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Plasma Fetuin-A concentration, genetic variation in the *AHSG* gene and risk of colorectal cancer

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Novelty and Impact: Fetuin-A is a liver protein known to induce insulin resistance.
Hyperinsulinemia is a possible risk factor for colorectal cancer, but whether fetuin-A plays a role
in the etiology of colorectal cancer is currently unclear. In this prospective study, we found a
modest linear association between fetuin-A and higher risk of colorectal cancer, but genetically
raised fetuin-A was unrelated to colorectal cancer risk, arguing against a direct role of fetuin-A in
colorectal carcinogenesis.

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Abstract

Fetuin-A, also referred to as α2-Heremans-Schmid glycoprotein (AHSG) is a liver protein known to inhibit insulin actions. Hyperinsulinemia is a possible risk factor for colorectal cancer, however, the role of fetuin-A in the development of colorectal cancer is unclear. We investigated the association between circulating fetuin-A and colorectal cancer risk in a nested case-control study within the European Prospective Investigation into Cancer and Nutrition. Fetuin-A concentrations were measured in pre-diagnostic plasma samples from 1,367 colorectal cancer cases and 1,367 matched controls. In conditional logistic regression models adjusted for potential confounders the estimated relative risk (95% CI) of colorectal cancer per 40 µg/mL higher fetuin-A concentrations (approximately one standard deviation) was 1.13 (1.02, 1.24) overall, 1.21 (1.05, 1.39) in men and 1.06 (0.93, 1.22) in women, 1.13 (1.00, 1.27) for colon cancer and 1.12 (0.94, 1.32) for rectal cancer. To improve causal inference in a Mendelian Randomization approach, 5 tagging single nucleotide polymorphisms of the *AHSG* gene were genotyped in a subset of 456 case-control pairs. The *AHSG* allele-score explained 21% of the inter-individual variation in plasma fetuin-A concentrations. In instrumental variable analysis, genetically raised fetuin-A was not associated with colorectal cancer risk (relative risk per 40 µg/mL genetically determined higher fetuin-A was 0.98, 95% CI 0.73, 1.33). The findings of our study indicate a modest linear association between fetuin-A concentrations and risk of colorectal cancer, but suggest that fetuin-A may not be causally related to colorectal cancer development.

Introduction

Fetuin-A, also referred to as α2-Heremans-Schmid glycoprotein (*AHSG*), is a protein that is almost exclusively expressed and secreted by the liver, particularly in nonalcoholic fatty liver disease (NAFLD) [1]. Fetuin-A plays a role in the insulin signaling pathway, acting as an inhibitor of insulin receptor tyrosine kinase [2]. Mice deficient for the *AHSG* gene displayed improved insulin sensitivity, lower body weight and were resistant to weight gain upon a high-fat diet [3, 4]. Further experimental studies in rodents have shown that fetuin-A induces insulin resistance [5]. These findings are supported by a number of human studies that observed positive associations between circulating fetuin-A and measures of insulin resistance [1, 6, 7]. In addition, a bidirectional Mendelian Randomization study suggested a causal relationship between fetuin-A and body mass index [8]. Recently, positive associations between fetuin-A concentrations and risk of type 2 diabetes [9-11] and cardiovascular disease [12, 13] have been observed in large prospective studies. Insulin resistance and associated metabolic states such as hyperinsulinemia, hyperglycemia, low-grade inflammation and hypoadiponectinemia have been associated with increased risk of colorectal cancer in several prospective studies [14] including the European Prospective Investigation into Cancer and Nutrition (EPIC) [15-18]. However, it is currently unknown whether fetuin-A plays a role in the development of colorectal cancer. Therefore, we aimed to investigate the association between circulating fetuin-A and risk of colorectal cancer in a prospective nested case-control study in EPIC. Because observational studies relating circulating biomarker concentrations to risk of disease may be affected by residual confounding and reverse causation, even in a prospective study design, we additionally aimed to examine potential causality by using single nucleotide polymorphisms (SNPs) in the *AHSG* gene as relatively unbiased proxies for fetuin-A concentration using a Mendelian Randomization approach [19].

Material and Methods

Study population

The EPIC study is an ongoing cohort study with over 520,000 participants from 23 centers in 10 Western European countries who were between 25 and 70 years old at study recruitment in the period between 1992 and 2000. Details on the methods of the EPIC study design were reported previously [20]. Briefly, standardized lifestyle and personal history questionnaires, anthropometric data and blood samples were collected from most participants at recruitment, prior to disease onset or diagnosis. Diet over the previous 12 months was assessed at recruitment by validated country-specific questionnaires. The EPIC study was approved by the ethics review board of the International Agency for Research on Cancer (IARC, Lyon, France) and the local review boards of the participating institutions.

Identification of colorectal cancer cases

Incident cancer cases were identified through record linkage with regional cancer registries in most participating countries (Denmark, Norway, the Netherlands, Spain, Sweden, UK and most of the Italian study centers). In France, Germany, Greece and Naples (Italy), follow-up was performed actively by the participants or their next of kin through mailed questionnaires. Self-reported cancer cases were then verified by study physicians using health insurance data, information from cancer and clinical registries, and medical records provided by treating physicians. The closure dates, which were defined as the latest date of complete follow-up for both cancer incidence and vital status, ranged from December 2002 to June 2005. Based on the

International Statistical Classification of Diseases, Injury and Causes of Death (10th Revision),

colorectal cancer was defined as a combination of tumors of the colon (C18.0-C18.7), tumors that

were overlapping or unspecified (C18.8-C18.9), and tumors of the rectum (C19-C20).

