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Total and High-Molecular Weight Adiponectin and Resistin in Relation to the Risk for Type 2 Diabetes in Women: A Prospective Study

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Abstract

Background—Adiponectin, particularly high-molecular weight adiponectin, and resistin are recently discovered adipokines that may provide a molecular link between adiposity and type 2 diabetes.

Objective—To evaluate whether total and high-molecular weight adiponectin and resistin are associated with the future risk for type 2 diabetes, independent of obesity and other known diabetes risk factors.

Design—Prospective, nested case-control study.

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Statistical code: Available subject to approval by the NHS committees.

Data set: Available subject to approval by the NHS committees

Settings—United States.

Participants—1,038 initially healthy women of the Nurses' Health Study who developed type 2 diabetes after blood sampling (1989–1990) through 2002 and 1,136 matched control subjects.

Measurements—Plasma concentrations of adipokines were measured by enzyme-linked immunosorbent assays.

Results—In multivariate models adjusting for diabetes risk factors including body-mass index (BMI), both higher total and high-molecular weight adiponectin levels were associated with a substantially lower risk for type 2 diabetes. The odds ratios comparing the highest with the lowest quintiles were 0.17 (95% CI, 0.12–0.25) for total adiponectin and 0.10 (CI, 0.06–0.15) for high-molecular weight adiponectin. In addition, a higher high-molecular weight-to-total adiponectin ratio was associated with a significantly lower risk even after adjusting for total adiponectin (odds ratio, 0.45 [CI, 0.31–0.65]). In the multivariate model adjusting for diabetes risk factors except BMI, high resistin levels were significantly associated with an increased diabetes risk for (odds ratio, 1.68 [CI, 1.25–2.25]). However, after additional adjustment for BMI, this association was no longer statistically significant (odds ratio, 1.28 [CI, 0.93–1.76]).

Limitations—Residual confounding by imperfectly or unmeasured confounders cannot be excluded.

Conclusions—These results indicate that adiponectin, but not resistin is strongly associated with diabetes risk independent of BMI. The high-molecular weight-to-total adiponectin ratio is related to diabetes risk independent of total adiponectin, suggesting an important role of the relative proportion of high-molecular weight adiponectin in diabetes pathogenesis.

Introduction

Excess adiposity is a major modifiable risk factor for the development of type 2 diabetes (1). It is now established that the adipose tissue is an important metabolically active endocrine organ that secretes a variety of hormones and cytokines, termed adipokines (2). These adipokines, particularly adiponectin and resistin, have been proposed to provide a molecular link between adiposity and type 2 diabetes (3, 4).

Adiponectin is a collagen-like protein that is exclusively synthesized by adipocytes (5) and circulates at relatively high concentrations of 2–30 $\mu\text{g/mL}$ in blood (3, 6). Unlike other adipokines, concentrations of adiponectin are paradoxically reduced in obese compared with lean subjects (3, 6). There is no clear explanation for this inverse association, but in vitro and in vivo studies suggest endogenous inflammatory cytokines such as interleukin-6, interleukin-8, and tumor necrosis factor- α , which are elevated in obesity, may inhibit the production of adiponectin (7). In addition, prospective studies have consistently found a decreased risk for type 2 diabetes with increasing concentrations of total adiponectin (8–18). However, adiponectin exists in plasma as a trimer, a hexamer, and a high-molecular weight form (2, 6). Recent evidence suggests that high-molecular weight adiponectin is the most biologically active form of the hormone (6), but few epidemiologic studies have investigated high-molecular weight adiponectin separately from total adiponectin (18–20).

Resistin is a cysteine-rich polypeptide that is expressed at relatively lower levels in human adipocytes, but higher levels in macrophages (4, 21). Initial experimental studies in rodents pointed to an important role of resistin as a mediator of obesity-associated insulin resistance (22). While the majority of recent animal studies further supports this hypothesis, the physiological relevance of resistin for obesity-related conditions in humans remains controversial (4), and inference from most human studies was limited by cross-sectional design and small sample size (23–33).

Therefore, we conducted a large, prospective case-control study nested within the Nurses' Health Study to evaluate whether total and high-molecular weight adiponectin and resistin are associated with the future risk for type 2 diabetes, independent of obesity and other known diabetes risk factors.

Methods

Study population

The Nurses' Health Study was initiated in 1976, when 121,700 female U.S. nurses aged 30–55 years responded to a questionnaire requesting health-related information. Since 1976, this cohort has been followed using a biennially mailed questionnaire to obtain updated information. Between 1989 and 1990, a subset of 32,826 consenting women provided blood samples. For the present study, we included those 1,059 women who were free of diabetes, coronary heart disease, stroke, and cancer at blood collection and developed type 2 diabetes at least one year after their blood sampling through June 2002. Control subjects had similar exclusion criteria at baseline and were matched to diabetes cases by age at blood draw (± 1 year), date of blood draw (± 3 month), fasting status (≥ 8 hours vs. < 8 hours since last meal), and race. To improve statistical control for obesity, additional control subjects were matched by these factors and by body-mass index (BMI; ± 1 unit) to diabetes cases in the upper decile of the BMI distribution, providing 1,164 control subjects (1,059 non-BMI matched and 105 BMI-matched control subjects). From this sample, we excluded all women, who had missing information on BMI ($n=4$) and missing values ($n=44$) or values below the detection limit ($n=1$) for the adipokines. The final analytical sample comprised 1,038 diabetes cases and 1,136 control subjects (1,032 non-BMI matched and 104 BMI-matched control subjects). The study was approved by the Institutional Review Board of the Brigham and Women's Hospital, Boston, Massachusetts.

