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Association between dietary factors and plasma fetuin-A concentrations in the general population

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Abstract

Circulating fetuin-A, a novel marker for hepatic fat accumulation, has been related to higher risk of type 2 diabetes and cardiovascular diseases in a growing number of prospective studies. However, little is known about dietary determinants of fetuin-A concentrations in the general population. Therefore, we aimed to investigate the association between dietary intake of energy, energy-providing nutrients, alcohol, and major food groups and plasma fetuin-A concentrations in the Bavarian Food Consumption Survey II. Dietary intake was assessed by three 24-hour dietary recalls and plasma concentrations of fetuin-A were measured in 558 adults (18-81 years). After multivariable adjustment for life-style factors and body fatness, higher energy intake was non-significantly associated with higher fetuin-A concentrations (per 500 kcal/day 3.7 µg/mL, 95% CI -0.5, 7.8 µg/mL). There was no clear association between energy-providing nutrients and fetuin-A concentrations. Higher alcohol intake was associated with lower fetuin-A concentrations (p-trend 0.003): mean (95% CI) fetuin-A concentrations were 324 (313, 335) µg/mL in nondrinkers, and with 293 (281, 306) µg/mL significantly lower in participants who drank ≥30 g alcohol per day. Mean (95% CI) fetuin-A concentrations decreased across quintiles of milk and dairy products intake (lowest quintile 319 (309; 330) µg/mL, highest quintile 304 (293, 314) µg/mL, p-trend 0.03) and each 150 g increment in milk/dairy products per day was associated with 5.6 (95% CI -9.6, 1.5) µg/mL lower fetuin-A. Dietary intakes of vegetables, meat or fish were not associated with fetuin-A concentrations. Due to the preventive potential of our findings further exploration is warranted.

Introduction

Fetuin-A, also referred to as α2-Heremans-Schmid glycoprotein (AHSG), is a protein synthesized and secreted by the liver, particularly in hepatic steatosis [1]. To a lesser degree fetuin-A is also secreted by placenta and tongue and recent findings suggest that it is also expressed and secreted by adipose tissue [1,2]. Fetuin-A is related to hepatic insulin resistance and subclinical inflammation and has been suggested as a novel marker for hepatic fat accumulation [1,3,4]. Mice deficient for the *AHSG* gene are resistant to weight gain upon a high-fat diet [5,6]. Observational studies have shown a positive association between fetuin-A and obesity [3,7] and a recent bidirectional Mendelian Randomization study suggests that fetuin-A is causally associated with higher body mass index [8]. In addition, there is growing evidence from prospective studies that high plasma fetuin-A concentrations are associated with higher risk of type 2 diabetes [4,9] and cardiovascular diseases [7]. Taken together fetuin-A plays a role in a number of metabolic conditions and chronic diseases. Therefore, knowledge of modifiable determinants of circulating fetuin-A has direct public health relevance. However, so far little is known about dietary determinants of fetuin-A concentrations in the general population. In a randomized clinical trial among 76 overweight diabetic women calorie restriction resulted in a decrease in fetuin-A concentrations[10]. In a cross-sectional analysis within a sub-cohort from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study, dietary intake of red meat or whole-grain were not associated with fetuin-A concentrations [11]. In a recent analysis of women from the Nurses' Health Study an inverse association between alcohol consumption and fetuin-A concentrations was observed, and fetuin-A explained a substantial proportion of the inverse association between alcohol consumption and risk of type 2 diabetes [12]. In both, the EPIC-Potsdam Study and the Nurses' Health Study, dietary intake was assessed using food frequency questionnaires (FFQ).

With this study, we aimed to investigate the association between energy intake, energy-providing nutrients, alcohol consumption, and major food groups and plasma fetuin-A concentrations in the Bavarian Food Consumption Survey II, a population-based survey, in which dietary intake was assessed by three 24-hour dietary recalls.

