Leptin and Soluble Leptin Receptor Levels in Plasma and Risk of Type 2 Diabetes in U.S. Women

A Prospective Study

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OBJECTIVE—We prospectively examined plasma levels of leptin and soluble leptin receptor (sOB-R), as well as their interactions with other diabetes risk factors, in relation to type 2 diabetes to elucidate the complex relation between these two biomarkers and diabetes risk.

RESEARCH DESIGN AND METHODS—Of 32,826 Nurses' Health Study participants who provided blood samples during 1989–1990, 1,054 incident case subjects of type 2 diabetes were identified and confirmed during 1990–2004 and 1,254 matched control subjects were selected. Plasma leptin and sOB-R levels were measured among these participants.

RESULTS—After multivariate adjustment for BMI, lifestyle practices, and dietary factors, sOB-R levels were significantly associated with a lower risk of type 2 diabetes. In comparison with women in the lowest quintile, the ORs (95% CI) of developing type 2 diabetes were 0.73 (0.55–0.96), 0.51 (0.38–0.68), 0.42 (0.31–0.57), and 0.39 (0.28–0.54; P for trend < 0.0001) for women in the second to fifth quintiles of sOB-R levels, respectively. In contrast, plasma leptin levels were not significantly associated with the risk of type 2 diabetes: The OR (95% CI) was 0.82 (0.62–1.10; P for trend = 0.46) comparing the highest with the lowest quintile of leptin levels. sOB-R levels were consistently associated with a decreased risk of type 2 diabetes at various levels of leptin or high-molecular-weight adiponectin.

CONCLUSIONS—These data suggest a strong inverse association between plasma sOB-R levels and risk of type 2 diabetes, independent of BMI, leptin, and adiponectin levels. ***Diabetes*** **59: 611–618, 2010**

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Leptin, a 16-kDa protein produced primarily in adipose tissue (1), is a pleiotropic hormone that is involved in body weight regulation, puberty, reproduction, and immune function (2–4). Although accumulating evidence suggests that leptin may also directly interact with insulin on glucose metabolism (3), conflicting results have been generated for the effects of leptin on insulin sensitivity in animal models and humans (5) and for circulating leptin levels in relation to type 2 diabetes in humans (6–14). These mixed results indicate that other regulatory factors may modulate the effects of leptin on insulin sensitivity or diabetes. Soluble leptin receptors (sOB-Rs) that provide the primary leptin-binding capacity in human circulation (15) have been suggested to be such a modulating factor because sOB-R acts as a buffer to maintain the bioavailability of free leptin in the circulation (16). Interestingly, several cross-sectional studies consistently showed that sOB-R was inversely correlated with adiposity and insulin resistance indexes in humans (17–20). However, no prospective data exist regarding the association between sOB-R and type 2 diabetes risk or the joint effects with leptin on diabetes risk. Therefore, we performed a prospective case-control study in the Nurses' Health Study (NHS) cohort to examine the associations among leptin, sOB-R, and risk of type 2 diabetes in U.S. women.

RESEARCH DESIGN AND METHODS

The NHS cohort was established in 1976 with 121,700 female registered nurses aged 30–55 years who were residing in 11 U.S. states and had completed a mailed questionnaire on their medical history and lifestyle characteristics. During 1989–1990, upon request, 32,826 women provided blood samples, the majority (97%) of which 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 the vapor phase of liquid nitrogen freezers at a temperature $\leq -130°C$.

Among the participants who provided blood samples and were free of diabetes, cardiovascular disease, and cancer at blood draw, we prospectively identified and confirmed 1,054 type 2 diabetes case subjects from the date of blood draw through June 2004. Using risk-set sampling, one or two control subjects were randomly selected for each case subject from the rest of the population who remained free of diabetes and matched to case subjects by age at blood draw (±1 year), date of blood draw (±3 months), fasting status (fasting for ≥8 h or not fasting), and race. After excluding eight control subjects with missing leptin or sOB-R data, 1,054 incident type 2 diabetes case subjects and 1,254 control subjects were available for the current analysis.

The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

Ascertainment of type 2 diabetes. In the NHS, women who reported a diagnosis of type 2 diabetes in the biennial follow-up questionnaires were sent supplementary questionnaires inquiring about symptoms, diagnostic tests, and

treatment for the purpose of confirmation. The self-report of diagnosis of type 2 diabetes has been proven to be accurate in a validation study (21). Of a random sample of 62 diabetic nurses initially confirmed by the supplementary questionnaire, 61 (98%) were confirmed by medical records reviewed by an endocrinologist blinded to the supplementary questionnaire information (21). In the current study, all case subjects were confirmed using the supplementary questionnaire. For case subjects diagnosed before 1998, we used the following criteria from the National Diabetes Data Group for the confirmation of type 2 diabetes diagnosis: *1*) an elevated glucose concentration (fasting plasma glucose ≥7.8 mmol/l, random plasma glucose ≥11.1 mmol/l, or plasma glucose ≥11.1 mmol/l after an oral glucose load) and at least one symptom (excessive thirst, polyuria, weight loss, or hunger) related to diabetes; *2*) no symptoms but elevated glucose concentrations on two occasions; and *3*) treatment with insulin or oral hypoglycemic medication. For case subjects of type 2 diabetes identified after 1998, the cutoff point used for fasting plasma glucose concentrations was lowered to 7.0 mmol/l according to the American Diabetes Association criteria.

Laboratory procedures. Each case-control pair or triplet was shipped in the same batch and analyzed in the same run. Within each batch, samples were assayed by the same technicians in a random sequence under identical conditions.

