity, menopausal status, and use of postmenopausal hormone therapy (HT) (e.g., oral estrogen alone, or combined estrogen and progesterone) were obtained using the mailed questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Diet was assessed using a validated semi-quantitative food frequency questionnaire. Nutrient intake was calculated based on responses to the food frequency questionnaire; the nutrient content of foods was derived from the Harvard Food Composition Database [15].

2.4. Statistical analysis

Questionnaire and laboratory data were summarized using means and standard deviations for continuous variables and percentages for

Table 1
Study population characteristics (n = 558).

	Mean ± SD or n (%)
Demographic and behavioral variables	
Age, years	56 ± 7
White/Caucasian (versus Other)	546 (97.9)
Postmenopausal	436 (78.1)
Current hormone use[a]	183 (32.8)
Current smoker	62 (11.2)
Body mass index ($kg/m^{2)}$	25.1 ± 4.3
Physical activity, MET-h/week	15.0 ± 17.5
Daily energy and macronutrient intake	
Energy intake, kcal	1766 ± 494
Alcohol intake, % kcal	2.1 ± 3.8
Carbohydrate, %kcal	50.4 ± 8.6
Protein, %kcal	18.9 ± 3.2
Dairy protein, % kcal	3.7 ± 1.9
Total fat, %kcal	31.2 ± 6.0
Saturated fat, % kcal	10.5 ± 2.5
Trans fat, % kcal	1.5 ± 0.6
Monounsaturated fat, %kcal	12.0 ± 2.7
Polyunsaturated fat, %kcal	5.9 ± 1.6
Biomarkers	
IGF-I, total, ng/mL	152.3 ± 50.7
IGF-I, free, ng/mL	0.5 ± 0.3
IGFBP-1, ng/mL	41.5 ± 28.1
IGFBP-2, ng/mL	593.2 ± 352.0
IGFBP-3, ng/mL	4676.8 ± 1001.7
CRP, mg/dL	1.4 ± 3.6
Adiponectin, μg/mL	18.3 ± 7.4
Resistin, ng/mL	17.5 ± 11.9
Leptin, ng/mL	19.5 ± 13.0
Soluble leptin receptor (sOB-R), ng/mL	34.9 ± 11.3
Insulin, μU/mL	6.8 ± 5.3
HbA1C, %	5.2 ± 0.4
IL-18, pg/mL	300.7 ± 187.3

[a] Current hormone therapy (HT) (versus past/never), body mass index (BMI).

Table 2
Percent difference in IGF-axis protein levels per unit increase (or per level increase) in sociodemographic/lifestyle characteristics using linear regression models (n = 558).