Experimental methods

Study design and population

The second Bavarian Food Consumption Survey (BVS II) is a cross-sectional study designed to be representative for the Bavarian population. The aim of the study was to investigate dietary and life-style habits in Bavaria. The study population comprises 1,050 German-speaking participants aged 13-80 years who were recruited in a three-stage random route sampling procedure between September 2002 and June 2003. Participants' characteristics, life-style factors, and medical history were collected during a computer-aided personal interview. Within two weeks after recruitment, trained interviewers contacted participants by telephone three times (two weekdays, one weekend day) to assess dietary intake by 24-hour dietary recalls. In addition to dietary information, physical activity on the previous day was assessed at the end of each telephone interview using standardized questions on type and duration of physical activity as well as duration of television/personal computer time and sleeping hours. The overall participation rate was 71%. Within six weeks after recruitment, all adults (≥18 years) who had completed the baseline interview and at least one dietary recall were invited to their nearest public health office for blood sampling and anthropometrical measurements. Out of 879 invited subjects, 568 persons followed the invitation (65% of eligible persons). The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the local ethics committee (Bavarian Ministry of Health) [13]. Written informed consent was obtained from all study participants.

Dietary intake assessment

The 24-hour dietary recalls were conducted using the EPIC-Soft software [14,15]. The data from the three recalls per person were weighted for weekday and weekend day to calculate the average daily food intake. The different foods reported during the 24-hour dietary recalls were grouped into 17 food groups. Nutrient intakes were calculated using food content data from the German food composition database "Bundeslebensmittelschluessel" (version II.3) [16].

Blood sampling and laboratory measurements

Venous blood was drawn into EDTA tubes or serum tubes, chilled at 4°C, and processed subsequently within three hours. Serum was separated from blood cells by centrifugation and samples were divided into aliquots. Samples were cooled for a maximum of one day until they

were stored at -80°C. Plasma concentrations of fetuin-A were measured by enzyme linked immunosorbent assays (BioVendor Human Fetuin-A ELISA; intra-assay coefficient of variation 3.9-6.5%, inter-assay coefficient of variation 2.6-5.1% according to the manufacturer) in the laboratory of Prof. Pischon, Molecular Epidemiology Group, Max Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Germany. Based on internal quality control samples (2 on each of the 8 analysis plates), inter-assay coefficient of variation was 7.7%.

Statistical analysis

After exclusion of participants with missing information on diet (n=7) or fetuin-A (n=3), 558 participants (235 men, 323 women) were included in the statistical analysis. Waist circumference was missing for a few (n=8) study participants. For statistical analysis, these missing values were replaced with sex-specific median values. In descriptive statistics, we compared participants' characteristics across sex-specific quintiles of fetuin-A concentrations. The association between total energy intake, energy-providing nutrients (dietary fat, carbohydrates, protein), alcohol, or major food groups (vegetables, fruit, milk/dairy products, unprocessed red meat, processed meat, poultry and fish) and fetuin-A concentrations was investigated using multivariable linear regression models with robust variance [17]. Results are presented as mean fetuin-A concentrations with corresponding confidence intervals (95% CI) in quintiles or categories of dietary intake. In addition, continuous estimates showing the increase or decrease in fetuin-A associated with a pre-specified increment in dietary intake are presented. Multivariable models were adjusted for age (continuous in years), sex, smoking status (never, former, current), social status (5 categories), physical activity (sex-specific quintiles of total activity in MET-hours/day), alcohol intake (except for models investigating alcohol intake; nondrinker or continuous in g/day) and non-alcohol energy intake (continuous in kcal/day with and without additional adjustment for body mass index (BMI, continuous, kg/m^2) and residuals of BMI-adjusted waist circumference (to avoid multicollinearity; continuous). Fasting status was not included in the multivariable models because it was not related to fetuin-A concentrations and inclusion of fasting status into the models did not alter parameter estimates of dietary variables substantially. For the analysis of dietary fat, carbohydrates or protein intake, we created multivariable energy-density models with nutrient intake expressed in percentage of total energy intake and mutual adjustment for energy

