Supplementary Material to:

Plasma concentrations of afamin are associated with prevalent and incident type 2 diabetes: a pooled analysis in more than 20,000 individuals

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Study Populations and Study Design

KORA F3 and KORA F4

The Cooperative Health Research in the Region of Augsburg (KOoperative Gesundheitsforschung in der Region Augsburg, KORA) Study incorporates **population-based cohort studies** drawn from equally sized ten year age-sex-strata of the target population which consists of all 25 to 74 year old German residents of the city of Augsburg, Germany and two surrounding counties, and was initiated as part of the WHO MONICA Study. A detailed description of the sampling methods is given elsewhere [(1)]. A standardized face-to-face interview and medical examinations including blood draw as well as anthropometric measurements were done by certified medical staff in all study participants [(1)]. Moreover, participants were asked to bring all product packages of currently used medication to the study centre.

The KORA F3 study is a follow-up investigation of the KORA S3 study conducted in 1994/1995 with a response rate of 75%. Of all 4,856 KORA S3 participants, 3,184 also participated in 2004/2005 in KORA F3. About 92% of the KORA F3 participants were non-fasting. Afamin data were available in 3,158 KORA F3 participants. Prevalent type 2 diabetes at KORA F3 was defined as self-reported and validated by hospital records or by questioning the responsible physician, or as current use of antidiabetic medication. Additionally, a validation of the diabetes type was requested. If no type validation, but also no contradicting information was given, diabetic participants were assumed to have type 2 diabetes.

Incident cases of type 2 diabetes were mainly assessed using follow-up questionnaire data collected in 2008/2009. Self-reported type 2 diabetes and the date of diagnosis were validated by hospital records or by questioning the responsible physician. Furthermore, hospital records of those deceased during the follow-up period were examined. The records were searched for a history of type 2 diabetes and the date of diagnosis. If a physician-diagnosis of type 2 diabetes was known from other sources, e.g. from the records of the population-based MONICA/KORA registry of acute myocardial infarction, this information was also used. In general, incident cases of type 2 diabetes, which had been diagnosed up to December 31, 2009, were included. In total, 13% of participants were lost to follow-up.

The KORA F4 study is a follow-up of the independent KORA S4 survey, conducted between 1999 and 2001 in the same geographical region as KORA S3, with a response rate of 67%. Of all 4,261 KORA S4 participants, 3,080 also participated between 2006 and 2008

in the follow-up study KORA F4. Afamin data were available in 3,059 KORA F4 participants. The second follow-up (KORA FF4) was conducted in 2013/2014 and 2,161 former F4 participants took part. Of them, 2,148 had data on afamin. Prevalent type 2 diabetes at KORA F4 was defined as self-reported and validated by hospital records or by questioning the responsible physician, or as current use of antidiabetic medication. Additionally, a validation of the diabetes type was requested. If no type validation, but also no contradicting information was given, diabetic participants were assumed to have type 2 diabetes. In the type 2 diabetes incidence analyses, only those participants who attended both the KORA F4 and KORA FF4 studies were included. The percentage of loss-to-follow up could be quantified with 30%. Incident type 2 diabetes in KORA FF4 was assessed and defined as specified for prevalent type 2 diabetes in KORA F4.

All KORA F4 participants without known diabetes were to receive a standard oral glucose tolerance test (OGTT), carried out in the morning (7:00 am to 11:00 am). Participants were asked to fast for 10h overnight, to avoid heavy physical activity on the day before examination and to refrain from smoking before and during the test. Exclusion criteria for the OGTT were: (i) consumption of foods or drinks containing calories within 8h before the fasting blood draw; (ii) medical contraindications such as gastrointestinal disease, fructose-intolerance, currant allergy, weakness, risk of hypoglycaemia, or pregnancy. Fasting venous blood was sampled for glucose determination and 75g of anhydrous glucose given (Dextro OGT, Boehringer Mannheim, Germany, containing currant extract). In order to keep type 2 diabetes definitions comparable across the investigated study populations, KORA F4 OGTT data were not used for type 2 diabetes definition in the current pooled study but for prediabetes definition and for calculation of the whole-body insulin sensitivity index ISI(composite) as well as risk discrimination and reclassification analyses that were done in KORA F4 only.

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or antihypertensive drug treatment in case the individual was aware of the disease.

In both cohorts, the cholesterol-esterase method (CHOL Flex, Dade-Behring, Germany) was applied to determine total cholesterol. For triglyceride and HDL cholesterol concentrations the TGL Flex and AHDL Flex method (Dade-Behring) and for LDL cholesterol a direct method (ALDL, Dade-Behring) was used, respectively. In KORA F4, fasting serum insulin was assessed by ELISA (Invitrogen, Darmstadt, Germany) and fasting serum glucose

using a hexokinase method (GLU Flex, Dade Behring, Deerfield, IL). The following formula was applied to calculate HOMA-IR: fasting insulin [µU/mL] * fasting glucose [mg/dL] / 405 [(2)]. The quantification of HbA1c was done in hemolyzed whole blood in KORA F4 with a cation-exchange HPLC photometric assay on an Adams HA-8160 Hemoglobin Analysis System (Arkray Inc., distributed by A. Menarini Diagnostics, Florence, Italy) and in KORA F3 with a turbidimetric immunoassay method (Tina-quant® Hämoglobin A1c) on a Dimension RXL instrument, Dade-Behring Inc., Newark U.S.A. High-sensitivity CRP (hs-CRP) was measured by immunonephelometry on a BN II analyzer using the CardioPhase assay from Siemens (Marburg, Germany) [(3,4)]

CoLaus Study

The CoLaus (Cohorte Lausannoise) Study was designed to examine the epidemiology and genetic determinants of cardiovascular disease. In total, 6,188 Caucasian participants, 3,251 females and 2,937 males aged between 35 and 75 years, were recruited using a **simple non-stratified random sample of the population registry of the city of Lausanne**, Switzerland [(5)]. The participation rate was 41% and all participants came to the outpatient clinic of the University Hospital of Lausanne in the morning after an overnight fast. Venous blood samples were drawn and routine clinical assays were performed at the Clinical Laboratory of the Centre Hospitalier Universitaire Vaudois (CHUV). Total cholesterol was measured by CHOD-PAP, HDL cholesterol by CHOD-PAP + PEG + cyclodextrin and triglycerides by GPO-PAP. LDL cholesterol was calculated based on the Friedewald formula only if triglycerides were <4.6 mmol/l. The measurement of high sensitive CRP (hsCRP) was carried out with a latex– enhanced HS immunoassay (Roche Diagnostics, CH). A solid-phase, two-site chemiluminescent immunometric assay by Diagnostic Products Corporation, Los Angeles, USA was applied for insulin and glucose dehydrogenase (Roche Diagnostics, CH) for glucose measurement. HOMA-IR was estimated as fasting serum insulin (mU/l) * fasting plasma glucose (mmol/l) / 22·5. Hba1c was not available. Afamin was measured in 4,773 participants. In CoLaus, type 2 diabetes was defined as fasting plasma glucose ≥7.0 mmol/L and/or oral hypoglycaemic or insulin treatment. In case of diabetes without self-reported type 1 diabetes, a participant was defined to have type 2 diabetes.

Cardiovascular Risk in Young Finns Study (YFS)

The YFS is a **prospective multicenter study** from Finland initiated in 1980 (n=3,596, baseline age range 3–18 years) with several follow-ups over a time period of 30 years. Main aim is the investigation of risk factors for cardiometabolic outcomes [(6,7)].

Detailed data were collected by questionnaires, physical measurements, and blood tests, including information on general health status, serum lipids, insulin, obesity indices, blood pressure, and smoking status. In addition, risk factors such as C-reactive protein (CRP) have been measured. After an overnight fast venous blood samples were drawn and stored at −70°C. Serum triglyceride concentration was measured using the enzymatic glycerol kinase–glycerol phosphate oxidase method (Triglyceride reagent, Beckman Coulter Biomedical, Ireland). Serum total cholesterol, HDL cholesterol (after precipitation of low density lipoprotein (LDL) and very low density lipoprotein levels were assessed with dextran sulfate–Mg2+ by the enzymatic cholesterol esterase–cholesterol oxidase method (Cholesterol reagent, Beckman Coulter Biomedical). An enzymatic hexokinase method (Glucose reagent, Beckman Coulter Biomedical) was applied to measure serum glucose concentrations. Serum insulin concentration was examined by microparticle enzyme immunoassay kit (Abbott Laboratories, Chicago, IL) [(8)]. LDL-cholesterol was determined by the Friedewald formula in participants with triglyceride concentrations <4.0 mmol/l. Afamin values are available from 2,270 individuals in the 2001 follow-up which served as our baseline investigation. The data for incident type 2 diabetes are taken from the 2007 or the 2011 follow-up investigations. Of included participants at baseline, 13% were lost to follow-up. Glycated hemoglobin A1c (HbA1c) was not yet available in 2001. Insulin resistance was estimated based on the HOMA index, i.e. the product of fasting glucose and insulin divided by the constant 22.5. The diagnosis of type 2 diabetes was based on fasting glucose concentrations ≥7mmol/l or HbA1c ≥6.5% or self-reported diabetes or use of medication [(9)].

NHLBI Family Heart Study (FamHS)

The Family Heart Study was initiated in 1992 with the ascertainment of 1,200 families with approximately 6,000 individuals, half randomly sampled, and half selected because of an excess of coronary heart disease (CHD) or risk factor abnormalities and funded by the National Heart, Lung, and Blood Institute (NHLBI) [(10)]. The FamHS is a **prospective study** that investigates the genetic and non-genetic determinates of atherosclerosis. Study participants belonging to the largest pedigrees were invited for a second clinical examination in 2002/03.

