Plasma Fetuin-A Levels and the Risk of Type 2 Diabetes

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OBJECTIVE—The liver-secreted protein fetuin-A induces insulin resistance in animals, and circulating fetuin-A is elevated in insulin resistance and fatty liver in humans. We investigated whether plasma fetuin-A levels predict the incidence of type 2 diabetes in a large prospective, population-based study.

RESEARCH DESIGN AND METHODS—A case-cohort study within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study comprising 27,548 subjects was designed. We randomly selected a subcohort of 2,500 individuals of whom 2,164 were diabetes free at baseline and had anamnestic, anthropometrical, and metabolic data for analysis. Of the 849 incident diabetic case subjects identified in the full cohort during 7 years of follow-up, 703 remained for analyses after similar exclusions.

RESULTS—Plasma fetuin-A levels were positively associated with diabetes risk after adjustment for age (relative risk [RR] for extreme quintiles 1.75 [95% CI 1.32–2.31]; RR for 10 μg/ml 1.04 [1.03–1.06]). The association remained significant after adjustment for sex, BMI, waist circumference, and lifestyle risk factors (RR for 10 μg/ml 1.03 [1.01–1.06]). Adjustment for glucose, triglycerides, HDL cholesterol, A1C, γ-glutamyltransferase, or high-sensitivity C-reactive protein or mutual adjustment for these biomarkers did not appreciably change this result (RR for 10 μg/ml full adjusted model 1.05 [1.02–1.07]). Furthermore, fetuin-A was associated with increased diabetes risk particularly in individuals with elevated plasma glucose.

CONCLUSIONS—Our data suggest that fetuin-A is an independent risk factor of type 2 diabetes. *Diabetes* 57:2762–2767, 2008

Type 2 diabetes represents a major global public health threat and, together with obesity, constitutes an important contributor to the predicted decline in life expectancy (1). The pathophysiology of type 2 diabetes is complex: In addition to impaired insulin secretion from β-cells, reduced insulin sensitivity was found to play a predominant role in the pathogenesis of the disease (2). Several circulating proteins have been shown to be involved in the regulation of insulin sensitivity such as adiponectin (3,4), retinol binding protein 4 (5,6), and fetuin-A (former name for the human protein α_2-Heremans-Schmid glycoprotein, AHSG). Fetuin-A is an endogenous inhibitor of the insulin-stimulated insulin receptor tyrosine kinase (7–9). Administration of fetuin-A to rodents inhibited insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 in rat liver and skeletal muscle (7). In addition, fetuin-A knockout mice exhibited increased insulin sensitivity and were resistant to the adipogenic effect of a high-fat diet (10), supporting the hypothesis that fetuin-A is involved in the pathophysiology of insulin resistance in rodents.

In agreement with these data, we and others have recently shown that high levels of circulating fetuin-A are associated with insulin resistance in humans (11,12), suggesting that fetuin-A may represent a novel mechanism involved in the pathophysiology of type 2 diabetes. In the present study, we investigated whether circulating fetuin-A predicted the incidence of type 2 diabetes, independently of established risk factors, in the large European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study.

RESEARCH DESIGN AND METHODS

Study population and diabetes ascertainment. The EPIC-Potsdam study is part of the multicenter prospective cohort study EPIC (13). In Potsdam, Germany, 27,548 subjects (16,644 women and 10,904 men) were recruited from the general population between 1994 and 1998. The age range was 35–65 years in women and 40–65 years in men. The baseline examination included anthropometric measurements, a self-administered validated food-frequency questionnaire, and a personal interview, including questions about prevalent diseases, and a questionnaire about sociodemographic and lifestyle characteristics. Follow-up questionnaires were sent out every 2–3 years to identify incident cases of type 2 diabetes. Complete questionnaires of follow-up round 1 were returned from 96%, of follow-up round 2 from 95%, and follow-up round 3 from 93% of the eligible participants. We also included all questionnaires of the 4th follow-up round sent out until 31 January 2005, of which 90% were returned by 31 August 2005. Informed consent was obtained from all participants, and approval was given by the Ethics Committee of the State of Brandenburg, Germany.