intake. The continuous estimates from these models estimate the change in circulating fetuin-A associated with a 5 percent higher energy intake provided by the nutrient under study in substitution with 5 percent of energy provided by carbohydrates (or fat). Because of the relatively large proportion of non-drinkers (10% in men and 19% in women), for the analysis of the association between alcohol consumption and fetuin-A we present adjusted mean fetuin-A concentrations in established categories for alcohol intake [18]. Similarly, due to the low consumption of poultry and fish, mean fetuin-A concentrations are presented by categories of these two variables (non-consumers, </≥40 g/day, which corresponds to the approximate median cut-offs). Tests for linear trends across dietary intake quintiles or categories were performed by modeling the median values in each quintile/category and evaluating this variable's statistical significance using the Wald's test. To correct for multiple hypothesis testing, we took the false-discovery rate into account, counting each dietary factor under investigation as an independent hypothesis test[19] (n=12). In pre-specified subgroup analyses, we tested for statistical interaction in the association between dietary factors and fetuin-A by sex using cross-product terms. Because no statistically significant interactions by sex were observed (all p-values >0.2), only combined associations in the whole study population are presented. However, because underreporting of energy intake has been shown to depend on sex and BMI [20,21] , we also describe the association between energy intake and fetuin-A concentrations stratified by sex and body mass index (</≥25 kg/m^2). In addition, to investigate the robustness of observed associations we conducted sensitivity analyses. First, we excluded participants who were suspected to be underreporters of dietary intake based on a low ratio of energy intake (EI) to estimated basal metabolic rate (BMR) (EI/BMR<0.8, n=18 men, n=28 women) [22]. Second, we excluded study participants with metabolic diseases (n=203), i.e. participants who were obese (BMI >=30 kg/m2, n=110) and/or had reported prevalent diabetes (n=37) or hypertension (n=138).

Results

Mean fetuin-A concentrations were 303.7 µg/mL in men, ranging from 177.6 to 462.7 µg/mL and in women the mean was 314.7 µg/mL, with a range from 182.4 to 531.9 µg/mL. The mean age of study participants decreased across quintiles of fetuin-A concentrations (**Table 1**). Body weight and body mass index slightly increased across fetuin-A quintiles, but no significant trend was

observed. Mean dietary intakes of energy, alcohol, energy-providing nutrients and major food groups in male and female BVSII participants are shown in supplemental table 1.

There was no clear association between energy intake or intake of energy-providing nutrients with fetuin-A concentrations (**table 2)**. We observed a slight suggestion of a positive association between total energy intake and circulating fetuin-A (per 500 kcal/day 3.7 µg/mL, 95% CI -0.5, 7.8 µg/mL in the multivariable model including body fatness), but the adjusted mean values across quintiles were not suggestive of a linear trend (p-trend 0.16).. The continuous estimate was slightly higher but still statistically non-significant (4.5 µg/mL, 95% CI -0.3, 9.2 µg/mL) when underreporters of energy intake were excluded (sample size after exclusion n=512). While a statistically significant association between energy intake and fetuin-A concentrations was observed in men (continuous estimate 6.4 µg/mL, 95% CI 1.2, 11.5 µg/mL, p-trend 0.02) there was no association in women (2.2 µg/mL, 95% CI -4.6, 9.1 µg/mL, p-trend 0.52; p-interaction 0.84). After stratification by body mass index, we observed a statistically significant continuous estimate (7.9 µg/mL, 95% CI 0.1, 15.6 µg/mL) and trend across quintiles (p-trend 0.02) in lean study participants (BMI<25 kg/m^2,;), but not in overweight participants (BMI≥25 kg/m^2, continuous estimate 2.0 µg/mL, 95% CI -2.8, 6.7 µg/mL, p-trend 0.87), although no multiplicative interaction was observed ($p_{interaction}$ 0.40). Mean fetuin-A concentrations increased slightly across quintiles of fat intake and decreased slightly across quintiles of carbohydrate or protein intake. However, there was no suggestion of any important association between energy-providing nutrient intake and fetuin-A concentrations. We observed an inverse association between alcohol consumption and fetuin-A concentrations (**table 3**). In the multivariable model including body fatness mean fetuin-A concentrations (95% CI) were 324 (313, 335) µg/mL in nondrinkers, 311 (302, 320) µg/mL in individuals with low alcohol consumption (<5 g/day), 314 (304, 323) µg/mL in individuals with low-moderate alcohol consumption (5-14.9 g/day), 303 (292, 314) µg/mL in moderate drinkers (15-29.9 g/day) and 293 (281, 306) in individuals in the highest alcohol consumption category (≥ 30 g/day) and a statistically significant trend across alcohol intake categories was observed (p-trend 0.003). The inverse association remained statistically significant after taking the false-discovery rate into account (adjusted p-value 0.04) The adjusted mean fetuin-A values across alcohol intake categories (data not shown) were similar in men (p-trend 0.04) and in women (p-trend 0.04), in lean participants (BMI<25 kg/m^2, p-trend

0.66 and overweight (BMI ≥25 kg/m^2, p-trend 0.05 participants, and after exclusion of participants with metabolic diseases (p-trend 0.003).

