Insulin-Like Growth Factor Axis and Risk of Type 2 Diabetes in Women

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IGF-I shares structural homology and in vitro metabolic activity with insulin. Laboratory models suggest that IGF-I and its binding proteins IGFBP-1 and IGFBP-2 have potentially beneficial effects on diabetes risk, whereas IGFBP-3 may have adverse effects. We therefore conducted a prospective nested case-control investigation of incident diabetes (n = 742 case subjects matched 1:1 to control subjects) and its associations with IGF-axis protein levels in the Nurses' Health Study, a cohort of middle-aged women. The median time to diabetes was 9 years. Statistical analyses adjusted for multiple risk factors, including insulin and C-reactive protein. Diabetes risk was fivefold lower among women with baseline IGFBP-2 levels in the top versus bottom quintile (odds ratio $[OR]_{q5–q1}$ = 0.17 [95% CI 0.08–0.35]; P trend < 0.0001) and was also negatively associated with IGFBP-1 levels ($OR_{q5–q1}$ = 0.37 [0.18–0.73]; P trend = 0.0009). IGFBP-3 was positively associated with diabetes ($OR_{q5–q1}$ = 2.05 [1.20–3.51]; P trend = 0.002). Diabetes was not associated with total IGF-I levels, but free IGF-I and diabetes had a significant association that varied (P interaction = 0.003) by insulin levels above the median ($OR_{q5–q1}$ = 0.48 [0.26–0.90]; P trend = 0.0001) versus below the median ($OR_{q5–q1}$ = 2.52 [1.05–6.06]; P trend < 0.05). Thus, this prospective study found strong associations of incident diabetes with baseline levels of three IGFBPs and free IGF-I, consistent with hypotheses that the IGF axis might influence diabetes risk.

Increasing evidence suggests that the insulin-like growth factor (IGF) axis may play a role in glucose homeostasis (1). IGF-I shares structural homology and downstream signaling pathways with insulin, and like insulin, IGF-I can promote glucose and fatty acid uptake in peripheral tissues (2). Administration of exogenous IGF-I decreases serum glucose levels and improves insulin sensitivity in individuals with and without type 2 diabetes (3,4). It has therefore been hypothesized that interindividual heterogeneity in endogenous IGF-I and IGF binding protein (IGFBP) levels may influence the risk of developing type 2 diabetes (5).

Consistent with this, cross-sectional studies by our group and others have found that individuals with impaired glucose tolerance or diabetes have lower IGFBP-1 and possibly higher IGFBP-3 levels than healthy individuals (6–8). A positive association between high IGFBP-3 and diabetes could be explained by its sequestration of IGF-I. In contrast, while IGFBP-1 also sequesters IGF-I, the production of IGFBP-1 is downregulated by insulin, thus resulting in low IGFBP-1 levels in patients with preexisting insulin resistance and hyperinsulinemia (8,9). These relationships are complicated by the fact that certain IGFBPs may also have important IGF-independent metabolic activity (5,10,11), including potentially beneficial effects of IGFBP-1 and IGFBP-2 on glucose uptake (12,13). IGF-I, conversely, may have certain adverse metabolic effects (e.g., promoting the proliferation of preadipocytes) in addition to its beneficial insulin-like activity (14). Hence, carefully conducted prospective studies are needed to determine the associations between IGF-axis proteins and the development of diabetes.

To date, few studies have prospectively investigated the relation of IGF-axis protein levels with incident diabetes. However, these initial investigations reported intriguing results (10,15–17). In particular, three of four studies found that low IGFBP-1 levels were associated with increased risk of incident diabetes (15–17). Associations between IGF-I and diabetes were also reported. Two studies found a positive relation of IGF-I levels with diabetes that were of borderline statistical significance (15,16). A third study, though, reported an inverse association of IGF-I levels with impaired glucose tolerance in subjects with low but not high IGFBP-1—data, suggesting a biologic interaction (10).

The reason for these conflicting IGF-I findings is unknown. However, prior investigations had relatively few diabetic case subjects and involved limited statistical control for established diabetes risk factors. Furthermore, despite the prospective collection of data, it is uncertain whether the associations of IGFBP-1 with diabetes in prior studies might have reflected "reverse causality," whereby a step in the disease process (high insulin levels) may have preceded and caused changes in the exposure of interest (low IGFBP-1 levels in the presence of hyperinsulinemia) (18).