Characteristic	Total IGF-I, %	Free IGF-I, %	IGFBP-1, %	IGFBP-2, %	IGFBP-3, %
Age					
Model 1	**−1.4 (−1.7 to −1.0)**	**−1.8 (−2.7 to −1.0)**	**1.6 (0.5 to 2.6)**	**0.9 (0.2 to 1.6)**	−0.2 (−0.4 to 0.1)
Model 2	**−0.8 (−1.2 to −0.3)**	−0.2 (−1.3 to 0.9)	0.1 (−0.9 to 1.0)	**1.3 (0.5 to 2.2)**	−0.2 (−0.5 to 0.1)
Model 3	**−0.8 (−1.3 to −0.4)**	−0.3 (−1.4 to 0.8)	−0.2 (−1.1 to 0.8)	**1.5 (0.7 to 2.2)**	−0.3 (−0.6 to 0)
Caucasian					
Model 1	4.9 (−18.0 to 34.2)	**−35.5 (−57.1 to −3.0)**	43.1 (−10.0 to 127.6)	**43.3 (2.0 to 101.2)**	21.2 (−5.0 to 54.6)
Model 2	5.8 (−15.6 to 32.7)	**−31.5 (−51.9 to −2.4)**	12.4 (−26.2 to 71.2)	30.3 (−6.1 to 80.8)	22.7 (−3.9 to 56.6)
Model 3	0.9 (−15.7 to 20.4)	**−35.4 (−53.7 to −10.0)**	18.8 (−17.7 to 71.5)	23.6 (−9.4 to 68.7)	21.3 (−1.4 to 49.2)
Postmenopausal					
Model 1	**−14.2 (−20.9 to −7.0)**	**−33.5 (−44.5 to −20.4)**	**42.6 (14.8 to 77.1)**	−10.7 (−23.8 to 4.6)	1.2 (−4.2 to 6.9)
Model 2	−5.9 (−13.3 to 2.2)	−16.5 (−30.3 to 0)	10.7 (−8.1 to 33.3)	−5.8 (−18.1 to 8.2)	5.2 (−0.7 to 11.4)
Model 3	−4.5 (−11.5 to 3.1)	−15.2 (−28.9 to 1.3)	10.5 (−6.5 to 30.5)	−2.5 (−13.4 to 9.9)	5.7 (0 to 11.8)
Current HT use					
Model 1	**−21.4 (−25.8 to −16.9)**	**−45.1 (−52.5 to −36.6)**	**82.7 (60.6 to 107.7)**	**−16.5 (−25.4 to −6.5)**	**−7.8 (−11.5 to −4.0)**
Model 2	**−20.7 (−25.3 to −15.8)**	**−42.1 (−50.3 to −32.6)**	**58.1 (40.8 to 77.6)**	**−22.4 (−30.4 to −13.4)**	**−8.2 (−12.1 to −4.0)**
Model 3	**−16.9 (−21.6 to −12.0)**	**−34.7 (−44.1 to −23.7)**	**40.9 (25.2 to 58.7)**	**−23.2 (−30.4 to −15.2)**	**−7.5 (−11.5 to −3.3)**
Current smoking					
Model 1	6.6 (−1.4 to 15.2)	**30.1 (8.5 to 55.9)**	−10.5 (−26.3 to 8.6)	**15.2 (0.1 to 32.6)**	0.3 (−5.0 to 5.9)
Model 2	5.8 (−1.5 to 13.6)	**26.5 (7.8 to 48.5)**	−6.3 (−19.1 to 8.6)	**14.8 (2.5 to 28.7)**	−0.2 (−5.4 to 5.4)
Model 3	5.6 (−2.4 to 14.2)	18.4 (−0.9 to 41.5)	11.0 (−4.8 to 29.5)	**25.4 (13.1 to 39.1)**	0.3 (−5.3 to 6.3)
BMI					
Model 1	0.1 (−0.6 to 0.7)	1.7 (0.2 to 3.1)	**−9.6 (−10.9 to −8.2)**	**−5.7 (−6.7 to −4.8)**	**0.6 (0.1 to 1.0)**
Model 2	−0.2 (−0.8 to 0.4)	0.8 (−0.5 to 2.1)	**−8.8 (−10.1 to −7.5)**	−6.1 (−7.1 to −5.0)	**0.5 (0.1 to 0.9)**
Model 3	−0.6 (−1.3 to 0.2)	0.2 (−1.6 to 2.1)	**−4.8 (−6.5 to −3.1)**	−0.9 (−2.3 to 0.6)	−0.1 (−0.7 to 0.6)
Physical activity					
Model 1	0.1 (0 to 0.3)	0 (−0.4 to 0.4)	**0.6 (0.2 to 1.0)**	0.3 (0 to 0.5)	0 (−0.1 to 0.1)
Model 2	0.1 (0 to 0.3)	0.1 (−0.3 to 0.4)	0.3 (0 to 0.7)	0.1 (−0.1 to 0.4)	0 (−0.1 to 0.1)
Model 3	0 (−0.1 to 0.2)	0 (−0.4 to 0.3)	0.2 (−0.1 to 0.5)	−0.1 (−0.3 to 0.1)	0 (−0.1 to 0.1)

Hormone therapy (HT) (versus past/never), body mass index (BMI), Model 1 adjusted for age and race/ethnicity; Model 2 adjusted for Model 1 + menopausal status (pre/post), current HT use (versus past/never), current smoking (versus past/never), BMI, and physical activity (continuous, METs/week); Model 3: Model 2 + CRP, adiponectin, resistin, leptin, sOb-R, insulin, HbA1C%, IL-18 + energy (kcal) + alcohol %kcal + dairy protein %kcal + fat %kcal (*trans* fat, saturated fat, polyunsaturated fat, monounsaturated fat).
The bold values denote statistical significance at $P < 0.05$.

categorical variables. Analysis of the associations between IGF-axis protein levels and other factors was conducted using linear regression models. In these models, IGF levels were ln-transformed to help normalize their distributions, and to allow the beta coefficients of the regression models to be interpreted as percent change in IGF-axis protein levels. For most variables, the percent change in IGF-axis proteins was determined per unit change in the exposure variable (e.g., age, physical activity, macronutrient consumption). Specifically, the following linear regression model was assumed:

$$\log Y = \beta_0 + \beta X + \gamma Z$$

where Z is a covariate vector. Therefore, for every unit increase in X (or one level increase for ordinal variables), the percent change in Y is $\delta = (e^{\beta} - 1) * 100$. For b units of change in X, the percent change in Y is $(e^{b\beta} - 1) * 100 = ((1 + \delta/100)^b - 1) * 100$.

As with the IGF-axis protein levels, all additional serologic data were ln-transformed. Thus, the percent change in IGF-axis proteins was determined per percent change in each serologic exposure variable.

Specifically, the following linear regression model was assumed:

$$\log Y = \beta_0 + \beta \log X + \gamma Z$$

where Z is a covariate vector. Therefore, for every percent increase in X, the percent change in Y is $\delta = (1.01^{\beta} - 1) * 100$. For θ percent increase in X, the percent change in Y is $((1 + \theta)^{\beta} - 1) * 100 = ((1 + \theta)^{\log(1 + \delta/100)/\log(1.01)} - 1) * 100$.

Multivariate linear regression was used to study the factors associated with IGF-axis protein levels. Three models are presented: *Model 1* included age and race/ethnicity as covariates; *Model 2* additionally included menopausal status, current hormone use, current smoking, BMI, and physical activity; and *Model 3* (the "fully adjusted" model) included all covariates in Model 2 as well as CRP, adiponectin, resistin, leptin, sOB-R, insulin, HbA1C%, IL-18, energy (kcal), alcohol (% of total energy), dairy protein (% of total energy), and fat (% of total energy).