The associations between dietary intake of major food groups and circulating fetuin-A are shown in **table 4**: We observed a statistically significant inverse association between milk and dairy products intake and circulating fetuin-A : Mean fetuin-A concentrations decreased across quintiles of milk and dairy products (p-trend 0.03) and each 150g increment in milk/dairy products per day was associated with 5.6 (95% CI -9.6, -1.5) µg/mL lower fetuin-A. This inverse association was slightly attenuated after additional adjustment for protein (-4.9 µg/mL, 95% CI -9.1, -0.7 µg/mL), but was not substantially altered after additional adjustment for fat (-5.4 µg/mL, 95% CI -9.4, -1.4 µg/mL, p-value 0.02) or calcium intake (-5.4 µg/mL, 95% CI -11.2, 0.4 µg/mL, p-value 0.07). Milk, yogurt and cheese were also individually inversely associated with fetuin-A (milk per 100 g/day -3.3 µg/mL, 95% -6.5, -0.2 µg/mL; yogurt per 50 g/day -2.0 µg/mL, 95% -5.6, 1.7 µg/mL; cheese per 30 g/day -2.4 µg/mL, 95% CI -7.5, 2.8). However, the inverse association between milk and dairy products intake and fetuin-A concentrations was statistically non-significant after accounting for the false-discovery rate (adjusted p-trend 0.18). Fetuin-A concentrations were not associated with dietary intake of vegetables, fruit, unprocessed red meat, processed meat, poultry or fish.

Discussion

To our knowledge this is the first observational study comprehensively investigating dietary determinants of fetuin-A. Total energy intake and energy-providing nutrients were not clearly associated with fetuin-A concentrations. We observed that alcohol intake was associated with lower fetuin-A concentrations. Among the major food groups, higher dietary intake of milk/dairy products was associated with lower circulating fetuin-A, but this association was not statistically significant after correction for multiple hypothesis testing. Dietary intakes of fruit, vegetables, meat or fish were not associated with fetuin-A concentrations. Due to the described association between fetuin-A and obesity, insulin resistance, diabetes and coronary heart disease, modulation of fetuin-A concentration by dietary guidance may be of public health relevance.

The here observed inverse association between alcohol intake and fetuin-A is largely supported by the existing literature: Univariable inverse associations between alcohol intake and fetuin-A have been observed in several epidemiological studies [4,7,9,23-25]. Similar to our investigation, higher alcohol consumption was associated with lower fetuin-A concentrations in women participating in the Nurses' Health Study after adjustment for demographic information and lifestyle variables including body mass index [12]. Furthermore, post-hoc analyses of three randomized crossover trials on alcohol intake revealed that moderate alcohol consumption decreased fetuin-A in men, although no significant association was observed in women [26]. As to date, the physiological mechanisms that may explain the inverse association between alcohol intake and fetuin-A remain unclear.

We also observed that higher milk/dairy product intake was statistically significantly associated with lower fetuin-A, although statistical significance was lost after accounting for the false-discovery rate. The inverse association was slightly attenuated after additional adjustment for protein, suggesting that protein may partly explain the association. However, adjustment for other nutrients found in dairy, such as fat or calcium did not alter associations remarkably. Several epidemiological studies have observed an inverse association between dairy consumption and presence of the metabolic syndrome [27,28]. In a prospective study among young adults, inverse associations between dairy consumption and the development of obesity and insulin resistance were observed [29]. Furthermore, a few intervention studies have shown that dairy consumption is associated with improved insulin sensitivity [30]. Considering the role of fetuin-A in the insulin signaling pathway [31], i.e. induction of insulin resistance through inhibition of insulin-receptor

tyrosin kinase [32], it is conceivable that fetuin-A may play a mediating role in the association between dairy consumption and improved insulin sensitivity. This warrants further exploration in prospective studies.