Ascertainment of type 2 diabetes

Cases of type 2 diabetes were identified by the biennially mailed questionnaires and confirmed by a validated supplementary questionnaire regarding symptoms, diagnostic laboratory test results, and diabetes treatment as described previously (34). Validation studies in subsamples of the Nurses' Health Study revealed a high validity of the self-reported diagnosis of diabetes through the supplementary questionnaire (98.4% were confirmed by medical records) and a low percentage of false negative self-reports (0.5%) (35).

Assessment of lifestyle and dietary information

Body weight was reported at the time of blood collection. BMI was calculated as the ratio of weight (in kg) to squared height (in m^2). The presence or absence of a family history of diabetes in a first-degree relative was reported in 1982 and 1988. Information on cigarette smoking, menopausal status, use of hormone replacement therapy, and physical activity was obtained from the questionnaire in 1988. Physical activity was computed as metabolic equivalents per week based on the average time spent per week on various leisure time activities, weighted by their intensity level (36). Dietary intake was expressed as average of the intakes obtained from semiquantitative food frequency questionnaires administered in 1986 and 1990. Values for nutrients were energy adjusted by the residual method (37). The validity of self-reported body weight, physical activity, and dietary intake in this cohort of women has been previously reported (38).

Laboratory procedures

The blood collection and processing procedure has been described in detail previously (34). Briefly, women consenting to provide blood samples were sent a phlebotomy kit. Blood samples were returned by overnight mail in a frozen water bottle, processed upon arrival, and stored in liquid nitrogen -130°C or lower until laboratory analysis.

Study samples were analyzed in randomly ordered case-control pairs and the laboratory personnel was blinded to the case-control status to reduce interassay variation and systematic bias. Resistin concentration was measured by an enzyme-linked immunosorbent assay (ELISA) (Linco Research, Inc., St. Charles, Missouri) with a minimum detectable limit of 0.16 ng/mL. The intraclass correlation for the reproducibility of resistin over one year was 0.75 ($n=35$). Total and high-molecular weight adiponectin were determined by ELISA (ALPCO Diagnostics, Salem, New Hampshire) with a sensitivity of 0.04 ng/mL. The recovery rate was 99–103% for total adiponectin and 97–105% for high-molecular weight adiponectin (20). In our study, the coefficient of variation for total adiponectin, high-molecular weight adiponectin, and resistin based on blinded quality control samples was 8.9%, 9.9%, and 2.5% respectively. Assays used for the measurement of C-reactive protein, interleukin-6, and soluble tumor necrosis factor- α receptor 2 and of fasting insulin, proinsulin, C-peptide, and HbA_{1c} were described elsewhere (34, 39).

Statistical analysis

Based on their distribution among control subjects, adipokine concentrations were divided into quintiles. One exception was the categorization into tertiles for the joint classification of adipokines due to low numbers of subjects in extreme joint categories. Category-specific odds ratios of type 2 diabetes were estimated using unconditional logistic regression adjusted for matching factors (age [years], date of blood draw [month], fasting status [8 hours vs. <8 hours since last meal], and ethnicity). We repeated the main analyses using conditional logistic regression and observed essentially the same results. Thus, we presented the results from unconditional regression for both main effects and subgroup analyses. In multivariate models, odds ratios were further adjusted for known and suggested diabetes risk factors (1) including physical activity (quintiles of metabolic equivalents/week), smoking (never, past, current 1–14 cigarettes/d, and current 15 cigarettes/d), family history of diabetes (yes/no), hormone replacement therapy (premenopause, never, past, current, and missing information), alcohol consumption (nondrinkers, 0.1–4.9, 5–10, and >10 g/d), glycemic load (quintiles), ratio of polyunsaturated to saturated fatty acids (quintiles), intakes (quintiles) of cereal fiber, trans-fatty acids, magnesium, caffeine, and total energy intake, and BMI (<23, 23–24.9, 25–26.9, 27–29.9, 30–34.9, and ≥ 35 kg/m²). Adjustment for BMI as a continuous instead of a categorized variable yielded nearly identical results. Trend tests were conducted by including the median adipokine concentration of each quintile as a continuous variable into the models. Potential interactions between adipokines and covariates were tested by using the likelihood ratio test (40). Akaike's information criterion was used to compare the goodness of fit for non-nested models (41).

We used restricted cubic spline regression with 4 knots according to the 5th, 35th, 65th, and 95th percentiles of the adipokine concentrations to examine a possible non-linear relation of the biomarkers to diabetes risk (42). Tests for non-linearity were conducted using the likelihood ratio test (40).