At baseline, the presence of type 2 diabetes was evaluated by a physician with information from self-reported medical diagnoses, medication records, and dieting behavior. In ambiguous cases, the diagnosis was confirmed by personal communication with the participant and/or the treating physician. All incident cases of diabetes identified during follow-up were verified by questionnaires that were mailed to the physicians. Thereby, information about the date and the type of diagnosis, about the diagnostic tests, and about the treatment was obtained. Only subjects having had a diagnosis of type 2 diabetes that was confirmed by a physician (ICD10: E11) and a diagnosis date after the baseline examination were considered as incident cases of type 2 diabetes. Within a mean follow-up time of 7.1 years, 849 subjects developed incident type 2 diabetes.

A prospective case-cohort study (14) within the EPIC-Potsdam study was designed. A random subcohort of 2,500 individuals was selected from all participants of the EPIC-Potsdam study population. Participants with prevalent diabetes ($n = 120$) or without follow-up ($n = 58$) were excluded from analyses. Although we considered missing anthropometry as exclusion criteria, none of the subcohort members qualified for exclusion here. Furthermore, we excluded 112 participants for whom not all biomarkers were available and 46 individuals with either unexplainable low plasma glucose concentrations (<50 mg/dl) or concentrations within the diabetic range (random glucose

TABLE 1
Baseline characteristics by quintiles of plasma fetuin-A among men and women, EPIC-Potsdam Study

| Characteristics | Quintiles of fetuin-A | | | | | P value |
	1	2	3	4	5	
Fetuin-A (μg/ml)	158	201	227	255	304	
Age (years)	51.0	50.4	49.9	48.8	47.6	<0.001
BMI (kg/m^2)	25.6	26.1	26.1	25.8	26.2	0.194
Waist circumference (cm)	84.7	86.3	86.1	84.9	84.7	0.562
Sport activities (h/week)	1.0	1.0	1.0	1.1	1.0	0.673
Alcohol consumption (g/day)	14.5	15.2	14.6	15.5	11.3	0.033
Men (%)	39.5	40.3	40.7	38.3	30.7	0.013
Random plasma glucose (mg/dl)	85.8	87.6	87.3	87.2	86.9	0.397
Fasting plasma glucose (mg/dl)*	82.3	87.2	85.9	85.8	86.9	0.006
Triglycerides (mg/dl)	93.9	95.4	99.4	94.3	96.9	0.518
Fasting triglycerides (mg/dl)*	76.7	84.4	82.9	83.5	86.0	0.126
HDL cholesterol (mg/dl)	45.3	46.4	46.6	47.5	48.0	<0.001
γ-Glutamyltransferase (units/l)	15.1	17.1	17.3	16.5	17.0	0.093
hs-CRP (mg/dl)	0.06	0.06	0.07	0.06	0.08	<0.001
A1C (%)	6.4	6.5	6.5	6.4	6.4	0.419

*Data are percent or means (geometric means for glucose, triglycerides, HDL cholesterol, hs-CRP, A1C, and γ-glutamyltransferase). *In the random subcohort, 246 men and 376 women did not eat for at least 8 h before blood drawing.

≥200 mg/dl or fasting glucose ≥126 mg/dl). Altogether, 2,164 participants were included in the random subcohort for analyses. The process of randomly selecting a subcohort, together with the appropriate statistics for this type of research design, renders the results generalizable without measurements of biomarkers in the entire cohort (15). Of the 849 incident case subjects identified in the full cohort, 703 remained for analyses after similar exclusion criteria were applied. Because the subcohort is representative of the full cohort at baseline in case-cohort studies, the subcohort in our analyses included 64 subjects who developed incident type 2 diabetes during follow-up. A total of 639 of the 703 incident case subjects were identified in the remaining part of the total cohort and constituted the "external" cases for analyses (14). A comparison of the randomly selected subcohort, before and after exclusions, and the full EPIC-Potsdam cohort is shown in supplementary Table 1 (available in an online appendix at http://dx.doi.org/10.2337/db08-0538). There were no significant differences in age, BMI, or sex distribution between full and subcohort before exclusions, suggesting that the subcohort is representative for the full cohort. Slight differences were observable between full cohort and subcohort after exclusions reflecting the selection of an at baseline diabetes-free population. Subjects in the subcohort were followed for an average of 7.0 years (range 0.3–10.3 years). Incident case subjects had on average a diagnosis date 4.0 years after the baseline assessment (range 0.1–9.7 years).

