MATERIAL AND METHODS

Study population

The Study of Health in Pomerania (SHIP)

The presented data were derived from the population-based Study of Health in Pomerania (SHIP). The study design and recruitment strategy have been described elsewhere. In brief, a random cluster sample (age range 20 to 81 years) was drawn from the population of West Pomerania, the north-eastern coastal region of Germany (response 68.8%). At the baseline examination, echocardiography was restricted to 2,517 individuals (1,237 women) who were 45 years or older. We excluded participants with missing values for aortic valve sclerosis (n=230), hepatic ultrasound (n=70), alanine aminotransferase (ALT, n=20) or any of the covariables (n=64) as well individuals with reported history of liver cirrhosis (n=6), aortic stenosis (n=23), aortic valve replacement (n=5), positive findings for hepatitis B surface antigen or presence of anti-hepatitis C virus antibodies (n=28). The final analytical sample comprised 2,212 subjects. All study participants gave written informed consent. The study was approved by the ethics committee of the University of Greifswald and complies with the Declaration of Helsinki.

Interview, medical and laboratory examination

Information on age, sex, physical activity, smoking status (never, former or current smoker), alcohol consumption and medication use was gathered by trained and certificated staff during a standardized computer-assisted interview. Individuals
who participated in leisure time physical training during summer or winter for less than one hour per week were classified as being physically inactive. Assessment of average daily alcohol consumption (in grams ethanol per day) was based on data regarding weekday and weekend consumption of beer, wine, and spirits. Heavy alcohol consumption was defined as consumption of ≥ 20 g of ethanol per day for women and ≥ 40 g for men. Information on medication was categorized according to the anatomical–therapeutical–chemical (ATC) code.

All participants underwent an extensive standardized medical examination as described elsewhere in detail. After a 5 minutes rest period, systolic and diastolic blood pressures were measured three times at the right arm of seated participants using a digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan) with a 3 minutes interval between consecutive measurements. The mean of the second and third measurements was used for the present analyses. Waist circumference was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the participant standing comfortably with weight distributed evenly on both feet. Waist-to-height ratio was calculated as the waist circumference divided by height measured in centimeters. A non-fasting venous blood sample was obtained from all study participants between 07:00 a.m. and 04:00 p.m. while sitting. Serum aliquots were stored at -80°C. Total serum cholesterol and high-density lipoprotein (HDL) cholesterol were measured photometrically (Hitachi 704, Roche, Mannheim, Germany). Triglycerides were determined enzymatically using reagents from Roche Diagnostics (Hitachi 717, Roche Diagnostics, Mannheim, Germany). Glycosylated hemoglobin was determined by high-performance liquid chromatography (Bio-Rad...
Diamat, Munich, Germany). The creatinine concentration (Jaffé method) was determined on a Hitachi 717 (Roche Diagnostics, Mannheim, Germany). The estimated glomerular filtration rate (eGFR) was estimated according to the CKD-EPI formula and expressed in mL/min/1.73 m². Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGT) were measured photometrically (Hitachi 704 and 171, Roche Diagnostics, Mannheim, Germany) and expressed as µmol/l x s ([µmol/l x s] x 60 = IU/L). The inter-assay coefficients of variations for ALT, AST and GGT were 4.2%, 3.9% and 3.9%, respectively. Serum concentrations of high-sensitive C-reactive protein (hs-CRP) were measured with commercially available reagents on a BN II analyzer (Dade Behring, Eschborn, Germany). Serum ferritin levels were determined by an immunoturbidimetric assay (Cobas Micra plus, F. Hoffmann-La Roche Ltd, Basel, Switzerland). White blood cells (WBC) count was measured within 60 minutes either at the hospital laboratory in Greifswald with a Coulter Max M analyzer (Coulter Electronics, Miami, USA) or at the hospital laboratory in Stralsund with a Coulter T660 analyzer (Coulter Electronics, Miami, USA). All assays were performed according to the manufacturers’ recommendations by skilled technical personnel. In addition, the laboratory participates in official quarterly German external proficiency testing programs. Diabetes mellitus was defined as self-reported by the individual and/or use of antidiabetic medication and/or glycosylated hemoglobin ≥ 6.5% and/or non-fasting glucose ≥ 11.1 mmol/l.

Ultrasound examination of the Liver
Sonographic examinations of the liver were performed by trained physicians using a 5 MHz transducer and a high-resolution instrument (Vingmed VST Gateway Santa Clara, CA) as described previously.\textsuperscript{13} The sonographers were blinded to the participant’s clinical and laboratory characteristics. The presence of an ultrasonographic bright liver, with evident contrast between hepatic and renal parenchyma was interpreted as ultrasonographic evidence for hepatic steatosis.\textsuperscript{13, 14}

In addition to our primary definition of hepatic steatosis (based on ultrasonographic evidence of hepatic steatosis), we performed sensitivity analyses, using an alternative definition of hepatic steatosis based on i) ultrasonographic evidence of hepatic steatosis as described above and ii) increased serum ALT levels (≥75th age- and sex-specific percentile).\textsuperscript{5, 14} Specifically, we defined the following groups:

1. Reference group: Participants without hyperechogenic liver and with serum ALT levels below the 75th age- and sex-specific percentile (US- and ALT-; n=1,161 individuals).

