Plasma Concentrations of Afamin Are Associated With Prevalent and Incident Type 2 Diabetes: A Pooled Analysis in More Than 20,000 Individuals

Diabetes Care 2017;40:1386–1393 | https://doi.org/10.2337/dc17-0201

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OBJECTIVE

The human vitamin E–binding glycoprotein afamin is primarily expressed in the liver and has been associated with prevalent and incident metabolic syndrome. These data were in line with observations in transgenic mice. We thus investigated whether afamin concentrations are associated with prediabetes, type 2 diabetes, and insulin resistance (IR).

RESEARCH DESIGN AND METHODS

Individual-level baseline (n = 20,136) and follow-up data (n = 14,017) of eight prospective cohort studies were investigated. Study-level data were combined using random-effects meta-analyses. Main outcomes were prevalent and incident type 2 diabetes, prediabetes, and IR. Discrimination and reclassification of participants was analyzed for incident type 2 diabetes.

RESULTS

Mean afamin concentrations between studies ranged from 61 to 73 mg/L. The eight studies included 1,398 prevalent and 585 incident cases of type 2 diabetes. Each increase of afamin by 10 mg/L was associated with prevalent type 2 diabetes (odds ratio [OR] 1.19 [95% CI 1.12–1.26], $P = 5.96 \times 10^{-8}$). Afamin was positively associated with IR assessed by HOMA-IR (β 0.110 [95% CI 0.089–0.132], $P = 1.37 \times 10^{-23}$). Most importantly, afamin measured at baseline was an independent predictor for 585 incident cases of type 2 diabetes (OR 1.30 [95% CI 1.23–1.38], $P = 3.53 \times 10^{-19}$) and showed a significant and valuable gain in risk classification accuracy when added to this extended adjustment model.

CONCLUSIONS

This pooled analysis in >20,000 individuals showed that afamin is strongly associated with IR, prevalence, and incidence of type 2 diabetes independent of major metabolic risk factors or parameters. Afamin might be a promising novel marker for the identification of individuals at high risk for the development of type 2 diabetes.

The worldwide number of adults with type 2 diabetes has quadrupled during the last 35 years. In 2014, the age-standardized prevalence rate was 9.0% for men and 7.9% for women and is predicted to increase to 12.8% and 10.8%, respectively, by 2025 (1). Most importantly, about a third to a half of individuals with diabetes remains

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undiagnosed (2,3). Besides the enormous annual costs of $825 billion worldwide, metabolic syndrome and diabetes increase the subsequent number of nonfatal and fatal outcomes (2,4,5). More than 2 million deaths every year can be attributed to diabetes and its macrovascular and microvascular complications (1). Thus, an in-depth understanding of the pathogenesis as well as the identification of early risk predictors is of major importance.

We recently demonstrated in a pooled analysis of three epidemiological studies (6) including >5,000 study participants that plasma afamin concentrations are predictive not only for the prevalence but also for the incidence of metabolic syndrome. In patients with polycystic ovary syndrome, afamin concentrations have been reported to be associated with insulin resistance (IR) (7), but data on the association between afamin and type 2 diabetes are still lacking.

Afamin was first described in 1994 as the fourth member of the human albumin gene family including albumin, α-fetoprotein, and vitamin D–binding protein (8,9). The human plasma glycoprotein afamin has a molecular mass of 87 kDa with 15% carbohydrate content (10) and 55% amino acid sequence similarity to albumin (8). It is primarily expressed in the liver (8) but also in tissues such as the brain, testes, ovaries, and kidney (www.proteinatlas.org). Knowledge about the (patho)physiological functions of this protein is still limited (11,12). Transgenic mice overexpressing the human afamin gene developed increased body weight and increased blood concentrations of lipids and glucose (6). Based on these findings and the epidemiological data on afamin and metabolic syndrome in humans (6), we aimed to investigate whether afamin is associated with the prevalence and incidence of type 2 diabetes in a pooled analysis in >20,000 individuals from mainly population-based cohorts. Furthermore, we evaluated whether afamin is also related to prediabetes and type 2 diabetes–related phenotypes such as IR.

RESEARCH DESIGN AND METHODS

Study Populations and Study Design

This investigation is based on eight prospective cohort studies: six of them were by definition population-based (Bruneck Study, KORA [Cooperative Health Research in the Region of Augsburg] F3 Study, KORA F4 Study, CoLaus [Cohorte Lausannoise] Study, YFS [Young Finns Study], and the NHLBI [National Heart, Lung, and Blood Institute] Family Heart Study [FamHS]); one study included unrelated healthy middle-aged men from nine general practices (NPHS-II [Second Northwick Park Heart Study]); and one study was based on a healthy working population (SAPHIR [Salzburg Atherosclerosis Prevention Program in subjects at High Individual Risk] Study). The baseline examination included a total of 20,136 individuals, and information was available on a follow-up examination that included 14,017 individuals. The baseline examination finally included a total of 20,094 individuals with prevalent type 2 diabetes, and the follow-up examination included 13,347 individuals with incident type 2 diabetes. The percentage of loss to follow-up varied between 3% (NPHS-II) and 36% (NHLBI FamHS). This frequency could not be calculated for the CoLaus Study since the follow-up collection of data on incident diabetes is still in progress. The average follow-up time in the eight studies ranged from 4.5 to 12.5 years (Supplementary Table 1). All studies were approved by the respective local ethics committees. The clinical investigations described were carried out according to the Declaration of Helsinki. All participants provided written informed consent. For more details on study design, recruitment, clinical assessment of laboratory parameters, and definition of outcomes, see the Supplementary Data.