Total leptin was measured by a radioimmunoassay (Millipore, Billerica, MA), the sensitivity of which is 0.5 ng/ml. sOB-R was measured by ELISA technique (R&D Systems, Minneapolis, MN) with a sensitivity of 0.06 ng/ml. Laboratory control samples ($n = 20$) were run along with the case-control samples. Based on the measurements of these control samples, the average intra-assay coefficient of variation was 7.7% for the leptin assay and 7.3% for the sOB-R assay. In addition to leptin and sOB-R, total and high-molecular-weight (HMW) adiponectin, resistin, C-reactive protein, tumor necrosis factor-α receptor 2, interleukin-6 (IL-6), interleukin-18 (IL-18), fasting insulin, and C-peptide were also measured for all or some of the case and control subjects. The assays for these biomarkers have been described elsewhere (22–24).

Assessment of lifestyle and dietary covariates. In baseline and/or subsequent biennial follow-up questionnaires, information on major lifestyle risk factors for chronic diseases, such as body weight, cigarette smoking, physical activity, family history of diabetes, menopausal status, and postmenopausal hormone use, was collected and updated in the NHS cohort. BMI as weight in kilograms divided by the square of height in meters (kg/m^2) was calculated to assess overall adiposity. We asked the participants to measure their waist circumference (at umbilicus) and hip circumference (the largest circumference) in 1986. From 1980, diet was assessed using a validated semiquantitative food frequency questionnaire every 2–4 years. Nutrient intake was calculated based on responses to the food frequency questionnaire and the nutrient content of foods was derived from the Harvard Food Composition Database.

Statistical methods. We evaluated the correlation of leptin and sOB-R with anthropometric measurements and plasma concentrations of inflammatory markers and adipokines among control subjects. Spearman partial correlation coefficients were calculated, adjusted for age at blood draw (years), date of blood draw, fasting status (8 h versus <8 h since last meal), race, BMI (kg/m^2), smoking status (never smoked, former smoker, or current smoker), postmenopausal status (yes/no), hormone use (never used, former user, current user), family history of diabetes (yes/no), and physical activity and alcohol intake (both in quintiles). Because we observed a strong correlation between leptin biomarkers and BMI (Table 2), we calculated the BMI-adjusted residuals of the biomarkers by regressing each biomarker on BMI at blood draw using linear regressions and used these residuals in the subsequent analysis. These residuals are statistically independent of BMI and, therefore, are subject to minimal residual confounding by BMI. This approach has been described in detail elsewhere (25).

We categorized the study population into quintiles according to the distribution of the BMI-adjusted levels of biomarkers among control subjects. We used both conditional and unconditional logistic regressions to examine the associations of interest, and because the findings were similar, we presented the results only from unconditional logistic regressions. In the multivariate analysis, we controlled for the aforementioned lifestyle covariates, as well as intake of alcohol, cereal fiber, heme iron, *trans* fat, magnesium, coffee, and red meat (all in quintiles). All lifestyle covariates were derived from the questionnaire administered in 1990 or the nearest year prior to 1990. Because we have multiple assessments of dietary factors, we calculated the average of nutrient intake in 1980, 1984, 1986, and 1990 to minimize measurement errors and better represent long-term diet (26). The chosen lifestyle and dietary covariates were either matching factors that should be adjusted when using unconditional regression to derive ORs in a nested case-control study (27) or established diabetes risk factors that were also correlated with leptin or sOB-R levels. *P* values for linear trend were calculated by entering an ordinal score based on the median value in each quintile of leptin biomarkers into the multivariate models. When we examined potential interactions between leptin biomarkers and lifestyle/dietary risk factors, we used tertiles of the biomarkers to preserve statistical power and to gain more stable estimates of the OR. We constructed interaction terms between biomarker tertiles and the interacting factors of interest and used likelihood ratio tests to assess the significance of these interaction terms. Likelihood ratio tests are calculated as the difference of −2 log likelihood in models with and without interaction terms and follow the χ^2 distribution with the degree of freedom equal to the number of parameters for the interaction terms. In addition, we used restricted cubic spline regressions with three knots (28) to examine possible nonlinear relationships between leptin biomarkers and risk of type 2 diabetes. Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms.

All *P* values were two-sided, and 95% CIs were calculated for ORs. Data were analyzed with the Statistical Analysis Systems software package, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Table 1 shows the baseline characteristics of study population. As expected, type 2 diabetes case subjects had a higher BMI, lower physical activity levels, higher probability of having family history of diabetes, and less healthy dietary intake at baseline than control subjects. Case subjects also had significantly higher leptin levels but lower sOB-R levels than control subjects. The distribution of other inflammatory biomarkers and adipokines in case and control subjects was consistent with our previous findings (22–24).

In control subjects, after adjustment for multiple covariates, plasma leptin levels were strongly correlated with BMI; the partial Spearman correlation coefficient (r) was 0.72 (Table 2). In contrast, sOB-R was significantly inversely correlated with BMI ($r = -0.43$). To better control for confounding by BMI, we used BMI-adjusted residuals of these biomarkers in the subsequent analyses. BMI-adjusted leptin and sOB-R were not correlated with waist circumference or waist-to-hip ratio. Significantly positive correlations were found between leptin and C-reactive protein ($r = 0.25$) and between sOB-R and HMW adiponectin ($r = 0.26$). In a subset of the control subjects with insulin and C-peptide data, we found no significant correlations with insulin but significant correlations for leptin ($r = 0.18$) and sOB-R ($r = -0.18$) with C-peptide.