Participants were not required to be fasted at the baseline assessment. However, about one-third of the participants did not eat for at least 8 h before the blood drawing. Thus, fasting blood samples were available in 622 subjects in the subcohort and in 192 incident case subjects.

Baseline anthropometrics and lifestyle characteristics. Waist circumference was measured midway between the lower rib margin and the superior anterior iliac spine to the nearest 0.5 cm with a nonstretching tape applied horizontally, the proper use of which was controlled with a mirror. Information on educational attainment, smoking, occupational activity level, and leisure time physical activity were assessed with a self-administered questionnaire and a personal interview. We considered sport activities and cycling as leisure time activities, both calculated as the average time spent per week during the 12 months before the baseline recruitment.

Measurement of biochemical variables. Plasma levels of glucose, HDL cholesterol, triglycerides, γ-glutamyltransferase, and fetuin-A and erythrocyte levels of A1C were measured with the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany). For determination of fetuin-A, an immunoturbidimetric method was used with specific polyclonal goat anti-human fetuin-A antibodies to human fetuin-A (BioVendor Laboratory Medicine, Modreci, Czech Republic). This method was evaluated in a side-by-side comparison with an enzyme-linked immunosorbent assay (intra-assay coefficient of variation [CV] 3.5% and interassay CV 5.4%; BioVendor Laboratory Medicine, Modreci, Czech Republic) (11,12) showing a r^2 of 0.88.

Statistical analysis. Associations between plasma fetuin-A levels and selected diabetes risk factors were examined in the subcohort using an age-adjusted Pearson partial correlation coefficient. Fetuin-A levels were categorized into quintiles based on subcohort participants. Hazard ratios as a measure of relative risk (RR) were computed using a weighted Cox propor-

tional hazards model, modified for the case-cohort design according to the Prentice method (16). Age was the underlying time variable in the counting processes, with entry defined as the subjects' age at the time of recruitment and exit defined as age at the diagnosis of diabetes, or censoring. We computed age-adjusted RRs for each quintile of fetuin-A compared with the lowest quintile. The significance of linear trends across quintiles of fetuin-A was tested by assigning each participant the median value for the quintile and modeling this value as a continuous variable. Because this analysis indicated no department from linearity, we considered fetuin-A as continuous variable estimating the RR associated with an increment of 10 μg/ml or per 1 SD in all further analyses. We used information on covariates obtained from the baseline examination in multivariate analyses, including sex, BMI (continuous), waist circumference (continuous), education (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), sports activity (0, 0.1–4, or >4 h/week), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h/week), smoking (never, past, or current <20 cigarettes/day or current ≥20 cigarettes/day), and alcohol intake (0, 0.1–5, 5.1–10, 10.1–20, 20.1–40, or >40 g/day). We furthermore examined the impact of potential intermediate biomarkers by adding log-transformed HDL cholesterol, glucose, triglyceride, A1C, γ-glutamyltransferase, and CRP levels as continuous variables to our models.

For stratified analysis, we also calculated the multivariable-adjusted RR associated with a difference in fetuin-A by 1 SD according to sex, fasting status, the presence of abdominal obesity (waist ≥102 cm in men and ≥88 cm in women) and elevated blood glucose (≥100 mg/dl). We tested interactions between fetuin-A levels and subgroups by evaluating the significance of cross-product terms.

All statistical analyses were performed with SAS release 9.1 (SAS Institute, Cary, NC). All P values presented are two-tailed; P < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics and metabolic traits across quintiles of fetuin-A of the random subcohort are presented in Table 1. Participants with higher fetuin-A concentrations were younger, were less likely to be men, and drank smaller amounts of alcohol compared with participants with lower fetuin-A concentrations. Supplementary Fig. 1 shows the frequency distribution for men and women across the range of plasma fetuin-A levels (14–442 μg/ml [mean ± SD 129 ± 53 μg/ml]).