We therefore conducted a large prospective, nested case-control investigation of the associations between total IGF-I, free (unbound) IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 and risk of incident type 2 diabetes in the Nurses' Health Study (NHS), a large cohort of middle-aged and older women (19). The study involved >700 case subjects, controlled for multiple diabetes risk factors, and involved several steps to address the possibility of reverse causality.

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RESEARCH DESIGN AND METHODS

Study population. The NHS is an ongoing longitudinal cohort initiated in 1976 that enrolled 121,700 female registered nurses aged 30–55 years. As described in detail elsewhere (19), these women are followed on a biennial basis with mailed questionnaires. During 1989 and 1990, blood specimens were obtained from 32,826 NHS women who were free of diabetes, coronary heart disease, stroke, or cancer. Women who subsequently reported a diagnosis of type 2 diabetes were mailed a supplementary questionnaire regarding relevant symptoms, diagnostic testing, and current treatment. Diabetes was considered confirmed if the responses matched the criteria of the National Diabetes Data Group (cases through 1997) or the American Diabetes Association (cases after 1997) (see Table 1 footnotes for detailed definitions) (20,21). In a validation study conducted among 84 randomly selected NHS subjects who met the above criteria, a diagnosis of diabetes was confirmed in 61 (98%) of the 62 individuals for whom medical records were obtained (22).

The current nested case-control investigation involved 742 incident diabetic case subjects individually matched 1:1 to control subjects using risk-set sampling (23) based on age (±1 year), date of blood draw (±3 months), fasting status (yes/no; 95% of case-control pairs fasted ≥8 h), and race/ethnicity. All women signed informed consent forms, and the study protocol was approved by the institutional review boards of the Brigham and Women's Hospital, the Harvard School of Public Health, and the Albert Einstein College of Medicine.

Laboratory assays. Laboratory personnel were masked to case-control status, and specimens from each case-control pair were tested in replicate in the same assay runs. Total IGF-I, free IGF-I, and IGFBP-1, -2 and -3 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. C-reactive protein (CRP), insulin, and glycosylated hemoglobin (HbA_{1c}) levels had been previously measured, as reported (24,25).

Statistical analysis. Case-control differences in selected baseline characteristics were initially assessed using *t* tests for continuous data and χ^2 tests for categorical data. Correlations between levels of IGF-axis proteins, insulin, CRP, waist circumference, and BMI were measured in control subjects using age- and race-adjusted partial Spearman correlation coefficients. Multivariable conditional logistic regression models were used to estimate the associations between IGF-axis protein levels and risk of incident diabetes, adjusted for relevant covariates (23). These covariates included BMI, cigarette smoking (never smoked, past smoker, or current smoker), menopausal status, hormone therapy use (never used, past user, or current user), family history of type 2 diabetes (yes/no), physical activity, and intake of alcohol, cereal fiber, heme iron, *trans* fat, magnesium, coffee, and red meat (all parameterized as quintiles). IGF-axis protein levels in these models were also parameterized as quintiles, with all quintiles defined according to the distribution of levels among the control subjects.

Our a priori multivariable models included total or free IGF-I, each of the three IGFBPs, and each of the above covariates. Insulin and CRP quintiles were also added to these models to assess their impact on the findings. Tests of linear trend were conducted by using the median value for each quintile and fitting this as a continuous variable in the models. Stratified subgroup analyses (defined by levels of HbA_{1c}, insulin, BMI, CRP, and age) were conducted using unconditional multivariable logistic regression. Two-sided statistical tests were used in all analyses.

RESULTS

Baseline characteristics. Table 1 shows selected characteristics of case and control subjects at baseline. Given the matched study design, the two groups, as expected, were similar with respect to age and race/ethnicity. Compared with control subjects, however, case subjects had higher mean BMI and waist circumference, reported lower physical activity and less alcohol consumption, and were less likely to use hormone therapy. Case subjects also were more likely than control subjects to report a family history of diabetes, and the two groups differed in their consumption of several dietary factors. Moreover, case subjects had significantly higher mean plasma levels of insulin, CRP, and IGFBP-3, as well as significantly lower levels of IGFBP-1 and IGFBP-2. Mean total and free IGF-I levels did not significantly differ between case and control subjects in these univariate analyses. The mean (median) time from blood draw to the development of incident diabetes was 8.4 (9.0) years.

