Accepted Preprint first posted on 8 February 2018 as Manuscript EJE-17-1053

Association of fetuin-A with incident type 2 diabetes: Results from the MONICA/KORA Augsburg study and a systematic meta-analysis

Short title: Fetuin-A with incident type 2 diabetes

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Keywords: type 2 diabetes; fetuin-A; alpha-2 Heremans Schmid glycoprotein; association; prospective; case-cohort study; meta-analysis

Word counts:

Abstract: 244 words

Main Text: 4,970 words (incl. title page, funding, author contributions and references)

Abstract

Objective We investigated the association of circulating fetuin-A with incident T2D particularly examining potential sex differences. Additionally, we determined whether putative associations were independent of subclinical inflammation, adiponectin and liver fat content.

Design Case-cohort study plus systematic meta-analysis.

Methods We investigated the association between baseline fetuin-A levels and incident T2D in the MONICA/KORA Augsburg study using Cox proportional hazards analyses. Furthermore, we conducted a systematic review within PUBMED and EMBASE and pooled association estimates of eligible studies with the MONICA/KORA Augsburg data using a DerSimonian-Laird random effects model.

Results Within MONICA/KORA Augsburg, 930 participants developed incident T2D (median follow-up: 14 years). We observed a significant association between fetuin-A and T2D risk after multivariable adjustment including C-reactive protein and adiponectin. The strength of the association was similar in males and females (*P*-value for sex interaction >0.55). Seven eligible published studies were identified in addition to the MONICA/KORA Augsburg study for the meta-analysis. The pooled hazard ratio (95% CI) for incident T2D per 1 standard deviation (SD) increment of fetuin-A was 1.24 (1.14–1.34) for the multivariable adjusted model. Our sex-stratified meta-analysis yielded relative risk estimates per 1 SD of 1.19 (1.04–1.38) in males and 1.29 (1.15–1.46) in females. Further individual adjustment for subclinical inflammation, adiponectin and liver fat content had almost no impact on the strength of the association.

Conclusions Higher fetuin-A levels are associated with incident T2D in both males and females independently of subclinical inflammation, adiponectin, and liver fat content.

Introduction

The number of people with diabetes is predicted to increase by nearly 60% from around 400 million in 2015 to over 640 million in 2040[1]. Diabetes is projected to become one of the leading causes of death and type 2 diabetes (T2D) is the most frequent type of diabetes[2]. There are several different mechanisms underlying the pathogenesis of T2D. Understanding its complex pathophysiology is important for management of the disease and the development of prevention strategies[3].

Human fetuin-A or alpha-2 Heremans Schmid glycoprotein is an endogenous glycoprotein secreted by the liver that plays a role in insulin resistance in T2D patients[4] and that is suggested to be a novel risk marker for T2D in non-diabetic populations[5-7]. However, some studies reported significant associations in females only[8,9]. Furthermore, previous studies used varying degrees of adjustment and it is not entirely clear whether observed associations are mediated or confounded by subclinical inflammation[5,8,10], adiponectin[10,11] or liver fat content[5,8,9,11].

The present study aimed to further investigate the association between fetuin-A and incident T2D, particularly examining potential sex differences and addressing the impact of subclinical inflammation, adiponectin and liver fat content on the observed associations. As a first step, we conducted a case-cohort study using unpublished data from the Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) Augsburg/Cooperative Health Research in the Region of Augsburg (KORA) study. As a second step, to assess the association between circulating fetuin-A and risk of T2D with the maximum of statistical power, we performed a systematic review and pooled data from MONICA/KORA Augsburg together with all eligible published studies.

Subjects and methods

The MONICA/KORA Augsburg case-cohort study

Study population

A prospective case-cohort study was conducted within the MONICA/KORA Augsburg study population including three independent cross-sectional population-based surveys, S1–S3, conducted between 1984 and 1995. The MONICA/KORA Augsburg study was designed to estimate the prevalence and distribution of cardiovascular risk factors among 13,427 men and women aged 25–74 years in the region of Augsburg, Southern Germany. The study design has been previously described in detail[12,13]. Informed consent was provided by all study participants, and the ethics committee of the Bavarian Chamber of Physicians approved the study protocol. The study complies with the principles outlined in the Declaration of Helsinki.

In the present MONICA/KORA Augsburg case-cohort study we restricted the source population to those aged 35–74 years (n=10,718 persons) due to the low incidence of T2D under the age of 35 years. The follow-up period was restricted until 2009. After exclusion of 1,187 participants without blood sample, 1 newly identified duplicate, 508 participants with prevalent T2D, 25 persons who developed other types of diabetes, 473 persons without follow-up information for T2D, and 55 persons with an observation time ≤365 days, the final source population comprised 8,469 participants. Out of these, a random sub-cohort of 1,991 persons was selected stratified by sex and survey. A total of 930 incident T2D cases (43.4% were females) were included; 217 of these cases stemmed from the sub-cohort. The study participants were followed for a median of 14.3 years (25^{th} and 75^{th} percentiles were 11.2 and 19.2 years, respectively).

Assessment of T2D risk factors

Sociodemographic, clinical and lifestyle parameters were assessed as previously described in detail[14]. Serum and plasma samples stored at -80°C were used to analyse biomarkers[15,16].

