Higher afamin concentrations are associated with increases in fatty liver indices: population-based KORA F4/FF4 study

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Table of contents

Supplementary methods..……...pp. 2-4

Supplementary table 1……..p. 5

Supplementary table 2……..p. 6

Supplementary figure 1……p. 7

Supplementary figure 2.....p. 8

Supplementary methods

Assessment of covariates:

Briefly, BMI was calculated as body weight (in kilograms) divided by [height (in m)]². Waist circumference was measured midway between the lower rib margin and the iliac crest.[1] Haemoglobin A_{1c} was measured using cation-exchange high-performance liquid chromatography photometric assays on an Adams HA 8160 Hemoglobin Analysis System (Arkray Inc., distributed by A. Menarini Diagnostics, Florence, Italy), and total cholesterol, high-density lipoprotein and triacylglycerols were determined using an enzymatic colorimetric CHOL Flex, ALDL Flex or TGL Flex assay on a Dimension RxL instrument (Dade Behring Inc., Newark, USA), respectively.[2] The assessment of the glucose tolerance status was performed using standardised oral glucose tolerance tests as described in detail elsewhere.[3] The following three glucose tolerance categories were defined according to the 1999 World Health Organisation diagnostic criteria[4]: normal glucose tolerance (NGT), prediabetes, and type 2 diabetes (T2D). Prediabetes was defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or combined IFG/IGT.

Kidney function was assessed from the estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) serum creatinine equation.[5]

Information on sex, age, smoking status, alcohol consumption and physical activity were obtained by standardised interviews. Participants were asked whether they were current smokers, past smokers or never smokers.[1] With respect to alcohol consumption, study participants were divided into individuals with or without alcohol intake. Alcohol consumers (>30 g/day for men and >20 g/day for women) were excluded from the analyses. Physical activity was defined as more than 1 h of activity per week during leisure time in summer and in winter.[6] Hypertension was defined by systolic blood pressure more than 140 mmHg or diastolic blood pressure more than 90 mmHg (with or without the use of anti-hypertensive medications). Using a database-supported computer software data on the regular use of non-steroidal anti-

inflammatory drugs (NSAIDs) and lipid-lowering drugs were collected.[1] The liver enzymes ALT, AST and GGT were analysed according to the recommendations of the International Federation of Clinical Chemistry from 1983 (confirmed and extended in 2002) as described.[7] Plasma concentrations of high-sensitivity C-reactive protein were measured using a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring) and plasma interleukin-18 levels were determined using the ELISA kits from MBL (Nagoya, Japan) as described before.[8]

References

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The following cut-off values were defined for the afamin quartiles (Q) (mg/L) at F4:

NAFLD liver fat score:
Q1 (male: 46.19-60.27; female: 46.19-57.65),
Q2 (male: 60.28-70.60; female: 57.66-67.73),
Q3 (male: 70.66-82.15; female: 67.73-79.07),
Q4 (male: 82.15-119.48; female: 79.07-119.48)

Hepatic steatosis index:
Q1 (male: 46.22-60.08; female: 46.22-57.65),
Q2 (male: 60.12-70.45; female: 57.66-67.73),
Q3 (male: 70.51-82.08; female: 67.73-79.06),
Q4 (male: 82.10-119.73; female: 79.07-119.73)

FIB-4 index:
Q1 (male: 27.90-59.91; female: 25.48-57.69),
Q2 (male: 59.91-69.63; female: 57.76-67.46),
Q3 (male: 69.67-81.87; female: 67.50-78.82),
Q4 (male 81.91- 119.56; female: 78.87-119.56)

The following cut-off values were defined for the afamin quartiles (Q) (mg/L) at FF4:

NAFLD liver fat score:
Q1 (male: 46.19-59.83; female: 46.19-56.72),
Q2 (male: 59.88-70.05; female: 56.72-66.65),
Q3 (male: 70.22-81.95; female: 66.67-78.24),
Q4 (male: 82.08-119.48; female: 78.27-119.48)

Hepatic steatosis index:
Q1 (male: 46.22-59.76; female: 46.22-56.81),
Q2 (male: 59.83-70.01; female: 56.86-67.00),
Q3 (male: 70.05-81.95; female: 67.02-78.31),
Q4 (male: 82.08-119.73; female: 78.39-119.73)

FIB-4 index:
Q1 (male: 27.90-59.91; female: 25.48-57.69),
Q2 (male: 59.91-69.63; female: 57.76-67.46),
Q3 (male: 69.67-81.87; female: 67.50-78.82),
Q4 (male 81.91- 119.56; female: 78.87-119.56)

Supplementary tables

Table S1 Cross-sectional associations between afamin and fatty liver index at KORA F4.