We next examined the association of fetuin-A levels with selected risk factors for type 2 diabetes (Table 2). After adjustment for age, fetuin-A was significantly positively correlated with fasting glucose, fasting triglycerides, HDL cholesterol, γ-glutamyltransferase, high-sensitivity C-

TABLE 2
Age-adjusted Pearson partial correlation coefficients between plasma fetuin-A levels and selected risk factors, EPIC-Potsdam Study, 819 men and 1,345 women

Risk factors	r	P value
Waist circumference	0.025	0.247
Random plasma glucose	0.039	0.068
Fasting plasma glucose*	0.098	0.015
Triglycerides	0.041	0.058
Fasting triglycerides*	0.093	0.021
HDL cholesterol	0.086	<0.001
γ-Glutamyltransferase	0.063	0.004
hs-CRP	0.122	<0.001
A1C	0.049	0.024

*In the random subcohort, 246 men and 376 women did not eat for at least 8 h before blood drawing.

reactive protein (hs-CRP), and A1C; however, correlation coefficients were overall weak.

Figure 1 presents the age-adjusted RRs of type 2 diabetes for quintiles of fetuin-A. Participants in the highest quintile of fetuin-A levels had a significantly increased risk of diabetes compared with the lowest quintile (RR 1.75 [95% CI 1.32–2.31]; P for trend <0.0001). Because there was no indication of nonlinearity and fetuin-A levels were approximately normally distributed, we considered fetuin-A as continuous variable on its natural scale in further analyses. Table 3 shows the estimated RRs of type 2 per 10 μg/ml fetuin-A levels at baseline for all subjects and according to fasting status. After adjustment for age, fetuin-A levels were significantly associated with increased risk of diabetes (1.04 [1.03–1.06]) among all participants. This association was similar after adjustment for sex, BMI, and waist circumference (1.04 [1.02–1.06]) and also after further adjustment for lifestyle risk factors (1.03 [1.01–1.06]) and remained significant. The association became slightly stronger after adjustment for glucose, triglycerides, HDL cholesterol, A1C, γ-glutamyltransferase, and hs-CRP (1.05 [1.02–1.07]). The association between fetuin-A and type 2 diabetes risk appeared to be somewhat stronger among fasting subjects (RR full adjusted model 1.09 [1.04–1.13]) compared with nonfasting subjects (1.04 [1.01–1.07]).

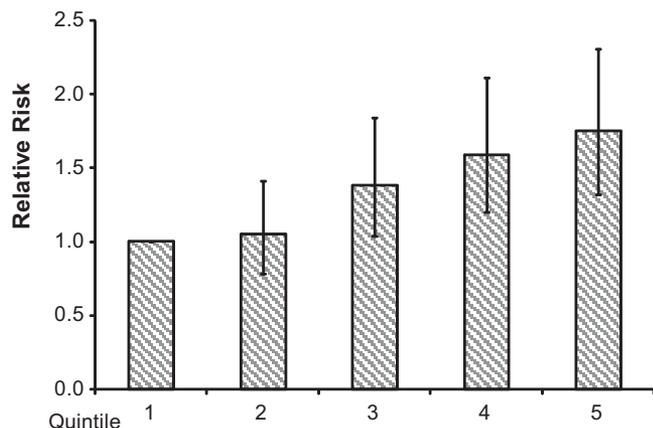Figure 2 shows the multivariable-adjusted RR of type 2

diabetes for 1 SD of fetuin-A per 1 SD with different adjustment for biochemical variables. The RR adjusted for age, sex, and anthropometric and lifestyle characteristics was 1.19 (95% CI 1.06–1.33). Adjustment for HDL cholesterol, triglycerides, glucose, A1C, γ-glutamyltransferase, or hs-CRP or mutual adjustment for all these biomarkers did not appreciably affect this result (RR for full adjusted model 1.28 [95% CI 1.13–1.46]). Similarly, adjustment for other biomarkers did not alter the observed association among fasted or nonfasted subjects (data not shown).