To assess the proportion of variability explained by significant determinants of IGF, we took the difference in R^2 between models including all statistically significant predictors of each IGF protein and models including age, race, and the IGF protein.

3. Results

Table 1 shows selected characteristics of the 558 subjects at the time of blood collection. The mean age of this population was 56 ± 7 years, 436 (78%) were postmenopausal and 183 (33%) reported current hormone therapy (HT) use. Half of the participants were overweight or obese, and the mean BMI was 25.1 ± 4.3 kg/m^2. There were 62 (11%) who reported current cigarette smoking. Table 1 also shows the mean macronutrient consumption, IGF-axis protein and adipocytokines levels, as well as HbA1C% and insulin levels.

Older age was inversely associated with levels of total and free IGF-I, and positively associated with IGFBP-1 and IGFBP-2 levels, after adjustment for race/ethnicity (Model 1; Table 2). Following adjustment for additional covariates (Models 2 and 3), however, only total IGF-I and IGFBP-2 were significantly related to age. There was also a strong positive association between current smoking and IGFBP-2 (25.4% higher vs. never/past smoking; 95% CI: 13.1 to 39.1) in Model 3, but smoking was not significantly associated with other IGF-axis proteins. BMI was inversely associated with IGFBP-1 (−4.8%; 95% CI: −6.5 to −3.1) in the fully adjusted model. No associations with physical activity were observed.

The strongest association of IGF-axis protein levels related to demographic and behavioral factors was with HT use. Every one of the IGF-axis proteins measured was significantly different among current HT-users versus past or non-users in the fully adjusted model, including a 34.7% (95% CI: 44.1 to 23.7) reduction in free IGF-I, as well as reductions in total IGF-I (16.9%; 95% CI: 21.6 to 12.0), IGFBP-2 (23.2%; 95% CI: 30.4 to 15.2), and IGFBP-3 (7.5%; 95% CI: 11.5 to 3.3), and a 40.9% (95% CI: 25.2 to 58.7) increase in IGFBP-1. Given these strong associations with HT use, we conducted sensitivity analyses for each of the other analyses presented here (including all demographic, behavioral, macronutrient, and molecular factors studied), in which we excluded women reporting current hormone use. However, the strength and significance of the associations were not meaningfully altered (data not shown).

Table 3 shows the percent change in IGF-axis protein levels per unit change in energy and macronutrient consumption. After accounting for other covariates, only the consumption of dairy protein, monounsaturated fat, and saturated fat, as a percentage of total calories, was significantly associated with IGF-axis protein levels. Most notably, each percent increase in calories derived from dairy protein (mean 3.7%kcal, SD ± 1.9%) was associated with a 3.8% (95% CI: 2.3 to 5.2%) higher total IGF-I and 2.5% (95% CI: 1.4 to 3.6%) higher IGFBP-3 level. Monounsaturated fat consumption (12.0%kcal, SD ± 2.7%) also had a positive association with IGF-I and IGFBP-3, whereas saturated fat consumption (10.5%kcal, SD ± 2.5%) was inversely associated these IGF-axis proteins, and was positively associated with IGFBP-2.

To provide greater perspective regarding the likely impact of changes in each macronutrient and serologic variable on IGF-axis protein levels, we graphically depicted these associations. Specifically, Fig. 1 summarizes the percent change in levels for a given IGF-axis protein per one SD increase from the mean in each of the serologic and macronutrient variables significantly associated with that IGF-axis protein. A one SD increase from the mean level of dairy protein consumption, for example, correlated with an increase of 7.3% in IGF-I and 4.8% in IGFBP-3 levels. There was also a similar effect on these proteins per SD change in monounsaturated fat consumption, and in the opposite direction per SD change in saturated fat consumption.

Several strong associations were observed between the serum analytes that were measured and IGF-axis proteins levels (Table 4). Most notably, sOB-R levels were broadly distributed (mean 34.9 ng/mL),

Table 3
Percent difference in IGF-axis protein levels per unit increase in dietary factors from linear regression models (n = 558).