All reported *P* values are two-sided. Statistical analyses were performed using SAS software 9.1 (SAS Institute Inc., Cary, NC).

Role of the funding sources

The funding sources had no role in the collection, analysis, and interpretation of the data and in the decision to submit the manuscript for publication.

Results

Women who developed type 2 diabetes during the follow-up had higher mean baseline values for BMI and waist circumference, were less physically active, and were more likely to have a family history of diabetes than those who remained free of diabetes (Table 1). In addition, women developing type 2 diabetes had significantly lower baseline concentrations of total and high-molecular weight adiponectin, a lower high-molecular weight-to-total adiponectin ratio, and higher concentrations of resistin than control subjects.

Among control subjects, both total and high-molecular weight adiponectin showed moderate inverse correlations with markers of obesity, inflammation, insulin secretion, and hyperglycemia, with the correlations being slightly stronger for high-molecular weight adiponectin (Appendix Table 1). Adjustment for BMI attenuated these correlations, although the correlations of total and high-molecular weight adiponectin with fasting insulin, proinsulin, and C-peptide remained significant. The high-molecular weight-to-total adiponectin ratio showed similar patterns of correlations; however, after adjustment for BMI, correlations with fasting proinsulin and C-peptide were no longer significant. Resistin also showed modest positive associations with BMI and with inflammatory and insulin markers. Adjustment for BMI nearly abolished the correlations between resistin and the markers of insulin secretion, although resistin remained significantly correlated with the inflammatory markers interleukin-6 and soluble tumor necrosis factor- α receptor 2.

In multivariate models adjusting for diabetes risk factors except BMI, both total and high-molecular weight adiponectin levels were strongly and inversely associated with the risk for type 2 diabetes (Table 2). These associations were only slightly attenuated after further adjusting for BMI; the odds ratios comparing the highest with the lowest quintiles were 0.17 (95% CI, 0.12–0.25) for total adiponectin and 0.10 (CI, 0.06–0.15) for high-molecular weight adiponectin. Results from conditional logistic regression were essentially the same; the odds ratios comparing extreme quintiles were 0.12 (95% CI, 0.08–0.20) for total adiponectin and 0.06 (CI, 0.04–0.12) for high-molecular weight adiponectin. Additional adjustment for weight and physical activity change between 1986 and baseline did not alter the results; the odds ratios comparing extreme quintiles were identical to those in the analysis without these potential confounder variables.

When both total and high-molecular weight adiponectin were included in the same multivariate model including BMI, the inverse associations with type 2 diabetes remained significant (P for trend=0.006 or <0.001 for total or high-molecular weight adiponectin, respectively). However, because of the collinearity between total and high-molecular weight adiponectin, odds ratios were attenuated and less precise (as indicated by wider confidence intervals): odds ratios across quintiles were 1.0, 0.63 (CI, 0.46–0.88), 0.48 (0.31–0.73), 0.38 (0.23–0.64), and 0.55 (0.29–1.04) for total adiponectin and 1.0, 0.56 (CI, 0.40–0.78), 0.63 (0.41–0.96), 0.52 (0.31–0.86), and 0.17 (0.09–0.35) for high-molecular weight adiponectin. The multivariate models with total and high-molecular weight adiponectin yielded values of 2423 and 2407 for the Akaike's information criterion, respectively, suggesting a slightly better model fit with high-molecular weight than total adiponectin. The hypothesis that adiponectin might be a molecular link between adiposity and type 2 diabetes was supported by results showing attenuated risk estimates of BMI after adjusting for adiponectin (odds ratio 1.11 [CI, 1.09–1.13] and 1.07 [1.05–1.09] for BMI as continuous variable in the multivariate model with and without total adiponectin, respectively).

The high-molecular weight-to-total adiponectin ratio also showed a significantly inverse association with diabetes risk even after adjusting for BMI and other diabetes risk factors (Table 2). This association was attenuated but remained significant after further adjustment for total adiponectin (odds ratio comparing extreme quintiles 0.45, [CI, 0.31–0.65]).

In a multivariate model adjusting for diabetes risk factors except BMI, resistin levels were significantly and positively associated with diabetes risk (Table 2). However, additional adjustment for BMI attenuated the risk estimates and the association was no longer significant; the odds ratio comparing extreme quintiles was 1.28 (CI, 0.93–1.76). Further adjustment for total or high-molecular weight adiponectin did not alter these results appreciably.

Joint classification of the adipokines indicated no additional role of resistin above total or high-molecular weight adiponectin in risk estimation (Figure 1A–B). In addition, joint analysis of total adiponectin and the high-molecular weight-to-total adiponectin ratio pointed to an independent association of the ratio with type 2 diabetes at both low and high total adiponectin levels (Figure 1C).

Spline regression models illustrated a deviation from linearity for the relation between total (P for non-linearity =0.005) and high-molecular weight adiponectin (P for non-linearity <0.001) and risk for type 2 diabetes, with a stronger decrease in risk within the lower than the higher range of adiponectin concentrations (Figure 2A–B). However, spline regression showed a strong linear association between the high-molecular weight-to-total adiponectin ratio and diabetes risk (P for non-linearity =0.71) (Figure 2C). Moreover, spline regression further confirmed the non-significant association between resistin and diabetes risk after accounting for confounder variables including BMI (Figure 2D).