After multivariate adjustment for established and potential lifestyle and dietary risk factors for type 2 diabetes, BMI-adjusted leptin levels were not significantly associated with diabetes risk (Table 3). In contrast, BMI-adjusted sOB-R levels were significantly associated with a lower risk of type 2 diabetes. Compared with women in the lowest quintile of sOB-R, women in the highest quintile had an OR of 0.39 (95% CI: 0.28–0.54; *P* for trend < 0.0001). This association was attenuated but remained statistically significant after further adjustment for HMW adiponectin; the OR (95% CI) was 0.67 (0.47–0.95; *P* for trend = 0.005). Further adjustment for other biomarkers, including inflammatory markers and other adipokines, did not change the point estimates of these associations materially, although the 95% CIs were much wider and *P* values less significant because only half of the study population had all of these biomarkers available (data not shown). Lastly, when we further adjusted for fasting insulin in a subset of the study participants (39% of total participants) with fasting insulin data, the associations for sOB-R levels were slightly attenuated, but a statistically significant trend persisted (data not shown).

We found no significant deviation from a linear relationship between sOB-R and risk of type 2 diabetes in

TABLE 1
Baseline characteristics of type 2 diabetic case and control subjects, the Nurses' Health Study, 1990

Characteristics*	Case subjects	Control subjects	P†
n	1,054	1,254	
Demography and lifestyle			
Age (years)‡	56.0 ± 6.9	56.1 ± 7.0	0.72
BMI (kg/m^2)	30.3 ± 5.5	25.9 ± 4.8	<0.0001
Physical activity (MET-h/week)	8.9 (3.8–19.0)	12.8 (5.9–22.8)	<0.0001
Alcohol (g/day)	1.1 (0.0–4.0)	2.3 (0.3–8.1)	<0.0001
Smoking status (%)			0.21
Never smoked	46.7	47.9	
Former smoker	40.1	40.8	
Current smoker	13.2	11.3	
Menopausal status (%)			<0.0001
Premenopausal	22.4	22.3	
Postmenopausal, never used hormone	14.2	12.3	
Postmenopausal, hormone past user	28.7	37.9	
Postmenopausal, hormone current user	34.7	27.5	
Family history of diabetes (%)	44.7	22.5	<0.0001
Fasting status (%)‡	64.3	64.1	0.92
Diet			
Total energy (kcal/day)	1,822.5 ± 558.5	1,765.9 ± 496.9	0.01
Coffee (cups/day)	2.1 ± 1.6	2.2 ± 1.6	0.02
Fruits and vegetables (servings/day)	5.2 ± 1.9	5.2 ± 1.9	0.99
Red meat (servings/day)	1.2 ± 0.5	1.1 ± 0.5	<0.0001
Fish (servings/week)	2.0 ± 1.2	1.8 ± 1.2	0.002
Glycemic load	96.9 ± 15.2	96.6 ± 16.4	0.66
Cereal fiber (g/day)	3.8 ± 1.6	4.2 ± 1.8	<0.0001
Whole grain (g/day)	14.7 ± 10.1	17.1 ± 11.8	<0.0001
Heme iron (mg/day)	1.3 ± 0.3	1.2 ± 0.3	<0.0001
Polyunsaturated-to-saturated fat ratio	0.50 ± 0.12	0.51 ± 0.14	0.004
Trans fat (g/day)	1.9 ± 0.5	1.8 ± 0.5	0.003
Magnesium (mg/day)	290.9 ± 55.6	299.2 ± 60.4	0.0006
Biomarker§			
Leptin (ng/ml)	29.2 ± 13.8	21.0 ± 13.3	<0.0001
sOB-R (ng/ml)	28.0 ± 8.2	34.1 ± 11.0	<0.0001
HMW adiponectin (μg/ml)	4.4 ± 3.0	8.0 ± 5.4	<0.0001
Resistin (ng/ml)	21.0 ± 16.9	17.9 ± 12.4	<0.0001
IL-18 (pg/ml)	349.1 ± 186.7	294.5 ± 161.5	<0.0001
TNFα-R2 (pg/ml)	2,762.7 ± 864.1	2,453.0 ± 792.7	<0.0001
IL-6 (ng/ml)	3.1 ± 2.7	2.4 ± 2.4	<0.0001
CRP (mg/l)	5.1 ± 5.3	2.4 ± 2.9	<0.0001

*For continuous variables, values were expressed as mean ± SD or median (interquartile range [IQR]); for categoric variables, % was used. †P values were based on Student t test for continuous variables expressed as mean ± SD, Wilcoxon rank-sum test for continuous variables expressed as median (IQR), or Pearson χ^2 test for categoric variables. ‡Matching factor. §For case subjects, tumor necrosis factor-α receptor 2 (TNFα-R2) was missing for 335, IL-6 was missing for 357, and C-reactive protein (CRP) was missing for 345; for control subjects, TNFα-R2 was missing for 730, IL-6 was missing for 749, and CRP was missing for 740. HMW adiponectin, resistin, and IL-18 were missing for 2 control subjects. MET-h, metabolic equivalent task-h.

spline regression models; the P value for nonlinearity is 0.35 (Fig. 1).

Joint associations for sOB-R and leptin, as well as HMW adiponectin, are shown in Fig. 2. We did not find a significant interaction between sOB-R and leptin (P for interaction = 0.09) or HMW adiponectin (P for interaction = 0.30). Within each tertile of leptin levels, sOB-R levels were consistently associated with a lower risk of type 2 diabetes. In our previous study, HMW adiponectin was significantly associated with a lower risk of type 2 diabetes (24). In the current analysis, sOB-R was associated with a lower risk within each HMW adiponectin level and vice versa. Women who were in the highest tertiles of both sOB-R and HMW adiponectin had the lowest odds of developing type 2 diabetes; the OR (95% CI) was 0.13 (0.09–0.20) in comparison with women in the lowest tertiles of these two markers.