Definition of Outcomes

Type 2 diabetes was defined either as self-reported and/or as a fasting glucose level ≥126 mg/dL (≥7 mmol/L) according to the 1997 American Diabetes Association (ADA) criteria (13) and/or receiving antidiabetic medication. Participants with a diagnosis of type 1 diabetes were excluded. More details on the specific definitions in each study can be found in the Supplementary Data.

Measures of IR such as HOMA-IR and whole-body insulin sensitivity index [ISI(composite)] were calculated as described in the Supplementary Data.

Prediabetes was specified according to the 1997 ADA definition (impaired fasting glucose defined as fasting glucose of ≥100–125 mg/dL (≥5.6–6.9 mmol/L) and impaired glucose tolerance as a 2-h glucose value between ≥140 and 199 mg/dL (≥7.8–11.0 mmol/L) (13).

Measurement of Afamin Plasma Concentrations

Afamin was quantified with a custom-made double-antibody sandwich ELISA as previously described (6,10,14,15). Within-run and between-run coefficients of variation were 3.3% and 6.2%, respectively (15). Afamin concentrations were measured in all studies in the laboratory at the Medical University of Innsbruck. Extended information on the quality control of laboratory work is given in the Supplementary Data.

Statistical Analyses in All Cohorts

At baseline, the association between afamin and prevalent type 2 diabetes was explored by logistic regression analysis. At the follow-up investigation, logistic regression modeling of the relation of afamin values measured at baseline with incident type 2 diabetes was performed and participants with type 2 diabetes at baseline were excluded. Because the exact dates of the diagnosis of type 2 diabetes were not known in all studies, logistic instead of Cox proportional hazard

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Received 27 January 2017 and accepted 9 July 2017.
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc17-0201/-/DC1.

regression was used for investigating incident type 2 diabetes. Both prevalent and incident type 2 diabetes were considered as primary outcomes. All further analyzed outcomes [fasting insulin and glucose concentrations, glycated hemoglobin (HbA_{1c}), HOMA-IR, whole-body ISI(composite) (in the KORA F4 Study only)] were considered as secondary outcomes. For all analyses performed, the first model was adjusted for age and sex and the second (referred to as the extended adjustment model) additionally for other potential major metabolic risk factors or parameters (HDL cholesterol; triglycerides; BMI; hypertension; and, in six of eight studies, glucose concentrations).

The linearity of afamin on all outcomes was tested by a penalized, age- and sex-adjusted regression spline approach in the large population-based in-house KORA F4 Study that served as a reference for all other studies included in the pooled analyses. In addition, results for afamin divided into quartiles are shown for primary outcomes.

Afamin concentrations are quite normally distributed (6). Whole-body ISI (composite), further continuous type 2 diabetes-related phenotypes (fasting insulin and glucose concentrations, HbA_{1c}, and HOMA-IR), and triglycerides were log-transformed based on the natural logarithm (ln) because of their skewed distribution.

To test heterogeneity between study-specific β estimates, the I^2 index as well as the χ^2-based *Q* statistic were calculated for each outcome according to the age- and sex-adjusted model (16). Since there was an indication for heterogeneity for prevalent diabetes (one of the two main outcomes) (Supplementary Table 2), a pooled effect size for the respective studies was calculated using random effects meta-analysis according to the study by DerSimonian and Laird (17).

Further Specific Statistical Analyses in the KORA F4 Study

For the primary outcome of incident diabetes, both a model additionally including glucose concentrations ≥100 mg/dL (100–125 mg/dL vs. <100 mg/dL = reference) in addition to major metabolic risk factors or parameters and a model considering glucose concentrations ≥100 mg/dL and a family history of diabetes were calculated. This cutoff of ≥100 mg/dL for glucose concentrations was defined according to the 1997 ADA definition for impaired fasting glucose (13).

Family history of diabetes in the KORA F4 Study included information about diabetes for all first-grade relatives and took age of onset into account (18). Variable selection in both adjustment models was based on the Framingham Risk Score for type 2 diabetes (19). Furthermore, logistic regression analyses were performed on the association of afamin with prediabetes, and linear regression analyses were performed on the association with whole-body ISI(composite). These latter analyses on whole-body ISI(composite) as well as the linear regression models on further continuous type 2 diabetes-related phenotypes described above (fasting insulin and glucose concentrations, HbA_{1c}, and HOMA-IR) were calculated excluding participants with prevalent type 2 diabetes at baseline. HOMA-IR and whole-body ISI(composite) were also analyzed divided by a cutoff of 2.5.