We next evaluated the associations between fetuin-A and the risk of type 2 diabetes in several subgroups (Fig. 3). The associations appeared to be stronger in men compared with women and among fasted compared with nonfasted participants. However, the tests for interactions were not significant. Similar associations were observable across strata of abdominal obesity. In contrast, glucose levels modified the association between fetuin-A levels and diabetes risk with a strong association observable among participants with elevated glucose (RR for 1 SD 1.62 [95% CI 1.31–2.00]), whereas there was no significant association among participants with normal glucose values (P value for interaction 0.023).

We repeated our analyses excluding all incident case subjects who were identified within the first 2 years of follow-up ($n = 163$). Fetuin-A remained positively associated with diabetes risk (RR for 1 SD 1.17 [95% CI 1.03–1.34]). In a further sensitivity analysis, we evaluated whether storage time had a potentially impact on our observation. Blood samples were stored on average 11.2 years (range 9.2–13.3 years) before fetuin-A measurement in December 2007. Fetuin-A was similarly positively associated among participants with blood storage time <11 years (RR for 1 SD 1.34 [1.08–1.65]) and those with storage time greater or equal 11 years (1.32 [1.12–1.54]).

DISCUSSION

Animal and human studies suggest that the protein fetuin-A induces insulin resistance (7–12), thus supporting the hypothesis that fetuin-A may also play a role in the pathophysiology of type 2 diabetes. To our knowledge, our study is the first to show that high plasma fetuin-A levels at baseline predicted the incidence of type 2 diabetes. This relationship remained statistically significant after adjustment for established risk factors of type 2 diabetes, such as sex, age, BMI, waist circumference, glucose, triglyceride, HDL cholesterol, A1C, γ-glutamyltransferase, and hs-CRP. Our findings, therefore, strongly indicate that fetuin-A independently predicts the risk of type 2 diabetes.

For a long time after its discovery, fetuin-A was considered to primarily play a role in the protection from vascular calcification by keeping calcium and phosphorus solubilized in serum (17). Besides these specific effects on the hydroxyapatite deposition in vessel walls, a new mechanism of fetuin-A action was detected. In a large study in humans, high serum fetuin-A levels were found to be positively associated with the metabolic syndrome and subclinical inflammation, suggesting that fetuin-A may be causally involved in the pathophysiology of these conditions (18). In agreement with that study, we found that fetuin-A levels correlated positively with hs-CRP levels, and extended the information on fetuin-A action by showing that fetuin-A promotes cytokine expression in monocytes and adipocytes and represses the production of the insulin-sensitizing adipokine adiponectin (19). Further

FIG. 1. Age-adjusted RR of type 2 diabetes by quintiles of plasma fetuin-A (μg/ml) among men and women in the EPIC-Potsdam Study. *P* for trend <0.001. Medians (ranges) for quintiles are as follows: quintile 1, 162 μg/ml (14–184); quintile 2, 202 μg/ml (185–214); quintile 3, 227 μg/ml (215–240); quintile 4, 255 μg/ml (241–271); quintile 5, 295 μg/ml (272–442).

TABLE 3
RR of type 2 diabetes for plasma fetuin-A (per 10 μg/ml) among men and women in the EPIC-Potsdam Study

Model	All subjects	Fasting subjects	Nonfasting subjects
Age adjusted	1.04 (1.03–1.06)	1.06 (1.03–1.10)	1.03 (1.01–1.05)
Adjusted for age, sex, BMI, and waist circumference	1.04 (1.02–1.06)	1.07 (1.03–1.11)	1.03 (1.00–1.05)
Multivariate adjusted*	1.03 (1.01–1.06)	1.08 (1.04–1.12)	1.02 (1.00–1.05)
Further adjustment for HDL cholesterol, triglycerides, glucose, A1C, γ-glutamyltransferase, and hs-CRP†	1.05 (1.02–1.07)	1.09 (1.04–1.13)	1.04 (1.01–1.07)