Nested case-control study

A total of 1,367 first incident colorectal cancer cases with available questionnaire data and blood

samples were included in the study. For each case one control was selected using an incidence

density sampling approach from all cohort members who were alive and free of cancer at the time

of diagnosis of the index case. Matching factors included sex, age at blood collection (2-months

to 4-year intervals), study center, time of blood collection (±4 hours) and fasting status (<3, 3-6,

or >6 hours). Women were additionally matched on menopausal status (premenopausal,

perimenopausal, postmenopausal, or surgically menopausal). Premenopausal women were

matched on phase of the menstrual cycle at blood collection (early follicular, late follicular,

ovulatory, early luteal, mid-luteal, or late luteal), and postmenopausal women were matched on

current use of hormone replacement therapy (yes/no). Of note, not all matching factors (e.g.

menstrual cycle) were relevant for the present study, since the case-control set was designed to be

used for several biomarker studies.

DNA was available for 1,110 participants (627 colorectal cancer cases, 483 control participants,

456 case-control pairs), largely due to unavailability of DNA samples from the Danish EPIC

centers (due to local technical and organizational circumstances that delayed sample retrieval)

and a proportion of empty DNA tubes throughout all study centers. Comparison of baseline

characteristics of control participants with DNA (n=483) with control participants excluded from

the genetic analyses in this study due to unavailable DNA (n=970) revealed that both groups

were similar with regards to gender, age, education, and body fatness.

Laboratory measurements

At baseline, blood samples were collected from participants using a standardized protocol and stored at the International Agency for Research on Cancer (Lyon, France) in liquid nitrogen at -196°C, with the exception of the Danish samples, which were stored in nitrogen vapor at −150°C, and Swedish samples, which were stored in -80°C freezers [20]. Plasma concentrations of fetuin-A were measured by enzyme linked immunosorbent assays (BioVendor Human Fetuin-A ELISA) in the laboratory of Prof. Pischon, Molecular Epidemiology Group, Max Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Germany. Based on quality control samples (n=40), inter-assay coefficient of variation was 8.1%.

Five tagging single nucleotide polymorphisms (SNPs) in the *AHSG* gene (rs2248690, rs2070633, rs2070635, rs4917 and rs6787344) were selected via HapMap applying stringent criteria (minor allele frequency >5% and pairwise $r^2 \geq 0.80$), and genotyped using TaqMan methodology. Genotype call rates were >99.2% for all assays.

Circulating concentrations of C-peptide, HbA1c, insulin-like growth factor 1 (IGF-1), total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), adiponectin, leptin, and soluble leptin receptor (sOB-R), were also available and methods for their measurement are described elsewhere [15, 17, 18, 21, 22].

Statistical analysis

Baseline characteristics between cases and controls were compared using paired t-test, Wilcoxon signed rank test, or Mc Nemar's test. Spearman partial correlation coefficients, adjusted for age and sex, were calculated in control participants to examine the relationship between fetuin-A and

age (sex-adjusted only), body mass index, waist circumference, physical activity, alcohol intake and metabolic biomarkers.

The association between circulating fetuin-A and risk of colorectal (overall, and separately by sex), colon or rectal cancer was evaluated using conditional logistic regression analysis calculating odds ratios (ORs) and 95% confidence intervals (95% CI). Because of the incidence density sampling, ORs approximate incidence rate ratios which can be interpreted as relative risks. Data were analyzed by quintiles of fetuin-A concentrations with cut-offs based on the distribution among control participants. Test for trend across quintiles was performed by entering the quintile medians as continuous variable to the conditional logistic regression model and evaluating its significance by using the Wald-test. We also examined fetuin-A as a continuous variable by increments of 40 µg/mL (approximately one standard deviation in fetuin-A concentration). We tested for potential non-linear associations between fetuin-A and risk of colorectal cancer using fractional polynomials [23]. ORs were evaluated in a crude model conditioned on matching factors only as well as in a multivariable model with adjustment for potentially confounding factors including education, Cambridge physical activity index [24], smoking status, alcohol intake, total energy intake, fiber intake, fruit and vegetable intake, red meat intake, processed meat intake and fish and shellfish intake (model 1). Because risk estimates from crude models did not differ appreciably from multivariable models, only multivariable estimates were presented. In a separate model, we added body mass index (BMI) and residuals of BMI-adjusted waist circumference (to avoid multicollinearity) as covariables (model 2) to examine whether adjustment for body fatness changed risk estimates. There were 128 participants with missing values on waist circumference in whom the waist circumference residuals were substituted with sex-specific median values. In addition, in four participants who had no information on dietary intake, sex-specific median dietary intake values were imputed for

analysis. Potential heterogeneity in the associations between fetuin-A and colon versus rectal cancer was determined using competing risk analysis [25]. Heterogeneity by sex was evaluated using the Q-statistic from the inverse variance method [26].

Additional analyses were performed with exclusion of cases (and their matched controls) diagnosed within 2 years after recruitment (n=217 cases, 16% of all cases) to evaluate whether our findings may be influenced by reverse causation due to preclinical disease.

Furthermore, we additionally adjusted for metabolic biomarkers which were significantly correlated with fetuin-A and have been shown to be independent risk factors for colorectal cancer in previous studies in EPIC [17, 18, 22], including C-peptide, HbA1c, and sOB-R. In order to examine C-peptide and HbA1c simultaneously, we added a factor score resulting from factor analysis on both variables to the model [27]. A dataset containing observations with available data on all these biomarkers was created, which included 465 case-control pairs (the lower number is because HbA1c and C-peptide were assayed only for part of the dataset).