Fasting triglyceride concentrations were assayed using triglyceride GB reagent and serum total cholesterol using a commercial cholesterol oxidase method on the Roche COBAS FARA centrifugal analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula in

case of triglyceride concentrations <4.5 mmol/L (400 mg/dL). Otherwise, LDL was measured by ultracentrifugation [(11)]. Fasting glucose was examined by a thin film adaptation of an enzymatic glucose-oxidase spectrophotometric procedure using the Vitros analyzer (Ortho Clinical Diagnostics, Rochester, NY) and insulin concentrations by the coated-tube radioimmunoassay method (Diagnostic Products Corporation, Los Angeles, CA) [(12)]. Type 2 diabetes was defined as intake of hypoglycaemic agents, participants reporting a previous clinical diagnosis of type 2 diabetes, or fasting glucose at or above 7 mmol/L. Individuals with type 1 diabetes and age of type 2 diabetes diagnosed before an age of 20 years were excluded. In the current analysis, 1,877 participants of Caucasian origin with available afamin values were included. Finally, 36% of participants were lost to follow-up.

Bruneck-Study

The **prospective, population-based** Bruneck Study was designed to investigate the epidemiology and pathogenesis of atherosclerosis [(13,14)]. In 1990, a random sample including 1,000 subjects of Caucasian origin recruited from the entire population of Bruneck was stratified according to sex and age with 125 subjects of each sex and 5th to 8th decade of age. The participation rate was 93.6% resulting in 919 subjects with complete data. In an interval of five years, follow-up examinations were performed. The baseline for this investigation was the 1995 examination and follow-up data were taken from the 2010 investigation. Of the 826 subjects included at baseline, all had afamin data and detailed information on prevalent and incident diabetes available. All laboratory measurements were determined in samples collected in 1995 and measured by validated standard laboratory methods as described previously [(14,15)]. HbA1c was determined by high performance liquid chromatography (DCCT-aligned assay and insulin resistance by homeostasis model assessment (HOMA-IR) applying the formula fasting plasma glucose in mmol/l × fasting serum insulin in mU/l divided by 22.5. Definition of type 2 diabetes was based on the 1997 American Diabetes Association criteria (fasting glucose ≥126 mg/dL, i.e. ≥7 mmol/L) and/or receiving anti-diabetic treatment and diabetes diagnosis validated through medical records [(16)].

SAPHIR-Study

The SAPHIR Study (Salzburg Atherosclerosis Prevention Program in subjects at High Individual Risk) is an **observational study** accomplished in the years 1999 to 2002 based on 1,770 **healthy unrelated Caucasian subjects**. The recruitment of study participants was

done through health screening programs in large companies in and around the city of Salzburg [17]. Clincial examinations were performed with a main focus on CVD risk factors and lipid metabolism. After an overnight fasting period, venous EDTA blood was collected. Plasma was gathered by low-speed centrifugation and stored at −70°C. Afamin was available in 1,499 participants at the baseline examination. Follow-up examinations were conducted between 2002 and 2008 with a mean follow-up time of 4.59 years; range: 2.10-8.42 years, 22% loss to follow-up. Type 2 diabetes was defined according to the 1997 American Diabetes Association criteria (fasting glucose ≥126 mg/dL) and/or receiving anti-diabetic treatment and diabetes diagnosis validated through medical records [17].

Second Northwick Park Heart Study (NPHS-II)

The **prospective** Second Northwick Park Heart Study (NPHS-II) included 3,052 **unrelated healthy middle-aged men from nine general practices** in the United Kingdom [18]. Baseline characteristics were obtained by questionnaire completed at study entry in 1989. Of the initial cohort, 3,012 men were Caucasian and 2,674 eligible men had afamin measured. These men were prospectively followed with the aim to comprehensively study CVD risk factors and outcomes. Only 3% of participants could not be included at follow-up. For all examinations, participants were non-fasting, but have avoided smoking, vigorous exercise or heavy meals from midnight the day before. Data on lifestyle habits, anthropometrics, blood pressure and various blood biomarkers were collected at the baseline and prospective follow-up investigations. Lipids, total cholesterol, and triglyceride concentrations were gathered with automated enzyme procedures. More details on recruitment and measurements have been reported elsewhere [19]. Prevalent diabetes was defined by self-report (answer to the question: have you ever had diabetes?) in the Second Northwick Park Heart Study (NPHS-II) and diagnosis of incident diabetes was validated through medical records (from a note search undertaken in 2005).

Measures of insulin resistance

Besides the homeostasis model assessment-estimated insulin resistance (HOMA-IR) we calculated the whole-body insulin sensitivity index (ISI(composite)) [20], a valid surrogate measure of data derived from euglycemic insulin clamp, based on the formula: ISI = 10,000 / sqrt ((fasting glucose (mg/dL) * fasting insulin ((µlU/ml))*(2-h glucose (mg/dL) * 2-h insulin (µlU/ml))) as recently applied in KORA F4.

HOMA-IR and whole-body ISI(composite) were also analysed divided by a cut-off of 2.5. Whole-body IS (composite) values ≥2.5 reflect insulin sensitivity, values <2.5 insulin

resistance [21]. For HOMA-IR values ≥2.5 refer to insulin resistance, and values <2.5 to insulin sensitivity. Data on whole-body ISI(composite) were only available in individuals ≥ 62 years of age [22].

Measurement of afamin plasma concentrations

As previously described [23,24] afamin was quantified with a custom-made double-antibody sandwich ELISA using an affinity-purified biotinylated polyclonal anti-afamin antibody for coating 96-well streptavidin-bound microtiter plates and peroxidase-conjugated monoclonal antibody N13 for detection (MicroCoat Biotechnologie GmbH, Bernried, Germany). Secondary plasma in serial dilutions that was initially calibrated with a primary standard served as the assay standard. Afamin purified to homogeneity from human plasma was originally used as the primary standard and the protein concentration of this standard was estimated by quantitative amino-acid compositional analysis. Within-run and between-run coefficients of variation were 3.3% and 6.2%, respectively (mean concentration 73 mg/L) [25]. The four same control samples were added to each assay plate using new aliquots each time which were thawed the first time. These control samples were used in all eight studies and were assayed in duplicates. These four samples were monitored throughout the entire project and the assay was repeated when more than one control samples showed a divergent result of more than 10% from the expected values. The intra-assay coefficient of variation (CV) was calculated from the mean and standard deviation (SD) of each of the measured four control samples using the formula CV (%) = SD * 100 / mean using 284 duplicate measurements. The inter-assay CV was calculated using the same formula using the values of the same four controls samples included in 71 runs over a period of six months. The samples of all study participants for each study were measured in a random way independent of a case-control status and the lab personnel was blinded to all variables except the study name. Afamin concentrations were measured for all studies in the laboratory at the Medical University of Innsbruck. A previous report on the assay evaluation described afamin as a robust, stable analyte that is virtually unchanged under different storage conditions. It is independent of sex, fasting state, and a daily and monthly rhythm [25]. In this pooled analysis, data on afamin concentrations was available in 20,136 individuals.

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SUPPLEMENTARY DATA

Supplementary Table 1: Clinical and laboratory data of participants with available afamin measurements in the Bruneck Study (n=826), KORA F3 Study (n=3,158), KORA F4 Study (n=3,059), SAPHIR Study (n=1,499), CoLaus Study (n=4,773), NPHS-II Study (n=2,674), YFS Study (n=2,270), and FamHS Study (n=1,877).

	Study population			
	Bruneck (n=826)	**KORA F3* (n=3,158)**	**KORA F4 (n=3,059)**	**SAPHIR (n=1,499)**
Age, yrs (minimum-maximum)	63±11 53/63/72 (45-85)	57±13 46/57/67 (35-84)	56±13 44/56/67 (32-81)	51±6 46/52/55 (39-67)
Gender: male/female: n, %	414/412 50.1/49.9	1533/1625 48.5/51.5	1477/1582 48.3/51.7	1010/489 67.4/32.6
Smoking (Non-smoker/Ex-smoker/Smoker): n, %	452/213/161 54.7/25.8/19.5	1325/1101/551 44.5/37.0/18.5	1349/1160/546 44.2/38.0/17.9	957/214/328 63.8/14.3/21.9
Follow-up time (years)	12.5 ± 4.3 10.3/15.0/15.0	4.5 ± 0.4 4.2/4.5/4.8	6.5 ± 0.3 6.3/6.4/6.6	4.6± 0.7 4.3/4.4/4.6
Afamin (mg/L)	62.6±15.3 52.1/61.5/71.7	71.4±17.1 59.3/69.7/81.4	70.6±17.2 58.8/68.7/80.6	66.2±14.3 56.4/64.1/73.9
Body mass index, kg/m²	25.7±3.9	27.6±4.6	27.6±4.8	26.8±4.1
Obesity (BMI ≥30: n, (%))	115 (13.9)	842 (26.9)	809 (26.6)	280 (18.7)
Systolic blood pressure (mmHg)	148±21	131±20	122±18	138±18
Diastolic blood pressure (mmHg)	87±9	82±11	75±10	86±12
Hypertension: n, %	564 (68)	1576 (50)	1169 (38)	821 (55)
Antihypertensive medication: n, %	230 (28)	996 (32)	944 (31)	212 (14)
Waist circumference (cm)	90±11	95±13	94±14	95±12
Total cholesterol, mg/dL	230±42	218±40	216±40	227±39
HDL cholesterol, mg/dL	59±16	59±17	56±14	59±16
LDL cholesterol, mg/dL	145±38	128±33	136±35	145±36
Triglycerides, mg/dL	132±81 81/111/158	165±126 88/135/201	125±89 72/104/151	126±89 72/101/151
Use of lipid lowering drugs: n, %	38 (4.6)	337 (10.7)	382 (12.5)	63 (4.2)
Type 2 Diabetes: n, %	93 (11.2)	260 (8.3)	245 (8.0)	41 (2.7)
Diabetes medication: n, %	38 (4.6)	205 (6.5)	179 (5.9)	23 (1.5)
HbA1c (%)†	5.5±0.7 5.1/5.4/5.8	5.4±0.5 5.1/5.3/5.5	5.6±0.6 5.2/5.5/5.7	5.6±0.6 5.4/5.6/5.7
HOMA-IR	4.0±5.3 2.1/3.0/4.3	NA	2.1±8.3 0.6/1.0/1.7	1.8±1.5 0.9/1.4/2.1
Fasting glucose (mg/dL)	102±24 91/97/107	NA	98±19 88/94/102	93±18 85/91/98
Fasting insulin (µlU/ml)	15±13 9/12/17	NA	9±34 3/4/7	7±5 4/6/9
eGFR (mL/min/1.73m²)	79±15	83±18	84±17	95±12
Hs-CRP (mg/L)	3.4±7.4 1.0/2.0/3.0	NA	2.5±5.3 0.6/1.2/2.6	2.8±6.6 0.8/1.5/2.9

Values are provided as mean and standard deviation and 25th, 50th and 75th percentile where appropriate and in case of non-normal distribution as not indicated otherwise or number, % (=valid percent considering missing values). To convert mg/dL in mmol/L multiply by 0.0555 for glucose, 0.0259 for cholesterol and 0.0113 for triglycerides. To convert µlU/ml in pmol/L for insulin, multiply by 7.175. * Participants (92.3%) non-fasting; † To convert % to mmol/mol the following formula is used: New (mmol/mol) = 10.93xOld (%) - 23.5 mmol/mol. Hypertension defined according to the JNC7 Criteria (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, and/or receiving antihypertensive treatment); Lipid lowering drugs includes statin and/or fibrate use; Glomerular filtration rate (eGFR) measured according to the CKD-EPI equation [(26)].