Data are RR (95% CI). *Adjusted for age, sex, BMI, waist circumference, education (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), sport activity (0, 0.1–4, or >4.0 h/week), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h/week), smoking (never, past, or current <20 cigarettes/day or current ≥20 cigarettes/day), and alcohol intake (0, 0.1–5, 5.1–10, 10.1–20, 20.1–40, or >40 g/day). †HDL cholesterol, triglycerides, glucose, A1C, γ-glutamyltransferase, and hs-CRP were log transformed.

support for a potential role of fetuin-A in the regulation of glucose and lipid metabolism is given by the fact that the gene encoding fetuin-A is located on chromosome 3q27, the chromosomal region that was previously mapped as a type 2 diabetes and metabolic syndrome susceptibility locus (20). The aforementioned findings and the animal studies showing that fetuin-A induces insulin resistance (7–10) resulted in the view that fetuin-A is an interesting candidate involved in the pathophysiology of type 2 diabetes. Genetic analyses revealing that single nucleotide polymorphisms in the fetuin-A gene were associated with type 2 diabetes in cross-sectional studies (21) further corroborated this hypothesis. However, the role of fetuin-A in the natural history of type 2 diabetes still remained obscure. Fetuin-A, besides the placenta, is ex-
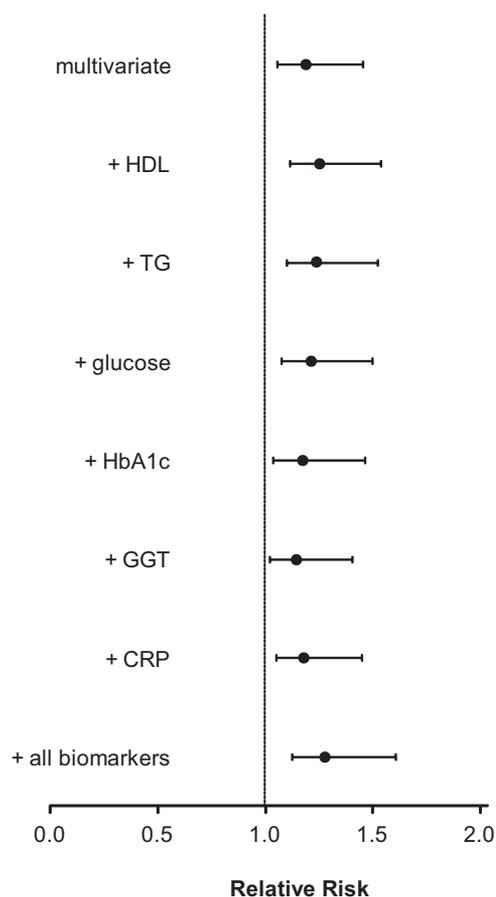
FIG. 2. RR of type 2 diabetes per 1 SD of plasma fetuin-A with varying adjustment for metabolic risk markers among men and women in the EPIC-Potsdam Study. RRs were adjusted for age, sex, BMI, waist circumference, education (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), sport activity (0, 0.1–4, or >4 h/week), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h/week), smoking (never, past, or current <20 cigarettes/day or current ≥20 cigarettes/day), and alcohol intake (0, 0.1–5, 5.1–10, 10.1–20, 20.1–40, or >40 g/day).
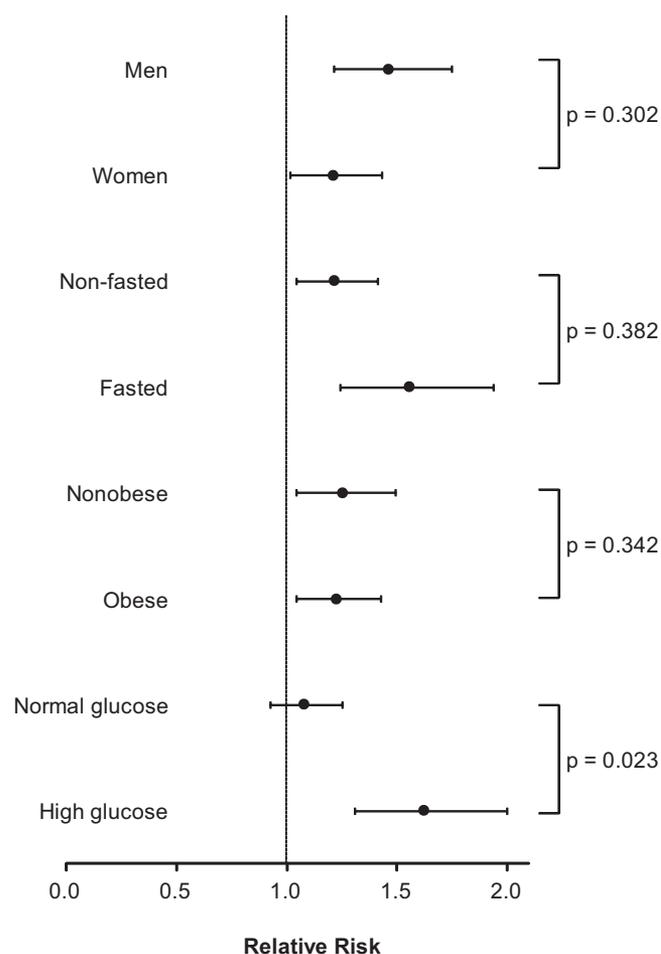