Fetuin-A measurement

Fetuin-A was measured in serum using ultrasensitive molecular counting technology (MCT; Singulex, Alameda, California, USA)[17]. The fetuin-A assay used a recombinant fetuin-A protein (1184-P1-050) from R&D Systems (Minneapolis, Minnesota, USA), a mouse monoclonal anti-human fetuin-A capture antibody (MAB1184) from R&D Systems and a polyclonal goat anti-fetuin-A detection antibody (AF1184; R&D Systems). The intra- and inter-assay coefficients of variation were 10% and 11%, respectively.

Assessment of incident type 2 diabetes

As described previously[13], incidence of T2D was initially assessed from patients' self-report of physician-diagnosed diabetes and the use of antidiabetic medication through written follow-up questionnaires sent to all MONICA/KORA S1-S3 study participants in 1997/1998, in 2002/2003 and in 2008/2009. Additionally, all participants from the first survey were invited to participate in a follow-up examination conducted in 1987/1988. All self-reports of physician-diagnosed diabetes were validated by contacting the treating physician or medical chart review and type of diabetes and the year of diagnosis were assessed.

Statistical analysis

Cox proportional hazards models modified for the case-cohort study design using Barlow's method[18] were used to evaluate the association between fetuin-A and incident T2D. Results are given as hazard ratios (HRs) with 95% confidence intervals (CIs) per sex-specific quartile of fetuin-A. We also evaluated HRs per 1 standard deviation (SD) increment of fetuin-A, considering natural log-transformed fetuin-A as a

continuous variable. Missing information on fetuin-A and other covariates were less than 22% and were imputed using 20-fold multiple imputation by chained equations (MICE)[19], performed using R version 3.2.3[20] and R package mice version 2.25[21]. Additional variation due to imputation was taken into account according to the Rubin's rules for multiple imputation[22].

Cox proportional hazards models were initially adjusted for age, sex and the three surveys (model 1). Then we further adjusted for classical T2D risk factors by adding body mass index, smoking status, alcohol intake, physical activity, high-density lipoprotein level, actual hypertension, and parental history of diabetes (model 2). We further adjusted for daily consumption of coffee, meat and whole grain products (model 3). In addition, CRP, as a marker of subclinical inflammation and adiponectin were separately added to the Cox models (all natural log-transformed) to assess their mediating or confounding effect on the association of fetuin-A and incident T2D (models 4 and 5). In addition to analyses in the total study sample, we examined sex-specific associations and formally tested for sex differences by inclusion of an interaction term between sex and level of fetuin-A.

Apart from multiple imputation, all statistical analyses were carried out with the statistical software SAS® 9.3 edition (SAS Institute Inc., Cary, North Carolina, USA). As far as nothing else is mentioned, we applied a significance level of 0.05 to all analyses.

Systematic meta-analysis

The systematic literature search strategy to retrieve all published eligible studies is reported in the Supplementary Text 1.

We pooled effect estimates with 95% CIs from the eligible studies using the DerSimonian and Laird random effects model assuming true heterogeneity between the reported individual study associations[23,24]. Inverse-variance fixed effects models[25] were calculated for comparison. A significance level of 0.05 was applied. Reported

HRs and odd ratios for incident T2D were assumed to approximate relative risks (RRs). We calculated pooled RR estimates adjusted for as many established risk factors as possible and for age and sex plus minimum number of other covariates only. If only one adjustment model was reported, the reported association was included in the minimum and in the multivariable adjusted model.

To investigate whether there were sex differences in the association between fetuin-A and risk of T2D, studies that reported sex-specific associations were pooled in a subgroup analysis. Further subgroup analyses were carried out by calculating pooled estimates defined by age (<65 years; ≥65 years), ethnicity (mixed; predominantly Caucasian), study size (<100 incident cases; ≥100 incident cases) and study quality using a quality score based on an adapted version of the Newcastle-Ottawa Scale (NOS) for cohort studies[26] (*see Supplementary Text 2*) (i.e. risk of bias: NOS <median score; NOS ≥median score). A sensitivity analysis was performed, excluding studies conducted in specific high-risk populations (such as pre-diabetic populations).

To establish whether the association between fetuin-A and T2D is independent of subclinical inflammation (assessed by CRP, Interleukin-6 (IL-6) and tumor necrosis factor (TNF)-alpha), adiponectin levels and liver fat content (assessed either directly through computed tomography (CT) imaging or indirectly through measurement of liver enzymes), we performed additional analyses including only studies that presented results with adjustment for these factors.

We carried out statistical tests for heterogeneity between study-specific association estimates using Chi-squared Cochran's Q-test for heterogeneity[27], applying a significance level of 0.10. The test examines the null hypothesis that included studies evaluate a common effect[28]. We quantified the heterogeneity with the I-squared statistic which describes the proportion of total variation between individual study estimates due to heterogeneity rather than due to chance or sampling error[29]. The value of I-squared statistic can be interpreted as low (about 25%), moderate (about 50%) and high (about 75%)[28].

To determine whether publication bias is present, funnel plot asymmetry was assessed visually and by Egger's regression test. Our meta-analysis was performed with SAS® 9.4 edition (SAS Institute Inc., Cary, North Carolina, USA).

Results

MONICA/KORA Augsburg case-cohort study

Baseline demographic, lifestyle, and clinical characteristics of the study participants who developed T2D during the follow-up period (cases) and those who remained free of diabetes (non-cases) are reported in Supplementary Table 1.