Correlations between biomarkers. Total and free IGF-I each had a significant negative correlation with CRP and a positive correlation with IGFBP-3 (Table 2), and these relationships were stronger for total IGF-I than for free IGF-I. Conversely, free IGF-I had a stronger negative correlation with IGFBP-1 than did total IGF-I, and neither free nor total IGF-I was significantly correlated with IGFBP-2. IGFBP-2, but not IGFBP-1, had a strong negative correlation with CRP. Both IGFBP-1 and IGFBP-2, however, had strong negative correlations with BMI, waist circumference, and insulin. IGFBP-3 and free IGF-I had weak positive correlations with BMI, waist circumference, and insulin that did not always reach statistical significance.

Associations with type 2 diabetes. Table 3 shows the results of multivariable conditional logistic regression models for the relation of IGF-axis protein levels with risk of diabetes. Total IGF-I was not significantly associated with incident diabetes in our a priori models that adjusted for the three IGFBPs in addition to multiple diabetes risk factors (see RESEARCH DESIGN AND METHODS). In contrast, free IGF-I was inversely associated with diabetes risk in these models (odds ratio [OR] comparing highest and lowest quintile [$OR_{q5–q1}$] = 0.57 [95% CI 0.30–1.08]; *P* trend = 0.01). In secondary data analyses, however, we observed a significant statistical interaction between free IGF-I and insulin (*P* interaction = 0.003) (Table 3 and Fig. 1). Specifically, among women with baseline insulin levels at or above the median (i.e., 4.6 μU/mL based on the values in control subjects), free IGF-I was inversely associated with diabetes risk ($OR_{q5–q1}$ = 0.48 [0.26–0.90]; *P* trend = 0.0001). In those with insulin levels below the median, free IGF-I was positively associated with incident diabetes ($OR_{q5–q1}$ = 2.52 [1.05–6.06]; *P* trend < 0.05). Additional adjustment for insulin (as a continuous variable within each of the two strata) and CRP quintile did not alter the findings of this subanalysis, nor did using insulin cut points other than the median. Excluding cases of diabetes that occurred within 2 years of baseline (i.e., a 2-year lag time) also had no meaningful affect on these results.

IGFBP-1 and IGFBP-2 levels had strong inverse associations with risk of diabetes in the same a priori models described above (Table 3). The $OR_{q5–q1}$ for IGFBP-1 was 0.37 (95% CI 0.18–0.73; *P* trend = 0.0009), and for IGFBP-2, the $OR_{q5–q1}$ was 0.17 (0.08–0.35; *P* trend < 0.0001)—a more than fivefold association with diabetes risk. IGFBP-3 levels were positively associated with development of incident type 2 diabetes ($OR_{q5–q1}$ = 2.05 [1.20–3.51]; *P* trend = 0.002). Additional adjustment for CRP and insulin did not meaningfully change the associations of diabetes with IGFBP-1, -2, or -3. Unlike for free IGF-I, the associations between diabetes and IGFBP-1, -2, and -3 also did not vary by insulin values above versus below the median (see Fig. 1). None of the IGFBP results were meaningfully changed by excluding cases of diabetes that occurred within 2 years of enrollment.

Because IGFBP-1 and IGFBP-2 are both partly regulated by insulin, we took additional steps to assess whether insulin resistance at baseline might have affected these findings. Specifically, we restricted analysis to women who not only had insulin levels below the median but also had HbA_{1c} levels within the normal range of <5.7% (26). The mean (median) time from blood draw to development of incident diabetes was 11.1 (11.2) years in these subjects. The associations of IGFBP-1 ($OR_{q5–q1}$ = 0.21 [95% CI 0.08–0.57]; *P* trend = 0.009) and IGFBP-2 ($OR_{q5–q1}$ = 0.16 [0.06–0.42]; *P* trend = 0.0001) were not, however, meaningfully altered.

TABLE 1
Baseline characteristics of women who developed incident type 2 diabetes* and matched control subjects in the NHS