We also conducted analyses stratified by median age ($</\geq$ 59 years), categories of BMI (<25, 25-30, $\geq$30 kg/m^2), waist circumference (<88 cm in women or <102 cm in men versus $\geq$88 cm in women or $\geq$102 cm in men), HbA1c (<5.7%, $\geq$5.7%), C-peptide (<4.5 ng/mL in men or <3.4 ng/mL in women versus $\geq$4.5 ng/mL in men or $\geq$3.4 ng/mL in women) and tertiles of sex-specific alcohol intake. Because numbers of case-control pairs were small in certain strata, stratified analyses were conducted using unconditional logistic regression models, adjusting for matching variables and all potential covariables included in model 2 (excluding the stratifying variable). Potential effect modification (statistical interaction on the multiplicative scale) was evaluated by including a product term of stratifying factors (median values of categories) and fetuin-A (continuously) in the model and evaluating its statistical significance using Wald's test.

For the Mendelian Randomization (MR) evaluation of the association between genetically raised fetuin-A and risk of colorectal cancer, we first examined whether selected SNPs were useful as instrumental variables, i.e. predicted fetuin-A concentrations. For this purpose, the association between SNPs and fetuin-A was estimated using univariate linear regression models with robust variance [28] in control participants. Mean fetuin-A concentrations and 95% confidence intervals (95% CI) by genotype and the estimated difference in fetuin-A per minor allele (with genotypes coded 0, 1 or 2 according to the number of minor alleles) were calculated. We created a weighted allele-score by summing each genotype of the *AHSG* SNPs that were individually associated with higher fetuin-A concentrations [29, 30] multiplied by its estimated coefficient from the linear regression, and then divided by the sum of weights. To examine whether the *AHSG*-score was independent of potentially confounding factors, we compared the baseline characteristics across tertiles of the *AHSG*-score. The Mendelian Randomization estimate for the association between genetically raised fetuin-A and colorectal cancer risk was quantified by instrumental variable analysis using two-stage least squares regression. The first stage comprised the linear regression of fetuin-A concentrations on the allele score, resulting in predicted values of fetuin-A concentrations. The second stage comprised a logistic regression of colorectal cancer on the predicted fetuin-A concentrations. Instrument strength for MR analysis was evaluated using the F-statistic [31, 32] from the first stage regression. Because according to the Mendelian Randomization concept *AHSG* genetic variants can be used as unbiased proxies for fetuin-A concentrations[19], the instrumental variable analyses were conducted without adjustment for potential confounders. However, in sensitivity analyses we additionally adjusted for matching factors and BMI.

All reported p-values are two-sided. Instrumental variable analyses were performed using the STATA SE 12 (StataCorp, College Station, Texas, USA). All other analyses were performed

using SAS Version 4.3- Graphical Software Interface - SAS Enterprise Guide (SAS Institute Inc., Cary, North Carolina, USA).

Results

Incident colorectal cancer cases had at baseline a higher BMI and a higher waist circumference and were more often physically inactive than matched control participants (Table 1). Dietary intake of fiber was lower in colorectal cancer cases than in control participants. Median pre-diagnostic blood concentrations of fetuin-A were 227.7 µg/mL in colorectal cancer cases and 225.1 µ/mL in matched control participants. Compared with their matched controls, colorectal cancer cases had higher pre-diagnostic blood concentrations of C-reactive protein, and lower concentrations of HDL cholesterol, adiponectin and sOB-R.

In control participants, plasma fetuin-A was significantly, albeit weakly, positively correlated with BMI (r=0.14, p-value <0.0001) and waist circumference (r=0.16, p-value <0.0001), and inversely correlated with age (r=-0.07, p-value 0.01) and alcohol consumption (-0.09, p-value 0.001). Of the metabolic biomarkers, C-peptide (r=0.08, p-value 0.05), HbA1c (r=0.12, p-value 0.01) and triglycerides (r=0.09, p-value 0.02) were weakly positively correlated with fetuin-A, and sOB-R (r=-0.09, p-value 0.02) was inversely correlated with fetuin-A (supplemental table 1). C-reactive protein, IGF-1, total cholesterol, adiponectin, and leptin were not significantly correlated with fetuin-A (all p-values >0.09).

Each 40 µg/mL fetuin-A increment (approximately one standard deviation) was significantly associated with a 13% higher risk of colorectal cancer (OR 1.13, 95% CI 1.02, 1.24) before, and a 11% higher risk of colorectal cancer (OR 1.11, 95% CI 1.01, 1.22) after adjustment for body

fatness (Table 2). No such association was seen when comparing the highest with the lowest quintile. Using fractional polynomials with between one and two degrees, we found no evidence that a non-linear function was statistically superior to the linear model (all p-values for non-linearity >0.10). In analyses by sex, plasma fetuin-A concentrations were significantly positively associated with colorectal cancer risk in men both in continuous and categorical analyses (Table 3). In contrast, no association was observed in women. However, we did not observe statistically significant heterogeneity between men and women (p-heterogeneity 0.21 in model 1 and 0.24 in model 2). After exclusion of the first two years of follow-up, the positive association between plasma fetuin-A and risk of colorectal cancer was slightly attenuated overall, but became slightly stronger in men (table 2). When performing analyses according to tumor location, a significant positive association between plasma fetuin-A and risk of colon cancer was observed in the multivariable model with fetuin-A as a continuous variable (OR per 40 µg/mL 1.13, 95% CI 1.00, 1.27), but associations were non-significant after adjustment for body fatness (OR 1.11, 95% CI 0.99, 1.25) (table 3). Comparing the highest with the lowest fetuin-A quintile, a non-significantly higher risk of colon cancer was observed. Non-significant positive associations between plasma fetuin-A and risk of rectal cancer were observed with both continuous and categorical analyses. Competing risk analysis revealed no significant differences in the associations between fetuin-A and colon versus rectal cancer (p-heterogeneity 0.08 in model 1 and 0.89 in model 2). After exclusion of the first two years of follow-up, the positive association between plasma fetuin-A and risk of colon cancer was attenuated, whereas the positive association between fetuin-A and risk of rectal cancer was slightly stronger, albeit statistically non-significant.