Lifestyle characteristic	Total IGF-I, %	Free IGF-I, %	IGFBP-1, %	IGFBP-2, %	IGFBP-3, %
Energy, kcal					
Model 1	3.8 (−5.9 to 14.5)	24.5 (−0.8 to 56.1)	−16.3 (−34.5 to 7.1)	−5.4 (−20.9 to 13.1)	2.8 (−3.7 to 9.7)
Model 2	2.9 (−6.6 to 13.3)	22.5 (−1.6 to 52.4)	−2.8 (−21.2 to 19.9)	0 (−14.9 to 17.5)	1.9 (−4.7 to 8.9)
Model 3	1.0 (−8.0 to 10.9)	17.2 (−5.4 to 45.3)	2.5 (−15.1 to 23.9)	1.0 (−12.2 to 16.1)	1.1 (−5.2 to 7.9)
Alcohol, % kcal					
Model 1	**−0.8 (−1.4 to −0.2)**	−1.4 (−3.3 to 0.6)	0.5 (−1.5 to 2.6)	0.8 (−0.4 to 2.0)	0 (−0.5 to 0.6)
Model 2	**−0.8 (−1.4 to −0.2)**	−1.5 (−3.1 to 0.2)	−0.3 (−1.9 to 1.4)	0.1 (−1.0 to 1.2)	0.1 (−0.5 to 0.6)
Model 3	−0.5 (−1.1 to 0.1)	−1.1 (−2.8 to 0.6)	−0.9 (−2.5 to 0.7)	−0.8 (−1.8 to 0.2)	0.3 (−0.2 to 0.8)
Carbohydrate, % kcal[a]					
Model 1	0.2 (−0.1 to 0.6)	0.5 (−0.3 to 1.3)	**0.9 (0.1 to 1.8)**	0.4 (−0.2 to 1.0)	−0.1 (−0.4 to 0.1)
Model 2	0.2 (−0.1 to 0.6)	0.7 (0 to 1.4)	0.4 (−0.2 to 1.1)	0.2 (−0.4 to 0.7)	−0.1 (−0.4 to 0.2)
Protein, total, % kcal[a]					
Model 1	0.6 (−0.3 to 1.5)	−1.4 (−3.5 to 0.7)	−0.2 (−2.3 to 2.0)	−1.7 (−3.2 to −0.2)	0.6 (−0.1 to 1.3)
Model 2	0.8 (0 to 1.7)	−0.9 (−2.8 to 1.0)	0 (−1.7 to 1.8)	−1.0 (−2.3 to 0.4)	0.6 (−0.1 to 1.3)
Protein, dairy, % kcal					
Model 1	**2.2 (0.7 to 3.6)**	−0.1 (−3.4 to 3.4)	3.5 (0 to 7.2)	1.0 (−1.7 to 3.8)	**1.2 (0.2 to 2.2)**
Model 2	**2.2 (0.8 to 3.6)**	0.4 (−2.9 to 3.8)	2.6 (−0.4 to 5.6)	0.6 (−2.0 to 3.3)	**1.2 (0.2 to 2.2)**
Model 3	**3.8 (2.3 to 5.2)**	2.1 (−1.7 to 6.0)	0.2 (−2.9 to 3.5)	−1.6 (−4.0 to 0.9)	**2.5 (1.4 to 3.6)**
Fat, total, % kcal[a]					
Model 1	−0.2 (−0.7 to 0.3)	0.1 (−1.0 to 1.1)	−2.0 (−3.1 to −0.8)	−0.5 (−1.3 to 0.3)	0 (−0.3 to 0.4)
Model 2	−0.2 (−0.7 to 0.2)	−0.3 (−1.3 to 0.7)	−0.8 (−1.8 to 0.2)	0 (−0.7 to 0.8)	0 (−0.3 to 0.3)
Fat, monounsaturated, %kcal					
Model 1	−0.4 (−1.5 to 0.6)	0.3 (−2.0 to 2.7)	**−4.4 (−6.8 to −1.8)**	−1.4 (−3.1 to 0.4)	0.2 (−0.5 to 1.0)
Model 2	−0.5 (−1.5 to 0.6)	−0.5 (−2.7 to 1.7)	−2.0 (−4.2 to 0.2)	−0.4 (−2.0 to 1.3)	0.1 (−0.6 to 0.9)
Model 3	**1.9 (0.1 to 3.8)**	0.9 (−3.2 to 5.3)	−2.9 (−6.9 to 1.4)	−2.2 (−5.1 to 0.7)	**1.8 (0.4 to 3.2)**
Fat, polyunsaturated, %kcal					
Model 1	1.3 (−0.5 to 3.2)	2.3 (−1.3 to 6.1)	−4.2 (−8.3 to 0)	−0.4 (−3.5 to 2.7)	0.8 (−0.4 to 2.0)
Model 2	1.3 (−0.4 to 3.0)	2.0 (−1.4 to 5.5)	−2.1 (−5.5 to 1.3)	0.6 (−2.4 to 3.8)	0.7 (−0.5 to 1.8)
Model 3	1.6 (−0.3 to 3.6)	1.8 (−2.2 to 6.0)	−0.9 (−4.9 to 3.2)	1.0 (−2.6 to 4.6)	0.4 (−1.0 to 1.8)
Fat, saturated, %kcal					
Model 1	−1.1 (−2.2 to 0.1)	−0.7 (−3.1 to 1.8)	**−3.3 (−6.0 to −0.4)**	−0.5 (−2.6 to 1.6)	−0.3 (−1.1 to 0.5)
Model 2	−1.2 (−2.3 to −0.1)	−1.7 (−4.0 to 0.5)	−0.6 (−2.9 to 1.7)	0.5 (−1.3 to 2.3)	−0.4 (−1.3 to 0.4)
Model 3	**−3.1 (−4.8 to −1.4)**	−3.7 (−7.5 to 0.2)	1.7 (−2.1 to 5.7)	**2.7 (0.2 to 5.4)**	**−2.2 (−3.4 to −0.9)**
Fat, *trans*, %kcal					
Model 1	−0.6 (−5.0 to 3.9)	**11.4 (0.2 to 23.8)**	**−16.7 (−26.3 to −5.8)**	−4.1 (−12.6 to 5.3)	0.1 (−3.1 to 3.4)
Model 2	−3.2 (−7.4 to 1.3)	2.0 (−8.1 to 13.1)	−3.6 (−13.3 to 7.3)	−2.0 (−9.7 to 6.5)	−1.0 (−4.1 to 2.3)
Model 3	−1.4 (−6.7 to 4.3)	6.7 (−5.4 to 20.4)	2.2 (−10.1 to 16.2)	−0.8 (−9.8 to 9.1)	−0.9 (−4.9 to 3.2)