Risk of incident T2D in the top quartile of fetuin-A was significantly higher than the risk in the bottom quartile after adjustment for age, sex and survey (HR [95%CI]: 1.68 [1.29–2.18]) (model 1, Table 1). This association was slightly attenuated in the model adjusting for established T2D risk factors (1.45 [1.08–1.96]) (model 2). Further adjustment for dietary habits did not influence the association (model 3). The association was similar in models that were individually adjusted for CRP and adiponectin (models 4 and 5). In model 3, an increment per 1 SD of log-transformed fetuin-A was associated with a 15% higher risk of T2D. Results were similar for models 4 and 5.

After sex-stratification elevated concentrations of fetuin-A were significantly associated with an increased risk of T2D among men and women in the models adjusting for age and survey. The HRs were 1.78 (1.24–2.57) in males and 1.57 (1.09–2.28) in females for the fourth quartile compared with the first quartile of fetuin-A (Table 1 model 1). Adjustment for established diabetes risk factors (model 2) hardly influenced the HRs in both sexes and the association between fetuin-A and incident T2D became statistically non-significant (HR females 1.30 [0.83–2.05]). Additional adjustment for dietary habits, CRP and adiponectin influenced the HRs only slightly (models 3-5). Overall, the interaction between fetuin-A and sex was not significant (*P*

for interaction in model 3=0.62), suggesting that the association between fetuin-A and T2D is similar in males and females.

Systematic meta-analysis

We identified 7 eligible published articles reporting the association of fetuin-A and incident T2D and included data from the present analysis of the MONICA/KORA case-cohort study in the meta-analysis (Supplementary Figure 1). Studies reporting only separate estimates for males and females[8,9] were considered as different studies when pooling the estimates. Supplementary Table 2 summarises the characteristics of eligible published articles. The results of quality assessment and rescaling are reported in Supplementary Tables 3 and 4, respectively.

Our meta-analysis included a total of 3,106 cases of T2D during a mean/median follow-up time of 2.7 to 14.3 years. Each SD increment in fetuin-A was associated with a 24% and 28% higher risk of T2D (P<0.001) using multivariable (Figure 1) and minimally (Supplementary Figure 2) adjusted estimates, respectively. There was moderate evidence of heterogeneity when combining study estimates from multivariable adjusted models (P_Q=0.025; I-sq=52.7%). Visual inspection of the funnel plot showed that studies with high and low precision of the estimates were distributed symmetrically around the pooled estimate (Supplementary Figure 3). Thus, no evidence of publication bias was suggested, which was further supported by the nonsignificant Egger's regression test (P=0.369).

To examine the sex-specific association between fetuin-A and incident T2D, we combined our sex-specific results with 4 previous studies reporting female-specific associations and 3 studies reporting male-specific associations. The pooled multivariable adjusted RRs (95% CIs) showed a significant positive association in both males and females. The pooled estimates per 1 SD increment of fetuin-A in males and females were 1.19 (1.04–1.38) and 1.29 (1.15–1.46), respectively (Figure 2).

We further meta-analysed 5 estimates from 4 studies (1 study reported 2 estimates for males and females separately) investigating the effect of inflammation on the associations. The pooled RRs were 1.26 (1.12–1.41)] with and 1.26 (1.13–1.40) without adjustment for inflammation (Figure 3).

Three estimates from 3 studies investigating the effect of adiponectin and 6 estimates from 4 studies (2 studies reporting 2 estimates for males and females separately) investigating the effect of liver fat content were also combined. The associations remained significant after adjustment for adiponectin and liver fat content separately. The pooled RRs were 1.17 (1.05–1.29) and 1.19 (1.01–1.39) with and without adjustment for adiponectin and they were 1.19 (1.08–1.32) and 1.24 (1.10–1.40) with and without adjustment for liver fat content, respectively (Figure 4). There was no evidence of heterogeneity between studies adjusted for adiponectin and liver fat content.

Subgroup analyses were performed to explore the heterogeneity in our meta-analysis (Supplementary Figure 4). Evidence of heterogeneity was no longer significant (*P*_Q=0.275) and I-sq was reduced from 52.7% to 20.3% after excluding studies that enrolled participants with mixed ethnicities other than Caucasian. The heterogeneity was also reduced by almost 10% to 43.0% with *P*_Q being 0.135 for studies including only older participants (≥65 years). We also grouped studies based on the study quality (NOS score) and the number of incident cases comparing those with more *versus* less than 100 incident cases. However, the results did not explain heterogeneity. Because most of the studies have more than 100 incident cases, we additionally compared those with more *versus* less cases than the mean number of incident cases. We found no evidence of heterogeneity (*P*_Q=0.560; I-sq=0%) in the 3 largest studies with more than the mean number of incident cases (310 cases).

We conducted sensitivity analyses to examine the robustness of our observations. First, we excluded a study including only pre-diabetic persons to investigate T2D incidence[30]. The result did not change substantially: the pooled RR

was 1.23 (1.13–1.33) and *P*_Q was 0.020. Second, we excluded the only study which adjusted for IL-6 and TNF-alpha and not for CRP[10] from our pooled analyses adjusted for inflammation. The pooled RR was 1.23 (1.10–1.38) and the evidence of heterogeneity remained significant (*P*_Q=0.022).