SUPPLEMENTARY DATA

Supplementary Table 1 (continuation): Clinical and laboratory data of participants in those with afamin measurements available in the Bruneck Study (n=826), KORA F3 Study (n=3,158), KORA F4 Study (n=3,059), SAPHIR Study (n=1,499), CoLaus Study (n=4,773), NPHS-II Study (n=2,674), YFS Study (n=2,270), and FamHS Study (n=1,877).

	Study population			
	CoLaus (n=4,773)	**NPHS-II* (n=2,674)**	**YFS (n=2,270)**	**FamHS (n=1,877)**
Age, yrs (minimum-maximum)	58±10 49/57/66 (40–82)	59±3 56/59/62 (50-66)	32±5 27/33/36 (24-39)	52±14 39/53/63 (25-89)
Gender: male/female: n, %	2235/2538 46.8/53.2	2674 (100)	1020/1250 (45/55)	863/1014 46/54
Smoking (Non-smoker/Ex-smoker/Smoker): n, %	1948/1785/1040 40.8/37.4/ 21.8	860/1072/742 32.2/40.1/27.8	1031/402/776 46.7/18.2/35.1	910/492/248 48.5/26.2/13.2
Follow-up time (years)	5.0±0.5 5.0/5.2/5.3	9.2±2.9 8.3/10.0/10.8	9.4±1.4 10.0/10.0/10.0	7.3±0.8 6.7/7.3/7.9
Afamin (mg/L)	73.1±16.6 61.3/71.3/82.8	67.0±15.8 55.9/65.4/76.3	61.4 ± 15.4 50.7/59.0/70.2	65.4±16.3 53.8/63.8/75.3
Body mass index, kg/m²	26.2±4.6	26.6±3.6	25.1±4.4	27.7±5.3
Obesity (BMI ≥30: n, (%)	816 (17.1)	418 (15.8)	276 (12.3)	462 (24.6)
Systolic blood pressure (mmHg)	126±18	134±18	117±13	117±18
Diastolic blood pressure (mmHg)	78±11	82±11	71±11	69±10
Hypertension: n, %	1969 (41.3)	1119 (41.9)	882 (39.3)	557 (29.7)
Antihypertensive medication: n, %	1292 (27.1)	232 (8.7)	51 (2.5)	455 (24.2)
Waist circumference (cm)	92±13	NA	84±12	97±15
Total cholesterol, mg/dL	220±40.1	218±38	200±38	205±39
HDL cholesterol, mg/dL	63±18	66±23	50±12	50±15
LDL cholesterol, mg/dL	133±36	119±39	127±33	125±34
Triglycerides, mg/dL	120±78 71/97/142	186±115 112/155/229	119±76 71/97/142	150±105 84/125/185
Use of lipid lowering drugs: n, %	877 (18.4)	NA	7 (0.3)	163 (8.7)
Type 2 Diabetes: n, %	503 (10.5)	68 (2.5)	17 (0.8)	171 (9.1)
Diabetes medication: n, %	261 (5.5)	26 (1.0)	0 (0)	84 (4.5)
HbA1c (%)†	NA	NA	NA	NA
HOMA-IR	2.5±7.1 1.1/1.6/2.7	NA	1.8±1.5 1.0/1.4/2.1	3.3±7.6 1.4/2.1/3.3
Fasting glucose (mg/dL)	106±20 95/103/110	NA	90±9 85/90/95	100±28 88/94/102
Fasting insulin (µlU/ml)	9±16 4/7/10	NA	8±6 5/6/9	12±15 6/9/14
eGFR (mL/min/1.73m²)	83±15	75±10	114± 6	84±16
Hs-CRP (mg/L)	2.5±3.6 0.7/1.3/2.8	3.9±3.9 1.2/3.5/5.1	1.9±3.9 0.3/0.8/1.9	NA

Values are provided as mean and standard deviation and 25th, 50th and 75th percentile where appropriate and in case of non-normal distribution as not indicated otherwise or number, % (=valid percent considering missing values). To convert mg/dL in mmol/L multiply by 0.0555 for glucose, 0.0259 for cholesterol and 0.0113 for triglycerides. To convert µlU/ml in pmol/L for insulin, multiply by 7.175. * The NPHS-II Study includes only males. † To convert % to mmol/mol the following formula is used: New (mmol/mol) = 10.93xOld (%) - 23.5 mmol/mol. Hypertension defined according to the JNC7 Criteria (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, and/or receiving antihypertensive treatment); Lipid lowering drugs includes statin and/or fibrate use; Glomerular filtration rate (eGFR) measured according to the CKD EPI equation [(26)].

SUPPLEMENTARY DATA

Supplementary Table 2: I^2 index and p value from chi-square based *Q* statistic based on an age- and sex-adjusted model

Outcome	All cohorts		Excluding KORA F3 and NPHS-II	
	I^2 index	p value (*Q* statistic)	I^2 index	p value (*Q* statistic)
Prevalent type 2 diabetes				
Afamin on a continuous scale	63.50	0.008	14.25	0.32
Afamin categorized by quartiles				
Afamin 2nd vs. 1st quartile	34.86	0.16	34.12	0.19
Afamin 3rd vs. 1st quartile	21.62	0.26	0 *	0.50
Afamin 4th vs. 1st quartile	50.13	0.06	0 *	0.41
Incident type 2 diabetes				
Afamin on a continuous scale	0 *	0.55	0 *	0.47
Afamin categorized by quartiles				
Afamin 2nd vs. 1st quartile	35.30	0.15	6.71	0.38
Afamin 3rd vs. 1st quartile	0 *	0.65	0 *	0.50
Afamin 4th vs. 1st quartile	13.89	0.32	21.05	0.28
Continuous type 2 diabetes-related phenotypes				
Ln-HbA1c (%) †	78.36	0.003	NA	NA
Ln-Glucose (mg/dL) ‡	95.21	<0.0001	NA	NA
Ln-Insulin (µlU/ml) ‡	93.57	<0.0001	NA	NA
Ln-HOMA Index ‡	94.43	<0.0001	NA	NA

Ln refers to log-transformation based on the natural logarithm (ln).

* In case of I^2 = 0, the random effects model equals the fixed effects model

† Cohorts included: Bruneck Study, SAPHIR Study, KORA F3 and KORA F4 Study (those without type 2 diabetes diagnosis at baseline). To convert % to mmol/mol the following formula is used: New (mmol/mol) = 10.93xOld (%) - 23.5 mmol/mol.

‡ Includes all cohorts except KORA F3 and NPHS-II (those without type 2 diabetes diagnosis at baseline)

NA, not applicable

SUPPLEMENTARY DATA

Supplementary Table 3: Logistic regression analysis of afamin (increment 10 mg/L) on prevalent and incident type 2 diabetes

Prevalent Type 2 Diabetes (total number): yes = 1,398, no = 18,696					
Study	**Type 2 Diabetes (1 = yes / 0 = no (=ref.)** [*]	**OR (95% CI)** [†]	**P**	**OR (95% CI)** [‡]	**P**
Bruneck Study	(1 = 93 / 0 = 733)	1.68 (1.45-1.95)	$8.47*10^{-12}$	1.42 (1.19-1.68)	$7.26*10^{-5}$
SAPHIR Study	(1 = 41 / 0 = 1,447)	1.54 (1.28-1.84)	$2.51*10^{-6}$	1.25 (1.01-1.55)	0.043
FamHS Study	(1 = 171 / 0 = 1,706)	1.38 (1.25-1.52)	$9.17*10^{-11}$	1.19 (1.06-1.34)	$3.70*10^{-3}$
YFS Study	(1 =17 / 0 = 2,253)	1.41 (1.11-1.78)	$4.42*10^{-3}$	1.18 (0.86-1.62)	0.293
NPHS-II Study	(1 = 68 / 0 = 2,606)	1.26 (1.10-1.44)	$7.19*10^{-4}$	1.06 (0.88-1.28)	0.535
KORA F4 Study	(1 = 245 / 0 = 2,805)	1.40 (1.30-1.51)	$2.60*10^{-19}$	1.23 (1.13-1.34)	$2.18*10^{-6}$
KORA F3 Study	(1 = 260 / 0 = 2,876)	1.25 (1.16-1.34)	$2.24*10^{-9}$	1.05 (0.96-1.15)	0.260
CoLaus Study	(1 = 503 / 0 = 4,270)	1.44 (1.36-1.52)	$3.05*10^{-38}$	1.21 (1.14-1.29)	$1.28*10^{-9}$
Meta-analysis including all studies [§]		**1.40 (1.31-1.48)**	**$2.54*10^{-27}$**	**1.19 (1.12-1.26)**	**$5.96*10^{-08}$**
Meta-analysis excluding KORA F3 & NPHS-II [‖]		**1.44 (1.38-1.50)**	**$5.64*10^{-61}$**	**1.23 (1.17-1.28)**	**$2.62*10^{-20}$**
Incident Type 2 Diabetes (total number): yes = 585, no = 12,762					
Study	**Type 2 Diabetes (1 = yes / 0 = no (=ref.)** [*]	**OR (95% CI)** [†]	**P**	**OR (95% CI)** [‡]	**P**
Bruneck Study	(1 = 52 / 0 = 681)	1.48 (1.21-1.82)	$1.28*10^{-4}$	1.32 (1.04-1.68)	0.025
SAPHIR Study	(1 = 78 / 0 = 1,087)	1.64 (1.41-1.92)	$2.28*10^{-10}$	1.33 (1.11-1.59)	$1.83*10^{-3}$
FamHS Study	(1 = 83 / 0 = 1,036)	1.47 (1.30-1.66)	$1.54*10^{-9}$	1.37 (1.18-1.59)	$3.54*10^{-5}$
YFS Study	(1 = 55 / 0 = 1,900)	1.54 (1.35-1.76)	$1.84*10^{-10}$	1.35 (1.13-1.61)	$9.55*10^{-4}$
NPHS-II Study	(1 = 135 / 0 = 2,391)	1.42 (1.29-1.57)	$1.34*10^{-12}$	1.18 (1.04-1.35)	0.013
KORA F4 Study	(1 = 86 / 0 = 1,925)	1.60 (1.42-1.80)	$7.67*10^{-15}$	1.40 (1.23-1.60)	$5.49*10^{-7}$
KORA F3 Study	(1 = 52 / 0 = 2,459)	1.46 (1.27-1.68)	$9.64*10^{-8}$	1.28 (1.09-1.50)	$2.70*10^{-3}$
CoLaus Study	(1 = 44 / 0 = 1,283)	1.32 (1.12-1.57)	$1.19*10^{-3}$	1.17 (0.96-1.42)	0.124
Meta-analysis including all studies [§]		**1.49 (1.42-1.56)**	**$5.97*10^{-62}$**	**1.30 (1.23-1.38)**	**$3.53*10^{-19}$**
Meta-analysis excluding KORA F3 & NPHS-II [‖]		**1.52 (1.43-1.61)**	**$4.52*10^{-45}$**	**1.34 (1.25-1.43)**	**$1.90*10^{-16}$**