Model 1: includes age and race/ethnicity.
Model 2: Model 1 + menopausal status (pre/post), current hormone use (versus past/never), current smoking (versus past/never), BMI, and physical activity (continuous, METs/week).
Model 3: Model 2 + CRP, adiponectin, resistin, leptin, sOb-R, insulin, HbA1C%, IL-18 + energy (kcal) + alcohol %kcal + dairy protein %kcal + fat %kcal (*trans* fat, saturated fat, polyunsaturated fat, monounsaturated fat).
The bold values denote statistical significance at $P < 0.05$.
[a] Carbohydrate, total protein, and total fat were not included in the final model (Model 3).

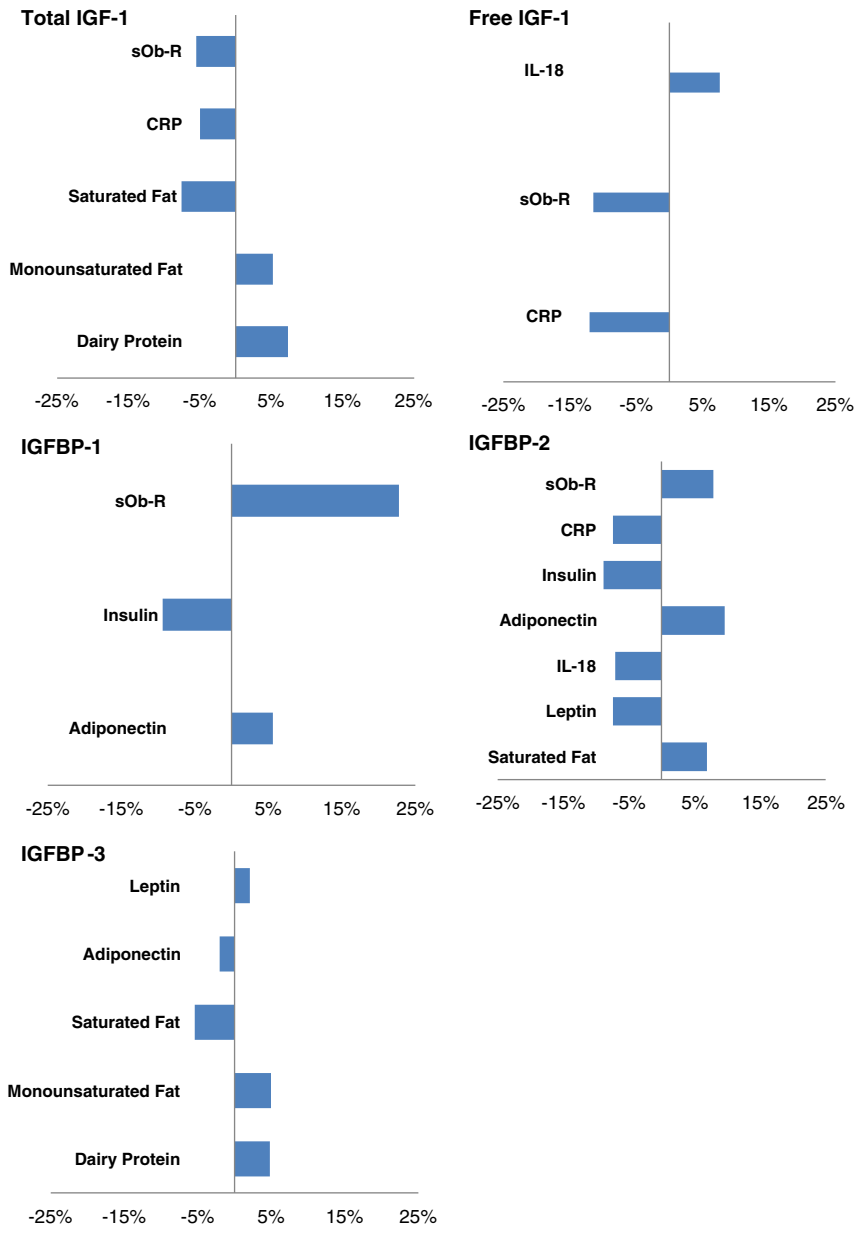

Fig. 1. Percent difference in IGF-axis protein levels per standard deviation difference in molecular and dietary factors, n = 558. Estimated from regression models adjusting for age, race/ethnicity, BMI, menopausal status, current hormone use, current smoking, BMI, physical activity, CRP, adiponectin, resistin, leptin, sOb-R, insulin, HbA1C%, IL-18 + energy (kcal) + alcohol %kcal + dairy protein %kcal + fat %kcal (*trans* fat, saturated fat, polyunsaturated fat, monounsaturated fat).