We considered incident type 2 diabetes as an outcome also taking an oral glucose tolerance test (OGTT) into account and performed a test of deviances on nested models to assess whether afamin significantly added to the extended adjustment model. Whether afamin concentrations contributed to a better classification of individuals into predefined categories of incident type 2 diabetes risk in addition to a model already including major metabolic risk factors or parameters (age; sex; HDL cholesterol; triglycerides; BMI; hypertension and *1*) fasting glucose concentrations ≥100 mg/dL [100–125 mg/dL vs. <100 mg/dL = reference] or *2*) fasting glucose concentrations ≥100 mg/dL [100–125 mg/dL vs. <100 mg/dL = reference]) and a family history of diabetes was also evaluated. The categorical net reclassification improvement (NRI) was calculated using the reclass function in R based on the risk categories <5%, 5–24%, and ≥25% for individuals in whom type 2 diabetes developed during a median follow-up period of 6.4 years (n = 132), for those who did not receive a diagnosis of type 2 diabetes (n = 1,718), as well as for the total group. SEs for categorical NRI were computed according to Pencina et al. (20). For comparison purposes, the continuous NRI was also calculated (again for case patients and control subjects as well as for the total group) with the function improveProb in R. The continuous NRI has the advantage over the categorical NRI that it does not depend on the choice of specific risk categories, and any change in predicted risk in the correct direction is considered appropriate.

For all analyses performed, a two-sided test P value <0.05 was considered statistically significant. Analyses were performed using SPSS for Windows, version 21.0 (IBM Corporation, Armonk, NY) and R for Windows, version 3.1.3 (Vienna, Austria).

RESULTS

Baseline Characteristics

Baseline characteristics of all eight studies included in this pooled analysis are shown in Supplementary Table 1. Mean afamin concentrations were lowest in the YFS (61.4 ± 15.4 mg/L) and highest in the CoLaus Study (73.1 ± 16.6 mg/L). Based on nonlinear P-splines, there was no evident deviation from linearity of afamin in the applied regression models either at baseline or at follow-up in KORA F4 Study (Supplementary Figs. 1–6). There was no effect of sex on associations of afamin with main outcomes (data not shown).

Association Between Afamin Concentrations and Prevalent Type 2 Diabetes (Primary Outcome)

The age- and sex-adjusted logistic regression analysis revealed an increased probability for prevalent type 2 diabetes per 10 mg/L increase in afamin concentrations (odds ratio [OR] 1.40 [95% CI 1.31–1.48], $P = 2.54 \times 10^{-27}$). The extended model was additionally adjusted for HDL cholesterol, triglycerides, BMI, and hypertension and still showed an OR of 1.19 (95% CI of 1.12–1.26) and P value of 5.96×10^{-8} (Fig. 1*A* and Supplementary Table 3). When afamin was categorized in quartiles, the association reached statistical significance in the age- and sex-adjusted model when the third and the fourth quartiles were compared with the first quartile (OR 1.74 [95% CI 1.38–2.20], $P = 3.47 \times 10^{-6}$; and OR 3.91 [95% CI 2.97–5.14], $P = 2.10 \times 10^{-22}$, respectively). This association was still significant for the fourth quartile after extended adjustment (OR 1.72 [95% CI 1.27–2.33], $P = 5.09 \times 10^{-4}$) (Fig. 1*B* and Supplementary Table 4). In a sensitivity analysis, we excluded the KORA-F3 Study and NPHS-II from the pooled analysis since their participants were not necessarily fasting. This reduced heterogeneity but led basically to the same results with slightly increased effect estimates.