[*] Numbers refer to the age- and sex-adjusted model

[†] Adjusted for age and sex;

[‡] Adjusted for age, sex, HDL cholesterol, triglycerides, BMI and hypertension

[§] Meta-analysis beta estimate (recalculated to an odds ratio and corresponding 95% CI) and P-values derived from a random effects model

[‖] Meta-analysis beta estimate (recalculated to an odds ratio and corresponding 95% CI) and P-values derived from a random effects model without KORA F3 and NPHS-II. These two studies did not ask participants to be fasting at their examination.

SUPPLEMENTARY DATA

Supplementary Table 4: Logistic regression analysis of afamin (divided into quartiles) on prevalent type 2 diabetes

Study [*]	Type 2 Diabetes (1 = yes / 0 = no (=ref.) [†]	OR (95% CI) [‡]	P	OR (95% CI) [§]	P
Bruneck Study	1st quartile (1 = 12 / 0 = 194)	Reference			
Bruneck Study	2nd quartile (1 = 13 / 0 = 195)	1.19 (0.52-2.72)	0.684	1.07 (0.46-2.52)	0.869
Bruneck Study	3rd quartile (1 = 23 / 0 = 183)	2.31 (1.09-4.88)	0.029	1.41 (0.62-3.19)	0.407
Bruneck Study	4th quartile (1 = 45 / 0 = 161)	5.29 (2.65-10.58)	$2.42*10^{-6}$	2.25 (1.02-5.00)	0.045
SAPHIR Study	1st quartile (1 = 4 / 0 = 371)	Reference			
SAPHIR Study	2nd quartile (1 = 3 / 0 = 369)	0.70 (0.15-3.16)	0.642	0.50 (0.11-2.33)	0.375
SAPHIR Study	3rd quartile (1 = 8 / 0 = 364)	1.71 (0.51-5.76)	0.388	0.98 (0.27-3.47)	0.970
SAPHIR Study	4th quartile (1 = 26 / 0 = 343)	6.01 (2.06-17.50)	$1.01*10^{-3}$	1.96 (0.60-6.41)	0.266
FamHS Study	1st quartile (1 = 22 / 0 = 447)	Reference			
FamHS Study	2nd quartile (1 = 22 / 0 = 447)	0.74 (0.39-1.41)	0.355	0.45 (0.22-0.90)	0.023
FamHS Study	3rd quartile (1 = 42 / 0 = 427)	1.38 (0.81-2.37)	0.238	0.87 (0.46-1.64)	0.670
FamHS Study	4th quartile (1 = 85 / 0 = 385)	3.11 (1.93-5.01)	$3.16*10^{-6}$	1.37 (0.75-2.50)	0.310
NPHS-II Study	1st quartile (1 = 12 / 0 = 657)	Reference			
NPHS-II Study	2nd quartile (1 = 8 / 0 = 660)	0.66 (0.27-1.62)	0.361	0.40 (0.15-1.08)	0.071
NPHS-II Study	3rd quartile (1 = 14 / 0 = 655)	1.18 (0.54-2.58)	0.674	0.40 (0.15-1.06)	0.066
NPHS-II Study	4th quartile (1 = 34 / 0 = 634)	2.93 (1.50-5.71)	$2.00*10^{-3}$	1.15 (0.51-2.59)	0.727
KORA F4 Study	1st quartile (1 = 25 / 0 = 736)	Reference			
KORA F4 Study	2nd quartile (1 = 34 / 0 = 728)	1.18 (0.69-2.04)	0.541	0.94 (0.53-1.66)	0.832
KORA F4 Study	3rd quartile (1 = 56 / 0 = 707)	1.88 (1.14-3.09)	0.013	1.13 (0.66-1.94)	0.651
KORA F4 Study	4th quartile (1 = 130 / 0 = 634)	4.53 (2.87-7.15)	$9.17*10^{-11}$	2.25 (1.34-3.77)	$2.05*10^{-3}$
KORA F3 Study	1st quartile (1 = 46 / 0 = 739)	Reference			
KORA F3 Study	2nd quartile (1 = 41 / 0 = 745)	0.94 (0.60-1.47)	0.786	0.76 (0.47-1.22)	0.249
KORA F3 Study	3rd quartile (1 = 64 / 0 = 721)	1.32 (0.88-1.98)	0.182	0.78 (0.49-1.21)	0.265
KORA F3 Study	4th quartile (1 = 109 / 0 = 671)	2.57 (1.76-3.74)	$8.71*10^{-7}$	1.07 (0.69-1.68)	0.756
CoLaus Study	1st quartile (1 = 48 / 0 = 1,146)	Reference			
CoLaus Study	2nd quartile (1 = 85 / 0 = 1,108)	1.74 (1.20-2.53)	$3.64*10^{-3}$	1.38 (0.94-2.04)	0.101
CoLaus Study	3rd quartile (1 = 119 / 0 = 1,074)	2.45 (1.71-3.50)	$7.42*10^{-7}$	1.59 (1.09-2.31)	0.016
CoLaus Study	4th quartile (1 = 251 / 0 = 942)	5.45 (3.92-7.58)	$7.50*10^{-24}$	2.46 (1.71-3.52)	$9.58*10^{-7}$
Meta-analysis including all studies [‖]					
	1st quartile (1 = 169 / 0 = 4,290)	Reference			
	2nd quartile (1 = 206 / 0 = 4,252)	**1.09 (0.81-1.46)**	**0.572**	**0.80 (0.56-1.16)**	**0.238**
	3rd quartile (1 = 326 / 0 = 4,131)	**1.74 (1.38-2.20)**	**$3.47*10^{-6}$**	**1.02 (0.74-1.40)**	**0.917**
	4th quartile (1 = 680 / 0 = 3,770)	**3.91 (2.97-5.14)**	**$2.10*10^{-22}$**	**1.72 (1.27-2.33)**	**$5.09*10^{-4}$**
Meta-analysis excluding KORA F3 & NPHS-II [¶]					
	1st quartile (1 = 111 / 0 = 2,894)	Reference			
	2nd quartile (1 = 157 / 0 = 2,847)	**1.21 (0.85-1.72)**	**0.296**	**0.89 (0.56-1.40)**	**0.609**
	3rd quartile (1 = 248 / 0 = 2,755)	**2.03 (1.60-2.57)**	**$4.82*10^{-9}$**	**1.29 (1.00-1.67)**	**0.052**
	4th quartile (1 = 537 / 0 = 2,465)	**4.66 (3.75-5.79)**	**$3.54*10^{-44}$**	**2.15 (1.68-2.74)**	**$1.14*10^{-9}$**

[*] The YFS Study is not included in these analyses due to low numbers of cases.

[†] Numbers refer to the age- and sex-adjusted model

[‡] Adjusted for age and sex

[§] Adjusted for age, sex, HDL cholesterol, triglycerides, body mass index and hypertension

[‖] Meta-analysis beta estimate (recalculated to an odds ratio and corresponding 95% CI) and P-values derived from a random effects model

[¶] Meta-analysis beta estimate (recalculated to an odds ratio and corresponding 95% CI) and P-values derived from a random effects model without KORA F3 and NPHS-II. These two studies did not ask participants to be fasting at their examination.