We further conducted stratified analysis to examine whether the association between plasma fetuin-A and colorectal cancer risk differed by age, body fatness (BMI and waist circumference),

alcohol consumption, and concentrations of HbA1c and C-peptide. No significant association between fetuin-A and risk of colorectal cancer was observed in any strata, and there was no indication of interaction by any of these factors (supplemental table 2).

In the subset of participants with available data on fetuin-A, C-peptide, HbA1c and soluble leptin receptor (465 cases and 465 matched controls), the risk estimate for the association between plasma fetuin-A and risk of colorectal cancer was the same as in the full sample, but the confidence interval was wider (OR per 40 µg/mL in multivariable model including body fatness 1.11, 95% CI 0.94, 1.32) and therefore the association lost statistical significance. Adding metabolic biomarkers to the model did not alter the association in the subset: OR (95% CI) per 40 µg/mL fetuin-A increment was 1.11 (0.94, 1.32) after adding log-transformed C-peptide, 1.11 (0.94-1.31) after adding log-transformed HbA1c, 1.11 (0.94, 1.31) after adding a factor score combining C-peptide and HbA1c, and 1.13 (0.96, 1.34) after adding log-transformed concentration of soluble leptin receptor.

Among control participants with available data on both plasma fetuin-A and *AHSG* SNPs (n=483), 4 of the 5 selected tagging SNPs in the *AHSG* gene were associated with plasma concentrations of fetuin-A (Figure 2). The weighted *AHSG*-score was associated with 30.4 µg/ml (95% CI 25.7; 35.2) higher plasma fetuin-A per score unit, which explained 21% of the inter-individual variation in plasma fetuin-A (F-value=287). Most potentially confounding or mediating lifestyle, dietary and metabolic factors did not differ substantially across *AHSG*-score tertiles (Supplemental table 3). However, higher *AHSG*-score appeared to be negatively associated with age at blood collection (p-trend 0.03) and soluble leptin-receptor (p-trend 0.02), but was positively associated with BMI (p-trend 0.01). When using the *AHSG*-score as instrumental variable in a Mendelian randomization analysis, no positive association between

genetically raised fetuin-A and risk of colorectal cancer was observed (OR per 40 µg/mL genetically determined higher fetuin-A 0.98, 95% CI 0.73, 1.33), arguing against a causal association. The estimate was not altered substantially after adjustment for matching factors including age (OR 0.99, 95% CI 0.73, 1.33), or additional adjustment for BMI (OR 0.94, 95% CI 0.69, 1.28). Genetically raised fetuin-A was unrelated to risk of colorectal cancer in men (OR 1.04, 95% CI 0.68, 1.60) and women (OR 0.93, 95% CI 0.93, 1.42). Mendelian Randomization estimates for genetically raised fetuin-A were 1.15 (95% CI 0.81, 1.63) for risk of colon cancer and 0.68 (95% CI 0.37, 1.24) for risk of rectal cancer. It should be noted that the sample size for these subgroup analyses was limited.

Discussion

In this prospective study, we found a modest positive association between plasma fetuin-A concentrations and risk of colorectal cancer, adjusting for a variety of potentially confounding factors. However, significant associations were only observed when fetuin-A was modeled as a continuous variable, and not when comparing quintiles of fetuin-A concentrations. Furthermore, associations were restricted to male participants, while no statistically significant associations were observed in women. The observed associations between fetuin-A and risk of colorectal cancer were only slightly attenuated after adjustment for body fatness and were not substantially influenced by additional adjustment (in a subset) for C-peptide or HbA1c. In contrast to these observations, genetically raised fetuin-A concentrations were not associated with a higher risk of colorectal cancer in the Mendelian Randomization analysis, arguing against the hypothesis that plasma fetuin-A concentrations play a causal role in colorectal carcinogenesis.

To our knowledge this is the first prospective study to examine circulating fetuin-A concentrations in relation to risk of colorectal cancer. Biological mechanisms for a positive

association between fetuin-A and risk of colorectal cancer are conceivable through fetuin-A's impact on obesity[8, 33, 34] and insulin resistance[1, 5-7], since markers of obesity, insulin resistance and hyperinsulinemia have been associated with higher risk of colorectal cancer in a number of prospective studies [14, 35], including findings from the EPIC cohort [17, 18, 36]. There is also some evidence showing that fetuin-A mediates the adhesion of tumor cells, which is an important step in tumor growth and motility as well as development of metastases [37]. While we found a positive association between measured fetuin-A and risk of colorectal cancer , the Mendelian Randomization analysis, [19, 29, 31], argues against a causal effect of fetuin-A on colorectal cancer risk: Genetically raised fetuin-A was not associated with risk of colorectal cancer. One possible explanation for this discrepancy is that pre-clinical colorectal cancer may have resulted in elevated fetuin-A concentrations. In fact, we found some evidence for such reverse causality in the observational analysis: the linear association between measured fetuin-A and risk of colorectal cancer was slightly attenuated after excluding the first two years of follow-up. However, the attenuation was small and may also be due to chance. It is currently unclear how preclinical colorectal cancer may result in an upregulation of fetuin-A expression, but in a recent proteomics study, a fetuin-A precursor was found to be up-regulated in post-diagnostic plasma of colorectal cancer patients as compared to healthy volunteers and authors suggested this precursor as potential diagnosis marker for colorectal cancer [38]. In contrast, in a small cross-sectional study comparing fetuin-A concentrations between 32 colorectal cancer patients and 30 control participants no differences were observed [39].