Discussion

Summary of findings

In this large case-cohort study, which included Caucasian participants from the region of Augsburg, Southern Germany, higher levels of fetuin-A were significantly associated with an elevated risk of incident T2D. The association tended to be slightly weaker among women where associations became non-significant after adjustment for T2D risk factors, but we did not observe any significant sex-differences. Adjustment for CRP and adiponectin had only a marginal impact on the strength of observed associations. Our own results from the MONICA/KORA Augsburg case-cohort study are largely in line with the results from the present meta-analysis combining data from 7 published studies and the MONICA/KORA Augsburg case-cohort study. In the meta-analysis, higher fetuin-A levels were significantly associated with risk of incident diabetes among both males and females and adjustment for subclinical inflammation, adiponectin or liver fat content did not explain the observed associations.

Comparison of results with other studies

The present case-cohort study is the largest individual study addressing the association between fetuin-A and incident T2D published to date in terms of included number of incident cases (>900). Thus, the present meta-analysis has a considerably larger sample size (more than 3,000 cases of T2D) and consequently a larger power than previous meta-analyses[8,11,31]. Furthermore, in extension to the most recent meta-analysis[31], we calculated pooled sex-specific risk estimates to address the unresolved issue of potential sex differences in the association between fetuin-A and incident T2D[8]

and we performed extensive further subgroup analyses to identify potential sources of heterogeneity. Furthermore, we specifically addressed the mediating/confounding impact of subclinical inflammation, adiponectin and liver fat content.

Overall, our findings support the results from previous meta-analyses indicating a positive association between fetuin-A and incident T2D[8,11,31]. However, while the meta-analysis of Aroner et al. suggested that the risk of T2D associated with fetuin-A is higher among women (effect estimate [95% CI] per 1 SD increment: 1.51 [1.32–1.74][8] than among men (1.12 [0.94–1.32]), we did not observe any significant sex differences.

We found that adjustment for CRP, a biomarker of inflammation, and for adiponectin did not affect the association between fetuin-A and incident T2D in the MONICA/KORA Augsburg case-cohort study. Likewise, in our meta-analysis after combining studies, which separately adjusted for inflammation, adiponectin and liver fat content, we found that fetuin-A remained significantly associated with T2D risk. Our meta-analysis is the first to systematically investigate whether inflammation, adiponectin or liver fat content mediate or confound the association between fetuin-A concentrations and T2D risk. Previous individual studies which addressed the impact of subclinical inflammation[5,8,10], adiponectin[10,11] and liver fat content assessed through CT scans[8] or liver enzymes[5,9,11] observed that these factors hardly attenuated the observed associations.

Heterogeneity in study results

Our meta-analysis revealed moderate evidence of heterogeneity when combining study estimates from multivariable adjusted models (*P*_Q=0.025; I-sq=52.7%). This heterogeneity was not explained by sex. However, further subgroup analysis demonstrated that different ethnic backgrounds and age explain a large proportion of the observed heterogeneity. Heterogeneity was reduced to 20.3% in predominantly Caucasian populations and to 43% in elderly populations. In the meta-analysis of

Roshanzamir et al.[31], heterogeneity was also moderate (*P*_Q=0.10; I-sq=46.1%, but no subgroup analyses were performed due to the limited number of studies.

Mechanisms

Stefan and Häring suggested that fetuin-A promotes insulin resistance by interacting with free fatty acids (FFAs)[32]. Fetuin-A is involved in lipid-induced inflammation by acting as an endogenous ligand between FFAs and Toll-like receptor 4 in adipocytes resulting in insulin resistance[33,34]. A recent study also found that in the presence of fatty liver, fetuin-A directly impairs glucose-induced insulin secretion without interacting with Toll-like receptor 4 by mediating metabolic crosstalk between fatty liver and pancreatic islets[35]. Furthermore, adiponectin, another protein secreted from adipose tissues which is also involved in the regulation of insulin sensitivity[7,36], was found to be inversely associated with fetuin-A[37].

Thus, it is plausible that inflammation, decreased adiponectin levels and increased liver fat content mediate or confound the link between fetuin-A and incident T2D. However, this hypothesis is not supported by our results, which demonstrated little change in the effect estimates for fetuin-A after adjustment for the above-mentioned pathways, suggesting that other yet unknown mechanisms explain the association between fetuin-A and incident T2D. Nonetheless, it should be noted that we cannot totally rule out that imprecision in the measurements is responsible for our results especially with regard to liver fat content. All studies included in our meta-analysis, except Aroner et al[8], used liver enzymes (alanine transaminase and gamma-glutamyltranspeptidase), a relatively crude measure of liver fat content[38], as surrogate measures of liver fat.

Strengths of the study

Our study has several strengths. The MONICA/KORA Augsburg case-cohort study has a considerably larger number of incident cases than previous studies. It is population-based and uses a prospective design with a long follow-up period. In our

meta-analysis, we included different levels of covariate adjustment, investigated sex-differences by separately pooling sex-specific estimates and investigated mediation by separately pooling estimates individually adjusted for potential mediators. We tried to explain heterogeneity by subgroup analyses. Furthermore, our meta-analysis strategy to convert the scales of the included estimates into per 1 SD increases of fetuin-A, leads to better comparable estimates than previous meta-analyses pooling quantile extremes without rescaling[11,31].

Limitations of the study

Limitations of this study also warrant consideration. The initial identification of incident cases of diabetes in the MONICA/KORA Augsburg case-cohort study was based on self-report only as no oral glucose tolerance tests have been performed. Although our meta-analysis pooled estimates using different levels of adjustment, residual confounding cannot be ruled out due to the nature of observational studies. The current case-cohort study as well as our meta-analysis identified evidence in predominantly Caucasian populations, thus we cannot generalize our results to other ethnicities.