2. Participants without hyperechogenic liver but with increased (>75th age- and sex-specific percentile) serum ALT levels (US- and ALT+; n=174 individuals).

3. Participants with hyperechogenic liver but with serum ALT levels below the 75th age- and sex-specific percentile (US+ and ALT-; n=532 individuals).

4. Participants with both, hyperechogenic liver and increased (>75th age- and sex-specific percentile) serum ALT levels (US+ and ALT+; n=345 individuals).
This definition was chosen as it may increase the sensitivity and reduce the risk of exposure-misclassification.\textsuperscript{15} In the \textbf{Supplementary Table I} we show the distribution of ALT (< or $\geq$75th percentile by sex and age-group) in the study sample.

\textbf{Echocardiography}

Two-dimensional, M-Mode and Doppler echocardiography were performed using the Vingmed CFM 800A system (GE Medical Systems, Waukesha, USA) as described in detail elsewhere.\textsuperscript{16} Aortic valve was scanned from the parasternal short and long axis as well as from the apical three and five chamber views. An abnormal irregular thickening and a focal or diffuse increase of the echogenicity of the leaflets with or without reduced systolic opening was defined as aortic valve sclerosis.\textsuperscript{16} Aortic stenosis was present if calcification of the leaflets with a reduced systolic opening and a Doppler gradient of at least 15 mm Hg were found.\textsuperscript{17} Measurements of left ventricular end-diastolic diameter (LVEDD), septal wall thickness (SWT), posterior wall thickness (PWT) and left atrial diameter were performed according to the guidelines of the American Society of Echocardiography.\textsuperscript{18} Left ventricular mass was calculated according to the formula: 

\[
LVM \ (g) = 0.8 \times \{1.04 \times [(LVEDD + SWT + PWT)^3 - LVEDD^3]\} + 0.6 \ \text{g}
\]

as described by Devereux and Reichek.\textsuperscript{19, 20} Left ventricular end-diastolic and end-systolic volumes (LVEDV, LVESV) were determined using the Teichholz\textsuperscript{21} equations: 

\[
LVEDV \ (mL) = (7/[2.4 + LVEDD]) \times LVEDD^3
\]

and 

\[
LVESV \ (mL) = (7/[2.4 + LVESD]) \times LVESD^3.
\]

The left ventricular ejection fraction (EF) was calculated as 

\[
EF \ (%) = (LVEDV - LVESV)/LVEDV.
\]

Certification examinations for inter-observer variations revealed an agreement with regard to aortic valve sclerosis of $>90\%$.\textsuperscript{16}
Statistical analysis

Data on quantitative characteristics are expressed as median (25th, 75th percentile). Data on qualitative characteristics are expressed as percent values. We selected factors known to be associated with hepatic steatosis and aortic valve sclerosis and performed regression-adjustment to control for confounding. Multiple logistic regressions were used to relate hepatic steatosis (exposure variable) to aortic valve sclerosis (outcome variable). Four models were estimated. The first model was adjusted for age and sex. The second model added waist-to-height ratio, smoking, alcohol consumption and physical activity. The third model also included systolic blood pressure, antihypertensive medication, total/HDL cholesterol ratio, lipid-lowering medication, glycosylated hemoglobin, antidiabetic medication, antiplatelet medication and estimated glomerular filtration rate (CKD EPI formula\textsuperscript{8}). Finally, we estimated an extra model (Model 4), which also included the inflammatory markers hs-CRP, ferritin and WBC.

To avoid a possibly strong influence of diabetes mellitus and heavy alcohol consumption on the outcome of our models, we performed supplementary analyses excluding individuals with one or both of these conditions. We also stratified the sample by age (< or =65 years old).

Furthermore, we performed sensitivity analyses to establish the robustness of our exposure definition, using an alternative definition of hepatic steatosis, based on ultrasonographic evidence and serum ALT levels, categorized in four groups as detailed above. We tested interactions of hepatic steatosis with sex, age and alcohol consumption on additive and multiplicative scales. Continuous covariables that did not follow a linear dose-response relation with aortic valve sclerosis were modeled
using restricted cubic splines with knots at the 5th, 50th, 65th and 95th percentiles.\textsuperscript{22} Since a moderate fraction (12.1\%) of the initial sample had missing values on at least one of the variables included in the multivariable regression analyses, we rerun our models using multiple imputation by chained equations.\textsuperscript{23} Stata 12.1 SE was used for statistical analyses (Stata Corporation, College Station, TX, USA).

REFERENCES


4. Ruckert IM, Heier M, Rathmann W, Baumeister SE, Doring A, Meisinger C. Association between markers of fatty liver disease and impaired glucose
regulation in men and women from the general population: The kora-f4-study.  
*PloS one.* 2011;6:e22932


concentration with inflammatory biomarkers - cross-sectional findings from the population-based study of health in pomerania. *Clinical endocrinology*. 2011;75:561-566