Within the limits of our study we tried to control for potential confounding as completely as possible in our analysis. However, an uncontrolled or insufficiently controlled environmental or lifestyle factor associated with both fetuin-A and colorectal cancer may have influenced our findings on plasma fetuin-A concentrations and risk of colorectal cancer. Therefore, besides

reverse causation, the observed significant association of measured circulating fetuin-A and colorectal cancer may also be explained by residual confounding. This is in contrast to the null association found in our Mendelian Randomization analysis, which circumvents reverse causation and uncontrolled confounding[18, 28, 30], thus suggesting that fetuin-A has no causal role for colorectal cancer.

The limited sample size for the Mendelian Randomization analysis is a limitation of our study. Instrumental variable estimates can be biased when both the variance explained by the instruments and the sample size are small [29]. However, the selected polymorphisms in the *AHSG* gene were strong predictors of fetuin-A concentrations, explaining a good proportion of inter-individual variation. Nevertheless, as reflected by the wide confidence interval of the instrumental variable estimate, our study had limited statistical power to detect small effects, and we cannot exclude that a modest association between genetically raised fetuin-A and colorectal cancer risk would have been detected in a Mendelian Randomization study with substantially larger sample size. Of note, in a previous Mendelian Randomization study in EPIC with a similar sample size, a significant association between genetically raised CRP and risk of colorectal cancer was observed, even though the instrument strength of the CRP-score was weaker than of the here applied *AHSG*-score [40]. It should also be noted that the *AHSG*-score was not completely independent of potentially confounding factors. Both BMI and soluble leptin-receptor differed significantly by tertiles of the *AHSG*-score. This finding, however, is consistent with studies showing that certain *AHSG* genotypes are more common in obese than in lean people [33]; With respect to the analysis of the association between measured circulating fetuin-A and risk of colorectal cancer, random measurement error may have attenuated observed associations. It also is a limitation of our study that fetuin-A was measured only once at baseline and thus may not

necessarily represent long-term values. Furthermore, we cannot exclude that storage time influenced fetuin-A values.

Strengths of our study include the prospective design, the ability to adjust for a variety of potentially confounding factors including metabolic biomarkers as well as the ability to investigate potential causality in a Mendelian Randomization approach using multiple *AHSG* genetic variants which were strongly associated with fetuin-A concentrations.

In conclusion, the findings of our study suggest that high fetuin-A concentrations are associated with a modest higher risk of colorectal cancer, but that fetuin-A may not be causally related to colorectal cancer development. Both, the basic association between circulating fetuin-A and risk of colorectal cancer as well as the lack of association between genetically raised fetuin-A and colorectal cancer risk warrant confirmation by further studies.

Acknowledgements

References

1. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Krober SM, Machicao F, Fritsche A, Haring HU. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes care 2006;29:853-7.
2. Srinivas PR, Wagner AS, Reddy LV, Deutsch DD, Leon MA, Goustin AS, Grunberger G. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. Mol Endocrinol 1993;7:1445-55.
3. Auberger P, Falquerho L, Contreres JO, Pages G, Le Cam G, Rossi B, Le Cam A. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. Cell 1989;58:631-40.
4. Mathews ST, Singh GP, Ranalletta M, Cintron VJ, Qiang X, Goustin AS, Jen KL, Charron MJ, Jahnen-Dechent W, Grunberger G. Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. Diabetes 2002;51:2450-8.
5. Hennige AM, Staiger H, Wicke C, Machicao F, Fritsche A, Haring HU, Stefan N. Fetuin-A induces cytokine expression and suppresses adiponectin production. PloS one 2008;3:e1765.
6. Rasul S, Wagner L, Kautzky-Willer A. Fetuin-A and angiopoietins in obesity and type 2 diabetes mellitus. Endocrine 2012;42:496-505.
7. Mori K, Emoto M, Yokoyama H, Araki T, Teramura M, Koyama H, Shoji T, Inaba M, Nishizawa Y. Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects. Diabetes care 2006;29:468.
8. Thakkinstian A, Chailurkit L, Warodomwichit D, Ratanachaiwong W, Yamwong S, Chanprasertyothin S, Attia J, Sritara P, Ongphiphadhanakul B. Causal relationship between body mass index and fetuin-A level in the asian population: a bidirectional mendelian randomization study. Clinical endocrinology 2013.
9. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, Schulze MB. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 2008;57:2762-7.
10. Sun Q, Cornelis MC, Manson JE, Hu FB. Plasma levels of fetuin-a and hepatic enzymes and risk of type 2 diabetes in women in the u.s. Diabetes 2013;62:49-55.
11. Ix JH, Wassel CL, Kanaya AM, Vittinghoff E, Johnson KC, Koster A, Cauley JA, Harris TB, Cummings SR, Shlipak MG. Fetuin-A and incident diabetes mellitus in older persons. Jama 2008;300:182-8.
12. Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost HG, Haring HU, Boeing H, Fritsche A. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation 2008;118:2555-62.
13. Jensen MK, Bartz TM, Mukamal KJ, Djousse L, Kizer JR, Tracy RP, Zieman SJ, Rimm EB, Siscovick DS, Shlipak M, Ix JH. Fetuin-A, Type 2 Diabetes, and Risk of Cardiovascular Disease in Older Adults: The Cardiovascular Health Study. Diabetes care 2012.
14. Pisani P. Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. Archives of physiology and biochemistry 2008;114:63-70.
15. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven FJ, Fedirko V, Rinaldi S, Romieu I, Riboli E, Romaguera D, Westphal S, et al. Total and high-molecular weight adiponectin and risk of colorectal cancer: the European Prospective Investigation into Cancer and Nutrition Study. Carcinogenesis 2012;33:1-8.
16. Aleksandrova K, Jenab M, Boeing H, Jansen E, Bueno-de-Mesquita HB, Rinaldi S, Riboli E, Overvad K, Dahm CC, Olsen A, Tjonneland A, Boutron-Ruault MC, et al. Circulating C-reactive protein concentrations and risks of colon and rectal cancer: a nested case-control study within the

European Prospective Investigation into Cancer and Nutrition. American journal of epidemiology 2010;172:407-18.