Conclusion

Higher fetuin-A levels are significantly associated with incident T2D in males and females. Our findings based on studies with mainly Caucasian participants suggest that higher fetuin-A levels increase the risk of T2D independently from subclinical inflammation, adiponectin and liver fat content. Further studies are necessary to explore this association among other ethnicities.

Conflicts of interest

None to declare.

Sources of funding

Cornelia Huth is supported by intramural funding for Translational & Clinical Projects of the Helmholtz Zentrum München–German Research Center for Environmental Health, Germany, which is funded by the BMBF, Germany, and the State of Bavaria. Data analyses of the MONICA/KORA Augsburg case-cohort study were supported by a grant from the Else Kröner-Fresenius-Stiftung. The KORA study was initiated and financed by the Helmholtz Zentrum München–German Research Center for Environmental Health, which is funded by the BMBF and the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. The German Diabetes Center is supported by the Ministry of Science and Research of the State of North Rhine-Westphalia (MIWF NRW) and the German Federal Ministry of Health (BMG). This study was supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD).

The sponsors had no role in study design, data collection, data analysis, data interpretation, writing of the report and decision to publish the manuscript.

Author Contributions

The protocol for systematic review and meta-analysis was developed by CS, BT and CHu. CS conducted the literature search. CS and SM independently conducted data extraction and quality assessment. BT, AP, CHe, WK and J S-K researched data in the MONICA/KORA Augsburg study. AZ performed statistical analyses for the MONICA/KORA Augsburg case-cohort study. CS performed statistical analyses for the meta-analysis and drafted the manuscript. All authors critically revised the manuscript and approved of the final version.

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Figure Legends

Figure 1. **Forest plot summarising individual study estimates and pooled estimates for the association of a 1 SD increment of fetuin-A level with incident T2D adjusted for as many classical T2D risk factors as possible**. I-sq, I-squared statistic; LCI and UCI, lower and upper confidence intervals; N(cases), number of T2D cases; N(total), number of total study participants; P_Q, p-value for Cochran's Q test; RR, relative risk; wRE, random effects weights. M and W indicate sex-specific associations in men and women, respectively. Diamonds represent the overall (pooled) association estimates. Bars indicate 95% confidence intervals. Dashed vertical line indicates the pooled random effects estimate. The size of the box illustrates the random effects weight. Studies were sorted according to the study's weight in the random effects meta-analysis.

Figure 2. Forest plot summarising sex-specific associations per 1 SD increment of fetuin-A level with incident T2D adjusted for as many classical risk factors as possible in males and females. I-sq indicates I-squared statistic; LCI and UCI, lower and upper confidence intervals; N(cases) indicates number of T2D cases; N(total), number of total study participants; P_Q, p-value for Cochran's Q test for heterogeneity; RR, relative risk; wRE, random effects weights. M and W indicate sex-specific association in men and women, respectively. Diamonds represent the overall (pooled) association estimates. Bars indicate 95% confidence intervals. The size of the box illustrates the random effects weight. Studies were sorted according to the study's weight in the random effects meta-analysis.

Figure 3. **Forest plot summarising the results of the main analyses**. I-sq indicates I-squared statistic, the proportion of heterogeneity; LCI and UCI, lower and upper confidence intervals; N(cases), number of T2D cases; N(studies) indicates number of

studies included; N(total), number of total study participants; P_Q, p-value for Cochran's Q test; RR, relative risk. Minimum adjustment indicates model adjusted for age, sex and minimal number of classical risk factors as possible. Multivariable adjustment indicates model adjusted for as many classical risk factors as possible. Bars indicate 95% confidence intervals.

Supplementary Figure Legends

Supplementary Figure 1. **A flow diagram showing the identification of eligible studies**. [a]41 unique articles were found within PubMed only, 275 within EMBASE only and 222 within PubMed and EMBASE. [b]43 duplicates were found within EMBASE and 222 duplicates between PubMed and EMBASE

Supplementary Figure 2. Forest plot summarising individual study estimates and pooled estimates for minimum adjusted model per 1 SD increment of fetuin-A. I-sq indicates I-squared statistic, the proportion of heterogeneity; LCI and UCI, lower and upper confidence intervals; N(cases) indicates number of T2D cases; N(total), number of total study participants; P_Q, p-value for Cochran's Q test; RR, relative risk; wRE, random effects weights. M and W indicate sex-specific association in men and women, respectively. Diamonds represent the overall (pooled) association estimates. Bars indicate 95% confidence intervals. Vertical dash line indicates the pooled random effects estimate. The size of the box illustrates the random effects weight.