Stratified and sensitivity analysis

To explore whether overweight status, physical activity level, family history of diabetes, or the C-reactive protein level modify the observed associations, we performed stratified analyses (Appendix Table 2). The observed associations were largely consistent across all subgroups and most interaction tests were not statistically significant. There was some evidence suggesting that the association between resistin and diabetes was stronger among normal weight than overweight women, but the P value for interaction was not significant.

To address the possibility of undiagnosed diabetes or of subclinical cardiovascular disease at baseline, we repeated the analysis after excluding women with baseline HbA_{1c} values >6.5%; the first 6 years of follow-up; or women who developed an incident myocardial infarction or stroke during the follow-up, respectively. The results from these sensitivity analyses remained essentially the same (Appendix Table 3). In addition, adjustment for waist circumference, history of hypertension and hyperlipidemia, or C-reactive protein did not appreciably change the results. Adjustment for fasting insulin (as a measure of insulin resistance) and the proinsulin-to-insulin ratio (as a measure of β -cell dysfunction) did not materially alter results. Sensitivity analyses were performed in subsamples of the study population with available waist or biomarker measurements.

Discussion

In this large, nested case-control study of initially healthy women, high baseline concentrations of total and high-molecular weight adiponectin were associated with a substantially reduced risk for developing type 2 diabetes over 12 years of follow-up. These associations were independent of several known diabetes risk factors including BMI and not explained by markers of insulin resistance or inflammation. A high ratio of high-molecular

weight to total adiponectin was also related to a decreased risk for type 2 diabetes, independently of total adiponectin. In contrast, higher concentrations of resistin were associated with a significantly increased diabetes risk, but this association was attenuated after adjusting for BMI.

Our results of the inverse association between total adiponectin and diabetes risk are in accordance with previous prospective studies among other populations such as Pima Indians (8), Europeans (9–13), Asians (14–17), and Japanese-Americans (18). However, inferences from some of the previous studies were hindered by highly selected study populations such as patients with impaired glucose tolerance or impaired fasting glucose (14, 17), coronary artery disease (14), and kidney transplantation (10) or a relatively short period of follow-up. Further, most of the previous studies did not account for lifestyle factors such as smoking and physical activity or dietary factors (8, 10, 14–18), which might confound the association between adiponectin and diabetes risk. Therefore, the results of the present study, with women free of major chronic diseases at baseline, a long follow-up period, and a comprehensive adjustment for most known diabetes risk factors, extend the evidence linking total adiponectin to the future risk for type 2 diabetes.

Adiponectin circulates in several forms, principally as a low-molecular weight hexamer (~180 kDa) and a high-molecular weight multimer (~360 kDa) (6). Recent evidence suggests that the high-molecular weight adiponectin complex may be the more biologically active form. For example, the proportion of high-molecular weight to total adiponectin was more strongly correlated with 2-hour glucose levels in glucose tolerance testing in Indo-Asian males (43) and was more predictive of insulin resistance or the metabolic syndrome in Japanese patients (44) than total adiponectin. Further, high-molecular weight adiponectin was shown to be more strongly related to multiple characteristics within the metabolic syndrome complex than total adiponectin (19). However, other data do not support a superiority of the high-molecular weight form over total adiponectin in identifying the presence of insulin resistance (20). It must be pointed out that all these studies were small and cross-sectional in nature. The only prospective study that was conducted in Japanese-Americans reported that high-molecular weight adiponectin was more closely related to incident type 2 diabetes than total adiponectin (18). In our study, both higher total and high-molecular weight adiponectin levels were strongly associated with a decreased risk for type 2 diabetes. Our data also suggest a strong association of the high-molecular weight-to-total adiponectin ratio with diabetes risk, independent of total adiponectin levels, providing evidence for an important role of the relative proportion of the high-molecular weight form in the development of type 2 diabetes.

Interestingly, the inverse association with diabetes risk appeared to be particularly pronounced within the lower ranges of total and high-molecular weight adiponectin distributions. This suggests that potentially high risk subjects with very low adiponectin levels could benefit most from interventions that increase adiponectin levels such as weight loss by lifestyle modifications or thiazolidinedione therapy (6). However, further studies are needed to confirm the markedly elevated diabetes risk within the low range of adiponectin levels observed in this study and to elucidate the underlying biological mechanisms.