Characteristic	Case subject (n = 742)	Control subject (n = 742)	P value†
Demography and lifestyle			
Age (years)	56.2 (7.0)	56.3 (7.0)	—§
White	81.9	83.0	—§
Current smoker	13.3	11.1	0.38
Postmenopausal	78.6	78.0	0.80
Current hormone use	29.5	41.2	<0.0001
Family history of diabetes	44.2	23.1	<0.0001
BMI (kg/m^2)	29.6 (5.3)	24.9 (4.1)	<0.0001
Waist circumference (cm)	88.2 (11.9)	77.1 (9.8)	<0.0001
Physical activity (MET hour/week)	14.7 (16.0)	17.8 (17.4)	0.0004
Alcohol (g/day)	4.0 (7.4)	6.4 (9.2)	<0.0001
Diet			
Fruits and vegetables (servings/day)	5.2 (1.9)	5.3 (2.0)	0.41
Red meat (servings/day)	1.2 (0.5)	1.0 (0.5)	<0.0001
Fish (servings/week)	0.28 (0.17)	0.25 (0.16)	0.003
Cereal fiber (g/day)	3.8 (1.6)	4.2 (1.7)	0.0002
Whole grain (g/day)	15.0 (10.5)	17.3 (11.7)	<0.0001
Total energy (kcal/day)	1,803 (534)	1,756 (482)	0.08
Biomarkers			
Insulin (μU/mL)	10.3 (7.9)	6.0 (4.1)	<0.0001
CRP (mg/dL)	0.51 (0.52)	0.24 (0.29)	<0.0001
Total IGF-I (ng/mL)	151 (50)	151 (50)	0.96
Free IGF-I (ng/mL)	0.48 (0.30)	0.46 (0.28)	0.16
IGFBP-1 (ng/mL)	24.2 (23.6)	42.9 (29.0)	<0.0001
IGFBP-2 (ng/mL)	335 (229)	584 (342)	<0.0001
IGFBP-3 (ng/mL)	5,060 (1,262)	4,641 (980)	<0.0001

For continuous variables, values are expressed as mean (SD); for categorical variables, values are proportions (%). *A self-reported case of diabetes was considered confirmed if in a supplementary questionnaire, the subject reported information consistent with the National Diabetes Data Group (NDDG) criteria (for those NHS cases reported through 1997) or consistent with the American Diabetes Association (ADA) criteria (for those NHS cases reported after 1997). Specifically, under the NDDG criteria, diabetes was diagnosed if the subject reported at least one of the following: *1*) polydipsia, polyuria, polyphagia, weight loss, or coma, in addition to a fasting glucose level ≥140 mg/dL or a random level ≥200 mg/dL; *2*) in the absence of symptoms, elevated plasma glucose concentrations on at least two occasions, including a fasting glucose ≥140 mg/dL, a random glucose ≥200 mg/dL, or after ≥2 h during a oral glucose test, a glucose ≥200 mg/dL; *3*) treatment with oral drugs for hyperglycemia or with insulin. For cases of diabetes reported after 1997, the diagnosis was confirmed using new recommendations of the ADA, which changed the fasting glucose cutoff to 126 mg/dL. †P values for differences between case and control subjects (unadjusted) were determined by Student t test for continuous variables and χ^2 test for categorical variables. §Matching factor.

Additional subanalyses. No significant variation (statistical interaction) in the associations between diabetes and IGF-axis protein levels were observed according to median age, BMI, or CRP levels (data not shown). The findings for total and free IGF-I did not vary by median IGFBP levels, and the findings for IGFBP-1, -2, and -3 did not vary by IGF-I levels. Only one potential interaction was observed: free IGF-I had an inverse association of borderline statistical significance with diabetes risk among women with IGFBP-1 levels below the median ($OR_{q5–q1}$ = 0.69 [95% CI 0.36–1.33]; P trend = 0.07) but had no association with diabetes when IGFBP-1 levels were at or above the median (P interaction = 0.09). No results in the study were meaningfully changed by restricting analysis to the 95% of case-control pairs who had fasted for at least 8 h before blood collection.

DISCUSSION

To our knowledge, this is the first large prospective study to assess multiple components of the IGF axis and their associations with risk of incident type 2 diabetes. Free IGF-I and IGFBP-1, IGFBP-2, and IGFBP-3 each had significant independent associations with diabetes risk. These associations remained significant even after control for important molecular and other established diabetes risk factors. Moreover, the OR estimates for all three IGFBPs were strong and showed a biologic gradient, gaining in strength with increasing quintile. There was, for example, a more than fivefold reduction in risk between the highest and lowest quintiles of IGFBP-2 and a nearly threefold reduction in risk associated with the IGFBP-1 quintile. Considering the large fraction of participants in high or moderate risk IGFBP strata, these relationships could have important clinical and public health implications.