17. Jenab M, Riboli E, Cleveland RJ, Norat T, Rinaldi S, Nieters A, Biessy C, Tjonneland A, Olsen A, Overvad K, Gronbaek H, Clavel-Chapelon F, et al. Serum C-peptide, IGFBP-1 and IGFBP-2 and risk of colon and rectal cancers in the European Prospective Investigation into Cancer and Nutrition. International journal of cancer 2007;121:368-76.

18. Rinaldi S, Rohrmann S, Jenab M, Biessy C, Sieri S, Palli D, Tumino R, Mattiello A, Vineis P, Nieters A, Linseisen J, Pischon T, et al. Glycosylated hemoglobin and risk of colorectal cancer in men and women, the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev 2008;17:3108-15.

19. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? International journal of epidemiology 2003;32:1-22.

20. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113-24.

21. van Duijnhoven FJ, Bueno-De-Mesquita HB, Calligaro M, Jenab M, Pischon T, Jansen EH, Frohlich J, Ayyobi A, Overvad K, Toft-Petersen AP, Tjonneland A, Hansen L, et al. Blood lipid and lipoprotein concentrations and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition. Gut 2011;60:1094-102.

22. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven FJ, Rinaldi S, Fedirko V, Romieu I, Riboli E, Gunter MJ, Westphal S, et al. Leptin and Soluble Leptin Receptor in Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition Cohort. Cancer research 2012.

23. Sauerbrei W, Meier-Hirmer C, Benner A, Royston P. Multivariable regression model building by using fractional polynomials: Description of SAS, STATA and R programs. Computational Statistics & Data Analysis 2006;50:3464-85.

24. Validity of a short questionnaire to assess physical activity in 10 European countries. European journal of epidemiology 2012;27:15-25.

25. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995;51:524-32.

26. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin.Trials 1986;7:177-88.

27. Dossus L, Lukanova A, Rinaldi S, Allen N, Cust AE, Becker S, Tjonneland A, Hansen L, Overvad K, Chabbert-Buffet N, Mesrine S, Clavel-Chapelon F, et al. Hormonal, metabolic, and inflammatory profiles and endometrial cancer risk within the EPIC cohort--a factor analysis. American journal of epidemiology 2013;177:787-99.

28. White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica 1980;48:817-38.

29. Palmer TM, Lawlor DA, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, Davey Smith G, Sterne JA. Using multiple genetic variants as instrumental variables for modifiable risk factors. Statistical methods in medical research 2012;21:223-42.

30. Lin X, Song K, Lim N, Yuan X, Johnson T, Abderrahmani A, Vollenweider P, Stirnadel H, Sundseth SS, Lai E, Burns DK, Middleton LT, et al. Risk prediction of prevalent diabetes in a Swiss population using a weighted genetic score--the CoLaus Study. Diabetologia 2009;52:600-8.

31. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Statistics in medicine 2008;27:1133-63.

32. Stock JHW, J.H.; . A Survey of Weak Instruments and Weak Identification in Generalized Method of Moments. J Bus Econ Stat 2002;20:12.

33. Lavebratt C, Wahlqvist S, Nordfors L, Hoffstedt J, Arner P. AHSG gene variant is associated with leanness among Swedish men. Human genetics 2005;117:54-60.

34. Mathews ST, Rakhade S, Zhou X, Parker GC, Coscina DV, Grunberger G. Fetuin-null mice are protected against obesity and insulin resistance associated with aging. Biochemical and biophysical research communications 2006;350:437-43.

35. World Cancer Research Fund / American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. Washington, DC, 2011.

36. Pischon T, Lahmann PH, Boeing H, Friedenreich C, Norat T, Tjonneland A, Halkjaer J, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Guernec G, Bergmann MM, et al. Body size and risk of colon and rectal cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). Journal of the National Cancer Institute 2006;98:920-31.

37. Watson K, Koumangoye R, Thompson P, Sakwe AM, Patel T, Pratap S, Ochieng J. Fetuin-A triggers the secretion of a novel set of exosomes in detached tumor cells that mediate their adhesion and spreading. FEBS letters 2012;586:3458-63.

38. Fan NJ, Kang R, Ge XY, Li M, Liu Y, Chen HM, Gao CF. Identification alpha-2-HS-glycoprotein precursor and tubulin beta chain as serology diagnosis biomarker of colorectal cancer. Diagnostic pathology 2014;9:53.

39. Dowling P, Clarke C, Hennessy K, Torralbo-Lopez B, Ballot J, Crown J, Kiernan I, O'Byrne KJ, Kennedy MJ, Lynch V, Clynes M. Analysis of acute-phase proteins, AHSG, C3, CLI, HP and SAA, reveals distinctive expression patterns associated with breast, colorectal and lung cancer. International journal of cancer 2012;131:911-23.