	Plasma afamin (per 10 mg/L increase)		**Sex-specific quartiles of serum afamin**				
			Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	p_{trend}
	beta (95% CI)	*p*	beta (95% CI)	beta (95% CI)	beta (95% CI)	beta (95% CI)	
Fatty liver index at F4							
Model 1	9.91 (9.33, 10.49)	**<0.001**	Ref	12.61 (9.90, 15.32)	23.87 (21.16, 26.58)	40.47 (37.76, 43.18)	**<0.001**
Model 2	5.52 (5.00, 6.03)	**<0.001**	Ref	7.96 (5.83, 10.09)	14.47 (12.29, 16.64)	23.40 (21.08, 25.71)	**<0.001**
Model 3	1.78 (1.47, 2.08)	**<0.001**	Ref	2.76 (1.56, 3.96)	4.27 (3.03, 5.52)	7.83 (6.48, 9.19)	**<0.001**
Model 4	1.73 (1.43, 2.04)	**<0.001**	Ref	2.59 (1.39, 3.79)	4.14 (2.89, 5.38)	7.57 (6.20, 8.94)	**<0.001**

Beta coefficients with their corresponding 95% confidence intervals (CI) and p-values were calculated using multivariable linear regression analysis. Significant differences ($p<0.05$) are highlighted in bold.

The following upper and lower limits were defined for the afamin quartiles (mg/L): Q1 (male: 46.42-59.91; female: 46.42-57.66), Q2 (male: 59.99-69.73; female: 57.67-67.44), Q3 (male: 69.73-81.87; female: 67.44-78.81), Q4 (male: 81.91-119.60; female: 78.82-119.60).

The following four models with adjustments for covariates were built: Model 1: crude. Model 2: adjusted for age, sex, smoking status, alcohol consumption, physical activity, hypertension, total cholesterol to high-density lipoprotein cholesterol ratio, diabetes, glomerular filtration rate, lipid-lowering medication, and regular use of non-steroidal anti-inflammatory drugs. Model 3: adjusted for model 2 and body mass index and triglycerides. Model 4: adjusted for model 3 and high-sensitivity C-reactive protein and interleukin-18.

CI, confidence interval; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; Ref, reference.

Table S2 Prospective associations of afamin levels at KORA F4 with fatty liver index at KORA FF4.

	Plasma afamin (per 10 mg/L increase)		**Sex-specific quartiles of serum afamin**				
			Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	p_{trend}
	beta (95% CI)	*p*	beta (95% CI)	beta (95% CI)	beta (95% CI)	beta (95% CI)	
Fatty liver index at FF4							
Model 1	-0.34 (-0.80, 0.12)	0.142	Ref	2.17 (0.39, 3.94)	0.90 (-0.93, 2.73)	-1.18 (-3.17, 0.81)	0.113
Model 2	-0.12 (-0.59, 0.36)	0.627	Ref	2.51 (0.70, 4.31)	1.42 (-0.45, 3.29)	-0.16 (-2.24, 1.93)	0.476
Model 3	0.05 (-0.42, 0.52)	0.828	Ref	2.49 (0.71, 4.27)	1.55 (-0.30, 3.40)	0.47 (-1.60, 2.53)	0.948
Model 4	0.12 (-0.36, 0.59)	0.633	Ref	2.68 (0.89, 4.47)	1.75 (-0.11, 3.61)	0.74 (-1.35, 2.83)	0.864

Beta coefficients with their corresponding 95% confidence intervals (CI) and p-values were calculated using multivariable linear regression analysis. Significant differences ($p<0.05$) are highlighted in bold.

The following upper and lower limits were defined for the afamin quarters (mg/L): Q1 (male: 46.42-59.76; female: 46.42-56.94), Q2 (male: 59.83-69.75; female: 56.94-66.45), Q3 (male: 69.79-81.58; female: 66.48-77.96), Q4 (male: 81.61-119.60; female: 77.97-119.60).

The following four models with adjustments for covariates were built: Model 1: adjusted for FLI index at F4. Model 2: adjusted for FLI index at F4 and age, sex, smoking status, alcohol consumption, physical activity, hypertension, total cholesterol to high-density lipoprotein cholesterol ratio, diabetes, glomerular filtration rate, lipid-lowering medication, and regular use of non-steroidal anti-inflammatory drugs. Model 3: adjusted for model 2 and body mass index and triglycerides. Model 4: adjusted for model 3 and high-sensitivity C-reactive protein and interleukin-18. CI, confidence interval; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; Ref, reference.