For resistin, initial experimental studies showed that circulating concentrations are markedly increased in both diet-induced and genetically obese mice and decreased after treatment with insulin-sensitizing peroxisome proliferator-activated receptor (PPAR)- γ agonists (22). Moreover, treatment with recombinant resistin induced insulin resistance in healthy mice, whereas immunoneutralization of resistin improved insulin sensitivity in obese, insulin-resistant mice (22). However, human studies are highly inconsistent. While several studies reported higher resistin concentrations in obese than in leaner persons (23–25, 45), others

did not observe significant differences (28–30, 46). Similarly, several studies demonstrated higher resistin concentrations in insulin-resistant or diabetic persons as compared with their healthy counterparts (23–27), whereas other studies did not find such a difference (28–33). These conflicting data may result from lack of adjustment for potential confounding variables, limited power due to small sample sizes, and the cross-sectional design. To our knowledge, the present study is the first prospective analysis on resistin and the risk for type 2 diabetes. Our results indicate that resistin is not significantly related to diabetes risk after adjusting for BMI, although resistin remained positively, albeit moderately, associated with several inflammatory markers. These findings suggest that resistin could be a marker of subclinical inflammation rather than an independent predictor of diabetes risk.

The apparent differences between rodents and humans in the role of resistin in the pathogenesis of type 2 diabetes could in part reflect different expression and regulatory mechanisms of resistin in these species. For example, murine resistin is almost exclusively expressed in adipocytes, whereas human resistin is expressed at significantly lower concentrations in adipocytes and readily detectable in other cell types, especially macrophages (47). Further, mouse and human resistin share only 59% identity at the amino acid level (47).

The strengths of the present study comprise the prospective design with a long follow-up, the large sample size, and detailed information on diet and lifestyle. Several limitations of this study should also be acknowledged. First, the results were based on single measurements of the adipokines and therefore, may not reflect long-term exposure to these hormones. However, single measurements of resistin and adiponectin have been suggested to be reliable for the risk assessment in longitudinal studies based on reported intraclass correlations of 0.95 for resistin and 0.73 for adiponectin over 3 years (48). Second, the present study was limited to predominantly Caucasian women. Because of ethnic and gender differences in the level of adiponectin and resistin (6, 47), the generalizability of our results to other populations may be limited. Finally, we adjusted for known confounding factors, but residual confounding by imperfectly or unmeasured confounders cannot be excluded.

In conclusion, we observed a strong and inverse association between total and high-molecular weight adiponectin and risk for type 2 diabetes in apparently healthy women independent of BMI and other diabetes risk factors. The high-molecular weight-to-total adiponectin ratio is related to type 2 diabetes risk independent of total adiponectin, suggesting an important role of the relative proportion of high-molecular weight adiponectin in diabetes pathogenesis. In contrast, the positive association between resistin and type 2 diabetes was largely explained by BMI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

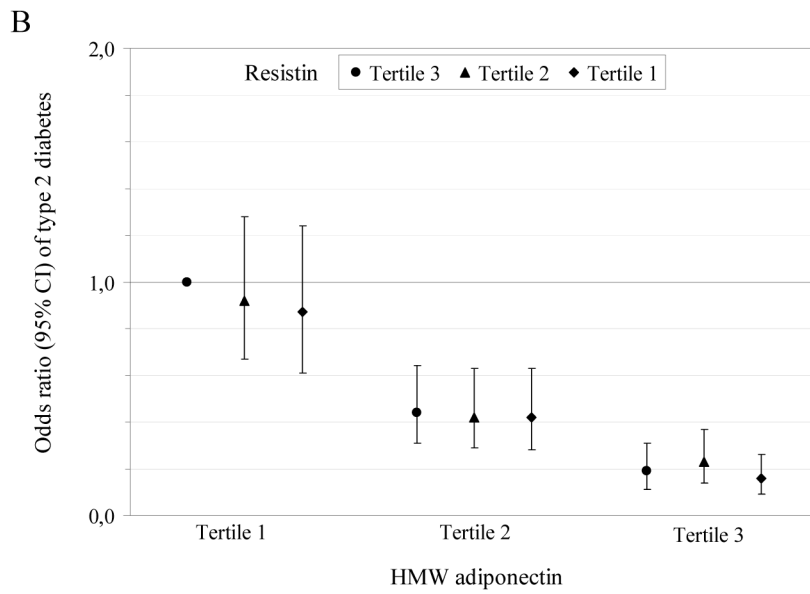
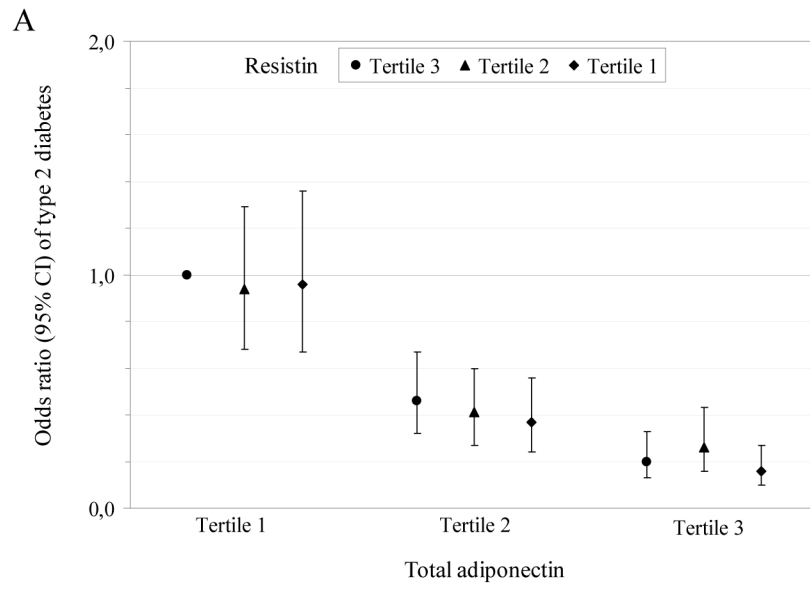
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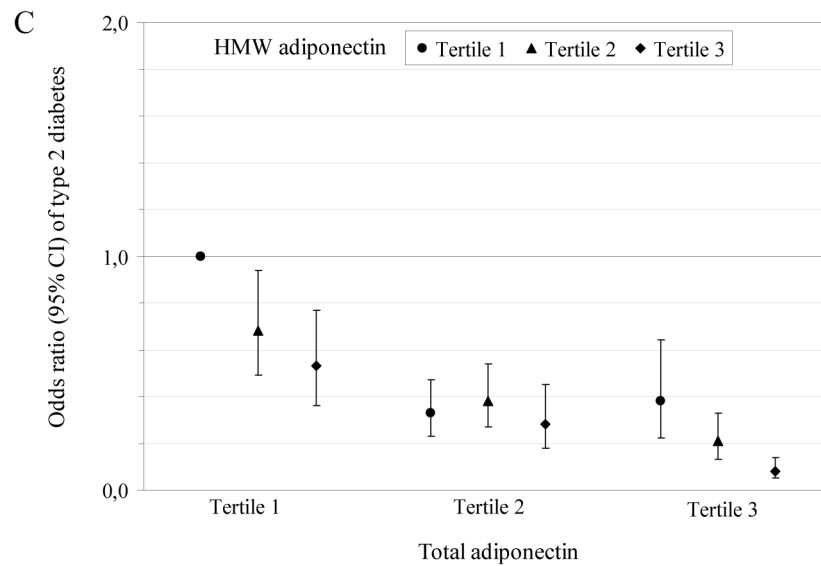