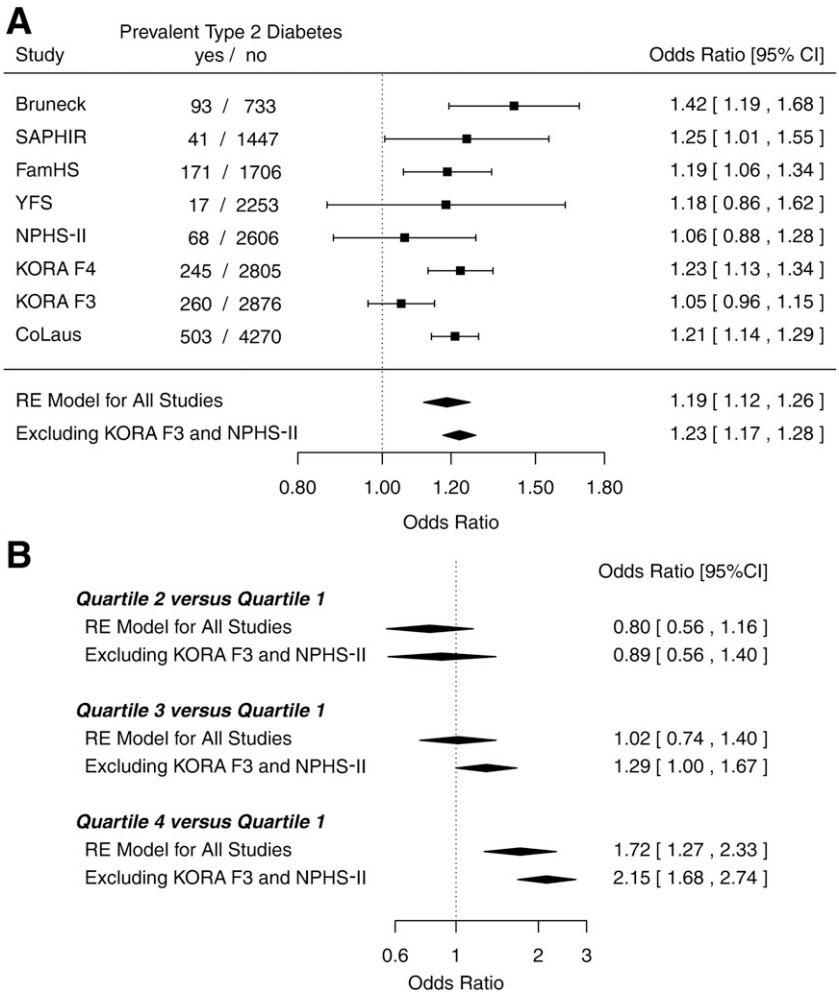

Figure 1—Forest plot illustrating the association of afamin with prevalent type 2 diabetes (extended adjustment model), based on a random-effects (RE) model for all eight studies as well as excluding the KORA F3 Study and NPHS-II since most participants in these studies were nonfasting. Panel *A* provides data for an afamin increment of 10 mg/L, and panel *B* provides data for afamin divided into quartiles. ORs and 95% CIs are shown for each study and the pooled analyses. Numbers for prevalent type 2 diabetes (yes/no) refer to the age- and sex-adjusted model.

Association Between Afamin Concentrations and Incident Type 2 Diabetes (Primary Outcome)

Afamin concentrations measured at baseline were also a significant predictor for the development of type 2 diabetes during follow-up. Each increase in afamin concentrations by 10 mg/L was significantly associated with 49% higher odds for the development of incident type 2 diabetes (OR 1.49 [95% CI 1.42–1.56], $P = 5.97 \times 10^{-62}$) in the age- and sex-adjusted model and with a 30% higher odds in the extended adjustment model (OR 1.30 [95% CI 1.23–1.38], $P = 3.53 \times 10^{-19}$) (Fig. 2*A* and Supplementary Table 3). When afamin concentrations were stratified in quartiles, the association was most pronounced for the fourth quartile with an OR of 5.28 (95% CI 3.83–7.27), $P = 2.64 \times 10^{-24}$, in the age- and sex-adjusted model and an OR of 2.33 (95% CI 1.61–3.36), $P = 6.66 \times 10^{-6}$, in the extended adjustment model. This association was already present but less pronounced in the third quartile (age- and sex-adjusted: OR = 2.56 [95% CI 1.88–3.49], $P = 2.25 \times 10^{-9}$; extended adjustment model: OR = 1.47 [95% CI 1.04–2.08], $P = 0.03$) (Fig. 2*B* and Supplementary Table 5). Again, excluding the KORA-F3 Study and NPHS-II revealed similar results with slightly increased effect estimates.

Association Between Afamin Concentrations and Continuous Type 2 Diabetes–Related Phenotypes (Secondary Outcomes)

Further analyses on continuous type 2 diabetes–related phenotypes such as HbA_{1c}, insulin, glucose, and HOMA-IR were performed excluding all participants who already had type 2 diabetes at baseline. Baseline afamin concentrations were positively associated with insulin concentrations and HOMA-IR in the age- and sex-adjusted model as well as in the extended adjustment model (Table 1 and Supplementary Table 6). An example of a forest plot is provided for HOMA-IR in Supplementary Fig. 7. These associations were less pronounced but still statistically significant in both adjustment models for glucose and HbA_{1c} as dependent variables (Table 1 and Supplementary Table 6).

Extended Analyses in the KORA F4 Study

Association Between Afamin and Prediabetes as Well as IR

Each increase of age- and sex-adjusted plasma afamin concentrations by 10 mg/L increased the probability for prediabetes based on the 1997 ADA definition in 2,635 individuals from the KORA F4 Study without type 2 diabetes at baseline (OR 1.41 [95% CI 1.33–1.49], $P = 1.66 \times 10^{-29}$). The same was observed for the extended adjustment model (OR 1.21 [95% CI 1.14–1.30], $P = 8.62 \times 10^{-9}$).

In addition to these findings, afamin was inversely related to IR based on whole-body ISI(composite) in both adjustment models in the KORA F4 Study (Table 1). When this IR measure was stratified by a cutoff of 2.5, each increase in afamin concentrations by 10 mg/L was associated with an increased probability for IR (OR 1.89 [95% CI 1.67–2.15], $P = 3.92 \times 10^{-23}$). This association remained highly significant in the extended adjustment model (OR 1.77 [95% CI 1.54–2.03], $P = 6.94 \times 10^{-16}$). The same association was found for HOMA-IR stratified by 2.5: each increase in afamin concentrations by 10 mg/L was related to a higher probability for IR in the age- and sex-adjusted model (OR 1.70 [95% CI 1.58–1.82], $P = 5.91 \times 10^{-91}$) and extended adjustment model (OR 1.47 [95% CI 1.34–1.56], $P = 1.45 \times 10^{-20}$), respectively.