Several limitations of this study should be noted. First of all, due to the cross-sectional study design in which the dietary exposure and the biomarker outcome both were assessed within a short time period, it is difficult to determine the direction of observed associations and we cannot make any causal inferences. We hypothesized that dietary factors would influence fetuin-A concentrations. It appears unlikely that fetuin-A concentrations directly influence dietary habits, but we cannot exclude that for example existing fatty liver disease reflected by high fetuin-A concentrations may have led to a change in dietary habits, due to dietary recommendations given by general practitioners. In addition, although through multivariable adjustment we tried to control for potential confounding as completely as possible, residual confounding cannot be excluded. We also cannot exclude the possibility that storage of plasma samples at -80°C for approximately 10 years may have affected fetuin-A concentrations. However, any such impact on fetuin-A measurement is unlikely to be differential according to participant's dietary intake, thus is unlikely to have introduced systematic bias. Furthermore, the absolute fetuin-A concentrations in our study were comparable to concentrations that have been observed in other observational studies in Germany [1,7]. In our study habitual dietary intake was assessed with three 24-hour dietary recalls involving detailed quantification of consumed foods including composition of mixed meals. As with all self-reported methods, the dietary assessment by three 24-hour recalls is prone to measurement error since it depends on the participants' memory and ability to recall their diet. In addition, because the 24-hour recall is an open-ended instrument, the interviewer is also a potential source of bias. However, the training of interviewers and the high standardization of the 24-hour recalls conducted with EPIC-soft limits this source of bias[15]. The here applied number of three 24-hour recalls has been shown to be sufficient for estimating total energy intake with energy intake assessment using the doubly labelled water (DLW) as reference method [33]. However, misclassification of diet, in particular underreporting of energy intake remains a concern when relating self-reported dietary intake to health outcomes. Underreporting of energy intake has been reported to be more prevalent individuals with a high BMI and in women [20,21]. A recent pooled analysis of five DLW validation studies demonstrated that underreporting of energy intake was approximately 10% with three averaged 24-hour recalls compared with 30% with FFQs [34]. Although dietary intake is reported more accurately with multiple 24-hour recalls

than with FFQs, underreporting of energy intake played a role also in the present study: by comparing the reported energy intake with the estimated basal metabolic rate we identified a proportion of underreporters of energy intake (n=46, 8%) in our sample. In the analysis of energy intake in relation to fetuin-A concentrations, continuous estimates were slightly stronger after exclusion of underreporters, and statistically significant only in lean study participants and in men. These observations point to the problem of misreporting. Thus, the analysis of energy intake in relation to fetuin-A should be interpreted with caution. Overall, we expect misreporting of diet in our study to be non-differential, i.e. misclassification independent of fetuin-A concentrations, which may have biased observed associations towards a null association. A further limitation of our study is related to the generalizability of our findings. Although the BVS II was designed as a representative study the here observed findings can be generalized to the adult Bavarian population only with caution, since the overall participation of adult study participants who also provided blood samples was 46% (71%x65%), thus compromising the representativeness of our study sample. Whether our findings may be generalized to other populations warrants further investigation but we expect that the associations found in our study should be comparable in populations with similar characteristics as in our study.

In conclusion, in this comprehensive investigation of dietary determinant of fetuin-A we observed that higher consumption of alcohol and dietary intake of milk/dairy products were associated with lower fetuin-A concentrations. These observations warrant confirmation by further observational studies or controlled feeding intervention studies. Nevertheless, our findings provide a first suggestion that fetuin-A concentrations may be influenced by targeted dietary interventions. Considering the role of fetuin-A in the development of obesity, insulin resistance, diabetes and coronary heart disease, this may be of direct public health importance. Whether previously observed associations between dietary intake and health outcomes are mediated by fetuin-A requires exploration in prospective cohort studies.

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Authorship

The authors' contributions were as follows – KN, TP, JL: contributed to the conception and design of the research; KN: performed statistical analyses, interpreted the data, and wrote the paper; JL was responsible for the concept and design of the BVSII as well as the acquisition of data; JJ: was responsible for the measurement of fetuin-A in plasma samples; all authors critically appraised the manuscript and approved the final version.

Conflict of Interest

None.