FIG. 3. RR of type 2 diabetes per 1 SD of plasma fetuin-A for subgroups of sex, fasting status, abdominal obesity, and glucose levels in the EPIC-Potsdam Study. RRs were adjusted for age, sex BMI, waist circumference, education (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), sport activity (0, 0.1–4, or >4 h/week), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h/week), smoking (never, past, or current <20 cigarettes/day or current ≥20 cigarettes/day), and alcohol intake (0, 0.1–5, 5.1–10, 10.1–20, 20.1–40, or >40 g/day), HDL cholesterol, triglycerides, glucose, A1C, γ-glutamyltransferase, and hs-CRP (all log transformed). Abdominal obesity was defined as waist ≥102 cm among men or ≥88 cm among women. High glucose was defined as ≥100 mg/dl.

clusively secreted from the liver (22). Expression of fetuin-A and plasma levels of the protein were found to be increased when there is fat accumulation in the liver (11,23) and circulating fetuin-A is increased in the metabolic syndrome (18), the condition that strongly associates with fatty liver (24). Based on these data and on the observation that fatty liver strongly predicts type 2 diabetes (25), the present findings suggest that fetuin-A may be a mediator of fatty liver–induced type 2 diabetes. In agreement with the latter hypothesis, we found that plasma fetuin-A levels in our study also correlated positively with the plasma γ-glutamyltransferase level, which is considered a weak surrogate marker of fatty liver (26). However, fetuin-A remained significantly associated with diabetes risk even after adjustment for γ-glutamyltransferase. Still, we were not able to adjust for more precise measures of liver fat, e.g., by localized proton magnetic resonance spectroscopy (11,24), and thus were not able to clarify the extent to which the risk associated with fetuin-A is explainable by liver fat content. If our hypothesis can be further substantiated by future studies, then fetuin-A, similar to the adipokines secreted from adipose tissue (27), may represent the first identified factor among other, yet-unknown secreted proteins from the liver (hepatokines), which regulate insulin signaling in insulin-sensitive tissues. Finally, whether fetuin-A also affects insulin secretory function of the β-cells and thereby influences the incidence of type 2 diabetes remains to be investigated.

Interestingly, the association between circulating fetuin-A and type 2 diabetes was modified by the existence of elevated glucose levels. A positive association was observed among participants with elevated plasma glucose levels within the nondiabetic range, whereas fetuin-A was not associated with diabetes risk among participants with normal glucose levels. Fasting hyperglycemia largely results from impaired function of the β-cells to secrete insulin, the major factor involved in the pathogenesis of type 2 diabetes (28). Our findings therefore support that fetuin-A itself or fetuin-A–induced insulin resistance may lead to a deterioration of insulin secretion and ultimately to a decompensation of glucose homeostasis, particularly in subjects who already display impaired β-cell function (Fig. 4). Thus, measurement of plasma fetuin-A may be particularly important for the evaluation of the individual risk of type 2 diabetes in these individuals who already have a high risk for the disease. Conversely, adequate β-cell function may protect individuals who are characterized by normal fasting glycemia from the detrimental effects of higher fetuin-A levels.