We subsequently examined potential interactions of leptin biomarkers with other risk factors in relation to type 2 diabetes risk (Table 4). Although we did not find significant interactions between leptin biomarkers and risk factors such as age, fasting status, and physical activity, the association for leptin with diabetes risk was significantly modified by BMI (P for interaction = 0.001). Leptin was significantly associated with a lower risk of type 2 diabetes in participants with a BMI ≥30 kg/m^2. In contrast, in lean participants (BMI <25 kg/m^2), leptin levels were associated with a significantly increased risk of type 2 diabetes. Additional adjustment for waist circumference or waist-to-hip ratio did not change these associations (data not shown).

DISCUSSION

In this nested case-control study conducted in middle-aged U.S. women, we found that high sOB-R levels were

TABLE 2
Partial Spearman correlation coefficients for anthropometric measurements, adipokines, and inflammatory markers among control subjects

	Leptin (ng/ml)	sOB-R (ng/ml)
Age (years)	0.07*	0.11*
BMI (kg/m^2)	0.72*	−0.43*
Waist circumference (cm)	0.07	−0.08†
Waist-to-hip ratio	−0.03	−0.09*
HMW adiponectin (μg/ml)	−0.07†	0.26*
Resistin (ng/ml)	0.06†	−0.07†
CRP (mg/dl)	0.25*	0.02
TNFα-R2 (pg/ml)	0.06	−0.01
IL-6 (ng/ml)	0.08	−0.07
IL-18 (pg/ml)	0.04	0.01
Insulin (μU/ml)‡	0.06	−0.08
C-peptide (pm/ml)‡	0.18*	−0.18*
Leptin (ng/ml)	—	−0.14*
sOB-R (ng/ml)	−0.14*	—

Spearman correlation coefficients between biomarkers were adjusted for age at blood draw, date of blood draw, fasting status (8 h vs. <8 h since last meal), race, BMI (kg/m^2), smoking status (never smoked, former smoker, or current smoker), postmenopausal status (yes, no), hormone use (never used, former user, current user), family history of diabetes (yes, no), and physical activity and alcohol intake (both in quintiles). BMI-adjusted residuals of leptin and sOB-R were used except for the correlations with BMI. n = 1,254 for leptin, sOB-R, age, and BMI; n = 892 for waist circumference and waist-to-hip ratio; n = 1,252 for HMW, resistin, and IL-18; n = 492 for C-reactive protein (CRP), tumor necrosis factor-α receptor 2 (TNFα-R2), and IL-6. *$P < 0.01$. †$P < 0.05$. ‡Fasting samples only, n = 344.

strongly associated with a lower risk of type 2 diabetes independent of baseline leptin or adiposity levels. On the other hand, BMI-adjusted leptin levels were not significantly associated with risk of type 2 diabetes.

Our study provided the first piece of evidence for an inverse association between sOB-R levels and risk of developing type 2 diabetes independent of plasma leptin levels. Leptin generates its central and peripheral effects by binding to its receptors on the cell surface and subsequently activating downstream signaling pathways (29). Through posttranscriptional alternative RNA splicing, several isoforms of leptin receptors with identical extracellular and transmembrane domains but variable intracellular domains are expressed in humans (30). The long form of leptin receptor with the full length of intracellular domain is expressed primarily in the hypothalamus, whereas the short forms of leptin receptor (OB-R_S) are expressed primarily in peripheral tissues (30). sOB-R, a special leptin receptor with the extracellular domain only, is formed by ectodomain shedding of leptin receptors on the cell surface (31), and provides the primary binding capacity in human circulation (15). The function of sOB-R is not entirely clear but believed to delay the clearance of leptin from the circulation and, thus, increase leptin's availability (16). In addition, there is evidence suggesting that sOB-R not only alters the clearance of leptin but also potentiates leptin action (32). This is an analogy to the observation that the action of IL-6 can be boosted by binding to its soluble receptor (33), which shares a homologous structure with leptin receptor (34). Furthermore, because sOB-R levels are highly correlated with the cell surface expression of leptin receptors (r = 0.80) (35), especially OB-R_S, sOB-R may represent the total amount or biological activity of OB-R_S expressed in peripheral tissues. Unlike long form of leptin receptor, OB-R_S does not contain the intracellular motifs required to activate the Janus kinase/signal transducers and activators of transcription signaling pathway that mediates the energy homeostasis effects of leptin (36). However, accumulating evidence suggests that OB-R_S may mediate the effects of leptin on insulin sensitivity and other peripheral effects through a distinct pathway involving insulin receptor substrate/phosphatidylinositol-3-OH kinase pathway (37–41). Although more data are needed to further elucidate sOB-R's functions, these mechanisms may underlie the inverse association with diabetes risk for sOB-R observed in the current study.

Consistent with a previous study (20), we found a significant, positive correlation between sOB-R and HMW adiponectin in the current study. Both biomarkers are correlated with lower levels of adiposity and decrease after weight loss (17,19), and their short-term variations are nearly identical (42), suggesting these two biomarkers may share some common regulatory factors. However, the inverse associations of these two biomarkers were independent of each other, and women with high levels of both biomarkers had dramatically lower risk of developing diabetes. Whether these two molecules share the same pathway or affect diabetes risk through distinct pathways warrants further investigation.