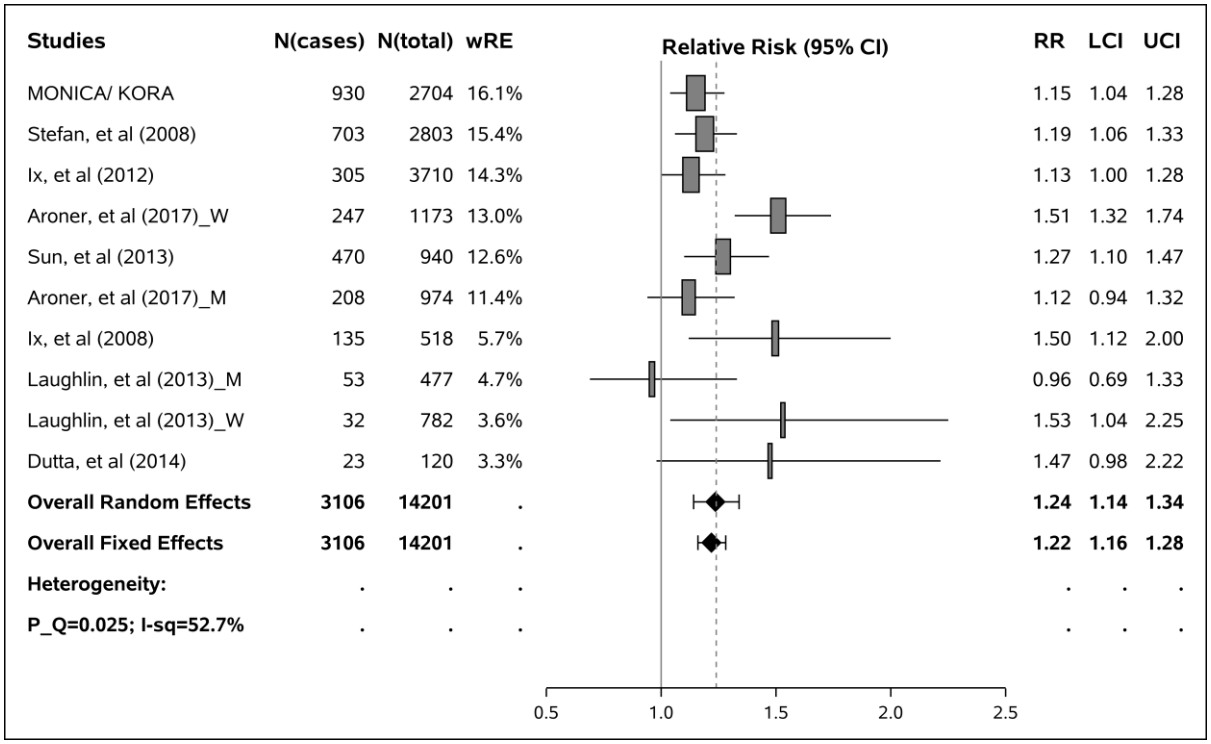
Supplementary Figure 3. Funnel plot for publication bias (Egger's linear regression test for publication bias *P*=0.369). The funnel plot plots the relative risks (RRs) of individual study [x-axis] against the study precision [y-axis]. Study precision is the inverse of the standard error; the higher the precision, the lower the standard error. The dots indicate multivariable-adjusted RRs of individual study per 1 SD increment of fetuin-A; the vertical dash line represents the pooled RR estimate with random effects model.

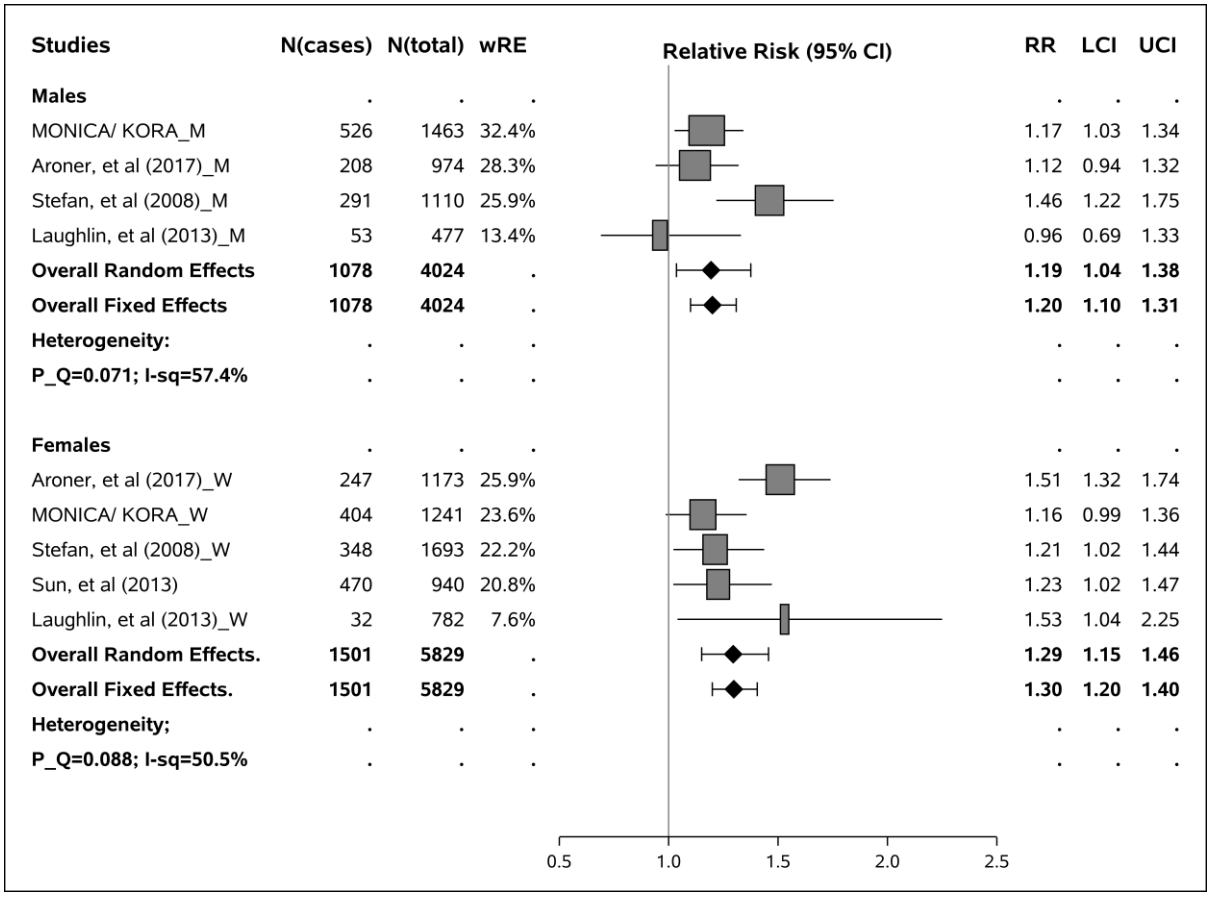
Supplementary Figure 4. Forest plot summarising the results of subgroup analyses for relative risk (RR) of T2D per 1 SD increment of fetuin-A levels. I-sq, I-squared statistic; LCI and UCI, lower and upper confidence intervals; N(studies) indicates number of studies included; NOS, Newcastle-Ottawa Scale; P_Q, p-value for Cohcran's Q test for heterogeneity; RR, relative risk. Multivariable adjustment indicates model adjusted for as many classical risk factors as possible; Median NOS score was 6. Bars indicate 95% confidence intervals.