TABLE 2
Age- and race-adjusted partial correlation coefficients between IGF-I, IGFBPs, and selected other risk factors for type 2 diabetes among the 742 control subjects

	Total IGF-I	Free IGF-I	IGFBP-1	IGFBP-2	IGFBP-3
BMI	0.02	0.09†	−0.47*	−0.42*	0.11†
Waist	0.04	0.08†	−0.36*	−0.26*	0.11†
Insulin	0.06	0.06	−0.36*	−0.32*	0.10†
CRP	−0.34*	−0.23*	−0.07	−0.43*	−0.10
IGFBP-3	0.62*	0.22*	−0.21*	−0.21*	—
IGFBP-2	0.01	0.02	0.32*	—	—
IGFBP-1	−0.28*	−0.41*	—	—	—
Free IGF-I	0.53*	—	—	—	—

*$P < 0.001$. †$P < 0.05$.

TABLE 3
ORs (95% CIs) for incident type 2 diabetes, according to quintile of IGF-I and its binding proteins*

	Quintile of baseline plasma level†					
	1	2	3	4	5	*P* trend‡
Total IGF-I						
Case subjects (*n*)	147	155	136	153	151	
Multivariable model	1.0	1.29 (0.80–2.08)	1.03 (0.63–1.70)	1.50 (0.92–2.44)	1.49 (0.87–2.54)	0.12
Model + IGFBP-1 + IGFBP-2 + IGFBP-3 (a priori model)	1.0	0.84 (0.47–1.49)	0.68 (0.36–1.27)	0.69 (0.36–1.35)	0.58 (0.28–1.22)	0.11
Model + IGFBP-1 + IGFBP-2 + IGFBP-3 + CRP + insulin	1.0	0.85 (0.46–1.56)	0.71 (0.36–1.41)	0.77 (0.37–1.60)	0.70 (0.31–1.58)	0.38
Free IGF-I						
Case subjects (*n*)	119	160	166	137	159	
Multivariable model	1.0	1.28 (0.79–2.09)	1.16 (0.71–1.90)	1.02 (0.59–1.76)	1.11 (0.66–1.85)	0.97
Model + IGFBP-1 + IGFBP-2 + IGFBP-3 (a priori model)	1.0	1.06 (0.61–1.84)	0.72 (0.40–1.29)	0.47 (0.24–0.92)	0.57 (0.30–1.08)	0.01
Model + IGFBP-1 + IGFBP-2 + IGFBP-3 + CRP + insulin	1.0	1.16 (0.64–2.09)	0.84 (0.45–1.57)	0.53 (0.26–1.08)	0.67 (0.34–1.34)	0.04
(<*Median* insulin level) Model + IGFBP-1 + IGFBP-2 + IGFBP-3§	1.0	1.12 (0.48–2.64)	1.50 (0.61–3.70)	1.53 (0.68–3.47)	2.52 (1.05–6.06)	<0.05
(≥*Median* insulin level) Model + IGFBP-1 + IGFBP-2 + IGFBP-3§	1.0	1.28 (0.72–2.28)	0.82 (0.46–1.46)	0.50 (0.27–0.92)	0.48 (0.26–0.90)	0.0001
IGFBP-1						
Case subjects (*n*)	400	153	73	73	43	
Multivariable model	1.0	0.51 (0.33–0.80)	0.39 (0.24–0.64)	0.30 (0.18–0.51)	0.22 (0.12–0.39)	<0.0001
Model + free IGF-I + IGFBP-2 + IGFBP-3 (a priori model)	1.0	0.72 (0.44–1.18)	0.58 (0.33–1.01)	0.46 (0.25–0.85)	0.37 (0.18–0.73)	0.0009
Model + free IGF-I + IGFBP-2 + IGFBP-3 + CRP + insulin	1.0	0.71 (0.43–1.20)	0.63 (0.35–1.16)	0.51 (0.26–1.00)	0.42 (0.20–0.90)	0.01
IGFBP-2						
Case subjects (*n*)	407	156	103	49	27	
Multivariable model	1.0	0.41 (0.26–0.63)	0.31 (0.19–0.51)	0.15 (0.08–0.27)	0.11 (0.06–0.22)	<0.0001
Model + free IGF-I + IGFBP-1 + IGFBP-3 (a priori model)	1.0	0.52 (0.33–0.82)	0.45 (0.26–0.76)	0.23 (0.12–0.43)	0.17 (0.08–0.35)	<0.0001
Model + free IGF-I + IGFBP-1 + IGFBP-3 + CRP + insulin	1.0	0.54 (0.32–0.90)	0.49 (0.28–0.88)	0.25 (0.13–0.50)	0.19 (0.09–0.41)	<0.0001
IGFBP-3						
Case subjects (*n*)	105	103	99	175	260	
Multivariable model	1.0	1.33 (0.78–2.25)	1.16 (0.68–1.99)	2.40 (1.47–3.91)	2.63 (1.64–4.22)	<0.0001
Model + free IGF-I + IGFBP-1 + IGFBP-2 (a priori model)	1.0	1.27 (0.71–2.26)	1.23 (0.69–2.20)	2.00 (1.16–3.42)	2.05 (1.20–3.51)	0.002
Model + free IGF-I + IGFBP-1 + IGFBP-2 + CRP + insulin	1.0	1.46 (0.78–2.72)	1.31 (0.72–2.40)	2.22 (1.25–3.96)	2.07 (1.16–3.71)	0.004