TABLE 3
ORs (95% CI) of type 2 diabetes for quintiles of plasma leptin and sOB-R levels

	Baseline plasma levels					*P* for trend
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Leptin (ng/ml), median (range)	12.7 (<15.0)	17.2 (15.0–19.1)	21.1 (19.2–23.3)	26.1 (23.4–29.7)	35.3 (>29.7)	—
Case/control subjects (n)	252/250	138/251	188/251	226/251	250/251	—
Model 1	1.0	0.54 (0.41–0.71)	0.75 (0.58–0.97)	0.89 (0.70–1.15)	0.99 (0.77–1.27)	0.11
Model 2	1.0	0.76 (0.55–1.04)	0.90 (0.67–1.21)	0.95 (0.71–1.28)	0.82 (0.62–1.10)	0.46
Model 2 + HMW adiponectin	1.0	0.73 (0.52–1.02)	0.85 (0.62–1.17)	0.90 (0.66–1.23)	0.80 (0.59–1.08)	0.39
sOB-R (ng/ml), median (range)	20.4 (<22.9)	25.0 (22.9–27.0)	28.9 (27.1–30.8)	33.9 (30.9–36.9)	42.0 (>36.9)	—
Case/control subjects (n)	268/250	260/251	205/251	192/251	129/251	—
Model 1	1.0	0.96 (0.75–1.22)	0.76 (0.59–0.97)	0.71 (0.55–0.92)	0.47 (0.36–0.62)	<0.0001
Model 2	1.0	0.73 (0.55–0.96)	0.51 (0.38–0.68)	0.42 (0.31–0.57)	0.39 (0.28–0.54)	<0.0001
Model 2 + HMW adiponectin	1.0	0.83 (0.62–1.12)	0.62 (0.46–0.84)	0.61 (0.44–0.84)	0.67 (0.47–0.95)	0.005

Multivariate model 1 was adjusted for matching factors only (age at blood draw, date of blood draw, fasting status [8 h vs. <8 h since last meal], and race). Model 2 was further adjusted for BMI (kg/m^2), smoking status (never smoked, past smoker, or current smoker), postmenopausal status (yes, no), hormone use (never used, past user, current user), family history of diabetes (yes, no), physical activity (in quintiles), intake of alcohol, cereal fiber, heme iron, *trans* fat, and magnesium, and coffee and red meat consumption (all in quintiles).

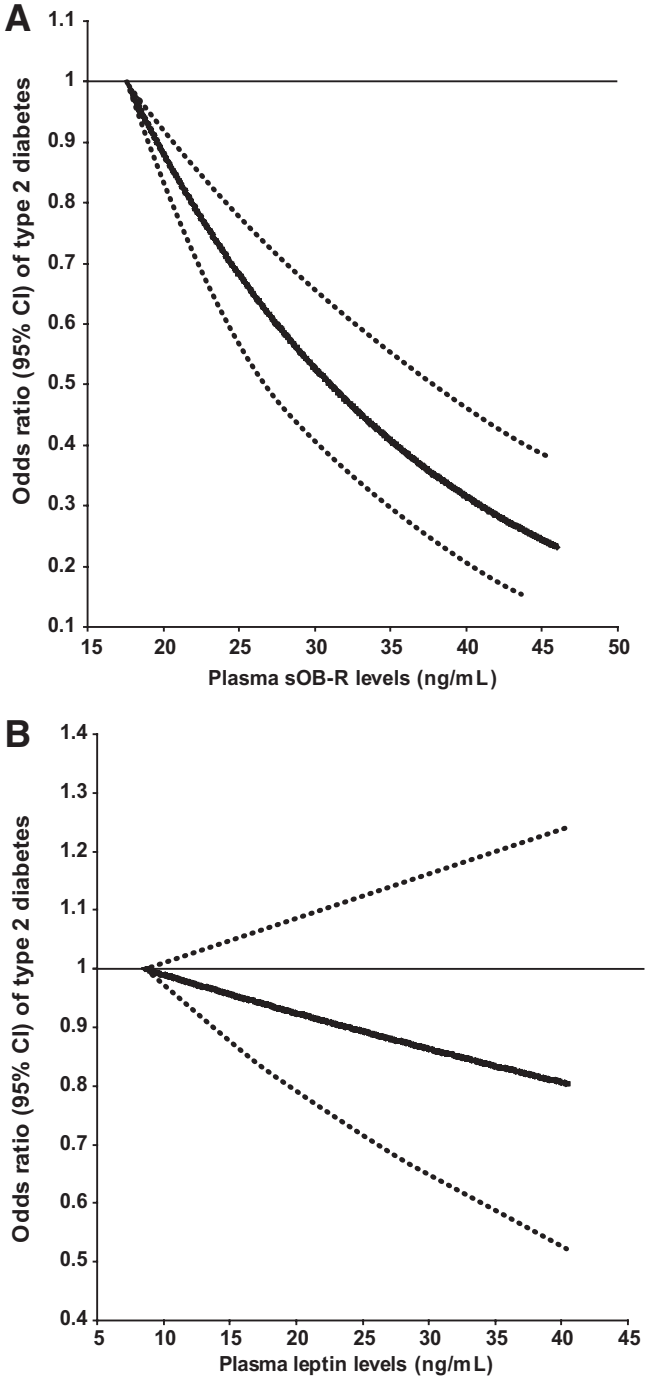

FIG. 1. ORs of type 2 diabetes according to plasma sOB-R and leptin levels. Participants with the lowest and highest 5% of leptin or sOB-R levels were excluded to minimize potential impact of outliers. Multivariate logistic regression models were adjusted for the same set of covariates for model 2 in Table 3. BMI as a continuous variable was further adjusted for in these models. Solid lines are ORs and dashed lines are 95% CIs. *A*: sOB-R. *B*: Leptin.