Figure 1. Multivariate-adjusted odds ratio (95% CI) of type 2 diabetes according to the joint classification of adipokines

Joint analysis of total adiponectin and resistin (A), high-molecular weight adiponectin and resistin (B), and total adiponectin and the high-molecular weight-to-total adiponectin ratio (C). Odds ratios are adjusted for the same variables as in the multivariate model including BMI of table 3. Tertiles were calculated among control subjects and defined as 12.55, 12.56–18.36, and >18.36 ng/mL for resistin, 14.67, 14.68–21.19, and >21.19 μ g/mL for total adiponectin, 4.97, 4.98–8.58, and >8.58 μ g/mL for high-molecular weight adiponectin, and 0.342, 0.343–0.442, and >0.442 for the high-molecular weight-to-total adiponectin ratio. HMW = high-molecular weight.

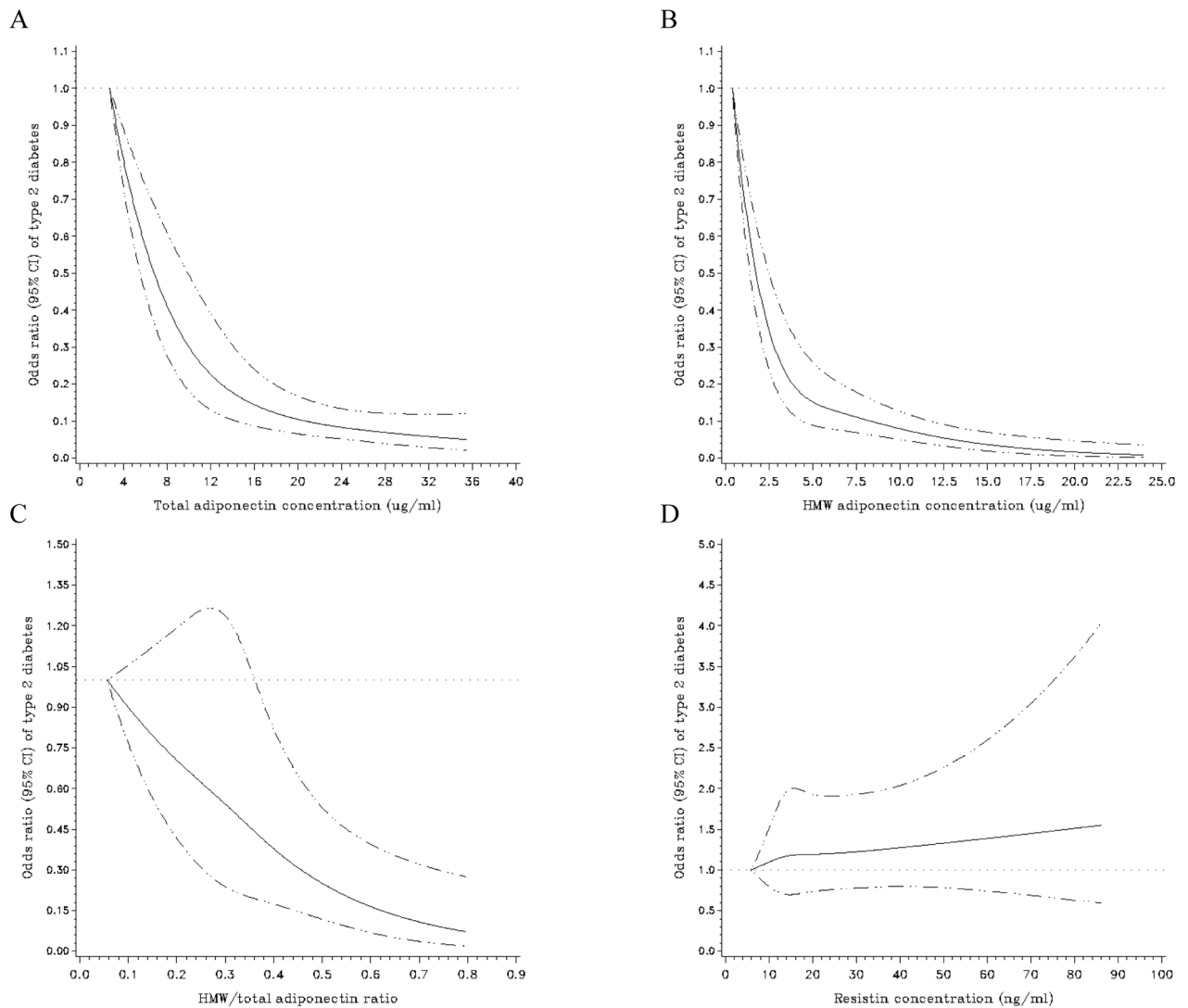


Figure 2. Multivariate-adjusted odds ratio (95% CI) of type 2 diabetes according to continuous adipokine concentrations

Spline regression models to examine the possible non-linear relation of total adiponectin (A), high-molecular weight adiponectin (B), the high-molecular weight-to-total adiponectin ratio (C), and resistin (D) to type 2 diabetes (adjusted for same variables as in the multivariate model including BMI of table 3). The solid black line represents odds ratios and the dotted lines represent 95% CIs. Women with extreme low or high adipokine concentrations (<1st or >99th percentile) were excluded from these analyses (n=41 for total adiponectin, n=42 for high-molecular weight adiponectin, n=43 for the high-molecular weight-to-total adiponectin ratio, and n=42 for resistin). HMW = high-molecular weight.

Table 1

Comparison of adipokine concentrations and risk factors for type 2 diabetes between case and control subjects at baseline. *