40. Nimptsch K, Aleksandrova K, Boeing H, Janke J, Lee YA, Jenab M, Bueno-De-Mesquita BH, Jansen EH, Tsilidis KK, Trichopoulou A, Weiderpass E, Wu C, et al. Association of CRP genetic variants with blood concentrations of C-reactive protein and colorectal cancer risk. International journal of cancer 2014.

Table 1. Baseline characteristics of incident colorectal cancer cases and matched control participants

	Cases (n=1,367)	Controls (n=1,367)	p-value
Female sex, n (%)	710 (51.9)	710 (51.9)	[1]
Age at blood donation, years, mean (SD)	58.2 (7.1)	58.2 (7.1)	[1]
Current smoking, n (%)	344 (25.2)	338 (24.7)	0.78
University degree, n (%)	232 (17.0)	239 (17.5)	0.71
Diabetes at baseline, n (%)	60 (4.5)	56 (4.2)	0.77
Body mass index, kg/m^2, mean (SD)	26.8 (4.1)	26.5 (3.9)	0.01
Waist circumference, cm, mean (SD)	90.7 (12.9)	88.9 (12.3)	<0.0001
Physically inactive, n (%)	363 (26.6)	316 (23.1)	0.02
Physical activity, METs/week, median (IQR)	73. 9 (44.5-115.6)	79.1 (46.3-121.0)	0.04
Alcohol consumption, g/day, median (IQR)	8.8 (1.5-24.1)	8.1 (1.7-22.1)	0.15
Dietary intake			
Energy intake, kcal/day, median (IQR)	2,074 (1,678-2,487)	2,037 (1,651-2,465)	0.72
Fiber intake, g/day, median (IQR)	22.0 (17.5-26.9)	22.9 (18.0-27.7)	0.01
Fruits and vegetables intake, g/day, median (IQR)	370.1 (248.6-529.4)	382.6 (249.1-561.0)	0.08
Red meat intake, g/day, median (IQR)	47.7 (25.1-75.0)	46.2 (24.8-74.0)	0.32
Processed meat intake, g/day, median (IQR)	25.4 (13.0-43.8)	23.9 (12.6-43.6)	0.19
Fish intake, g/day, median (IQR)	28.2 (15.2-49.3)	29.7 (14.6-51.5)	0.08
Biomarker concentration			
Fetuin-A, µg/mL, median (IQR)	227.7 (202.0-255.1)	225.1 (200.2-251.8)	0.02
CRP, mg/L, median (IQR)	2.79 (1.15-5.05)	2.35 (1.04-4.69)	0.04
C-peptide, ng/mL, median (IQR)	3.98 (2.84-6.04)	3.98 (2.71-5.92)	0.08
HbA1c, %, median (IQR)	5.70 (5.50-6.00)	5.70 (5.50-6.00)	0.10
IGF1, ng/mL, median (IQR)	210.0 (165.3-255.3)	205.9 (165.3-247.0)	0.96
Total cholesterol, mmol/L, median (IQR)	6.33 (5.56-7.08)	6.40 (5.80-7.23)	0.30
LDL cholesterol, mmol/L, median (IQR)	4.17 (3.50-4.84)	4.20 (3.55-4.91)	0.65
HDL cholesterol, mmol/L, median (IQR)	1.40 (1.14-1.69)	1.43 (1.18-1.77)	0.02
Triglycerides, mmol/L, median (IQR)	1.52 (1.04-2.19)	1.48 (1.00-2.16)	0.35
Total adiponectin, µg/mL, median (IQR)	6.37 (4.61-8.98)	6.87 (5.09-9.10)	0.004
Leptin, ng/mL, median (IQR)	8.33 (4.08-15.70)	7.25 (3.50-15.60)	0.07
Soluble Leptin Receptor, ng/mL, median (IQR)	20.3 (16.1-24.4)	21.0 (17.4-26.0)	0.003

P-values for the difference between cases and controls based on Mc Nemar's test for variables expressed as %, student's paired t-test for variables expressed as means, and Wilcoxon's two-sample test for variables expressed as medians

[1] matching variable

SD, standard deviation; IQR, inter-quartile range; CRP, C-reactive protein; IGF1, insulin-like growth factor 1; LDL, low-density lipoprotein; HDL, high-density lipoprotein

Table 2. Association between plasma fetuin-A concentrations and risk of colorectal cancer