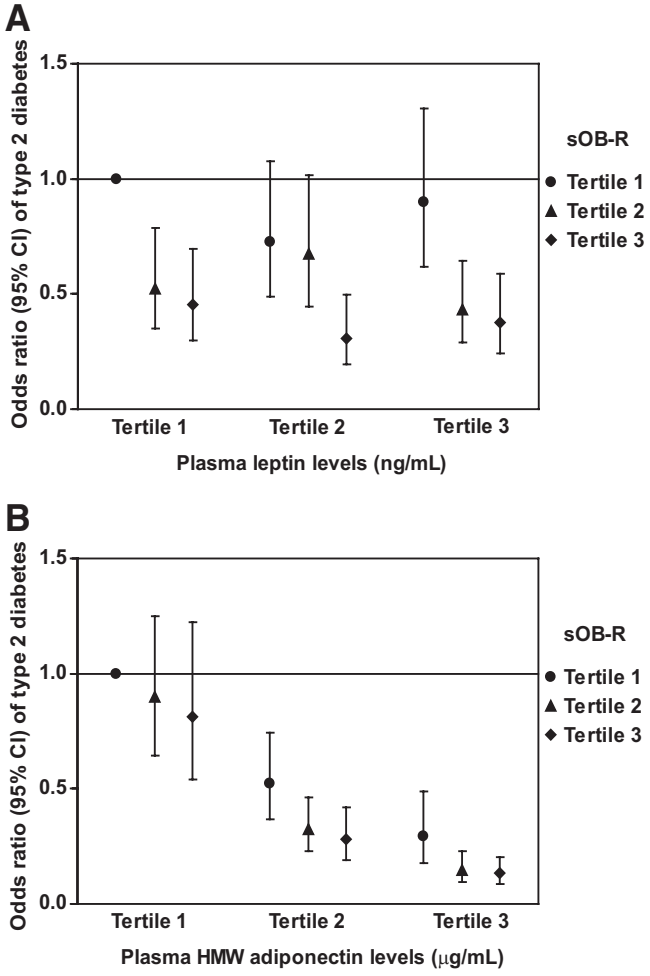

FIG. 2. Joint effects of sOB-R, leptin, and HMW adiponectin on type 2 diabetes risk. Multivariate logistic regression models were adjusted for the same set of covariates for model 2 in Table 3. BMI as a continuous variable was further adjusted for in these models. *A*: sOB-R and leptin. *B*: sOB-R and HMW adiponectin.

Although intervention studies conducted in leptin-deficient animal models and humans supported a beneficial effect of leptin on insulin sensitivity or type 2 diabetes (43–47), prospective epidemiologic data on circulating leptin levels and future risk of type 2 diabetes have been mixed in subjects with normal leptin secretion ability (6–14). In humans, leptin levels are strongly correlated with subcutaneous adiposity, reflecting leptin resistance in obese individuals. To remove the confounding effects of BMI, we examined BMI-adjusted leptin levels in relation to diabetes risk. In our study, BMI-adjusted leptin levels were not significantly associated with diabetes risk. However, our data suggest that the association between leptin and type 2 diabetes was modulated by BMI. Leptin levels tended to be associated with a lower risk among relatively obese subjects. Two previous studies also found a similar association (8,14). One possible explanation for the positive association between leptin and diabetes risk in lean participants could be that high leptin levels reflected the amount of adipose tissue that was not captured by BMI, although further adjustment for waist circumference did not change our observation. The beneficial effects of high leptin levels on diabetes risk in overweight or obese people may be related to the peripheral effects of leptin on insulin sensitivity rather than its effects on weight regulation (5) because in the cerebrospinal fluid free leptin levels are already saturated at low levels of circulating leptin levels (48). Whether high leptin levels in lean people represent a deteriorating metabolic status needs further examination.

The current study has several strengths. The prospective study design made it unlikely that disease status or treatment may influence the leptin or sOB-R levels (i.e., that reverse causation occurred). In addition, we used

TABLE 4
ORs (95% CI) of type 2 diabetes for tertiles of plasma leptin and sOB-R levels by lifestyle factors

	Baseline plasma levels				
	T1 (lowest)	T2	T3 (highest)	*P* for trend	*P* for interaction
Leptin (ng/ml)					
Age at blood draw*					0.11
<60 (case subject = 713, control subject = 837)	1.0	0.82 (0.61–1.11)	1.03 (0.77–1.37)	0.72	—
≥60 (case subject = 341, control subject = 417)	1.0	1.10 (0.70–1.70)	0.87 (0.57–1.31)	0.39	—
Fasting status (≥ 8 h since last meal)					0.63
Fasting (case subject = 376, control subject = 450)	1.0	0.80 (0.54–1.20)	0.91 (0.62–1.35)	0.70	—
Nonfasting (case subject = 678, control subject = 804)	1.0	1.00 (0.73–1.36)	0.95 (0.71–1.28)	0.70	—
BMI at baseline (kg/m^2)*					0.001
<25 (case subject = 182, control subject = 653)	1.0	1.24 (0.78–1.97)	1.67 (1.01–2.76)	0.04	—
25.0–29.9 (case subject = 363, control subject = 365)	1.0	0.98 (0.63–1.52)	0.88 (0.60–1.28)	0.47	—
≥30 (case subject = 509, control subject = 236)	1.0	0.78 (0.50–1.24)	0.61 (0.40–0.93)	0.02	—
Physical activity at baseline (MET-h)*					0.78
<10 (case subject = 569, control subject = 515)	1.0	0.84 (0.58–1.21)	0.91 (0.64–1.29)	0.68	—
≥10 (case subject = 485, control subject = 739)	1.0	0.94 (0.67–1.32)	0.97 (0.70–1.33)	0.86	—
sOB-R (ng/ml)					
Age at blood draw*					0.04
<60 (case subject = 713, control subject = 837)	1.0	0.66 (0.50–0.88)	0.37 (0.27–0.52)	<0.0001	—
≥60 (case subject = 341, control subject = 417)	1.0	0.49 (0.32–0.74)	0.55 (0.36–0.84)	0.01	—
Fasting status (≥ 8 h since last meal)					0.55
Fasting (case subject = 376, control subject = 450)	1.0	0.60 (0.41–0.89)	0.35 (0.22–0.54)	<0.0001	—
Nonfasting (case subject = 678, control subject = 804)	1.0	0.59 (0.44–0.78)	0.48 (0.35–0.65)	<0.0001	—
BMI at baseline (kg/m^2)*					0.76
<25 (case subject = 182, control subject = 653)	1.0	0.70 (0.44–1.10)	0.52 (0.32–0.83)	0.006	—
25.0–29.9 (case subject = 363, control subject = 365)	1.0	0.55 (0.38–0.80)	0.51 (0.33–0.79)	0.002	—
≥30 (case subject = 509, control subject = 236)	1.0	0.55 (0.36–0.84)	0.44 (0.27–0.72)	0.001	—
Physical activity at baseline (MET-h)*					0.14
<10 (case subject = 569, control subject = 515)	1.0	0.68 (0.49–0.95)	0.35 (0.24–0.52)	<0.0001	—
≥10 (case subject = 485, control subject = 739)	1.0	0.55 (0.40–0.76)	0.52 (0.37–0.72)	0.0002	—