Data are OR (95% CI) unless otherwise indicated. *The initial multivariable model conditioned on matching factors and adjusted for BMI, smoking status (never smoked, past smoker, or current smoker), postmenopausal status (yes/no), hormone use (never used, past user, or current user), family history of diabetes (yes/no), physical activity (in quintiles), and intake of alcohol, cereal fiber, heme iron, *trans* fat, magnesium, coffee, and red meat (all in quintiles). †Quintiles were determined using the distribution among control subjects, and quintile 1 served as a reference group for the estimation of each OR. ‡Tests of linear trend were conducted by assigning the median value for each quintile and fitting this as a continuous variable in the models. §These analyses were stratified by insulin levels less than vs. equal to or above the median measured in control subjects (i.e., 4.6 μU/mL) and were conducted using unconditional multivariate logistic regression models that incorporated the matching factors as well as all the above covariates.

IGFBP-1 has long been hypothesized to play a role in glucose homeostasis in part because IGFBP-1 production is downregulated by insulin. IGFBP-2 production is also inhibited by insulin, although the effect occurs more slowly (11,27). Consistent with this, our data showed negative correlations between levels of insulin and those of IGFBP-1 and IGFBP-2. These correlations, though, also raised the possibility of reverse causality (i.e., that the apparent associations of low IGFBP-1 and IGFBP-2 levels with diabetes might be explained by a greater frequency of high insulin levels, for example, related to insulin resistance, among case subjects than control subjects at baseline, rather than by a biologic role of these IGFBPs in diabetes pathogenesis). In the current study, however, we showed that the strong relation of IGFBP-1 and IGFBP-2 with diabetes was essentially unchanged by restricting the analysis to women who at baseline had both low insulin and normal HbA_{1c} levels—a subgroup with very low probability of preexisting insulin resistance. In these women, diabetes occurred a median of 11 years after blood draw.

Furthermore, the current IGFBP-1 results are consistent with those of prior epidemiologic studies. Three of four earlier prospective studies reported inverse associations between high IGFBP-1 levels and risk of diabetes (15–17). While no prior prospective studies examine the relation of IGFBP-2 or IGFBP-3 with diabetes, we previously reported cross-sectional data showing a possible positive association of IGFBP-3 levels with diabetes and impaired glucose tolerance (6).