Risk factor	Cases (n=1,038)	Controls (n=1,136)	P Value
Age, y (matching factor)	56.1 (7.0)	55.9 (7.0)	0.49
Body-mass index, kg/m ²	30.1 (5.4)	26.4 (6.1)	<0.001
Waist circumference, in	35.1 (4.8)	31.2 (4.7)	<0.001
Physical activity, metabolic equivalents/week	12.2 (14.9)	15.5 (24.7)	<0.001
Family history of diabetes, %	45.2	23.0	<0.001
Current smoking, %	13.4	12.2	0.42
Postmenopausal, %	62.7	60.7	0.32
Current postmenopausal hormone use, %	18.9	22.0	0.07
Dietary intake			
Cereal fiber, g/day	4.6 (2.2)	5.0 (2.6)	<0.001
Glycemic load	102 (16)	102 (18)	0.82
<i>Trans</i> fatty acids, g/d	2.6 (0.8)	2.5 (0.8)	0.003
Polyunsaturated/saturated fatty acids ratio	0.56 (0.15)	0.57 (0.18)	0.04
Magnesium, mg/d	297 (62)	307 (70)	<0.001
Caffeine, mg/d	237 (185)	257 (197)	0.02
Alcohol, g/day	3.4 (7.3)	5.7 (9.1)	<0.001
Total energy, kcal/day	1832 (510)	1775 (472)	0.007
Total adiponectin, µg/mL (median [IQR])	11.4 [7.6–16.3]	17.8 [12.9–22.7]	<0.001
High-molecular weight adiponectin, µg/mL (median [IQR])	3.6 [2.2–5.8]	6.6 [4.4–9.8]	<0.001
High-molecular weight/total adiponectin ratio (median [IQR])	0.33 [0.27–0.40]	0.39 [0.32–0.47]	<0.001
Resistin, ng/mL (median [IQR])	16.4 [12.4–23.6]	15.1 [11.5–21.2]	<0.001

* All data are presented as mean (SD) or percentage unless otherwise specified. Characteristics of case and control subjects were compared by χ^2 test (for percentages), t test (for means), or Wilcoxon rank-sum test (for medians). For waist circumference: 799 controls, 635 cases; for physical activity: 1,118 controls, 1,024 cases; and for dietary intake: 1,125 controls, 1,027 cases.

IQR = interquartile range.

Table 2

Odds ratio (95% CI) of type 2 diabetes according to quintiles of adipokine concentration.