SUPPLEMENTARY DATA

Supplementary Table 5: Logistic regression analysis of afamin (divided into quartiles) on incident type 2 diabetes

Study	Type 2 Diabetes (1=yes / 0=no (=ref.) *	OR (95% CI) †	P	OR (95% CI) ‡	P
Bruneck Study	1st quartile (1 = 6 / 0 = 188)	Reference			
Bruneck Study	2nd quartile (1 = 10 / 0 = 185)	1.69 (0.60-4.76)	0.320	1.55 (0.54-4.40)	0.412
Bruneck Study	3rd quartile (1 = 14 / 0 = 169)	2.59 (0.97-6.91)	0.057	1.96 (0.70-5.49)	0.202
Bruneck Study	4th quartile (1 = 22 / 0 = 139)	4.96 (1.96-12.57)	0.001	3.14 (1.11-8.89)	0.031
SAPHIR Study	1st quartile (1 = 10 / 0 = 297)	Reference			
SAPHIR Study	2nd quartile (1 = 10 / 0 = 285)	0.97 (0.40-2.37)	0.942	0.61 (0.24-1.54)	0.294
SAPHIR Study	3rd quartile (1 = 17 / 0 = 282)	1.62 (0.72-3.62)	0.240	0.69 (0.29-1.64)	0.396
SAPHIR Study	4th quartile (1 = 41 / 0 = 223)	5.05 (2.46-10.35)	$9.71*10^{-6}$	1.61 (0.72-3.61)	0.250
FamHS Study	1st quartile (1 = 9 / 0 = 280)	Reference			
FamHS Study	2nd quartile (1 = 13 / 0 = 269)	1.28 (0.53-3.10)	0.591	1.03 (0.42-2.50)	0.955
FamHS Study	3rd quartile (1 = 23 / 0 = 268)	2.13 (0.98-4.64)	0.056	1.46 (0.63-3.40)	0.383
FamHS Study	4th quartile (1 = 38 / 0 = 219)	4.08 (1.93-8.63)	$2.00*10^{-4}$	2.49 (1.08-5.79)	0.033
YFS Study	1st quartile (1 = 4 / 0 = 486)	Reference			
YFS Study	2nd quartile (1 = 10 / 0 = 478)	2.69 (0.84-8.65)	0.097	2.10 (0.64-6.90)	0.220
YFS Study	3rd quartile (1 = 9 / 0 = 479)	2.44 (0.74-7.99)	0.141	1.39 (0.41-4.75)	0.600
YFS Study	4th quartile (1 = 32 / 0 = 457)	8.89 (3.11-25.44)	$4.57*10^{-5}$	2.84 (0.87-9.22)	0.083
NPHS-II Study	1st quartile (1 = 17 / 0 = 611)	Reference			
NPHS-II Study	2nd quartile (1 = 10 / 0 = 634)	0.57 (0.26-1.25)	0.157	0.42 (0.18-0.96)	0.041
NPHS-II Study	3rd quartile (1 = 44 / 0 = 593)	2.68 (1.51-4.74)	$7.18*10^{-4}$	1.24 (0.65-2.38)	0.509
NPHS-II Study	4th quartile (1 = 64 / 0 = 553)	4.15 (2.40-7.18)	$3.40*10^{-7}$	1.46 (0.77-2.78)	0.250
KORA F4 Study	1st quartile (1 = 3 / 0 = 545)	Reference			
KORA F4 Study	2nd quartile (1 = 11 / 0 = 507)	3.45 (0.95-12.48)	0.059	2.78 (0.76-10.16)	0.122
KORA F4 Study	3rd quartile (1 = 24 / 0 = 487)	7.40 (2.21-24.84)	$1.12*10^{-3}$	4.72 (1.37-16.29)	0.014
KORA F4 Study	4th quartile (1 = 48 / 0 = 386)	17.05 (5.25-55.37)	$2.37*10^{-6}$	8.31 (2.44-28.28)	$7.09*10^{-4}$
KORA F3 Study	1st quartile (1 = 3 / 0 = 654)	Reference			
KORA F3 Study	2nd quartile (1 = 8 / 0 = 648)	2.66 (0.70-10.08)	0.150	1.70 (0.43-6.72)	0.447
KORA F3 Study	3rd quartile (1 = 13 / 0 = 608)	4.20 (1.19-14.85)	0.026	2.44 (0.67-8.82)	0.176
KORA F3 Study	4th quartile (1 = 28 / 0 = 549)	9.95 (3.00-32.99)	$1.71*10^{-4}$	4.01 (1.13-14.28)	0.032
CoLaus Study	1st quartile (1 = 7 / 0 = 352)	Reference			
CoLaus Study	2nd quartile (1 = 5 / 0 = 376)	0.69 (0.22-2.19)	0.526	0.52 (0.16-1.68)	0.273
CoLaus Study	3rd quartile (1 = 14 / 0 = 296)	2.29 (0.91-5.78)	0.079	1.54 (0.58-4.07)	0.381
CoLaus Study	4th quartile (1 = 18 / 0 = 259)	3.27 (1.34-7.99)	0.009	1.69 (0.63-4.52)	0.295
Meta-analysis including all studies §					
	1st quartile (1 = 59 / 0 = 3,413)	Reference			
	2nd quartile (1 = 77 / 0 = 3,382)	**1.31 (0.83-2.06)**	**0.251**	**1.00 (0.62-1.62)**	**0.996**
	3rd quartile (1 = 158 / 0 = 3,182)	**2.56 (1.88-3.49)**	$\mathbf{2.25*10^{-9}}$	**1.47 (1.04-2.08)**	**0.030**
	4th quartile (1 = 291 / 0 = 2,785)	**5.28 (3.83-7.27)**	$\mathbf{2.64*10^{-24}}$	**2.33 (1.61-3.36)**	$\mathbf{6.66*10^{-6}}$
Meta-analysis excluding KORA F3 & NPHS-II ‖					
	1st quartile (1 = 39 / 0 = 2,148)	Reference			
	2nd quartile (1 = 59 / 0 = 2,100)	**1.43 (0.92-2.22)**	**0.112**	**1.12 (0.68-1.86)**	**0.652**
	3rd quartile (1 = 101 / 0 = 1,981)	**2.40 (1.64-3.52)**	$\mathbf{7.47*10^{-6}}$	**1.51 (0.94-2.44)**	**0.087**
	4th quartile (1 = 199 / 0 = 1,683)	**5.46 (3.63-8.22)**	$\mathbf{4.07*10^{-16}}$	**2.54 (1.66-3.89)**	$\mathbf{9.68*10^{-6}}$

* Numbers refer to the age and sex adjusted model

† Adjusted for age and sex; ‡ Adjusted for age, sex, HDL cholesterol, triglycerides, body mass index and hypertension

§ Meta-analysis beta estimate (recalculated to an odds ratio and corresponding 95% CI) and P-values derived from a random effects model; ‖ Meta-analysis beta estimate (recalculated to an odds ratio and corresponding 95% CI) and P-values derived from a random effects model without KORA F3 and NPHS-II. These two studies did not ask participants to be fasting at their examination.

SUPPLEMENTARY DATA

Supplementary Table 6: Linear regression analysis 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	
Parameter / Study (n individuals)	**ß (95% CI) [*]**	**P**	**ß (95% CI) [†]**	**P**
Ln-HbA1c				
Bruneck Study (n=733)	0.006 (0.002-0.010)	$4.50*10^{-3}$	0.003 (-0.001-0.008)	0.166
SAPHIR Study (n=1,425)	0.007 (0.004-0.009)	$3.92*10^{-9}$	0.004 (0.002-0.007)	$1.65*10^{-3}$
KORA F3 Study (n=2,869)	0.005 (0.003-0.006)	$3.80*10^{-13}$	0.002 (0.0003-0.003)	0.018
KORA F4 Study (n=2,801)	0.008 (0.007-0.009)	$1.01*10^{-31}$	0.005 (0.003-0.006)	$8.37*10^{-10}$
CoLaus Study	---	---	---	---
NPHS-II Study	---	---	---	---
YFS Study	---	---	---	---
FamHS Study	---	---	---	---
Meta-analysis [‡]	**0.006 (0.004-0.008)**	**$4.41*10^{-10}$**	**0.003 (0.002-0.005)**	**$3.09*10^{-4}$**
Ln-HOMA Index				
Bruneck Study (n=733)	0.144 (0.121-0.168)	$1.46*10^{-33}$	0.082 (0.056-0.109)	$1.11*10^{-9}$
SAPHIR Study (n=1,441)	0.249 (0.231-0.268)	$3.90*10^{-157}$	0.152 (0.134-0.171)	$6.37*10^{-59}$
FamHS Study (n=1,706)	0.171 (0.151-0.190)	$1.37*10^{-64}$	0.104 (0.084-0.124)	$3.40*10^{-25}$
YFS Study (n=2,252)	0.159 (0.145-0.173)	$1.32*10^{-97}$	0.079 (0.064-0.093)	$8.66*10^{-26}$
KORA F4 Study (n=2,751)	0.222 (0.202-0.243)	$1.71*10^{-101}$	0.134 (0.112-0.156)	$7.21*10^{-33}$
CoLaus Study (n=4,270)	0.176 (0.165-0.187)	$8.77*10^{-210}$	0.111 (0.100-0.122)	$1.05*10^{-91}$
KORA F3 Study	---	---	---	---
NPHS-II Study	---	---	---	---
Meta-analysis [‡]	**0.187 (0.158-0.216)**	**$3.00*10^{-36}$**	**0.110 (0.089-0.132)**	**$1.37*10^{-23}$**
Ln-Insulin				
Bruneck Study (n=733)	0.129 (0.106-0.151)	$8.82*10^{-30}$	0.071 (0.046-0.097)	$2.74*10^{-8}$
SAPHIR Study (n=1,441)	0.228 (0.211-0.245)	$2.77*10^{-150}$	0.136 (0.119-0.154)	$1.35*10^{-54}$
KORA F3 Study	---	---	---	---
KORA F4 Study (n=2,754)	0.203 (0.183-0.222)	$3.34*10^{-90}$	0.121 (0.100-0.143)	$9.44*10^{-29}$
CoLaus Study (n=4,270)	0.164 (0.153-0.174)	$2.51*10^{-202}$	0.104 (0.094-0.115)	$2.47*10^{-89}$
NPHS-II Study	---	---	---	---
YFS Study (n=2,252)	0.153 (0.140-0.166)	$1.81*10^{-102}$	0.077 (0.063-0.090)	$1.28*10^{-27}$
FamHS Study (n=1,706)	0.155 (0.136-0.174)	$4.29*10^{-59}$	0.094 (0.075-0.113)	$1.67*10^{-22}$
Meta-analysis [‡]	**0.172 (0.146-0.198)**	**$3.32*10^{-39}$**	**0.101 (0.083-0.120)**	**$1.51*10^{-26}$**
Ln-Glucose				
Bruneck Study (n=733)	0.016 (0.011-0.021)	$7.21*10^{-10}$	0.011 (0.005-0.017)	$3.38*10^{-4}$
SAPHIR Study (n=1,442)	0.022 (0.018-0.025)	$2.39*10^{-31}$	0.016 (0.012-0.020)	$1.84*10^{-13}$
KORA F3 Study	---	---	---	---
KORA F4 Study (n=2,779)	0.020 (0.017-0.022)	$6.04*10^{-68}$	0.012 (0.010-0.015)	$1.01*10^{-21}$
CoLaus Study (n=4,270)	0.012 (0.011-0.014)	$3.08*10^{-49}$	0.007 (0.005-0.009)	$3.73*10^{-14}$
NPHS-II Study	---	---	---	---
YFS Study (n=2,253)	0.006 (0.004-0.008)	$2.64*10^{-07}$	0.002 (-0.0004-0.005)	0.108
FamHS Study (n=1,706)	0.015 (0.012-0.018)	$8.30*10^{-24}$	0.010 (0.006-0.013)	$8.90*10^{-9}$
Meta-analysis [‡]	**0.015 (0.010-0.020)**	**$4.68*10^{-10}$**	**0.009 (0.006-0.013)**	**$7.48*10^{-7}$**