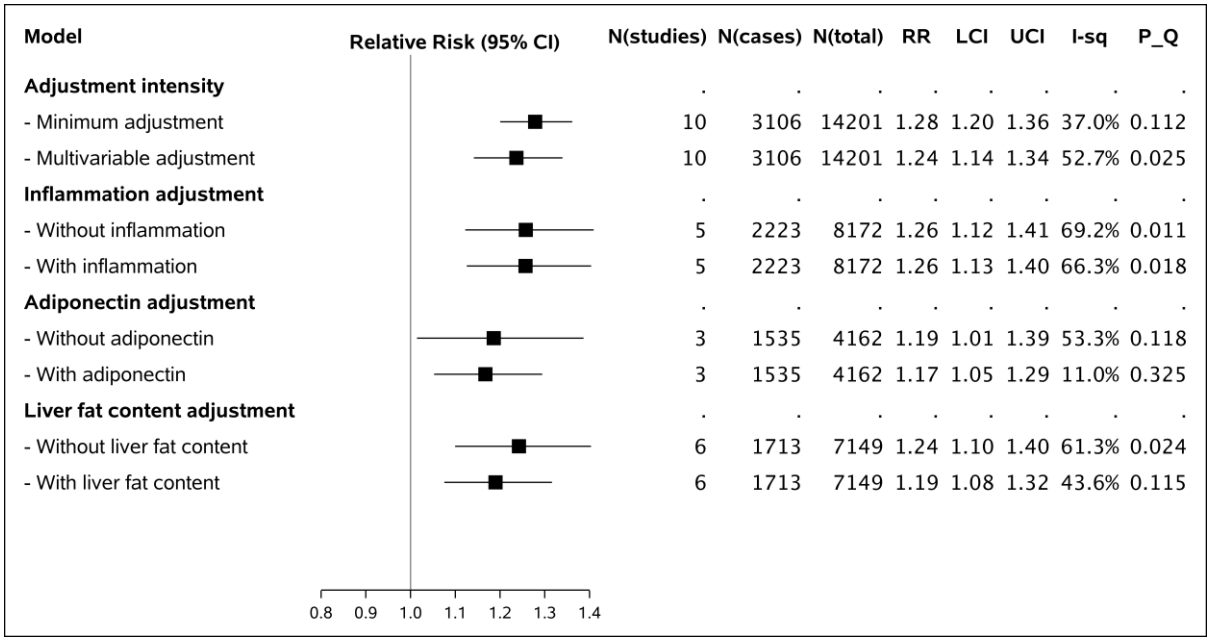
Table 1. Results from MONICA/KORA Augsburg case-cohort study

Model	Fetuin-A quartiles				Continuous
	I	II	III	IV	per 1 SD
All (n=2,704)					
Model 1	(ref.)	1.20 (0.92–1.57)	1.37 (1.04–1.78)	1.68 (1.29–2.18)	1.19 (1.09–1.30)
Model 2	(ref.)	1.09 (0.81–1.47)	1.27 (0.93–1.72)	1.45 (1.08–1.96)	1.15 (1.04–1.28)
Model 3	(ref.)	1.09 (0.81–1.47)	1.27 (0.94–1.73)	1.45 (1.08–1.95)	1.15 (1.04–1.28)
Model 4	(ref.)	1.10 (0.81–1.49)	1.28 (0.94–1.74)	1.47 (1.09–1.98)	1.16 (1.04–1.28)
Model 5	(ref.)	1.05 (0.77–1.44)	1.24 (0.91–1.69)	1.47 (1.08–1.99)	1.17 (1.05–1.30)
Females (n=1,241)					
Model 1	(ref.)	1.06 (0.73–1.55)	1.30 (0.89–1.90)	1.57 (1.09–2.28)	1.23 (1.08–1.40)
Model 2	(ref.)	0.94 (0.59–1.50)	1.17 (0.74–1.86)	1.30 (0.83–2.05)	1.16 (0.99–1.36)
Model 3	(ref.)	0.95 (0.60–1.51)	1.17 (0.74–1.87)	1.31 (0.84–2.07)	1.16 (0.99–1.36)
Model 4	(ref.)	0.97 (0.61–1.54)	1.18 (0.73–1.89)	1.33 (0.84–2.09)	1.16 (0.99–1.37)
Model 5	(ref.)	0.90 (0.55–1.47)	1.17 (0.73–1.89)	1.35 (0.85–2.13)	1.19 (1.01–1.40)
Males (n=1,463)					
Model 1	(ref.)	1.31 (0.91–1.90)	1.39 (0.95–2.04)	1.78 (1.24–2.57)	1.17 (1.04–1.32)
Model 2	(ref.)	1.30 (0.86–1.97)	1.38 (0.89–2.15)	1.75 (1.17–2.62)	1.17 (1.03–1.34)
Model 3	(ref.)	1.29 (0.85–1.96)	1.39 (0.90–2.15)	1.74 (1.16–2.61)	1.17 (1.03–1.34)
Model 4	(ref.)	1.30 (0.86–1.98)	1.40 (0.90–2.17)	1.75 (1.16–2.63)	1.18 (1.03–1.35)
Model 5	(ref.)	1.25 (0.82–1.91)	1.29 (0.82–2.03)	1.68 (1.11–2.54)	1.18 (1.02–1.35)