Association Between Afamin and Incident Type 2 Diabetes Based on Variable Selection According to the Framingham Risk Score for Type 2 Diabetes

Further adjustment models on the development of type 2 diabetes were performed. When fasting glucose concentrations of ≥100 mg/dL (100–125 mg/dL

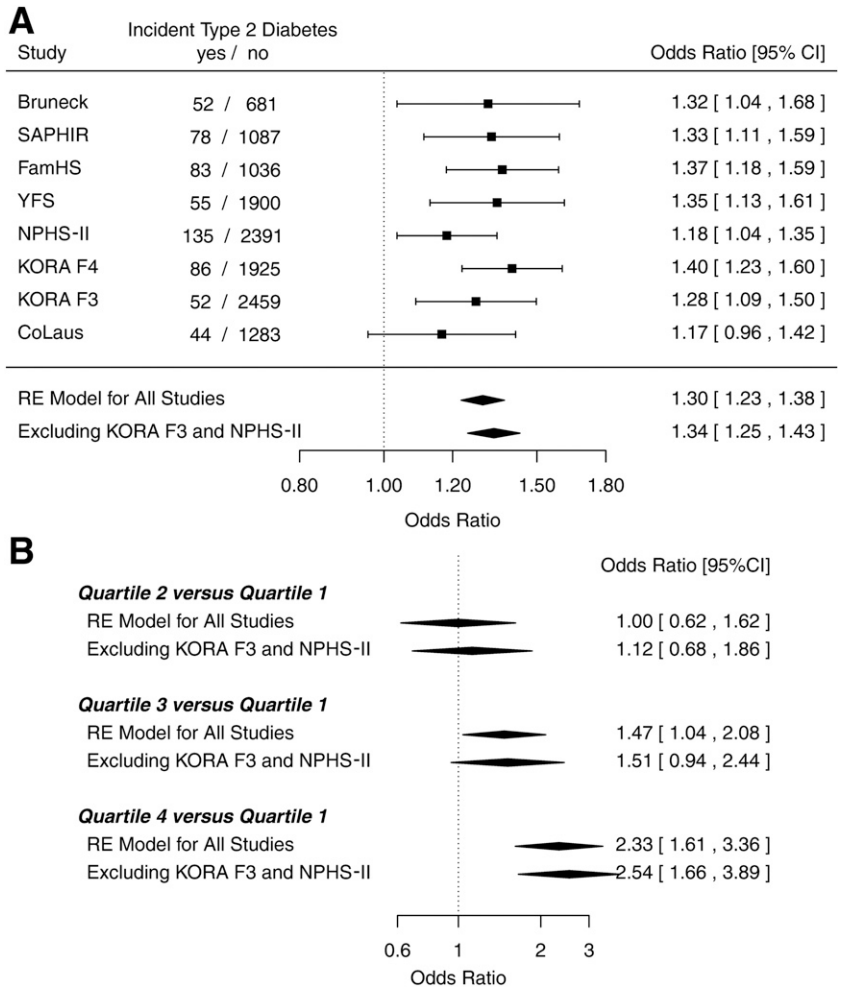

Figure 2—Forest plot illustrating the association of afamin (increment 10 mg/L) with incident type 2 diabetes (extended adjustment model), based on a random-effects (RE) model for all eight studies as well as excluding the KORA F3 Study and NPHS-II. Panel *A* provides data for an afamin increment of 10 mg/L, and panel *B* provides data for afamin divided into quartiles. ORs and 95% CIs are shown for each study and the pooled analyses. Numbers for incident type 2 diabetes (yes/no) refer to the age- and sex-adjusted model.

vs. <100 mg/dL = reference) were additionally included in the extended adjustment model, afamin concentrations measured at baseline were still a significant predictor for the development of type 2 diabetes (OR 1.35 [95% CI 1.17–1.57], $P = 6.19 \times 10^{-5}$). When all cohorts were taken into account where fasting plasma glucose concentrations were available, pooled effect estimates for afamin in these six studies did only marginally differ when compared with the single analysis in the KORA F4 Study (with glucose concentrations as categorical variable [100–125 mg/dL vs. <100 mg/dL = reference]) (OR 1.27 [95% CI 1.18–1.36], $P = 5.09 \times 10^{-10}$). Furthermore, when glucose concentrations were included in the model on a continuous scale, the effect estimate was almost unchanged (OR 1.21 [95% CI 1.11–1.30], $P = 2.87 \times 10^{-6}$) (for more details, see Supplementary Table 7).

Even when in addition to glucose concentrations ≥100 mg/dL the family history of diabetes was taken into account, each increase of 10 mg/L in afamin concentrations still showed a significantly higher probability for incident type 2 diabetes (OR 1.33 [95% CI 1.13–1.56], $P = 0.001$).

Various further adjustment models for primary and secondary outcomes were performed. No matter whether we added either smoking, alcohol intake, physical activity, waist circumference (instead of BMI), family history of diabetes, fasting glucose concentrations, fasting insulin concentrations, or HOMA-IR (where appropriate) to the extended adjustment model, the effect estimates of afamin remained highly significant (OR range 1.20–1.43, all *P* values ≤0.001). Similar results were found for type 2 diabetes–related phenotypes, which did not show major changes in the β estimates for all outcomes (data not shown).