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Table 1. Basic characteristics (mean values and standard deviations or numbers and percentages) by quintiles of circulating fetuin-A in 558 men and women who participated in the Bavarian Food Consumption Survey II

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
N	111	112	112	112	111
Female sex, n (%)	64 (57.7)	65 (58.0)	65 (58.0)	65 (58.0)	64 (57.7)
Age, years, mean (SD)	50.4 (13.8)	49.3 (15.9)	51.2 (15.9)	46.8 (14.5)	43.5 (14.7)
Current smoking, n (%)	60 (54.1)	58 (51.8)	60 (53.6)	61 (54.5)	52 (46.8)
Physical activity (MET/hours per day)	13.4 (13.6)	10.4 (8.4)	11.4 (8.8)	12.2 (8.9)	11.6 (10.4)
Waist circumference, cm, mean (SD)	92.4 (14.6)	94.6 (14.4)	95.8 (14.6)	93.9 (12.5)	94.9 (14.6)
BMI, kg/m^2, mean (SD)	26.0 (4.9)	26.5 (4.8)	27.3 (5.3)	26.9 (4.6)	26.6 (4.9)
Obese, n (%)	17 (15.3)	26 (23.2)	23 (20.5)	21 (18.8)	23 (20.7)
Prevalent diabetes, n (%)	10 (9.0)	9 (8.0)	10 (8.9)	4 (3.6)	4 (3.6)
Prevalent hypertension, n (%)	28 (25.2)	26 (23.2)	36 (32.1)	25 (22.3)	23 (20.7)

Quintile cutoffs were 264, 291, 314 and 344µg/mL in men and 265, 299, 325, and 358 µg/mL in women.

SD, standard deviation

Table 2. Multivariable adjusted mean fetuin-A concentrations (95% CI) by quintiles of energy intake, energy-providing nutrient intake in 558 men and women who participated in the Bavarian Food Consumption Survey II

	Quintiles of energy intake or energy-providing nutrients						
	Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	**Continuous estimate** [§]	**p-trend**
	Mean Fetuin-A (95% CI)						
Energy							
Age- and sex-adjusted	303 (293; 313)	317 (307; 327)	301 (291; 310)	317 (307; 327)	313 (302; 323)	2.7 (-1.4; 6.8)	0.31
Multivariable*	301 (291; 311)	316 (306; 327)	301 (291; 311)	318 (308; 327)	312 (302; 323)	3.3 (-0.8; 7.4)	0.20
Multivariable* plus body fatness	300 (290; 310)	316 (305; 326)	301 (291; 311)	318 (308; 327)	313 (302; 323)	3.7 (-0.5; 7.8)	0.16
Fat (percent of energy)							
Age- and sex-adjusted	305 (295; 315)	306 (296; 315)	311 (301; 320)	315 (304; 325)	315 (305; 325)	3.0 (-0.1; 6.2)	0.09
Multivariable (carbohydrate substitution model)[†]	306 (295; 317)	307 (297; 318)	310 (301; 320)	313 (302; 323)	312 (302; 323)	1.8 (-1.6; 5.2)	0.33
Multivariable plus body fatness[‡]	306 (296; 317)	308 (298; 318)	311 (302; 321)	312 (301; 322)	311 (300; 321)	1.6 (-1.8; 4.9)	0.47
Carbohydrates (percent of energy)							
Age- and sex-adjusted	315 (303; 326)	306 (296; 315)	307 (298; 317)	312 (302; 322)	311 (301; 321)	0.3 (-2.7; 3.4)	0.89
Multivariable (fat substitution model)[†]	320 (308; 331)	307 (297; 318)	306 (296; 316)	309 (298; 319)	306 (295; 316)	-1.8 (-5.2; 1.6)	0.14
Multivariable plus body fatness**	319 (307; 330)	307 (297; 317)	306 (297; 316)	309 (299; 319)	306 (296; 316)	-1.6 (-4.9; 1.8)	0.17
Protein (percent of energy)							
Age- and sex-adjusted	319 (309; 329)	309 (299; 320)	304 (295; 312)	307 (297; 317)	311 (301; 322)	-2.4 (-10.5; 5.6)	0.31
Multivariable (carbohydrate substitution model)[l]	319 (308; 330)	308 (298; 318)	304 (295; 313)	306 (296; 317)	312 (301; 323)	-2.2 (-10.6; 6.1)	0.35
Multivariable plus body fatness[l]	321 (310; 332)	309 (298; 319)	303 (294; 312)	306 (296; 316)	309 (298; 320)	-4.9 (-13.3; 3.6)	0.11