Multivariate models were adjusted for the same set of covariates for model 2 in Table 3. *We further adjusted for the interacting variable in the continuous form to control for residual confounding in the stratified analysis.

BMI-adjusted residuals of leptin biomarkers in the current analysis to more completely control for strong confounding by BMI. Other strengths include a large sample size, long follow-up period, validated approach for confirming type 2 diabetes case subjects, and adjustment for a multitude of risk factors for type 2 diabetes.

Several limitations merit discussion as well. First, in observational studies, residual confounding, especially the confounding by adiposity not captured by BMI and waist circumference in the current study, cannot be entirely ruled out. In addition, some covariates were assessed at different time points, which may lead to potentially incomplete controlling for confounding by these factors. Second, a single baseline measurement of leptin or sOB-R levels may not represent the long-term levels of these markers. However, leptin levels have been shown to be quite stable over time (49). Similar to adiponectin, the short-term variation of sOB-R levels was relatively small (50). Third, because our study sample included only middle-aged women who were predominantly white, it is unclear whether the results can be generalized to men and other ethnic groups.

In conclusion, we found a strong, inverse association between circulating soluble leptin receptor levels and risk of type 2 diabetes, independent of obesity and leptin levels. On the other hand, the association between leptin and diabetes risk may be modified by adiposity. Biological mechanisms underlying these novel observations need to be further elucidated.

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REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–432
2. Farooqi IS, O'Rahilly S. Leptin: a pivotal regulator of human energy homeostasis. Am J Clin Nutr 2009;89:980S–984S
3. Harris RB. Leptin—much more than a satiety signal. Annu Rev Nutr 2000;20:45–75

4. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–770
5. Ceddia RB, Koistinen HA, Zierath JR, Sweeney G. Analysis of paradoxical observations on the association between leptin and insulin resistance. Faseb J 2002;16:1163–1176
6. Ley SH, Harris SB, Connelly PW, Mamakeesick M, Gittelsohn J, Hegele RA, Retnakaran R, Zinman B, Hanley AJ. Adipokines and incident type 2 diabetes in an Aboriginal Canadian [corrected] population: the Sandy Lake Health and Diabetes Project. Diabetes Care 2008;31:1410–1415
7. Boyko EJ, de Courten M, Zimmet PZ, Chitson P, Tuomilehto J, Alberti KG. Features of the metabolic syndrome predict higher risk of diabetes and impaired glucose tolerance: a prospective study in Mauritius. Diabetes Care 2000;23:1242–1248
8. Wannamethee SG, Lowe GD, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and risk of type 2 diabetes in older men. Diabetes Care 2007;30:1200–1205
9. Soderberg S, Zimmet P, Tuomilehto J, Chitson P, Gareeboo H, Alberti KG, Shaw JE. Leptin predicts the development of diabetes in Mauritian men, but not women: a population-based study. Int J Obes (Lond) 2007;31:1126–1133
10. Welsh P, Murray HM, Buckley BM, de Craen AJ, Ford I, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Sattar N. Leptin predicts diabetes but not cardiovascular disease: results from a large prospective study in an elderly population. Diabetes Care 2009;32:308–310
11. McNeely MJ, Boyko EJ, Weigle DS, Shofer JB, Chessler SD, Leonnetti DL, Fujimoto WY. Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. Diabetes Care 1999;22:65–70
12. Snijder MB, Dekker JM, Bouter LM, Heine RJ, Stehouwer CD, Seidell JC. Comment on: Schmidt MI, Duncan BB, Vigo A et al. (2006) Leptin and incident type 2 diabetes: risk or protection? Diabetologia 2006;49:2086–2096. Diabetologia 2007;50(1):234–236; author reply 237–238; discussion 239–240
13. Kanaya AM, Wassel Fyr C, Vittinghoff E, Harris TB, Park SW, Goodpaster BH, Tylavsky F, Cummings SR. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. Arch Intern Med 2006;166:350–356
14. Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G, ARIC Investigators. Leptin and incident type 2 diabetes: risk or protection? Diabetologia 2006;49:2086–2096
15. Lammert A, Kiess W, Bottner A, Glasow A, Kratzsch J. Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun 2001;283:982–988
16. Huang L, Wang Z, Li C. Modulation of circulating leptin levels by its soluble receptor. J Biol Chem 2001;276:6343–6349
17. Ogier V, Ziegler O, Méjean L, Nicolas JP, Stricker-Krongrad A. Obesity is associated with decreasing levels of the circulating soluble leptin receptor in humans. Int J Obes Relat Metab Disord 2002;26:496–503
18. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR. Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. Obes Res 2003;11:760–768
19. Laimer M, Ebenbichler CF, Kaser S, Sandhofer A, Weiss H, Nehoda H, Aigner F, Patsch JR. Weight loss increases soluble leptin receptor levels and the soluble receptor bound fraction of leptin. Obes Res 2002;10:597–601
20. Ogawa T, Hirose H, Yamamoto Y, Nishikai K, Miyashita K, Nakamura H, Saito I, Saruta T. Relationships between serum soluble leptin receptor level and serum leptin and adiponectin levels, insulin resistance index, lipid profile, and leptin receptor gene polymorphisms in the Japanese population. Metabolism 2004;53:879–885
21. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH, Speizer FE. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 1991;338:774–778
22. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004;53:693–700
23. Schulze MB, Solomon CG, Rifai N, Cohen RM, Sparrow J, Hu FB, Manson JE. Hyperproinsulinaemia and risk of type 2 diabetes mellitus in women. Diabet Med 2005;22:1178–1184
24. Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworoger SS, Mantzoros CS, Hu FB. Total and high-molecular-weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. Ann Intern Med 2008;149:307–316
25. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S–1228S; discussion 1229S–1231S
26. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol 1999;149:531–540
27. Kleinbaum DG, Kupper LL, Muller KE, Nizam A. *Applied Regression Analysis and Other Multivariable Methods*. Boston, MA, Duxbury Press, 1998
28. Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989;8:551–561
29. Kronenberg HM, Melmed S, Larsen PR, Polonsky KS. *Williams Textbook of Endocrinology*. Philadelphia, PA, Elsevier Health Sciences, 2008
30. Tartaglia LA. The leptin receptor. J Biol Chem 1997;272:6093–6096
31. Ge H, Huang L, Pourbahrami T, Li C. Generation of soluble leptin receptor by ectodomain shedding of membrane-spanning receptors in vitro and in vivo. J Biol Chem 2002;277:45898–45903
32. Cohen SE, Kokkotou E, Biddinger SB, Kondo T, Gebhardt R, Kratzsch J, Mantzoros CS, Kahn CR. High circulating leptin receptors with normal leptin sensitivity in liver-specific insulin receptor knock-out (LIRKO) mice. J Biol Chem 2007;282:23672–23678
33. Maione D, Di Carlo E, Li W, Musiani P, Modesti A, Peters M, Rose-John S, Della Rocca C, Tripodi M, Lazzaro D, Taub R, Savino R, Ciliberto G. Coexpression of IL-6 and soluble IL-6R causes nodular regenerative hyperplasia and adenomas of the liver. EMBO J 1998;17:5588–5597
34. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. Cell 1995;83:1263–1271
35. Maamra M, Bidlingmaier M, Postel-Vinay MC, Wu Z, Strasburger CJ, Ross RJ. Generation of human soluble leptin receptor by proteolytic cleavage of membrane-anchored receptors. Endocrinology 2001;142:4389–4393
36. Bjørbaek C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. J Biol Chem 1997;272:32686–32695
37. Huan JN, Li J, Han Y, Chen K, Wu N, Zhao AZ. Adipocyte-selective reduction of the leptin receptors induced by antisense RNA leads to increased adiposity, dyslipidemia, and insulin resistance. J Biol Chem 2003;278:45638–45650
38. Morton GJ, Gelling RW, Niswender KD, Morrison CD, Rhodes CJ, Schwartz MW. Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. Cell Metab 2005;2:411–420
39. Zhao AZ, Shinohara MM, Huang D, Shimizu M, Eldar-Finkelman H, Krebs EG, Beavo JA, Bornfeldt KE. Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. J Biol Chem 2000;275:11348–11354
40. Anderwald C, Müller G, Koca G, Fürnsinn C, Waldhäusl W, Roden M. Short-term leptin-dependent inhibition of hepatic gluconeogenesis is mediated by insulin receptor substrate-2. Mol Endocrinol 2002;16:1612–1628
41. Huang W, Dedousis N, Bhatt BA, O'Doherty RM. Impaired activation of phosphatidylinositol 3-kinase by leptin is a novel mechanism of hepatic leptin resistance in diet-induced obesity. J Biol Chem 2004;279:21695–21700
42. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004;50:1511–1525
43. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540–543
44. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A. Leptin-replacement therapy for lipodystrophy. N Engl J Med 2002;346:570–578
45. Andreev VP, Paz-Filho G, Wong ML, Licinio J. Deconvolution of insulin secretion, insulin hepatic extraction post-hepatic delivery rates and sensitivity during 24-hour standardized meals: time course of glucose homeostasis in leptin replacement treatment. Horm Metab Res 2009;41:142–151
46. Paz-Filho G, Esposito K, Hurwitz B, Sharma A, Dong C, Andreev V, Delibasi T, Erol H, Ayala A, Wong ML, Licinio J. Changes in insulin sensitivity during leptin replacement therapy in leptin-deficient patients. Am J Physiol Endocrinol Metab 2008;295:E1401–E1408
47. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest 2002;109:1345–1350

48. Brabant G, Horn R, von zur Mühlen A, Mayr B, Wurster U, Heidenreich F, Schnabel D, Grüters-Kieslich A, Zimmermann-Belsing T, Feldt-Rasmussen U. Free and protein bound leptin are distinct and independently controlled factors in energy regulation. Diabetologia 2000;43:438–442
49. Lee SA, Kallianpur A, Xiang YB, Wen W, Cai Q, Liu D, Fazio S, Linton MF, Zheng W, Shu XO. Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. Cancer Epidemiol Biomarkers Prev 2007;16:2464–2470
50. Gavrila A, Peng CK, Chan JL, Mietus JE, Goldberger AL, Mantzoros CS. Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. J Clin Endocrinol Metab 2003;88:2838–2843