Afamin and Type 2 Diabetes Risk Discrimination and Reclassification Analysis

To assess whether afamin contributes to better discrimination between individuals in whom type 2 diabetes has developed and those who remained free of type 2 diabetes during the prospective follow-up in the KORA F4 Study, two statistical concepts were applied: *1*) deviances and *2*) categorical as well as continuous NRI. For these analyses, we applied a more accurate definition for incident type 2 diabetes available in the KORA F4 Study by further using an OGTT (according to the 1997 ADA criteria) (13). The effect estimate of afamin did not change compared with the diabetes definition without OGTT as used in the pooled analysis according to the extended adjustment model (OR 1.48 [95% CI 1.32–1.66], $P = 5.96 \times 10^{-11}$ vs. OR 1.40 [95% CI 1.23–1.60], $P = 5.49 \times 10^{-7}$). The model including afamin (deviance = 694.69) showed a significantly improved model fit compared with the extended risk model including glucose concentrations ≥100 mg/dL (100–125 mg/dL vs. <100 mg/dL = reference) (deviance = 726.90) (difference in deviance −32.21, $P < 0.0001$). When in addition to glucose concentrations ≥100 mg/dL a family history of diabetes was included in the extended adjustment model (deviance = 602.71), the model also containing afamin (deviance = 577.07) still indicated a significantly improved model fit (difference in deviance −25.64, $P < 0.0001$). Furthermore, the categorical NRI was applied to test whether the inclusion of afamin into a model containing known metabolic risk factors or parameters significantly adds to type 2 diabetes risk reclassification. Based on predefined risk categories (<5%, 5–24%, ≥25%), as shown in Table 2, the NRI for cases was 0.114 (95% CI 0.031–0.221), $P = 0.002$, and for controls 0.021 (95% CI 0.006–0.036), $P = 0.008$. Overall NRI for the total group was 0.135 (95% CI 0.048–0.221), $P = 0.002$. Of the 132 individuals in whom type 2 diabetes developed, 24 (18.2%) were correctly reclassified and thus moved to a higher risk category. Of those individuals

Table 1—Pooled results from study-specific linear regression analyses of afamin (increment 10 mg/L) on type 2 diabetes–related phenotypes at the baseline investigation excluding those with type 2 diabetes at baseline

	Adjustment for age and sex		Extended adjustment	
Parameters/(*n* individuals)	β (95% CI)*‡	*P*	β (95% CI)†‡	*P*
Ln-HbA_{1c} (%) (*n* = 7,828)§	0.006 (0.004–0.008)	4.41×10^{-10}	0.003 (0.002–0.005)	3.09×10^{-4}
Ln-insulin (μIU/mL) (*n* = 13,156)‖	0.172 (0.146–0.198)	3.32×10^{-39}	0.101 (0.083–0.120)	1.51×10^{-26}
Ln-glucose (mg/dL) (*n* = 13,183)‖	0.015 (0.010–0.020)	4.68×10^{-10}	0.009 (0.006–0.013)	7.48×10^{-7}
Ln-HOMA-IR (*n* = 13,153)‖	0.187 (0.158–0.216)	3.00×10^{-36}	0.110 (0.089–0.132)	1.37×10^{-23}
Ln-ISI(composite) (*n* = 926)¶	−0.246 (−0.278 to −0.214)	2.18×10^{-50}	−0.171 (−0.204 to −0.137)	4.53×10^{-24}

Ln, log-transformation based on the ln; *n*, age- and sex-adjusted model. *Adjusted for age and sex. †Adjusted for age, sex, HDL cholesterol, triglycerides, BMI, and hypertension. ‡Meta-analysis β estimate, 95% CI, and *P* values derived from a random-effects model. §Studies included: Bruneck Study, SAPHIR Study, KORA F3 Study, and KORA F4 Study. ‖Studies included: Bruneck Study, SAPHIR Study, KORA F4 Study, CoLaus Study, YFS, and FamHS. ¶Study included: KORA F4 Study.

who remained free of type 2 diabetes (*n* = 1,718), 110 (6.4%) moved to a lower risk category and can be considered as correctly reclassified based on adding afamin to the risk model. In subjects at intermediate risk (5% to <24%), the addition of afamin to the risk model resulted in a correct reclassification of 17 cases (24.3%) and 84 controls (19.9%), respectively (Table 2 and Supplementary Fig. 8). Even when additionally adding family history of diabetes to the risk model, afamin still contributed to an improved type 2 diabetes risk reclassification (see Supplementary Table 8 and Supplementary Fig. 9). Results based on continuous NRI showed a significant gain in classification accuracy when afamin was added to the risk model (NRI for cases 0.197 [95% CI 0.030–0.364], $P = 0.02$; NRI for controls 0.354 (95% CI 0.310–0.398), $P < 0.0001$). Overall continuous NRI for the total group was 0.551 (95% CI 0.378–0.724), $P < 0.0001$. This means that in approximately three of five subjects assignment to the case or control status has been enforced by adding afamin to the risk model. The same conclusion holds true when family history of diabetes was also included in the risk classification calculations because absolute NRI values did not change for the total group (0.491 [95% CI 0.298–0.685], $P < 0.0001$) and for controls (0.351 [95% CI 0.305–0.398], $P < 0.0001$) and were only slightly attenuated for cases (0.140 [95% CI 0.047–0.328], $P = 0.14$).