with a standard deviation (±11.3) equal to 32% of the mean, and were associated with all IGF-axis proteins except IGFBP-3. In multivariate models, each 1% increase in sOB-R was associated with 0.20% (95% CI 0.31 to 0.09) lower total IGF-I and 0.42% (95% CI 0.68 to 0.17) lower free IGF-I, as well as 0.73% (95% CI 0.51 to 0.95) higher IGFBP-1 and 0.27% (95% CI 0.09 to 0.45) IGFBP-2. As shown in Fig. 1, a one SD increase from the mean sOB-R level was associated with 5.5% and 11.4% lower total and free IGF-I, respectively, as well as 22.8% and 7.9% greater IGFBP-1 and IGFBP-2.

Similar to sOB-R, adiponectin (18.3 μg/mL ± 7.4) was positively associated with IGFBP-1 and -2 levels, and also had a modest inverse association with IGFBP-3 (Table 4 and Fig. 1). Conversely, leptin (19.5 ng/mL ± 13.0) was inversely associated with IGFBP-2 levels, and had a modest positive association with IGFBP-3. IL-18 (300.7 pg/mL ± 187.3) was significantly associated with free IGF-I and with IGFBP-2. Resistin had no significant associations with IGF-axis proteins. Insulin (6.8 μU/mL ± 5.3) was inversely associated with both IGFBP-1 (−0.17%; 95% CI: −0.27% to −0.08%) and IGFBP-2 (−0.16%; 95% CI: −0.23% to −-0.09%), whereas CRP (mean 1.4 mg/dL ± 3.6) had inverse associations with total and free IGF-I and IGFBP-2.

The R^2 values for models including statistically significant predictors of each IGF protein were 0.29 for IGF-I, 0.21 for free IGF-I, 0.46 for IGFBP-1, 0.42 for IGFBP-2, and 0.10 for IGFBP-3. The proportion of variability in each biomarker explained by the significant predictors other than age and race, calculated as the difference in R^2 with models including only age and race, was 0.21 for IGF-I, 0.18 for free IGF-I, 0.44 for IGFBP-1, 0.40 for IGFBP-2, and 0.09 for IGFBP-3.

4. Discussion

This is the first study, to our knowledge, to conduct a detailed assessment of the factors associated with circulating levels of IGFBP-1 and -2, and free IGF-I, three IGF-axis proteins that are strongly associated with risk of incident type 2 diabetes, according to recent studies [11,16–18].

Table 4
Percent difference in IGF-axis protein levels per percent increase in biomarker estimated from linear regression models (n = 558).