| Fetuin-A | Ca/Co | OR[1] | (95% CI) | OR[2] | (95% CI) | First 2 years of follow-up excluded | | |
						Ca/Co	OR[2]	(95% CI)
Colorectal cancer, overall								
Quintile 1	268/271	1.00	Reference	1.00	Reference	221/210	1.00	Reference
Quintile 2	254/281	0.93	(0.73-1.19)	0.94	(0.73-1.21)	211/235	0.89	(0.67-1.17)
Quintile 3	274/283	0.98	(0.76-1.25)	0.96	(0.75-1.24)	226/244	0.86	(0.65-1.14)
Quintile 4	266/269	1.03	(0.78-1.34)	1.01	(0.77-1.33)	228/230	0.96	(0.71-1.30)
Quintile 5	305/263	1.27	(0.95-1.70)	1.22	(0.91-1.63)	264/231	1.11	(0.80-1.53)
p-trend			0.08		0.16			0.43
per 40 µg/ml (1 SD)		**1.13**	**(1.02-1.24)**	**1.11**	**(1.01-1.22)**		1.09	(0.98-1.21)
Colorectal cancer, men								
Quintile 1	131/134	1.00	Reference	1.00	Reference	101/103	1.00	Reference
Quintile 2	124/123	1.12	(0.76-1.63)	1.13	(0.77-1.65)	102/97	1.19	(0.77-1.84)
Quintile 3	116/148	0.82	(0.56-1.20)	0.81	(0.55-1.20)	90/126	0.75	(0.48-1.16)
Quintile 4	124/129	1.02	(0.68-1.53)	1.01	(0.67-1.51)	105/109	1.06	(0.68-1.68)
Quintile 5	162/123	**1.62**	**(1.04-2.51)**	1.55	(1.00-2.41)	141/104	**1.66**	**(1.01-2.72)**
p-trend			0.06		0.09			0.08
per 40 µg/ml (1 SD)		**1.21**	**(1.05-1.39)**	**1.18**	**(1.03-1.37)**		**1.21**	**(1.03-1.42)**
Colorectal cancer, women								
Quintile 1	137/137	1.00	Reference	1.00	Reference	120/107	1.00	Reference
Quintile 2	130/158	0.79	(0.56-1.12)	0.80	(0.57-1.13)	109/138	0.68	(0.47-1.00)
Quintile 3	158/135	1.11	(0.79-1.58)	1.10	(0.77-1.56)	136/118	0.94	(0.64-1.38)
Quintile 4	142/140	1.00	(0.69-1.46)	0.99	(0.68-1.44)	123/121	0.86	(0.56-1.30)
Quintile 5	143/140	1.02	(0.68-1.53)	0.99	(0.66-1.49)	123/127	0.81	(0.51-1.29)
p-trend			0.58		0.71			0.72
per 40 µg/ml (1 SD)		1.06	(0.93-1.22)	1.05	(0.92-1.21)		1.00	(0.86-1.17)
p for heterogeneity by sex [3]			0.21		0.24			0.18

Quintile cut-offs were 195.2, 216.3, 235.8 and 259.0.

[1]Conditioned on matching factors and adjusted for education (no degree/primary, technical/professional, secondary, university, not specified), physical activity (sex-specific quartiles), smoking status (never, past, current, unknown), nondrinker, alcohol intake (continuous, g/day), energy intake (continuous, kcal/day), fiber intake, fruit and vegetable intake, red meat intake, processed meat intake, fish intake (continuous, g/day)

[2] additionally adjusted for body mass index and waist circumference residuals

[3] p-value for heterogeneity by sex derived from inverse variance method (Q-statistic for heterogeneity)

Ca/Co, Number of cases/number of controls; OR, Odds ratio

Table 3. Association betweeen plasma fetuin-A concentrations and risk of colon and rectal cancer

Fetuin-A	Ca/Co	OR[1]	(95% CI)	OR[2]	(95% CI)	First 2 years of follow-up excluded Ca/Co	OR[2]	(95% CI)
Colon cancer								
Quintile 1	177/181	1.00	Reference	1.00	Reference	149/139	1.00	Reference
Quintile 2	158/183	0.88	(0.65-1.20)	0.90	(0.66-1.23)	137/155	0.83	(0.59-1.17)
Quintile 3	173/181	0.95	(0.69-1.31)	0.94	(0.68-1.29)	144/156	0.82	(0.58-1.17)
Quintile 4	176/170	1.05	(0.75-1.48)	1.05	(0.75-1.48)	152/150	0.91	(0.63-1.33)
Quintile 5	188/161	1.28	(0.90-1.82)	1.23	(0.86-1.75)	159/144	1.00	(0.67-1.49)
p-trend			0.11		0.19			0.85
per 40 µg/ml (1 SD)		**1.13**	**(1.00-1.27)**	1.11	(0.99-1.25)		1.04	(0.92-1.19)
Rectal cancer								
Quintile 1	91/90	1.00	Reference	1.00	Reference	72/71	1.00	Reference
Quintile 2	96/98	1.04	(0.67-1.62)	1.04	(0.67-1.61)	74/80	1.02	(0.62-1.68)
Quintile 3	101/102	0.98	(0.63-1.52)	0.96	(0.62-1.49)	82/88	0.94	(0.58-1.52)
Quintile 4	90/99	0.96	(0.60-1.53)	0.93	(0.58-1.49)	76/80	1.05	(0.62-1.77)
Quintile 5	117/102	1.26	(0.75-2.13)	1.21	(0.71-2.05)	105/87	1.46	(0.82-2.61)
p-trend			0.49		0.61			0.23
per 40 µg/ml (1 SD)		1.12	(0.94-1.32)	1.10	(0.92-1.30)		1.18	(0.98-1.43)
p for heterogeneity by location[3]			0.08		0.89			0.57

Quintile cut-offs were 195.2, 216.3, 235.8 and 259.0.

[1] Conditioned on matching factors and adjusted for education (no degree/primary, technical/professional, secondary, university, not specified), physical activity (sex-specific quartiles), smoking status (never, past, current, unknown), nondrinker, alcohol intake (continuous, g/day), energy intake (continuous, kcal/day), fiber intake, fruit and vegetable intake, red meat intake, processed meat intake, fish intake (continuous, g/day)

[2] additionally adjusted for body mass index and waist circumference residuals

[3] p for heterogeneity by location based on competing risk test

Ca/Co, Number of cases/number of controls; OR, Odds ratio

Figure Legends

Figure 1. Association between *AHSG* genetic variation (individual *AHSG* SNPs and combined score) and fetuin-A concentration among controls with available data on both fetuin-A and *AHSG* genotypes (n=483)

Bars indicate 95% confidence intervals. P-values for trend were <0.0001 except for rs6787344 (p for trend 0.08). Models were unadjusted.

* Incorporated in *AHSG*-score (weighted allele-score, created by summing each genotype multiplied by its estimated coefficient from the linear regression, divided by the sum of weights).

[†] Continuous estimate (95% CI) for mean difference in fetuin-A (µg/mL)

Figure 1

Fetuin-A (μg/mL)