CONCLUSIONS

This is the first analysis in >20,000 individuals from mainly population-based studies that describes novel associations of afamin with prevalent and incident type 2 diabetes and type 2 diabetes–related phenotypes. The main findings were as follows: *1*) increased afamin concentrations were significantly associated with prediabetes and type 2 diabetes at baseline and with type 2 diabetes-related phenotypes such as IR defined by HOMA-IR and whole-body ISI(composite); *2*) afamin concentrations at baseline significantly predicted the development of type 2 diabetes during follow-up, and all of these associations were independent from major metabolic risk factors or parameters; and *3*) afamin showed a significantly improved model fit and gain in classification accuracy for incident type 2 diabetes when added to an extended-adjustment model including major metabolic risk factors or parameters.

Previously, we showed that afamin concentrations measured at baseline were significantly related to all components of the metabolic syndrome, with one of the strongest associations found with elevated waist circumference at both the baseline and follow-up investigation (6). Elevated waist circumference and BMI are measures of increased body fat and are well-established risk factors for the metabolic syndrome and type 2 diabetes (21–23). Furthermore, this increase in body fat elevates not only the risk for type 2 diabetes but also for IR. Most importantly, in our large analysis afamin was associated with prediabetes, measures of IR, as well as the prevalence and incidence of type 2 diabetes independent of major metabolic factors or parameters. Taken together, the findings on incident type 2 diabetes and prediabetes strongly suggest that afamin might be a valid marker to predict a high risk for the development of type 2 diabetes. Novel mechanisms and pathways besides those related to metabolic syndrome might be involved.

Adipose tissue can affect the development of IR in other tissues such as liver by producing free fatty acids and several other proinflammatory and anti-inflammatory factors (24). IR causes hyperinsulinemia and leads to steatosis via various mechanisms such as increased hepatic de novo lipogenesis (24), inflammation, and lipotoxicity (25). There is evidence that nonalcoholic fatty liver disease might also be a risk factor for future type 2 diabetes and that the increased risk for nonalcoholic fatty liver disease might not only be secondary to diabetes (26). As afamin is primarily expressed in the liver, the liver might indeed play an important role in contributing to elevated afamin concentrations and thus to the development of type 2 diabetes.

In general, afamin seems to have heterogeneous effects depending on the site of action. It has been shown that afamin might have binding properties for two of the major forms of antioxidative vitamin E, α-tocopherol and γ-tocopherol (14). The antioxidative function of vitamin E remains controversial (27). Our previous work (10) has demonstrated that plasma afamin concentrations are not associated with those of vitamin E, indicating that afamin does not play a major role in binding and transporting vitamin E in plasma (in fact, vitamin E is mostly carried by the lipoprotein system). Thus, the proposed vitamin E–binding role of afamin might be of functional relevance for diseases such as type 2 diabetes and metabolic syndrome only in extravascular fluids or tissues. The possible mechanisms for such a scenario remain unknown.

The causality of the association of afamin with type 2 diabetes as well as possible underlying mechanisms remains to be elucidated. The preliminary findings of a hyperglycemic phenotype in mice transgenic for the human afamin gene are supportive for a causal role of afamin in the development of type 2 diabetes

Table 2—Reclassification of individuals into low, medium, and high risk categories for development of type 2 diabetes within the study period in the KORA F4 Study (median follow-up 6.4 years) when additionally considering afamin in the risk model

Individuals with incident type 2 diabetes (*n* = 132)

		Baseline model plus afamin		
Baseline model	Total	<5% risk	5–24% risk	≥25% risk
<5% risk	17	10 (58.8)‡	7 (41.2)*	0 (0.0)*
5–24% risk	70	4 (5.7)†	49 (70.0)‡	17 (24.3)*
≥25% risk	45	0 (0.0)†	5 (11.1)†	40 (88.9)‡
Total	132	14	61	57

Individuals without incident type 2 diabetes (*n* = 1,718)

		Baseline model plus afamin		
Baseline model	Total	<5% risk	5–24% risk	≥25% risk
<5% risk	1,202	1,156 (96.2)‡‡	45 (3.7)††	1 (0.08)††
5–24% risk	422	84 (19.9)**	310 (73.5)‡‡	28 (6.6)††
≥25% risk	94	0 (0.0)**	26 (27.7)**	68 (72.3)‡‡
Total	1,718	1,240	381	97

Values are presented as *n* (row percentage). The baseline model includes the risk factors or parameters age, sex, HDL cholesterol, triglycerides, BMI, hypertension, and glucose concentrations ≥100 mg/dL (100–125 mg/dL vs. <100 mg/dL = reference). Categorical NRI in this table is calculated for 132 individuals with and for 1,718 individuals without type 2 diabetes. Overall NRI for the total group: 0.135 (95% CI 0.048–0.221), $P = 0.002$. *Moved to higher risk category that is correctly reclassified, $n = 24$. †Moved to lower risk category that is wrongly reclassified, $n = 9$. ‡Stayed in the same risk category, $n = 99$. NRI for cases 0.114 (95% CI 0.031–0.221), $P = 0.002$. **Moved to lower risk category that is correctly reclassified, $n = 110$. ††Moved to higher risk category that is wrongly reclassified, $n = 74$. ‡‡Stayed in the same risk category, $n = 1,534$. NRI for controls 0.021 (95% CI 0.006–0.036), $P = 0.008$.