Biomarker, % difference	Total IGF-I, % difference	Free IGF-I, % difference	IGFBP-1, % difference	IGFBP-2, % difference	IGFBP-3, % difference
CRP					
Model 1	**−0.05 (−0.06 to −0.03)**	**−0.10 (−0.14 to −0.06)**	−0.03 (−0.07 to 0.01)	**−0.10 (−0.13 to −0.08)**	0 (−0.02 to 0.01)
Model 2	**−0.04 (−0.05 to −0.02)**	**−0.09 (−0.13 to −0.06)**	0.02 (−0.02 to 0.05)	**−0.05 (−0.08 to −0.03)**	−0.01 (−0.02 to 0.01)
Model 3	**−0.04 (−0.06 to −0.02)**	**−0.10 (-0.14 to −0.06)**	0.02 (−0.01 to 0.06)	**−0.06 (−0.09 to −0.03)**	−0.01 (−0.02 to 0)
Adiponectin					
Model 1	**−0.06 (−0.12 to −0.01)**	**−0.18 (-0.30 to −0.05)**	**0.64 (0.50 to 0.78)**	**0.49 (0.38 to 0.59)**	**−0.07 (−0.11 to −0.03)**
Model 2	−0.05 (−0.11 to 0)	−0.09 (−0.22 to 0.04)	**0.33 (0.21 to 0.45)**	**0.37 (0.27 to 0.47)**	**−0.06 (−0.10 to −0.02)**
Model 3	−0.03 (−0.09 to 0.02)	−0.02 (−0.15 to 0.11)	**0.16 (0.05 to 0.28)**	**0.27 (0.18 to 0.36)**	**−0.06 (−0.10 to −0.02)**
Resistin					
Model 1	−0.02 (−0.08 to 0.04)	0.05 (−0.08 to 0.18)	**−0.24 (−0.38 to −0.09)**	**−0.14 (−0.24 to −0.04)**	−0.01 (−0.05 to 0.03)
Model 2	−0.02 (−0.08 to 0.03)	0 (−0.12 to 0.12)	0 (−0.14 to 0.13)	−0.03 (−0.14 to 0.08)	−0.02 (−0.07 to 0.02)
Model 3	−0.02 (−0.07 to 0.03)	−0.01 (−0.13 to 0.11)	0.03 (−0.10 to 0.15)	0.01 (−0.08 to 0.10)	−0.02 (−0.06 to 0.03)
Leptin					
Model 1	0 (−0.04 to 0.03)	0.04 (−0.05 to 0.12)	**−0.46 (−0.54 to 0.37)**	**−0.38 (−0.43 to −0.32)**	**0.04 (0.01 to 0.06)**
Model 2	0.01 (−0.03 to 0.06)	0 (−0.11 to 0.11)	**−0.15 (−0.24 to −0.05)**	**−0.25 (−0.33 to −0.18)**	**0.04 (0 to 0.07)**
Model 3	0.02 (−0.02 to 0.07)	0.02 (−0.09 to 0.13)	−0.06 (−0.15 to 0.04)	**−0.15 (0.22 to −0.07)**	**0.04 (0 to 0.08)**
sOb-R					
Model 1	**−0.21 (−0.30 to −0.12)**	**−0.58 (−0.81 to −0.35)**	**1.46 (1.27 to 1.66)**	**0.65 (0.49 to 0.81)**	**−0.10 (−0.17 to −0.04)**
Model 2	**−0.17 (−0.27 to −0.06)**	**−0.33 (−0.58 to −0.08)**	**0.84 (0.64 to 1.04)**	**0.52 (0.34 to 0.71)**	−0.06 (−0.13 to 0.01)
Model 3	**−0.20 (−0.31 to −0.09)**	**−0.42 (−0.68 to −0.17)**	**0.73 (0.51 to 0.95)**	**0.27 (0.09 to 0.45)**	−0.04 (−0.11 to 0.04)
Insulin					
Model 1	0.01 (−0.03 to 0.05)	0.06 (−0.03 to 0.16)	**−0.44 (−0.56 to −0.32)**	**−0.33 (−0.40 to −0.26)**	**0.02 (0 to 0.05)**
Model 2	0.01 (−0.04 to 0.05)	0.03 (−0.06 to 0.11)	**−0.24 (−0.32 to −0.15)**	**−0.23 (−0.30 to −0.16)**	0.01 (−0.02 to 0.04)
Model 3	−0.03 (−0.07 to 0.02)	−0.07 (−0.18 to 0.04)	**−0.17 (−0.27 to −0.08)**	**−0.16 (−0.23 to −0.09)**	0 (−0.04 to 0.03)
HbA1C%					
Model 1	0.33 (−0.08 to 0.74)	**1.58 (0.62 to 2.56)**	**−2.67 (−3.68 to −1.64)**	**−1.68 (−2.45 to −0.90)**	0.16 (−0.12 to 0.45)
Model 2	0.25 (−0.16 to 0.65)	**1.16 (0.23 to 2.10)**	**−1.12 (−1.94 to −0.28)**	**−1.08 (−1.77 to −0.40)**	0.05 (−0.25 to 0.34)
Model 3	−0.07 (−0.56 to 0.41)	0.58 (−0.54 to 1.72)	0.14 (−0.84 to 1.13)	−0.58 (−1.29 to 0.14)	−0.07 (−0.42 to 0.29)
IL-18					
Model 1	−0.02 (−0.08 to 0.04)	0.09 (−0.06 to 0.23)	**−0.20 (−0.36 to −0.04)**	**−0.25 (−0.36 to −0.13)**	0.01 (−0.03 to 0.05)
Model 2	−0.01 (−0.07 to 0.05)	0.09 (−0.06 to 0.23)	−0.06 (−0.19 to 0.08)	**−0.14 (−0.25 to −0.03)**	0 (−0.04 to 0.04)
Model 3	0.02 (−0.04 to 0.08)	**0.15 (0.01 to 0.29)**	−0.10 (−0.23 to 0.02)	**−0.15 (−0.24 to −0.05)**	0.01 (−0.04 to 0.05)

Model 1: includes age and race/ethnicity.
Model 2: Model 1 + menopausal status (pre/post), current hormone use (versus past/never), current smoking (versus past/never), BMI, and physical activity (continuous, METs/week).
Model 3: Model 2 + CRP, adiponectin, resistin, leptin, sOb-R, insulin, HbA1C%, IL-18 + energy (kcal) + alcohol %kcal + dairy protein %kcal + fat %kcal (*trans* fat, saturated fat, polyunsaturated fat, monounsaturated fat).
The bold values denote statistical significance at $P < 0.05$.

While correlates of total IGF-I and IGFBP-3 levels have been previously reported, few prior studies concurrently examined a wide range of molecular, macronutrient, and behavioral correlates [16]. This is important because it offers insight into the relative magnitude of difference in IGF-axis protein levels related to each factor.