Some possible limitations of our findings must be considered. All potential cases in our study were verified by a physician. Although we considered only clinically apparent type 2 diabetes and did not screen our study population during follow-up, we excluded participants with plasma glucose values within the diabetic range at baseline, and fetuin-A remained positively associated with diabetes risk after exclusion of case subjects diagnosed within the first 2 years of follow-up. Thus, it is unlikely that prevalent but undiagnosed cases of diabetes remained in our analyses. The potential of residual confounding applies to our study as it does to observational studies in general. We adjusted for a large variety of known risk factors, among them age, sex, anthropometry, alcohol consumption, activity patterns, and metabolic risk markers. Although fetuin-A remained significantly associated with diabetes risk, we cannot rule out that other unmea-
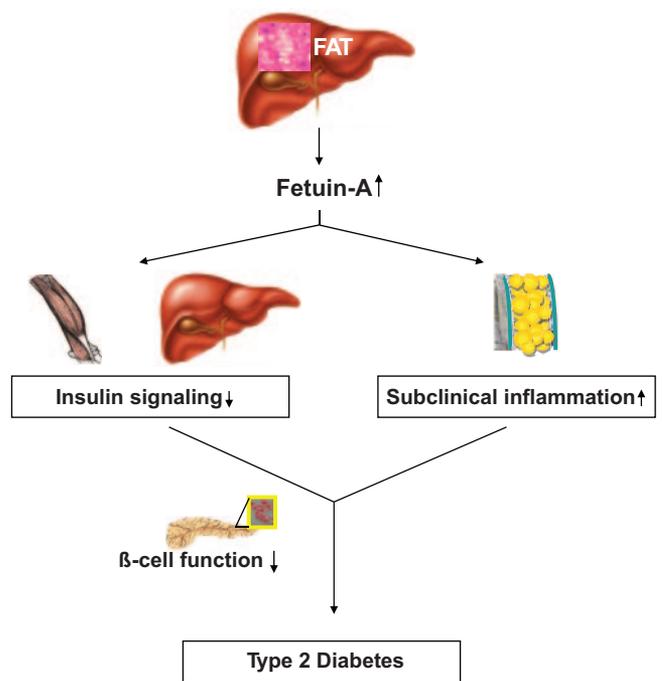

FIG. 4. Schematic overview of the effects of circulating fetuin-A on target tissues. Increase in obesity, particularly in visceral fat mass, results in an increase of liver fat. By yet-unknown mechanisms, this is associated with increased production of fetuin-A (11,23). Elevated circulating fetuin-A directly inhibits insulin signaling in muscle and liver (7–9) and results in systemic subclinical and adipose tissue inflammation (19). Under a normal β-cell function, fetuin-A–induced insulin resistance and effects of subclinical inflammation on the β-cells may be compensated; however, the existence of impaired β-cell function results in fetuin-A–induced type 2 diabetes.

sured factors or that imprecision in the measurement of covariates explain this observation. Also, we only had a single blood drawing, which might have introduced random measurement errors in determining fetuin-A and other biochemical variables. The lack of repeated measurements may have led to an underestimation of the observed associations. Most participants in our study were nonfasted at blood drawing. Although statistically not significantly different, the association between fetuin-A and diabetes risk appeared to be stronger among fasted individuals compared with nonfasted. Fasted subjects were on average 2 years older than nonfasted participants and slightly more likely to be men (40 vs. 37%). Given the higher incidence rates observed in our study for men compared with women and with increasing age (29), a higher baseline risk among fasted participants might therefore be an explanation for the difference. Finally, because of the observational design of our study, we cannot unequivocally prove whether the relationship between circulating fetuin-A levels and type 2 diabetes risk is causal.

In conclusion, our finding that high plasma fetuin-A levels predict the incidence of type 2 diabetes independently of other established risk factors supports the hypothesis that fetuin-A may play a role in the development of type 2 diabetes.

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