(6). A direct role of afamin in glucose metabolism was very recently shown by Shen et al. (28) in a thyroid carcinoma cell line transfected with human afamin. Afamin was found to upregulate several key enzymes and metabolites of glucose metabolism, revealing possible new insights into the molecular functions of afamin. Since the transgenic animals as well as the transfected cell line model are of only limited relevance for the pathogenesis of type 2 diabetes in humans, both models have to be considered with caution as valid models for a functional and a causal role for afamin in type 2 diabetes.

Our results are in accordance with a recently reported study demonstrating a strong association between concentrations of microRNA (miRNA)-122 and the incidence of metabolic syndrome and type 2 diabetes in the Bruneck Study (29). miRNA-122 was also highly significantly associated with afamin analyzed by the proteomics approach. miRNAs play a key role in the epigenetic regulation of gene expression. miRNA-122 is the predominant miRNA in the liver and regulates a number of genes involved in cholesterol and fatty acid metabolism (for review, see Willeit et al. [30]). Willeit et al. (29) therefore investigated in a mouse model the expressed hepatic proteome after antisense targeting of miRNA-122. Afamin was not differentially expressed when comparing untreated mice with mice lacking miRNA-122, suggesting no gene regulatory function of miRNA-122 for afamin, at least in mice.

Finally, the question remains of whether afamin adds information to well-known risk predictors for incident type 2 diabetes. All measures of discrimination and reclassification (i.e., deviance, continuous and categorical NRI) suggested a significant and valuable gain in model fit and classification accuracy in the population-based KORA F4 Study when afamin measured at baseline was added to a risk model including age, sex, metabolic risk factors or parameters, glucose concentrations ≥100 mg/dL, and a positive family history of diabetes. This is even more impressive because most of these metabolic risk factors or parameters are major components of the metabolic syndrome.

A main strength of the study is that data were generated from eight independent populations, the great majority of them being population-based. In addition, we had follow-up data on incident type 2 diabetes available in all of these studies. It might be considered a limitation that we performed the extended analyses and adjusted for potential confounders or risk factors, such as smoking, alcohol intake, physical activity, waist circumference, or fasting glucose concentrations and a family history of diabetes, mainly in the large population-based in-house KORA F4 Study, which had all of these variables available and included only fasting participants. Moreover, data on family history of diabetes besides the power issue could be susceptible to inaccuracies. However, including those data showed results that were very similar to those in the presented main pooled analyses. In addition, an analysis was added further adjusting for fasting glucose concentrations in six of the eight cohorts that had fasting glucose concentrations available, and results remained highly consistent.

Statistical concepts for risk reclassification such as categorical NRI have known limitations such as the arbitrary choice of risk categories if no recommended risk thresholds exist. Therefore, we also applied the continuous NRI that does not rely on predefined risk categories. Moreover, the result of the test on deviances was in line with the results of both NRI analyses. Thus, the model performance of afamin was consistent over all applied statistical concepts of risk prediction and discrimination. Marginal differences in NRI analyses when family history of diabetes was further added to the risk model were most probably caused by limited statistical power; however, the main conclusion drawn that afamin improved type 2 diabetes risk reclassification did not change. Moreover, as in most epidemiological studies, we cannot exclude that results are to some extent biased by residual and unmeasured confounding as well as by loss to follow-up. Finally, the analyses were performed only in Caucasians, and thus it has to be elucidated whether these findings can be replicated in individuals of other ethnicities.

In summary, this large analysis of mainly population-based studies demonstrated that afamin is highly significantly associated with prediabetes, IR, prevalence of type 2 diabetes, as well as the development of type 2 diabetes independent of major metabolic risk factors or parameters. Increased plasma afamin

concentrations may therefore indicate the development of type 2 diabetes at a very early stage. Because the number of individuals in whom diabetes is diagnosed has been steadily increasing for decades and according to the World Health Organization global diabetes prevalence has doubled since 1980, finding crucial markers contributing to the development of type 2 diabetes is indispensable for an adequate and rapid identification of affected patients or of patients at high risk as well as for the elucidation of the pathogenesis of this disease.

Funding. This study was supported by grants from the Standortagentur Tirol and the Austrian Heart Fund to F.K. and from the Austrian Research Fund (P19969-B11) to H.D. Funding information for each study is provided in the Supplementary Data.

Duality of Interest. H.D. is owner of and shareholder of Vitateq Biotechnology GmbH, a spin-off company of Medical University of Innsbruck, and holds several patents related to research described in this article. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. B.K. and F.K. designed the study; performed the analyses; interpreted the findings; wrote and revised the report; and were involved in the design, recruitment, phenotyping, data collection, data preparation, and data management of the singular cohorts. C.L. designed the study, performed the analyses, interpreted the findings, and wrote and revised the report. C.Hu., C.M., C.He., L.K., J.W., B.T., D.D., D.S., K.W., M.R., W.R., B.P., A.P., M.K., T.L., O.T.R., S.E.H., and P.V. were involved in the design, recruitment, phenotyping, data collection, data preparation, and data management of the singular cohorts. P.M.-V. and J.C. performed the analyses, interpreted the findings, and wrote and revised the report. S.K., I.S., and S.C.H. were involved in the design, recruitment, phenotyping, data collection, data preparation, and data management of the singular cohorts; performed the analyses; interpreted the findings; and wrote and revised the report. H.D. designed the study. All authors contributed to critical reading and revision of the draft report. F.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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