N refer to the age and sex adjusted model

[*] Adjusted for age and sex

[†] Adjusted for age, sex, HDL cholesterol, triglycerides, body mass index and hypertension

[‡] Meta-analysis beta estimate, 95% CI and P-values derived from a random effects model

SUPPLEMENTARY DATA

Supplementary Table 7: Logistic regression analysis of afamin (increment 10 mg/L) on incident type 2 diabetes in 6 out of 8 cohorts additionally including glucose concentrations.

Study	Type 2 Diabetes (1 = yes / 0 = no (=ref.)	OR (95% CI) *	P	OR (95% CI) †	P
Bruneck Study	(1=52 / 0=681)	1.26 (0.98-1.62)	0.069	1.21 (0.94-1.56)	0.144
YFS Study	(1=55 / 0=1,837)	1.34 (1.11-1.61)	0.002	1.31 (1.08-1.58)	0.005
FamHS Study	(1=79 / 0=978)	1.28 (0.09-1.50)	0.002	1.21 (1.03-1.42)	0.021
KORA F4 Study	(1=71 / 0=1,900)	1.35 (1.17-1.57)	$6.19*10^{-5}$	1.27 (1.09-1.49)	0.003
CoLaus Study	(1=44 / 0=1,283)	1.15 (0.94-1.40)	0.166	1.07 (0.87-1.31)	0.531
SAPHIR Study	(1=72 / 0=1,075)	1.14 (0.93-1.41)	0.201	1.09 (0.89-1.35)	0.398
Meta-analysis	(1=373 / 0=7,754)	1.27 (1.18-1.36)	$5.09*10^{-10}$	1.21 (1.11-1.30)	$2.87*10^{-6}$

* Adjusted for age, sex, HDL cholesterol, triglycerides, BMI, hypertension and glucose concentrations ≥100 mg/dL (100-125 mg/dL vs. <100 mg/dL = reference)

† Adjusted for age, sex, HDL cholesterol, triglycerides, BMI, hypertension and logarithmized glucose concentrations

SUPPLEMENTARY DATA

Supplementary Table 8: 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. 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) and family history of diabetes.

Individuals with incident type 2 diabetes (n=107)				
		Baseline model plus afamin		
Baseline model	Total	<5% risk	5-24% risk	>=25% risk
<5% risk	14	10 (71.4)	4 (28.6) *	0 (0.0) *
5-24% risk	62	2 (3.2) †	46 (74.1)	14 (22.6) *
>=25% risk	31	0 (0.0) †	3 (9.7) †	28 (90.3)
Total	107	12	53	42

* Moved to higher risk which is correctly reclassified (light gray), n =18; † Moved to lower risk which is wrongly reclassified (dark gray), n =5; stayed in the same risk category (medium grey), n=84; **NRI 0.121 (95%CI 0.037-0.206), p=0.005.**

Individuals without incident type 2 diabetes (n=1,563)				
		Baseline model plus afamin		
Baseline model	Total	<5% risk	5-24% risk	>=25% risk
<5% risk	1,137	1,097 (96.5)	39 (3.4) †	1 (0.09) †
5-24% risk	355	61 (17.2) *	272 (76.6)	22 (6.2) †
>=25% risk	71	0 (0.0) *	20 (28.2) *	51 (71.8)
Total	1,563	1,158	331	74

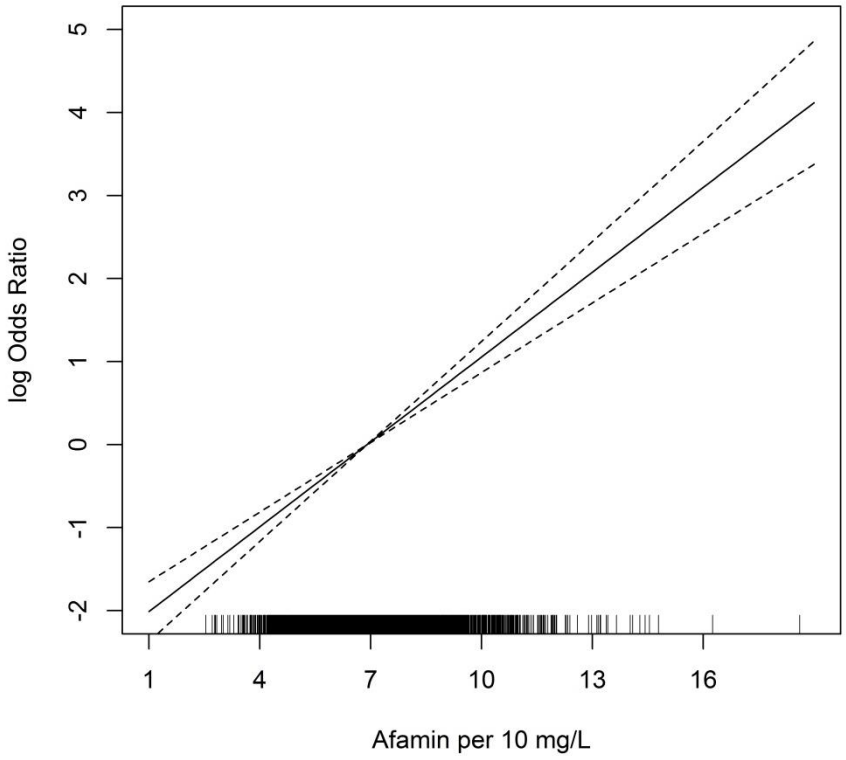
* Moved to lower risk category which is correctly reclassified (light gray), n =81; † Moved to higher risk category which is wrongly reclassified (dark gray), n=62; stayed in the same risk category (medium grey); n = 1,420; **NRI 0.012 (95%CI -0.003-0.027), p=0.115.**

Values are presented as n (row percent).

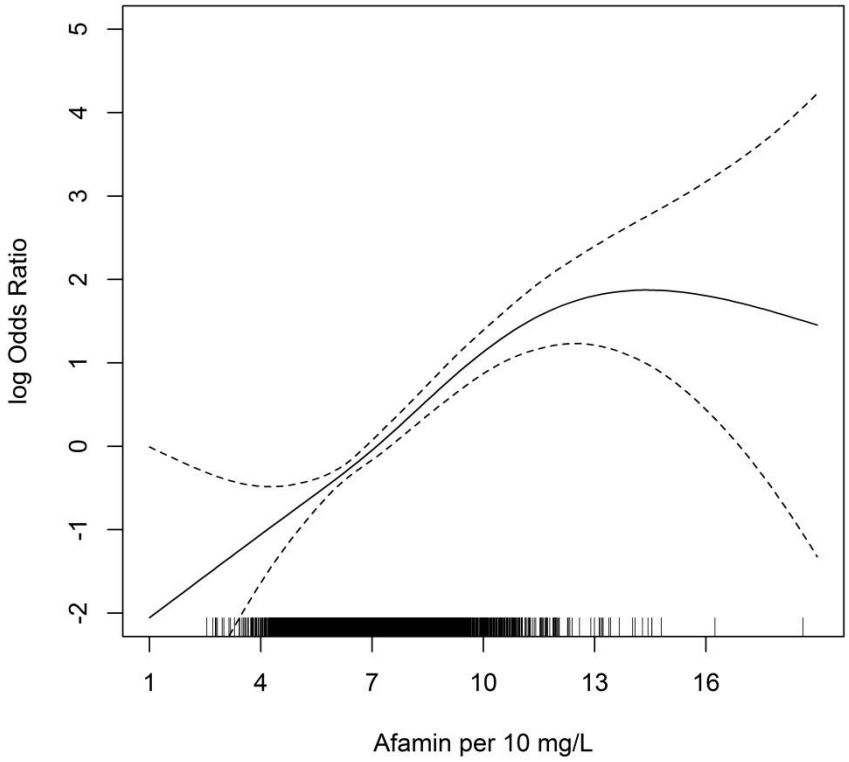
Categorical net reclassification improvement (NRI) in this table is calculated for 107 individuals with and for 1,563 individuals without type 2 diabetes. **Overall NRI for the total group: 0.134 (95%CI 0.044-0.223), p=0.003.**

SUPPLEMENTARY DATA

Supplementary Figure 1: Nonlinear P-Spline for afamin concentrations (per 10 mg/L) on prediabetes in the age and sex-adjusted logistic regression model in KORA F4. The dashed lines correspond to 95% confidence bands.