[§] Increments are: 500 kcal/day for total energy; 5% of energy for fat, carbohydrates, and protein;

[*] Multivariabe adjusted for age, sex, smoking status, social status, physical activity, alcohol intake (nondrinker or g/day); NOTE: energy intake refers to non-alcohol energy intake

[†] Multivariabe adjusted for age, sex, smoking status, social status, physical activity, energy intake, alcohol intake (nondrinker or % of energy), protein intake (% of energy)

[l] Multivariabe adjusted for age, sex, smoking status, social status, physical activity, energy intake, alcohol intake (nondrinker or % of energy), fat intake (% of energy), BMI and waist circumference residuals

NOTE: energy intake refers to non-alcohol energy intake; plus body fatness refers to additional adjustment for body mass index (BMI) and BMI-adjusted waist circumference residuals

Table 3. Multivariable adjusted mean fetuin-A concentrations (95% CI) by categories of alcohol consumption in 558 men and women who participated in the Bavarian Food Consumption Survey II

	Alcohol intake categories					
	Nondrinker	**<5 g/d**	**5-<15 g/d**	**15-<30 g/d**	**≥30 g/d**	**p-trend**
	Mean Fetuin-A (95% CI)					
n	86	161	134	94	83	
Age- and sex-adjusted	325 (314; 335)	311 (302; 319)	314 (305; 323)	302 (291; 313)	297 (285; 308)	0.004
Multivariable adjusted†	326 (315; 337)	311 (302; 320)	313 (303; 323)	302 (290; 313)	295 (282; 307)	0.003
Multivariable adjusted† plus body fatness	324 (313; 335)	311 (302; 320)	314 (304; 323)	303 (292; 314)	293 (281; 306)	0.003

† Multivariabe adjusted for age, sex, smoking status, social status, physical activity, and energy intake (excluding energy from alcoholic beverages)
NOTE: plus body fatness refers to additional adjustment for body mass index (BMI) and BMI-adjusted waist circumference residuals

Table 4. Multivariable adjusted mean fetuin-A concentrations (95% CI) by quintiles or categories of major food groups in 558 men and women who participated in the Bavarian Food Consumption Survey II

	Quintiles or categories of food intake						
	Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	**Continuous estimate ***	**p-trend**
	Mean Fetuin-A (95% CI)						
Vegetables							
Age- and sex-adjusted	320 (310; 330)	312 (301; 323)	301 (291; 311)	307 (298; 317)	310 (300; 320)	-2.5 (-7.5; 2.44)	0.22
Multivariable*	320 (309; 331)	312 (302; 323)	301 (291; 312)	307 (297; 317)	308 (298; 318)	-3.3 (-8.2; 1.64)	0.13
Multivariable* plus body fatness	320 (309; 331)	313 (302; 323)	301 (291; 311)	307 (297; 317)	308 (298; 318)	-3.2 (-8.1; 1.70)	0.13
Fruit							
Age- and sex-adjusted	314 (304; 325)	305 (294; 316)	314 (304; 324)	310 (300; 320)	307 (298; 316)	-1.5 (-6.3; 3.36)	0.52
Multivariable*	314 (303; 324)	307 (296; 318)	314 (304; 325)	309 (299; 319)	303 (294; 313)	-3.3 (-8.1; 1.52)	0.16
Multivariable* plus body fatness	313 (302; 323)	306 (295; 317)	316 (305; 326)	307 (297; 317)	307 (297; 317)	-1.6 (-6.6; 3.30)	0.46
Dairy							
Age- and sex-adjusted	318 (309; 328)	310 (300; 319)	314 (304; 324)	304 (293; 316)	304 (294; 314)	**-4.6 (-8.8; -0.43)**	**0.05**
Multivariable*	320 (309; 330)	310 (301; 319)	314 (304; 325)	304 (293; 315)	302 (291; 312)	**-5.9 (-9.9; -1.8)**	**0.02**
Multivariable* plus body fatness	319 (309; 330)	311 (302; 320)	312 (302; 323)	304 (293; 315)	304 (293; 314)	**-4.8 (-9.0; -0.55)**	**0.03**
Unprocessed red meat							
Age- and sex-adjusted	313 (306; 320)	329 (308; 351)	307 (297; 317)	308 (299; 318)	307 (297; 318)	-0.47 (-6.0; 5.00)	0.36
Multivariable*	313 (305; 321)	330 (309; 351)	305 (295; 315)	307 (297; 317)	308 (296; 319)	-0.46 (-6.1; 5.13)	0.38
Multivariable* plus body fatness	314 (306; 322)	329 (308; 350)	306 (296; 316)	308 (298; 318)	305 (294; 316)	-1.9 (-7.5; 3.67)	0.17
Processed meat							
Age- and sex-adjusted	316 (306; 327)	304 (294; 313)	310 (301; 319)	311 (300; 322)	310 (300; 320)	-0.14 (-4.1; 3.87)	0.82
Multivariable*	315 (305; 326)	303 (293; 312)	311 (302; 320)	311 (300; 323)	309 (298; 319)	-0.30 (-4.4; 3.84)	0.77
Multivariable* plus body fatness	317 (306; 327)	304 (295; 314)	310 (301; 320)	311 (299; 322)	307 (297; 318)	-1.6 (-5.8; 2.71)	0.49
	Non-consumers	**<40 g/day**	**≥40 g/day**				
Poultry							
n	382	76	90				
Age- and sex-adjusted	311 (306; 316)	307 (294; 319)	309 (298; 321)			-0.55 (-4.7; 3.61)	0.72
Multivariable*	310 (304; 316)	307 (295; 319)	310 (298; 322)			-0.15 (-4.4; 4.14)	0.91
Multivariable* plus body fatness	310 (304; 317)	307 (295; 319)	309 (298; 321)			-0.40 (-4.7; 3.90)	0.77