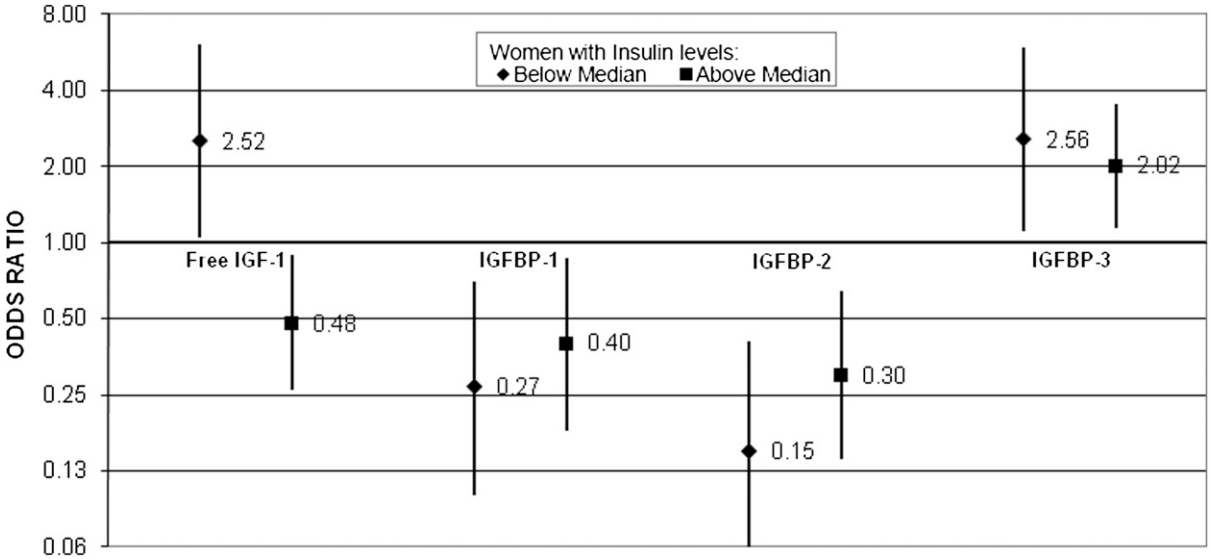

FIG. 1. Associations of incident diabetes with IGF-axis protein levels (highest vs. lowest quintile) stratified by insulin levels above and below the median. All associations (ORs) were adjusted for covariates using multivariable logistic regression models (as reported in Table 3). Median insulin level was determined using values among control subjects (i.e., 4.61 μU/mL). Vertical lines represent 95% CIs.

Laboratory studies also provide evidence consistent with the current findings. For example, in vitro models show that IGFBP-1 and IGFBP-2 inhibit preadipocyte proliferation and differentiation (11,13), and in mouse models, the overexpression of IGFBP-2 was associated with decreased susceptibility to obesity and improved insulin sensitivity (12,13). IGFBP-1 overexpression also lowered susceptibility to obesity in rodent models (28,29) and was associated with decreased risk of insulin resistance in a mouse model that accounted for the lower IGF affinity of nonphosphorylated than phosphorylated IGFBP-1 (a difference that is found in humans but not rodents) (11,30,31). Furthermore, IGFBP-1 and IGFBP-2 have both been shown to bind $\alpha_5\beta_1$-integrin receptors on cell surfaces, thereby activating the phosphatidylinositol 3-kinase and protein kinase B signaling pathway, resulting in intracellular translocation of GLUT4 and increased glucose uptake (11,32,33).

IGFBP-3 had a positive association with diabetes risk in this study and this too was in keeping with laboratory data. IGFBP-3 is the most abundant IGFBP in circulation and binds >90% of all circulating IGF-I (34). Furthermore, IGFBP-3 binds cellular proteins involved in the regulation of glucose metabolism, such as 9-*cis* retinoic acid receptor-α (34–36), and can induce hepatic insulin resistance and lower glucose uptake in muscle and adipose tissue (37,38).

Free IGF-I, unlike IGFBP-1, -2, and -3, had an association with diabetes that varied with insulin and to a lesser extent, with IGFBP-1 levels; these results shared similarity with previously reported IGF-I data. Sandhu et al. (10), for example, found that high IGF-I levels were associated with lower risk of impaired glucose tolerance among those with IGFBP-1 levels below but not above the median. In the current study, we observed an inverse association of borderline statistical significance between free IGF-I and diabetes that likewise was limited to women with IGFBP-1 levels below the median. In a separate model, we also observed a significant inverse association of free IGF-I and diabetes limited to women with insulin levels above the median. Given that insulin downregulates IGFBP-1 production, these two interactions could be related, albeit even larger studies or data from multiple cohorts would be needed to examine both interactions simultaneously. Lewitt et al. (15,16), in contrast, reported positive associations of borderline significance between IGF-I and risk of diabetes (10). It is notable, therefore, that among women with insulin levels below the median, we found a positive association of free IGF-I and diabetes risk, unlike the inverse association found when insulin levels were greater than the median (discussed above). Thus, the effects of IGF-I may vary according to the baseline characteristics of the patient population (e.g., baseline insulin levels).