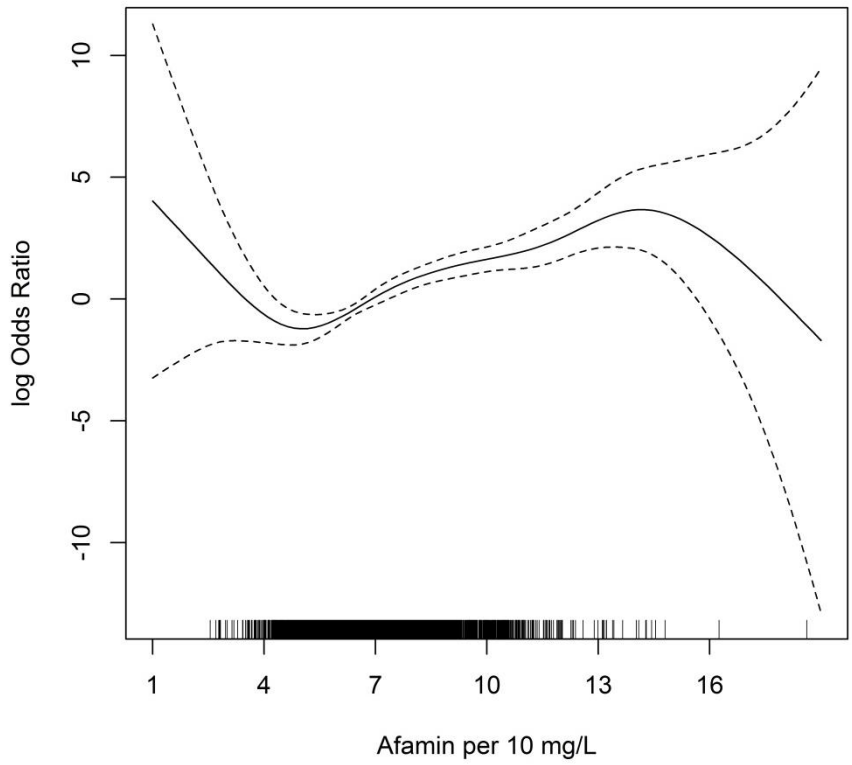


Supplementary Figure 2: Nonlinear P-Spline for afamin concentrations (per 10 mg/L) on prevalent type 2 diabetes in the age- and sex-adjusted logistic regression model in KORA F4. The dashed lines correspond to 95% confidence bands.

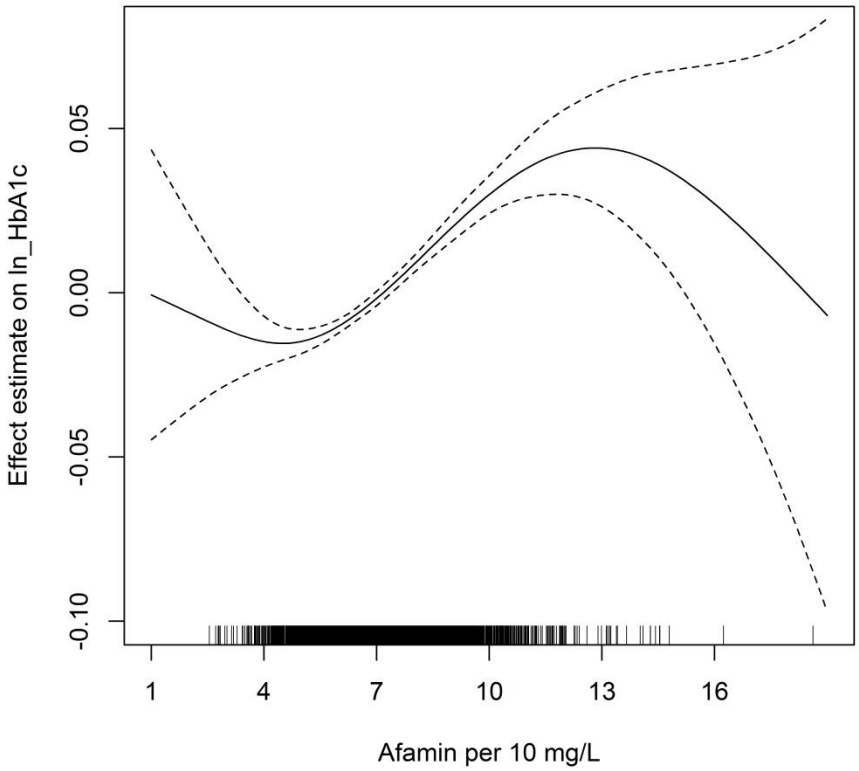


SUPPLEMENTARY DATA

Supplementary Figure 3: Nonlinear P-Spline for afamin concentrations (per 10 mg/L) on incident type 2 diabetes in the age- and sex-adjusted logistic regression model in KORA F4. The dashed lines correspond to 95% confidence bands.

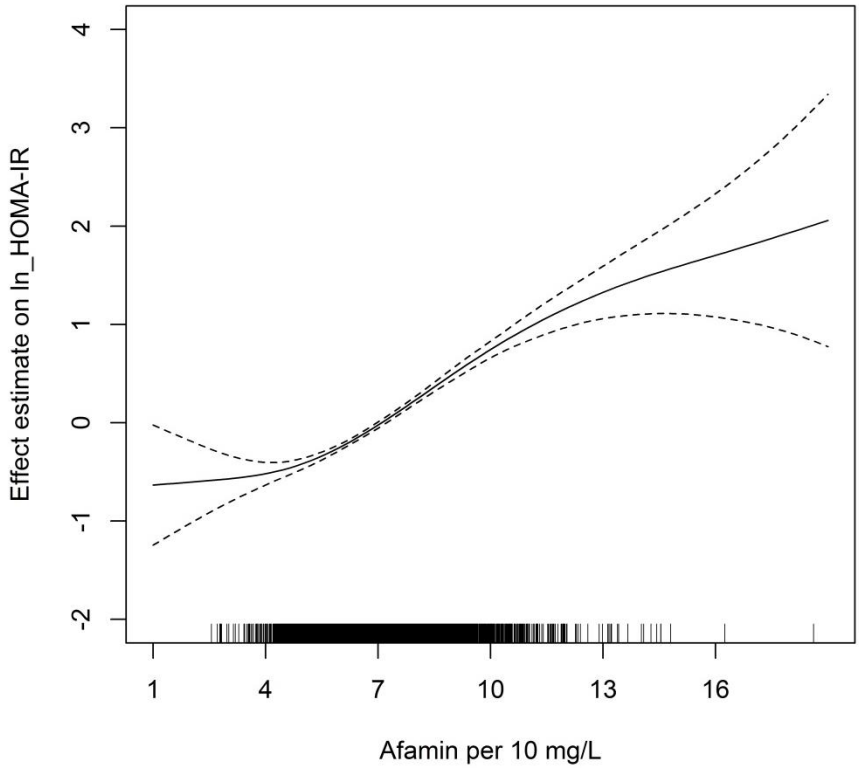


Supplementary Figure 4: Nonlinear P-Spline for afamin concentrations (per 10 mg/L) on logarithmized HbA1c in the age- and sex-adjusted linear regression model in KORA F4 in those without type 2 diabetes at baseline. The dashed lines correspond to 95% confidence bands.

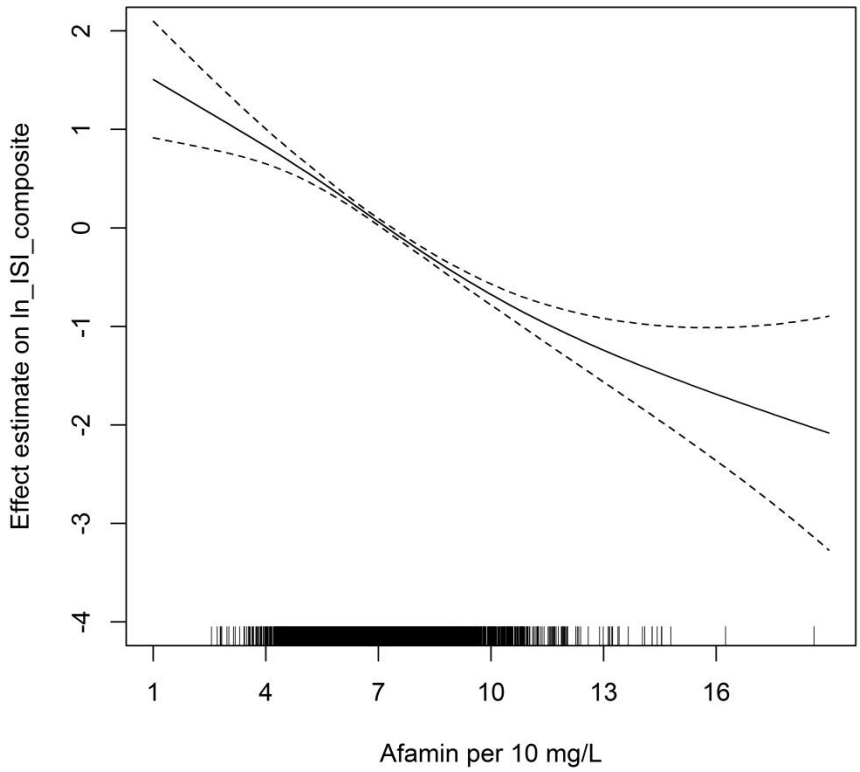


SUPPLEMENTARY DATA

Supplementary Figure 5: Nonlinear P-Spline for afamin concentrations (per 10 mg/L) on logarithmized HOMA-IR in the age- and sex-adjusted linear regression model in KORA F4 in those without type 2 diabetes at baseline. The dashed lines correspond to 95% confidence bands