Supplementary figures

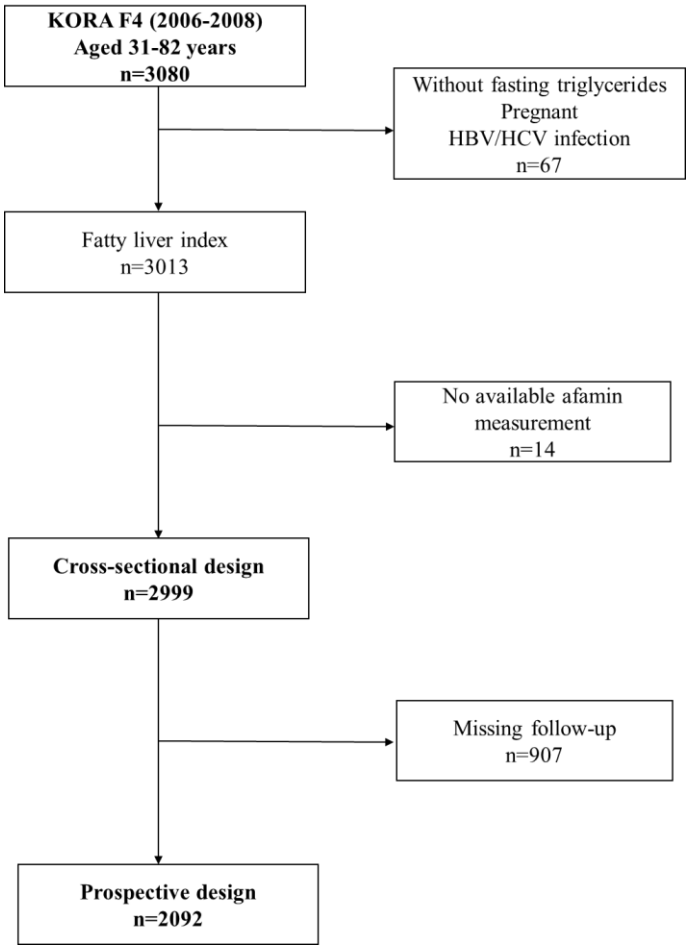

Fig. S1 Flow chart of the inclusion process of the study participants for the analyses of fatty liver index.
HBV, hepatitis B virus; HCV, hepatitis C virus; KORA, Cooperative Health Research in the Region of Augsburg.

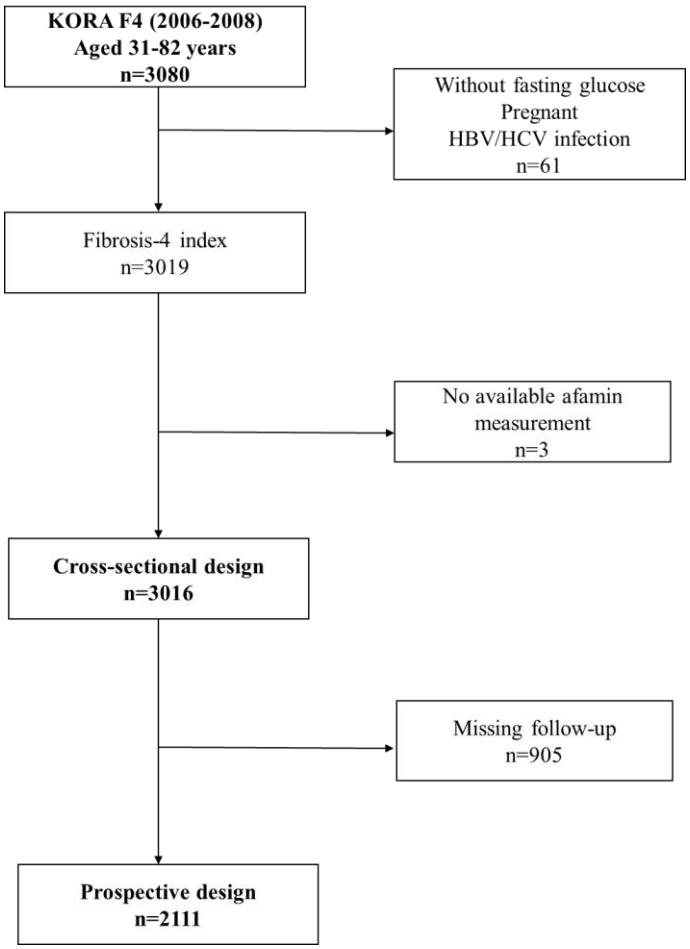

Fig. S2 Flow chart of the inclusion process of the study participants for the analyses of fibrosis-4 index.
HBV, hepatitis B virus; HCV, hepatitis C virus; KORA, Cooperative Health Research in the Region of Augsburg.