Data are hazard ratios (95% confidence intervals). Abbreviations: SD, standard deviation. Model 1: adjusted for age, sex and survey. Model 2: Model 1 + body mass index, smoking status, alcohol consumption, physical activity, high density lipoprotein levels, hypertension, parental history of diabetes. Model 3: Model 2 + daily coffee consumption, frequency of red meat consumption per day, frequency of whole grain consumption per day. Model 4: Model 3 + C-reactive protein levels. Model 5: Model 3 + adiponectin.


Studies
N(cases)
N(total)
wRE
Relative Risk (95% CI)
RR
LCI
UCI
MONICA/ KORA 930 2704 16.1% 1.15 1.04 1.28
Stefan, et al (2008) 703 2803 15.4% 1.19 1.06 1.33
Ix, et al (2012) 305 3710 14.3% 1.13 1.00 1.28
Aroner, et al (2017)_W 247 1173 13.0% 1.51 1.32 1.74
Sun, et al (2013) 470 940 12.6% 1.27 1.10 1.47
Aroner, et al (2017)_M 208 974 11.4% 1.12 0.94 1.32
Ix, et al (2008) 135 518 5.7% 1.50 1.12 2.00
Laughlin, et al (2013)_M 53 477 4.7% 0.96 0.69 1.33
Laughlin, et al (2013)_W 32 782 3.6% 1.53 1.04 2.25
Dutta, et al (2014) 23 120 3.3% 1.47 0.98 2.22
Overall Random Effects 3106 14201 . 1.24 1.14 1.34
Overall Fixed Effects 3106 14201 . 1.22 1.16 1.28
Heterogeneity: . . . . . .
P_Q=0.025; I-sq=52.7% . . . . . .
0.5
1.0
1.5
2.0
2.5


Studies
N(cases)
N(total)
wRE
Relative Risk (95% CI)
RR
LCI
UCI
Males . . . . . .
MONICA/ KORA_M 526 1463 32.4% 1.17 1.03 1.34
Aroner, et al (2017)_M 208 974 28.3% 1.12 0.94 1.32
Stefan, et al (2008)_M 291 1110 25.9% 1.46 1.22 1.75
Laughlin, et al (2013)_M 53 477 13.4% 0.96 0.69 1.33
Overall Random Effects 1078 4024 . 1.19 1.04 1.38
Overall Fixed Effects 1078 4024 . 1.20 1.10 1.31
Heterogeneity: . . . . . .
P_Q=0.071; I-sq=57.4% . . . . . .
Females . . . . . .
Aroner, et al (2017)_W 247 1173 25.9% 1.51 1.32 1.74
MONICA/ KORA_W 404 1241 23.6% 1.16 0.99 1.36
Stefan, et al (2008)_W 348 1693 22.2% 1.21 1.02 1.44
Sun, et al (2013) 470 940 20.8% 1.23 1.02 1.47
Laughlin, et al (2013)_W 32 782 7.6% 1.53 1.04 2.25
Overall Random Effects. 1501 5829 . 1.29 1.15 1.46
Overall Fixed Effects. 1501 5829 . 1.30 1.20 1.40
Heterogeneity; . . . . . .
P_Q=0.088; I-sq=50.5% . . . . . .
0.5
1.0
1.5
2.0
2.5



Model	N(studies)	N(cases)	N(total)	RR	LCI	UCI	I-sq	P_Q
Adjustment intensity	.	.	.	.	.	.	.	.
- Minimum adjustment	10	3106	14201	1.28	1.20	1.36	37.0%	0.112
- Multivariable adjustment	10	3106	14201	1.24	1.14	1.34	52.7%	0.025
Inflammation adjustment	.	.	.	.	.	.	.	.
- Without inflammation	5	2223	8172	1.26	1.12	1.41	69.2%	0.011
- With inflammation	5	2223	8172	1.26	1.13	1.40	66.3%	0.018
Adiponectin adjustment	.	.	.	.	.	.	.	.
- Without adiponectin	3	1535	4162	1.19	1.01	1.39	53.3%	0.118
- With adiponectin	3	1535	4162	1.17	1.05	1.29	11.0%	0.325
Liver fat content adjustment	.	.	.	.	.	.	.	.
- Without liver fat content	6	1713	7149	1.24	1.10	1.40	61.3%	0.024
- With liver fat content	6	1713	7149	1.19	1.08	1.32	43.6%	0.115