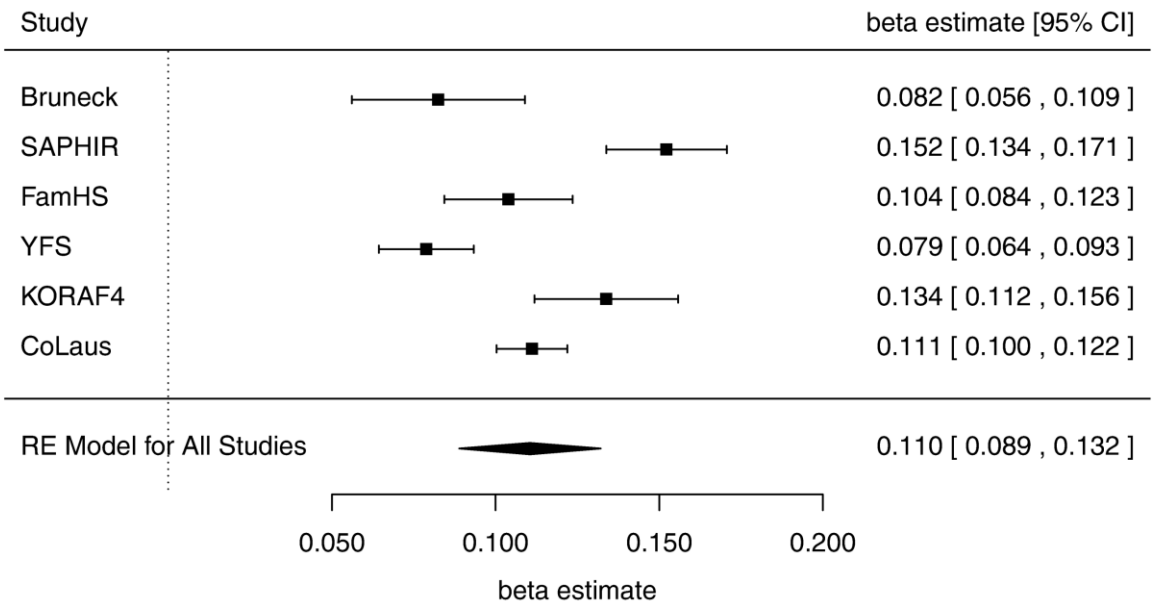


Supplementary Figure 6: Nonlinear P-Spline for afamin concentrations (per 10 mg/L) on logarithmized whole-body ISI(composite) in the age- and sex-adjusted linear regression model in KORA F4 in those without type 2 diabetes at baseline. The dashed lines correspond to 95% confidence bands



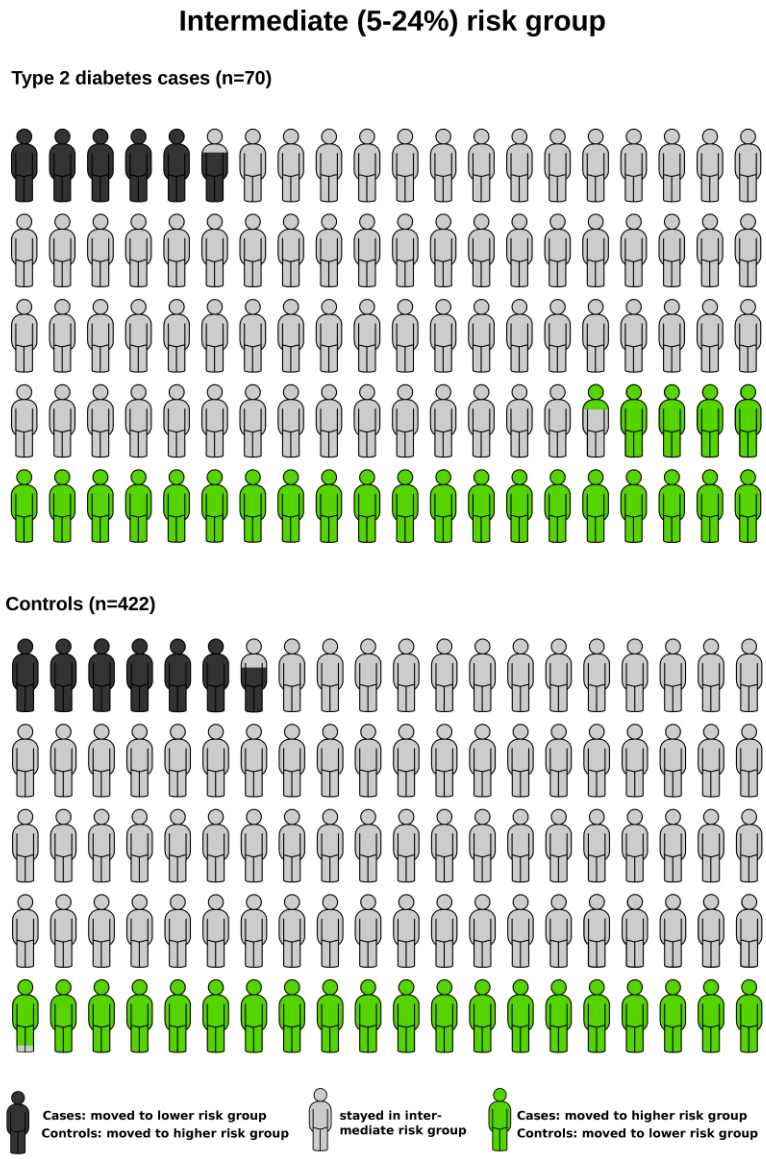
SUPPLEMENTARY DATA

Supplementary Figure 7: Forest plot illustrating the association of afamin (increment 10 mg/L) with logarithmized insulin resistance index (HOMA-IR) (extended adjustment model), based on a random effects (RE) model for all 6 studies with available HOMA-IR measurements. Beta estimates and 95% confidence intervals are shown for each study and the pooled analysis.



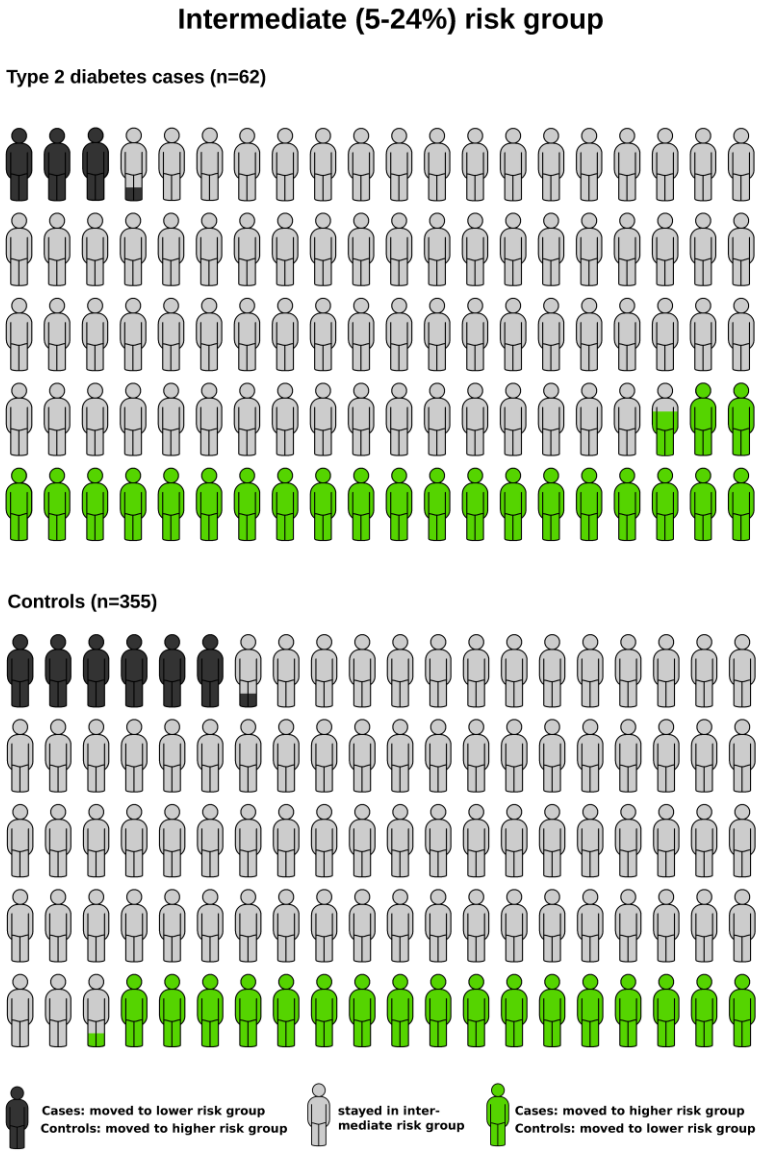
SUPPLEMENTARY DATA

Supplementary Figure 8: Reclassification of individuals (70 cases with type 2 diabetes and 422 controls) predicted to be at intermediate risk (5-24%) for the development of type 2 diabetes during follow-up (median 6.4 years) based on an additional inclusion of afamin concentrations in the KORA F4 extended risk model as compared to a risk model including age, sex and major metabolic risk factors or parameters (HDL cholesterol, triglycerides, BMI, hypertension, and fasting plasma glucose concentrations ≥100 mg/dL (100-125 mg/dL vs. <100 mg/dL=reference)). Adding afamin to the risk model resulted in a reclassification of 30.0% of patients and 26.5% of controls. Proportions are shown for 1) type 2 diabetes cases (70.0%) and controls (73.5%) that stayed in the intermediate risk group (illustrated in grey), and 2) type 2 diabetes cases that were correctly reclassified and thus moved to a higher risk category (24.3%) and controls that moved to a lower risk category (19.9%), respectively (illustrated in green) and 3) type 2 diabetes cases that were wrongly reclassified and thus moved to a lower risk category (5.7%) and controls that moved to a higher risk category (6.6%), respectively (illustrated in black).



SUPPLEMENTARY DATA

Supplementary Figure 9: Reclassification of individuals (62 cases with type 2 diabetes and 355 controls) predicted to be at intermediate risk (5-24%) for the development of type 2 diabetes during follow-up (median 6.4 years) based on an additional inclusion of afamin concentrations in the KORA F4 extended risk model as compared to a risk model including age, sex and major metabolic risk factors or parameters (HDL cholesterol, triglycerides, BMI, hypertension, plasma glucose concentrations ≥100 mg/dL (100-125 mg/dL vs. <100 mg/dL=reference) and family history of diabetes). Adding afamin to the risk model resulted in a reclassification of 25.8% of patients and 23.4% of controls. Proportions are shown for 1) type 2 diabetes cases (74.1%) and controls (76.6%) that stayed in the intermediate risk group (illustrated in grey), and 2) type 2 diabetes cases that were correctly reclassified and thus moved to a higher risk group (22.6%) and controls that moved to a lower risk group (17.2%), respectively (illustrated in green) and 3) type 2 diabetes cases that were wrongly reclassified and thus moved to a lower risk group (3.2%) and controls that moved to a higher risk group (6.2%), respectively (illustrated in black).



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