Overall, the collective data raise the possibility that the relation of IGF-I with diabetes risk may be bimodal. For example, among patients with preexisting insulin resistance, the insulin-like activity of IGF-I on glucose and free fatty acid uptake may play an important role in glucose homeostasis. Consistent with this, laboratory studies have shown that in the presence of insulin resistance, there is upregulation of insulin/IGF hybrid receptor expression in muscle (39,40). These receptors are largely responsive to IGF-I (40), and additional data suggest that their binding could be as potent in stimulating peripheral glucose uptake as insulin binding with its receptor (41). Correspondingly, transgenic mouse studies have shown that a muscle-specific dominant-negative IGF-I receptor mutation results in defective hybrid receptors and the development of diabetes by 6 weeks of age (42–44). It is noteworthy, therefore, that obesity may be associated with moderate elevation of free IGF-I levels (5,8,45,46), as reflected by the positive correlation of free IGF-I and BMI in the current study. This can occur despite the relation of obesity with hyposecretion of growth hormone (GH) (47), the primary regulator of IGF-I production by the liver (48), because of IGF-I production by adipocytes (49) and the stimulation of hepatic IGF-I synthesis by insulin (27). Collectively, high circulating levels of free IGF-I and upregulation of hybrid receptor expression in muscle tissue in obese compared with normal weight individuals could accentuate the beneficial metabolic effects of

IGF signaling. Furthermore, free IGF-I downregulates GH production via a negative-feedback loop, and by reducing circulating GH levels, it may reduce the anti-insulin effects of GH (45,50). Through either one or both of these mechanisms, free IGF-I may therefore have beneficial effects on glucose homeostasis in obese and other patients with preexisting insulin resistance (5).

Prior to the development of insulin resistance, however, the adverse metabolic effects of IGF-I may predominate. For example, IGF-I promotes preadipocyte differentiation and proliferation through activation of the insulin receptor substrate and mitogen-activated protein kinase pathways (14,29). Mouse models, concordantly, have shown that reduction of IGF-I receptor expression in adipose tissue (29,51) or targeted inactivation of hepatic IGF-I expression (52) results in a reduction in adipose tissue. Furthermore, the relationship between IGF-axis signaling and adiposity may be depot specific, with a greater affect of IGF-I on the differentiation of visceral than subcutaneous adipocytes (53). Insulin resistance is more strongly associated with visceral than subcutaneous adiposity (54). Thus, in women and men who are not currently obese, IGF-I signaling could promote visceral adiposity and increased risk of diabetes.

Despite several areas of concordance between our study and prior epidemiologic and laboratory data, there are limitations to the current investigation that must be considered in its interpretation. First, unlike both Sandhu et al. (10) and Lewitt et al. (15,16), we did not observe a relation of diabetes with total IGF-I, only a relation of diabetes with free IGF-I. Conversely, none of the prior prospective studies measure free IGF-I, and it is free (unbound) IGF-I that is thought to be the most bioactive component of total IGF-I (55). We had in fact predicted stronger, more consistent associations with free IGF-I than with total IGF-I because our earlier studies of cancer found disease associations with free but not total IGF-I (56,57). Other limitations must also be considered. Although we assessed HbA_{1c} and fasting insulin, we did not measure other indicators of normoglycemia or insulin resistance, such as homeostasis model assessment–estimated insulin resistance or oral glucose tolerance. Despite our efforts to address confounding and reverse causality, we cannot exclude the possibility that one or both may have affected our results. We also cannot exclude the possibility that chance findings might explain the statistical interaction between free IGF-I and insulin. Lastly, the current study included predominantly Caucasian women, and the results may not be generalizable to men or to other racial/ethnic groups.

In conclusion, the findings of this large prospective study suggest that IGFBP-1 and IGFBP-2 have strong inverse associations with risk of type 2 diabetes risk in women, whereas IGFBP-3 is associated with increased risk, and the relation of free IGF-I and diabetes may vary by insulin levels. These results provide important new evidence that circulating IGF-axis proteins may have a biologic role in the pathogenesis of type 2 diabetes. Moreover, if these strong associations are confirmed, IGF-axis proteins may be useful in diabetes risk stratification (e.g., in combination with other biomarkers) and could represent targets for the development of new chemoprevention or treatment strategies.

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S.N.R. contributed to the study design, helped obtain funding, and wrote the manuscript. M.H. and Q.S. managed and analyzed data. R.C.K. contributed to the design of the analysis and to discussion. R.M., T.E.R., and M.J.G. provided input regarding the relevant molecular pathways and edited the manuscript. M.P. led laboratory testing and contributed to discussion. M.K. helped determine the statistical methods, oversaw the analyses, and edited the manuscript. J.E.P., J.B., and J.W.-R. reviewed the manuscript and contributed to discussion. F.B.H. helped design the overall study, acquired laboratory and clinical data used in the study, and reviewed and edited the manuscript. H.D.S. helped design the overall study and obtain funding and wrote the manuscript. S.N.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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