Several strong, statistically significant associations were observed. In particular, for each SD higher sOB-R there was a decrease in total and free IGF-I of −5.5% and −11.4% respectively, while IGFBP-1 increased 22.8% and IGFBP-2 increased 7.9%. These relationships are especially noteworthy, since prior epidemiologic data suggest that higher sOB-R levels may be inversely related to diabetes risk [19], an association similar to those that were reported for IGFBP-1 and -2 [11]. Most circulating IGF-axis proteins and sOB-R are produced in the liver [20]. Thus, it will be important for future studies to concurrently assess the effects of IGF-axis proteins and sOB-R on the risk of diabetes, as well as additional factors discussed below that have strong associations with both IGF-axis protein levels and diabetes risk.

In contrast to sOB-R, its ligand, leptin, had no association with total or free IGF-I or with IGFBP-1, and had a negative correlation with IGFBP-2, opposite to that of sOB-R. Prior studies similarly reported no association of leptin with total or free IGF-I in women [21,22]. Leptin also had a modest positive association with IGFBP-3 in this analysis; though this contrasts with a cross-sectional study reporting an inverse correlation ($r = -0.21$ among 134 women aged 15–70); albeit, those prior results were not adjusted for age or other potential confounders [22]. The relation of IGFBP-2 and leptin has not to our knowledge been previously studied in healthy adult women [20].

Adiponectin was similar to sOB-R in that it had significant positive associations with IGFBP-1 and -2 levels and an inverse association with IGFBP-3 levels. A 1% increase in adiponectin was associated with an increase of 0.16% in IGFBP-1 and 0.27% in IGFBP-2 and a decrease of −0.06% in IGFBP-3, whereas it was not associated with total or free IGF-I, all of which is consistent with prior reports [23,24]. Insulin levels were inversely associated with IGFBP-1 and -2, but were not associated with other IGF-axis proteins. In the Women's Health Initiative, insulin was not associated with total or free IGF-I or IGFBP-3 among non-HT users, but correlated modestly with IGFBP-3 among those using HT [25].

We also studied two biomarkers of systemic inflammation, CRP and IL-18. Levels of CRP had an inverse association with total and free IGF-I, and IGFBP-2. Negative correlations between IGF-I, IGFBP-2, IGFBP-3, and inflammatory factors are consistent with prior reports [11,26]. IL-18 was also associated with free IGF-I and IGFBP-2, but not with other IGF-axis proteins, and for both CRP and IL-18 the significant associations were of modest size.

In addition to molecular factors, one behavioral factor had particularly strong associations with IGF-axis protein levels — the use of HT in postmenopausal women. Specifically, HT use was associated with substantial reductions in total and free IGF-I levels, IGFBP-2 and

IGFBP-3. Similar results showing a reduction in IGF-axis protein levels related to HT use have been reported before by our group and others [25,27], and is thought to reflect the "first pass effect" of a large bolus of oral estrogen on liver protein production [28]. Curiously, though, IGFBP-1 levels were higher (not lower) in women using HT. Whether and to what extent the impact of HT use on IGF-axis proteins might help explain some of the effects of HT use on health and disease may warrant further investigation.

Few associations between macronutrient intake and the IGF axis were observed, but these were similar in strength to those found for many of the serum analytes studied. In particular, dairy protein was positively and saturated fat was inversely associated with total IGF-I and IGFBP-3. Prior research within NHS [29] and other studies [30–32] also reported a positive association of dairy protein consumption with IGF-I levels [33–36].

Certain limitations must be considered in interpreting these findings. In particular, cross-sectional data cannot provide information regarding the temporality of the observed associations, and the generalizability of our findings can only be determined after additional similar studies in other populations, as all subjects were female health professionals of primarily European ancestry free of diabetes, coronary heart disease, stroke and cancer at the time of blood draw. On the other hand, our results regarding total IGF-I and IGFBP-3 were consistent with earlier reports [26,29,30,37], and it will be left to future studies to replicate our analyses of other IGF-axis proteins and to ascertain whether parsing total IGFBP-3 into intact and fragmented molecules alters associations with BMI, physical activity, and disease risk [38]. While the concurrent analysis of multiple different exposure variables could result in over-adjustment (e.g., due to factors in the same pathway) this was a large study and our tables present models with incrementally increasing numbers of covariates, making it possible for the reader to independently evaluate this issue. However, another concern is multiple comparisons, which leads to an increased the likelihood of type I errors, and therefore verification of these results in other cohorts is needed.

In summary, the current data regarding multiple different molecular and macronutrient variables provide several new insights into the factors that may influence IGF-axis protein levels — an important hormonal axis which plays a major role in diabetes risk, and numerous other important health outcomes. Most notably, sOB-R had strong inverse associations with IGF-I and free IGF-I, and strong positive associations with IGFBP-1 and IGFBP-2, independent of adiposity and other biomarkers such as leptin and insulin. Given the inter-individual heterogeneity of sOB-R levels (reflected by its standard deviation), sOB-R itself or signaling pathways correlated with this factor, could have a substantial impact on the IGF-axis. Adiponectin, insulin, leptin, inflammatory factors, and consumption of dairy protein, monounsaturated and saturated fats, were also significantly correlated with certain IGF-axis protein levels. Mechanistic studies are needed to elucidate pathways underlying these and other associations reported in this study in order to inform approaches for modulating risk of metabolic disorders such as diabetes.

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