Fish					
n	365	90	93		
Age- and sex-adjusted	311 (305; 316)	305 (295; 316)	313 (302; 324)	1.66 (-2.0; 5.27)	0.78
Multivariable*	310 (304; 316)	305 (294; 315)	313 (302; 325)	1.87 (-1.9; 5.62)	0.66
Multivariable* plus body fatness	310 (304; 317)	305 (295; 315)	312 (301; 324)	-1.6 (-5.8; 2.71)	0.49

* Multivariabe adjusted for age, sex, smoking status, social status, physical activity, alcohol intake (nondrinker or g/day), non-alcohol energy intake
NOTE: plus body fatness refers to additional adjustment for body mass index (BMI) and BMI-adjusted waist circumference residuals
Statistically significant results ($p<0.05$) are shown in **bold**.
Portion sizes based on approximate standard deviations

Supplemental table 1. Mean (standard deviation) dietary intake in 558 men and women who participated in the Bavarian Food Consumption Survey II

		Men (n=235) Mean (SD)	Women (n=323) Mean (SD)
Total energy	kcal/day	2348 (640)	1732 (485)
Energy excluding energy from alcohol	kcal/day	2199 (633)	1680 (476)
Alcohol	g ethanol/day	21.2 (20.8)	7.4 (10.2)
	% of total energy	6.5 (6.8)	3.0 (4.1)
Fat	g/day	95.5 (34.5)	70.6 (24.8)
	% of total energy	36.7 (7.5)	36.9 (7.2)
Carbohydrates	g/day	244 (81.8)	195 (68.7)
	% of total energy	42.0 (7.9)	45.4 (8.0)
Protein	g/day	85.7 (26.2)	61.6 (17.3)
	% of total energy	14.9 (2.9)	14.7 (3.1)
Vegetables	g/day	140 (104)	133 (83.6)
Fruit	g/day	124 (153)	147 (145)
Dairy products	g/day	235 (110)	171 (67.3)
Milk	g/day	168 (166)	189 (147)
Cheese	g/day	89.8 (146)	102 (114)
Yogurt	g/day	31.3 (57.7)	43.1 (69.3)
Unprocessed red meat	g/day	30.2 (29.8)	27.8 (26.0)
Processed meat	g/day	50.2 (54.4)	28.5 (32.2)
Poultry	g/day	87.4 (67.9)	44.8 (43.3)
Fish	g/day	17.0 (33.6)	13.6 (26.9)

SD, standard deviation.