Variable	Quintile of adipokine concentration					P for Trend
	1 (lowest)	2	3	4	5 (highest)	
Total adiponectin, µg/mL						
Median	8.1	13.9	17.9	21.7	28.4	
Range	0.05–11.6	11.7–16.0	16.1–20.0	20.1–24.3	24.4–42.1	
Cases, <i>n</i> /controls, <i>n</i>	548/227	221/228	139/227	73/227	57/227	
Crude odds ratio (95% CI) *	1.0	0.39 (0.30–0.49)	0.24 (0.19–0.32)	0.13 (0.09–0.17)	0.10 (0.07–0.13)	<0.001
Multivariate odds ratio (95% CI) †	1.0	0.42 (0.32–0.54)	0.26 (0.20–0.35)	0.15 (0.11–0.21)	0.12 (0.08–0.17)	<0.001
Multivariate odds ratio + BMI (95% CI) ‡	1.0	0.46 (0.35–0.60)	0.31 (0.23–0.42)	0.21 (0.15–0.30)	0.17 (0.12–0.25)	<0.001
High-molecular weight adiponectin, µg/mL						
Median	2.4	4.7	6.5	9.1	14.7	
Range	0.2–3.9	4.0–5.5	5.6–7.7	7.8–11.0	11.1–43.7	
Cases, <i>n</i> /controls, <i>n</i>	556/227	203/227	150/228	97/227	32/227	
Crude odds ratio (95% CI) *	1.0	0.36 (0.28–0.45)	0.26 (0.20–0.34)	0.16 (0.12–0.22)	0.05 (0.04–0.08)	<0.001
Multivariate odds ratio (95% CI) †	1.0	0.37 (0.28–0.48)	0.28 (0.22–0.37)	0.19 (0.14–0.26)	0.07 (0.04–0.10)	<0.001
Multivariate odds ratio + BMI (95% CI) ‡	1.0	0.41 (0.31–0.53)	0.35 (0.26–0.47)	0.26 (0.19–0.36)	0.10 (0.06–0.15)	<0.001
High-molecular weight/total adiponectin ratio						
Median	0.25	0.33	0.39	0.45	0.55	
Range	0.02–0.30	0.31–0.36	0.37–0.41	0.42–0.49	0.50–0.94	
Cases, <i>n</i> /controls, <i>n</i>	390/227	243/226	189/228	139/228	77/227	
Crude odds ratio (95% CI) *	1.0	0.63 (0.49–0.80)	0.48 (0.37–0.62)	0.35 (0.27–0.46)	0.20 (0.14–0.27)	<0.001
Multivariate odds ratio (95% CI) †	1.0	0.63 (0.49–0.82)	0.53 (0.40–0.69)	0.39 (0.29–0.52)	0.23 (0.16–0.31)	<0.001
Multivariate odds ratio + BMI (95% CI) ‡	1.0	0.73 (0.56–0.97)	0.61 (0.46–0.82)	0.47 (0.35–0.64)	0.33 (0.23–0.47)	<0.001
Multivariate odds ratio + BMI + total adiponectin (95% CI) §	1.0	0.84 (0.63–1.12)	0.76 (0.56–1.03)	0.62 (0.45–0.85)	0.45 (0.31–0.65)	<0.001
Resistin, ng/mL						
Median	9.0	12.2	15.0	19.2	30.3	
Range	2.3–10.7	10.8–13.4	13.5–16.7	16.8–22.7	22.8–183.7	
Cases, <i>n</i> /controls, <i>n</i>	147/228	191/226	205/229	220/226	275/227	

Variable	Quintile of adipokine concentration					P for Trend
	1 (lowest)	2	3	4	5 (highest)	
Crude odds ratio (95% CI) *	1.0	1.31 (0.99–1.74)	1.40 (1.06–1.85)	1.52 (1.15–2.00)	1.91 (1.45–2.50)	<0.001
Multivariate odds ratio (95% CI) †	1.0	1.24 (0.92–1.69)	1.36 (1.00–1.83)	1.35 (1.00–1.82)	1.68 (1.25–2.25)	0.001
Multivariate odds ratio + BMI (95% CI) ‡	1.0	1.08 (0.78–1.50)	1.13 (0.82–1.57)	1.04 (0.75–1.44)	1.28 (0.93–1.76)	0.15

* Adjusted for matching factors (age, date of blood draw, fasting status, and ethnicity).

† Additionally adjusted for physical activity (quintiles of metabolic equivalents/week), smoking (never, past, current 1–14 cigarettes/d, and current 15 cigarettes/d), family history of diabetes (yes/no), hormone replacement therapy (premenopause, never, past, current, and missing information), alcohol consumption (nondrinkers, 0.1–4.9, 5–10, and >10 g/d), glycemic load (quintiles), ratio of polyunsaturated fat to saturated fat (quintiles), and intake (quintiles) of cereal fiber, trans-fat, magnesium, and total energy.

‡ Additionally adjusted for BMI (<23, 23–24.9, 25–26.9, 27–29.9, 30–34.9, and 35 kg/m²).

§ Additionally adjusted for total adiponectin (quintiles).

BMI = body-mass index.